ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2015 August 21; 21(31): 9233-9456

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

A peer-reviewed, online, open-access journal of gastroenterology and hepatology

Editorial Board

2014-2017

The *World Journal of Gastroenterology* Editorial Board consists of 1380 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 68 countries, including Algeria (2), Argentina (7), Australia (31), Austria (9), Belgium (11), Brazil (20), Brunei Darussalam (1), Bulgaria (2), Cambodia (1), Canada (26), Chile (4), China (165), Croatia (2), Cuba (1), Czech (6), Denmark (2), Egypt (9), Estonia (2), Finland (6), France (20), Germany (58), Greece (31), Guatemala (1), Hungary (15), Iceland (1), India (33), Indonesia (2), Iran (10), Ireland (9), Israel (18), Italy (194), Japan (151), Jordan (1), Kuwait (1), Lebanon (7), Lithuania (1), Malaysia (1), Mexico (11), Morocco (1), Netherlands (5), New Zealand (4), Nigeria (3), Norway (6), Pakistan (6), Poland (12), Portugal (8), Puerto Rico (1), Qatar (1), Romania (10), Russia (3), Saudi Arabia (2), Singapore (7), Slovenia (2), South Africa (1), South Korea (69), Spain (51), Sri Lanka (1), Sudan (1), Sweden (12), Switzerland (5), Thailand (7), Trinidad and Tobago (1), Tunisia (2), Turkey (55), United Kingdom (49), United States (181), Venezuela (1), and Vietnam (1).

Gordon Stanley Howarth, *Roseworthy*
Seungha Kang, *Brisbane*
Alfred King Lam, *Gold Coast*
Ian C Lawrance, *PerthFremantle*
Barbara Anne Leggett, *Brisbane*
Daniel A Lemberg, *Sydney*
Rupert W Leong, *Sydney*
Finlay A Macrae, *Victoria*
Vance Matthews, *Melbourne*
David L Morris, *Sydney*
Reme Mountifield, *Bedford Park*
Hans J Netter, *Melbourne*
Nam Q Nguyen, *Adelaide*
Liang Qiao, *Westmead*
Rajvinder Singh, *Adelaide*
Ross Cyril Smith, *StLeonards*
Kevin J Spring, *Sydney*
Debbie Trinder, *Fremantle*
Daniel R van Langenberg, *Box Hill*
David Ian Watson, *Adelaide*
Desmond Yip, *Garran*
Li Zhang, *Sydney*

Austria

Felix Aigner, *Innsbruck*
Gabriela A Berlakovich, *Vienna*
Herwig R Cerwenka, *Graz*
Peter Ferenci, *Wien*
Alfred Gangl, *Vienna*
Kurt Lenz, *Linz*
Markus Peck-Radosavljevic, *Vienna*
Markus Raderer, *Vienna*
Stefan Riss, *Vienna*

Belgium

Michael George Adler, *Brussels*
Benedicte Y De Winter, *Antwerp*
Mark De Ridder, *Jette*
Olivier Detry, *Liege*
Denis Dufrane Dufrane, *Brussels*
Sven M Francque, *Edegem*
Nikos Kotzampassakis, *Liège*
Geert KMM Robaeys, *Genk*
Xavier Sagaert, *Leuven*
Peter Starkel, *Brussels*
Eddie Wisse, *Keerbergen*

Brazil

SMP Balzan, *Santa Cruz do Sul*
JLF Caboclo, *Sao jose do rio preto*
Fábio Guilherme Campos, *Sao Paulo*
Claudia RL Cardoso, *Rio de Janeiro*
Roberto J Carvalho-Filho, *Sao Paulo*
Carla Daltro, *Salvador*
José Sebastiao dos Santos, *Ribeirao Preto*
Eduardo LR Mello, *Rio de Janeiro*
Sthela Maria Murad-Regadas, *Fortaleza*
Claudia PMS Oliveira, *Sao Paulo*
Júlio C Pereira-Lima, *Porto Alegre*
Marcos V Perini, *Sao Paulo*
Vietla Satyanarayana Rao, *Fortaleza*
Raquel Rocha, *Salvador*
AC Simoes e Silva, *Belo Horizonte*
Mauricio F Silva, *Porto Alefre*
Aytan Miranda Sipahi, *Sao Paulo*
Rosa Leonôra Salerno Soares, *Niterói*
Cristiane Valle Tovo, *Porto Alegre*
Eduardo Garcia Vilela, *Belo Horizonte*

Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*

Bulgaria

Tanya Kirilova Kadiyska, *Sofia*
Mihaela Petrova, *Sofia*

Cambodia

Francois Rouet, *Phnom Penh*

Canada

Brian Bressler, *Vancouver*
Frank J Burczynski, *Winnipeg*
Wangxue Chen, *Ottawa*
Francesco Crea, *Vancouver*
Mirko Diksic, *Montreal*
Jane A Foster, *Hamilton*
Hugh J Freeman, *Vancouver*
Shahrokh M Ghobadloo, *Ottawa*
Yuewen Gong, *Winnipeg*
Philip H Gordon, *Quebec*
Rakesh Kumar, *Edmonton*
Wolfgang A Kunze, *Hamilton*
Patrick Labonte, *Laval*
Zhikang Peng, *Winnipeg*
Jayadev Raju, *Ottawa*
Maitreyi Raman, *Calgary*
Giada Sebastiani, *Montreal*
Maida J Sewitch, *Montreal*
Eldon A Shaffer, *Alberta*
Christopher W Teshima, *Edmonton*
Jean Sévigny, *Québec*
Pingchang Yang, *Hamilton*
Pingchang Yang, *Hamilton*
Eric M Yoshida, *Vancouver*
Bin Zheng, *Edmonton*

Chile

Marcelo A Beltran, *La Serena*
Flavio Nervi, *Santiago*
Adolfo Parra-Blanco, *Santiago*
Alejandro Soza, *Santiago*

China

Zhao-Xiang Bian, *Hong Kong*
San-Jun Cai, *Shanghai*
Guang-Wen Cao, *Shanghai*
Long Chen, *Nanjing*
Ru-Fu Chen, *Guangzhou*
George G Chen, *Hong Kong*
Li-Bo Chen, *Wuhan*
Jia-Xu Chen, *Beijing*
Hong-Song Chen, *Beijing*
Lin Chen, *Beijing*
Yang-Chao Chen, *Hong Kong*
Zhen Chen, *Shanghai*
Ying-Sheng Cheng, *Shanghai*
Kent-Man Chu, *Hong Kong*
Zhi-Jun Dai, *Xi'an*
Jing-Yu Deng, *Tianjin*
Yi-Qi Du, *Shanghai*
Zhi Du, *Tianjin*
Hani El-Nezami, *Hong Kong*
Bao-Ying Fei, *Hangzhou*
Chang-Ming Gao, *Nanjing*
Jian-Ping Gong, *Chongqing*
Zuo-Jiong Gong, *Wuhan*
Jing-Shan Gong, *Shenzhen*
Guo-Li Gu, *Beijing*
Yong-Song Guan, *Chengdu*
Mao-Lin Guo, *Luoyang*
Jun-Ming Guo, *Ningbo*
Yan-Mei Guo, *Shanghai*
Xiao-Zhong Guo, *Shenyang*
Guo-Hong Han, *Xi'an*
Ming-Liang He, *Hong Kong*
Peng Hou, *Xi'an*
Zhao-Hui Huang, *Wuxi*
Feng Ji, *Hangzhou*
Simon Law, *Hong Kong*
Yu-Yuan Li, *Guangzhou*
Meng-Sen Li, *Haikou*
Shu-De Li, *Shanghai*
Zong-Fang Li, *Xi'an*
Qing-Quan Li, *Shanghai*
Kang Li, *Lasa*
Han Liang, *Tianjin*
Xing'e Liu, *Hangzhou*
Zheng-Wen Liu, *Xi'an*
Xiao-Fang Liu, *Yantai*
Bin Liu, *Tianjin*
Quan-Da Liu, *Beijing*
Hai-Feng Liu, *Beijing*
Fei Liu, *Shanghai*
Ai-Guo Lu, *Shanghai*
He-Sheng Luo, *Wuhan*
Xiao-Peng Ma, *Shanghai*
Yong Meng, *Shantou*
Ke-Jun Nan, *Xi'an*
Siew Chien Ng, *Hong Kong*
Simon SM Ng, *Hong Kong*
Zhao-Shan Niu, *Qingdao*
Bo-Rong Pan, *Xi'an*
Di Qu, *Shanghai*
Ying-Mo Shen, *Beijing*
Rui-Hua Shi, *Nanjing*
Bao-Min Shi, *Shanghai*
Xiao-Dong Sun, *Hangzhou*
Si-Yu Sun, *Shenyang*
Guang-Hong Tan, *Haikou*
Wen-Fu Tang, *Chengdu*
Anthony YB Teoh, *Hong Kong*
Wei-Dong Tong, *Chongqing*
Eric Tse, *Hong Kong*
Hong Tu, *Shanghai*
Rong Tu, *Haikou*
Jian-She Wang, *Shanghai*
Kai Wang, *Jinan*
Xiao-Ping Wang, *Xianyang*
Xiu-Yan Wang, *Shanghai*

Dao-Rong Wang, *Yangzhou*
De-Sheng Wang, *Xi'an*
Chun-You Wang, *Wuhan*
Ge Wang, *Chongqing*
Xi-Shan Wang, *Harbin*
Wei-hong Wang, *Beijing*
Zhen-Ning Wang, *Shenyang*
Wai Man Raymond Wong, *Hong Kong*
Chun-Ming Wong, *Hong Kong*
Jian Wu, *Shanghai*
Sheng-Li Wu, *Xi'an*
Wu-Jun Wu, *Xi'an*
Qing Xia, *Chengdu*
Yan Xin, *Shenyang*
Dong-Ping Xu, *Beijing*
Jian-Min Xu, *Shanghai*
Wei Xu, *Changchun*
Ming Yan, *Jinan*
Xin-Min Yan, *Kunming*
Yi-Qun Yan, *Shanghai*
Feng Yang, *Shanghai*
Yong-Ping Yang, *Beijing*
He-Rui Yao, *Guangzhou*
Thomas Yau, *Hong Kong*
Winnie Yeo, *Hong Kong*
Jing You, *Kunming*
Jian-Qing Yu, *Wuhan*
Ying-Yan Yu, *Shanghai*
Wei-Zheng Zeng, *Chengdu*
Zong-Ming Zhang, *Beijing*
Dian-Liang Zhang, *Qingdao*
Ya-Ping Zhang, *Shijiazhuang*
You-Cheng Zhang, *Lanzhou*
Jian-Zhong Zhang, *Beijing*
Ji-Yuan Zhang, *Beijing*
Hai-Tao Zhao, *Beijing*
Jian Zhao, *Shanghai*
Jian-Hong Zhong, *Nanning*
Ying-Qiang Zhong, *Guangzhou*
Ping-Hong Zhou, *Shanghai*
Yan-Ming Zhou, *Xiamen*
Tong Zhou, *Nanchong*
Li-Ming Zhou, *Chengdu*
Guo-Xiong Zhou, *Nantong*
Feng-Shang Zhu, *Shanghai*
Jiang-Fan Zhu, *Shanghai*
Zhao-Hui Zhu, *Beijing*

Croatia

Tajana Filipec Kanizaj, *Zagreb*
Mario Tadic, *Zagreb*

Cuba

Damian Casadesus, *Havana*

Czech

Jan Bures, *Hradec Kralove*
Marcela Kopacova, *Hradec Kralove*
Otto Kucera, *Hradec Kralove*
Marek Minarik, *Prague*
Pavel Soucek, *Prague*
Miroslav Zavoral, *Prague*

Denmark

Vibeke Andersen, *Odense*
E Michael Danielsen, *Copenhagen*

Egypt

Mohamed MM Abdel-Latif, *Assiut*
Hussein Atta, *Cairo*
Ashraf Elbahrawy, *Cairo*
Mortada Hassan El-Shabrawi, *Cairo*
Mona El Said El-Raziky, *Cairo*
Elrashdy M Redwan, *New Borg Alrab*
Zeinab Nabil Ahmed Said, *Cairo*
Ragaa HM Salama, *Assiut*
Maha Maher Shehata, *Mansoura*

Estonia

Margus Lember, *Tartu*
Tamara Vorobjova, *Tartu*

Finland

Marko Kalliomäki, *Turku*
Thomas Kietzmann, *Oulu*
Kaija-Leena Kolho, *Helsinki*
Eija Korkeila, *Turku*
Heikki Makisalo, *Helsinki*
Tanja Pessi, *Tampere*

France

Armando Abergel Clermont, *Ferrand*
Elie K Chouillard, *Polssy*
Pierre Cordelier, *Toulouse*
Pascal P Crenn, *Garches*
Catherine Daniel, *Lille*
Fanny Daniel, *Paris*
Cedric Dray, *Toulouse*
Benoit Foligne, *Lille*
Jean-Noel Freund, *Strasbourg*
Hervé Guillou, *Toulouse*
Nathalie Janel, *Paris*
Majid Khatib, *Bordeaux*
Jacques Marescaux, *Strasbourg*
Jean-Claude Marie, *Paris*
Driffa Moussata, *Pierre Benite*
Hang Nguyen, *Clermont-Ferrand*
Hugo Perazzo, *Paris*
Alain L Servin, *Chatenay-Malabry*
Chang Xian Zhang, *Lyon*

Germany

Stavros A Antoniou, *Monchengladbach*
Erwin Biecker, *Siegburg*
Hubert E Blum, *Freiburg*
Thomas Bock, *Berlin*
Katja Breitkopf-Heinlein, *Mannheim*
Elke Cario, *Essen*
Güralp Onur Ceyhan, *Munich*
Angel Cid-Arregui, *Heidelberg*
Michael Clemens Roggendorf, *München*
Christoph F Dietrich, *Bad Mergentheim*
Valentin Fuhrmann, *Hamburg*
Nikolaus Gassler, *Aachen*
Andreas Geier, *Wuerzburg*
Markus Gerhard, *Munich*
Anton Gillessen, *Muenster*
Thorsten Oliver Goetze, *Offenbach*
Daniel Nils Gotthardt, *Heidelberg*
Robert Grützmann, *Dresden*
Thilo Hackert, *Heidelberg*
Claus Hellerbrand, *Regensburg*
Harald Peter Hoensch, *Darmstadt*
Jens Hoeppner, *Freiburg*
Richard Hummel, *Muenster*
Jakob Robert Izbicki, *Hamburg*
Gernot Maximilian Kaiser, *Essen*
Matthias Kapischke, *Hamburg*
Michael Keese, *Frankfurt*
Andrej Khandoga, *Munich*
Jorg Kleeff, *Munich*
Alfred Koenigsrainer, *Tuebingen*
Peter Christopher Konturek, *Saalfeld*
Michael Linnebacher, *Rostock*
Stefan Maier, *Kaufbeuren*
Oliver Mann, *Hamburg*
Marc E Martignoni, *Munic*
Thomas Minor, *Bonn*
Oliver Moeschler, *Osnabrueck*
Jonas Mudter, *Eutin*
Sebastian Mueller, *Heidelberg*
Matthias Ocker, *Berlin*
Andreas Ommer, *Essen*
Albrecht Piiper, *Frankfurt*
Esther Raskopf, *Bonn*
Christoph Reichel, *Bad Brückenau*
Elke Roeb, *Giessen*
Udo Rolle, *Frankfurt*
Karl-Herbert Schafer, *Zweibrücken*
Peter Schemmer, *Heidelberg*
Andreas G Schreyer, *Regensburg*
Manuel A Silva, *Penzberg*
Georgios C Sotiropoulos, *Essen*
Ulrike S Stein, *Berlin*
Dirk Uhlmann, *Leipzig*
Michael Weiss, *Halle*
Hong-Lei Weng, *Mannheim*
Karsten Wursthorn, *Hamburg*

Greece

Alexandra Alexopoulou, *Athens*
Nikolaos Antonakopoulos, *Athens*
Stelios F Assimakopoulos, *Patras*
Grigoris Chatzimavroudis, *Thessaloniki*
Evangelos Cholongitas, *Thessaloniki*
Gregory Christodoulidis, *Larisa*
George N Dalekos, *Larissa*
Urania Georgopoulou, *Athens*
Eleni Gigi, *Thessaloniki*
Stavros Gourgiotis, *Athens*
Leontios J Hadjileontiadis, *Thessaloniki*
Thomas Hyphantis, *Ioannina*
Ioannis Kanellos, *Thessaloniki*
Stylianos Karatapanis, *Rhodes*
Michael Koutsilieris, *Athens*
Spiros D Ladas, *Athens*

Theodoros K Liakakos, *Athens*
Emanuel K Manesis, *Athens*
Spilios Manolakopoulos, *Athens*
Gerassimos John Mantzaris, *Athens*
Athanasios D Marinis, *Piraeus*
Nikolaos Ioannis Nikiteas, *Athens*
Konstantinos X Papamichael, *Athens*
George Sgourakis, *Athens*
Konstantinos C Thomopoulos, *Patras*
Konstantinos Triantafyllou, *Athens*
Christos Triantos, *Patras*
Georgios Zacharakis, *Athens*
Petros Zezos, *Alexandroupolis*
Demosthenes E Ziogas, *Ioannina*

Guatemala

Carlos Maria Parellada, *Guatemala*

Hungary

Mihaly Boros, *Szeged*
Tamás Decsi, *Pécs*
Gyula Farkas, *Szeged*
Andrea Furka, *Debrecen*
Y vette Mandi, *Szeged*
Peter L Lakatos, *Budapest*
Pal Miheller, *Budapest*
Tamás Molnar, *Szeged*
Attila Olah, *Gyor*
Maria Papp, *Debrecen*
Zoltan Rakonczay, *Szeged*
Ferenc Sipos, *Budapest*
Miklós Tanyi, *Debrecen*
Tibor Wittmann, *Szeged*

Iceland

Tryggvi Bjorn Stefánsson, *Reykjavík*

India

Brij B Agarwal, *New Delhi*
Deepak N Amarapurkar, *Mumbai*
Shams ul Bari, *Srinagar*
Sriparna Basu, *Varanasi*
Runu Chakravarty, *Kolkata*
Devendra C Desai, *Mumbai*
Nutan D Desai, *Mumbai*
Suneela Sunil Dhaneshwar, *Pune*
Radha K Dhiman, *Chandigarh*
Pankaj Garg, *Mohali*
Uday C Ghoshal, *Lucknow*
Kalpesh Jani, *Vadodara*
Premashis Kar, *New Delhi*
Jyotdeep Kaur, *Chandigarh*
Rakesh Kochhar, *Chandigarh*
Pradyumna K Mishra, *Mumbai*
Asish K Mukhopadhyay, *Kolkata*
Imtiyaz Murtaza, *Srinagar*
P Nagarajan, *New Delhi*
Samiran Nundy, *Delhi*
Gopal Pande, *Hyderabad*
Benjamin Perakath, *Vellore*
Arun Prasad, *New Delhi*
D Nageshwar Reddy, *Hyderabad*
Lekha Saha, *Chandigarh*
Sundeep Singh Saluja, *New Delhi*
Mahesh Prakash Sharma, *New Delhi*
Sadiq Saleem Sikora, *Bangalore*
Sarman Singh, *New Delhi*
Rajeev Sinha, *Jhansi*
Rupjyoti Talukdar, *Hyderabad*
Rakesh Kumar Tandon, *New Delhi*
Narayanan Thirumoorthy, *Coimbatore*

Indonesia

David Handojo Muljono, *Jakarta*
Andi Utama, *Jakarta*

Iran

Arezoo Aghakhani, *Tehran*
Seyed Mohsen Dehghani, *Shiraz*
Ahad Eshraghian, *Shiraz*
Hossein Khedmat, *Tehran*
Sadegh Massarrat, *Tehran*
Marjan Mohammadi, *Tehran*
Roja Rahimi, *Tehran*
Farzaneh Sabahi, *Tehran*
Majid Sadeghizadeh, *Tehran*
Farideh Siavoshi, *Tehran*

Ireland

Gary Alan Bass, *Dublin*
David J Brayden, *Dublin*
Ronan A Cahill, *Dublin*
Glen A Doherty, *Dublin*
Liam J Fanning, *Cork*
Barry Philip McMahon, *Dublin*
RossMcManus, *Dublin*
Dervla O'Malley, *Cork*
Sinead M Smith, *Dublin*

Israel

Dan Carter, *Ramat Gan*
Jorge-Shmuel Delgado, *Metar*
Eli Magen, *Ashdod*
Nitsan Maharshak, *Tel Aviv*
Shaul Mordechai, *Beer Sheva*
Menachem Moshkowitz, *Tel Aviv*
William Bahij Nseir, *Nazareth*
Shimon Reif, *Jerusalem*
Ram Reifen, *Rehovot*
Ariella Bar-Gil Shitrit, *Jerusalem*
Noam Shussman, *Jerusalem*
Igor Sukhotnik, *Haifa*
Nir Wasserberg, *Petach Tiqwa*
Jacob Yahav, *Rehovot*
Doron Levi Zamir, *Gedera*
Shira Zelber-Sagi, *Haifa*
Romy Zemel, *Petach-Tikva*

Italy

Ludovico Abenavoli, *Catanzaro*
Luigi Elio Adinolfi, *Naples*
Carlo Virginio Agostoni, *Milan*
Anna Alisi, *Rome*
Piero Luigi Almasio, *Palermo*
Donato Francesco Altomare, *Bari*
Amedeo Amedei, *Florence*
Pietro Andreone, *Bologna*
Imerio Angriman, *Padova*
Vito Annese, *Florence*
Paolo Aurello, *Rome*
Salavtore Auricchio, *Naples*
Gian Luca Baiocchi, *Brescia*
Gianpaolo Balzano, *Milan*
Antonio Basoli, *Rome*
Gabrio Bassotti, *San Sisto*
Mauro Bernardi, *Bologna*
Alberto Biondi, *Rome*
Ennio Biscaldi, *Genova*
Massimo Bolognesi, *Padua*
Luigi Bonavina, *Milano*
Aldo Bove, *Chieti*
Raffaele Bruno, *Pavia*
Luigi Brusciano, *Napoli*
Giuseppe Cabibbo, *Palermo*
Carlo Calabrese, *Bologna*
Daniele Calistri, *Meldola*
Vincenza Calvaruso, *Palermo*
Lorenzo Camellini, *Reggio Emilia*
Marco Candela, *Bologna*
Raffaele Capasso, *Naples*
Lucia Carulli, *Modena*
Renato David Caviglia, *Rome*
Luigina Cellini, *Chieti*
Giuseppe Chiarioni, *Verona*
Claudio Chiesa, *Rome*
Michele Cicala, *Roma*
Rachele Ciccocioppo, *Pavia*
Sandro Contini, *Parma*
Gaetano Corso, *Foggia*
Renato Costi, *Parma*
Alessandro Cucchetti, *Bologna*
Rosario Cuomo, *Napoli*
Giuseppe Currò, *Messina*
Paola De Nardi, *Milano*
Giovanni D De Palma, *Naples*
Raffaele De Palma, *Napoli*
Giuseppina De Petro, *Brescia*
Valli De Re, *Aviano*
Paolo De Simone, *Pisa*
Giuliana Decorti, *Trieste*
Emanuele Miraglia del Giudice, *Napoli*
Isidoro Di Carlo, *Catania*
Matteo Nicola Dario Di Minno, *Naples*
Massimo Donadelli, *Verona*
Mirko D'Onofrio, *Verona*
Maria Pina Dore, *Sassari*
Luca Elli, *Milano*
Massimiliano Fabozzi, *Aosta*
Massimo Falconi, *Ancona*
Ezio Falletto, *Turin*
Silvia Fargion, *Milan*
Matteo Fassan, *Verona*
Gianfranco Delle Fave, *Roma*
Alessandro Federico, *Naples*
Francesco Feo, *Sassari*
Davide Festi, *Bologna*
Natale Figura, *Siena*
Vincenzo Formica, *Rome*
Mirella Fraquelli, *Milan*

Marzio Frazzoni, *Modena*
Walter Fries, *Messina*
Gennaro Galizia, *Naples*
Andrea Galli, *Florence*
Matteo Garcovich, *Rome*
Eugenio Gaudio, *Rome*
Paola Ghiorzo, *Genoa*
Edoardo G Giannini, *Genova*
Luca Gianotti, *Monza*
Maria Cecilia Giron, *Padova*
Alberto Grassi, *Rimini*
Gabriele Grassi, *Trieste*
Francesco Greco, *Bergamo*
Luigi Greco, *Naples*
Antonio Grieco, *Rome*
Fabio Grizzi, *Rozzano*
Laurino Grossi, *Pescara*
Simone Guglielmetti, *Milan*
Tiberiu Hershcovici, *Jerusalem*
Calogero Iacono, *Verona*
Enzo Ierardi, *Bari*
Amedeo Indriolo, *Bergamo*
Raffaele Iorio, *Naples*
Paola Iovino, *Salerno*
Angelo A Izzo, *Naples*
Loreta Kondili, *Rome*
Filippo La Torre, *Rome*
Giuseppe La Torre, *Rome*
Giovanni Latella, *L'Aquila*
Salvatore Leonardi, *Catania*
Massimo Libra, *Catania*
Anna Licata, *Palermo*
C armela Loguercio, *Naples*
Amedeo Lonardo, *Modena*
Carmelo Luigiano, *Catania*
Francesco Luzza, *Catanzaro*
Giovanni Maconi, *Milano*
Antonio Macrì, *Messina*
Mariano Malaguarnera, *Catania*
Francesco Manguso, *Napoli*
Tommaso Maria Manzia, *Rome*
Daniele Marrelli, *Siena*
Gabriele Masselli, *Rome*
Sara Massironi, *Milan*
Giuseppe Mazzarella, *Avellino*
Michele Milella, *Rome*
Giovanni Milito, *Rome*
Antonella d'Arminio Monforte, *Milan*
Fabrizio Montecucco, *Genoa*
Giovanni Monteleone, *Rome*
Mario Morino, *Torino*
Vincenzo La Mura, *Milan*
Gerardo Nardone, *Naples*
Riccardo Nascimbeni, *Brescia*
Gabriella Nesi, *Florence*
Giuseppe Nigri, *Rome*
Erica Novo, *Turin*
Veronica Ojetti, *Rome*
Michele Orditura, *Naples*
Fabio Pace, *Seriate*
Lucia Pacifico, *Rome*
Omero Alessandro Paoluzi, *Rome*
Valerio Pazienza, *San Giovanni Rotondo*
Rinaldo Pellicano, *Turin*
Adriano M Pellicelli, *Rome*
Nadia Peparini, *Ciampino*
Mario Pescatori, *Rome*
Antonio Picardi, *Rome*
Alberto Pilotto, *Padova*
Alberto Piperno, *Monza*
Anna Chiara Piscaglia, *Rome*
Maurizio Pompili, *Rome*
Francesca Romana Ponziani, *Rome*
Cosimo Prantera, *Rome*
Girolamo Ranieri, *Bari*
Carlo Ratto, *Tome*
Barbara Renga, *Perugia*
Alessandro Repici, *Rozzano*
Maria Elena Riccioni, *Rome*
Lucia Ricci-Vitiani, *Rome*
Luciana Rigoli, *Messina*
Mario Rizzetto, *Torino*
Ballarin Roberto, *Modena*
Roberto G Romanelli, *Florence*
Claudio Romano, *Messina*
Luca Roncucci, *Modena*
Cesare Ruffolo, *Treviso*
L ucia Sacchetti, *Napoli*
Rodolfo Sacco, *Pisa*
Lapo Sali, *Florence*
Romina Salpini, *Rome*
Giulio Aniello, *Santoro Treviso*
Armando Santoro, *Rozzano*
Edoardo Savarino, *Padua*
Marco Senzolo, *Padua*
Annalucia Serafino, *Rome*
Giuseppe S Sica, *Rome*
Pierpaolo Sileri, *Rome*
Cosimo Sperti, *Padua*
Vincenzo Stanghellini, *Bologna*
Cristina Stasi, *Florence*
Gabriele Stocco, *Trieste*
Roberto Tarquini, *Florence*
Mario Testini, *Bari*
Guido Torzilli, *Milan*
Guido Alberto Massimo, *Tiberio Brescia*
Giuseppe Toffoli, *Aviano*
Alberto Tommasini, *Trieste*
Francesco Tonelli, *Florence*
Cesare Tosetti Porretta, *Terme*
Lucio Trevisani, *Cona*
Guglielmo M Trovato, *Catania*
Mariapia Vairetti, *Pavia*
Luca Vittorio Valenti, *Milano*
Mariateresa T Ventura, *Bari*
Giuseppe Verlato, *Verona*
Marco Vivarelli, *Ancona*
Giovanni Li Volti, *Catania*
Giuseppe Zanotti, *Padua*
Vincenzo Zara, *Lecce*
Gianguglielmo Zehender, *Milan*
Anna Linda Zignego, *Florence*
Rocco Antonio Zoccali, *Messina*
Angelo Zullo, *Rome*

Japan

Yasushi Adachi, *Sapporo*
Takafumi Ando, *Nagoya*
Masahiro Arai, *Tokyo*
Makoto Arai, *Chiba*
Takaaki Arigami, *Kagoshima*
Itaru Endo,*Yokohama*
Munechika Enjoji, *Fukuoka*
Shunji Fujimori, *Tokyo*
Yasuhiro Fujino, *Akashi*
Toshiyoshi Fujiwara, *Okayama*
Yosuke Fukunaga, *Tokyo*
Toshio Fukusato, *Tokyo*
Takahisa Furuta, *Hamamatsu*
Osamu Handa, *Kyoto*
Naoki Hashimoto, *Osaka*
Yoichi Hiasa, *Toon*
Masatsugu Hiraki, *Saga*
Satoshi Hirano, *Sapporo*
Keiji Hirata, *Fukuoka*
Toru Hiyama, *Higashihiroshima*
Akira Hokama, *Nishihara*
Shu Hoteya, *Tokyo*
Masao Ichinose, *Wakayama*
Tatsuya Ide, *Kurume*
Masahiro Iizuka, *Akita*
Toshiro Iizuka, *Tokyo*
Kenichi Ikejima, *Tokyo*
Tetsuya Ikemoto, *Tokushima*
Hiroyuki Imaeda, *Saitama*
Atsushi Imagawa, *Kan-onji*
Hiroo Imazu, *Tokyo*
Shuji Isaji, *Tsu*
Toru Ishikawa, *Niigata*
Toshiyuki Ishiwata, *Tokyo*
Soichi Itaba, *Kitakyushu*
Yoshiaki Iwasaki, *Okayama*
Tatehiro Kagawa, *Isehara*
Satoru Kakizaki, *Maebashi*
Naomi Kakushima, *Shizuoka*
Terumi Kamisawa, *Tokyo*
Akihide Kamiya, *Isehara*
Osamu Kanauchi, *Tokyo*
Tatsuo Kanda, *Chiba*
Shin Kariya, *Okayama*
Shigeyuki Kawa, *Matsumoto*
Takumi Kawaguchi, *Kurume*
Takashi Kawai, *Tokyo*
Soo Ryang Kim, *Kobe*
Shinsuke Kiriyama, *Gunma*
Tsuneo Kitamura, *Urayasu*
Masayuki Kitano, *Osakasayama*
Hirotoshi Kobayashi, *Tokyo*
Hironori Koga, *Kurume*
Takashi Kojima, *Sapporo*
Satoshi Kokura, *Kyoto*
Shuhei Komatsu, *Kyoto*
Tadashi Kondo, *Tokyo*
Yasuteru Kondo, *Sendai*
Yasuhiro Kuramitsu, *Yamaguchi*
Yukinori Kurokawa, *Osaka*
Shin Maeda, *Yokohama*
Koutarou Maeda, *Toyoake*
Hitoshi Maruyama, *Chiba*
Atsushi Masamune, *Sendai*
Hiroyuki Matsubayashi, *Suntogun*
Akihisa Matsuda, *Inzai*
Hirofumi Matsui, *Tsukuba*
Akira Matsumori, *Kyoto*
Yoichi Matsuo, *Nagoya*
Y Matsuzaki, *Ami*
Toshihiro Mitaka, *Sapporo*
Kouichi Miura, *Akita*
Shinichi Miyagawa, *Matumoto*
Eiji Miyoshi, *Suita*
Toru Mizuguchi, *Sapporo*
Nobumasa Mizuno, *Nagoya*
Zenichi Morise, *Nagoya*

Tomohiko Moriyama, *Fukuoka*
Kunihiko Murase, *Tusima*
Michihiro Mutoh, *Tsukiji*
Akihito Nagahara, *Tokyo*
Hikaru Nagahara, *Tokyo*
Hidenari Nagai, *Tokyo*
Koichi Nagata, *Shimotsuke-shi*
Masaki Nagaya, *Kawasaki*
Hisato Nakajima, *Nishi-Shinbashi*
Toshifusa Nakajima, *Tokyo*
Hiroshi Nakano, *Kawasaki*
Hiroshi Nakase, *Kyoto*
Toshiyuki Nakayama, *Nagasaki*
Takahiro Nakazawa, *Nagoya*
Shoji Natsugoe, *Kagoshima City*
Tsutomu Nishida, *Suita*
Shuji Nomoto, *Naogya*
Sachiyo Nomura, *Tokyo*
Takeshi Ogura, *Takatsukishi*
Nobuhiro Ohkohchi, *Tsukuba*
Toshifumi Ohkusa, *Kashiwa*
Hirohide Ohnishi, *Akita*
Teruo Okano, *Tokyo*
Satoshi Osawa, *Hamamatsu*
Motoyuki Otsuka, *Tokyo*
Michitaka Ozaki, *Sapporo*
Satoru Saito, *Yokohama*
Chouhei Sakakura, *Kyoto*
Naoaki Sakata, *Sendai*
Ken Sato, *Maebashi*
Toshiro Sato, *Tokyo*
Tomoyuki Shibata, *Toyoake*
H Shimada, *Tokyo*
Tomohiko Shimatani, *Kure*
Yukihiro Shimizu, *Nanto*
Tadashi Shimoyama, *Hirosaki*
Masayuki Sho, *Nara*
Ikuo Shoji, *Kobe*
Atsushi Sofuni, *Tokyo*
Takeshi Suda, *Niigata*
M Sugimoto, *Hamamatsu*
Ken Sugimoto, *Hamamatsu*
Haruhiko Sugimura, *Hamamatsu*
Shoichiro Sumi, *Kyoto*
Hidekazu Suzuki, *Tokyo*
Masahiro Tajika, *Nagoya*
Hitoshi Takagi, *Takasaki*
Toru Takahashi, *Niigata*
Yoshihisa Takahashi, *Tokyo*
Shinsuke Takeno, *Fukuoka*
Akihiro Tamori, *Osaka*
Kyosuke Tanaka, *Tsu*
Shinji Tanaka, *Hiroshima*
Atsushi Tanaka, *Tokyo*
Yasuhito Tanaka, *Nagoya*
Shinji Tanaka, *Tokyo*
Minoru Tomizawa, *Yotsukaido City*
Kyoko Tsukiyama-Kohara, *Kagoshima*
Takuya Watanabe, *Niigata*
Kazuhiro Watanabe, *Sendai*
Satoshi Yamagiwa, *Niigata*
Takayuki Yamamoto, *Yokkaichi*
Hiroshi Yamamoto, *Otsu*
Kosho Yamanouchi, *Nagasaki*
Ichiro Yasuda, *Gifu*
Yutaka Yata, *Maebashi-city*
Shin-ichi Yokota, *Sapporo*
Norimasa Yoshida, *Kyoto*
Hiroshi Yoshida, *Tama-City*
Hitoshi Yoshiji, *Kashihara*
Kazuhiko Yoshimatsu, *Tokyo*
Kentaro Yoshioka, *Toyoake*
Nobuhiro Zaima, *Nara*

Jordan
Khaled Ali Jadallah, *Irbid*

Kuwait
Islam Khan, *Kuwait*

Lebanon
Bassam N Abboud, *Beirut*
Kassem A Barada, *Beirut*
Marwan Ghosn, *Beirut*
Iyad A Issa, *Beirut*
Fadi H Mourad, *Beirut*
AIa Sharara, *Beirut*
Rita Slim, *Beirut*

Lithuania
Antanas Mickevicius, *Kaunas*

Malaysia
Huck Joo Tan, *Petaling Jaya*

Mexico
Richard A Awad, *Mexico City*
Carlos R Camara-Lemarroy, *Monterrey*
Norberto C Chavez-Tapia, *Mexico City*
Wolfgang Gaertner, *Mexico City*
Diego Garcia-Compean, *Monterrey*
Arturo Panduro, *Guadalajara*
OT Teramoto-Matsubara, *Mexico City*
Felix Tellez-Avila, *Mexico City*
Omar Vergara-Fernandez, *Mexico City*
Saúl Villa-Trevino, *Cuidad de México*

Morocco
Samir Ahboucha, *Khouribga*

Netherlands
Robert J de Knegt, *Rotterdam*
Tom Johannes Gerardus Gevers, *Nijmegen*
Menno Hoekstra, *Leiden*
BW Marcel Spanier, *Arnhem*
Karel van Erpecum, *Utrecht*

New Zealand
Leo K Cheng, *Auckland*
Andrew Stewart Day, *Christchurch*
Jonathan Barnes Koea, *Auckland*
Max Petrov, *Auckland*

Nigeria
Olufunmilayo Adenike Lesi, *Lagos*
Jesse Abiodun Otegbayo, *Ibadan*
Stella Ifeanyi Smith, *Lagos*

Norway
Trond Berg, *Oslo*
Trond Arnulf Buanes, *Krokkleiva*
Thomas de Lange, *Rud*
Magdy El-Salhy, *Stord*
Rasmus Goll, *Tromso*
Dag Arne Lihaug Hoff, *Aalesund*

Pakistan
Zaigham Abbas, *Karachi*
Usman A Ashfaq, *Faisalabad*
Muhammad Adnan Bawany, *Hyderabad*
Muhammad Idrees, *Lahore*
Saeed Sadiq Hamid, *Karachi*
Yasir Waheed, *Islamabad*

Poland
Thomas Brzozowski, *Cracow*
Magdalena Chmiela, *Lodz*
Krzysztof Jonderko, *Sosnowiec*
Anna Kasicka-Jonderko, *Sosnowiec*
Michal Kukla, *Katowice*
Tomasz Hubert Mach, *Krakow*
Agata Mulak, *Wroclaw*
Danuta Owczarek, *Kraków*
Piotr Socha, *Warsaw*
Piotr Stalke, *Gdansk*
Julian Teodor Swierczynski, *Gdansk*
Anna M Zawilak-Pawlik, *Wroclaw*

Portugal
Marie Isabelle Cremers, *Setubal*
Ceu Figueiredo, *Porto*
Ana Isabel Lopes, *LIsbon*
M Paula Macedo, *Lisboa*
Ricardo Marcos, *Porto*
Rui T Marinho, *Lisboa*
Guida Portela-Gomes, *Estoril*
Filipa F Vale, *Lisbon*

Puerto Rico
Caroline B Appleyard, *Ponce*

Qatar
Abdulbari Bener, *Doha*

Romania
Mihai Ciocirlan, *Bucharest*
Dan LucianDumitrascu, *Cluj-Napoca*
Carmen Fierbinteanu-Braticevici, *Bucharest*

Romeo G Mihaila, *Sibiu*
Lucian Negreanu, *Bucharest*
Adrian Saftoiu, *Craiova*
Andrada Seicean, *Cluj-Napoca*
Ioan Sporea, *Timisoara*
Letiţia Adela Maria Streba, *Craiova*
Anca Trifan, *Iasi*

Russia

Victor Pasechnikov, *Stavropol*
Vasiliy Ivanovich Reshetnyak, *Moscow*
Vitaly Skoropad, *Obninsk*

Saudi Arabia

Abdul-Wahed N Meshikhes, *Dammam*
M Ezzedien Rabie, *Khamis Mushait*

Singapore

Brian KP Goh, *Singapore*
Richie Soong, *Singapore*
Ker-Kan Tan, *Singapore*
Kok-Yang Tan, *Singapore*
Yee-Joo Tan, *Singapore*
Mark Wong, *Singapore*
Hong Ping Xia, *Singapore*

Slovenia

Matjaz Homan, *Ljubljana*
Martina Perse, *Ljubljana*

South Korea

Sang Hoon Ahn, *Seoul*
Seung Hyuk Baik, *Seoul*
Soon Koo Baik, *Wonju*
Soo-Cheon Chae, *Iksan*
Byung-Ho Choe, *Daegu*
Suck Chei Choi, *Iksan*
Hoon Jai Chun, *Seoul*
Yeun-Jun Chung, *Seoul*
Young-Hwa Chung, *Seoul*
Ki-Baik Hahm, *Seongnam*
Sang Young Han, *Busan*
Seok Joo Han, *Seoul*
Seung-Heon Hong, *Iksan*
Jin-Hyeok Hwang, *Seoungnam*
Jeong Won Jang, *Seoul*
Jin-Young Jang, *Seoul*
Dae-Won Jun, *Seoul*
Young Do Jung, *Kwangju*
Gyeong Hoon Kang, *Seoul*
Sung-Bum Kang, *Seoul*
Koo Jeong Kang, *Daegu*
Ki Mun Kang, *Jinju*
Chang Moo Kang, *Seodaemun-gu*
Gwang Ha Kim, *Busan*
Sang Soo Kim, *Goyang-si*
Jin Cheon Kim, *Seoul*
Tae Il Kim, *Seoul*
Jin Hong Kim, *Suwon*
Kyung Mo Kim, *Seoul*
Kyongmin Kim, *Suwon*
Hyung-Ho Kim, *Seongnam*
Seoung Hoon Kim, *Goyang*
Sang Il Kim, *Seoul*
Hyun-Soo Kim, *Wonju*
Jung Mogg Kim, *Seoul*
Dong Yi Kim, *Gwangju*
Kyun-Hwan Kim, *Seoul*
Jong-Han Kim, *Ansan*
Sang Wun Kim, *Seoul*
Ja-Lok Ku, *Seoul*
Kyu Taek Lee, *Seoul*
Hae-Wan Lee, *Chuncheon*
Inchul Lee, *Seoul*
Jung Eun Lee, *Seoul*
Sang Chul Lee, *Daejeon*
Song Woo Lee, *Ansan-si*
Hyuk-Joon Lee, *Seoul*
Seong-Wook Lee, *Yongin*
Kil Yeon Lee, *Seoul*
Jong-Inn Lee, *Seoul*
Kyung A Lee, *Seoul*
Jong-Baeck Lim, *Seoul*
Eun-Yi Moon, *Seoul*
SH Noh, *Seoul*
Seung Woon Paik, *Seoul*
Won Sang Park, *Seoul*
Sung-Joo Park, *Iksan*
Kyung Sik Park, *Daegu*
Se Hoon Park, *Seoul*
Yoonkyung Park, *Gwangju*
Seung-Wan Ryu, *Daegu*
Il Han Song, *Cheonan*
Myeong Jun Song, *Daejeon*
Yun Kyoung Yim, *Daejeon*
Dae-Yeul Yu *Daejeon*

Spain

Mariam Aguas, *Valencia*
Raul J Andrade, *Málaga*
Antonio Arroyo, *Elche*
Josep M Bordas, *Barcelona*
Lisardo Boscá, *Madrid*
Ricardo Robles Campos, *Murcia*
Jordi Camps, *Reus*
Carlos Cervera *Barcelona*
Alfonso Clemente, *Granada*
Pilar Codoner-Franch, *Valencia*
Fernando J Corrales, *Pamplona*
Fermin Sánchez de Medina, *Granada*
Alberto Herreros de Tejada, *Majadahonda*
Enrique de-Madaria, *Alicante*
JE Dominguez-Munoz, *Santiago de Compostela*
Vicente Felipo, *Valencia*
CM Fernandez-Rodriguez, *Madrid*
Carmen Frontela-Saseta, *Murcia*
Julio Galvez, *Granada*
Maria Teresa García, *Vigo*
MI Garcia-Fernandez, *Málaga*
Emilio Gonzalez-Reimers, *La Laguna*
Marcel Jimenez, *Bellaterra*
Angel Lanas, *Zaragoza*
Juan Ramón Larrubia, *Guadalajara*
Antonio Lopez-Sanroman, *Madrid*
Vicente Lorenzo-Zuniga, *Badalona*
Alfredo J Lucendo, *Tomelloso*
Vicenta Soledad Martinez-Zorzano, *Vigo*
José Manuel Martin-Villa, *Madrid*
Julio Mayol, *Madrid*
Manuel Morales-Ruiz, *Barcelona*
Alfredo Moreno-Egea, *Murcia*
Albert Pares, *Barcelona*
Maria Pellise, *Barcelona*
José Perea, *Madrid*
Miguel Angel Plaza, *Zaragoza*
María J Pozo, *Cáceres*
Enrique Quintero, *La Laguna*
Jose M Ramia, *Madrid*
Francisco Rodriguez-Frias, *Barcelona*
Silvia Ruiz-Gaspa, *Barcelona*
Xavier Serra-Aracil, *Barcelona*
Vincent Soriano, *Madrid*
Javier Suarez, *Pamplona*
Carlos Taxonera, *Madrid*
M Isabel Torres, *Jaén*
Manuel Vazquez-Carrera, *Barcelona*
Benito Velayos, *Valladolid*
Silvia Vidal, *Barcelona*

Sri Lanka

Arjuna Priyadarsin De Silva, *Colombo*

Sudan

Ishag Adam, *Khartoum*

Sweden

Roland G Andersson, *Lund*
Bergthor Björnsson, *Linkoping*
Johan Christopher Bohr, *Örebro*
Mauro D'Amato, *Stockholm*
Thomas Franzen, *Norrkoping*
Evangelos Kalaitzakis, *Lund*
Riadh Sadik, *Gothenburg*
Per Anders Sandstrom, *Linkoping*
Ervin Toth, *Malmö*
Konstantinos Tsimogiannis, *Vasteras*
Apostolos V Tsolakis, *Uppsala*

Switzerland

Gieri Cathomas, *Liestal*
Jean Louis Frossard, *Geneve*
Christian Toso, *Geneva*
Stephan Robert Vavricka, *Zurich*
Dominique Velin, *Lausanne*

Thailand

Thawatchai Akaraviputh, *Bangkok*
P Yoysungnoen Chintana, *Pathumthani*
Veerapol Kukongviriyapan, *Muang*
Vijittra Leardkamolkarn, *Bangkok*
Varut Lohsiriwat, *Bangkok*
Somchai Pinlaor, *Khaon Kaen*
D Wattanasirichaigoon, *Bangkok*

Trinidad and Tobago

B Shivananda Nayak, *Mount Hope*

Tunisia

Ibtissem Ghedira, *Sousse*
Lilia Zouiten-Mekki, *Tunis*

Turkey

Inci Alican, *Istanbul*
Mustafa Altindis, *Sakarya*
Mutay Aslan, *Antalya*
Oktar Asoglu, *Istanbul*
Yasemin Hatice Balaban, *Istanbul*
Metin Basaranoglu, *Ankara*
Yusuf Bayraktar, *Ankara*
Süleyman Bayram, *Adiyaman*
Ahmet Bilici, *Istanbul*
Ahmet Sedat Boyacioglu, *Ankara*
Züleyha Akkan Cetinkaya, *Kocaeli*
Cavit Col, *Bolu*
Yasar Colak, *Istanbul*
Cagatay Erden Daphan, *Kirikkale*
Mehmet Demir, *Hatay*
Ahmet Merih Dobrucali, *Istanbul*
Gülsüm Ozlem Elpek, *Antalya*
Ayse Basak Engin, *Ankara*
Eren Ersoy, *Ankara*
Osman Ersoy, *Ankara*
Yusuf Ziya Erzin, *Istanbul*
Mukaddes Esrefoglu, *Istanbul*
Levent Filik, *Ankara*
Ozgur Harmanci, *Ankara*
Koray Hekimoglu, *Ankara*
Abdurrahman Kadayifci, *Gaziantep*
Cem Kalayci, *Istanbul*
Selin Kapan, *Istanbul*
Huseyin Kayadibi, *Adana*
Sabahattin Kaymakoglu, *Istanbul*
Metin Kement, *Istanbul*
Mevlut Kurt, *Bolu*
Resat Ozaras, *Istanbul*
Elvan Ozbek, *Adapazari*
Cengiz Ozcan, *Mersin*
Hasan Ozen, *Ankara*
Halil Ozguc, *Bursa*
Mehmet Ozturk, *Izmir*
Orhan V Ozkan, *Sakarya*
Semra Paydas, *Adana*
Ozlem Durmaz Suoglu, *Istanbul*
Ilker Tasci, *Ankara*
Müge Tecder-ünal, *Ankara*
Mesut Tez, *Ankara*
Serdar Topaloglu, *Trabzon*
Murat Toruner, *Ankara*
Gokhan Tumgor, *Adana*
Oguz Uskudar, *Adana*
Mehmet Yalniz, *Elazig*
Mehmet Yaman, *Elazig*
Veli Yazisiz, *Antalya*
Yusuf Yilmaz, *Istanbul*
Ozlem Yilmaz, *Izmir*
Oya Yucel, *Istanbul*
Ilhami Yuksel, *Ankara*

United Kingdom

Nadeem Ahmad Afzal, *Southampton*
Navneet K Ahluwalia, *Stockport*
Yeng S Ang, *Lancashire*
Ramesh P Arasaradnam, *Coventry*
Ian Leonard Phillip Beales, *Norwich*
John Beynon, *Swansea*
Barbara Braden, *Oxford*
Simon Bramhall, *Birmingham*
Geoffrey Burnstock, *London*
Ian Chau, *Sutton*
Thean Soon Chew, *London*
Helen G Coleman, *Belfast*
Anil Dhawan, *London*
Sunil Dolwani, *Cardiff*
Piers Gatenby, *London*
Anil T George, *London*
Pasquale Giordano, *London*
Paul Henderson, *Edinburgh*
Georgina Louise Hold, *Aberdeen*
Stefan Hubscher, *Birmingham*
Robin D Hughes, *London*
Nusrat Husain, *Manchester*
Matt W Johnson, *Luton*
Konrad Koss, *Macclesfield*
Anastasios Koulaouzidis, *Edinburgh*
Simon Lal, *Salford*
John S Leeds, *Aberdeen*
JK K Limdi, *Manchester*
Hongxiang Liu, *Cambridge*
Michael Joseph McGarvey, *London*
Michael Anthony Mendall, *London*
Alexander H Mirnezami, *Southampton*
J Bernadette Moore, *Guildford*
Claudio Nicoletti, *Norwich*
Savvas Papagrigoriadis, *London*
Sylvia LF Pender, *Southampton*
David Mark Pritchard, *Liverpool*
James A Ross, *Edinburgh*
Kamran Rostami, *Worcester*
Xiong Z Ruan, *London*
Frank I Tovey, *London*
Dhiraj Tripathi, *Birmingham*
Vamsi R Velchuru, *Great Yarmouth*
Nicholas T Ventham, *Edinburgh*
Diego Vergani, *London*
Jack Westwood Winter, *Glasgow*
Terence Wong, *London*
Ling Yang, *Oxford*

United States

Daniel E Abbott, *Cincinnati*
Ghassan K Abou-Alfa, *New York*
Julian Abrams, *New York*
David William Adelson, *Los Angeles*
Jonathan Steven Alexander, *Shreveport*
Tauseef Ali, *Oklahoma City*
Mohamed R Ali, *Sacramento*
Rajagopal N Aravalli, *Minneapolis*
Hassan Ashktorab, *Washington*
Shashi Bala, *Worcester*
Charles F Barish, *Raleigh*
P Patrick Basu, *New York*
Robert L Bell, *Berkeley Heights*
David Bentrem, *Chicago*
Henry J Binder, *New Haven*
Joshua Bleier, *Philadelphia*
Wojciech Blonski, *Johnson City*
Kenneth Boorom, *Corvallis*
Brian Boulay, *Chicago*
Carla W Brady, *Durham*
Kyle E Brown, *Iowa City*
Adeel A Butt, *Pittsburgh*
Weibiao Cao, *Providence*
Andrea Castillo, *Cheney*
Fernando J Castro, *Weston*
Adam S Cheifetz, *Boston*
Xiaoxin Luke Chen, *Durham*
Ramsey Cheung, *Palo Alto*
Parimal Chowdhury, *Little Rock*
Edward John Ciaccio, *New York*
Dahn L Clemens, *Omaha*
Yingzi Cong, *Galveston*
Laura Iris Cosen-Binker, *Boston*
Joseph John Cullen, *Lowa*
Mark J Czaja, *Bronx*
Mariana D Dabeva, *Bronx*
Christopher James Damman, *Seattle*
Isabelle G De Plaen, *Chicago*
Punita Dhawan, *Nashville*
Hui Dong, *La Jolla*
Wael El-Rifai, *Nashville*
Sukru H Emre, *New Haven*
Paul Feuerstadt, *Hamden*
Josef E Fischer, *Boston*
Laurie N Fishman, *Boston*
Joseph Che Forbi, *Atlanta*
Temitope Foster, *Atlanta*
Amy E Foxx-Orenstein, *Scottsdale*
Daniel E Freedberg, *New York*
Shai Friedland, *Palo Alto*
Virgilio George, *Indianapolis*
Ajay Goel, *Dallas*
Oliver Grundmann, *Gainesville*
Stefano Guandalini, *Chicago*
Chakshu Gupta, *St. Joseph*
Grigoriy E Gurvits, *New York*
Xiaonan Han, *Cincinnati*
Mohamed Hassan, *Jackson*
Martin Hauer-Jensen, *Little Rock*
Koichi Hayano, *Boston*
Yingli Hee, *Atlanta*
Samuel B Ho, *San Diego*
Jason Ken Hou, *Houston*
Lifang Hou, *Chicago*
K-Qin Hu, *Orange*
Jamal A Ibdah, *Columbia*
Robert Thomas Jensen, *Bethesda*
Huanguang "Charlie" Jia, *Gainesville*
Rome Jutabha, *Los Angeles*
Andreas M Kaiser, *Los Angeles*
Avinash Kambadakone, *Boston*
David Edward Kaplan, *Philadelphia*
Randeep Kashyap, *Rochester*
Rashmi Kaul, *Tulsa*
Ali Keshavarzian, *Chicago*
Amir Maqbul Khan, *Marshall*
Nabeel Hasan Khan, *New Orleans*
Sahil Khanna, *Rochester*
Kusum K Kharbanda, *Omaha*

World Journal of Gastroenterology

Contents

Weekly Volume 21 Number 31 August 21, 2015

Contents

World Journal of Gastroenterology
Volume 21 Number 31 August 21, 2015

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
August 21, 2015

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9233

World J Gastroenterol 2015 August 21; 21(31): 9233-9238
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Iron deficiency anemia in celiac disease

Hugh James Freeman

Hugh James Freeman, Department of Medicine (Gastroenterology), University of British Columbia, Vancouver, BC V6T 1W5, Canada

Author contributions: Freeman HJ contributed all to this paper.

Conflict-of-interest statement: Freeman HJ declares no conflict of interest related to this publication.

Correspondence to: Hugh James Freeman, MD, CM, FRCPC, FACP, Professor, Department of Medicine (Gastroenterology), University of British Columbia, UBC Hospital, 2211 Wesbrook Mall, Vancouver, BC V6T 1W5, Canada. hugfree@shaw.ca
Telephone: +1-604-8227216

Received: December 24, 2014
Peer-review started: December 25, 2014
First decision: March 10, 2015
Revised: March 30, 2015
Accepted: June 16, 2015
Article in press: June 16, 2015
Published online: August 21, 2015

Abstract

Iron is an important micronutrient that may be depleted in celiac disease. Iron deficiency and anemia may complicate well-established celiac disease, but may also be the presenting clinical feature in the absence of diarrhea or weight loss. If iron deficiency anemia occurs, it should be thoroughly evaluated, even if celiac disease has been defined since other superimposed causes of iron deficiency anemia may be present. Most often, impaired duodenal mucosal uptake of iron is evident since surface absorptive area in the duodenum is reduced, in large part, because celiac disease is an immune-mediated disorder largely focused in the proximal small intestinal mucosa. Some studies have also suggested that blood loss may occur in celiac disease, sometimes from superimposed small intestinal disorders, including ulceration or neoplastic diseases, particularly lymphoma. In addition, other associated gastric or colonic disorders may be responsible for blood loss. Rarely, an immune-mediated hemolytic disorder with increased urine iron loss may occur that may respond to a gluten-free diet. Reduced expression of different regulatory proteins critical in iron uptake has also been defined in the presence and absence of anemia. Finally, other rare causes of microcytic anemia may occur in celiac disease, including a sideroblastic form of anemia reported to have responded to a gluten-free diet.

Key words: Anemia; Iron deficiency; Autoimmune hemolysis; Celiac disease; Iron absorption; Ferroportin; Hepcidin; Divalent metal transporter; Enterocyte

Core tip: Iron is a critical micronutrient that may be deficient in well-established celiac disease or be the presenting clinical feature even in the absence of diarrhea or weight loss. Most often, impaired duodenal mucosal uptake of iron is evident since surface absorptive area in the duodenum in reduced, in large part, because celiac disease is an immune-mediated disorder largely focused in the proximal small intestine. Other superimposed small intestinal complications of celiac disease may be responsible causing blood loss, including ulceration or neoplasia. Finally, associated gastric or colonic causes of blood loss, immune-mediated hemolysis and reduced expression of different regulatory proteins critical in iron uptake may be present.

Freeman HJ. Iron deficiency anemia in celiac disease. *World J Gastroenterol* 2015; 21(31): 9233-9238 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9233.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9233

INTRODUCTION

Anemia, associated with iron deficiency, is most often due to increased blood loss, or impaired iron absorption. Iron-deficiency anemia is often recorded in newly diagnosed celiac disease[1] and may persist for variable periods after initiation of a gluten-free diet[2]. Iron deficiency anemia in children and adults may also be the presenting clinical feature of celiac disease, and may be the only finding present[3-5]. Unfortunately, this interesting relationship between iron deficiency anemia and celiac disease has been poorly appreciated, even among subspecialty physicians, including practicing hematologists[6].

IRON DEFICIENCY AND ANEMIA

From a global perspective, iron-deficiency anemia is probably the most common cause of microcytic anemia while other causes include thalassemia, anemia associated with chronic inflammation and sideroblastic anemia[7]. Iron plays a crucial role in the heme group of hemoglobin in order to transport oxygen. Iron is also detected in other proteins including cytochromes and myoglobin and may play a role in fatigue associated with iron deficiency alone in the absence of anemia, especially in females[8-10]. Due to menses, premenopausal females are at increased risk for iron deficiency and pregnancy further increases daily iron requirements. A separate risk group for iron deficiency includes athletes. Blood loss from the gastrointestinal tract has been documented in athletes along with low grade intravascular hemolysis causing increased urinary loss of iron[11,12]. Inflammation caused by exercise may also cause iron deficiency and impaired performance[13,14]. Each of these risk factors recognized particularly in healthy women (and healthy athletes) may occur if underlying celiac disease is also present. As a result, the degree of iron deficiency may be exacerbated. Although detailed epidemiological data on iron deficiency in celiac disease are limited, some recent studies suggest that iron deficiency may be significant in both children[15,16] and adults[17] with celiac disease.

IRON ABSORPTION

Iron enters the epithelial cell of the duodenal mucosa in ferrous form through an apical or brush border membrane transport protein termed the divalent metal transporter (DMT1). DMT1 is a protein that spans the entire brush border membrane. It has the capacity to transport iron as well as several other divalent metals[18]. Normally, this carrier protein functions in a co-transport mode with univalent protons[19]. Microcytic anemia caused by DMT1 mutations have also been identified in human subjects[20]. Iron transport by DMT1 also requires conversion of ferric iron, the predominant dietary form, to ferrous iron by means of ferric reductases at the apical surface of the intestinal enterocyte prior to cellular uptake. An acidic apical surface membrane microenvironment is also required. Inside the enterocyte, ferritin within the cytoplasmic compartment appears to be able to store large amounts of iron and may be important for controlled delivery of iron to the basolateral membrane.

Iron exits the duodenal enterocyte by means of a different carrier protein, ferroportin, also termed the metal transporter 1 (MTP 1). Studies have demonstrated that this critical protein is localized to the basolateral membrane. Iron then binds a separate glycosylated protein, transferrin, to permit iron delivery through the bloodstream to developing red cells. Transferrin has the capacity to transport two ferric ions to distant target tissues. Prior to transferrin binding, any ferrous ions must be initially converted to ferric ions by ferroxidases (*e.g.*, hephaestin, ceruloplasmin)[21]. A deficiency of these latter oxygen-dependent enzymes impedes iron uptake into cells. Excessive iron can also be stored within the liver, and made available later for transport to maturing red blood cells.

REGULATION OF IRON ABSORPTION

Body iron storage levels are well maintained in a consistent range. With iron deficiency, iron absorption may be increased, but with iron overload, decreased[22]. Iron homeostasis is regulated by hepcidin discovered almost 2 decades ago[23].

Hepcidin is actively synthesized by hepatocytes and then hepatocyte secrete this protein into circulating plasma. The concentration of hepcidin is affected by both iron stores. Hepcidin is found mostly in free form in the bloodstream, and is eventually filtered by the kidneys. Hepcidin regulates iron flow into the blood plasma by controlling the membrane protein, ferroportin. Hepcidin first appears to bind to ferroportin. Then, endocytosis of ferroportin occurs followed by destruction of the hepcidin within the cell in lysosomes. This process impairs iron absorption. As a result, release of iron into the circulation is prevented from both the enterocyte and hepatocyte stores. Mutations in this ferroportin protein have been described[24,25] that prevent hepcidin binding and lead to iron overload. Hepcidin may also be controlled by a bone marrow suppressor of hepcidin that responds to increased erythropoietin caused by hypoxia or significant bleeding[26,27]. Other possible homeostatic

mechanisms that may not be reliant on hepcidin include hypoxia and cellular iron deficiency. Both may result in an increase in ferroportin that may be independent of hepcidin control. Further information on role of hepcidin and details on systemic iron homeostasis can be found elsewhere[28,29].

IRON DEFICIENCY IN CELIAC DISEASE

Reduced duodenal iron absorption

Clinical and other features of celiac disease have been extensively reviewed in earlier reports in this journal[30,31]. Iron deficiency anemia is itself an independent clinical manifestation of either well established celiac disease, or may lead to its initial recognition, especially if other causes, such as a colonic cancer, have been excluded and iron deficiency appears refractory to oral iron treatment[32]. In part, this reflects the prominent duodenal mucosal geographic distribution of celiac disease and concurrence with the principal site of enterocyte uptake of iron by proximal small intestinal enterocytes. As a result of disease localized in the proximal small intestinal mucosa, impaired duodenal iron absorption can be expected, even if there is provision of added oral iron. Recently, more and more emphasis has been placed on micronutrient deficiency as a diagnostic clue to occult celiac disease, particularly for iron, and iron deficiency anemia[33]. In children, iron deficiency anemia in celiac disease is common and further screening with tissue transglutaminase antibodies has been strongly recommended[16]. Interestingly, pica may be the presenting clinical symptom of celiac disease coupled with iron deficiency anemia in children[34].

Gastrointestinal blood loss

Reports have also appeared in celiac disease with occult gastrointestinal bleeding as a cause of iron deficiency anemia[35,36]. In a study of young males presenting with iron deficiency anemia, peptic ulcer disease was the most common finding in 30%[37-39]. However, in this report, malignant causes were not detected but celiac disease was subsequently diagnosed in 4%. In celiac disease, added common causes of blood loss should be considered. Most experts would recommend a thorough evaluation, including endoscopic and radiologic imaging studies[40]. Routine duodenal biopsies obtained during upper endoscopic evaluation for iron deficiency[41] or other upper gastrointestinal symptoms[42] could lead to recognition of histopathologic changes of untreated celiac disease in the proximal small intestinal mucosa. In a recent report, celiac disease with iron deficiency anemia was more likely to be observed in Caucasians, than non-Caucasians[43]. Moreover, celiacs initially manifesting with anemia appeared to have more severe disease than celiacs presenting with diarrhea[44]. It should also be noted, however, that celiac disease may be complicated by a superimposed ulcerative small bowel disorder. Either benign mucosal ulcers, so-called non-granulomatous ulcerative jejunitis, or malignant ulcers due to a malignant lymphoma in celiac disease may cause occult or overt blood loss, positive fecal occult blood tests, and if chronically present over time, iron deficiency and anemia.

Iron deficiency and hemolysis

Intravascular hemolysis, usually related to an associated autoimmune disorder, with increased urinary iron losses should be considered. Although rare[45,46], improvement with a gluten-free diet has been recorded, even when prior steroid treatment failed[46]. To screen for hemosiderin, colorimetric methods on a collected urine sample may be considered along with hematopathological review of the peripheral blood smear. These initial studies may lead to a more detailed hematologic evaluation.

Unusual causes or associations of microcytic anemia

Occasionally, microcytic anemia may be due to other rare conditions in celiac disease. These include occasional anemias associated with co-existent chronic inflammatory diseases along with a rare sideroblastic anemia associated with pyridoxine deficiency[47]. Interestingly, the hematologic disorder in this initial case report of sideroblastic anemia responded completely to a gluten-free diet. Further screening studies for celiac disease in patients with sideroblastic anemia should be considered. A rare disorder of iron deficiency anemia in children with celiac disease and pulmonary hemosiderosis has been detailed, the so-called Lane-Hamilton syndrome[48]. In this series, the authors reported on improvement with a gluten-free diet. This contrasted with a larger series from France of 25 pediatric cases with idiopathic pulmonary hemosiderosis and hemoptysis. In these, 28% had celiac disease antibodies. Most children in this study required corticosteroids and immunosuppressants[49]. Finally, gastric changes have been reported in studies of children[50] and adults[51,52] with celiac disease, and some believe that these are complicated by superimposed *Helicobacter pylori* infection and be responsible for iron deficiency anemia.

REGULATION OF IRON ABSORPTION IN CELIAC DISEASE

Related to reduced mucosal surface absorptive area

Modern studies on the absorption of inorganic iron have been limited in celiac disease. Initial, essentially historically reported studies[53] evaluated the absorption of iron from a 5 mg dose of ferrous iron using a total body counter and confirmed that ferrous iron absorption was limited in untreated celiac disease, particularly if already iron deficient. However, improved

absorption resulted from a gluten free diet. In celiac disease, iron deficiency has generally been attributed to reduced surface area, particularly in the proximal small intestine.

Studies of iron regulatory proteins in celiac disease

Interestingly, iron regulatory proteins have been evaluated in celiacs compared to controls and iron deficient patients using duodenal biopsies[54,55]. Results showed that DMT1, ferroportin, hephaestin and transferrin receptor protein mRNA were increased while body iron stores were reduced in celiac disease. These different iron regulatory proteins were also increased with iron deficiency (unrelated to celiac disease) suggesting that the upregulation in iron absorption capacity that appears to occur in celiac disease is primarily due to iron deficiency *per se*, while increased enterocyte proliferation in celiac disease does not have a specific effect on iron uptake regulation[54]. In contrast, a recent study showed that expression of DMT1 and ferroportin are increased in celiac disease with or without iron deficiency[55]. In this study, ferritin expression was also found to be increased in celiac disease, but only in those with iron deficiency.

CONCLUSION

Iron is a key micronutrient that may be depleted in children and adults with celiac disease. Iron deficiency anemia may also complicate well-defined celiac disease, or actually represent the initial extra-intestinal clinical feature. Iron deficiency anemia should lead to careful exclusion of other common causes, such as colon cancer. Even with well-established celiac disease, other superimposed causes should be excluded, including occult lymphoma. In celiac disease, impaired iron uptake from the duodenal lumen is the most likely cause, even if other common features of classical celiac disease, such as diarrhea or weight loss, are absent. Although reduced duodenal mucosal surface area in unrecognized and untreated celiac disease may be present, recent studies have also evaluated duodenal biopsies from celiac disease patients in the presence and absence of anemia and documented reduced expression of important iron regulatory proteins.

REFERENCES

1 **Halfdanarson TR**, Litzow MR, Murray JA. Hematologic manifestations of celiac disease. *Blood* 2007; **109**: 412-421 [PMID: 16973955 DOI: 10.1182/blood-2006-07-031104]

2 **Annibale B**, Severi C, Chistolini A, Antonelli G, Lahner E, Marcheggiano A, Iannoni C, Monarca B, Delle Fave G. Efficacy of gluten-free diet alone on recovery from iron deficiency anemia in adult celiac patients. *Am J Gastroenterol* 2001; **96**: 132-137 [PMID: 11197242 DOI: 10.1111/j.1572-0241.2001.03463.x]

3 **Hoffbrand AV**. Anaemia in adult coeliac disease. *Clin Gastroenterol* 1974; **3**: 71-89 [PMID: 4599261]

4 **Unsworth DJ**, Lock FJ, Harvey RF. Iron-deficiency anaemia in premenopausal women. *Lancet* 1999; **353**: 1100 [PMID: 10199377 DOI: 10.1016/S0140-6736(05)76459-X]

5 **Bottaro G**, Cataldo F, Rotolo N, Spina M, Corazza GR. The clinical pattern of subclinical/silent celiac disease: an analysis on 1026 consecutive cases. *Am J Gastroenterol* 1999; **94**: 691-696 [PMID: 10086653 DOI: 10.1016/S0002-9270(98)00819-3]

6 **Smukalla S**, Lebwohl B, Mears JG, Leslie LA, Green PH. How often do hematologists consider celiac disease in iron-deficiency anemia? Results of a national survey. *Clin Adv Hematol Oncol* 2014; **12**: 100-105 [PMID: 24892255]

7 **DeLoughery TG**. Microcytic anemia. *N Engl J Med* 2014; **371**: 1324-1331 [PMID: 25271605 DOI: 10.1056/NEJMra1215361]

8 **Vaucher P**, Druais PL, Waldvogel S, Favrat B. Effect of iron supplementation on fatigue in nonanemic menstruating women with low ferritin: a randomized controlled trial. *CMAJ* 2012; **184**: 1247-1254 [PMID: 22777991 DOI: 10.1503/cmaj.110950]

9 **Verdon F**, Burnand B, Stubi CL, Bonard C, Graff M, Michaud A, Bischoff T, de Vevey M, Studer JP, Herzig L, Chapuis C, Tissot J, Pécoud A, Favrat B. Iron supplementation for unexplained fatigue in non-anaemic women: double blind randomised placebo controlled trial. *BMJ* 2003; **326**: 1124 [PMID: 12763985 DOI: 10.1136/bmj.326.7399.1124]

10 **Krayenbuehl PA**, Battegay E, Breymann C, Furrer J, Schulthess G. Intravenous iron for the treatment of fatigue in nonanemic, premenopausal women with low serum ferritin concentration. *Blood* 2011; **118**: 3222-3227 [PMID: 21705493 DOI: 10.1182/blood-2011-04-346304]

11 **Peeling P**, Dawson B, Goodman C, Landers G, Trinder D. Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. *Eur J Appl Physiol* 2008; **103**: 381-391 [PMID: 18365240 DOI: 10.1007/s00421-008-0726-6]

12 **Robertson JD**, Maughan RJ, Davidson RJ. Faecal blood loss in response to exercise. *Br Med J* (Clin Res Ed) 1987; **295**: 303-305 [PMID: 3115419 DOI: 10.1136/bmj.295.6593.303]

13 **Pasricha SR**, Low M, Thompson J, Farrell A, De-Regil LM. Iron supplementation benefits physical performance in women of reproductive age: a systematic review and meta-analysis. *J Nutr* 2014; **144**: 906-914 [PMID: 24717371 DOI: 10.3945/jn.113.189589]

14 **Mei Z**, Cogswell ME, Parvanta I, Lynch S, Beard JL, Stoltzfus RJ, Grummer-Strawn LM. Hemoglobin and ferritin are currently the most efficient indicators of population response to iron interventions: an analysis of nine randomized controlled trials. *J Nutr* 2005; **135**: 1974-1980 [PMID: 16046725]

15 **Gokce S**, Arslantas E. Changing face and clinical features of celiac disease in children. *Pediatr Int* 2015; **57**: 107-112 [PMID: 25040342 DOI: 10.1111/ped.12448]

16 **Ertekin V**, Tozun MS, Küçük N. The prevalence of celiac disease in children with iron-deficiency anemia. *Turk J Gastroenterol* 2013; **24**: 334-338 [PMID: 24254265]

17 **Cannizzaro R**, Da Ponte A, Tabuso M, Mazzucato M, De Re V, Caggiari L, Fornasarig M, Maiero S, Orzes E, Canzonieri V. Improving detection of celiac disease patients: a prospective study in iron-deficient blood donors without anemia in north Italy. *Eur J Gastroenterol Hepatol* 2014; **26**: 721-724 [PMID: 24841904 DOI: 10.1097/MEG.0000000000000100]

18 **Illing AC**, Shawki A, Cunningham CL, Mackenzie B. Substrate profile and metal-ion selectivity of human divalent metal-ion transporter-1. *J Biol Chem* 2012; **287**: 30485-30496 [PMID: 22736759 DOI: 10.1074/jbc.M112.364208]

19 **Gunshin H**, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 1997; **388**: 482-488 [PMID: 9242408 DOI: 10.1038/41343]

20 **Iolascon A**, De Falco L. Mutations in the gene encoding DMT1: clinical presentation and treatment. *Semin Hematol* 2009; **46**: 358-370 [PMID: 19786204]

21 **Cherukuri S**, Potla R, Sarkar J, Nurko S, Harris ZL, Fox PL. Unexpected role of ceruloplasmin in intestinal iron absorption.

Cell Metab 2005; **2**: 309-319 [PMID: 16271531 DOI: 10.1016/j.cmet.2005.10.003]
22 **Finch C**. Regulators of iron balance in humans. *Blood* 1994; **84**: 1697-1702 [PMID: 8080980]
23 **Ganz T**. Hepcidin and iron regulation, 10 years later. *Blood* 2011; **117**: 4425-4433 [PMID: 21346250 DOI: 10.1182/blood-2011-01-258467]
24 **Sham RL**, Phatak PD, Nemeth E, Ganz T. Hereditary hemochromatosis due to resistance to hepcidin: high hepcidin concentrations in a family with C326S ferroportin mutation. *Blood* 2009; **114**: 493-494 [PMID: 19589941 DOI: 10.1182/blood-2009-04-216226]
25 **Sham RL**, Phatak PD, West C, Lee P, Andrews C, Beutler E. Autosomal dominant hereditary hemochromatosis associated with a novel ferroportin mutation and unique clinical features. *Blood Cells Mol Dis* 2005; **34**: 157-161 [PMID: 15727899 DOI: 10.1016/j.bcmd.2004.12.002]
26 **Liu Q**, Davidoff O, Niss K, Haase VH. Hypoxia-inducible factor regulates hepcidin via erythropoietin-induced erythropoiesis. *J Clin Invest* 2012; **122**: 4635-4644 [PMID: 23114598 DOI: 10.1172/JCI63924]
27 **Mastrogiannaki M**, Matak P, Mathieu JR, Delga S, Mayeux P, Vaulont S, Peyssonnaux C. Hepatic hypoxia-inducible factor-2 down-regulates hepcidin expression in mice through an erythropoietin-mediated increase in erythropoiesis. *Haematologica* 2012; **97**: 827-834 [PMID: 22207682 DOI: 10.3324/haematol.2011.056119]
28 **Ganz T**. Systemic iron homeostasis. *Physiol Rev* 2013; **93**: 1721-1741 [PMID: 24137020 DOI: 10.1152/physrev.00008.2013]
29 **Gulec S**, Anderson GJ, Collins JF. Mechanistic and regulatory aspects of intestinal iron absorption. *Am J Physiol Gastrointest Liver Physiol* 2014; **307**: G397-G409 [PMID: 24994858 DOI: 10.1152/ajpgi.00348.2013]
30 **Freeman HJ**, Chopra A, Clandinin MT, Thomson AB. Recent advances in celiac disease. *World J Gastroenterol* 2011; **17**: 2259-2272 [PMID: 21633592 DOI: 10.3748/wjg.v17.i18.2259]
31 **Gujral N**, Freeman HJ, Thomson AB. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. *World J Gastroenterol* 2012; **18**: 6036-6059 [PMID: 23155333 DOI: 10.3748/wjg.v18.i42.6036]
32 **Annibale B**, Capurso G, Chistolini A, D'Ambra G, DiGiulio E, Monarca B, DelleFave G. Gastrointestinal causes of refractory iron deficiency anemia in patients without gastrointestinal symptoms. *Am J Med* 2001; **111**: 439-445 [PMID: 11690568 DOI: 10.1016/S0002-9343(01)00883-X]
33 **Oxentenko AS**, Murray JA. Celiac Disease: Ten Things That Every Gastroenterologist Should Know. *Clin Gastroenterol Hepatol* 2015; **13**: 1396-1404 [PMID: 25051511 DOI: 10.1016/j.cgh.2014.07.024]
34 **Korman SH**. Pica as a presenting symptom in childhood celiac disease. *Am J Clin Nutr* 1990; **51**: 139-141 [PMID: 2305699]
35 **Kosnai I**, Kuitunen P, Siimes MA. Iron deficiency in children with coeliac disease on treatment with gluten-free diet. Role of intestinal blood loss. *Arch Dis Child* 1979; **54**: 375-378 [PMID: 314273 DOI: 10.1136/adc.54.5.375]
36 **Fine KD**. The prevalence of occult gastrointestinal bleeding in celiac sprue. *N Engl J Med* 1996; **334**: 1163-1167 [PMID: 8602182 DOI: 10.1056/NEJM199605023341804]
37 **Shamir R**, Levine A, Yalon-Hacohen M, Shapiro R, Zahavi I, Rosenbach Y, Lerner A, Dinari G. Faecal occult blood in children with coeliac disease. *Eur J Pediatr* 2000; **159**: 832-834 [PMID: 11079196 DOI: 10.1007/PL00008348]
38 **Mant MJ**, Bain VG, Maguire CG, Murland K, Yacyshyn BR. Prevalence of occult gastrointestinal bleeding in celiac disease. *Clin Gastroenterol Hepatol* 2006; **4**: 451-454 [PMID: 16616349 DOI: 10.1016/j.cgh.2005.12.010]
39 **Carter D**, Levi G, Tzur D, Novis B, Avidan B. Prevalence and predictive factors for gastrointestinal pathology in young men evaluated for iron deficiency anemia. *Dig Dis Sci* 2013; **58**: 1299-1305 [PMID: 23192647 DOI: 10.1007/s10620-012-2496-6]
40 **Milano A**, Balatsinou C, Filippone A, Caldarella MP, Laterza F, Lapenna D, Pierdomenico SD, Pace F, Cuccurullo F, Neri M. A prospective evaluation of iron deficiency anemia in the GI endoscopy setting: role of standard endoscopy, videocapsule endoscopy, and CT-enteroclysis. *Gastrointest Endosc* 2011; **73**: 1002-1008 [PMID: 21396638 DOI: 10.1016/j.gie.2011.01.006]
41 **Emami MH**, Karimi S, Kouhestani S. Is routine duodenal biopsy necessary for the detection of celiac disease in patients presenting with iron deficiency anemia? *Int J Prev Med* 2012; **3**: 273-277 [PMID: 22624084]
42 **Freeman HJ**. Detection of adult celiac disease with duodenal screening biopsies over a 30-year period. *Can J Gastroenterol* 2013; **27**: 405-408 [PMID: 23862172]
43 **Murray JA**, McLachlan S, Adams PC, Eckfeldt JH, Garner CP, Vulpe CD, Gordeuk VR, Brantner T, Leiendecker-Foster C, Killeen AA, Acton RT, Barcellos LF, Nickerson DA, Beckman KB, McLaren GD, McLaren CE. Association between celiac disease and iron deficiency in Caucasians, but not non-Caucasians. *Clin Gastroenterol Hepatol* 2013; **11**: 808-814 [PMID: 23416278 DOI: 10.1016/j.cgh.2013.02.009]
44 **Abu Daya H**, Lebwohl B, Lewis SK, Green PH. Celiac disease patients presenting with anemia have more severe disease than those presenting with diarrhea. *Clin Gastroenterol Hepatol* 2013; **11**: 1472-1477 [PMID: 23756221 DOI: 10.1016/j.cgh.2013.05.030]
45 **Miller DG**. Coeliac disease with autoimmune haemolytic anaemia. *Postgrad Med J* 1984; **60**: 629-630 [PMID: 6483707 DOI: 10.1136/pgmj.60.707.629]
46 **Ivanovski P**, Nikolić D, Dimitrijević N, Ivanovski I, Perišić V. Erythrocytic transglutaminase inhibition hemolysis at presentation of celiac disease. *World J Gastroenterol* 2010; **16**: 5647-5650 [PMID: 21105201 DOI: 10.3748/wjg.v16.i44.5647]
47 **Dawson AM**, Holdsworth CD, Pitcher CS. Sideroblastic anaemia in adult coeliac disease. *Gut* 1964; **5**: 304-308 [PMID: 14209912 DOI: 10.1136/gut.5.4.304]
48 **Hendrickx GF**, Somers K, Vandenplas Y. Lane-Hamilton syndrome: case report and review of the literature. *Eur J Pediatr* 2011; **170**: 1597-1602 [PMID: 21947219 DOI: 10.1007/s00431-011-1568-5]
49 **Taytard J**, Nathan N, de Blic J, Fayon M, Epaud R, Deschildre A, Troussier F, Lubrano M, Chiron R, Reix P, Cros P, Mahloul M, Michon D, Clement A, Corvol H. New insights into pediatric idiopathic pulmonary hemosiderosis: the French RespiRare(®) cohort. *Orphanet J Rare Dis* 2013; **8**: 161 [PMID: 24125570 DOI: 10.1186/1750-1172-9-161]
50 **Nenna R**, Magliocca FM, Tiberti C, Mastrogiorgio G, Petrarca L, Mennini M, Lucantoni F, Luparia RP, Bonamico M. Endoscopic and histological gastric lesions in children with celiac disease: mucosal involvement is not only confined to the duodenum. *J Pediatr Gastroenterol Nutr* 2012; **55**: 728-732 [PMID: 22773062 DOI: 10.1097/MPG.0b013e318266aa9e]
51 **Wolber R**, Owen D, DelBuono L, Appelman H, Freeman H. Lymphocytic gastritis in patients with celiac sprue or spruelike intestinal disease. *Gastroenterology* 1990; **98**: 310-315 [PMID: 2295386]
52 **Arnason T**, Brown IS, Goldsmith JD, Anderson W, O'Brien BH, Wilson C, Winter H, Lauwers GY. Collagenous gastritis: a morphologic and immunohistochemical study of 40 patients. *Mod Pathol* 2015; **28**: 533-544 [PMID: 25234289 DOI: 10.1038/modpathol.2014.119]
53 **Anand BS**, Callender ST, Warner GT. Absorption of inorganic and haemoglobin iron in coeliac disease. *Br J Haematol* 1977; **37**: 409-414 [PMID: 603771 DOI: 10.1111/j.1365-2141.1977.tb01012.x]
54 **Barisani D**, Parafioriti A, Bardella MT, Zoller H, Conte D, Armiraglio E, Trovato C, Koch RO, Weiss G. Adaptive changes of duodenal iron transport proteins in celiac disease. *Physiol Genomics* 2004; **17**: 316-325 [PMID: 15054143 DOI: 10.1152/

physiolgenomics.00211.2003]
55 **Sharma N**, Begum J, Eksteen B, Elagib A, Brookes M, Cooper BT, Tselepis C, Iqbal TH. Differential ferritin expression is associated with iron deficiency in coeliac disease. *Eur J Gastroenterol Hepatol* 2009; **21**: 794-804 [PMID: 19404207 DOI: 10.1097/MEG.0b013e328308676b]

P- Reviewer: Pellegrino S **S- Editor**: Yu J **L- Editor**: A
E- Editor: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9239

World J Gastroenterol 2015 August 21; 21(31): 9239-9244
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Oral *Campylobacter* species: Initiators of a subgroup of inflammatory bowel disease?

Li Zhang

Li Zhang, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW 2052, Australia

Author contributions: Zhang L solely contributed to this paper.

Conflict-of-interest statement: The author has no conflict of interest.

Correspondence to: Li Zhang, MBBS, PhD, Senior Lecturer (Medical Microbiology and Immunology), School of Biotechnology and Biomolecular Sciences, University of New South Wales, High St, Kensington, Sydney, NSW 2052, Australia. l.zhang@unsw.edu.au
Telephone: +61-1-93852042
Fax: +61-2-93851483

Received: March 15, 2015
Peer-review started: March 16, 2015
First decision: April 23, 2015
Revised: May 8, 2015
Accepted: July 3, 2015
Article in press: July 3, 2015
Published online: August 21, 2015

Abstract

In recent years, a number of studies detected a significantly higher prevalence of *Campylobacter* species such as *Campylobacter concisus* (*C. concisus*) in intestinal biopsies and fecal samples collected from patients with inflammatory bowel disease (IBD) compared to controls. Most of these *Campylobacter* species are not of zoonotic origin but are human oral *Campylobacter* species. Bacterial species usually cause diseases in the location where they colonize. However, *C. concisus* and other oral *Campylobacter* species are associated with IBD occurring at the lower parts of the gastrointestinal tract, suggesting that these *Campylobacter* species may have unique virulence factors that are expressed in the lower parts of the gastrointestinal tract.

Key words: *Campylobacter concisus*; Oral *Campylobacter* species; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis

Core tip: The human oral cavity is a reservoir of a number of *Campylobacter* species. Accumulated evidence suggests that some oral *Campylobacter* species such as *Campylobacter concisus* may be initiators of a subgroup of human inflammatory bowel disease.

Zhang L. Oral *Campylobacter* species: Initiators of a subgroup of inflammatory bowel disease? *World J Gastroenterol* 2015; 21(31): 9239-9244 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9239.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9239

INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is a group of chronic relapsing inflammatory diseases of the gastrointestinal tract. The most common clinical types of IBD are Crohn's disease (CD) and Ulcerative colitis (UC); the two forms of IBD differ in clinical presentation, distribution of inflammation in the gastrointestinal tract, endoscopic appearance and histology[1]. The cause of IBD is not well understood. It is thought

that the disease occurs in individuals with genetic predisposition when triggered by environmental factors[2]. The incidence of IBD is increasing worldwide[3]. Epidemiological studies suggest that environmental factors play a particularly important role in the increased incidence of IBD[4,5].

BACTERIAL FACTORS ASSOCIATED WITH IBD

Studies have shown that microbes in the gastrointestinal tract play a key role in the development of IBD. Colitis did not occur in animal models of IBD when raised germ-free, and the intestinal inflammation resolved in patients with CD after faecal stream diversion[6,7].

Extensive research has been conducted to search for the identities of the bacterial species that might contribute to the development of IBD. These include analyses of gut microbiome and investigations of associations between individual bacterial species and IBD. Studies of gut microbiome, which were performed by sequencing 16S rRNA genes, detected reduced bacterial species diversity and changed relative abundance (dysbiosis) in inflamed mucosal tissues of patients with IBD[8,9]. Recent studies suggest that such changes were due to the impact of host inflammatory responses on the resident microbes[10,11]. Indeed, bacterial species have different abilities in resisting the responses of the immune system and some may even use the by-products of inflammatory responses to boost their growth[10,12].

A number of individual bacterial species were found to be associated with patients with IBD, which were summarized in a recent review by Hold *et al*[13]. Whether the gut bacterial dysbiosis and individual bacterial species that are associated with IBD contribute to the pathogenesis of IBD are still under investigation.

DIFFERENTIAL ROLE OF BACTERIAL SPECIES IN THE PATHOGENESIS OF IBD

The generally accepted concept is that IBD is caused by multiple bacterial species. This concept may require some refinement in order to more clearly define the role of different bacterial species in the pathogenesis of IBD.

It may be more informative to divide bacterial species that are involved in the pathogenesis of IBD into two broad categories, initiators and exacerbators. The initiator bacterial species are those that instigate the inflammation in the early stage of IBD, while the exacerbator bacterial species are those that contribute to the on-going inflammation after the intestinal epithelial barrier is breached by a direct action of the initiators or by the inflammation initiated by the initiators. The dominant intestinal resident bacterial species, which have co-evolved with the host's mucosal immune system, most likely are exacerbators in the pathogenesis of IBD.

IBD is a group of diseases that have similar clinical manifestation and histopathology. Given this, the initiators of IBD may consist of several agents that have some common virulence factors. Individual cases may be initiated predominantly by one initiator bacterial species or by more than one initiator. The chronic and recurring nature of IBD suggests that IBD patients are frequently exposed to these initiators. CD can occur at any part of the gastrointestinal tract, suggesting that some initiators are present in the upper gastrointestinal tract. Accumulated evidence suggests that *Campylobacter concisus* (*C. concisus)* and a number of other oral *Campylobacter* species are possible initiators of a subgroup of human IBD.

MOST OF THE *CAMPYLOBACTER* SPECIES DETECTED IN PATIENTS WITH IBD ARE NOT OF ZOONOTIC ORIGIN BUT ARE HUMAN ORAL *CAMPYLOBACTER* SPECIES

To date, four studies have examined the intestinal prevalence of *Campylobacter* species in patients with CD and controls using *Campylobacter* genus PCR; three of which have detected a significantly higher intestinal prevalence of *Campylobacter* species in patients with CD as compared with the controls[14-17]. Three studies have examined the intestinal prevalence of *Campylobacter* species in patients with UC and controls using *Campylobacter* genus PCR, two of which detected a significantly higher intestinal prevalence of *Campylobacter* species in patients with UC compared with the controls[16-18].

At the single species level, three studies found a significantly higher intestinal prevalence of *C. concisus* in patients with CD as compared to controls[14-16]. Two studies found a significantly higher intestinal prevalence of *C. concisus* in patients with UC as compared to controls[16,18]. Furthermore, Mukhopadhya *et al*[18]. detected a significantly higher intestinal prevalence of *C. ureolyticus* in patients with UC as compared to controls.

In these studies, a total of eight *Campylobacter* species were detected, including *C. concisus*, *Campylobacter showae*, *Campylobacter hominis*, *Campylobacter gracilis*, *Campylobacter rectus*, *Campylobacter jejuni, Campylobacter curvus* and *Campylobacter ureolyticus*[14]. *C. concisus* was the most commonly detected species[14-18]. The majority of *Campylobacter* species detected in these studies were not of zoonotic origin but were previously reported human oral *Campylobacter* species (Table 1). These *Campylobacter* species do not have strong abilities in resisting the antimicrobial effects of bile, suggesting that the

Table 1 Most of *Campylobacter* species detected in the intestinal biopsies and fecal samples collected from patients with inflammatory bowel disease and controls are not of zoonotic origin but are human oral *Campylobacter* species

Campylobacter species	Human oral bacteria	2% ox-bile resistance	Urease activity	Motile
C. concisus	Yes	14%-50%	-	Yes
C. showae	Yes	-	-	Yes
C. hominis	No	60%-93%	-	No
C. gracilis	Yes	-	-	No
C. jejuni	No	60%-93%	-	Yes
C. ureolyticus	Yes	-	50%-100%	No
C. curvus	Yes	-	-	Yes
C. rectus	Yes	-	-	Yes

Information was obtained from[14-18,23,27,32,33]. *Campylobacter jejuni* (*C. jejuni*) refers to *C. jejuni* subsp. *jejuni*. *C. ureolyticus* has been isolated from various clinical sources including dental samples. - indicates 0%-11% positivity.

intestinal tract is not an optimal colonization site for these *Campylobacter* species in general (Table 1). *C. concisus* has a better ability in resisting bile compared to other oral *Campylobacter* species, about half of the *C. concisus* strains were able to grow in the presence of 2% ox bile (Table 1). Some *C. ureolyticus* strains are urease positive (Table 1). Of the six oral *Campylobacter* species detected in intestinal tissues, four species are motile (Table 1).

PATHOGENIC MECHANISMS OF *C. CONCISUS* AND OTHER ORAL *CAMPYLOBACTER* SPECIES

We hypothesized that some oral *C. concisus* strains may play a role in the development of IBD in 2010 and conducted continuous research to investigate this in the following years[19]. If *C. concisus* is involved in IBD, it is most likely an initiator. Indeed *C. concisus* does not appear to have strong abilities in resisting an inflammatory environment; there was a lower prevalence of this bacterium in areas with more severe inflammation compared to areas with less severe inflammation[14,20]. However, these *Campylobacter* species live in the human oral cavity, they may repeatedly colonize the lower parts of the intestinal tract.

Studies suggest that the enteric pathogenicity of *C. concisus* may be determined by both the characteristics of individual strains and an individual's intestinal environment. *C. concisus* normally colonizes the human oral cavity[19,21], some individuals are colonized with multiple oral *C. concisus* strains, which was more often seen in patients with active IBD[22]. *C. concisus* strains are very sensitive to low pH; most of the swallowed *C. concisus* bacteria are likely to have been killed by the acidic gastric juice. Bile is also a great inhibitor to the growth of *C. concisus*[23]. These observations in part explain the low isolation rate of *C. concisus* from fecal samples despite the bacterium being transported from the oral cavity to the lower parts of the gastrointestinal tract through swallowed saliva or food[24,25]. The inhibitory effect of bile to *C. concisus* growth is dose dependent[23]. This may be one of the reasons why *C. concisus* was more often detected in intestinal biopsies collected from descending colon and rectum of patients with IBD in a previous study by Mahendran *et al*[16]. A small number of oral *C. concisus* strains have greater abilities in resisting the antimicrobial effects of low pH and bile. The association between these strains and different phenotypic variants of IBD are under investigation.

In addition to gastric acid and bile, another environmental factor that may affect the colonization of *C. concisus* in the intestinal tract is H_2 gas. H_2 gas has a great impact on *C. concisus* growth. In laboratory cultivation, *C. concisus* does not grow under microaerobic conditions but has a very slow growth under anaerobic conditions[26,27]. The presence of H_2 gas enables *C. concisus* to grow under microaerobic conditions and markedly increases its growth under anaerobic conditions[26]. The atmospheric conditions in the human intestinal tract are microaerobic to anaerobic. Given this, *C. concisus* is likely to establish an intestinal colonization in individuals whose intestinal environment is able to provide a constantly available H_2 for *C. concisus* to use in their growth.

Normally, bacterial species cause disease in the location where they colonize. In contrast, *C. concisus* has an unusual disease association; it uses the human oral cavity as its natural colonization site, but is associated with IBD occurring at the lower parts of the gastrointestinal tract[14-16,18]. This unusual disease association pattern suggests that *C. concisus* may have unique virulence factors that are expressed in the intestinal environment. We previously identified a number of putative prophages in *C. concisus* genome[21], one of which is CON_phi2. CON_phi2 contains a gene that encodes zonula occludens toxin (Zot)[21]. Recently we detected the expression of Zot in *C. concisus* and found that *C. concisus* Zot has biological effects on Caco2 cells[28]. Whether enteric environmental factors affect the release of *C. concisus* Zot toxin and the pathogenic mechanisms of *C. concisus* Zot are current under investigation, which will shed lights on further understanding why *C. concisus*, an oral commensal bacterium, may contribute to inflammatory diseases in the lower parts of the gastrointestinal tract. The *zot* gene was also detected in *C. ureolyticus* strains isolated from amniotic fluid and vagina[29]. Whether *C. ureolyticus* strains isolated from the oral cavity of patients with IBD have the *zot* gene and the pathogenicity of *C. ureolyticus* Zot are currently under investigation, which may reveal a common pathogenic mechanism shared by a number of *Campylobacter* species.

KOCH'S POSTULATES AND THE ROLE OF *C. CONCISUS* IN IBD

A question that we have often encountered in examining the role of *C. concisus* in IBD was whether the relationship between this bacterium and IBD has fulfilled Koch's postulates. In 1880s, Robert Koch proposed some criteria, which were called Koch's postulates, to determine the causative relationship between a microbe and a disease. Despite its contribution to the development of microbiology, these postulates have limitations, which have been discussed by other researchers[30]. IBD is not a single disease and *C. concisus* is not a typical pathogen. *C. concisus* is a bacterium that is present in everyone's oral cavity and some strains have acquired additional virulence factors such as toxins encoded by prophages. The pathogenicity of this bacterium is determined not only by the virulence of individual strains but also an individual's gastrointestinal environmental factors. Given this, Koch's postulates are not suitable to assess the relationship between *C. concisus* and IBD.

IMPORTANCE OF IDENTIFYING BACTERIAL SPECIES THAT INITIATE IBD AND SUGGESTIONS TO CONSIDER *C. CONCISUS* AND OTHER ORAL *CAMPYLOBACTER* SPECIES AS A TARGET IN MANAGEMENT OF HUMAN IBD

Due to the unknown identities of bacterial species that initiate the disease, the treatment of IBD is predominantly symptomatic management, involving anti-inflammation and suppression of patient's immune system. As a result, relapse in IBD is frequent and in some cases, surgery is required. As IBD is a group of diseases, it is important to identify initiators that are responsible for individual cases, which will enable the development of treatment strategies that are suitable for individual patients to reduce relapse and surgery.

Some strategies targeting *C. concisus* and other oral *Campylobacter* species may be incorporated into IBD management. One suggestion is to reduce the load of *C. concisus* and other *Campylobacter* species in the oral cavity using topical treatments. Oral cavity is the natural colonization site of *C. concisus* and a number of other *Campylobacter* species detected in the intestinal tissues of patients with IBD. Reduction of the load of *C. concisus* and other *Campylobacter* species in the oral cavity reduces the possibility of these bacteria colonizing the lower parts of the gastrointestinal tract. The main advantage of this strategy is that it is non-invasive and unlikely to disturb the balance of intestinal microbiota.

The second suggestion is to eradicate *C. concisus* and other oral *Campylobacter* species using antibiotics in patients with IBD, particularly in patients with frequent relapses and multiple surgeries. We previously found that *C. concisus* was not detected in saliva samples collected from IBD children who received metronidazole or ciprofloxacin one month prior[31]. However, *C. concisus* was detected in most of saliva samples (6/7, 86%) collected from IBD children who had antibiotics treatment two months prior[31]. These data showed that the two antibiotics that were used in treatment of some cases of IBD, metronidazole and ciprofloxacin, only had inhibited the growth of oral *C. concisus* or eradicated it from the oral cavity temporarily. An effective antibiotic therapy that can be used to eradicate *C. concisus* needs to be developed. Prior to using a given antibiotic to treat patients with IBD, whether the antibiotic induces *C. concisus* and other oral *Campylobacter* species to produce prophage toxins should also be examined.

FUTURE STUDIES

Accumulated evidence suggests that translocation of *C. concisus* and other *Campylobacter* species from their natural colonization site, the oral cavity, to the lower parts of the gastrointestinal tract may initiate mucosal inflammation there. Further studies investigating the unique pathogenic mechanisms of *C. concisus* and other oral *Campylobacter* species are needed, which will shed light on the understanding of how oral *Campylobacter* species may initiate the development of chronic mucosal inflammatory conditions such as IBD. Diagnostic methods that can accurately identify IBD cases which are caused by translocation of *C. concisus* or other oral *Campylobacter* species should be developed. In addition, effective therapies in reducing or eradicating oral *Campylobacter* species should be established. These strategies will provide useful information in assisting the clinical management of individual IBD cases.

REFERENCES

1 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429 [PMID: 12167685 DOI: 10.1056/NEJMra020831]

2 **Sartor RB**. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 390-407 [PMID: 16819502 DOI: 10.1038/ncpgasthep0528]

3 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]

4 **Jayanthi V**, Probert CS, Pinder D, Wicks AC, Mayberry JF. Epidemiology of Crohn's disease in Indian migrants and the

indigenous population in Leicestershire. *Q J Med* 1992; **82**: 125-138 [PMID: 1620813]
5 **Montgomery SM**, Morris DL, Pounder RE, Wakefield AJ. Asian ethnic origin and the risk of inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 1999; **11**: 543-546 [PMID: 10755259 DOI: 10.1097/00042737-199905000-00013]
6 **Mizoguchi A**. Animal models of inflammatory bowel disease. In: Conn PM, editor. Animal Models of Molecular Pathology, 2012: 263-320
7 **Rutgeerts P**, Goboes K, Peeters M, Hiele M, Penninckx F, Aerts R, Kerremans R, Vantrappen G. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. *Lancet* 1991; **338**: 771-774 [PMID: 1681159 DOI: 10.1016/0140-6736(91)90663-A]
8 **Gevers D**, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, Schwager E, Knights D, Song SJ, Yassour M, Morgan XC, Kostic AD, Luo C, González A, McDonald D, Haberman Y, Walters T, Baker S, Rosh J, Stephens M, Heyman M, Markowitz J, Baldassano R, Griffiths A, Sylvester F, Mack D, Kim S, Crandall W, Hyams J, Huttenhower C, Knight R, Xavier RJ. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* 2014; **15**: 382-392 [PMID: 24629344 DOI: 10.1016/j.chom.2014.02.005]
9 **Walker AW**, Sanderson JD, Churcher C, Parkes GC, Hudspith BN, Rayment N, Brostoff J, Parkhill J, Dougan G, Petrovska L. High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. *BMC Microbiol* 2011; **11**: 7 [PMID: 21219646 DOI: 10.1186/1471-2180-11-7]
10 **Winter SE**, Winter MG, Xavier MN, Thiennimitr P, Poon V, Keestra AM, Laughlin RC, Gomez G, Wu J, Lawhon SD, Popova IE, Parikh SJ, Adams LG, Tsolis RM, Stewart VJ, Bäumler AJ. Host-derived nitrate boosts growth of E. coli in the inflamed gut. *Science* 2013; **339**: 708-711 [PMID: 23393266 DOI: 10.1126/science.1232467]
11 **Craven M**, Egan CE, Dowd SE, McDonough SP, Dogan B, Denkers EY, Bowman D, Scherl EJ, Simpson KW. Inflammation drives dysbiosis and bacterial invasion in murine models of ileal Crohn's disease. *PLoS One* 2012; **7**: e41594 [PMID: 22848538 DOI: 10.1371/journal.pone.0041594]
12 **Rautemaa R**, Jarvis GA, Marnila P, Meri S. Acquired resistance of Escherichia coli to complement lysis by binding of glycophosphoinositol-anchored protectin (CD59). *Infect Immun* 1998; **66**: 1928-1933 [PMID: 9573071]
13 **Hold GL**, Smith M, Grange C, Watt ER, El-Omar EM, Mukhopadhya I. Role of the gut microbiota in inflammatory bowel disease pathogenesis: what have we learnt in the past 10 years? *World J Gastroenterol* 2014; **20**: 1192-1210 [PMID: 24574795 DOI: 10.3748/wjg.v20.i5.1192]
14 **Zhang L**, Man SM, Day AS, Leach ST, Lemberg DA, Dutt S, Stormon M, Otley A, O'Loughlin EV, Magoffin A, Ng PH, Mitchell H. Detection and isolation of Campylobacter species other than C. jejuni from children with Crohn's disease. *J Clin Microbiol* 2009; **47**: 453-455 [PMID: 19052183 DOI: 10.1128/JCM.01949-08]
15 **Man SM**, Zhang L, Day AS, Leach ST, Lemberg DA, Mitchell H. Campylobacter concisus and other Campylobacter species in children with newly diagnosed Crohn's disease. *Inflamm Bowel Dis* 2010; **16**: 1008-1016 [PMID: 19885905 DOI: 10.1002/ibd.21157]
16 **Mahendran V**, Riordan SM, Grimm MC, Tran TA, Major J, Kaakoush NO, Mitchell H, Zhang L. Prevalence of Campylobacter species in adult Crohn's disease and the preferential colonization sites of Campylobacter species in the human intestine. *PLoS One* 2011; **6**: e25417 [PMID: 21966525 DOI: 10.1371/journal.pone.0025417]
17 **Hansen R**, Berry SH, Mukhopadhya I, Thomson JM, Saunders KA, Nicholl CE, Bisset WM, Loganathan S, Mahdi G, Kastner-Cole D, Barclay AR, Bishop J, Flynn DM, McGrogan P, Russell RK, El-Omar EM, Hold GL. The microaerophilic microbiota of de-novo paediatric inflammatory bowel disease: the BISCUIT study. *PLoS One* 2013; **8**: e58825 [PMID: 23554935 DOI: 10.1371/journal.pone.0058825]
18 **Mukhopadhya I**, Thomson JM, Hansen R, Berry SH, El-Omar EM, Hold GL. Detection of Campylobacter concisus and other Campylobacter species in colonic biopsies from adults with ulcerative colitis. *PLoS One* 2011; **6**: e21490 [PMID: 21738679 DOI: 10.1371/journal.pone.0021490]
19 **Zhang L**, Budiman V, Day AS, Mitchell H, Lemberg DA, Riordan SM, Grimm M, Leach ST, Ismail Y. Isolation and detection of Campylobacter concisus from saliva of healthy individuals and patients with inflammatory bowel disease. *J Clin Microbiol* 2010; **48**: 2965-2967 [PMID: 20519479 DOI: 10.1128/jcm.02391-09]
20 **Macuch PJ**, Tanner AC. Campylobacter species in health, gingivitis, and periodontitis. *J Dent Res* 2000; **79**: 785-792 [PMID: 10728981 DOI: 10.1177/00220345000790021301]
21 **Zhang L**, Lee H, Grimm MC, Riordan SM, Day AS, Lemberg DA. Campylobacter concisus and inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 1259-1267 [PMID: 24574800 DOI: 10.3748/wjg.v20.i5.1259]
22 **Mahendran V**, Tan YS, Riordan SM, Grimm MC, Day AS, Lemberg DA, Octavia S, Lan R, Zhang L. The prevalence and polymorphisms of zonula occluden toxin gene in multiple Campylobacter concisus strains isolated from saliva of patients with inflammatory bowel disease and controls. *PLoS One* 2013; **8**: e75525 [PMID: 24086553 DOI: 10.1371/journal.pone.0075525]
23 **Ma R**, Sapwell N, Chung HK, Lee H, Mahendran V, Leong RW, Riordan SM, Grimm MC, Zhang L. Investigation of the effects of pH and bile on the growth of oral Campylobacter concisus strains isolated from patients with inflammatory bowel disease and controls. *J Med Microbiol* 2015; **64**: 438-445 [PMID: 25657299 DOI: 10.1099/jmm.0.000013]
24 **Engberg J**, On SL, Harrington CS, Gerner-Smidt P. Prevalence of Campylobacter, Arcobacter, Helicobacter, and Sutterella spp. in human fecal samples as estimated by a reevaluation of isolation methods for Campylobacters. *J Clin Microbiol* 2000; **38**: 286-291 [PMID: 10618103]
25 **Nielsen HL**, Ejlertsen T, Engberg J, Nielsen H. High incidence of Campylobacter concisus in gastroenteritis in North Jutland, Denmark: a population-based study. *Clin Microbiol Infect* 2013; **19**: 445-450 [PMID: 22512739 DOI: 10.1111/j.1469-0691.2012.03852.x]
26 **Lee H**, Ma R, Grimm MC, Riordan SM, Lan R, Zhong L, Raftery M, Zhang L. Examination of the Anaerobic Growth of Campylobacter concisus Strains. *Int J Microbiol* 2014; **2014**: 476047 [PMID: 25214843 DOI: 10.1155/2014/476047]
27 **Vandamme P**, Dewhirst FE, Paster BJ, On SLW. Genus I. Campylobacter. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. Bergey's Manual of Syst Bacteriol. 2 ed. New York: Springer, 2005: 1147-1160
28 **Lee H**, Liu F, Mahendran V, Zhang L. Detection of the zonula occludens toxin expressed in Campylobacter concisus strains. *J Gastroenterol Hepatol* 2014; **29**: 10-10
29 **Bullman S**, Lucid A, Corcoran D, Sleator RD, Lucey B. Genomic investigation into strain heterogeneity and pathogenic potential of the emerging gastrointestinal pathogen Campylobacter ureolyticus. *PLoS One* 2013; **8**: e71515 [PMID: 24023611 DOI: 10.1371/journal.pone.0071515]
30 **Fredricks DN**, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clin Microbiol Rev* 1996; **9**: 18-33 [PMID: 8665474]
31 **Wong SM**, Day A, Lemberg D, Riordan S, Grimm MC, Budiman V, Mitchell H, Ismail Y, Zhang L. Antibiotic susceptibility of Campylobacter concisus isolated from patients with inflammatory bowel disease. *J Gastroenterol Hepatol* 2010; **25**: A80-A80
32 **Duerden BI**, Eley A, Goodwin L, Magee JT, Hindmarch JM, Bennett KW. A comparison of Bacteroides ureolyticus isolates from different clinical sources. *J Med Microbiol* 1989; **29**: 63-73 [PMID: 2724326]

33 **Lastovica AJ**, On SLW, Zhang L. The Family Campylobacteraceae. In: Rosenberg E, Delong EF, Loy S, Stackebrandt E, Thomas F, editors. The Prokaryotes. Fourth Edition ed. London: Springer, 2014: 308-326

P- Reviewer: Boscá L, Hibi T, Wang CC, Wang DS **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9245

World J Gastroenterol 2015 August 21; 21(31): 9245-9252
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Treatment of hemorrhoids: A coloproctologist's view

Varut Lohsiriwat

Varut Lohsiriwat, Division of Colon and Rectal Surgery, Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

Author contributions: Lohsiriwat V solely contributed to this article.

Conflict-of-interest statement: The author declare no conflict of interest

Supported by Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Correspondence to: Varut Lohsiriwat, MD, PhD, Associate Professor of Surgery, Division of Colon and Rectal Surgery, Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wang-Lung Road, Bangkok Noi, Bangkok 10700, Thailand. bolloon@hotmail.com
Telephone: +66-2419-8005
Fax: +66-2412-1370

Received: January 26, 2015
Peer-review started: January 27, 2015
First decision: April 14, 2015
Revised: April 21, 2015
Accepted: July 3, 2015
Article in press: July 3, 2015
Published online: August 21, 2015

Abstract

Hemorrhoids is recognized as one of the most common medical conditions in general population. It is clinically characterized by painless rectal bleeding during defecation with or without prolapsing anal tissue. Generally, hemorrhoids can be divided into two types: internal hemorrhoid and external hemorrhoid. External hemorrhoid usually requires no specific treatment unless it becomes acutely thrombosed or causes patients discomfort. Meanwhile, low-graded internal hemorrhoids can be effectively treated with medication and non-operative measures (such as rubber band ligation and injection sclerotherapy). Surgery is indicated for high-graded internal hemorrhoids, or when non-operative approaches have failed, or complications have occurred. Although excisional hemorrhoidectomy remains the mainstay operation for advanced hemorrhoids and complicated hemorrhoids, several minimally invasive operations (including Ligasure hemorrhoidectomy, doppler-guided hemorrhoidal artery ligation and stapled hemorrhoidopexy) have been introduced into surgical practices in order to avoid post-hemorrhiodectomy pain. This article deals with some fundamental knowledge and current treatment of hemorrhoids in a view of a coloproctologist - which includes the management of hemorrhoids in complicated situations such as hemorrhoids in pregnancy, hemorrhoids in immunocompromised patients, hemorrhoids in patients with cirrhosis or portal hypertension, hemorrhoids in patients having antithrombotic agents, and acutely thrombosed or strangulated hemorrhoids. Future perspectives in the treatment of hemorrhoids are also discussed.

Key words: Hemorrhoids; Pathophysiology; Treatment; Outcome; Complication

Core tip: Hemorrhoids is a very common anorectal disease defined as the symptomatic enlargement and/or distal displacement of anal cushions. Apart from abnormally dilated vascular channel and destructive changes in supporting tissue within anal cushions, there is emerging evidence that hemorrhoids is associated with hyperperfusion state of anorectal

region and some degree of tissue inflammation. This article comprehensively and thoroughly reviews the pathophysiology, clinical diagnosis, and current treatment of hemorrhoids - which includes dietary and lifestyle modification, pharmacological approach, office-based procedures and operations for hemorrhoids (such as hemorrhoidectomy and other non-excisional surgery). The management of hemorrhoids in complicated situations is also addressed.

Lohsiriwat V. Treatment of hemorrhoids: A coloproctologist's view. *World J Gastroenterol* 2015; 21(31): 9245-9252 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9245.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9245

INTRODUCTION

Hemorrhoids is a very common anorectal disease defined as the symptomatic enlargement and/or distal displacement of anal cushions[1,2], which are prominences of anal mucosa formed by loose connective tissue, smooth muscle, arterial and venous vessels[3]. The true prevalence of hemorrhoids is unknown; however, recent evidence has suggested an increasing prevalence of hemorrhoids over time. In 1990, an epidemiologic study of hemorrhoids in the United State revealed a prevalence rate of 4.4%, whereas some reports in the 21st century from South Korea and Austria yielded a prevalence of hemorrhoids in adult population of 14.4%[4] and 38.9%[5], respectively. It has been estimated that 25% of British people and 75% of American citizens will experience hemorrhoids at some time in their lives[6,7], especially in pregnant women and elderly adults.

People with hemorrhoids, and those wrongly thought to have hemorrhoids, had a tendency to use self-medication rather than to seek proper medical attention[8]. According to the Google's annual roundup in 2012 (Google Zeitgeist), hemorrhoids was the top trending heath issue in the United State, ahead of gastroesophageal reflux disease and sexually transmitted disease. Unfortunately, the quality of information about hemorrhoids treatment on the internet was greatly variable and almost 50% of websites were of poor quality[9]. Clinicians should therefore advise and treat patients with hemorrhoids with evidence-based medicine and the standard of care. Practically, most patients with low-graded hemorrhoids can be effectively treated with non-operative measures by either primary care physician, gastroenterologist or general surgeon in an outpatient setting. Surgery is indicated for high-graded hemorrhoids, or when non-operative approaches have failed, or complications have occurred[2]. This article deals with some fundamental knowledge and current treatment of uncomplicated and complicated hemorrhoids in a view of a coloproctologist.

CONTEMPORARY PATHOPHYSIOLOGY OF HEMORRHOIDS

The exact pathophysiology of hemorrhoids is poorly understood. Currently, hemorrhoids is the pathologic term describing symptomatic and abnormally downward displacement of normal anal cushions[2]. As a result of destructive changes in the supporting connective tissue and abnormal blood circulation within anal cushions, the sliding anal cushions embrace abnormal dilation and distortion of hemorrhoid plexus. A recent study of morphology and hemodynamic of arterial supply to the anal canal revealed a hyperperfusion state of hemorrhoidal plexus in patients with hemorrhoids[10], suggesting the dysregulation of vascular tone within hemorrhoid tissue[1,2]. Moreover, it was evident that hemorrhoidal tissue contained some inflammatory cells[11] and newly-formed microvessels[12]. For circumferential prolapsing hemorrhoids, these might be related to an internal rectal prolapse[13]. In conclusion, although the true pathophysiology of hemorrhoid development is unknown, it is likely to be multifactorial[2] - including sliding anal cushion, hyperperfusion of hemorrhoid plexus, vascular abnormality, tissue inflammation and internal rectal prolapse (rectal redundancy). The different philosophies of hemorrhoid development may lead to different approaches to the treatment of hemorrhoids[2].

RISK FACTORS FOR HEMORRHOIDS

Several risk factors have been claimed to be the etiologies of hemorrhoid development including aging, obesity, abdominal obesity, depressive mood and pregnancy[4]. Meanwhile, some conditions related to increased intraabdominal pressure, such as constipation and prolonged straining, are widely believed to cause hemorrhoids as a result of compromised venous drainage of hemorrhoid plexus[14]. Some types of food and lifestyle, including low fiber diet, spicy foods and alcohol intake, was reported to link with the development of hemorrhoids and the aggravation of acute hemorrhoid symptoms[15].

DIAGNOSIS AND CLASSIFICATION OF HEMORRHOIDS

The most common presentation of hemorrhoids is painless rectal bleeding during defecation with or without prolapsing anal tissue. The blood is normally not mixed in stool but instead coated on the outer surface of stool, or it is seen during cleansing after bowel movement. The blood is typically bright red since hemorrhoid plexus has direct arteriovenous communication[10]. Patients with complicated hemorrhoids such as acutely thrombosed external hemorrhoids and strangulated internal hemorrhoids may present with anal pain and lump at the anal verge. It is uncommon that patients

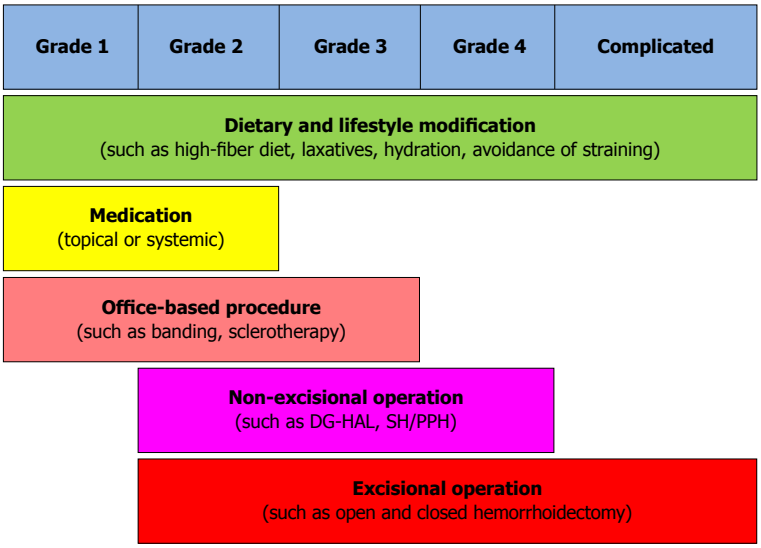


Figure 1 Current treatment of internal hemorrhoids based on their severity and degree of prolapse. DG-HAL: Doppler-guided hemorrhoidal artery ligation; SH: Stapled hemorrhoidopexy; PPH: Procedure for prolapse and hemorrhoids.

with uncomplicated hemorrhoid manifest any anal pain. In fact, severe anal pain in patient with hemorrhoids is more likely due to anal fissure and anorectal abscess[2].

A precise history and thorough physical examination, including digital rectal examination and anoscopy, are imperative for the diagnosis of hemorrhoids. Unless bright red blood is clearly seen from hemorrhoids, any patients with rectal bleeding should be scheduled for flexible sigmoidoscopy or colonoscopy, especially those being at risk of colorectal cancer[1,2].

Hemorrhoids are generally classified by their location; internal (originates above the dentate line and covered by anal mucosa), external (originates below the dentate line and covered by anoderm) and mixed type. Internal hemorrhoids are further graded based on their appearance and degree of prolapse: (1) Grade Ⅰ: non-prolapsing hemorrhoids; (2) Grade Ⅱ: prolapsing hemorrhoids on straining but reduce spontaneously; (3) Grade Ⅲ: prolapsing hemorrhoids requiring manual reduction; and (4) Grade Ⅳ: non-reducible prolapsing hemorrhoids which include acutely thrombosed, incarcerated hemorrhoids[16].

MANAGEMENT OF HEMORRHOIDS

Treatment options mainly depend on the type and severity of hemorrhoids, patient's preference and the expertise of physicians. Low-graded internal hemorrhoids are effectively treated with dietary and lifestyle modification, medical treatment and/or office-based procedures such as rubber band ligation and sclerotherapy (Figure 1). An operation is usually indicated in low-graded hemorrhoids refractory to non-surgical treatment, high-graded hemorrhoids, and strangulated hemorrhoids[2]. Meanwhile, external hemorrhoid requires no specific treatment unless it becomes acutely thrombosed or causes patient discomfort.

Dietary and lifestyle modification

A meta-analysis of 7 clinical trials comprising of 378 patients with hemorrhoids showed that fiber supplement had a consistent benefit of relieving symptom and minimizing risk of bleeding by approximately 50%[17]. Although there is relatively little information of the efficacy of dietary and lifestyle modification on the treatment of hemorrhoids, many physicians include advice on dietary and lifestyle modification as a part of conservative treatment of hemorrhoids and as a preventive measure. The advice usually includes increasing the intake of dietary fiber and oral fluid, having regular exercise, refraining from straining and reading on the toilet, and avoiding drug causing constipation or diarrhea.

Medical treatment

The main goal of medical treatment is to control acute symptoms of hemorrhoids rather than to cure the underlying hemorrhoids. There are several modern drugs and traditional medicine used which are available in a variety of format including pill, suppository, cream and wipes. However, the published literature lacks strong evidence supporting the true efficacy of topical treatment for symptomatic hemorrhoids. For an oral preparation, flavonoids are the most common phlebotonic agent used for treating hemorrhoids[18]. It is apparent that flavonoids could increase vascular tone, reduce venous capacity, decrease capillary permeability, facilitate lymphatic drainage and has anti-inflammatory effects[2]. A large meta-analysis of phlebotonics for hemorrhoids in 2012 showed that phlebotonics had significant beneficial effects on bleeding, pruritus, discharge and overall symptom

improvement. Phlebotonics also alleviated post-hemorrhoidectomy symptoms[19].

Office-based procedures

Many office-based procedures (such as rubber band ligation, injection sclerotherapy, infrared coagulation, cryotherapy, radiofrequency ablation and laser therapy) are effectively performed for grade Ⅰ-Ⅱ hemorrhoids and some cases of grade Ⅲ hemorrhoids with or without local anesthesia. Among several office-based procedures, rubber band ligation (RBL) appeared to have the lowest incidence of recurrent symptom and the need for retreatment[20]. RBL is also the most popular non-surgical intervention for hemorrhoids performed by surgeons[21]. It is a relatively safe and painless procedure with minimal complication. However, RBL is contraindicated in patient with anticoagulants or bleeding disorder, and those with concurrent anorectal sepsis. With a technical note, the proper position of rubber band should be at the base of hemorrhoid bundle or over the bleeding site, but not too close to the dentate line. Vacuum suction ligator may offer clearer visualisation of hemorrhoids and more precise placement of banding when compared to a traditional forcep ligator[22]. Multiple sites and serial sessions of banding may be required for large internal hemorrhoids.

Operative treatment

Surgical intervention is usually required in low-graded hemorrhoids refractory to non-surgical treatment, high-graded symptomatic hemorrhoids, and hemorrhoids with complication such as strangulation and thrombosis. An operation for hemorrhoids may be performed if patient has other concomitant anorectal conditions requiring surgery, or due to patient's preference.

An ideal operation for hemorrhoids should remove internal and external component of hemorrhoids completely, have minimal postoperative pain and complication, demonstrate less recurrence, and are easy to learn and perform. The procedure could be cheap and cost-effective too. Unfortunately, none of the currently available operation achieves all the ideal conditions. So far, excisional hemorrhoidectomy is the mainstay operation for grade Ⅲ-Ⅳ hemorrhoids and complicated hemorrhoids. Of note, closed (Ferguson) hemorrhoidectomy and open (Milligan-Morgan) hemorrhoidectomy were equally effective and safe[23,24], but the Ferguson method was superior to the Milligan-Morgan method in term of long time patient satisfaction and continence[25]. Nevertheless, both techniques may lead to severe postoperative pain[26]. In order to minimize or avoid post-hemorrhoidectomy pain, more recent approaches including Ligasure hemorrhoidectomy, doppler-guided hemorrhoidal artery ligation and stapled hemorrhoidopexy have been adopted into the surgical treatment of hemorrhoids. In addition, perioperative care for hemorrhoids has been significantly improved[1,27].

Surgical excision of hemorrhoids can be done by a variety of instrument such as a scalpel, scissors (Figure 2A), a cautery device, and more recently Ligasure™ - a vessel sealing device (Figure 2B). A recent Cochrane Review demonstrates that Ligasure hemorrhoidectomy resulted in shorter operative time, less postoperative pain, and shorter convalescence period when compared to conventional hemorrhoidectomy[28]. Meanwhile, there was no significant difference in postoperative complications and long-term outcomes between the two techniques. Excisional hemorrhoidectomy can be performed safely in a day-case basis under the perianal infiltration of local anesthetics[29], or regional anesthesia, or general anesthesia. It is evident that some medications could decrease post-hemorrhoidectomy pain such as perioperative analgesia with oral non-steroidal anti-inflammatory drugs[30] and gabapentin[31], topical administration of sucralfate[32] or metronidazole[33], and postoperative administration of phlebotonic drugs[19].

Non-excisional operation for hemorrhoids includes doppler-guided hemorrhoidal artery ligation (DG-HAL) or known as transanal hemorrhoidal dearterialization (THD), and plication of hemorrhoids (or known as ligation anopexy or mucopexy). DG-HAL has been introduced into a surgical practice to cut off the blood supply to hemorrhoids without the need of hemorrhoid removal. It involves the surgical ligation of terminal branches of superior hemorrhoidal artery causing shrinkage of hemorrhoid bundles. Plication of hemorrhoids is often performed with DG-HAL to control the prolapse more effectively. However, the recurrence rate following DG-HAL was up to 60% for grade Ⅳ hemorrhoids. DG-HAL is therefore considered as one of the effective operations only for grade Ⅱ-Ⅲ hemorrhoids with a one-year recurrence rate of 10% for prolapse and 10% for bleeding[34]. Notably, DG-HAL is not a totally painless operation as approximately 20% of patients experienced postoperative pain especially during the defecation[34]. Meanwhile, a ligation anopexy or mucopexy was also demonstrated to be a good alternative to excisional hemorrhoidectomy for grade Ⅱ-Ⅲ hemorrhoids, with shorter operative time and lower postoperative pain[35]. Given the fact that there is the possibility of revascularization and recurrent prolapse, further studies on the long-term outcomes of non-excisional operations for hemorrhoids are needed.

Stapled hemorrhoidopexy, also known as a procedure for prolapse and hemorrhoids (PPH), is an alternative operation for treating advanced internal hemorrhoids. A circular staple device is used to excise a ring of redundant rectal mucosa just above hemorrhoid bundles - not hemorrhoids *per se*. By doing this, prolapsing hemorrhoids will be repositioning (hemorrhoidopexy) and shrinking (due to a partial

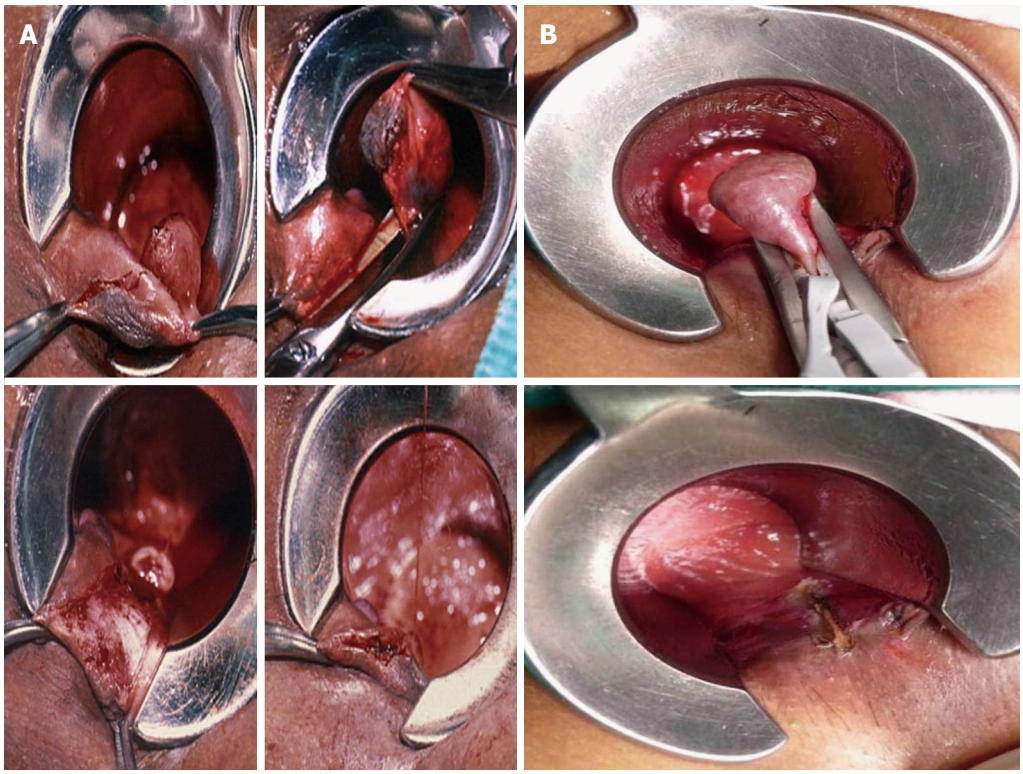


Figure 2 Hemorrhoidectomy by (A) scissors and (B) Ligasure ™ - a vessel sealing device.

interruption of blood supply to hemorrhoid plexus). A recent systematic review of 27 randomized controlled trials demonstrated that, compared with conventional hemorrhoidectomy, stapled hemorrhoidopexy had less pain, shorter operative time, and quicker patient' s recovery of patient, but a significantly higher rate of prolapse and reintervention for prolapse[36]. Interestingly, the latest meta-analysis comparing surgical outcomes between stapled hemorrhoidopexy and Ligasure hemorrhoidectomy in 2013 revealed that both surgical techniques were practically comparable - with a slightly favorable immediate postoperative results and technical advantages for Ligasure hemorrhoidectomy[37].

Given the fact that stapled hemorrhoidopexy did not offer any significant advantages over Ligasure hemorrhoidectomy[37] and it is a relatively expensive operation which may cause serious postoperative complications such as rectal stricture and rectal perforation[38] as well as severe chronic anal pain[39], stapled hemorrhoidopexy should be reserved for patients with circumferential prolapsing hemorrhoids and it must be performed by a well-trained surgeon[2].

SPECIFIC CONSIDERATION

Acutely thrombosed or strangulated internal hemorrhoids

Patients with acutely thrombosed or strangulated internal hemorrhoids usually present with severely painful and irreducible hemorrhoids. The incarcerated hemorrhoids may become necrotic and drain. This situation is quite difficult to treat particularly in a case of extensive strangulation or thrombosis (Figure 3A), or the presence of underlying circumferential prolapse of high-graded hemorrhoids. Manual reduction of the hemorrhoid masses, with or without intravenous analgesia or sedation, might help reducing pain and tissue congestion. Urgent hemorrhoidectomy is usually required in these circumstances. Unless the tissues are necrotic, mucosa and anoderm should be preserved as much as possible to prevent postoperative anal stricture. In expert hands, surgical outcomes of urgent hemorrhoidectomy were comparable to those of elective hemorrhoidectomy[40].

Acutely thrombosed external hemorrhoids

Acutely thrombosed external hemorrhoids often develop in patients with acute constipation, or those with a recent history of prolonged straining. A painful bluish-colored lump at the anal verge is a paramount finding (Figure 3B). The severity of pain is most intense within the first 24-48 h of onset. After that, the thrombosis will be gradually absorbed and patients will experience less pain. As a result, surgical removal of acute thrombus or excisional hemorrhoidectomy may be offered if patients experience severe pain especially within the first 48 h of onset. Otherwise, conservative measure will be exercised including pain control, warm sitz baths, and avoidance of constipation or straining. A resolving thrombosed external hemorrhoid could leave behind as a residual perianal skin tag -which may or

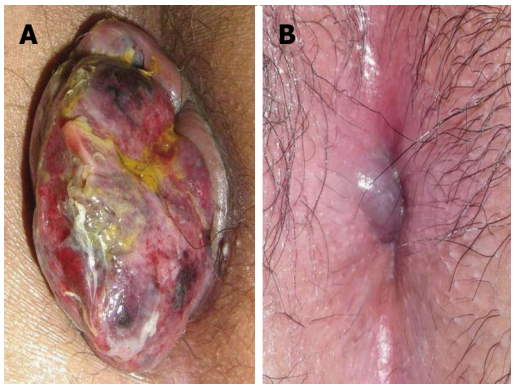


Figure 3 Complicated hemorrhoids. A: Strangulated internal hemorrhoid; B: Acutely thrombosed external hemorrhoid.

may not require a subsequent excision.

Hemorrhoids in pregnancy

Hemorrhoids are very common during pregnancy especially in the third trimester[41]. Acute crisis such as profound bleeding and irreducible prolapsing may be found in pregnant women with pre-existing hemorrhoids. Since hemorrhoids and its symptoms will gradually resolve after giving birth, the primary goal of treatment is to relief acute symptoms related to hemorrhoids - mostly by means of dietary and lifestyle modification. Kegel exercises, lying on left side, and avoidance of constipation could reduce the episode and severity of bleeding and prolapse. Fiber supplement, stool softener and mild laxatives are generally safe for pregnant women. Topical medication or oral phlebotonics may be used with special caution because the strong evidence of their safety and efficacy in pregnancy is lacking. In case of massive bleeding, anal packing could be a simple and useful maneuver. Hemorrhoidectomy is reserved in strangulated or extensively thrombosed hemorrhoids, and hemorrhoids with intractable bleeding.

Hemorrhoids in immunocompromised patients

In general any intervention or operation should be avoided, or performed with a careful consideration in immunocompromised patients because of an increases risk of anorectal sepsis and poor tissue healing in such cases[42]. A conservative measure is the mainstay for the treatment of hemorrhoids in this group of patients. If required, injection sclerotherapy appeared to be a better and safer alternative to banding and hemorrhoidectomy for treating bleeding hemorrhoids[43,44]. Antibiotic prophylaxis is always given before performing any intervention, even a minor office-based procedure, due to the possibility of bacteremia.

Hemorrhoids in patients with cirrhosis or portal hypertension

A clinician must differentiate bleeding hemorrhoids form bleeding anorectal varices because the latter can be managed by suture ligation along the course of varices, transjugular intrahepatic portosystemic shunt, or pharmacological treatment of portal hypertension[1]. Since a majority of bleeding hemorrhoids in such patients is not life threatening, conservative measure with the correction of any coagulopathy is a preferential initial approach. Of note, rubber band ligation is generally contraindicated in patients with advanced cirrhosis due to the risk of profound secondary bleeding following the procedure. Injection sclerotherapy is an effective and safe procedure for treating bleeding hemorrhoids in this situation. In a refractory case, suture ligation at the bleeder is advised. Hemorrhoidectomy is indicated when bleeding hemorrhoids are refractory to other approaches.

Hemorrhoids in patients having anticoagulant or antiplatelet drugs

Anticoagulant or antiplatelet drugs may promote anorectal bleeding in patients with hemorrhoids and increase risk of bleeding after banding or surgery[45]. Unless the bleeding is persistent or profound, the discontinuity of antithrombotic drugs may be unnecessary because most of the bleeding episodes are self-limited and stop spontaneously. Conservative measure is therefore the mainstay treatment in these patients. Injection sclerotherapy is a preferential treatment for bleeding low-graded hemorrhoids refractory to medical treatment. Rubber band ligation is not recommended in patients with the current use of anticoagulant or antiplatelet drugs due to the risk of secondary bleeding. If banding or any form of surgery for hemorrhoids is scheduled, the cessation of anticoagulant or antiplatelet drugs about 5-7 d before and after the procedure is suggested[46].

FUTURE PERSPECTIVES IN THE TREATMENT OF HEMORRHOIDS

To date, it is obvious that, apart from oral flavonoids-based phlebotonic drugs, currently available medication for hemorrhoids has no or limited beneficial effects on bleeding and prolapsing[19]. Since emerging evidence has suggested that perivascular inflammation, dysregulation of the vascular tone and vascular hyperplasia could play an important role in the development of hemorrhoids[2], the microcirculatory system of hemorrhoid tissue could be a potential and robust target for medical treatment. The combinations of vasoconstrictive and venoconstrictive agents, with or without anti-inflammatory drugs, might be a new pharmacological approach for hemorrhoids.

If an intervention, either office-based procedure or surgery - is indicated, evidence-based approaches must be exercised. Day-case operation or ambulatory surgery should be fully developed together with an effective program for peri-operative care[30]. Despite

advances in office-based procedures and better surgical approaches, post-procedural pain and disease recurrence remain the most challenging problems in the treatment of hemorrhoids. Consequently, future researches and novel management of hemorrhoids may focus on how to minimize pain following a procedure and how to prevent recurrent hemorrhoids. Meanwhile, long-term results of newly or recently developed interventions are definitely required.

In conclusion, the better understanding of the pathophysiology of hemorrhoids would prompt the development of effective treatments for hemorrhoids. Preventive measures, by means of dietary and lifestyle modification, may be the best treatment of hemorrhoids. Once hemorrhoids develop, its treatment options mainly depend on the type and severity of hemorrhoids, patient's preference and the expertise of physicians. Post-procedural pain and disease recurrence remain the most challenging problems in the treatment of hemorrhoids.

REFERENCES

1 **Lohsiriwat V**. Approach to hemorrhoids. *Curr Gastroenterol Rep* 2013; **15**: 332 [PMID: 23715885]
2 **Lohsiriwat V**. Hemorrhoids: from basic pathophysiology to clinical management. *World J Gastroenterol* 2012; **18**: 2009-2017 [PMID: 22563187]
3 **Thomson WH**. The nature and cause of haemorrhoids. *Proc R Soc Med* 1975; **68**: 574-575 [PMID: 1197343]
4 **Lee JH**, Kim HE, Kang JH, Shin JY, Song YM. Factors associated with hemorrhoids in korean adults: korean national health and nutrition examination survey. *Korean J Fam Med* 2014; **35**: 227-236 [PMID: 25309703]
5 **Riss S**, Weiser FA, Schwameis K, Riss T, Mittlböck M, Steiner G, Stift A. The prevalence of hemorrhoids in adults. *Int J Colorectal Dis* 2012; **27**: 215-220 [PMID: 21932016]
6 **Tucker H**, George E, Barnett D, Longson C. NICE Technology Appraisal on Stapled Haemorrhoidopexy for the Treatment of Haemorrhoids. *Ann R Coll Surg Engl* 2008; **90**: 82-84 [DOI: 10.1308/003588408X242178b]
7 **Baker H**. Hemorrhoids. In: Longe JL, editor Gale Encyclopedia of Medicine. 3rd ed. Detriot: Thomson Gale, 2006: 1766-1769
8 **Rohde H**, Christ H. [Haemorrhoids are too often assumed and treated. Survey of 548 patients with anal discomfort]. *Dtsch Med Wochenschr* 2004; **129**: 1965-1969 [PMID: 15375737]
9 **Yeung TM**, D'Souza ND. Quality analysis of patient information on surgical treatment of haemorrhoids on the internet. *Ann R Coll Surg Engl* 2013; **95**: 341-344 [PMID: 23838496]
10 **Aigner F**, Gruber H, Conrad F, Eder J, Wedel T, Zelger B, Engelhardt V, Lametschwandtner A, Wienert V, Böhler U, Margreiter R, Fritsch H. Revised morphology and hemodynamics of the anorectal vascular plexus: impact on the course of hemorrhoidal disease. *Int J Colorectal Dis* 2009; **24**: 105-113 [PMID: 18766355 DOI: 10.1007/s00384-008-0572-3]
11 **Morgado PJ**, Suárez JA, Gómez LG, Morgado PJ. Histoclinical basis for a new classification of hemorrhoidal disease. *Dis Colon Rectum* 1988; **31**: 474-480 [PMID: 3378471]
12 **Chung YC**, Hou YC, Pan AC. Endoglin (CD105) expression in the development of haemorrhoids. *Eur J Clin Invest* 2004; **34**: 107-112 [PMID: 14764073 DOI: 10.1111/j.1365-2362.2004.01305.x]
13 **Corman ML**, Gravié JF, Hager T, Loudon MA, Mascagni D, Nyström PO, Seow-Choen F, Abcarian H, Marcello P, Weiss E, Longo A. Stapled haemorrhoidopexy: a consensus position paper by an international working party - indications, contra-indications and technique. *Colorectal Dis* 2003; **5**: 304-310 [PMID: 12814406]
14 **Loder PB**, Kamm MA, Nicholls RJ, Phillips RK. Haemorrhoids: pathology, pathophysiology and aetiology. *Br J Surg* 1994; **81**: 946-954 [PMID: 7922085 DOI: 10.1002/bjs.1800810707]
15 **Pigot F**, Siproudhis L, Allaert FA. Risk factors associated with hemorrhoidal symptoms in specialized consultation. *Gastroenterol Clin Biol* 2005; **29**: 1270-1274 [PMID: 16518286 DOI: 10.1016/S0399-8320(05)82220-1]
16 **Clinical Practice Committee**, American Gastroenterological Association. American Gastroenterological Association medical position statement: Diagnosis and treatment of hemorrhoids. *Gastroenterology* 2004; **126**: 1461-1462 [PMID: 15131806 DOI: 10.1053/j.gastro.2004.03.001]
17 **Alonso-Coello P**, Mills E, Heels-Ansdell D, López-Yarto M, Zhou Q, Johanson JF, Guyatt G. Fiber for the treatment of hemorrhoids complications: a systematic review and meta-analysis. *Am J Gastroenterol* 2006; **101**: 181-188 [PMID: 16405552 DOI: 10.1111/j.1572-0241.2005.00359.x]
18 **Misra MC**. Drug treatment of haemorrhoids. *Drugs* 2005; **65**: 1481-1491 [PMID: 16134260 DOI: 10.2165/00003495-200565110-00003]
19 **Perera N**, Liolitsa D, Iype S, Croxford A, Yassin M, Lang P, Ukaegbu O, van Issum C. Phlebotonics for haemorrhoids. *Cochrane Database Syst Rev* 2012; **8**: CD004322 [PMID: 22895941]
20 **MacRae HM**, McLeod RS. Comparison of hemorrhoidal treatment modalities. A meta-analysis. *Dis Colon Rectum* 1995; **38**: 687-694 [PMID: 7607026]
21 **Beattie GC**, Wilson RG, Loudon MA. The contemporary management of haemorrhoids. *Colorectal Dis* 2002; **4**: 450-454 [PMID: 12790918 DOI: 10.1046/j.1463-1318.2002.00371.x]
22 **Ramzisham AR**, Sagap I, Nadeson S, Ali IM, Hasni MJ. Prospective randomized clinical trial on suction elastic band ligator versus forceps ligator in the treatment of haemorrhoids. *Asian J Surg* 2005; **28**: 241-245 [PMID: 16234072]
23 **Ho YH**, Buettner PG. Open compared with closed haemorrhoidectomy: meta-analysis of randomized controlled trials. *Tech Coloproctol* 2007; **11**: 135-143 [PMID: 17510742]
24 **Arbman G**, Krook H, Haapaniemi S. Closed vs. open hemorrhoidectomy--is there any difference? *Dis Colon Rectum* 2000; **43**: 31-34 [PMID: 10813120]
25 **Jóhannsson HO**, Påhlman L, Graf W. Randomized clinical trial of the effects on anal function of Milligan-Morgan versus Ferguson haemorrhoidectomy. *Br J Surg* 2006; **93**: 1208-1214 [PMID: 16952213 DOI: 10.1002/bjs.5408]
26 **Yeo D**, Tan KY. Hemorrhoidectomy - making sense of the surgical options. *World J Gastroenterol* 2014; **20**: 16976-16983 [PMID: 25493010]
27 **Lohsiriwat V**. Update on common anorectal diseases. *Tech Coloproctol* 2014; **18**: 87-88 [PMID: 24178950]
28 **Nienhuijs S**, de Hingh I. Conventional versus LigaSure hemorrhoidectomy for patients with symptomatic Hemorrhoids. *Cochrane Database Syst Rev* 2009; (**1**): CD006761 [PMID: 19160300 DOI: 10.1002/14651858.CD006761]
29 **Lohsiriwat D**, Lohsiriwat V. Outpatient hemorrhoidectomy under perianal anesthetics infiltration. *J Med Assoc Thai* 2005; **88**: 1821-1824 [PMID: 16518980]
30 **Lohsiriwat V**, Lohsiriwat D. Ambulatory anorectal surgery under perianal anesthetics infiltration: analysis of 222 cases. *J Med Assoc Thai* 2007; **90**: 278-281 [PMID: 17375632]
31 **Poylin V**, Quinn J, Messer K, Nagle D. Gabapentin significantly decreases posthemorrhoidectomy pain: a prospective study. *Int J Colorectal Dis* 2014; **29**: 1565-1569 [PMID: 25269619]
32 **Gupta PJ**, Heda PS, Kalaskar S, Tamaskar VP. Topical sucralfate decreases pain after hemorrhoidectomy and improves healing: a randomized, blinded, controlled study. *Dis Colon Rectum* 2008; **51**: 231-234 [PMID: 18095028]
33 **Ala S**, Saeedi M, Eshghi F, Mirzabeygi P. Topical metronidazole can reduce pain after surgery and pain on defecation in postoperative hemorrhoidectomy. *Dis Colon Rectum* 2008; **51**: 235-238 [PMID: 18176825]
34 **Giordano P**, Overton J, Madeddu F, Zaman S, Gravante G. Transanal hemorrhoidal dearterialization: a systematic review. *Dis*

Colon Rectum 2009; **52**: 1665-1671 [PMID: 19690499]
35 **Elshazly WG**, Gazal AE, Madbouly K, Hussen A. Ligation anopexy versus hemorrhoidectomy in the treatment of second- and third-degree hemorrhoids. *Tech Coloproctol* 2015; **19**: 29-34 [PMID: 25421703]
36 **Burch J**, Epstein D, Sari AB, Weatherly H, Jayne D, Fox D, Woolacott N. Stapled haemorrhoidopexy for the treatment of haemorrhoids: a systematic review. *Colorectal Dis* 2009; **11**: 233-243; discussion 243 [PMID: 18637932 DOI: 10.1111/j.1463-1318.2008.01638.x]
37 **Yang J**, Cui PJ, Han HZ, Tong DN. Meta-analysis of stapled hemorrhoidopexy vs LigaSure hemorrhoidectomy. *World J Gastroenterol* 2013; **19**: 4799-4807 [PMID: 23922480]
38 **Faucheron JL**, Voirin D, Abba J. Rectal perforation with life-threatening peritonitis following stapled haemorrhoidopexy. *Br J Surg* 2012; **99**: 746-753 [PMID: 22418745]
39 **Ielpo B**, Venditti D, Balassone V, Favetta U, Buonomo O, Petrella G. Proctalgia as a late complication of stapled hemorrhoidectomy. Report of our case series. *Int J Surg* 2010; **8**: 648-652 [PMID: 20797456]
40 **Pattana-arun J**, Wesarachawit W, Tantiphlachiva K, Atithansakul P, Sahakitrungruang C, Rojanasakul A. A comparison of early postoperative results between urgent closed hemorrhoidectomy for prolapsed thrombosed hemorrhoids and elective closed hemorrhoidectomy. *J Med Assoc Thai* 2009; **92**: 1610-1615 [PMID: 20043562]
41 **Gojnic M**, Dugalic V, Papic M, Vidaković S, Milićević S, Pervulov M. The significance of detailed examination of hemorrhoids during pregnancy. *Clin Exp Obstet Gynecol* 2005; **32**: 183-184 [PMID: 16433160]
42 **Morandi E**, Merlini D, Salvaggio A, Foschi D, Trabucchi E. Prospective study of healing time after hemorrhoidectomy: influence of HIV infection, acquired immunodeficiency syndrome, and anal wound infection. *Dis Colon Rectum* 1999; **42**: 1140-1144 [PMID: 10496553]
43 **Scaglia M**, Delaini GG, Destefano I, Hultén L. Injection treatment of hemorrhoids in patients with acquired immunodeficiency syndrome. *Dis Colon Rectum* 2001; **44**: 401-404 [PMID: 11289287]
44 **Buchmann P**, Seefeld U. Rubber band ligation for piles can be disastrous in HIV-positive patients. *Int J Colorectal Dis* 1989; **4**: 57-58 [PMID: 2708884]
45 **Nelson RS**, Thorson AG. Risk of bleeding following hemorrhoidal banding in patients on antithrombotic therapy. *Gastroenterol Clin Biol* 2009; **33**: 463-465 [PMID: 19524384 DOI: 10.1016/j.gcb.2009.05.007]
46 **Mounsey AL**, Halladay J, Sadiq TS. Hemorrhoids. *Am Fam Physician* 2011; **84**: 204-210 [PMID: 21766771]

P- Reviewer: Li W, Peng JS, Rutegard J **S- Editor**: Yu J
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9253

World J Gastroenterol 2015 August 21; 21(31): 9253-9261
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Lynch syndrome and Lynch syndrome mimics: The growing complex landscape of hereditary colon cancer

John M Carethers, Elena M Stoffel

John M Carethers, Elena M Stoffel, Division of Gastroenterology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109-5368, United States

John M Carethers, Elena M Stoffel, Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI 48109-5368, United States

Author contributions: Both authors contributed to this manuscript.

Supported by The United States Public Health Service (DK067287 and CA162147); and the A. Alfred Taubman Medical Research Institute of the University of Michigan.

Conflict-of-interest statement: No potential conflicts of interest are disclosed. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Correspondence to: John M Carethers, MD, Division of Gastroenterology, Department of Internal Medicine, University of Michigan, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-5368, United States. jcarethe@umich.edu
Telephone: +1-734-6151717
Fax: +1-734-6152645

Received: May 9, 2015
Peer-review started: May 12, 2015
First decision: June 2, 2015
Revised: June 13, 2015
Accepted: July 8, 2015
Article in press: July 8, 2015
Published online: August 21, 2015

Abstract

Hereditary non-polyposis colorectal cancer (HNPCC) was previously synonymous with Lynch syndrome; however, identification of the role of germline mutations in the DNA mismatch repair (MMR) genes has made it possible to differentiate Lynch syndrome from other conditions associated with familial colorectal cancer (CRC). Broadly, HNPCC may be dichotomized into conditions that demonstrate defective DNA MMR and microsatellite instability (MSI) *vs* those conditions that demonstrate intact DNA MMR. Conditions characterized by MMR deficient CRCs include Lynch syndrome (germline MMR mutation), Lynch-like syndrome (biallelic somatic MMR mutations), constitutional MMR deficiency syndrome (biallelic germline MMR mutations), and sporadic MSI CRC (somatic biallelic methylation of *MLH1*). HNPCC conditions with intact DNA MMR associated with familial CRC include polymerase proofreading associated polyposis and familial colorectal cancer type X. Although next generation sequencing technologies have elucidated the genetic cause for some HNPCC conditions, others remain genetically undefined. Differentiating between Lynch syndrome and the other HNPCC disorders has profound implications for cancer risk assessment and surveillance of affected patients and their at-risk relatives. Clinical suspicion coupled with molecular tumor analysis and testing for germline mutations can help differentiate the clinical mimicry within HNPCC and facilitate diagnosis and management.

Key words: Hereditary non-polyposis colorectal cancer; Lynch syndrome; Lynch-like syndrome; Familial colorectal cancer; DNA mismatch repair; Microsatellite instability; Familial colorectal cancer type X; Constitutional mismatch repair deficiency syndrome; Hereditary colorectal cancer

Core tip: Clinical criteria and phenotypic presentation

of patients and families with hereditary non-polyposis colorectal cancer (HNPCC) do not adequately differentiate the several genetic diseases now classified under HNPCC. Tumor analysis for microsatellite instability (MSI) can dichotomize for the clinician conditions with MSI or without MSI, allowing a focused differential diagnosis. Individual or panel germline genetic testing can further differentiate HNPCC into its genetically defined syndromes or its phenocopies.

Carethers JM, Stoffel EM. Lynch syndrome and Lynch syndrome mimics: The growing complex landscape of hereditary colon cancer. *World J Gastroenterol* 2015; 21(31): 9253-9261 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9253.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9253

INTRODUCTION

About one-third of patients diagnosed with colorectal cancer (CRC) have a family history of cancer, placing them and other family members at elevated risk for this disease. Only 5% of all patients with CRC have identifiable causes for their cancer predisposition; most of which are inherited mutations in genes that regulate growth processes in colonic stem cells, and/or are caretakers of the genome to ensure the fidelity of DNA passed onto progeny cells. The most common of these inherited CRC syndromes is Lynch syndrome, identified and defined by heritable germline mutations of DNA mismatch repair (MMR) genes[1-5]. The term hereditary non-polyposis colorectal cancer (HNPCC), previously used interchangeably with Lynch syndrome, now refers to a broader spectrum of familial CRC encompassing disorders that can mimic some clinical features of Lynch syndrome, but without germline mutations in MMR genes characteristic of Lynch syndrome (Figure 1).

Distinguishing among the HNPCC disorders is important clinically, as the approach to surveillance for patients and their at-risk family members differs according to risks for colonic and extracolonic cancers associated with each syndrome[5]. Health care providers should be observant for "red flags" suggestive of genetic predisposition to CRC, such as strong personal or family history of cancer, diagnoses of colorectal neoplasia at young ages, and histopathologic and molecular tumor features that are characteristic of specific syndromes. Screening of CRC tumors for microsatellite instability (MSI) and expression of DNA MMR genes (Figure 1) is an effective strategy to facilitate identification of patients at risk for Lynch syndrome[6]. Individuals whose personal and/or family history raises suspicion for a hereditary cancer syndrome should undergo clinical genetic evaluation, which includes genetic counseling and evaluation of patient health records[5,7]. Even if a genetic mutation is not identified, the outcome of the genetic evaluation may help guide decision making regarding surveillance and other interventions to reduce future cancer risk.

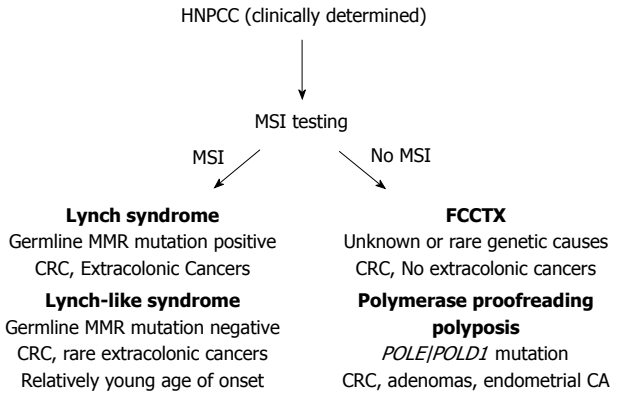


Figure 1 Hereditary non-polyposis colorectal cancer conditions can be dichotomized *via* microsatellite instability testing and/or DNA mismatch repair protein immunohistochemistry. When MSI [or loss of mismatch repair (MMR) protein expression] is present in the colorectal cancer indicating loss of functional DNA MMR, Lynch syndrome and Lynch-like syndrome remain in the differential. Germline testing for DNA MMR gene mutation can differentiate these two syndromes. When MSI is absent meaning DNA MMR remains intact and functional, consideration for polymerase proofreading associated polyposis and familial colorectal cancer type X should be undertaken. Performing germline testing for *POLE* and *POLD1* mutations might help differentiate these two syndromes. HNPCC: Hereditary non-polyposis colorectal cancer; MSI: Microsatellite instability; CRC: Colorectal cancer.

Here, we review several HNPCC conditions that may mimic Lynch syndrome and present distinguishing features and tests that can help differentiate among them. Next generation sequencing approaches will facilitate discovery of novel genetic events that will define the clinical and molecular phenotypes of familial CRC without germline MMR gene mutations.

FAMILIAL CRC WITH DEFECTIVE DNA MISMATCH REPAIR

The DNA MMR system provides recognition of post-DNA synthetic polymerase mistakes in the DNA strand at single base mispairs, chemotherapy-induced nucleotide alterations, and slippage mistakes at repetitive sequences termed microsatellites[1,3,8-12]. The DNA MMR recognition complexes, MutSα and MutSβ, consists of heterodimers of the MMR proteins MSH2-MSH6 and MSH2-MSH3, respectively, with MutSα recognizing single base mispairs and short insertion/deletion loops (I/D loops) of $\leqslant$ 2 nucleotides, and MutSβ recognizing $\geqslant$ 2 I/D loops[1,12,13]. Once a mispair or I/D loop is recognized, the execution complex MutLα (heterodimer of the MMR proteins MLH1 and PMS2) binds to MutSα or MutSβ to signal other proteins for excision and re-synthesis of the affected DNA, or commits the cell to programmed cell death if repair is futile[1,13,14].

There are several mechanisms that can inactivate

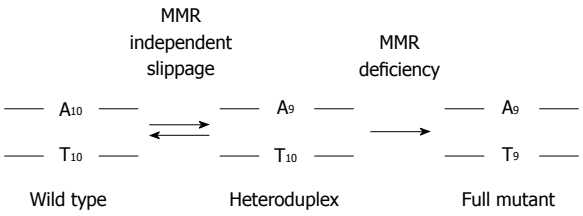


Figure 2 Loss of DNA mismatch repair forces polymerase slippage mistakes to become permanent frameshift mutations at microsatellite sequences. Depicted is a mononucleotide microsatellite of 10 adenines. During DNA replication, occasional polymerase mistakes allow slippage at microsatellite sequences, creating a heteroduplex structure often with one nucleotide as a loop. With intact DNA mismatch repair (MMR), the deletion loop is recognized, excised, and resynthesized correctly such that daughter cells will maintain fidelity of the proper microsatellite length. In the absence of DNA MMR, the deletion loop becomes a permanent frameshift in daughter cells. Frameshift mutations can occur in non-coding as well as in coding microsatellites; coding frameshifts cause the transcription and ultimate translation of truncated proteins that can act as neoantigens to the immune system.

DNA MMR function[1,4,13,15-29]. Abrogation of DNA MMR function generates a hypermutated tumor that accumulates hundreds of random point mutations and frameshifts in the cell's genome[30], and transition from an adenoma to CRC occurs at a rapid pace compared to MMR-intact tumors (1-3 years *vs* 1-2 decades, respectively)[1,3,31]. The biomarker assay that is used to determine loss of MMR function clinically is microsatellite instability (MSI)[1,3,32], which detects acquired new frameshift length changes of microsatellites in neoplastic tissue compared to non-neoplastic tissue when MMR function is defective (Figure 2). Additionally, the use of immunohistochemistry (IHC) to detect expression of DNA MMR proteins in neoplastic tissue is highly correlative to MMR function, with absence of MMR proteins predictive of MSI within the tumor[1,3,32]. The finding of MSI and/or absence of DNA MMR protein expression identifies that a tumor has lost DNA MMR function, and is the basis for differentiating familial CRC cases associated with Lynch syndrome from other HNPCC conditions (Figure 1).

CRCs with defective DNA MMR comprise only 15% of all CRCs and are associated with specific clinicopathologic features. MSI tumors are more likely to be located in the colon proximal to the splenic flexure, often exhibit poor differentiation and mucinous features, possess sub-epithelial lymphoid aggregates as response to neoantigens induced by truncated proteins produced from frameshifted coding microsatellite mutations (Figure 2), and demonstrate better survival compared to same-staged patients without MSI CRCs[1-3,33-37]. Because intact DNA MMR can recognize chemotherapy-induced nucleotide alterations to trigger cell demise, and in particular incorporated 5-fluorodeoxyuracil as a result of 5-fluorouracil (5-FU) therapy, loss of DNA MMR function renders the CRC resistant to 5-FU, and 5-FU treatment does not improve survival of patients with MSI CRCs[8-12,38-42]. However, the MSI CRC somatic mutational load is high, making it more susceptible to immune killing when the immune checkpoint inhibitor to PD-1 is administered to patients[43]. While the majority of MSI CRCs represent sporadic tumors which develop as a consequence of somatic events (*BRAF* mutation, *MLH1* promoter hypermethylation), a minority develop as a consequence of germline mutations in MMR genes associated with Lynch syndrome.

Lynch syndrome

Lynch syndrome can be identified in 2%-3% of all CRC patients, and approximately 2% of all endometrial cancer patients, the two most common cancers observed with this syndrome[2,44,45]. Lifetime risk for CRC approaches 80% and lifetime risk for endometrial cancer in women approaches 50%[2,5]. Patients can develop synchronous and metachronous cancers at relatively young ages, and Lynch-associated CRCs demonstrate accelerated neoplastic progression, with reports of cancers developing within 3 years after colonoscopy[46]. While CRCs are the most common tumors, risks for malignancies of the endometrium and ovaries, gastrointestinal tract (stomach and small intestine, pancreas, biliary tract), urinary tract, brain (glioblastomas), and skin (keratoacanthomas and sebaceous adenomas) are also increased[2,5].

Lynch syndrome is associated with germline mutations in one of the DNA MMR genes (*MSH2, MLH1, MSH6, PMS2, EPCAM*) (Table 1), and is transmitted in an autosomal dominant fashion[2,4,5]. Germline testing for mutations in the MMR genes is the gold standard for characterizing Lynch syndrome, and can be identified in > 80% of Lynch kindreds. The two most commonly mutated genes, *MSH2* and *MLH1*, account for approximately 90% of mutations found in Lynch kindreds, and can be point mutations, deletions, or rearrangements. *MSH2* and *MLH1* are critical components of the MMR recognition complexes and MMR execution complexes, respectively. Germline mutation of *MSH6* and *PMS2* are identified in < 10% of Lynch kindreds, and deletion of *EPCAM*, upstream of *MSH2* on chromosome 2 that causes allele specific methylation of the promoter of *MSH2*, is a relatively rare cause for loss of *MSH2* expression and Lynch syndrome[2,4,5]. Germline *MSH3* mutations have only rarely been identified in any families with Lynch syndrome[13,47]. Specific mutations of DNA MMR genes are associated with differences in phenotype of Lynch patients. For instance, *MLH1* and *MSH2* mutation carriers present with cancers at younger ages (40-50 years) whereas *MSH6* mutation carriers tend to be older at CRC diagnosis (age 50-65 years) with higher prevalence of endometrial cancer[2,3,5].

Family history-based clinical criteria, such as the Amsterdam criteria (3 relatives with CRC, across 2 generations, with one case diagnosed at age < 50 years) and/or Bethesda guidelines, have limited sensitivity and identify only a portion of MMR mutation

Table 1 Germline and cancer-specific genetic and epigenetic features for hereditary non-polyposis colorectal cancer conditions

	Lynch syndrome	CMMRD	Lynch-like syndrome	Sporadic MSI CRC and sessile serrated polyps	FCCTX	PPAP	HBOC
Germline mutation	One allele of a MMR gene: *MSH2, MLH1, MSH6, PMS2, EPCAM*	Both alleles of a MMR gene: *MSH2, MLH1, MSH6, PMS2, EPCAM*	None	None	*RPS20, SEMA4A, HNRNPA0, WIF1,* likely others	*POLE* (*L424V*) *POLD1* (*S478N*) (other exonuclease domain mutations)	*BRCA1* or *BRCA2*
Somatic mutation	2nd allele of MMR gene	None	Both alleles of a MMR gene	*BRAF*	Various	2nd allele of *POLE, POLD1* and > 100 × somatic mutations (hypermutated) compared with other MSS tumors	2nd allele of *BRCA1* or *BRCA2*
Tumor MMR phenotype	MMR deficient (MSI)	MMR deficient (MSI)	MMR deficient (MSI)	MMR deficient, (MSI)	MMR proficient (MSS)	MMR proficient (MSS)	MMR proficient (MSS)
Epigenetic	Germline deletion in 3′ end of *EPCAM* leads to Somatic Allele-specific *MSH2* methylation in tissues	None reported	None reported	Somatic biallelic promoter methylation for *MLH1*	None reported	None reported	None reported

MSS: Microsatellite stable; CMMRD: Constitutional mismatch repair-deficiency; FCCTX: Familial colorectal cancer type X; PPAP: Polymerase proofreading associated polyposis syndrome; HBOC: Hereditary breast and ovarian cancer syndrome; MSI: Microsatellite instability; CRC: Colorectal cancer.

carriers[2,5,44]. Given that intensive surveillance with colonoscopy every 1-2 years has been shown to decrease morbidity and mortality in families with Lynch syndrome, universal testing of all CRC tumors for MMR deficiency has been proposed as a cost-effective strategy to screen for Lynch syndrome[44,48]. Tumors associated with germline MMR gene mutations can be differentiated from sporadic MSI CRCs on the basis of the absence of somatic *BRAF* mutations and absence of methylation of *MLH1*)[1,3,5,33]. Loss of expression of MSH2 and/or MSH6 was previously thought to be diagnostic of germline mutations in the MMR gene corresponding to the absent protein, but recent data demonstrate that absent expression of these MMR proteins can also be observed in Lynch-like CRCs (Table 1).

Lynch-like syndrome

Lynch-like syndrome may account for as many as 60%-70% of cases in which Lynch syndrome is clinically suspected, but genetic testing fails to identify a germline MMR gene mutation[4]. Patients with Lynch-like syndrome resemble those with Lynch syndrome in that their tumors manifest MSI and immunohistochemical absence of a DNA MMR protein. Patients with Lynch-like syndrome present with cancer at younger ages, similar to Lynch syndrome (53.7 years *vs* 48.5 years of age), fueling the speculation that undiagnosed germline mutations may be implicated in at least some of these cases[22,23,49]. However, analysis of cancers among probands and families demonstrate heterogeneity in risks, with standardized incidence ratio (SIR) for CRC lower in Lynch-like (2.12) compared to Lynch syndrome (6.04), and with SIR for extracolonic cancers also lower in Lynch-like (1.69) *vs* Lynch syndrome (2.81) families[23].

From a clinical perspective, the key feature differentiating Lynch syndrome from Lynch-like syndrome is that the former is associated with presence of germline DNA MMR gene mutations, while the latter lacks identifiable germline mutations. There are several potential explanations for Lynch-like syndrome. First, it is possible that some Lynch-like patients could actually have Lynch syndrome, as there may be some germline mutations in DNA MMR genes that are not detectable by current testing, such as those occurring in areas of intronic sequences and promoters[4]. However, an alternative explanation is that there are other mechanisms that inactivate DNA MMR (aside from germline mutation in MMR genes) which could also result in tumor phenotype that closely resembles Lynch syndrome. Unlike sporadic MSI-H CRCs, Lynch-like CRCs do not show epigenetic inactivation of the DNA MMR gene *MLH1* or mutation in *BRAF*. However, 50%-60% of Lynch-like CRCs do exhibit the biallelic somatic inactivation of DNA MMR genes within the tumor[20,22,23,49-51] (Table 1). Somatic mutation in any one allele of the DNA MMR genes coupled with loss of heterozygosity (LOH) of the other allele is the most common pattern (with second most common mechanism being two somatic sequence mutations). Finally, it is also possible that patients with Lynch-like syndrome may harbor germline mutations in genes other than the DNA MMR genes known to be associated with Lynch syndrome. Consequently, Lynch-like syndrome cases might represent a spectrum of HNPCC conditions with heterogeneous etiologies; however until we have more information regarding cancer risks and rates of neoplastic progression, intensive surveillance

for cancer similar to Lynch syndrome guidelines is recommended.

Constitutional mismatch repair deficiency syndrome

Constitutional mismatch repair-deficiency (CMMRD) is a rare condition in which biallelic germline mutations in DNA MMR genes predispose to development of multiple cancers, often at very early ages[26,27] (Table 1). Affected individuals inherit a germline MMR mutation from each parent, with *PMS2* and *MSH6* mutations most commonly implicated. Although initial descriptions of CMMRD cases involved consanguineous families, a significant proportion of cases involve offspring of unrelated parents not previously diagnosed with Lynch syndrome. CMMRD patients often present in childhood, with brain tumors, colorectal and/or other gastrointestinal cancers (including in some cases multiple colonic adenomas), with hematological malignancies such as leukemias and lymphomas also commonly reported[27]. Other tumors such as rhabdomyosarcoma, Wilms tumor, and neuroblastomas have also been reported[27,52]. The presentation can be variable, but the scope of tumors and the extremely young age at presentation can provide clues to the diagnosis. Since nearly all CMMRD patients exhibit cutaneous café-au-lait spots[27], there may be some phenotypic overlap with other syndromes such as neurofibromatosis type 1, Li-Fraumeni syndrome, and familial adenomatous polyposis[27,53].

The diagnosis of CMMRD is confirmed with detection of biallelic germline mutations in MMR genes[27] (Table 1); however the diagnosis is not always straightforward. *PMS2* in particular has approximately 20 pseudogenes that make identifying one allele mutation, let alone both alleles, challenging. Additionally, variants of uncertain significances (VUSs) in one or both alleles of *MSH6* or *PMS2* or any other MMR gene are found in over 30% of suspected CMMRD patients, making the genetic confirmation of this syndrome difficult[3,5,27].

Like tumors which develop in Lynch syndrome and Lynch-like syndrome, CMMRD CRCs display MSI, and immunohistochemistry will demonstrate absence of staining of the mutated MMR protein. A differentiating feature of CMMRD patients is that the surrounding normal colon tissue may also demonstrate absence of the MMR protein corresponding with the germline mutation[27], given that both alleles are inactivated in every cell of the CMMRD patient's body even before cancer formation.

Sporadic microsatellite unstable CRC

Microsatellite unstable (MSI) is found in approximately 15% of all sporadic CRCs with MSI tumors more likely to develop at older ages (≥ 70 years of age) and more often in females[1,3,34]. Most of these sporadic MSI CRCs are associated with biallelic hypermethylation of the promoter of the *MLH1* gene (Table 1), which prevents its transcription[15-19]. An additional common finding among sporadic MSI CRCs is the presence of $BRAF^{V600E}$, an activating mutation that causes incessant mitogenic pathway signaling[1,3,5,33] (Table 1). The older presentation for CRC, lack of family history of cancer, as well as the presence of $BRAF^{V600E}$ and/or methylation of *MLH1* help distinguish patients with sporadic MSI CRC from those with Lynch or Lynch-like syndrome. Likewise, patients with sessile serrated polyps and adenomas (SSAs) exhibit multiple methylated DNA loci including that of the *MLH1* promoter, and manifest MSI and $BRAF^{V600E}$[33,54]. Although patients with SSAs often present with proximal colon location of the lesion similar to Lynch syndrome tumors, the *MLH1* hypermethylation and presentation of $BRAF^{V600E}$ show that the SSA is not part of Lynch syndrome.

FAMILIAL CRC WITH INTACT DNA MISMATCH REPAIR

Polymerase proofreading associated polyposis syndrome

Polymerase proofreading associated polyposis syndrome (PPAP) is a rare autosomal dominantly inherited syndrome in which the exonuclease domain of *POLE* (encoding DNA polymerase ε) or *POLD1* (encoding DNA polymerase δ1) is mutated in the germline[55,56]. Two highly penetrant mutations are described (POLE p.Leu424Val and POLD1 p.Ser478Asn)[55], but other mutations in the exonucleoase domain could also be causal[57] (Table 1). Individuals with germline *POLE* mutations exhibit colonic oligopolyposis (generally between 5-70 adenomas) as early as 20 years of age, CRCs, and duodenal adenomas and carcinomas[55,56]. *POLD1* mutation carriers exhibit colonic oligopolyposis (generally 3-50 adenomas) and CRC as young as 20 years of age as well, but in addition exhibit increased risk for endometrial cancers and brain tumors[55] which overlaps with the spectrum of tumors seen in Lynch syndrome. Indeed, the clinical phenotype of PPAP patients can be highly variable, ranging from an attenuated polyposis picture resembling that seen in association with germline mutations in *APC* or *MYH* to oligopolyposis or non-polyposis resembling HNPCC and Lynch syndrome[55,56].

Interestingly, although CRCs from PPAP patients are hypermuted or ultramutated (with 100-fold more mutations than sporadic microsatellite stable tumors) due to loss in polymerase function that then generates multiple random mutations in the cancer cell genome, these tumors are microsatellite stable and do not exhibit loss of expression of DNA MMR proteins[30,55,56,58] (Table 1). The absence of MSI in these CRCs is a distinguishing feature, and should raise the clinical suspicion of PPAP in families with cancer histories suggestive of Lynch syndrome.

Familial colorectal cancer type X syndrome

Familial colorectal cancer type X (FCCTX) is the

designation for patients with family history of CRC meeting Amsterdam Criteria for Lynch syndrome, but whose tumors lack MSI and whose germline lacks DNA MMR gene mutations[59]. Nearly half of Amsterdam Criteria-positive CRC families fit the description of FCCTX. Clinically, however, CRC risk among FCCTX patients is increased approximately 2-fold over the general population (compared to > 6-fold for Lynch syndrome patients), and FCCTX families lack extracolonic cancers[59].

As the "type X" label implies, the genetic etiology for FCCTX is largely unknown. Recent investigations suggest that FCCTX may be a heterogeneous condition, since gene finding studies have uncovered mutations in several genes, each affecting only one or a few families. Germline mutations in *RPS20*, encoding an rRNA maturation protein[60], as well as in *SEMA4A*, *HNRNPA0* and *WIF1*, whose encoded proteins are involved in the regulation of PI3 Kinases and MAPK/ERK signaling and NAD biosynthesis, have been identified[61,62] (Table 1). There is potential for phenotypic overlap between FCCTX and other known genetic conditions (such as PPAP), and germline mutations in *BMPR1A* (associated with juvenile polyposis) have been identified in some individuals with the clinical diagnosis of FCCTX[63]. Even so, genetic testing is clinically uninformative in the vast majority of patients with MMR proficient tumors without polyposis phenotypes.

Potential overlap with other hereditary cancer syndromes: Hereditary breast and ovarian cancer syndrome

CRCs are common, and thus may be seen in association with other hereditary cancer syndromes not typically associated with increased risk for colorectal neoplasia. Patients with hereditary breast and ovarian cancer syndrome (HBOC) possess a germline mutation in *BRCA1* or *BRCA2*, two genes involved in DNA double strand break repair (Table 1). The spectrum of cancers in kindreds with HBOC can include not only breast and ovarian cancer, but also pancreatic cancer and prostate cancer[64]. Additionally, some reports suggest that breast and prostate cancers may also be overrepresented in Lynch syndrome kindreds[64,65]. Thus, there is potential for phenotypic overlap between HBOC and Lynch syndrome, especially with regard to ovarian and pancreatic cancers[66,67], with Lynch syndrome potentially accounting for 13%-15% of hereditary ovarian cancers[68]. The lifetime risk for ovarian cancer in Lynch syndrome patients is approximately 8%[68-70]. Consequently, Lynch syndrome and HBOC should each be considered in the differential diagnosis for kindreds with ovarian and/or pancreatic cancers.

In evaluating suspected Lynch syndrome families, gene panel testing covering 25 cancer-causing genes yielded 9% of suspected families with germline DNA MMR gene mutations[71]. Surprisingly, another 1% demonstrated germline mutations in *BRCA1* or *BRCA2*, with 93% of these patients meeting NCCN guidelines for Lynch syndrome testing while only 33% meeting NCCN guidelines for BRCA1 or BRCA2 analysis[71]. This study demonstrates the phenotypic overlap between HBOC and HNPCC. This study also demonstrates that the use of broader panel testing can be highly informative in differentiating these syndrome genetically.

CONCLUSION

HNPCC encompasses a spectrum of conditions that have significant phenotypic overlap, and making a genetic diagnosis in familial CRC cases can be clinically challenging. Since risks for CRC and extracolonic cancers differ among the various conditions, genetic confirmation of the diagnosis can help direct surveillance recommendations for the patient and their at-risk family members. As clinical criteria have limited sensitivity and specificity, analysis of tumor tissue for the presence or absence of MSI can be effective for identifying individuals at risk for Lynch syndrome. Genetic testing for germline mutations in individual genes (or panels of genes) can further differentiate HNPCC into specific, defined syndromes. Even in the absence of an informative genetic test result, clinical suspicion should remain high, and specialized surveillance is justified for at-risk individuals from families affected with HNPCC. Next generation sequencing will likely uncover additional genetic defects in HNPCC kindreds. Documentation of phenotypes and cancer incidence through longitudinal studies will provide valuable clinical information regarding the natural history of disease which will help differentiate the Lynch syndrome mimics and guide diagnosis and management for the heterogeneous conditions currently grouped under the category of familial CRC.

REFERENCES

1 **Grady WM**, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. *Gastroenterology* 2008; **135**: 1079-1099 [PMID: 18773902 DOI: 10.1053/j.gastro.2008.07.076]

2 **Boland CR**, Koi M, Chang DK, Carethers JM. The biochemical basis of microsatellite instability and abnormal immunohistochemistry and clinical behavior in Lynch syndrome: from bench to bedside. *Fam Cancer* 2008; **7**: 41-52 [PMID: 17636426]

3 **Boland CR**, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* 2010; **138**: 2073-2087.e3 [PMID: 20420947 DOI: 10.1053/j.gastro.2009.12.064]

4 **Carethers JM**. Differentiating Lynch-like from Lynch syndrome. *Gastroenterology* 2014; **146**: 602-604 [PMID: 24468183 DOI: 10.1053/j.gastro.2014.01.041]

5 **Stoffel EM**, Kastrinos F. Familial colorectal cancer, beyond Lynch syndrome. *Clin Gastroenterol Hepatol* 2014; **12**: 1059-1068 [PMID: 23962553 DOI: 10.1016/j.cgh.2013.08.015]

6 **Palomaki GE**, McClain MR, Melillo S, Hampel HL, Thibodeau SN. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. *Genet Med* 2009; **11**: 42-65 [PMID: 19125127 DOI: 10.1097/GIM.0b013e31818fa2db]

7 **Carethers JM**. DNA testing and molecular screening for colon

cancer. *Clin Gastroenterol Hepatol* 2014; **12**: 377-381 [PMID: 24355100 DOI: 10.1016/j.cgh.2013.12.007]
8 **Tajima A**, Hess MT, Cabrera BL, Kolodner RD, Carethers JM. The mismatch repair complex hMutS alpha recognizes 5-fluorouracil-modified DNA: implications for chemosensitivity and resistance. *Gastroenterology* 2004; **127**: 1678-1684 [PMID: 15578504]
9 **Tajima A**, Iwaizumi M, Tseng-Rogenski S, Cabrera BL, Carethers JM. Both hMutSα and hMutSß DNA mismatch repair complexes participate in 5-fluorouracil cytotoxicity. *PLoS One* 2011; **6**: e28117 [PMID: 22164234 DOI: 10.1371/journal.pone.0028117]
10 **Iwaizumi M**, Tseng-Rogenski S, Carethers JM. DNA mismatch repair proficiency executing 5-fluorouracil cytotoxicity in colorectal cancer cells. *Cancer Biol Ther* 2011; **12**: 756-764 [PMID: 21814034 DOI: 10.4161/cbt.12.8.17169]
11 **Iwaizumi M**, Tseng-Rogenski S, Carethers JM. Acidic tumor microenvironment downregulates hMLH1 but does not diminish 5-fluorouracil chemosensitivity. *Mutat Res* 2013; **747-748**: 19-27 [PMID: 23643670 DOI: 10.1016/j.mrfmmm.2013.04.006]
12 **Hamaya Y**, Guarinos C, Tseng-Rogenski SS, Iwaizumi M, Das R, Jover R, Castells A, Llor X, Andreu M, Carethers JM. Efficacy of Adjuvant 5-Fluorouracil Therapy for Patients with EMAST-Positive Stage II/III Colorectal Cancer. *PLoS One* 2015; **10**: e0127591 [PMID: 25996601 DOI: 10.1371/journal.pone.0127591]
13 **Carethers JM**, Koi M, Tseng-Rogenski SS. EMAST is a Form of Microsatellite Instability That is Initiated by Inflammation and Modulates Colorectal Cancer Progression. *Genes* (Basel) 2015; **6**: 185-205 [PMID: 25836926 DOI: 10.3390/genes6020185]
14 **Carethers JM**, Hawn MT, Chauhan DP, Luce MC, Marra G, Koi M, Boland CR. Competency in mismatch repair prohibits clonal expansion of cancer cells treated with N-methyl-N'-nitro-N-nitrosoguanidine. *J Clin Invest* 1996; **98**: 199-206 [PMID: 8690794]
15 **Miyakura Y**, Sugano K, Konishi F, Ichikawa A, Maekawa M, Shitoh K, Igarashi S, Kotake K, Koyama Y, Nagai H. Extensive methylation of hMLH1 promoter region predominates in proximal colon cancer with microsatellite instability. *Gastroenterology* 2001; **121**: 1300-1309 [PMID: 11729109]
16 **Nakagawa H**, Nuovo GJ, Zervos EE, Martin EW, Salovaara R, Aaltonen LA, de la Chapelle A. Age-related hypermethylation of the 5' region of MLH1 in normal colonic mucosa is associated with microsatellite-unstable colorectal cancer development. *Cancer Res* 2001; **61**: 6991-6995 [PMID: 11585722]
17 **Veigl ML**, Kasturi L, Olechnowicz J, Ma AH, Lutterbaugh JD, Periyasamy S, Li GM, Drummond J, Modrich PL, Sedwick WD, Markowitz SD. Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. *Proc Natl Acad Sci USA* 1998; **95**: 8698-8702 [PMID: 9671741]
18 **Herman JG**, Umar A, Polyak K, Graff JR, Ahuja N, Issa JP, Markowitz S, Willson JK, Hamilton SR, Kinzler KW, Kane MF, Kolodner RD, Vogelstein B, Kunkel TA, Baylin SB. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci USA* 1998; **95**: 6870-6875 [PMID: 9618505]
19 **Kane MF**, Loda M, Gaida GM, Lipman J, Mishra R, Goldman H, Jessup JM, Kolodner R. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 1997; **57**: 808-811 [PMID: 9041175]
20 **Mensenkamp AR**, Vogelaar IP, van Zelst-Stams WA, Goossens M, Ouchene H, Hendriks-Cornelissen SJ, Kwint MP, Hoogerbrugge N, Nagtegaal ID, Ligtenberg MJ. Somatic mutations in MLH1 and MSH2 are a frequent cause of mismatch-repair deficiency in Lynch syndrome-like tumors. *Gastroenterology* 2014; **146**: 643-646.e8 [PMID: 24333619 DOI: 10.1053/j.gastro.2013.12.002]
21 **Diouf B**, Cheng Q, Krynetskaia NF, Yang W, Cheok M, Pei D, Fan Y, Cheng C, Krynetskiy EY, Geng H, Chen S, Thierfelder WE, Mullighan CG, Downing JR, Hsieh P, Pui CH, Relling MV, Evans WE. Somatic deletions of genes regulating MSH2 protein stability cause DNA mismatch repair deficiency and drug resistance in human leukemia cells. *Nat Med* 2011; **17**: 1298-1303 [PMID: 21946537 DOI: 10.1038/nm.2430]
22 **Haraldsdottir S**, Hampel H, Tomsic J, Frankel WL, Pearlman R, de la Chapelle A, Pritchard CC. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. *Gastroenterology* 2014; **147**: 1308-1316.e1 [PMID: 25194673 DOI: 10.1053/j.gastro.2014.08.041]
23 **Rodríguez-Soler M**, Pérez-Carbonell L, Guarinos C, Zapater P, Castillejo A, Barberá VM, Juárez M, Bessa X, Xicola RM, Clofent J, Bujanda L, Balaguer F, Reñé JM, de-Castro L, Marín-Gabriel JC, Lanas A, Cubiella J, Nicolás-Pérez D, Brea-Fernández A, Castellví-Bel S, Alenda C, Ruiz-Ponte C, Carracedo A, Castells A, Andreu M, Llor X, Soto JL, Payá A, Jover R. Risk of cancer in cases of suspected lynch syndrome without germline mutation. *Gastroenterology* 2013; **144**: 926-932.e1; quiz e13-e14 [PMID: 23354017 DOI: 10.1053/j.gastro.2013.01.044]
24 **Li F**, Mao G, Tong D, Huang J, Gu L, Yang W, Li GM. The histone mark H3K36me3 regulates human DNA mismatch repair through its interaction with MutSα. *Cell* 2013; **153**: 590-600 [PMID: 23622243 DOI: 10.1016/j.cell.2013.03.025]
25 **Crépin M**, Dieu MC, Lejeune S, Escande F, Boidin D, Porchet N, Morin G, Manouvrier S, Mathieu M, Buisine MP. Evidence of constitutional MLH1 epimutation associated to transgenerational inheritance of cancer susceptibility. *Hum Mutat* 2012; **33**: 180-188 [PMID: 21953887 DOI: 10.1002/humu.21617]
26 **Scott RH**, Mansour S, Pritchard-Jones K, Kumar D, MacSweeney F, Rahman N. Medulloblastoma, acute myelocytic leukemia and colonic carcinomas in a child with biallelic MSH6 mutations. *Nat Clin Pract Oncol* 2007; **4**: 130-134 [PMID: 17259933]
27 **Bakry D**, Aronson M, Durno C, Rimawi H, Farah R, Alharbi QK, Alharbi M, Shamvil A, Ben-Shachar S, Mistry M, Constantini S, Dvir R, Qaddoumi I, Gallinger S, Lerner-Ellis J, Pollett A, Stephens D, Kelies S, Chao E, Malkin D, Bouffet E, Hawkins C, Tabori U. Genetic and clinical determinants of constitutional mismatch repair deficiency syndrome: report from the constitutional mismatch repair deficiency consortium. *Eur J Cancer* 2014; **50**: 987-996 [PMID: 24440087 DOI: 10.1016/j.ejca.2013.12.005]
28 **Tseng-Rogenski SS**, Chung H, Wilk MB, Zhang S, Iwaizumi M, Carethers JM. Oxidative stress induces nuclear-to-cytosol shift of hMSH3, a potential mechanism for EMAST in colorectal cancer cells. *PLoS One* 2012; **7**: e50616 [PMID: 23226332 DOI: 10.1371/journal.pone.0050616]
29 **Tseng-Rogenski SS**, Hamaya Y, Choi DY, Carethers JM. Interleukin 6 alters localization of hMSH3, leading to DNA mismatch repair defects in colorectal cancer cells. *Gastroenterology* 2015; **148**: 579-589 [PMID: 25461668 DOI: 10.1053/j.gastro.2014.11.027]
30 **Cancer Genome Atlas Network**. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]
31 **Tsao JL**, Yatabe Y, Salovaara R, Järvinen HJ, Mecklin JP, Aaltonen LA, Tavaré S, Shibata D. Genetic reconstruction of individual colorectal tumor histories. *Proc Natl Acad Sci USA* 2000; **97**: 1236-1241 [PMID: 10655514]
32 **Boland CR**, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998; **58**: 5248-5257 [PMID: 9823339]
33 **Carethers JM**. One colon lumen but two organs. *Gastroenterology* 2011; **141**: 411-412 [PMID: 21708155 DOI: 10.1053/j.gastro.2011.06.029]
34 **Carethers JM**, Murali B, Yang B, Doctolero RT, Tajima A, Basa R, Smith EJ, Lee M, Janke R, Ngo T, Tejada R, Ji M, Kinseth M, Cabrera BL, Miyai K, Keku TO, Martin CF, Galanko JA, Sandler RS, McGuire KL. Influence of race on microsatellite instability and CD8+ T cell infiltration in colon cancer. *PLoS One* 2014; **9**: e100461 [PMID: 24956473 DOI: 10.1371/journal.pone.0100461]
35 **Carethers JM**. Screening for colorectal cancer in African Americans: determinants and rationale for an earlier age to commence screening. *Dig Dis Sci* 2015; **60**: 711-721 [PMID: 25540085 DOI: 10.1007/s10620-014-3443-5]

36 **Popat S**, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005; **23**: 609-618 [PMID: 15659508]

37 **Schwitalle Y**, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel HP, Tariverdian M, Benner A, von Knebel Doeberitz M. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. *Gastroenterology* 2008; **134**: 988-997 [PMID: 18395080 DOI: 10.1053/j.gastro.2008.01.015]

38 **Carethers JM**, Chauhan DP, Fink D, Nebel S, Bresalier RS, Howell SB, Boland CR. Mismatch repair proficiency and in vitro response to 5-fluorouracil. *Gastroenterology* 1999; **117**: 123-131 [PMID: 10381918]

39 **Jo WS**, Carethers JM. Chemotherapeutic implications in microsatellite unstable colorectal cancer. *Cancer Biomark* 2006; **2**: 51-60 [PMID: 17192059]

40 **Carethers JM**, Smith EJ, Behling CA, Nguyen L, Tajima A, Doctolero RT, Cabrera BL, Goel A, Arnold CA, Miyai K, Boland CR. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* 2004; **126**: 394-401 [PMID: 14762775]

41 **Ribic CM**, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE, Tu D, Redston M, Gallinger S. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003; **349**: 247-257 [PMID: 12867608]

42 **Chung H**, Chaudhry J, Lopez CG, Carethers JM. Cyclin E and histone H3 levels are regulated by 5-fluorouracil in a DNA mismatch repair-dependent manner. *Cancer Biol Ther* 2010; **10**: 1147-1156 [PMID: 20930505]

43 **Le DT**, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015; **372**: 2509-2520 [PMID: 26028255]

44 **Hampel H**, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Nakagawa H, Sotamaa K, Prior TW, Westman J, Panescu J, Fix D, Lockman J, Comeras I, de la Chapelle A. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med* 2005; **352**: 1851-1860 [PMID: 15872200]

45 **Hampel H**, Frankel W, Panescu J, Lockman J, Sotamaa K, Fix D, Comeras I, La Jeunesse J, Nakagawa H, Westman JA, Prior TW, Clendenning M, Penzone P, Lombardi J, Dunn P, Cohn DE, Copeland L, Eaton L, Fowler J, Lewandowski G, Vaccarello L, Bell J, Reid G, de la Chapelle A. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. *Cancer Res* 2006; **66**: 7810-7817 [PMID: 16885385]

46 **Järvinen HJ**, Aarnio M, Mustonen H, Aktan-Collan K, Aaltonen LA, Peltomäki P, De La Chapelle A, Mecklin JP. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology* 2000; **118**: 829-834 [PMID: 10784581]

47 **Yang X**, Wu J, Lu J, Liu G, Di G, Chen C, Hou Y, Sun M, Yang W, Xu X, Zhao Y, Hu X, Li D, Cao Z, Zhou X, Huang X, Liu Z, Chen H, Gu Y, Chi Y, Yan X, Han Q, Shen Z, Shao Z, Hu Z. Identification of a comprehensive spectrum of genetic factors for hereditary breast cancer in a Chinese population by next-generation sequencing. *PLoS One* 2015; **10**: e0125571 [PMID: 25927356 DOI: 10.1371/journal.pone.0125571]

48 **Giardiello FM**, Allen JI, Axilbund JE, Boland CR, Burke CA, Burt RW, Church JM, Dominitz JA, Johnson DA, Kaltenbach T, Levin TR, Lieberman DA, Robertson DJ, Syngal S, Rex DK. Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the US Multi-Society Task Force on colorectal cancer. *Gastroenterology* 2014; **147**: 502-526 [PMID: 25043945 DOI: 10.1053/j.gastro.2014.04.001]

49 **Geurts-Giele WR**, Leenen CH, Dubbink HJ, Meijssen IC, Post E, Sleddens HF, Kuipers EJ, Goverde A, van den Ouweland AM, van Lier MG, Steyerberg EW, van Leerdam ME, Wagner A, Dinjens WN. Somatic aberrations of mismatch repair genes as a cause of microsatellite-unstable cancers. *J Pathol* 2014; **234**: 548-559 [PMID: 25111426 DOI: 10.1002/path.4419]

50 **Sourrouille I**, Coulet F, Lefevre JH, Colas C, Eyries M, Svrcek M, Bardier-Dupas A, Parc Y, Soubrier F. Somatic mosaicism and double somatic hits can lead to MSI colorectal tumors. *Fam Cancer* 2013; **12**: 27-33 [PMID: 22987205 DOI: 10.1007/s10689-012-9568-9]

51 **Kang SY**, Park CK, Chang DK, Kim JW, Son HJ, Cho YB, Yun SH, Kim HC, Kwon M, Kim KM. Lynch-like syndrome: characterization and comparison with EPCAM deletion carriers. *Int J Cancer* 2015; **136**: 1568-1578 [PMID: 25110875 DOI: 10.1002/ijc.29133]

52 **Kratz CP**, Holter S, Etzler J, Lauten M, Pollett A, Niemeyer CM, Gallinger S, Wimmer K. Rhabdomyosarcoma in patients with constitutional mismatch-repair-deficiency syndrome. *J Med Genet* 2009; **46**: 418-420 [PMID: 19293170 DOI: 10.1136/jmg.2008.064212]

53 **Jasperson KW**, Samowitz WS, Burt RW. Constitutional mismatch repair-deficiency syndrome presenting as colonic adenomatous polyposis: clues from the skin. *Clin Genet* 2011; **80**: 394-397 [PMID: 21039432 DOI: 10.1111/j.1399-0004.2010.01543.x]

54 **Leggett B**, Whitehall V. Role of the serrated pathway in colorectal cancer pathogenesis. *Gastroenterology* 2010; **138**: 2088-2100 [PMID: 20420948 DOI: 10.1053/j.gastro.2009.12.066]

55 **Palles C**, Cazier JB, Howarth KM, Domingo E, Jones AM, Broderick P, Kemp Z, Spain SL, Guarino E, Salguero I, Sherborne A, Chubb D, Carvajal-Carmona LG, Ma Y, Kaur K, Dobbins S, Barclay E, Gorman M, Martin L, Kovac MB, Humphray S, Lucassen A, Holmes CC, Bentley D, Donnelly P, Taylor J, Petridis C, Roylance R, Sawyer EJ, Kerr DJ, Clark S, Grimes J, Kearsey SE, Thomas HJ, McVean G, Houlston RS, Tomlinson I. Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat Genet* 2013; **45**: 136-144 [PMID: 23263490 DOI: 10.1038/ng.2503]

56 **Spier I**, Holzapfel S, Altmüller J, Zhao B, Horpaopan S, Vogt S, Chen S, Morak M, Raeder S, Kayser K, Stienen D, Adam R, Nürnberg P, Plotz G, Holinski-Feder E, Lifton RP, Thiele H, Hoffmann P, Steinke V, Aretz S. Frequency and phenotypic spectrum of germline mutations in POLE and seven other polymerase genes in 266 patients with colorectal adenomas and carcinomas. *Int J Cancer* 2015; **137**: 320-331 [PMID: 25529843 DOI: 10.1002/ijc.29396]

57 **Valle L**, Hernández-Illán E, Bellido F, Aiza G, Castillejo A, Castillejo MI, Navarro M, Seguí N, Vargas G, Guarinos C, Juarez M, Sanjuán X, Iglesias S, Alenda C, Egoavil C, Segura Á, Juan MJ, Rodriguez-Soler M, Brunet J, González S, Jover R, Lázaro C, Capellá G, Pineda M, Soto JL, Blanco I. New insights into POLE and POLD1 germline mutations in familial colorectal cancer and polyposis. *Hum Mol Genet* 2014; **23**: 3506-3512 [PMID: 24501277 DOI: 10.1093/hmg/ddu058]

58 **Church DN**, Briggs SE, Palles C, Domingo E, Kearsey SJ, Grimes JM, Gorman M, Martin L, Howarth KM, Hodgson SV, Kaur K, Taylor J, Tomlinson IP. DNA polymerase ε and δ exonuclease domain mutations in endometrial cancer. *Hum Mol Genet* 2013; **22**: 2820-2828 [PMID: 23528559 DOI: 10.1093/hmg/ddt131]

59 **Lindor NM**, Rabe K, Petersen GM, Haile R, Casey G, Baron J, Gallinger S, Bapat B, Aronson M, Hopper J, Jass J, LeMarchand L, Grove J, Potter J, Newcomb P, Terdiman JP, Conrad P, Moslein G, Goldberg R, Ziogas A, Anton-Culver H, de Andrade M, Siegmund K, Thibodeau SN, Boardman LA, Seminara D. Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency: familial colorectal cancer type X. *JAMA* 2005; **293**: 1979-1985 [PMID: 15855431]

60 **Nieminen TT**, O'Donohue MF, Wu Y, Lohi H, Scherer SW, Paterson AD, Ellonen P, Abdel-Rahman WM, Valo S, Mecklin JP, Järvinen HJ, Gleizes PE, Peltomäki P. Germline mutation of RPS20, encoding a ribosomal protein, causes predisposition to hereditary nonpolyposis colorectal carcinoma without DNA mismatch repair

deficiency. *Gastroenterology* 2014; **147**: 595-598.e5 [PMID: 24941021 DOI: 10.1053/j.gastro.2014.06.009]

61 **Schulz E**, Klampfl P, Holzapfel S, Janecke AR, Ulz P, Renner W, Kashofer K, Nojima S, Leitner A, Zebisch A, Wölfler A, Hofer S, Gerger A, Lax S, Beham-Schmid C, Steinke V, Heitzer E, Geigl JB, Windpassinger C, Hoefler G, Speicher MR, Richard Boland C, Kumanogoh A, Sill H. Germline variants in the SEMA4A gene predispose to familial colorectal cancer type X. *Nat Commun* 2014; **5**: 5191 [PMID: 25307848 DOI: 10.1038/ncomms6191]

62 **Wei C**, Peng B, Han Y, Chen WV, Rother J, Tomlinson GE, Boland CR, Chaussabel D, Frazier ML, Amos CI. Mutations of HNRNPA0 and WIF1 predispose members of a large family to multiple cancers. *Fam Cancer* 2015; **14**: 297-306 [PMID: 25716654 DOI: 10.1007/s10689-014-9758-8]

63 **Nieminen TT**, Abdel-Rahman WM, Ristimäki A, Lappalainen M, Lahermo P, Mecklin JP, Järvinen HJ, Peltomäki P. BMPR1A mutations in hereditary nonpolyposis colorectal cancer without mismatch repair deficiency. *Gastroenterology* 2011; **141**: e23-e26 [PMID: 21640116 DOI: 10.1053/j.gastro.2011.03.063]

64 **Mersch J**, Jackson MA, Park M, Nebgen D, Peterson SK, Singletary C, Arun BK, Litton JK. Cancers associated with BRCA1 and BRCA2 mutations other than breast and ovarian. *Cancer* 2015; **121**: 269-275 [PMID: 25224030 DOI: 10.1002/cncr.29041]

65 **Buerki N**, Gautier L, Kovac M, Marra G, Buser M, Mueller H, Heinimann K. Evidence for breast cancer as an integral part of Lynch syndrome. *Genes Chromosomes Cancer* 2012; **51**: 83-91 [PMID: 22034109 DOI: 10.1002/gcc.20935]

66 **Raymond VM**, Mukherjee B, Wang F, Huang SC, Stoffel EM, Kastrinos F, Syngal S, Cooney KA, Gruber SB. Elevated risk of prostate cancer among men with Lynch syndrome. *J Clin Oncol* 2013; **31**: 1713-1718 [PMID: 23530095 DOI: 10.1200/JCO.2012.44.1238]

67 **Kastrinos F**, Mukherjee B, Tayob N, Wang F, Sparr J, Raymond VM, Bandipalliam P, Stoffel EM, Gruber SB, Syngal S. Risk of pancreatic cancer in families with Lynch syndrome. *JAMA* 2009; **302**: 1790-1795 [PMID: 19861671 DOI: 10.1001/jama.2009.1529]

68 **Nakamura K**, Banno K, Yanokura M, Iida M, Adachi M, Masuda K, Ueki A, Kobayashi Y, Nomura H, Hirasawa A, Tominaga E, Aoki D. Features of ovarian cancer in Lynch syndrome (Review). *Mol Clin Oncol* 2014; **2**: 909-916 [PMID: 25279173]

69 **South SA**, Vance H, Farrell C, DiCioccio RA, Fahey C, Piver MS, Rodabaugh KJ. Consideration of hereditary nonpolyposis colorectal cancer in BRCA mutation-negative familial ovarian cancers. *Cancer* 2009; **115**: 324-333 [PMID: 19117025 DOI: 10.1002/cncr.24012]

70 **Watson P**, Vasen HF, Mecklin JP, Bernstein I, Aarnio M, Järvinen HJ, Myrhøj T, Sunde L, Wijnen JT, Lynch HT. The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. *Int J Cancer* 2008; **123**: 444-449 [PMID: 18398828 DOI: 10.1002/ijc.23508]

71 **Yurgelun MB**, Allen B, Kaldate RR, Bowles KR, Judkins T, Kaushik P, Roa BB, Wenstrup RJ, Hartman AR, Syngal S. Identification of a Variety of Mutations in Cancer Predisposition Genes in Patients with Suspected Lynch Syndrome. *Gastroenterology* 2015; Epub ahead of print [PMID: 25980754 DOI: 10.1053/j.gastro.2015.05.006]

P- Reviewer: Lakatos PL, Roncucci L **S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9262

World J Gastroenterol 2015 August 21; 21(31): 9262-9272
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Role of phytochemicals in colorectal cancer prevention

Yu-Hua Li, Yin-Bo Niu, Yang Sun, Feng Zhang, Chang-Xu Liu, Lei Fan, Qi-Bing Mei

Yu-Hua Li, Yang Sun, Feng Zhang, Lei Fan, Qi-Bing Mei, Key Laboratory of Gastrointestinal Pharmacology of Chinese Materia Medica of the State Administration of Traditional Chinese Medicine, Collaborative Innovation Center for Chinese Medicine in Qinba Mountains, Department of Pharmacology, School of Pharmacy, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China

Yu-Hua Li, No. 181 Hospital of PLA, Guilin 541002, Guangxi Zhuang Autonomous Region, China

Yin-Bo Niu, Key Laboratory for Space Bioscience and Biotechnology, School of Life Sciences, Northwestern Polytechnical University, Xi'an 710072, Shaanxi Province, China

Chang-Xu Liu, No. 303 Hospital of PLA, Nanning 530022, Guangxi Zhuang Autonomous Region, China

Author contributions: Li YH and Mei QB did problem formulation; Niu YB, Sun Y and Fan L performed literature search; Li YH, Zhang F, and Liu CX wrote the paper.

Supported by Postdoctoral Science Foundation of China, No. 2012M512102, No. 2013T60964; and National Nature Science Foundation of China, No. 81302787.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

Correspondence to: Qi-Bing Mei, PhD, Key Laboratory of Gastrointestinal Pharmacology of Chinese Materia Medica of the State Administration of Traditional Chinese Medicine, Collaborative Innovation Center for Chinese Medicine in Qinba Mountains, Department of Pharmacology, School of Pharmacy, Fourth Military Medical University, No. 169 Changle West Road, Xi'an 710032, Shaanxi Province, China. qbmei53@hotmail.com

Telephone: +86-29-84779212
Fax: +86-29-84779212

Received: March 27, 2015
Peer-review started: March 28, 2015
First decision: April 28, 2014
Revised: May 9, 2015
Accepted: July 15, 2015
Article in press: July 15, 2015
Published online: August 21, 2015

Abstract

Although the incidence of colorectal cancer (CRC) has been declining in recent decades, it remains a major public health issue as a leading cause of cancer mortality and morbidity worldwide. Prevention is one milestone for this disease. Extensive study has demonstrated that a diet containing fruits, vegetables, and spices has the potential to prevent CRC. The specific constituents in the dietary foods which are responsible for preventing CRC and the possible mechanisms have also been investigated extensively. Various phytochemicals have been identified in fruits, vegetables, and spices which exhibit chemopreventive potential. In this review article, chemopreventive effects of phytochemicals including curcumin, polysaccharides (apple polysaccharides and mushroom glucans), saponins (Paris saponins, ginsenosides and soy saponins), resveratrol, and quercetin on CRC and the mechanisms are discussed. This review proposes the need for more clinical evidence for the effects of phytochemicals against CRC in large trials. The conclusion of the review is that these phytochemicals might be therapeutic candidates in the campaign against CRC.

Key words: Phytochemicals; Fruits; Vegetables; Spices; Colorectal cancer; Cancer prevention

Core tip: Colorectal cancer (CRC) remains a major public health issue as a leading cause of cancer mortality and morbidity worldwide. Chemoprevention is one milestone for this disease. A diet consisting of fruits, vegetables, and spices has the potential to prevent CRC. This manuscript reviews the phytochemicals in these dietary foods that are responsible for preventing CRC and the possible mechanisms. Various phytochemicals have been identified in fruits, vegetables, and spices which exhibit chemopreventive potential. This work will further promote the importance of phytochemicals in CRC prevention.

Li YH, Niu YB, Sun Y, Zhang F, Liu CX, Fan L, Mei QB. Role of phytochemicals in colorectal cancer prevention. *World J Gastroenterol* 2015; 21(31): 9262-9272 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9262.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9262

INTRODUCTION

Colorectal cancer (CRC) continues to be a worldwide killer, despite that the past decade has witnessed enormous amount of research and rapid development. According to recent statistics, CRC is the fourth most common malignant tumor worldwide, with an annual incidence of 1.2 million new cases and over 600000 deaths[1]. CRC mortality has been increasing in many Western countries[2]. In 2014, there were an estimated 136830 new cases of CRC and 50310 patients died from CRC in the United States[1]. In several areas at low risk historically, including Spain, and some countries in Eastern Europe and Eastern Asia, the incidence of CRC is rapidly increasing[2,3]. The number of CRC cases and the mortality are increasing in China as well, where more than 170000 new cases of CRC are diagnosed each year[4].

Considerable advances in neoadjuvant chemotherapy and surgical techniques have been achieved in the past decades. *e.g.*, the median survival period of CRC patients of stage Ⅳ was prolonged to 17.9 mo by adding bevacizumab to the program of 5-fluorouracil/calcium folinate[5]. However, the 5-year survival rate of colon cancer of stage Ⅳ was only 8.1% after treatments[6].

Anand *et al*[7] pointed out that only 5%-10% of all cancer cases are caused by genetic defects and the remaining 90%-95% are caused by environment and lifestyle, providing great opportunities for cancer prevention. Therefore, chemoprevention, which is defined as the intake of foreign agents in order to restrain induction, prevent or slow the progression of cancer, or reverse carcinogenesis at a premalignant stage has drawn more and more attention from both the scientific community and the general public[8]. Among cancers, CRC is a good candidate for chemoprevention owing to the long precancerous stage that provides individuals with an opportunity to interfere before adenomas develop into cancer. Various pharmacologic or dietary agents have been evaluated for their chemopreventive effects against cancer[9,10]. Unlike other cancer cells, CRC cells are exposed directly to the agents. Then, there is a growing interest to investigate how these agents are associated with CRC. Celecoxib, a cyclooxgenase-2 (COX-2) inhibitor, is approved for preventing familial adenomatous polyposis (FAP). However, the chemopreventive benefit of celecoxib is at the expense of its serious cardiovascular adverse effect[11]. The serious side effects of the FDA approved chemopreventive drugs are usually paid particular attention when taking into account long-term use of a drug in healthy people who may or may not develop cancer. This clearly indicates the need for agents, which are effective as well as safe in preventing CRC. Diet derived phytochemicals will be potential candidates for this purpose. It is widely believed that a diet high in fruits, vegetables, spices, and grains possesses beneficial effects on the intestine, particularly the colon. The substances in these dietary foods that are responsible for preventing CRC and the mechanisms by which they achieve this have been extensively studied. A large number of studies have shown that a proper diet can help protect against CRC. Many phytochemicals have been isolated and identified in fruits, vegetables, spices, and grains that show chemopreventive potential[12-15]. Below is a description of selected phytochemicals which have been studied extensively to determine their role in CRC prevention.

NATURAL COMPOUNDS WITH CHEMOPREVENTIVE POTENTIAL

CURCUMIN

Curcumin is a hydrophobic polyphenol derived from turmeric, the rhizome of the herb Curcuma longa, and has a potential in suppressing inflammation and inhibiting the growth of neoplastic cells[16]. Many *in vitro* studies have proven that curcumin could be used as a therapeutic agent for CRC through affecting numerous target molecules.

Both survivin and insulin like growth factor-1 (IGF-1) could lead to inhibition of apoptosis and prolonged survival of colon cancer cells by suppressing the mitochondria-mediated pathway. Curcumin down-regulates the expression of survivin and IGF-1 by activating the expression of p53 and reducing tumor necrosis factor-α (TNF-α) levels, leading to activation of apoptotic signal[17]. Guo *et al*[18] assessed the effects of curcumin and investigated its mechanism in LoVo cells. Cells incubated with 2.5-30 μg/mL of curcumin for 24, 48 or 72 h had a significantly decreased growth rate. Curcumin treatment not only induced cell cycle arrest of LoVo cells at S phase and apoptosis

accompanied by ultra-structural changes and release of lactate dehydrogenase in a dose-dependent manner, but also decreased the mitochondrial membrane potential and activated caspase-3 and caspase-9 in a dose- and time-dependent manner. Furthermore, curcumin increased Bax and p53 and reduced Bcl-2 and survivin expression, and triggered the release of cytochrome c in LoVo cells. The results indicate that curcumin suppressed the growth of LoVo cells, at least in part, by inducing apoptosis through a mitochondria-mediated pathway. Nuclear factor-kappa B (NF-κB) is one of the most important molecules involved in innate immunity and inflammation and has emerged as an important endogenous tumor promoter[19]. NF-κB plays an important role of supervision in controlling the transformation of inflammation in the context of inflammatory cells and cancer. Under resting condition, the NF-κB dimmers reside in the cytoplasm. Upon activation, it translocates to the nucleus, where it triggers the expression of more than 200 genes that exhibit the ability of suppressing apoptosis and inducing proliferation, cellular transformation, invasion, metastasis, inflammation, radio-resistance, and chemo-resistance. NF-κB activation in cancer cells would lead to inflammation-induced tumor growth, and inhibition of NF-κB activation could prevent tumor growth[20]. Various studies have demonstrated the pivotal role of NF-κB in tumor initiation and progression in CRC[21]. Curcumin could mediate its therapeutic effects partly through regulating the transcription factor NF-κB and NF-κB-regulated gene products including cyclin D1, TNF-α, Bcl-2, Bcl-XL, inducible nitric oxide synthase and matrix metallo-preteinases (MMPs)[22]. Curcumin inhibited the TNF-induced activation of inhibitor of nuclear factor kappa-B kinase (IKK), bringing about the suppression of TNF-dependent phosphorylation, degradation of IκBα and translocation of the p65 subunit. Curcumin also blocked hydrogen peroxide- and phorbol ester-induced activation of NF-κB[23].

COX-2 is one of the most important molecules involved in inflammation and cancer. Elevated expression of COX-2 has been detected in the majority of colorectal carcinomas[24-28], and in a subset of adenomas. Inhibition of COX-2 activity, either by genetic disruption or pharmacological methods, leads to reduced size and number of adenomas in murine models of intestinal tumorigenesis[29,30]. Curcumin markedly inhibited the mRNA and protein expression of COX-2, but not COX-1. Lev-Ari *et al*[31] conducted a study to investigate whether curcumin enhances the anti-growth effects of celecoxib, a specific COX-2 inhibitor, in human colon cancer cell line HT-29. This study revealed that in the presence of 10-15 μmol/L of curcumin, a physiologic dose of celecoxib at 5 μmol/L was sufficient to restrain cell growth by suppressing proliferation and promoting apoptosis through the COX-2-dependent and -independent pathways. This effect was similar to what can be achieved by using a 10-fold higher concentration of celecoxib (50 μmol/L) when administrated alone. Curcumin potentiated the growth inhibitory effect of celecoxib by shifting the dose-response curve to the left. The clinical importance of this effect lies in the fact that it can be achieved in the serum of patients treated with standard anti-inflammatory (200-400 mg) or antineoplastic (400-800 mg) doses of celecoxib[31]. The phosphorylated form of cortactin (cortical actin binding protein) or CTTN, a monomeric protein located in the cytoplasm of cells (pTyr^{421}) plays a crucial role in cancer cell migration and invasion. Upregulated pTyr^{421}-cortactin has been detected in colon cancer. Curcumin significantly downregulated pTyr^{421}-CTTN in HCT116 cells and SW480 cells, but had no effect in HT-29 cells. Curcumin physically interacted with PTPN1 tyrosine phosphatases to increase its activity, which resulted in dephosphorylation of pTyr^{421}-CTTN. PTPN1 inhibition could abolish the effects of curcumin on pTyr^{421}-CTTN. Curcumin decreased migration of HCT116 and SW480 cells which highly express PTPN1, but not of HT-29 cells with significantly reduced endogenous PTPN1 level. Adenovirally encoded CTTN increased migration of all three cell lines. Further study showed that curcumin significantly reduced the physical interaction of CTTN and pTyr^{421}-CTTN with p120 catenin (CTNND1). In summary, curcumin mediates the activity of PTPN1 phosphatase to reduce cortactin phosphorylation and interaction with CTNND1, and finally to reduce cell motility in colon cancer[32].

Curcumin, therefore, is a promising chemopreventive natural agent with multiple targets and no reported adverse or toxic events.

The pharmacodynamic and pharmacokinetic effects of oral curcuma extract in patients with CRC have been investigated. In one study, curcumin was administered to 126 patients with CRC before undergoing surgery. Patients either received 360 mg of curcumin or placebo three times daily. Results demonstrated that curcumin treatment was accompanied by increased body weight, decreased serum TNF-α level, modulated tumor cell apoptosis and enhanced p53 expression[17]. A dose-escalation pilot study of Curcuma extract at doses of 0.45-3.6 g in 15 patients with advanced CRC for a period of 4 mo showed a dose dependent inhibition of COX-2 activity, measured as basal and lipopolysaccharide (LPS)-mediated prostaglandin E_2 (PGE_2) production in blood, demonstrating the efficacy of curcumin in CRC. A daily dose of 3.6 g of curcumin was related to a 62% and 57% decrease of inducible PGE_2 on days 1 and 29, respectively ($P < 0.05$)[33]. In one pharmacodynamic and pharmacokinetic study of oral Curcuma extract, 15 patients with advanced CRC refractory to standard chemotherapies were given Curcuma extract at doses ranging from 440 to 2200 mg/d. Curcuma extract including 36-180 mg of curcumin was given for 4 mo. The doses were well tolerated with no obvious toxicity observed.

Table 1 Current clinical trials of curcumin and resveratrol

ClinicalTrials.gov Identifier	Title	Design/phase	Intervention	Primary purpose
NCT01294072	Study investigating the ability of plant exosomes to deliver curcumin to normal and colon cancer tissue	Phase Ⅰ	Dietary supplement: curcumin Dietary supplement: curcumin conjugated with plant exosomes Other: no intervention	Treatment
NCT00295035	Phase Ⅲ trial of gemcitabine, curcumin and celebrex in patients with metastatic colon cancer	Phase Ⅲ	Drug: celecoxib Drug: curcumin	Treatment
NCT00027495	Curcumin for the prevention of colon cancer	Phase Ⅰ	Dietary supplement: curcumin	Prevention
NCT00973869	Curcumin in preventing colorectal cancer in patients undergoing colorectal endoscopy or colorectal surgery	Phase Ⅰ	Dietary supplement: curcumin Other: high performance liquid chromatography Other: laboratory biomarker analysis Other: pharmacological study Procedure: diagnostic endoscopic procedure Procedure: therapeutic conventional surgery	Prevention
NCT00365209	Phase ⅡA trial of curcumin among patients with prevalent subclinical neoplastic lesions (aberrant crypt foci)	Phase Ⅱ	Other: laboratory biomarker analysis Other: pharmacological study Drug: curcumin	Prevention
NCT01490996	Combining curcumin with FOLFOX chemotherapy in patients with inoperable colorectal cancer	Phase Ⅰ Phase Ⅱ	Drug: oral complex C3 curcumin+ chemotherapy Drug: chemotherapy only	Treatment
NCT01859858	A prospective evaluation of the effect of curcumin on dose limiting toxicity and pharmacokinetics of irinotecan in patients with solid tumors	Phase Ⅰ	Dietary supplement: curcumin Drug: Irinotecan	Basic science
NCT00118989	Curcumin for the chemoprevention of colorectal cancer	Phase Ⅱ	4 g curcumin daily for 4 mo or placebo	Prevention
NCT00256334	Resveratrol for patients with colon cancer	Phase Ⅰ	Drug: resveratrol	Treatment
NCT00578396	Phase I biomarker study of dietary grape-derived low dose resveratrol for colon cancer prevention	Phase Ⅰ	Dietary supplement: grapes	Prevention Phase Ⅰ
NCT00433576	Resveratrol in treating patients with colorectal cancer that can be removed by surgery	Phase Ⅰ	Patients with colorectal adenocarcinomas received resveratrol for days 1 to 8 and on day 9 underwent colorectomy Tumor biopsy will be retrieved	

Ingestion of 440 mg for 29 d was correlated with a 59% decrease in lymphocytic glutathione S-transferase activity. This effect was not seen at higher doses. Neither curcumin nor its metabolites were detected in blood or urine. Despite the results, it is difficult to figure out whether curcumin at this dose is an effective chemopreventive agent in CRC because of the small number of subjects[34].

In another study, curcumin capsules (450, 1800, and 3600 mg) were given to patients with CRC daily for 7 d. Trace levels of curcumin were found outside the circulation. Level of M1G, a marker of DNA adduct formation, was significantly reduced by curcumin at a dose of 3600 mg. The study showed that curcumin at a dose of 3.6 g for daily use is pharmacologically efficacious[35]. In a phase IIa clinical trial, curcumin at doses of 2 g or 4 g was administered to 44 eligible smokers with 8 or more aberrant crypt foci (ACF) over a 30-d period. Results demonstrated that at a dose of 4 g, curcumin was able to reduce the ACF number by 40% ($P < 0.005$), whereas at a dose of 2 g, curcumin did not show the effect[36]. Other clinical trials of curcumin are listed in Table 1.

POLYSACCHARIDES

Polysaccharides are a structurally diverse class of biological macromolecules, which are composed of monosaccharide polymers linked through glycosidic bonds. They range in structure from linear to highly branched form, and are used extensively as foods and pharmaceuticals. Furthermore, the enormous variety of polysaccharides has resulted in a constantly evolving group of potential bioactive compounds.

Apple polysaccharides

Apples are a kind of healthy food, and the consumption of which may decrease the risk of CRC[37]. It has been found that cloudy apple juice could decrease ACF development, hyperproliferation, and DNA damage in the distal colon of dimethylhydrazine (DMH)-initiated rats[38]. One component that makes the apple juice cloudy is saccharide. We thus extracted polysaccharides from apple and evaluated their effects on CRC. A microarray was used to investigate the effects of apple polysaccharides (AP) on human colon carcinoma cells (HT-29). Treatment of HT-29 cells with AP caused

the expression of 333 genes over the cutoff value (⩾ 2-fold). Cell cycle pathways were mainly influenced. At concentrations from 0.001 to 0.1 mg/mL, AP induced a G_0/G_1 phase arrest in HT-29 cells dose-dependently. Administration of AP could protect ICR mice against CRC effectively. The results of Western blot suggested that AP induced cell-cycle block in a p53 independent manner[39]. Galectin-3, a member of the family of β-galactoside-binding lectins, is involved in different stages of inflammation, generally viewed as a promoter of inflammatory response[40-42], as well as the processes of tumorigenesis and metastasis[43-45]. Galectin-3 is a prognostic marker, and its alteration is correlated with tumor pathogenesis, progression and/or metastasis in various kinds of cancers including CRC[46,47]. In our previous study, we have found that AP moderately triggered apoptosis of colonic epithelial cells, indicating that the anti-CRC potency of AP was probably due to its apoptosis inducing ability. Western blot analysis revealed that galectin-3 changed in both the nucleus and the cytoplasm during the process from colitis to colon cancer in the model. Furthermore, AP could suppress the binding of galectin-3 to its ligands, which is partly the possible mechanism for AP to enhance apoptosis and prevent tumorigenesis[48].

LPS, a glycolipid from the outer membrane of Gram-negative bacteria, activates toll-like receptor 4 (TLR4) to stimulate intracellular signaling cascades including NF-κB pathways and mitogen activated protein kinases (MAPKs), and results in a substantial increase in the production of chemokines, cytokines, and the synthesis of a wide group of lipid inflammatory mediators[49,50]. By observing the effect of AP on LPS-activated HT-29 and SW-620 CRC cells and an azoxymethane/dextran sodium sulfate (AOM/DSS) induced mouse model, we found that AP reduced AOM/DSS caused toxicities, prevented carcinogenesis, and downregulated the expression of TLR4, MD2, myeloid differentiation protein88 (MyD88), TRIF-related adapter molecule (TRAM) interferon-β, interleukin-6, and TNF-α. The protective effects of AP may be associated with the suppression of TLR4/MD2-mediated signaling, including MyD88 and TRIF, as well as the inhibition of NF-κB-mediated inflammatory signaling pathways.

The data above may provide another molecular basis for understanding how apple acts to prevent CRC and suggest that AP has the potential in treating colitis and preventing carcinogenesis[51].

Mushroom glucans

Medicinal mushrooms have been traditionally used as a healthy food or supplement for the prevention and treatment of several health statuses or diseases, *e.g.*, cancer, atherosclerosis, hypertension, and diabetes. Polysaccharides extracted from many edible mushrooms show promising effects in preventing and treating CRC. It has been found the expression of the proliferating-associated marker proliferating cell nuclear antigen (PCNA) in colorectal adenocarcinomas of mice was significantly reduced by oral administration of *P. pulmonarius* glucans. This indicates that *P. pulmonarius* glucans inhibited colorectal carcinogenesis by suppressing the abnormal proliferative activity of preneoplastic and neoplastic cells[52]. Xie *et al*[53] reported that *Ganoderma lucidum* glucan extract inhibited proliferation of CRC SW480 cells. A soluble α-glucan from *P. ostreatus* got by Lavi *et al*[54] suppressed colon cancer cell proliferation *via* direct interaction of the glucan with the colon cancer cells and their apoptosis induction.

Induction of cell apoptosis has been a target mechanism for cancer treatments[55]. Certain mushroom polysaccharides have proapoptotic functions in many tumor cell lines *in vitro*. Hu *et al*[56] showed that the mushroom *Inonotus obliquus* induced apoptosis with differing sensitivity in human colon cancer DLD-1 cells. Lavi *et al*[52] observed that feeding mice with *P. pulmonarius* glucans increased the expression of active-caspase-3 and Bax. And these effects were found in *P. pulmonarius* glucans treated colon carcinoma cell lines (HCT-116 and HT29) as well. In the *P. pulmonarius* glucans treated colon carcinoma cell lines, there was an increase in the level of cytosolic cytochrome c and a decrease of the anti-apoptotic protein Bcl-2.

SAPONINS

Saponins, a major family of secondary metabolites containing a sugar moiety glycosidically linked to a hydrophobic aglycone (sapogenin), are a class of bioactive compounds naturally present in particular abundance in various plant species[57,58], including ginseng or red ginseng (Panax, Araliaceae) in a form called ginsenosides. This class of chemical compounds are found in different parts of the plant: stems, roots, bulbs, leaves, blossom and fruit[59]. Saponins of several plants are known to induce apoptosis in some cancer cells[60].

Paris saponins

We investigated the growth inhibitory effect of *Trillium tschonoskii* steroidal saponins (TTS), which were extracted from *Trillium tschonoskii* Maxim, in a mouse model of colitis-associated CRC and HT-29 cells. Forty male ICR mice were administered with 1, 2-dimethyl-hydrazine (DMH) and dextran sodium sulfate (DSS). Ten mice were given no further treatment, and the rest were administered with different doses of TTS (5, 10, and 20 mg/kg) orally, every three days from the 9th week to the 20th week. TTS effectively protected ICR mice from DMH/DSS-caused tumorigenesis. The incidence of tumor development was 90% (9/10) in the mice treated with DMH/DSS, but that was reduced to 50% (5/10), 40% (4/10), and 20% (2/10), respectively, in the mice treated with 5%,

10%, and 20% of TTS. Results of Ki-67 staining, TUNEL assay and caspase-3 activity assay revealed that TTS moderately decreased abnormal proliferation and increased apoptosis of colonic epithelial cells. TTS inhibited the growth and triggered the apoptosis of HT-29 cells, partly through suppressing MAPKs and triggering mitochondrial-mediated apoptotic pathway. Further, we isolated a monomer, namely, Paris saponin VII (PSVII), from TTS and evaluated its effect on human CRC cell lines HT-29 and SW-620. The results showed that PSVII inhibited growth of these cells effectively. It could not only induce cell cycle arrest in G_1 phase, but also trigger apoptosis in a caspase-3-dependent manner. One possible mechanism may be through inhibition of Ras activity by PSVII. We further proved that PSVII effectively prevented colitis associated-colorectal carcinogenesis in an ICR mouse model, and significantly reduced xenograft tumor size in a murine CRC model. These preclinical studies suggest that PSVII has potentials in the treatment of CRC[61].

Ginsenosides

Ginseng, a plant of the Araliaceae family named scientifically Panax ginseng[62], has a great reputation in the treatment of cancer. Growing evidence has shown that ginseng, especially the ingredients of which, namely, ginsenosides, possesses beneficial effects in the treatment and prevention of CRC[63-65]. Studies on ginsenosides especially Rh2 and Rg3 demonstrated that they are most effective anti-cancer compounds identified in ginseng[66-68].

Treatment of HCT116 and SW480 cells with ginsenoside Rh2 activated the p53 pathway, upregulated the level of the pro-apoptotic regulator Bax, and downregulated the level of anti-apoptotic protein Bcl-2. The anti-cancer effect of Rh2 could be enhanced by antioxidants[69]. Ginsenoside Rg3 is a single compound isolated from American ginseng (Panax quinquefolius L., Araliaceae) and Asian ginseng (Panax ginseng C. A. Meyer). Rg3 inhibited cell proliferation and viability of cancer cells *in vitro*. Allelic deletion of the oncogenic β-catenin in HCT116 cells made the cells more sensitive to Rg3-induced growth inhibition. He *et al*[70] also demonstrated that Rg3 effectively inhibited the growth of HCT116 xenograft tumors . Histologic examination revealed that Rg3 inhibited cancer cell proliferation, decreased PCNA expression and diminished nuclear staining intensity of β-catenin. The possible mechanisms could be partly attributed to Rg3 blocking nuclear translocation of the β-catenin protein and hence inhibiting β-catenin/Tcf transcriptional activity. Yuan *et al*[71] found that Rg3 induced apoptosis of HT-29 cells indicated by DNA fragmentation and cleavage of poly(ADP-ribose) polymerase (PARP). Rg3 downregulated the expression of anti-apoptotic protein Bcl-2, upregulated the expression of pro-apoptotic proteins p53 and Bax, and induced the release of mitochondrial cytochrome c, PARP, caspase-9 and caspase-3. However, suppression of AMPK with its inhibitor compound C or small interfering RNA for AMPK (siAMPK) completely abolished Rg3-induced apoptosis. In addition, STO-609 (CaMKKβ inhibitor) attenuated Rg3-induced AMPK activation and apoptosis. These results suggest that Rg3-induced apoptosis in HT-29 cells is mediated *via* the AMPK signaling pathway.

Rh2 and Rg3 exhibited anti-proliferative and anti-angiogenesis effects *in vivo* and *in vitro* through inhibition of the NF-κB pathway, suppression of cell proliferation and induction of apoptosis. This beneficial effect of ginsenosides might be due to cell cycle arrest in cancer. It seems that the G_1 phase and G_1/S checkpoint were blocked by different mechanisms of ginsenosides[72,73], which involved upregulation of p53 and p21 and downregulation of cyclin and CDK including CDK2, cyclin E and cyclin D1 in G_1 phase and G_1/S checkpoint[73].

Soy saponins

Frequent consumption of soy and soy-based products is related with reduced cancer incidence particularly for colon cancer. Kim *et al*[74] examined the effect of crude soy saponins extract on PMA (phorbol 12-myristate 13-acetate)-induced inflammatory responses. They found that crude saponin extract suppressed cell growth in a dose- and time-dependent manner. In addition, crude soy saponins extract suppressed the degradation of IKKBα in PMA-stimulated cells, while COX-2 and PKC expression was significantly downregulated.

Tsai *et al*[75] treated WiDr human colon cancer cells, the same cell line as HT-29 with 150, 300, 600 or 1200 ppm of soy saponin. They found that soy saponin decreased the number of viable cells in a dose-dependent manner and suppressed PMA-induced PKC activity. Cells treated with saponins developed cytoplasmic vesicles with the cell membrane being rougher and more irregular in a dose-dependent manner, and eventually disassembled. At 600 and 1200 ppm, the activity of AP was increased ($P < 0.05$). These findings provide another molecular basis for understanding how soy acts to prevent inflammation and cancer.

RESVERATROL

Resveratrol (*trans*-3,5,40-trihydroxystilbene) is a phytoalexin found in plants including berries, grapes, and peanuts. Numerous *in vitro* studies have shown that resveratrol has anti-CRC effects by inhibiting both tumor initiation and progression. For example, resveratrol could suppress inflammatory responses through decreasing nitric oxide levels and inhibiting the phosphorylation of the IκB complex, thus suppressing the activation of NF-κB dependent mechanisms[76].

Serra *et al*[77] conducted a study by pretreating HT-29 colon epithelial cells with 25 mM of resveratrol and/or 500 mM of 5-aminosalicylic acid, and then exposing the cells to a combination of cytokines (IL-1α, TNF-α, IFN-c) for a certain period of time. The data demonstrated that resveratrol, at a concentration 20 times lower than 5-aminosalicylic acid, could significantly decrease PGE_2 and NO production, iNOS and COX-2 expression and reactive oxidant species formation induced by the cytokine challenge. And resveratrol downregulated JAK-STAT pathway by decreasing the levels of activated STAT1 in the nucleus. Liu *et al*[78] suggested that resveratrol exhibited growth inhibitory effects in human colon cancer cells by regulating separately the PTEN/PI3K/Akt and Wnt/β-catenin signaling. They found that resveratrol inhibited the proliferation and promoted apoptosis of HCT116 cells, and suppressed the xenograft tumor growth of colon cancer as well. Resveratrol upregulated the expression of phosphatase and tensin homolog (PTEN) and decreased the phosphorylation of Akt1/2. The exogenous expression of PTEN inhibited the PI3K/Akt signal and promoted the growth inhibitory effects of resveratrol in HCT116 cells. Knockdown of PTEN increased PI3K/Akt signal but reduced the growth inhibitory function of resveratrol. The mRNA and protein expression of β-catenin was both reduced by resveratrol in a concentration dependent way. Resveratrol could activate caspases-3 and -8 and increase the Bax/Bcl-2 ratio[79]. The process through which resveratrol promoted the cleavage of caspases-3 and -8 was by reactive oxygen species triggered autophagy[80]. Several *in vivo* studies have demonstrated that resveratrol possesses beneficial effects in preventing and treating colon tumor formation as well. Azoxymethane was used to induce colon tumorigenesis in 344 male Fisher rats, and then, resveratrol (daily intake calculated to be 200 mg/kg BW) was added into their drinking water for 100 d[81]. Tessitore *et al*[81] found that compared with control water treatment, resveratrol treatment reduced the appearance of ACF precursors for colon cancer. Resveratrol also decreased the appearance of large-sized ACF and increased the expression of Bax. In another study, adult male Wistar rats were given 1,2-dimethylhydrazine (DMH) once weekly for the first 15 wk. The DMH-treated rats were divided into three groups and then administrated daily with resveratrol (8 mg/kg BW; intragastric administration). The rats of group 1 were supplemented with resveratrol every day starting 2 wk before carcinogen treatment for the first 15 wk; the rats of group 2 were supplemented with resveratrol 2 d after the last injection of the carcinogen and continued to the end of the experiment; and the rats of group 3 were supplemented with resveratrol from the day of carcinogen treatment and continued to the end of the entire experimental period of 30 wk. All the rats were sacrificed 30 wk after the initial exposed DMH injection. In comparison to vehicle-treated DMH rats, resveratrol treatment decreased tumor incidence and the number of ACF[82]. Resveratrol could also reduce COX-2 activity and expression, decrease ornithine decarboxylase that is highly expressed in cells during cell proliferation and tumor promotion, and increase the level of cleaved caspase-3[83]. A genetically engineered mouse model for sporadic CRC, in which the APC locus was knocked out and KRAS was activated specifically in the distal colon, was used to investigate the effects of resveratrol in preventing and treating CRC. Feeding the mice with a diet containing 150 or 300 ppm resveratrol (105 and 210 mg daily human equivalent dose, respectively) before tumors were visible by colonoscopy, resulted in a 60% inhibition of tumor production. In the 40% of mice that developed tumors, KRAS expression was lost in the tumors. In a therapeutic assay where tumors were allowed to develop prior to treatment, feeding tumor bearing mice with resveratrol led to a complete remission in 33% of the mice and a 97% decrease in tumor size in the remaining mice. It was shown by the analysis of miRNA expression that resveratrol treatment caused increased expression of miR-96, a miRNA previously shown to regulate KRAS translation in non-tumoral and tumoral colonic tissues. These results indicate that resveratrol is able to inhibit the formation and growth of colorectal tumors by downregulating KRAS expression[84].

Many clinical pilot studies have shown that large doses of resveratrol are comparatively safe. Twenty CRC patients were given resveratrol at a dose of 0.5 g or 1.0 g orally for 8 d prior to surgery. The results showed that resveratrol was well tolerated. Resveratrol and its metabolites were detected in CRC resection tissue. Resveratrol (0.5 g or 1.0 g) decreased tumor cell proliferation by 5% (P = 0.005) and was enough to elicit anticarcinogenic effects in colon tumors[85]. In this study, serum resveratrol concentrations of 86 and 674 nmol/mL were observed at 0.5 and 1.0 g dose levels. Moreover, parent resveratrol accounted for a much larger proportion of resveratrol species in colorectal tissue than in plasma. These data support the notion that the colorectum is a suitable target for chemoprevention by resveratrol. Resveratrol at doses of 0.5 and 1.0 g has the capacity to induce pharmacological effects in the gastrointestinal tract[86]. Other clinical trials of resveratrol are listed in Table 1.

QUERCETIN

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the major dietary flavonoid and polyphenol found in several fruits, vegetables and beverages such as tea and wine. It has been shown that quercetin plays a role in inhibiting tumorigenesis in colon cells through antioxidant, anti-inflammatory, antiproliferative, and pro-apoptotic mechanisms. Quercetin downregulated Bcl-2 through inhibition of NF-κB[86] and inhibited phosphorylation of EGFR, thus

suppressing downstream signaling in colon carcinoma cells[87]. Mutoh *et al*[88] used a reporter gene assay to investigate the inhibitory effect of 12 flavonoids on the transcriptional activity of *COX-2* gene in human colon cancer DLD-1 cells. They observed that quercetin was the most potent suppressor of COX-2 transcription with an IC_{50} value of 10.5 mmol/L. Dysregulation of Wnt/beta-catenin pathway plays a key role in colorectal carcinogenesis. Park *et al*[89] indicated that quercetin may reduce nuclear beta-catenin and Tcf-4 proteins to downregulate β-catenin/Tcf transcriptional activity in SW480 cells. They found that quercetin downregulated the transcriptional activity of β-catenin/Tcf both in SW480 and in HEK293 cells which were transiently transfected with constitutively active mutant β-catenin gene. Both the electrophoretic mobility shift assay and immunoprecipitation results showed that binding of the Tcf complexes to its specific DNA-binding sites was significantly suppressed by quercetin. Results of Western blot suggested the decreased binding induced by quercetin was caused by decreased levels of β-catenin and Tcf-4 products in the nucleus. In another study, Shan *et al*[90] found that quercetin reduced cell viability in a dose- and time-dependent manner in SW480 cells. The percentages of apoptotic cells and cells in G_2/M phase were increased obviously after treatment with quercetin. Treatment with quercetin at a concentration of 160 μmol/L reduced β-catenin/Tcf transcriptional activity by about 18-fold. Quercetin reduced cyclin D1 and survivin expression in a dose-dependent manner at both mRNA and protein levels. Activation of AMP-activated protein kinase (AMPK), a physiological cellular energy sensor, strongly inhibits cell proliferation in tumor cells. Kim *et al*[91] found that treatment of HT-29 cells with quercetin significantly decreased cell viability, induced cell cycle arrest in the G1 phase and increased the expression of apoptosis-related proteins, such as AMPK, p53, and p21. *In vivo* studies demonstrated that quercetin treatment for over 6 wk led to a significant reduction in tumor volume. The study suggested that quercetin may trigger apoptosis *via* AMPK activation and p53-dependent apoptotic cell death in HT-29 cells.

CONCLUSION

Regular consumption of fruits and vegetables might protect against cancer. The protective role of certain components (phytochemicals) in fruits and vegetables against cancers occurring in different anatomical sites is now well supported. The objectives of this review were to document the chemopreventive effects of several phytochemicals including curcumin, polysaccharides (AP, and mushroom glucans), saponins (Paris saponins, ginsenosides and soy saponins), resveratrol, and quercetin on CRC and the possible mechanisms. These phytochemicals have advantages because they are comparatively safe and usually target multiple cell signaling pathways. As research continues, interventions in CRC using various phytochemicals might eventually become a specific treatment of choice.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Center MM**, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. *CA Cancer J Clin* 2009; **59**: 366-378 [PMID: 19897840 DOI: 10.3322/caac.20038]

3 **Center MM**, Jemal A, Ward E. International trends in colorectal cancer incidence rates. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1688-1694 [PMID: 19505900 DOI: 10.1158/1055-9965.EPI-09-0090]

4 **Wan DS**. [Epidemiologic trend of and strategies for colorectal cancer]. *Ai Zheng* 2009; **28**: 897-902 [PMID: 19728903]

5 **Kabbinavar FF**, Hambleton J, Mass RD, Hurwitz HI, Bergsland E, Sarkar S. Combined analysis of efficacy: the addition of bevacizumab to fluorouracil/leucovorin improves survival for patients with metastatic colorectal cancer. *J Clin Oncol* 2005; **23**: 3706-3712 [PMID: 15867200 DOI: 10.1200/JCO.2005.00.232]

6 **O'Connell JB**, Maggard MA, Ko CY. Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. *J Natl Cancer Inst* 2004; **96**: 1420-1425 [PMID: 15467030 DOI: 10.1093/jnci/djh275]

7 **Anand P**, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. *Pharm Res* 2008; **25**: 2097-2116 [PMID: 18626751 DOI: 10.1007/s11095-008-9661-9]

8 **Sporn MB**, Suh N. Chemoprevention: an essential approach to controlling cancer. *Nat Rev Cancer* 2002; **2**: 537-543 [PMID: 12094240 DOI: 10.1038/nrc844]

9 **Shukla S**, Meeran SM, Katiyar SK. Epigenetic regulation by selected dietary phytochemicals in cancer chemoprevention. *Cancer Lett* 2014; **355**: 9-17 [PMID: 25236912 DOI: 10.1016/j.canlet.2014.09.017]

10 **Jaganathan SK**, Vellayappan MV, Narasimhan G, Supriyanto E. Role of pomegranate and citrus fruit juices in colon cancer prevention. *World J Gastroenterol* 2014; **20**: 4618-4625 [PMID: 24782614 DOI: 10.3748/wjg.v20.i16.4618]

11 Available from: URL: http://www.fda.gov/cder/drug/infopage/cox2/NSAIDdecision Memo

12 **Divisi D**, Di Tommaso S, Salvemini S, Garramone M, Crisci R. Diet and cancer. *Acta Biomed* 2006; **77**: 118-123 [PMID: 17172193]

13 **Steinmetz KA**, Potter JD. Vegetables, fruit, and cancer prevention: a review. *J Am Diet Assoc* 1996; **96**: 1027-1039 [PMID: 8841165 DOI: 10.1016/S0002-8223(96)00273-8]

14 **Greenwald P**. Lifestyle and medical approaches to cancer prevention. *Recent Results Cancer Res* 2005; **166**: 1-15 [PMID: 15648179]

15 **Vainio H**, Weiderpass E. Fruit and vegetables in cancer prevention. *Nutr Cancer* 2006; **54**: 111-142 [PMID: 16800779 DOI: 10.1207/s15327914nc5401_13]

16 **Temraz S**, Mukherji D, Shamseddine A. Potential targets for colorectal cancer prevention. *Int J Mol Sci* 2013; **14**: 17279-17303 [PMID: 23975167 DOI: 10.3390/ijms140917279]

17 **He ZY**, Shi CB, Wen H, Li FL, Wang BL, Wang J. Upregulation of p53 expression in patients with colorectal cancer by administration of curcumin. *Cancer Invest* 2011; **29**: 208-213 [PMID: 21314329 DOI: 10.3109/07357907.2010.550592]

18 **Guo LD**, Chen XJ, Hu YH, Yu ZJ, Wang D, Liu JZ. Curcumin inhibits proliferation and induces apoptosis of human colorectal cancer cells by activating the mitochondria apoptotic pathway. *Phytother Res* 2013; **27**: 422-430 [PMID: 22628241 DOI: 10.1002/ptr.4731]

19 **Mantovani A**, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008; **454**: 436-444 [PMID: 18650914 DOI: 10.1038/nature07205]

20 **Luo JL**, Maeda S, Hsu LC, Yagita H, Karin M. Inhibition of NF-kappaB in cancer cells converts inflammation- induced tumor growth mediated by TNFalpha to TRAIL-mediated tumor regression. *Cancer Cell* 2004; **6**: 297-305 [PMID: 15380520 DOI: 10.1016/j.ccr.2004.08.012]

21 **Greten FR**, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 2004; **118**: 285-296 [PMID: 15294155 DOI: 10.1016/j.cell.2004.07.013]

22 **Shishodia S**, Sethi G, Aggarwal BB. Curcumin: getting back to the roots. *Ann N Y Acad Sci* 2005; **1056**: 206-217 [PMID: 16387689 DOI: 10.1196/annals.1352.010]

23 **Singh S**, Aggarwal BB. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. *J Biol Chem* 1995; **270**: 24995-25000 [PMID: 7559628]

24 **Eberhart CE**, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994; **107**: 1183-1188 [PMID: 7926468]

25 **Elder DJ**, Baker JA, Banu NA, Moorghen M, Paraskeva C. Human colorectal adenomas demonstrate a size-dependent increase in epithelial cyclooxygenase-2 expression. *J Pathol* 2002; **198**: 428-434 [PMID: 12434411 DOI: 10.1002/path.1232]

26 **Kargman SL**, O'Neill GP, Vickers PJ, Evans JF, Mancini JA, Jothy S. Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res* 1995; **55**: 2556-2559 [PMID: 7780968]

27 **Sano H**, Kawahito Y, Wilder RL, Hashiramoto A, Mukai S, Asai K, Kimura S, Kato H, Kondo M, Hla T. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. *Cancer Res* 1995; **55**: 3785-3789 [PMID: 7641194]

28 **Sheehan KM**, Sheahan K, O'Donoghue DP, MacSweeney F, Conroy RM, Fitzgerald DJ, Murray FE. The relationship between cyclooxygenase-2 expression and colorectal cancer. *JAMA* 1999; **282**: 1254-1257 [PMID: 10517428 DOI: 10.1001/jama.282.13.1254]

29 **Oshima M**, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 1996; **87**: 803-809 [PMID: 8945508 DOI: 10.1016/S0092-8674(00)81988-1]

30 **Jacoby RF**, Seibert K, Cole CE, Kelloff G, Lubet RA. The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. *Cancer Res* 2000; **60**: 5040-5044 [PMID: 11016626]

31 **Lev-Ari S**, Strier L, Kazanov D, Madar-Shapiro L, Dvory-Sobol H, Pinchuk I, Marian B, Lichtenberg D, Arber N. Celecoxib and curcumin synergistically inhibit the growth of colorectal cancer cells. *Clin Cancer Res* 2005; **11**: 6738-6744 [PMID: 16166455 DOI: 10.1158/1078-0432.CCR-05-0171]

32 **Radhakrishnan VM**, Kojs P, Young G, Ramalingam R, Jagadish B, Mash EA, Martinez JD, Ghishan FK, Kiela PR. pTyr421 cortactin is overexpressed in colon cancer and is dephosphorylated by curcumin: involvement of non-receptor type 1 protein tyrosine phosphatase (PTPN1). *PLoS One* 2014; **9**: e85796 [PMID: 24465712 DOI: 10.1371/journal.pone.0085796]

33 **Sharma RA**, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, Marczylo TH, Morgan B, Hemingway D, Plummer SM, Pirmohamed M, Gescher AJ, Steward WP. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res* 2004; **10**: 6847-6854 [PMID: 15501961 DOI: 10.1158/1078-0432.CCR-04-0744]

34 **Sharma RA**, McLelland HR, Hill KA, Ireson CR, Euden SA, Manson MM, Pirmohamed M, Marnett LJ, Gescher AJ, Steward WP. Pharmacodynamic and pharmacokinetic study of oral Curcuma extract in patients with colorectal cancer. *Clin Cancer Res* 2001; **7**: 1894-1900 [PMID: 11448902]

35 **Garcea G**, Berry DP, Jones DJ, Singh R, Dennison AR, Farmer PB, Sharma RA, Steward WP, Gescher AJ. Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 120-125 [PMID: 15668484]

36 **Carroll RE**, Benya RV, Turgeon DK, Vareed S, Neuman M, Rodriguez L, Kakarala M, Carpenter PM, McLaren C, Meyskens FL, Brenner DE. Phase IIa clinical trial of curcumin for the prevention of colorectal neoplasia. *Cancer Prev Res* (Phila) 2011; **4**: 354-364 [PMID: 21372035 DOI: 10.1158/1940-6207.CAPR-10-0098]

37 **Jedrychowski W**, Maugeri U. An apple a day may hold colorectal cancer at bay: recent evidence from a case-control study. *Rev Environ Health* 2009; **24**: 59-74 [PMID: 19476292]

38 **Barth SW**, Fähndrich C, Bub A, Dietrich H, Watzl B, Will F, Briviba K, Rechkemmer G. Cloudy apple juice decreases DNA damage, hyperproliferation and aberrant crypt foci development in the distal colon of DMH-initiated rats. *Carcinogenesis* 2005; **26**: 1414-1421 [PMID: 15802299 DOI: 10.1093/carcin/bgi082]

39 **Li Y**, Mei L, Niu Y, Sun Y, Huang H, Li Q, Kong X, Liu L, Li Z, Mei Q. Low molecular weight apple polysaccharides induced cell cycle arrest in colorectal tumor. *Nutr Cancer* 2012; **64**: 439-463 [PMID: 22429028 DOI: 10.1080/01635581.2012.658951]

40 **Rabinovich GA**, Baum LG, Tinari N, Paganelli R, Natoli C, Liu FT, Iacobelli S. Galectins and their ligands: amplifiers, silencers or tuners of the inflammatory response? *Trends Immunol* 2002; **23**: 313-320 [PMID: 12072371 DOI: 10.1016/S1471-4906(02)02232-9]

41 **Rubinstein N**, Ilarregui JM, Toscano MA, Rabinovich GA. The role of galectins in the initiation, amplification and resolution of the inflammatory response. *Tissue Antigens* 2004; **64**: 1-12 [PMID: 15191517 DOI: 10.1111/j.1399-0039.2004.00278.x]

42 **Liu FT**, Hsu DK. The role of galectin-3 in promotion of the inflammatory response. *Drug News Perspect* 2007; **20**: 455-460 [PMID: 17992268 DOI: 10.1358/dnp.2007.20.7.1149628]

43 **Song YK**, Billiar TR, Lee YJ. Role of galectin-3 in breast cancer metastasis: involvement of nitric oxide. *Am J Pathol* 2002; **160**: 1069-1075 [PMID: 11891203 DOI: 10.1016/S0002-9440(10)64927-9]

44 **Honjo Y**, Nangia-Makker P, Inohara H, Raz A. Down-regulation of galectin-3 suppresses tumorigenicity of human breast carcinoma cells. *Clin Cancer Res* 2001; **7**: 661-668 [PMID: 11297262]

45 **Bresalier RS**, Mazurek N, Sternberg LR, Byrd JC, Yunker CK, Nangia-Makker P, Raz A. Metastasis of human colon cancer is altered by modifying expression of the beta-galactoside-binding protein galectin 3. *Gastroenterology* 1998; **115**: 287-296 [PMID: 9679034 DOI: 10.1016/S0016-5085(98)70195-7]

46 **Endo K**, Kohnoe S, Tsujita E, Watanabe A, Nakashima H, Baba H, Maehara Y. Galectin-3 expression is a potent prognostic marker in colorectal cancer. *Anticancer Res* 2005; **25**: 3117-3121 [PMID: 16080575]

47 **Tsuboi K**, Shimura T, Masuda N, Ide M, Tsutsumi S, Yamaguchi S, Asao T, Kuwano H. Galectin-3 expression in colorectal cancer: relation to invasion and metastasis. *Anticancer Res* 2007; **27**: 2289-2296 [PMID: 17695516]

48 **Li Y**, Liu L, Niu Y, Feng J, Sun Y, Kong X, Chen Y, Chen X, Gan H, Cao S, Mei Q. Modified apple polysaccharide prevents against tumorigenesis in a mouse model of colitis-associated colon cancer: role of galectin-3 and apoptosis in cancer prevention. *Eur J Nutr* 2012; **51**: 107-117 [PMID: 21516492 DOI: 10.1007/s00394-011-0194-3]

49 **Pålsson-McDermott EM**, O'Neill LA. Signal transduction by the lipopolysaccharide receptor, Toll-like receptor-4. *Immunology* 2004; **113**: 153-162 [PMID: 15379975 DOI: 10.1111/j.1365-2567.2004.01976.x]

50 **Bals R**, Hiemstra PS. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *Eur Respir J* 2004; **23**: 327-333 [PMID: 14979512 DOI: 10.1183/09031936.03.00098803]

51 **Zhang D**, Li YH, Mi M, Jiang FL, Yue ZG, Sun Y, Fan L, Meng J, Zhang X, Liu L, Mei QB. Modified apple polysaccharides suppress the migration and invasion of colorectal cancer cells induced by lipopolysaccharide. *Nutr Res* 2013; **33**: 839-848 [PMID: 24074742

DOI: 10.1016/j.nutres.2013.06.004]
52 **Lavi I**, Nimri L, Levinson D, Peri I, Hadar Y, Schwartz B. Glucans from the edible mushroom Pleurotus pulmonarius inhibit colitis-associated colon carcinogenesis in mice. *J Gastroenterol* 2012; **47**: 504-518 [PMID: 22187166 DOI: 10.1007/s00535-011-0514-7]
53 **Xie JT**, Wang CZ, Wicks S, Yin JJ, Kong J, Li J, Li YC, Yuan CS. Ganoderma lucidum extract inhibits proliferation of SW 480 human colorectal cancer cells. *Exp Oncol* 2006; **28**: 25-29 [PMID: 16614703]
54 **Lavi I**, Friesem D, Geresh S, Hadar Y, Schwartz B. An aqueous polysaccharide extract from the edible mushroom Pleurotus ostreatus induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells. *Cancer Lett* 2006; **244**: 61-70 [PMID: 16413114 DOI: 10.1016/j.canlet.2005.12.007]
55 **Shanmugam MK**, Kannaiyan R, Sethi G. Targeting cell signaling and apoptotic pathways by dietary agents: role in the prevention and treatment of cancer. *Nutr Cancer* 2011; **63**: 161-173 [PMID: 21294053 DOI: 10.1080/01635581.2011.523502]
56 **Hu H**, Zhang Z, Lei Z, Yang Y, Sugiura N. Comparative study of antioxidant activity and antiproliferative effect of hot water and ethanol extracts from the mushroom Inonotus obliquus. *J Biosci Bioeng* 2009; **107**: 42-48 [PMID: 19147108 DOI: 10.1016/j.jbiosc.2008.09.004]
57 **Hostettmann K**, Marston A. Saponins. Cambridge: Cambridge University Press, 1995
58 **Liener**, Irvin E. Toxic constituents of plant foodstuffs. New York City: Academic Press, 1980
59 Available from: URL: http://sun.ars-grin.gov:8080/npgspub/xsql/duke/plantdisp.xsql?taxon=691
60 **Ali R**, Mirza Z, Ashraf GM, Kamal MA, Ansari SA, Damanhouri GA, Abuzenadah AM, Chaudhary AG, Sheikh IA. New anticancer agents: recent developments in tumor therapy. *Anticancer Res* 2012; **32**: 2999-3005 [PMID: 22753764]
61 **Li Y**, Sun Y, Fan L, Zhang F, Meng J, Han J, Guo X, Zhang D, Zhang R, Yue Z, Mei Q. Paris saponin VII inhibits growth of colorectal cancer cells through Ras signaling pathway. *Biochem Pharmacol* 2014; **88**: 150-157 [PMID: 24462916 DOI: 10.1016/j.bcp.2014.01.018]
62 **Kennedy DO**, Scholey AB. Ginseng: potential for the enhancement of cognitive performance and mood. *Pharmacol Biochem Behav* 2003; **75**: 687-700 [PMID: 12895687 DOI: 10.1016/S0091-3057(03)00126-6]
63 **Yun TK**. Experimental and epidemiological evidence on non-organ specific cancer preventive effect of Korean ginseng and identification of active compounds. *Mutat Res* 2003; **523-524**: 63-74 [PMID: 12628504 DOI: 10.1016/S0027-5107(02)00322-6]
64 **Yun TK**, Choi SY. Preventive effect of ginseng intake against various human cancers: a case-control study on 1987 pairs. *Cancer Epidemiol Biomarkers Prev* 1995; **4**: 401-408 [PMID: 7655337]
65 **Yun TK**, Choi SY, Yun HY. Epidemiological study on cancer prevention by ginseng: are all kinds of cancers preventable by ginseng? *J Korean Med Sci* 2001; **16** Suppl: S19-S27 [PMID: 11748373]
66 **Kim HS**, Lee EH, Ko SR, Choi KJ, Park JH, Im DS. Effects of ginsenosides Rg3 and Rh2 on the proliferation of prostate cancer cells. *Arch Pharm Res* 2004; **27**: 429-435 [PMID: 15180309 DOI: 10.1007/BF02980085]
67 **Helms S**. Cancer prevention and therapeutics: Panax ginseng. *Altern Med Rev* 2004; **9**: 259-274 [PMID: 15387718]
68 **Liu TG**, Huang Y, Cui DD, Huang XB, Mao SH, Ji LL, Song HB, Yi C. Inhibitory effect of ginsenoside Rg3 combined with gemcitabine on angiogenesis and growth of lung cancer in mice. *BMC Cancer* 2009; **9**: 250 [PMID: 19624862 DOI: 10.1186/1471-2407-9-250]
69 **Li B**, Zhao J, Wang CZ, Searle J, He TC, Yuan CS, Du W. Ginsenoside Rh2 induces apoptosis and paraptosis-like cell death in colorectal cancer cells through activation of p53. *Cancer Lett* 2011; **301**: 185-192 [PMID: 21194832 DOI: 10.1016/j.canlet.2010.11.015]
70 **He BC**, Gao JL, Luo X, Luo J, Shen J, Wang L, Zhou Q, Wang YT, Luu HH, Haydon RC, Wang CZ, Du W, Yuan CS, He TC, Zhang BQ. Ginsenoside Rg3 inhibits colorectal tumor growth through the down-regulation of Wnt/ß-catenin signaling. *Int J Oncol* 2011; **38**: 437-445 [PMID: 21152855 DOI: 10.3892/ijo.2010.858]
71 **Yuan HD**, Quan HY, Zhang Y, Kim SH, Chung SH. 20(S)-Ginsenoside Rg3-induced apoptosis in HT-29 colon cancer cells is associated with AMPK signaling pathway. *Mol Med Rep* 2010; **3**: 825-831 [PMID: 21472321 DOI: 10.3892/mmr.2010.328]
72 **Qi LW**, Wang CZ, Yuan CS. American ginseng: potential structure-function relationship in cancer chemoprevention. *Biochem Pharmacol* 2010; **80**: 947-954 [PMID: 20599804 DOI: 10.1016/j.bcp.2010.06.023]
73 **Park JW**, Lee JC, Ann S, Seo DW, Choi WS, Yoo YH, Park SK, Choi JY, Um SH, Ahn SH, Han JW. A fermented ginseng extract, BST204, inhibits proliferation and motility of human colon cancer cells. *Biomolecules and Therapeutics* 2011; **19**: 211-217 [DOI: 10.4062/biomolther.2011.19.2.211]
74 **Kim HY**, Yu R, Kim JS, Kim YK, Sung MK. Antiproliferative crude soy saponin extract modulates the expression of IkappaBalpha, protein kinase C, and cyclooxygenase-2 in human colon cancer cells. *Cancer Lett* 2004; **210**: 1-6 [PMID: 15172114 DOI: 10.1016/j.canlet.2004.01.009]
75 **Tsai CY**, Chen YH, Chien YW, Huang WH, Lin SH. Effect of soy saponin on the growth of human colon cancer cells. *World J Gastroenterol* 2010; **16**: 3371-3376 [PMID: 20632438 DOI: 10.3748/wjg.v16.i27.3371]
76 **Panaro MA**, Carofiglio V, Acquafredda A, Cavallo P, Cianciulli A. Anti-inflammatory effects of resveratrol occur via inhibition of lipopolysaccharide-induced NF-κB activation in Caco-2 and SW480 human colon cancer cells. *Br J Nutr* 2012; **108**: 1623-1632 [PMID: 22251620 DOI: 10.1017/S0007114511007227]
77 **Serra D**, Rufino AT, Mendes AF, Almeida LM, Dinis TC. Resveratrol modulates cytokine-induced Jak/STAT activation more efficiently than 5-aminosalicylic acid: an in vitro approach. *PLoS One* 2014; **9**: e109048 [PMID: 25271420 DOI: 10.1371/journal.pone.0109048]
78 **Liu YZ**, Wu K, Huang J, Liu Y, Wang X, Meng ZJ, Yuan SX, Wang DX, Luo JY, Zuo GW, Yin LJ, Chen L, Deng ZL, Yang JQ, Sun WJ, He BC. The PTEN/PI3K/Akt and Wnt/β-catenin signaling pathways are involved in the inhibitory effect of resveratrol on human colon cancer cell proliferation. *Int J Oncol* 2014; **45**: 104-112 [PMID: 24756222 DOI: 10.3892/ijo.2014.2392]
79 **Fouad MA**, Agha AM, Merzabani MM, Shouman SA. Resveratrol inhibits proliferation, angiogenesis and induces apoptosis in colon cancer cells: calorie restriction is the force to the cytotoxicity. *Hum Exp Toxicol* 2013; **32**: 1067-1080 [PMID: 23536519 DOI: 10.1177/0960327113475679]
80 **Miki H**, Uehara N, Kimura A, Sasaki T, Yuri T, Yoshizawa K, Tsubura A. Resveratrol induces apoptosis via ROS-triggered autophagy in human colon cancer cells. *Int J Oncol* 2012; **40**: 1020-1028 [PMID: 22218562 DOI: 10.3892/ijo.2012.1325]
81 **Tessitore L**, Davit A, Sarotto I, Caderni G. Resveratrol depresses the growth of colorectal aberrant crypt foci by affecting bax and p21(CIP) expression. *Carcinogenesis* 2000; **21**: 1619-1622 [PMID: 10910967 DOI: 10.1093/carcin/21.8.1619]
82 **Sengottuvelan M**, Nalini N. Dietary supplementation of resveratrol suppresses colonic tumour incidence in 1,2-dimethylhydrazine-treated rats by modulating biotransforming enzymes and aberrant crypt foci development. *Br J Nutr* 2006; **96**: 145-153 [PMID: 16870003 DOI: 10.1079/BJN20061789]
83 **Sengottuvelan M**, Deeptha K, Nalini N. Influence of dietary resveratrol on early and late molecular markers of 1,2-dimethylhydrazine-induced colon carcinogenesis. *Nutrition* 2009; **25**: 1169-1176 [PMID: 19818280 DOI: 10.1016/j.nut.2009.03.009]
84 **Saud SM**, Li W, Morris NL, Matter MS, Colburn NH, Kim YS, Young MR. Resveratrol prevents tumorigenesis in mouse model of Kras activated sporadic colorectal cancer by suppressing oncogenic Kras expression. *Carcinogenesis* 2014; **35**: 2778-2786 [PMID: 25280562 DOI: 10.1093/carcin/bgu209]
85 **Patel KR**, Brown VA, Jones DJ, Britton RG, Hemingway D, Miller AS, West KP, Booth TD, Perloff M, Crowell JA, Brenner DE, Steward WP, Gescher AJ, Brown K. Clinical pharmacology of resveratrol

and its metabolites in colorectal cancer patients. *Cancer Res* 2010; **70**: 7392-7399 [PMID: 20841478 DOI: 10.1158/0008-5472.CAN-10-2027]

86 **Priego S**, Feddi F, Ferrer P, Mena S, Benlloch M, Ortega A, Carretero J, Obrador E, Asensi M, Estrela JM. Natural polyphenols facilitate elimination of HT-29 colorectal cancer xenografts by chemoradiotherapy: a Bcl-2- and superoxide dismutase 2-dependent mechanism. *Mol Cancer Ther* 2008; **7**: 3330-3342 [PMID: 18852136 DOI: 10.1158/1535-7163.MCT-08-0363]

87 **Fridrich D**, Teller N, Esselen M, Pahlke G, Marko D. Comparison of delphinidin, quercetin and (-)-epigallocatechin-3-gallate as inhibitors of the EGFR and the ErbB2 receptor phosphorylation. *Mol Nutr Food Res* 2008; **52**: 815-822 [PMID: 18618485 DOI: 10.1002/mnfr.200800026]

88 **Mutoh M**, Takahashi M, Fukuda K, Komatsu H, Enya T, Matsushima-Hibiya Y, Mutoh H, Sugimura T, Wakabayashi K. Suppression by flavonoids of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells: structure-activity relationship. *Jpn J Cancer Res* 2000; **91**: 686-691 [PMID: 10920275]

89 **Park CH**, Chang JY, Hahm ER, Park S, Kim HK, Yang CH. Quercetin, a potent inhibitor against beta-catenin/Tcf signaling in SW480 colon cancer cells. *Biochem Biophys Res Commun* 2005; **328**: 227-234 [PMID: 15670774 DOI: 10.1016/j.bbrc.2004.12.151]

90 **Shan BE**, Wang MX, Li RQ. Quercetin inhibit human SW480 colon cancer growth in association with inhibition of cyclin D1 and survivin expression through Wnt/beta-catenin signaling pathway. *Cancer Invest* 2009; **27**: 604-612 [PMID: 19440933 DOI: 10.1080/07357900802337191]

91 **Kim HJ**, Kim SK, Kim BS, Lee SH, Park YS, Park BK, Kim SJ, Kim J, Choi C, Kim JS, Cho SD, Jung JW, Roh KH, Kang KS, Jung JY. Apoptotic effect of quercetin on HT-29 colon cancer cells via the AMPK signaling pathway. *J Agric Food Chem* 2010; **58**: 8643-8650 [PMID: 20681654 DOI: 10.1021/jf101510z]

P- Reviewer: Atoum MF, Temraz S **S- Editor**: Yu J
L- Editor: Wang TQ **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9273

World J Gastroenterol 2015 August 21; 21(31): 9273-9285
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Gastrointestinal Endoscopy

Endoscopic full-thickness resection: Current status

Arthur Schmidt, Benjamin Meier, Karel Caca

Arthur Schmidt, Benjamin Meier, Karel Caca, Department of Gastroenterology and Oncology, Klinikum Ludwigsburg, 71640 Ludwigsburg, Germany

Author contributions: Schmidt A drafted the manuscript, collected and analysed the data together with Meier B; and Caca K reviewed and edited the manuscript.

Supported by the Bundesministerium für Bildung und Forschung (BMBF, KMU-innovativ: OntoVigilance SWS365-065, FKZ 01|S12038A) within a subcontract with novineon GmbH (partly).

Conflict-of-interest statement: Arthur Schmidt and Karel Caca have received lectures fees from Ovesco Endoscopy for full-thickness resection device training courses. The authors have no conflict of interest related to the manuscript.

Correspondence to: Arthur Schmidt, MD, Department of Gastroenterology and Oncology, Klinikum Ludwigsburg, Posilipo-Str. 1-4, 71640 Ludwigsburg, Germany. karel.caca@kliniken-lb.de
Telephone: +49-7141-9967201
Fax: +49-7141-9967209

Received: April 4, 2015
Peer-review started: April 7, 2015
First decision: April 23, 2015
Revised: May 16, 2015
Accepted: July 3, 2015
Article in press: July 3, 2015
Published online: August 21, 2015

Abstract

Conventional endoscopic resection techniques such as endoscopic mucosal resection or endoscopic submucosal dissection are powerful tools for treatment of gastrointestinal neoplasms. However, those techniques are restricted to superficial layers of the gastrointestinal wall. Endoscopic full-thickness resection (EFTR) is an evolving technique, which is just about to enter clinical routine. It is not only a powerful tool for diagnostic tissue acquisition but also has the potential to spare surgical therapy in selected patients. This review will give an overview about current EFTR techniques and devices.

Key words: Endoscopic full-thickness resection; Over-the-scope-clip; Colorectal adenoma; Colorectal carcinoma; Endoscopic gastrointestinal surgery

Core tip: Endoscopic full-thickness resection is an evolving technique, which is just about to enter clinical routine. It is not only a powerful tool for diagnostic tissue acquisition but also has the potential to spare surgical therapy in selected patients. This review gives an overview about the current status of endoscopic full-thickness resection. General principles, indications and resection techniques and -devices will be discussed in detail on the basis of currently available literature.

Schmidt A, Meier B, Caca K. Endoscopic full-thickness resection: Current status. *World J Gastroenterol* 2015; 21(31): 9273-9285 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9273.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9273

INTRODUCTION

Flexible endoscopy was initially established as pure diagnostic procedure but has evolved to an important therapeutic modality over the last years. As a therapeutic instrument, Conway *et al*[1] recently called

endoscopy "truly the queen of minimally invasive interventions, being less morbid than surgery and without the radiation exposure of interventional radiologic interventions". Advanced techniques like endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) are well investigated methods for endoscopic resection of gastrointestinal neoplasms[2,3]. However, those techniques are limited to the superficial layers of the gastrointestinal (GI) wall, namely mucosa and submucosa. Although "superficial" resection may be sufficient for the majority of indications (*e.g.*, colorectal adenomas), full-thickness resection of the GI wall may be necessary in a subset of cases. For example, non-lifting lesions or neoplasms arising from deeper layers than the submucosa are difficult if not impossible to treat with conventional techniques due to the increased risk of perforation. Endoscopic full-thickness resection (EFTR) with secure defect closure may offer a safe and -compared to surgical resection- minimally invasive approach for those lesions. Moreover, diagnostic yield of full thickness resection specimen may be higher compared to endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD), *e.g.*, in case of T1-carcinomas. Hence, there is a clinical need for EFTR. However, for many years, it has not entered routine endoscopic practice due to the lack of safe techniques and devices. But what qualifies an EFTR device for broad clinical use? Basic requirements of such a device are: (1) safe and reliable defect closure; and (2) good endoluminal maneuverability. The ideal device would also be relatively easy use not only in the hands of experts. The very first device for flexible endoluminal full-thickness resection was introduced back in 2001 by Schurr and colleagues[4]. This prototype over-the-scope device had a flexible shaft and a multifunctional front-end incorporating tissue retractors and a stapling/cutting mechanism. Although it has been used successfully in the left-sided colon in animal experiments, the device has never entered clinical practice, probably due to its large dimensions and the limited endoluminal maneuverability. In the following couple of years, no major innovations had been published concerning EFTR devices suitable for routine clinical use. However, extensive experimental research on Natural Orifice Transluminal Endoscopic Surgery (NOTES) has led to substantial progress in the field of conservative and endoscopic management of GI wall defects[5]. This resulted not only in improvements in the therapy of accidental perforations, but also has opened the door for clinical use of EFTR. One major innovation for example was the introduction of the over-the-scope-clips (OTSC). Based on this closure technique, an over-the-scope full-thickness resection device (FTRD, Ovesco Endoscopy, Tuebingen, Germany, see below) was developed and recently CE-marked. Compared to the very first FTRD, the device is much smaller, easier to use and suitable for resections in the entire colon. With those and other recent developments, EFTR is now just about to enter clinical practice and may be the next logical step towards more extended endoscopic resections[6]. This review will give an overview over the current status of experimental and clinical EFTR techniques and devices.

LITERATURE SEARCH

The MEDLINE database was searched for articles describing endoscopic full-thickness resection. Keywords included "EFTR", "Endoscopic full-thickness resection" and "gastrointestinal AND endoscopic full-thickness resection". Additionally, we created ontology-based search queries on EFTR and used the "Ontovigilance" search engine (more information at http://www.ontovigilance.org) to search for reports not listed in MEDLINE.

The "Ontovigilance" search engine is an innovative prototype system for semantic search based on ontologies. Here, a so called Search Ontology, specifically designed for the identification of EFRT-related content, was applied. The specific ontology allows the expert the formal specification of domain concepts (*e.g.*, EFTR; Over-the-scope clip *etc.*) search terms associated to the domain, and rules describing domain concepts in order to generate complex search queries connected with Boolean operators[7]. The following search queries were used: ("endoscopic full thickness resection" OR eFTR) ("endoscopic full thickness resection" OR eFTR) AND ("over the scope" OR Ovesco OR OTSC OR FTRD); ("endoscopic full thickness resection" OR eFTR) AND (suture OR T-Tag OR Plicator OR Overstitch OR GERDX); ("endoscopic full thickness resection" OR eFTR) AND (Stapler). To avoid duplicate search results, the following databases were excluded: Synmed, Slideshare, Mdlinx, Eventscribe, Bioportfolio, EM-consulte, and Researchgate). Articles dealing with non-gastrointestinal endoscopy and articles in other than English or German language were also excluded. We further excluded articles on laparoscopic or combined endoscopic/laparoscopic procedures. Animal studies as well as human case reports and studies were included.

CURRENT INDICATIONS FOR EFTR

Indications for EFTR substantially differ in the upper and lower GI tract. Although not yet clinical "routine", all mentioned indications are already applicable in clinical practice with existing EFTR techniques and devices. The indications for EFTR in the colorectum are mainly suitable for resections with the FTRD System.

Upper GI tract

Subepithelial tumors (SET) arising from (or infiltrating) the muscularis propria are the most frequent indication for EFTR. Those tumors are difficult, if not impossible to resect with other endoscopic techniques due to the high risk of perforation.

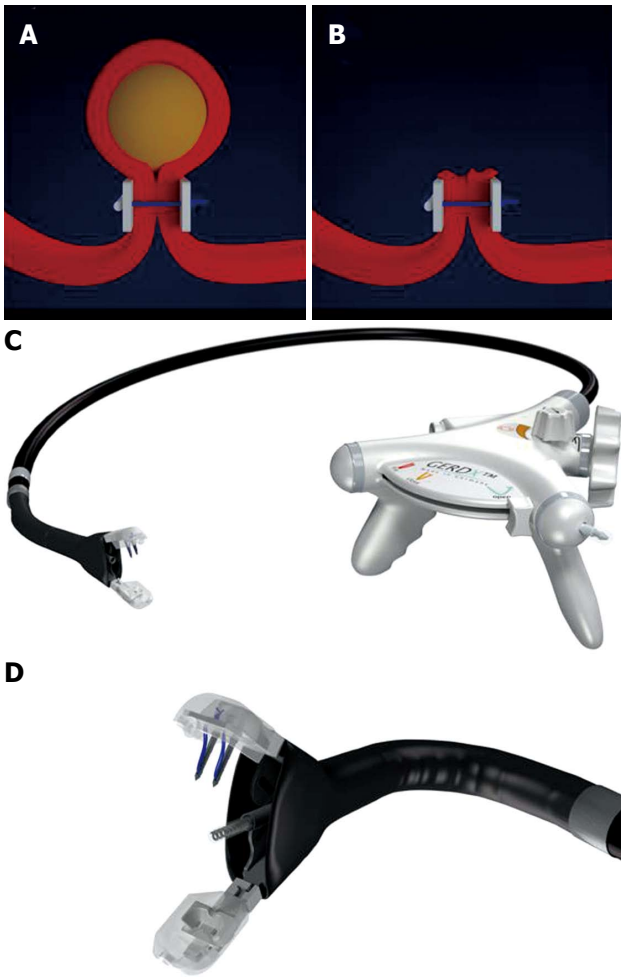


Figure 1 Endoscopic full-thickness resection with prior transmural suturing. A: Transmural sutures are placed underneath a subepithelial tumor (schematic illustration); B: The sutures are securing gastric wall patency after full-thickness resection; C: The GERDX™ device (G-Surg, Seeon, Germany); D: The tip of GERDX™ device with opened branches and the central tissue retractor.

Non-lifting recurrent or previously untreated non-ampullary duodenal adenomas may also be effectively resected by EFTR. However, this can not yet considered to be a routine indication because data on duodenal EFTR is very limited.

Lower GI tract

Recurrent adenomas with negative lifting sign. Although recurrency rate after EMR has recently described to be low[3], endoscopic re-treatment of those lesions is difficult. ESD has been reported to be effective for those lesions[2] but is technically challenging and harbours a substantial risk of perforation due to scarring even in the hands of experienced endoscopists. EFTR, especially with one-step closure/resection devices, may be technically easier, more time effective and safer. However, comparative studies are not yet available.

Incomplete resected non-lifting adenomas. Similar to recurrent adenomas, scarring represents a major problem for endoscopic re-treatment. EFTR may be a good indication for resection of those residual lesions.

Non-lifting adenomas without previous treatment. Those lesions are suspicious for invasive carcinoma. In those cases, EFTR may be done as primarily diagnostic resection. Compared to ESD, a full-thickness resection may increase the diagnostic yield and help to stratify in low-risk or high-risk situation. In particular, submucosal infiltration depth may be determined more accurately by the pathologist.

Re-resection of T1-carcinomas. When a T1 carcinoma is incidentally diagnosed in a lesion, which has been resected with conventional endoscopic methods and R-status and/or submucosal infiltration depth can not be determined accurately by the pathologist, diagnostic EFTR may be the method of choice to obtain a full-thickness resection. In case of low-risk lesions (submucosal infiltration depth < 1000 μm, G1 or G2, no lymphatic vessel invasion, R0-resection), it is even therapeutic. Compared to ESD, EFTR may also be the more radical approach resulting in lower recurrency rates. However, comparative studies are lacking.

Adenomas at difficult anatomic locations not suitable for "conventional" endoscopic resection. Adenomas involving a diverticulum can be effectively resected by EFTR[8]. Moreover, EFTR has been proposed for adenomas involving the appendical orifice[9]. In those cases EFTR may be a minimally invasive alternative for surgical resection.

Subepithelial tumors (SET). Although a rare indication, EFTR has been reported to be feasible for resection of small subepithelial lesions such as neuroendocrine tumors[10,11]. Conventional resection techniques like ESD harbour a significant risk of perforation, especially when the tumor infiltrates or (arises from) the muscularis propria.

Diagnostic resections in patients with suspected motility disorders. Compared to standard full-thickness biopsies, EFTR may increase the diagnostic yield in patients with suspected aganglionosis such as Hirschsprung's disease[12-14].

GENERAL PRINCIPLES OF EFTR

Full thickness resection naturally results in GI wall defect. Hence, the mainstay of EFTR is secure defect closure. Generally, there are two different approaches combining EFTR and defect closure: (1) Full thickness resection followed by closure of the wall defect; and (2) Securing GI wall patency by creation of GI wall duplication (with serosa-to-serosa apposition) before resection (Figure 1).

EFTR followed by subsequent perforation closure has been described to be feasible and effective[15-17]. However, reliable sealing of large (> 2 cm) GI wall defects may be difficult even with modern closure devices like Over-the-scope Clips[18]. Another potential problem is that with creation of a large wall defect, loss of air/CO_2 may lead to collapse of the GI lumen

and failure to maintain a reasonable operative field. Several studies investigating non-insufflation techniques and countertraction devices/platforms have addressed this problem, but all devices are still at the stage of prototypes and far away from clinical use[19-21]. Therefore, securing GI wall patency before resection may be a potentially safer and easier approach[22]. In analogy to surgical standards, this resection technique is based on creating a GI wall duplication with serosa-to-serosa apposition and therefore requires not only a mucosal/submucosal but rather a transmural closure mechanism. Depending on the anatomic resection site (stomach *vs* colonic) and thickness of the GI wall, transmural suturing techniques or closure with over-the-scope clips prior to resection have been described to be clinically feasible[9,23-26], whereas endoluminal stapler-assisted methods are still in the experimental stage of development[27]. Another potential advantage of prior closure is that in case of tumor fragmentation during resection [*e.g.*, in case or large gastric gastro-intestinal stromal tumors (GIST)], tumor seeding into the abdominal cavity may be avoided.

EFTR followed by perforation closure may lead to spillage of gastrointestinal content into the abdominal cavity, which may result in intraperitoneal infection. However, extensive research on NOTES has clearly demonstrated that abdominal infection during transgastric interventions is uncommon. Moreover, experimental studies have shown that the degree of peritoneal bacterial contamination can be reduced by antibiotics and decontamination of endoscopic entry routes. Also, bacterial contamination of the peritoneum does not necessarily correlate with relevant infection[28,29]. However, data on transcolonic interventions is limited and risk of bacterial seeding is likely to be higher for this access route[30,31]. Therefore, at least for colonic resections, reliable single-step closure/resection devices may be preferable compared to step-by-step closure modalities in order to minimize exposure of the peritoneal cavity to the bowel lumen[32].

EFTR TECHNIQUES

Submucosal endoscopic tumor resection

The concept of "submucosal tunnelling" in the esophagus was initially introduced by Inoue *et al*[33] for peroral endoscopic myotomy (POEM). Only a few years later, this technique was applied for resection of esophageal subepithelial tumors (SET) arising from the muscularis propria[34,35]. In analogy to the POEM procedure, a mucosal incision at least 5 cm proximal to the tumor is created and the endoscope is introduced into the submucosal space. The tumor is subsequently enucleated in ESD technique. After extracting the tumor from the tunnel, the mucosal incision is finally closed with through-the-scope (TTS) clips. The beauty of this technique is that even if a full thickness resection has been performed, the intact mucosal layer over the resection site covers the perforation and protects from mediastinitis or peritonitis. Hence, in contrast to other EFTR techniques, endoscopic closure of the resection site is not required. The largest study published to date included 85 SET (60 esophageal and 9 gastric). The tumors were mainly arising from the superficial muscularis propria (MP, 88.2%) and had a mean size of 19.2 mm (range 10-30 mm). Complete resection was achieved in 100% of cases with a mean procedure time of 57.2 min. Pneumothorax occurred in 7.1%, subcutaneous emphysema in 9.4% and pneumoperitoneum im 4.7%, respectively[36]. Other smaller studies reported success rates between 78% and 100% and complication rates between 13% and 33%[35,37,38]. The most common complications reported are pneumothorax, subcutaneous and mediastinal emphysema and pneumoperitoneum. While occurrence of pneumothorax generally requires a chest drain, air leakage into the mediastinum, the abdominal cavity and the subcutaneous tissue may not be considered as a "complication" rather than a natural consequence when the MP is perforated/resected. As long as the covering mucosa over the perforation is preserved, leakage of esophageal or gastric content is prevented. In the clinical studies published to date, no severe intraabdominal or mediastinal infections have been reported. Hence, submucosal endoscopic tumor resection using a tunnelling technique is feasible and relatively safe for tumors originating from the MP in the esophagus and cardia.

EFTR with subsequent clip closure

Gastric resections: Multiple asian studies reported on pure endoscopic full-thickness resection of gastric SET with subsequent defect closure. A study by Zhou *et al*[15] reported full thickness resection of 26 gastric SETs with a mean tumor sizes of 2.8 cm (1.2-4.5 cm) arising from the muscularis propria. The tumors were resected using ESD technique and the gastric wall defect was closed with standard through-the-scope clips. Complete resection rate was 100% with a mean procedure time of 105 min; no major complications were reported. Two other groups recently confirmed these results in a similar studies on 35 and 48 patients[16,17]. Another more recent retrospective study reported on a similar resection technique in 20 patients with gastric SETs. In this study, the wall defects were closed with clips and endoloops, severe complications where not reported, en bloc resection rate was as high as 100%[39]. Ye *et al*[40] recently also showed excellent results with a similar closure technique in 51 patients. There are two studies comparing laparoscopic resection *vs* EFTR with secondary clip closure. Both studies did not show significant differences in complete resection rate, operation time and length of hospital stay, indicating that the endoscopic approach may be an alternative strategy for such lesions[17,41].

Although the studies mentioned report excellent

results with no serious complications, it must be emphasized that defect closure with standard clips is usually only possible for small gastric perforations. Moreover, concerns have been raised whether closure of only the mucosal layer is sufficient after EFTR[42]. A porcine study compared closure of NOTES gastrostomies by either TTS clips or over-the-scope clips (OTSC)[43]. In the TTS-clip group, 3 minor and 1 major leaks were observed and four pigs developed peritonitis. No leaks occurred in the OTSC group, and necropsy with microscopic evaluation of the perforation sites showed that OTSC led to a deeper defect closure within the submucosal or muscular layer, respectively. Another potential advantage of OTSC closure is that OTSC deployment is a single-step procedure compared to step-by-step closure with TTS clips (+/- Endoloops) which may reduce the time of peritoneal exposure to gastric contents. Multiple clinical studies have shown high effectivity of OTSC for treatment of GI wall perforations, fistulas and leaks[10,44-47]. EFTR followed by defect closure with OTSC was clinically evaluated in the EndoResect study[48]. 20 patients with gastric SET ⩽ 3 cm were enrolled; 14 of them were resected using a double channel endoscope, a tissue retractor and a monofilament snare. Perforation occurred in six patients, all of which could be closed by OTSC application; mean procedure time was 44 min. Although this approach is very interesting because of its technical simplicity, most of the procedures in the study were done under laparoscopic control. Guo *et al*[49] most recently reported on EFTR of gastric SETs with subsequent OTSC defect closure. All interventions were done without laparoscopic assistance and successful defect closure was achieved in 100% of cases. Mean time for OTSC closure was as low as 4.9 min, reflecting the simplicity of the procedure. However, all tumors in this study had a size of ⩽ 2 cm, so the resulting wall defects should have been relatively small. Although OTSCs are preferable over TTS-Clips for gastric defects > 1 cm[5], secure closure requires apposition of the borders of the defect which may be difficult if impossible in case of larger perforations.

Colonic resections: Endoscopic resection of the colonic wall can generally be achieved in 3 ways: (1) Traction of the colonic with a forceps or an anchoring device and snare resection using a 2-channel endoscope; (2) suction of the colonic wall into a cap followed by snare resection; and (3) cutting of the wall with a knife in ESD-technique. Defect closure can then be performed with TTS-Clips or OTSCs.

Ahmed *et al*[50] experimentally compared traction-*vs* suction to retract the colonic wall for EFTR. The suction-resection technique resulted in larger resection specimen but was associated with more injury to the adjacent viscera compared to the traction technique; closure of the defect was not attempted in this study.

Raju *et al*[51] published a porcine experimental study where EFTR was performed with a band-ligation-resection device. Transverse closure of the circular perforations with TTS clips was unsuccessful in 3/11 cases, whereas longitudinal closure resulted in a leak proof seal in 6 of 7 cases. EFTR using a ESD-like technique with subsequent TTS-clip closures has been reported to be clinically feasible for resection of colonic SET[52]. However, in 2 of 16 patients required laparoscopic closure of the colonic wall defect and 2 patients developed signs of peritonitis. Apart from this report, there are no other clinical studies available following this approach[53].

von Renteln *et al*[54] evaluated a grasp-and-snare technique using a double channel endoscope and tissue anchor (Ovesco endoscopy, Tuebingen) in a porcine study. Resection yielded specimen up to 5.5 cm. Secure OTSC closure in the pigs with large defects) 2.4-5-5 cm in only 9 of 20 cases. In contrast, when an endoloop was used to secure the resection base before EFTR, resection specimen were smaller (1.2-2.2 cm) and OTSC closure led to efficient sealing of the defects in all cases. This indeed does reflect the clinical experience that colonic perforations up to approximately 2.5-3 cm can be closed sufficiently with OTSC whereas bigger defects can often not be sealed reliably.

EFTR with subsequent suturing

While transmural suturing is a hallmark procedure in open and laparoscopic GI surgery, endoluminal suturing with flexible instruments is technically much more difficult and still an area of intensive research. Roughly, there are 3 categories of endoscopic suturing methods: dedicated suturing devices, through-the-scope catheter based devices and multitasking platforms[1]. The Over- Stitch suturing device (Apollo Endosurgery Inc, Austin, Tex) is a commercially available device which is mounted on the tip of a endoscope and which was designed to create single-knot sutures. There are reports on successful closures of post-ESD mucosal defects[55] and a gastric fistula[56]. Chiu *et al*[57] demonstrated feasibility of EFTR using a master and slave transluminal endoscopic robot and closed the gastric perforations successfully with the Apollo Overstitch in two live porcine models. There are alos publications on the EagleClaw suturing device which uses a similar principle and has been used for closure of gastrostomies and other various indications[1,58,59]. However, to our knowledge there are no studies which further investigated EFTR with defect closure using these over-the-scope suturing devices.

Our group reported on defect closure after resection of gastric GIST by means of full-thickness suturing with the Plicator™, Suturing device (NDO Surgical, Inc, Mansfield, Mass)[23]. This device was originally designed for endoscopic antireflux therapy and deploys transmural PTFE (Polytetraflourethylene)-

pledgeted sutures. Although resection and closure of the gastric wall defect were successful in both cases we followed a "suture first, cut later" approach for the future cases (see below)[24].

Ikeda *et al*[60] were the first to investigate gastric EFTR with perforation closure using T-Tags. The tissue apposition system (TAS, Ethicon, Blue Ash, Ohio, United States) is a through-the-scope instrument and consists of a needle, which is used to transmurally place T-tags at the edges of the perforation and a knot-tying device. T-Tags have been used to to close wide colon perforations, esophageal, gastric and duodenal defects[61-65]. Raju *et al*[66] reported on colonic EFTR with subsequent closure using T-Tags. Suture closure of 2 cm defects was successful in 19/20 pigs with a median duration of 41 min for 4 sutures. Eighteen animals survived without signs of clinical distress and well-healed scars without peritonitis on necropsy at two weeks. One animal failed to thrive and necropsy revealed mild peritonitis, small abscesses, distant adhesions and 2 mm insufficiency at the suture site, respectively. Although T-Tag closure seemed to be promising in numerous studies, the TAS has been withdrawn form the market and is no longer commercially available.

Mori *et al*[21] demonstrated the experimental use of a Double-arm-bar Suturing System (DBSS) to close gastric defects after EFTR. Similar to the OverStich system, the device is mounted on an endoscope and allow serial single-stitch sutures. Closures were compared with hand-sewn sutures and OTSCs. No significant difference was found in the leak tests between the hand-sewn group and the DBSS, while burst pressures were significantly higher in the DBSS and hand-sewn group *vs* the OTSC group. The utility of the device was also demonstrated in two porcine video case reports recently[20,67]. It is noteworthy, that all interventions with the DBSS were done without air/CO2-insufflation, a mechanical countertraction device was used to maintain an operative field. Both the countertraction device and the DBSS are still in the stage of early prototypes and are not clinically approved.

EFTR with prior transmural suturing

In 2008, our group reported on the concept of applying transmural sutures underneath gastric SETs prior to EFTR[23]. We used a device originally designed for endoscopic anti-refllux therapy (Plicator™,NDO Surgical, Inc, Mansfield, Mass) to place two non-resorbable transmural PTFE-pledgeted sutures underneath the tumor. Thereby, a full-thickness duplication with serosa-to-serosa apposition was created and the "Pseudopolyp" was then resected with a snare above the suture (Figures 1 and 2). In 2011, a second series with four patients undergoing successful EFTR after deploying resorbable sutures was published[24]. Recently, our group reported on EFTR of gastric SET in a series of 31 patients using this "suture-first-cut-later" technique[25]. Mean tumor size was 20.5 mm (range 8-48 mm). Macroscopically complete en bloc resection could be achieved in 100 %, R0-resection rate was 90.3% with a median procedure time of 60 min. Perforation occurred in three patients; in all cases, the perforation was successfully closed with additional transmural sutures. When compared to OTSC application before resection (see below), this method is applicable for tumors up to a size of about 4 cm. The suturing device was originally designed to work in retroflex position, so technique is especially suitable for tumors in the proximal corpus, cardia and even in the fundus. In comparison to the clip closure techniques described above, patency of the gastric wall is secured not only by mucosal closure but rather by full- thickness suturing with serosa-to-serosa apposition. This technique meets surgical standards for defect closure and may result in a more secure and durable gastric wall repair especially for resection of large lesions. A major limitation of this method the need of special endoscopic equipment. Moreover, the devices are relatively large and can exclusively be used in the stomach. Handling of the devices also require a certain experience, *e.g.*, in endoscopic anti-reflux therapy. The Plicator™ device from NDO is not any more commercially available. However, a new CE-marked single-use device is available in Europe now (GERDX™, G-Surg, Seeon, Germany) (Figure 1). It uses the same suturing technique as the Plicator™ but works with a hydraulic closure mechanism. This device was used for the last two cases in our series and seems to be as effective as the Plicator™. The device is currently being evaluated in a prospective study initiated by our group.

EFTR with flexible stapler devices

The very first report on a one-step endoluminal full thickness resection device was published as early as 2001 by Schurr *et al*[4]. The device was a combination of a semicircular stapler and scalpel; it was also equipped with flexible tissue graspers. The device had a central lumen to accommodate a conventional flexible endoscope. For resection, the tissue graspers were used to pull the colonic wall into a resection chamber at the head of the device, thereby creating a full-thickness duplication. After application of 11 staples, the tissue above the staples was cut with the integrated scalpel and the resection specimen was retrieved within the resection chamber. This was the very first study to show that full thickness bowel wall resection was feasible with a flexible one-step instrument. The results of this study were confirmed by Rajan *et al*[68] in animal experiments, yielding resection specimen with a mean diameter of 3.6 cm. This novel device technique represented a major advantage in EFTR as the concept of a one-step closure-and-cut technique without exposition of the peritoneal cavity

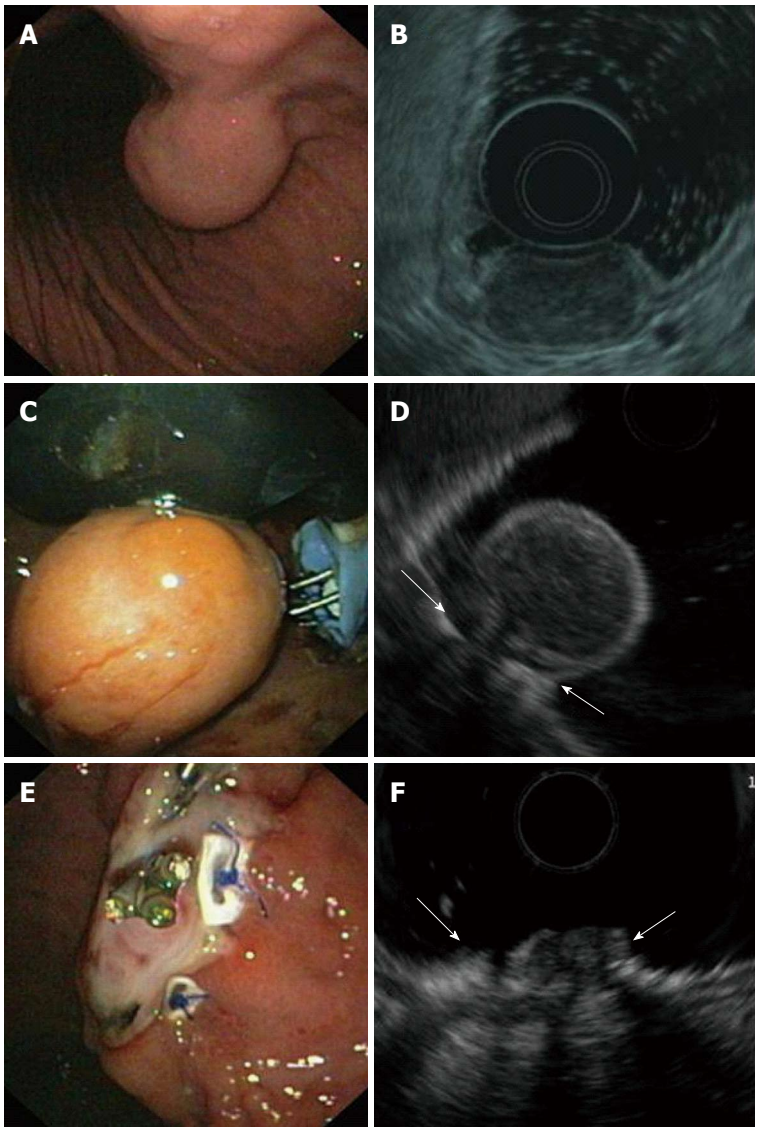


Figure 2 Endoscopic full-thickness resection of a gastric gastrointestinal stromal tumors after prior transmural suturing. A: Endoscopic image of the subepithelial tumor in the gastric corpus; B: EUS-image showing an inhomogeneous tumor arising from the muscularis propria; C: Two transmural sutures are deployed underneath the tumor using the PlicatorTM suturing device; D: EUS image after suturing. The PTFE pledges are indicated with arrows; E: Resection site. The transmural sutures are securing gastric wall patency; F: EUS image of the resection site. The PTFE pledges are indicated with arrows. EUS: Endoscopic ultrasonography.

to the bowel lumen and content. However, due to the technical requirements of a stapling mechanism, the device was quite big with limited maneuverability and could not be advanced beyond the left-sided colon. Therefore, the stapling closure approach was left and replaced by a more simple OTSC-based technique which resulted in the recently CE marked FTRD (Full Thickness Resection Device, see below)[69].

In 2006, Kaehler *et al*[27] demonstrated EFTR with the SurgAssist System (Power Medical Interventions Deutschland GmbH). The SurgAssist combines a 20 cm long flexible shaft with a linear stapling device and allows electronically controlled remote release of conventional stapler magazines. An endoscope is introduced simultaneously to allow endoscopic vision and to retract the gastric wall. In the study, the technique was shown to be feasible for EFTR in the gastric corpus in 3 human exenterates. The technique has also been applied in 2 clinical cases for successful full thickness resection of a T1 carcinoma and a carcinoid tumor in the gastric corpus[70]. There is also a porcine study reporting on successful closure of gastric defects after NOTES[71]. The SurgAssist device was also investigated in a non-survival porcine study by Evans *et al*[72]. EFTR was successful in only 2 cases. In the other 2, resection was limited to the submucosa. Furthermore, parallel introduction of the endoscope and the stapler caused one severe tear in the esophagus. In two animals, the endoscope was introduced through a gastrostomy port, which allowed better visualisation and also better countertraction of the tissue. All studies claimed limited intragastric maneuverability of the device; moreover, parallel introduction of scope and stapler device through

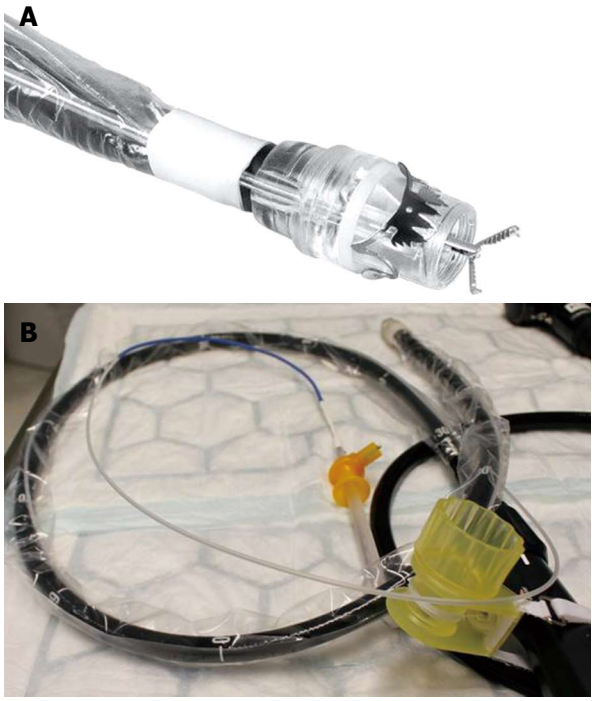


Figure 3 Full thickness resection device (Ovesco Endoscopy, Tuebingen, Germany). A: Tip of a colonoscope with the mounted FTRD. A grasping forceps is advanced through the working channel of the scope; B: The assembled FTRD on a colonoscopy. FTRD: Full thickness resection device.

the esophagus may be difficult and risky. There is a more recent study reporting on successful *ex vivo* closures of NOTES colostomies using a novel flexible stapler (Covidien North Haven, CT, United States)[73]. Although all studies mentioned show that endoscopic full-thickness stapling is feasible, this technique has not been followed consequently and is still far away from routine use for full thickness resection. The main reason may be that due the technical requirements, currently available stapling devices are too large in diameter, not flexible enough and show limited intraluminal maneuverability. Further technical improvements including miniaturisation seem to be necessary before use in clinical routine.

EFTR after OTSC application/FTRD

The concept of OTSC application followed by snare resection above the clip was reported in several clinical retrospective studies. An American group reported about resection of small subepithelial tumors in different locations with a mean tumor size of 13.4 mm[74]. Lesions were located in the duodenum, in the esophagus, in the stomach and in the rectum. After OTSC-deployment, all lesions were resected successfully. R0-resection was achieved in all but one cases, respectively. A recent retrospective German series included 17 patients with a variety of indications including SET and relapsed or R1-resected colonic adenomas/carcinomas; 17/17 resections were done in the lower GI tract[11]. Technical success was 94% with a R0 resection rate of 100%. A drawback of this technique is that the size of the cap limits the maximum size of the lesion.

The novel "Full thickness resection device" (FTRD, Ovesco Endoscopy, Tübingen, Germany) was designed for one-step colonic EFTR after OTSC application. Similar to the OTSC system, it can be mounted over a standard colonoscope and consists of a long transparent applicator cap carrying a modified 14 mm OTSC. Compared to the conventional OTSC system, the cap is much longer (23 mm *vs* 6 mm) and can therefore incorporate more tissue. A 13 mm monofilament high frequency (HF) snare is a preloaded in the tip of the cap. The handle of the snare runs on the outer surface of the scope underneath a plastic sheath (Figure 3). For resection, a grasping forceps (or a tissue anchor) is advanced through the working channel of the scope, the lesion is pulled into the cap thereby creating a full thickness duplication of the colonic wall (Figures 4 and 5). Immediately after clip deployment, the tissue above the clip is resected with the snare above the clip. The device was firstly introduced in 2011 and evaluated in several porcine studies[69,75-77]. In the most recent study, EFTR was done in 11 pigs at one or two sites, divided into three study sessions/groups, respectively[69]. Animals were euthanized after 7 or 28 d. The colonic resections were carried out without complications yielding specimen with an average diameter between 3.1 and 5.4 cm. No immediate or delayed perforations or leakages were observed, the serosa had primarily healed after 28 d in all cases. To date, there are three published reports on clinical use of the device. Our group was the first to report on successful EFTR of 3 recurrent non-lifting colonic adenomas[26]. At the same time, a Swiss group published a video case demonstrating successful EFTR of an adenoma arising from a diverticulum[8]. Furthermore, we recently reported on 25 patients who underwent EFTR in the colorectum at two centers[9]. The majority of indications were non-lifting adenomas, resection sites were spread throughout the colorectum with 40% being in the right-sided colon. Technical success was 83.3% and R0-resection rate 75%, respectively. In this study, we did not observe any immediate or delayed perforation or major bleeding. However, two patients developed a post-polypectomy syndrome after coecal resections which may reflect local serositis after the transmural intervention. This data suggests that EFTR with the FTRD is feasible, effective and safe. The major limitation of the system is the maximum size of the lesion to resect. This strongly depends of the mobility of the colonic wall; whereas resection specimen up to 5.4 cm have been reported in experiments with healthy porcine colon[69], the median diameter in the mentioned clinical study was 24 mm (range 12-40 mm)[9]. Moreover, the long cap limits endoscopic view and flexibility of the endoscope tip so that advancement of the scope

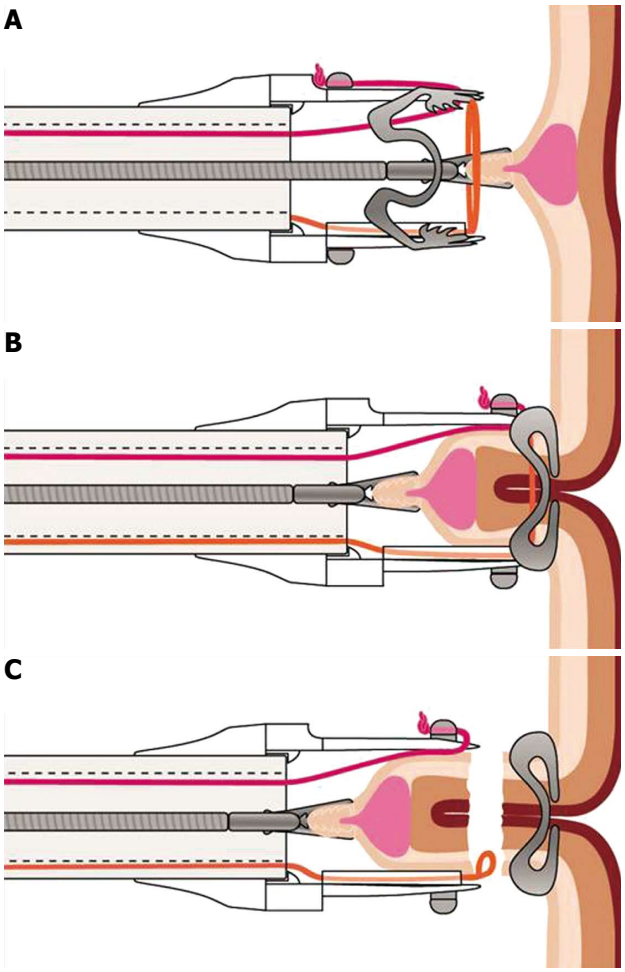


Figure 4 Schematic image of the resection procedure with the full-thickness resection device. A: The lesion is grasped with a forceps and pulled into the cap thereby creating a full-thickness duplication of the colonic wall; B: The over-the-scope clip is deployed; C: The tissue above the clip is resected with the integrated snare.

thorough the sigmoid or beyond colonic flexures can be difficult. The device was just recently CE marked for colonic EFTR and is commercially available in Europe. Although it has also been used for duodenal resections (Schmidt *et al*, manuscript accepted) we would like to stress that it is currently not approved for use in the upper GI tract. Full thickness resection in the stomach may not be possible due to the thickness of the gastric wall. Furthermore, the outer diameter (21 mm) of the device and its sharp edges limit peroral introducability, so that modifications of the device seem to be necessary before routine use in the upper GI tract.

LIMITATIONS OF EFTR AND FUTURE DIRECTIONS

With the development of secure endoscopic closure techniques, major progress has recently been made in (at least partly) transferring an experimental technique into clinical routine. The best example is surely the OTSC-based FTRD which will truly change the clinical management of colorectal non-lifting lesions and will obviate the need for surgical therapy in selected patients. This over-the-scope system shows nicely how existing endoscopic components can be put together to create a safe, highly efficient and easy to use one-step resection device. Another example of applying existent devices for new indications is the suture-first-cut-later technique with the Plicator/GERDX device, which was originally designed for endoscopic anti-reflux therapy. Both techniques also demonstrate that the concept of securing GI wall patency before resection may be a safer and -with the current endoscopic techniques- easier approach compared to secondary defect closure. However, for more extended and complex resections, neither OTSC-assisted nor rather cumbersome suturing devices will suffice to reach the precision of a laparoscopic or open surgical operation. To achieve this, several developments seem to be necessary. More sophisticated and miniaturized stapler devices may facilitate secure and precise wall resections in the future. In our view, one-step stapler devices may be even more important than single-step endoluminal suturing instruments. More extended wall resections as well as suturing require countertraction. In the instruments available, this is achieved by rather primitive tissue retractors running through the working channel of the scope or through the suturing instrument itself. The ideal endoluminal EFTR device suitable for extended resections would be equipped two arms which can be moved independently of each other enabling traction and countertraction like in laparoscopic surgery. Although such prototype platforms have been investigated[57,78], those devices currently still seem quite far away from clinical use.

Looking at recent advances in the field of resection and closure techniques, it is tempting to state that EFTR is "the next logical step towards more extended oncological resections"[6]. However, it is noteworthy to say that all those innovative techniques still need to be investigated systematically. The majority of studies cited in this review are preclinical studies with a very limited amount of animal models or retrospective non-controlled clinical series. Although most resection and closure techniques seem to be feasible and safe, there is still a significant lack of prospective clinical trials. At least for the FTRD System, two prospective German trials have been initiated. The "WALL RESECT" study (NCT02362126) is a single-arm multicentre study investigating efficacy and safety of the device for resection of (mainly non-lifting) lesions in the colorectum. The "FIRE" study (NCT02353533) is a randomized monocentric trial investigating EFTR *vs* EMR for "difficult-to-resect" colorectal adenomas. There is also a prospective uncontrolled study investigating efficacy and safety of GERDX-mediated resection of gastric SETs ("FROST" study, NCT Nr pending), which has just started to recruit patients. The next step to would certainly be to directly compare those techniques with the surgical standard.

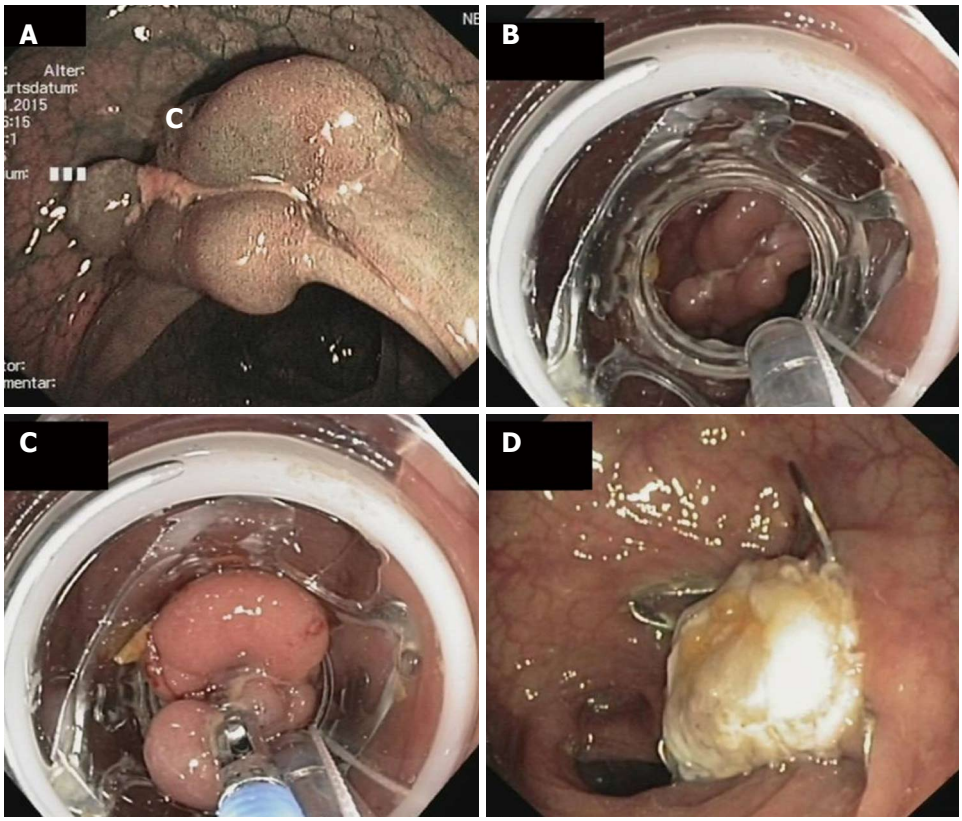


Figure 5 Endoscopic full thickness resection of a non-lifting recurrent adenoma in a patient with a polyposis syndrome. A: Endoscopic image showing a polypoid and centrally depressed non-lifting adenoma (2.5 cm) in the sigmoid (Narrow band imaging mode); B: View with the mounted full thickness resection device (FTRD, Ovesco Endoscopy, Tübingen, Germany); C: The lesion is pulled into the cap with a forceps; D: Resection site. The over-the-scope clip is securing gastric wall patency.

Furthermore, at this early stage of development it is not yet clear to which extend endoscopic interventions will be able to replace oncologic surgical resections. A minimal-invasive endoluminal approach may be ideal for lesions with low risk of tumor seeding like advanced adenomas, "small" mesenchymal tumors or even a subset of early carcinomas. For more advanced lesions, more extended resection including lymph node dissection is generally necessary, and at least at this point of development, this is not possible with the endoscopic-endoluminal approach.

In summary, recent developments have finally brought EFTR into clinical routine for selected indications. This progress has again pushed the frontiers of endoluminal resections towards transmural interventions. However, prospective clinical trials as well as technical improvements regarding resection/closure devices and - platforms are necessary for further development of this evolving technique.

ACKNOWLEDGMENTS

We thank Timo Weiland from Novineon CRO (Tübingen, Germany) for creating the search queries for the Ontovigilance Software. We thank Ovesco Endoscopy (Tübingen, Germany) and G-Surg (Seeon, Germany) for providing the images.

REFERENCES

1 **Conway NE**, Swanström LL. Endoluminal flexible endoscopic suturing for minimally invasive therapies. *Gastrointest Endosc* 2015; **81**: 262-269.e19 [PMID: 25440675 DOI: 10.1016/j.gie.2014.09.013]

2 **Kuroki Y**, Hoteya S, Mitani T, Yamashita S, Kikuchi D, Fujimoto A, Matsui A, Nakamura M, Nishida N, Iizuka T, Yahagi N. Endoscopic submucosal dissection for residual/locally recurrent lesions after endoscopic therapy for colorectal tumors. *J Gastroenterol Hepatol* 2010; **25**: 1747-1753 [PMID: 21039836 DOI: 10.1111/j.1440-1746.2010.06331.x]

3 **Moss A**, Williams SJ, Hourigan LF, Brown G, Tam W, Singh R, Zanati S, Burgess NG, Sonson R, Byth K, Bourke MJ. Long-term adenoma recurrence following wide-field endoscopic mucosal resection (WF-EMR) for advanced colonic mucosal neoplasia is infrequent: results and risk factors in 1000 cases from the Australian Colonic EMR (ACE) study. *Gut* 2015; **64**: 57-65 [PMID: 24986245 DOI: 10.1136/gutjnl-2013-305516]

4 Full thickness resection device (FTRD) for endoluminal removal of large bowel tumours: development of the instrument and related experimental studies. *Minim Invasive Ther Allied Technol* 2001; **10**: 301-309 [PMID: 16754033]

5 **Paspatis GA**, Dumonceau JM, Barthet M, Meisner S, Repici A, Saunders BP, Vezakis A, Gonzalez JM, Turino SY, Tsiamoulos ZP, Fockens P, Hassan C. Diagnosis and management of iatrogenic endoscopic perforations: European Society of Gastrointestinal Endoscopy (ESGE) Position Statement. *Endoscopy* 2014; **46**: 693-711 [PMID: 25046348 DOI: 10.1055/s-0034-1377531]

6 **Meining A**. Endoscopic full-thickness resection: the logical step toward more extended endoscopic oncologic resections? *Endoscopy* 2015; **47**: 101-102 [PMID: 25635729 DOI: 10.1055/

s-0034-1391372]
7 **Uciteli A**, Goller C, Burek P, Siemoleit S, Faria B, Galanzina H, Weiland T, Drechsler-Hake D, Bartussek W, Herre H. Search Ontology, a new approach towards Semantic Search. *GI Ed Proc LNI* 2014; **232**: 667-672
8 **Valli PV**, Kaufmann M, Vrugt B, Bauerfeind P. Endoscopic resection of a diverticulum-arisen colonic adenoma using a full-thickness resection device. *Gastroenterology* 2014; **147**: 969-971 [PMID: 25093544 DOI: 10.1053/j.gastro.2014.07.053]
9 **Schmidt A**, Bauerfeind P, Gubler C, Damm M, Bauder M, Caca K. Endoscopic full-thickness resection in the colorectum with a novel over-the-scope device: first experience. *Endoscopy* 2015; Epub ahead of print [PMID: 25763833]
10 **Mönkemüller K**, Peter S, Toshniwal J, Popa D, Zabielski M, Stahl RD, Ramesh J, Wilcox CM. Multipurpose use of the 'bear claw' (over-the-scope-clip system) to treat endoluminal gastrointestinal disorders. *Dig Endosc* 2014; **26**: 350-357 [PMID: 23855514 DOI: 10.1111/den.12145]
11 **Fähndrich M**, Sandmann M. Endoscopic full-thickness resection for gastrointestinal lesions using the over-the-scope clip system: a case series. *Endoscopy* 2015; **47**: 76-79 [PMID: 25221859 DOI: 10.1055/s-0034-1377975]
12 **Fritscher-Ravens A**, Milla P, Ellrichmann M, Hellwig I, Böttner M, Hadeler KG, Wedel T. A novel endoscopic prototype device for gastric full-thickness biopsy for the histopathologic diagnosis of GI neuromuscular pathology: in vivo porcine long-term survival study (with videos). *Gastrointest Endosc* 2013; **77**: 262-271 [PMID: 23199648 DOI: 10.1016/j.gie.2012.09.013]
13 **Rajan E**, Gostout CJ, Aimore Bonin E, Moran EA, Locke RG, Szarka LA, Talley NJ, Deters JL, Miller CA, Knipschield MA, Lurken MS, Stoltz GJ, Bernard CE, Grover M, Farrugia G. Endoscopic full-thickness biopsy of the gastric wall with defect closure by using an endoscopic suturing device: survival porcine study. *Gastrointest Endosc* 2012; **76**: 1014-1019 [PMID: 23078926 DOI: 10.1016/j.gie.2012.07.010]
14 **Rajan E**, Gostout CJ, Lurken MS, Talley NJ, Locke GR, Szarka LA, Sumiyama K, Bakken TA, Stoltz GJ, Knipschield MA, Farrugia G. Endoscopic "no hole" full-thickness biopsy of the stomach to detect myenteric ganglia. *Gastrointest Endosc* 2008; **68**: 301-307 [PMID: 18262183 DOI: 10.1016/j.gie.2007.10.017]
15 **Zhou PH**, Yao LQ, Qin XY, Cai MY, Xu MD, Zhong YS, Chen WF, Zhang YQ, Qin WZ, Hu JW, Liu JZ. Endoscopic full-thickness resection without laparoscopic assistance for gastric submucosal tumors originated from the muscularis propria. *Surg Endosc* 2011; **25**: 2926-2931 [PMID: 21424195 DOI: 10.1007/s00464-011-1644-y]
16 **Feng Y**, Yu L, Yang S, Li X, Ding J, Chen L, Xu Y, Shi R. Endolumenal endoscopic full-thickness resection of muscularis propria-originating gastric submucosal tumors. *J Laparoendosc Adv Surg Tech A* 2014; **24**: 171-176 [PMID: 24555874 DOI: 10.1089/lap.2013.0370]
17 **Huang LY**, Cui J, Lin SJ, Zhang B, Wu CR. Endoscopic full-thickness resection for gastric submucosal tumors arising from the muscularis propria layer. *World J Gastroenterol* 2014; **20**: 13981-13986 [PMID: 25320536 DOI: 10.3748/wjg.v20.i38.13981]
18 **Weiland T**, Fehlker M, Gottwald T, Schurr MO. Performance of the OTSC System in the endoscopic closure of iatrogenic gastrointestinal perforations: a systematic review. *Surg Endosc* 2013; **27**: 2258-2274 [PMID: 23340813 DOI: 10.1007/s00464-012-2754-x]
19 **Mori H**, Kobara H, Fujihara S, Nishiyama N, Rafiq K, Oryu M, Fujiwara M, Suzuki Y, Masaki T. Feasibility of pure EFTR using an innovative new endoscopic suturing device: the Double-arm-bar Suturing System (with video). *Surg Endosc* 2014; **28**: 683-690 [PMID: 24202707 DOI: 10.1007/s00464-013-3266-z]
20 **Mori H**, Rafiq K, Kobara H, Tsushimi T, Fujihara S, Nishiyama N, Matsunaga T, Ayaki M, Yachida T, Tani J, Miyoshi H, Masaki T. Development of pure endoscopic full-thickness resection with mechanical countertraction and double-armed bar suturing systems. *Gastrointest Endosc* 2014; **79**: 24-25 [PMID: 24112590 DOI: 10.1016/j.gie.2013.08.031]
21 **Mori H**, Rafiq K, Kobara H, Fujihara S, Nishiyama N, Oryuu M, Suzuki Y, Masaki T. Innovative noninsufflation EFTR: sufficient endoscopic operative field by mechanical counter traction device. *Surg Endosc* 2013; **27**: 3028-3034 [PMID: 23407912 DOI: 10.1007/s00464-013-2846-2]
22 **Schmidt A**, Bauder M, Riecken B, Caca K. Endoscopic resection of subepithelial tumors. *World J Gastrointest Endosc* 2014; **6**: 592-599 [PMID: 25512768 DOI: 10.4253/wjge.v6.i12.592]
23 **von Renteln D**, Schmidt A, Riecken B, Caca K. Gastric full-thickness suturing during EMR and for treatment of gastric-wall defects (with video). *Gastrointest Endosc* 2008; **67**: 738-744 [PMID: 18291389 DOI: 10.1016/j.gie.2007.10.051]
24 **Walz B**, von Renteln D, Schmidt A, Caca K. Endoscopic full-thickness resection of subepithelial tumors with the use of resorbable sutures (with video). *Gastrointest Endosc* 2011; **73**: 1288-1291 [PMID: 21481864 DOI: 10.1016/j.gie.2011.01.052]
25 **Schmidt A**, Bauder M, Riecken B, von Renteln D, Muehleisen H, Caca K. Endoscopic full-thickness resection of gastric subepithelial tumors: a single-center series. *Endoscopy* 2015; **47**: 154-158 [PMID: 25380509 DOI: 10.1055/s-0034-1390786]
26 **Schmidt A**, Damm M, Caca K. Endoscopic full-thickness resection using a novel over-the-scope device. *Gastroenterology* 2014; **147**: 740-742.e2 [PMID: 25083605 DOI: 10.1053/j.gastro.2014.07.045]
27 **Kaehler GF**, Langner C, Suchan KL, Freudenberg S, Post S. Endoscopic full-thickness resection of the stomach: an experimental approach. *Surg Endosc* 2006; **20**: 519-521 [PMID: 16437279 DOI: 10.1007/s00464-005-0147-0]
28 **Fritscher-Ravens A**, Arlt A. Safety notes: how to avoid infections in natural orifice transluminal endoscopic surgery. *Endoscopy* 2011; **43**: 58-62 [PMID: 21234843 DOI: 10.1055/s-0030-1256033]
29 **Eickhoff A**, Vetter S, von Renteln D, Caca K, Kähler G, Eickhoff JC, Jakobs R, Riemann JF. Effectivity of current sterility methods for transgastric NOTES procedures: results of a randomized porcine study. *Endoscopy* 2010; **42**: 748-752 [PMID: 20669093 DOI: 10.1055/s-0030-1255597]
30 **Meining A**, Feussner H, Swain P, Yang GZ, Lehmann K, Zorron R, Meisner S, Ponsky J, Martiny H, Reddy N, Armengol-Miro JR, Fockens P, Fingerhut A, Costamagna G. Natural-orifice transluminal endoscopic surgery (NOTES) in Europe: summary of the working group reports of the Euro-NOTES meeting 2010. *Endoscopy* 2011; **43**: 140-143 [PMID: 21229472 DOI: 10.1055/s-0030-1256128]
31 **Meining A**, Spaun G, Fernández-Esparrach G, Arezzo A, Wilhelm D, Martinek J, Spicak J, Feussner H, Fuchs KH, Hucl T, Meisner S, Neuhaus H. NOTES in Europe: summary of the working group reports of the 2012 EURO-NOTES meeting. *Endoscopy* 2013; **45**: 214-217 [PMID: 23446668 DOI: 10.1055/s-0032-1326205]
32 **Kopelman Y**, Siersema PD, Bapaye A, Kopelman D. Endoscopic full-thickness GI wall resection: current status. *Gastrointest Endosc* 2012; **75**: 165-173 [PMID: 22196814 DOI: 10.1016/j.gie.2011.08.050]
33 **Inoue H**, Kudo SE. [Per-oral endoscopic myotomy (POEM) for 43 consecutive cases of esophageal achalasia]. *Nihon Rinsho* 2010; **68**: 1749-1752 [PMID: 20845759 DOI: 10.1055/s-0029-1244080]
34 **Inoue H**, Ikeda H, Hosoya T, Onimaru M, Yoshida A, Eleftheriadis N, Maselli R, Kudo S. Submucosal endoscopic tumor resection for subepithelial tumors in the esophagus and cardia. *Endoscopy* 2012; **44**: 225-230 [PMID: 22354822 DOI: 10.1055/s-0031-1291659]
35 **Gong W**, Xiong Y, Zhi F, Liu S, Wang A, Jiang B. Preliminary experience of endoscopic submucosal tunnel dissection for upper gastrointestinal submucosal tumors. *Endoscopy* 2012; **44**: 231-235 [PMID: 22354823 DOI: 10.1055/s-0031-1291720]
36 **Ye LP**, Zhang Y, Mao XL, Zhu LH, Zhou X, Chen JY. Submucosal tunneling endoscopic resection for small upper gastrointestinal subepithelial tumors originating from the muscularis propria layer. *Surg Endosc* 2014; **28**: 524-530 [PMID: 24013472 DOI: 10.1007/s00464-013-3197-8]
37 **Xu MD**, Cai MY, Zhou PH, Qin XY, Zhong YS, Chen WF, Hu JW, Zhang YQ, Ma LL, Qin WZ, Yao LQ. Submucosal tunneling

endoscopic resection: a new technique for treating upper GI submucosal tumors originating from the muscularis propria layer (with videos). *Gastrointest Endosc* 2012; **75**: 195-199 [PMID: 22056087 DOI: 10.1016/j.gie.2011.08.018]

38 **Lee SH**, Kim SJ, Lee TH, Chung IK, Park SH, Kim EO, Lee HJ, Cho HD. Human applications of submucosal endoscopy under conscious sedation for pure natural orifice transluminal endoscopic surgery. *Surg Endosc* 2013; **27**: 3016-3020 [PMID: 23397506 DOI: 10.1007/s00464-013-2844-4]

39 **Shi Q**, Chen T, Zhong YS, Zhou PH, Ren Z, Xu MD, Yao LQ. Complete closure of large gastric defects after endoscopic full-thickness resection, using endoloop and metallic clip interrupted suture. *Endoscopy* 2013; **45**: 329-334 [PMID: 23468195 DOI: 10.1055/s-0032-1326214]

40 **Ye LP**, Yu Z, Mao XL, Zhu LH, Zhou XB. Endoscopic full-thickness resection with defect closure using clips and an endoloop for gastric subepithelial tumors arising from the muscularis propria. *Surg Endosc* 2014; **28**: 1978-1983 [PMID: 24619327 DOI: 10.1007/s00464-014-3421-1]

41 **Zhang B**, Huang LY, Wu CR, Cui J, Jiang LX, Zheng HT. Endoscopic full-thickness resection of gastric stromal tumor arising from the muscularis propria. *Chin Med J* (Engl) 2013; **126**: 2435-2439 [PMID: 23823814 DOI: 10.3760/cma.j.issn.0366-6999.20130881]

42 **Zhang Y**, Fan Z. Is closure of only the mucosal layer really sufficient? *Endoscopy* 2014; **46**: 82 [PMID: 24353126 DOI: 10.1016/0002-9610(92)90118-B]

43 **von Renteln D**, Vassiliou MC, Rothstein RI. Randomized controlled trial comparing endoscopic clips and over-the-scope clips for closure of natural orifice transluminal endoscopic surgery gastrotomies. *Endoscopy* 2009; **41**: 1056-1061 [PMID: 19899033 DOI: 10.1055/s-0029-1215241]

44 **Weiland T**, Fehlker M, Gottwald T, Schurr MO. Performance of the OTSC System in the endoscopic closure of gastrointestinal fistulae--a meta-analysis. *Minim Invasive Ther Allied Technol* 2012; **21**: 249-258 [PMID: 22694247 DOI: 10.3109/13645706.2012.694367]

45 **Kirschniak A**, Subotova N, Zieker D, Königsrainer A, Kratt T. The Over-The-Scope Clip (OTSC) for the treatment of gastrointestinal bleeding, perforations, and fistulas. *Surg Endosc* 2011; **25**: 2901-2905 [PMID: 21424197 DOI: 10.1007/s00464-011-1640-2]

46 **Baron TH**, Song LM, Ross A, Tokar JL, Irani S, Kozarek RA. Use of an over-the-scope clipping device: multicenter retrospective results of the first U.S. experience (with videos). *Gastrointest Endosc* 2012; **76**: 202-208 [PMID: 22726484 DOI: 10.1016/j.gie.2012.03.250]

47 **Voermans RP**, Le Moine O, von Renteln D, Ponchon T, Giovannini M, Bruno M, Weusten B, Seewald S, Costamagna G, Deprez P, Fockens P. Efficacy of endoscopic closure of acute perforations of the gastrointestinal tract. *Clin Gastroenterol Hepatol* 2012; **10**: 603-608 [PMID: 22361277 DOI: 10.1016/j.cgh.2012.02.005]

48 **Schlag C**, Wilhelm D, von Delius S, Feussner H, Meining A. EndoResect study: endoscopic full-thickness resection of gastric subepithelial tumors. *Endoscopy* 2013; **45**: 4-11 [PMID: 23254401 DOI: 10.1055/s-0032-1325760]

49 **Guo J**, Liu Z, Sun S, Liu X, Wang S, Ge N, Wang G, Qi Y. Endoscopic full-thickness resection with defect closure using an over-the-scope clip for gastric subepithelial tumors originating from the muscularis propria. *Surg Endosc* 2015; Epub ahead of print [PMID: 25701060 DOI: 10.1007/s00464-015-4076-2]

50 **Ahmed I**, Shibukawa G, Groce R, Poussard A, Brining D, Raju GS. Study of full-thickness endoluminal segmental resection of colon in a porcine colon model (with videos). *Gastrointest Endosc* 2007; **65**: 696-702 [PMID: 17383466 DOI: 10.1016/j.gie.2006.10.051]

51 **Raju GS**, Ahmed I, Shibukawa G, Poussard A, Brining D. Endoluminal clip closure of a circular full-thickness colon resection in a porcine model (with videos). *Gastrointest Endosc* 2007; **65**: 503-509 [PMID: 17321256 DOI: 10.1016/j.gie.2006.06.085]

52 **Xu M**, Wang XY, Zhou PH, Li QL, Zhang Y, Zhong Y, Chen W, Ma L, Ishaq S, Qin W, Hu J, Yao L. Endoscopic full-thickness resection of colonic submucosal tumors originating from the muscularis propria: an evolving therapeutic strategy. *Endoscopy* 2013; **45**: 770-773 [PMID: 23897399 DOI: 10.1055/s-0033-1344225]

53 **Brigic A**, Symons NR, Faiz O, Fraser C, Clark SK, Kennedy RH. A systematic review regarding the feasibility and safety of endoscopic full thickness resection (EFTR) for colonic lesions. *Surg Endosc* 2013; **27**: 3520-3529 [PMID: 23588710 DOI: 10.1007/s00464-013-2946-z]

54 **von Renteln D**, Schmidt A, Vassiliou MC, Rudolph HU, Caca K. Endoscopic full-thickness resection and defect closure in the colon. *Gastrointest Endosc* 2010; **71**: 1267-1273 [PMID: 20598252 DOI: 10.1016/j.gie.2009.12.056]

55 **Kantsevoy SV**, Bitner M, Mitrakov AA, Thuluvath PJ. Endoscopic suturing closure of large mucosal defects after endoscopic submucosal dissection is technically feasible, fast, and eliminates the need for hospitalization (with videos). *Gastrointest Endosc* 2014; **79**: 503-507 [PMID: 24332082 DOI: 10.1016/j.gie.2013.10.051]

56 **Kantsevoy SV**, Thuluvath PJ. Successful closure of a chronic refractory gastrocutaneous fistula with a new endoscopic suturing device (with video). *Gastrointest Endosc* 2012; **75**: 688-690 [PMID: 21762902 DOI: 10.1016/j.gie.2011.04.031]

57 **Chiu PW**, Phee SJ, Wang Z, Sun Z, Poon CC, Yamamoto T, Penny I, Wong JY, Lau JY, Ho KY. Feasibility of full-thickness gastric resection using master and slave transluminal endoscopic robot and closure by Overstitch: a preclinical study. *Surg Endosc* 2014; **28**: 319-324 [PMID: 23990156 DOI: 10.1007/s00464-013-3149-3]

58 **Chiu PW**, Lau JY, Ng EK, Lam CC, Hui M, To KF, Sung JJ, Chung SS. Closure of a gastrotomy after transgastric tubal ligation by using the Eagle Claw VII: a survival experiment in a porcine model (with video). *Gastrointest Endosc* 2008; **68**: 554-559 [PMID: 18635172 DOI: 10.1016/j.gie.2008.03.1110]

59 **Liu L**, Chiu PW, Teoh AY, Lam CC, Ng EK, Lau JY. Endoscopic suturing is superior to endoclips for closure of gastrotomy after natural orifices translumenal endoscopic surgery (NOTES): an ex vivo study. *Surg Endosc* 2014; **28**: 1342-1347 [PMID: 24196557 DOI: 10.1007/s00464-013-3280-1]

60 **Ikeda K**, Fritscher-Ravens A, Mosse CA, Mills T, Tajiri H, Swain CP. Endoscopic full-thickness resection with sutured closure in a porcine model. *Gastrointest Endosc* 2005; **62**: 122-129 [PMID: 15990831 DOI: 10.1016/S0016-5107(05)00517-1]

61 **Sumiyama K**, Gostout CJ, Rajan E, Bakken TA, Deters JL, Knipschield MA. Endoscopic full-thickness closure of large gastric perforations by use of tissue anchors. *Gastrointest Endosc* 2007; **65**: 134-139 [PMID: 17185093 DOI: 10.1016/j.gie.2006.01.050]

62 **Raju GS**, Shibukawa G, Ahmed I, Brining D, Poussard A, Xiao SY, Coe J, Cropper M, Martin D, Hull J. Endoluminal suturing may overcome the limitations of clip closure of a gaping wide colon perforation (with videos). *Gastrointest Endosc* 2007; **65**: 906-911 [PMID: 17350009 DOI: 10.1016/j.gie.2006.08.048]

63 **Fritscher-Ravens A**, Hampe J, Grange P, Holland C, Olagbeye F, Milla P, von Herbay A, Jacobsen B, Seehusen F, Hadeler KG, Mannur K. Clip closure versus endoscopic suturing versus thoracoscopic repair of an iatrogenic esophageal perforation: a randomized, comparative, long-term survival study in a porcine model (with videos). *Gastrointest Endosc* 2010; **72**: 1020-1026 [PMID: 21034902 DOI: 10.1016/j.gie.2010.07.029]

64 **Ikeda K**, Mosse CA, Park PO, Fritscher-Ravens A, Bergström M, Mills T, Tajiri H, Swain CP. Endoscopic full-thickness resection: circumferential cutting method. *Gastrointest Endosc* 2006; **64**: 82-89 [PMID: 16813808 DOI: 10.1016/j.gie.2005.12.039]

65 **Park PO**, Bergström M, Ikeda K, Fritscher-Ravens A, Mosse S, Kochman M, Swain P. Endoscopic pyloroplasty with full-thickness transgastric and transduodenal myotomy with sutured closure. *Gastrointest Endosc* 2007; **66**: 116-120 [PMID: 17451701 DOI: 10.1016/j.gie.2006.10.018]

66 **Raju GS**, Malhotra A, Ahmed I. Colonoscopic full-thickness resection of the colon in a porcine model as a prelude to endoscopic

surgery of difficult colon polyps: a novel technique (with videos). *Gastrointest Endosc* 2009; **70**: 159-165 [PMID: 19559838 DOI: 10.1016/j.gie.2009.02.022]

67 **Mori H**, Kobara H, Kazi R, Fujihara S, Nishiyama N, Masaki T. Balloon-armed mechanical counter traction and double-armed bar suturing systems for pure endoscopic full-thickness resection. *Gastroenterology* 2014; **147**: 278-280.e1 [PMID: 24973723 DOI: 10.1053/j.gastro.2014.06.030]

68 **Rajan E**, Gostout CJ, Burgart LJ, Leontovich ON, Knipschiel MA, Herman LJ, Norton ID. First endoluminal system for transmural resection of colorectal tissue with a prototype full-thickness resection device in a porcine model. *Gastrointest Endosc* 2002; **55**: 915-920 [PMID: 12024155 DOI: 10.1067/mge.2002.124099]

69 **Schurr MO**, Baur FE, Krautwald M, Fehlker M, Wehrmann M, Gottwald T, Prosst RL. Endoscopic full-thickness resection and clip defect closure in the colon with the new FTRD system: experimental study. *Surg Endosc* 2015; **29**: 2434-2441 [PMID: 25318369 DOI: 10.1007/s00464-014-3923-x]

70 **Kaehler G**, Grobholz R, Langner C, Suchan K, Post S. A new technique of endoscopic full-thickness resection using a flexible stapler. *Endoscopy* 2006; **38**: 86-89 [PMID: 16429361]

71 **Meireles OR**, Kantsevoy SV, Assumpcao LR, Magno P, Dray X, Giday SA, Kalloo AN, Hanly EJ, Marohn MR. Reliable gastric closure after natural orifice translumenal endoscopic surgery (NOTES) using a novel automated flexible stapling device. *Surg Endosc* 2008; **22**: 1609-1613 [PMID: 18401658 DOI: 10.1007/s00464-008-9750-1]

72 **Evans JA**, Rosato FE, Ginsberg GG. Gastrostomy port assisted full-thickness gastric resection by using the peroral SurgASSIST introduced via an oroesophageal overtube in a porcine model. *Gastrointest Endosc* 2007; **65**: 684-687 [PMID: 17383464 DOI: 10.1016/j.gie.2006.09.020]

73 **Sodergren M**, Clark J, Beardsley J, Bryant T, Horton K, Darzi A, Teare J. A novel flexible endoluminal stapling device for use in NOTES colotomy closure: a feasibility study using an ex vivo porcine model. *Surg Endosc* 2011; **25**: 3266-3272 [PMID: 21533972 DOI: 10.1007/s00464-011-1703-4]

74 **Sarker S**, Gutierrez JP, Council L, Brazelton JD, Kyanam Kabir Baig KR, Mönkemüller K. Over-the-scope clip-assisted method for resection of full-thickness submucosal lesions of the gastrointestinal tract. *Endoscopy* 2014; **46**: 758-761 [PMID: 24830398 DOI: 10.1055/s-0034-1365513]

75 **Schurr MO**, Baur F, Ho CN, Anhoeck G, Kratt T, Gottwald T. Endoluminal full-thickness resection of GI lesions: a new device and technique. *Minim Invasive Ther Allied Technol* 2011; **20**: 189-192 [PMID: 21574825 DOI: 10.3109/13645706.2011.582119]

76 **von Renteln D**, Kratt T, Rösch T, Denzer UW, Schachschal G. Endoscopic full-thickness resection in the colon by using a clip-and-cut technique: an animal study. *Gastrointest Endosc* 2011; **74**: 1108-1114 [PMID: 21944313 DOI: 10.1016/j.gie.2011.07.003]

77 **von Renteln D**, Rösch T, Kratt T, Denzer UW, El-Masry M, Schachschal G. Endoscopic full-thickness resection of submucosal gastric tumors. *Dig Dis Sci* 2012; **57**: 1298-1303 [PMID: 22370915 DOI: 10.1007/s10620-012-2039-1]

78 **Thompson CC**, Ryou M, Soper NJ, Hungess ES, Rothstein RI, Swanstrom LL. Evaluation of a manually driven, multitasking platform for complex endoluminal and natural orifice transluminal endoscopic surgery applications (with video). *Gastrointest Endosc* 2009; **70**: 121-125 [PMID: 19394008 DOI: 10.1016/j.gie.2008.11.007]

P- Reviewer: Arezzo A, Imaeda H, Mann O
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9286

World J Gastroenterol 2015 August 21; 21(31): 9286-9296
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Gastrointestinal Endoscopy

Local excision by transanal endoscopic surgery

Luis J García-Flórez, Jorge L Otero-Díez

Luis J García-Flórez, Jorge L Otero-Díez, General and Digestive Surgery Service, Colorectal Unit, Hospital San Agustín, 33401 Avilés, Spain

Author contributions: García-Flórez LJ and Otero-Díez JL contributed equally to this work, designed, performed and wrote the review.

Conflict-of-interest statement: All authors have no conflict of interest.

Correspondence to: Luis J García-Flórez, MD, PhD, Associate Professor, General and Digestive Surgery Service, Colorectal Unit, Hospital San Agustín, 33401 Avilés, Spain. luisjgf@gmail.com
Telephone: +34-985-123000
Fax: +34-985-123010

Received: March 13, 2015
Peer-review started: March 16, 2015
First decision: April 23, 2015
Revised: May 10, 2015
Accepted: July 3, 2015
Article in press: July 3, 2015
Published online: August 21, 2015

Abstract

Transanal endoscopic surgery (TES) consists of a series of anorectal surgical procedures using different devices that are introduced into the anal canal. TES has been developed significantly since it was first used in the 1980s. The key point for the success of these techniques is how accurately patients are selected. The main indication was the resection of endoscopically unresectable adenomas. In recent years, these techniques have become more widespread which has allowed them to be applied in conservative rectal procedures for both benign diseases and selected cases of rectal cancer. For more advanced rectal cancers it should be considered palliative or, in some controlled trials, experimental. The role of newer endoscopic techniques available has not yet been defined. TES may allow for new strategies in the treatment of rectal pathology, like transanal natural orifice transluminal endoscopic surgery or total mesorectal excision.

Key words: Transanal endoscopic surgery; Rectal adenoma; Early rectal cancer; Chemoradiotherapy; Rectal polyps

Core tip: In recent years, the diffusion of transanal endoscopic surgery techniques has allowed the application of conservative rectal procedures in both benign diseases and selected cases of early rectal cancer. For more advanced rectal cancers it should be considered palliative or, in some controlled trials, experimental and may allow for new strategies in the treatment of rectal pathologies.

García-Flórez LJ, Otero-Díez JL. Local excision by transanal endoscopic surgery. *World J Gastroenterol* 2015; 21(31): 9286-9296 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9286.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9286

INTRODUCTION

Transanal endoscopic surgery (TES) consists of a series of anorectal surgical procedures using different devices

Table 1 Applications of transanal endoscopic surgery

Pelvic abscess
Benign rectal stenosis
Rectal Dieulafoy's lesion
Rectourethral fistula
Gastrointestinal stromal tumour
Rectal condylomata acuminata
Rectal prolapse
Impacted fecaloma
Rectal perforations
Presacral tumor
Foreign body
Neuroendocrine tumour
Transanal total mesorectal excision

that are introduced into the anal canal. These devices allow the creation of a stable pneumorectum and offer a number of working channels that enable the introduction of optics and instruments for maneuvers of dissection, cutting, coagulation and suture. In recent years, the greater spread of these techniques has allowed the application of conservative rectal procedures in both benign diseases and selected cases of rectal cancer. The purpose of this article is to review the current indications of local excision, technical options available, and outcomes.

INDICATIONS

The key point for the success of these techniques lies in how accurately patients are selected. Preoperative workup includes physical exploration with digital rectal examination, fecal incontinence score, endoscopy, endoscopic rectal ultrasound (ERUS), pelvic magnetic resonance imaging (MRI) and abdominal CT in case of malignancies. Digital examination plays a key role. Magnification chromoendoscopy, ERUS and MRI are complementary staging modalities. TES has developed significantly since it was first used in the 1980s. The main indication was the resection of endoscopically unresectable adenomas. Local excision with TES has also shown benefits treating selected early rectal cancers. For more advanced rectal cancers it should be considered palliative or, in some controlled trials, experimental. With the development of the technique and the experience of surgeons, the indications have been increased. There are many applications beyond local excision, the most important in recent years is the development of transanal total mesorectal excision (TME)[1-3] (Table 1). Nowadays, we can consider these platforms as an important part of the colorectal surgeon's armamentarium, available for solving complex proctologic diseases, and may offer new strategies in the treatment of rectal cancer.

TECHNICAL OPTIONS

Rigid platforms

The first specifically designed device for these procedures was developed by Buess *et al*[4] in the mid 1980s. (Transanal Endoscopic Microsurgery, TEM, Richard Wolf, Germany). TEM instruments consist of a 3D optic viewing system with specific operating instruments and an endosurgical unit. The operating instruments include the operating rectoscope (4 cm in diameter, 12 or 20 cm in length), the stereoscope and instruments for dissection, excision and suturing. The endosurgical unit provides insufflation, suction, irrigation and continuous monitoring of intrarectal pressure. The rectoscope and its attachments are secured to the operating room table using a multi-jointed clamp, Martin's arm (Figure 1A). It was costly equipment and required specific training, so its spread, mainly in high-volume colorectal surgery units, was slow.

A few years later a cheaper alternative was introduced. The transanal endoscopic operation (TEO, Karl Storz, Germany), which was a newer and simpler system, has become widely implemented. It does not use the 3D optic system and has a shorter rectoscope (4 cm in diameter, 7.5 or 15 cm in length). Standard laparoscopic instruments, equipment and set up costs are lower, potentially opening the technique to any surgeon with previous laparoscopic experience. The main difference is a lack of binocular vision (Figure 1B). Several studies have compared TEO with TEM for benign and malignant lesions and have shown satisfactory outcomes[5,6].

Soft platforms

In recent years, the procedure known under the name of Transanal Minimally Invasive Surgery (TAMIS) has become increasingly more popular. Reported by Atallah *et al*[7] in 2010, the technique stems from the use of a single port initially designed for abdominal surgery. TAMIS is defined as the use of any multichannel port combined with ordinary laparoscopic instruments, a laparoscopic camera lens (preferably 5 mm and 30°), and a standard laparoscopic insufflator. Currently in the United States there are two ports with Food and Drug Administration (FDA) approval for TAMIS. They are the single-incision assisted laparoscopic surgery (SILS) Port (Covidien, United States) and the GelPOINT Path Transanal Access Platform (Applied Medical, United States). The latter is the only multichannel port specifically designed for TAMIS procedures (Figures 1C and 2). Standard laparoscopic instruments available at any operating theatre allow any experienced colorectal laparoscopic surgeon to perform the procedure without additional investment. It is also possible to use a flexible endoscope as a camera and offers an additional way to grasp or retract the bowel with an endoscopic grasper (eTAMIS)[8].

A variety of multichannel ports can be applied transanally[9-14] (Table 2). Since the inception of TAMIS, at least 390 procedures were reported worldwide from 2010 to 2013[15]. Robotic-TAMIS have also been reported, but with limited data. Success with robotic-

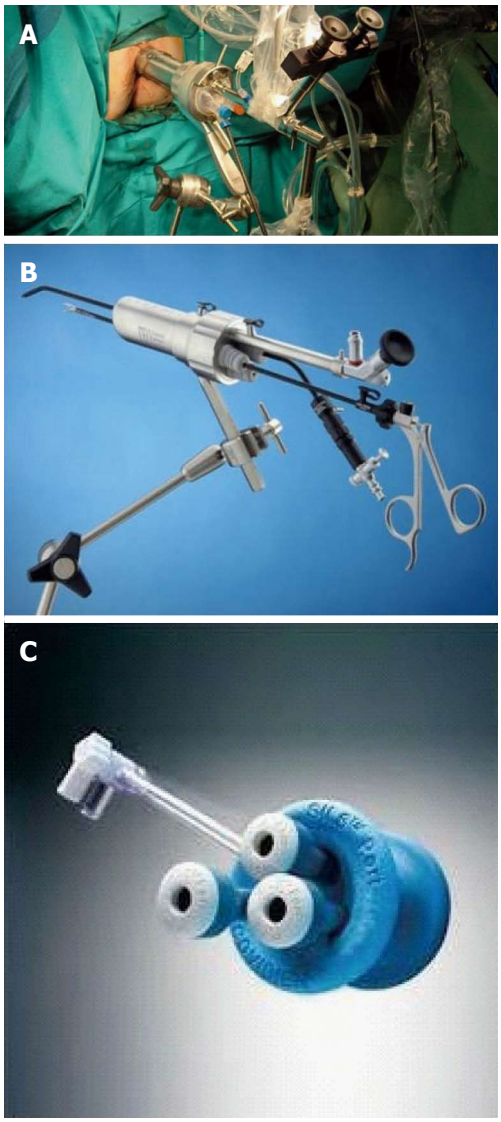


Figure 1 Transanal endoscopic microsurgery (A), transanal endoscopic operation (B) and single-incision assisted laparoscopic surgery (C) port.

TAMIS has been demonstrated with various patient positions and using a glove port[16-18]. Regardless of which platform is used, the basic principles of the procedure remain the same. Although alternate synonyms for TAMIS exist, it could be a valid generic term for all procedures using multichannel disposable ports regarding transanal minimal access surgery.

OPERATIVE TECHNIQUE

Mechanical bowel preparation, antibiotic and anti-thrombotic prophylaxis are usually recommended. Anesthesia may be general or spinal. Lee *et al*[19] have reported a series of 25 TAMIS using spinal anesthesia. In TEM the surgeon works with the tumour visible in the lower part of the rectoscope at all times, so the positioning of the patient depends on the location of the rectal tumour. In TAMIS the majority of lesions can be excised in a lithotomy position. However, we still recommend turning the patient for large anterior lesions, especially if the distance from the anal verge is in a range where there might be a risk of opening the peritoneum.

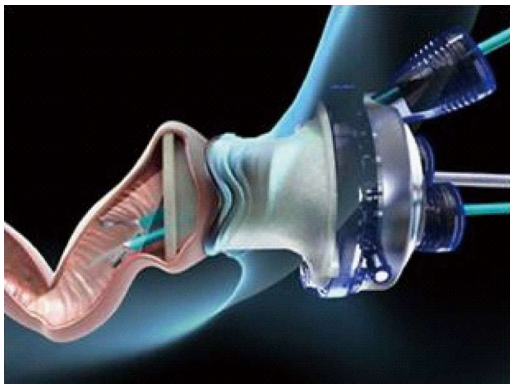


Figure 2 GelPOINT Path transanal port.

The pneumorectum is maintained at a constant pressure. Rectal distension created in this way exposes the tumour and the rectal wall. Some groups are now using the AirSeal system insufflator (SurgiQuest, United States) to maintain a stable pneumorectum during TAMIS[20]. Right-angle camera cords can improve ergonomics and decrease instrument collision[21]. Care must be taken to avoid entering the abdominal cavity whenever it is possible. As usual, we recommend beginning the dissection making a dotted line with the monopolar scalpel about 10 mm from the tumour. We then open the mucosa over the dotted line and begin the full-thickness excision of the rectal wall reaching the mesorectal fat using an ultrasound scalpel (Ultracision, Ethicon Endo-Surgery, United States) allowing a good hemostasis. Conventional laparoscopic instruments are suitable for TAMIS, but advanced laparoscopic instruments can be employed, like linear staplers, vessel-sealing systems or articulating instruments[7,9]. We usually wash the rectal defect with diluted povidone, and we recommend exsufflating the rectum after complete resection, then wait 3-5 min and reinsufflate in order to assure good hemostasis. The defect is sutured transversally to avoid stenosis of the rectal lumen and postoperative bleeding. Suturing in this area is sometimes difficult for technical reasons as the working space is limited. For defect closure different techniques are frequently used, such as clip-fixated sutures. If the defect cannot be completely closed, it should be reduced to the maximum, especially in the upper rectum, due to the risk of perforation. We are now using barbed sutures, that display the same bursting pressure as monofilament sutures, and their use for rectal wall closure seems feasible[22]. Suture line dehiscence is described in up to one-third of patients but mainly remains clinically unrecognized[23]. The dehiscence is presumably related to the wider size of the residual cavity. Obliteration of the residual perirectal space with a hemostatic agent and by the

Table 2 Transanal ports

SILS port (Covidien, United States)
Gel POINT Path (Applied Medical, United States)
SSL port (Ethicon, United States)
Triport (Olympus, Japan)
Glove port
Long Gel POINT Path (Applied Medical, United States)
Endorec (Aspide, France)
Single ballon trocar (pediatric use)

SILS: Single-incision assisted laparoscopic surgery.

introduction of gauzes into the rectal ampulla, may reduce the risk of postoperative perirectal abscess, and thus reduce the suture line dehiscence rate[24].

In centres with TEM experience, the average duration of surgery ranges from 45 to 120 min. Also, a number of studies have reported significantly decreased duration of surgery for TEM compared with radical surgery[25]. Several large studies have reported hospital stays of 4-5 d with low readmission rates. Some articles have also shown that 23-h discharge is safe, although the number of patients in these studies is small[26].

BENEFITS AND LIMITATIONS

Most experience with TES is derived from TEM and TEO. In a recent prospective randomized clinical trial, no technical or clinical differences were observed between the results obtained with the two systems except lower cost with TEO[6]. For some authors, the introduction of the TAMIS port into the anal canal is more complex than in TEM or TEO[9]. A SILS port can be used in patients with narrow or fibrotic anal canals which do not allow the GelPOINT Path transanal access device to be introduced. A further disadvantage of TAMIS is that the rectoscope cannot be mobilized at the site of the lesion; rectal lesions located behind a rectal haustral valve may be more difficult to access and remove. The longer channels associated with TEM and TEO equipments facilitate intraluminal rectal retraction. A new disposable port (GelPOINT Path Long Channel) can reach lesions up to 15 cm from the anal verge[27]. In addition, an assistant is required to hold and manipulate the laparoscope during the TAMIS procedure.

Traditionally, the upper limit of dissection is 10 cm for anterior tumours, 12 for lateral and 15 cm for posterior tumours. The limit for low lesions is the anal verge itself, but air-tightness of the insufflation system may be compromised for tumours less than 4 cm from the anal verge and therefore traditional transanal local excision (TAE) is sometimes better for these lower tumours. Nonetheless, the operation can begin by TAE and then be converted to TES to finish. In experienced hands, TES is capable of providing high quality local excision with a reach triple to that of Park' s TAE. TEM offers excellent magnified exposure of the operative site, especially for the upper and deep limits of the tumour, enhancing monobloc excision. TAE was associated with a significantly increased risk of fragmentation and R1 resection, leading to a higher risk of local recurrence (LR)[28,29]. A meta-analysis has confirmed a higher rate of R0 resection and disease-free survival after TEM[30]. Recently reported by Elmessiry *et al*[31], when TAE and TEM were compared, the latter resulted in a greater number of tumour-free excision margins, especially at the deep margin, and enabled a full-thickness whole specimen rather than a fragmented one. There was, however, no significant difference in LR or survival between the two techniques.

The limits of these techniques lie chiefly in the size and the circumferential extension of the lesions. Classically, the technique is recommended for superficial rectal tumours up to 3 cm in diameter and involving up to 40% of the rectal circumference. Nowadays we can consider that there are very few limits in terms of the location (anterior, lateral) of the lesion. In fact all four quadrants can sometimes be reached if the lesions are not particularly wide and if the size does not exceed the height permitted. It is possible to excise adenomatous lesions that cover even more than three quadrants of the circumference.

POSTOPERATIVE COMPLICATIONS

Whichever technique is used, morbidity and mortality are lower than for radical surgery. Operative mortality is less than 0.5% and morbidity ranges from 4% to 30% in large series, depending on the inclusion of minor complications. Bignell *et al*[32] found that the use of the harmonic scalpel reduces the complication rate. The most frequent complications include acute urinary retention (0%-11%), bleeding requiring re-operation (0.7%-9%), entry into the peritoneum (6%-20%) and recto-vaginal fistula (0.3%-1.4%)[33]. Kumar *et al*[34] found that the size of the tumour was associated with a risk of bleeding and anterior and lateral location was associated with a risk of peritoneal violation and acute urinary retention. Kreissler-Haag *et al*[35] assessed the anatomical variables of rectal neoplasia as well as surgeon experience on postoperative complications in patients undergoing TEM, they found 0.3% mortality and a 9% overall complication rate, including bleeding, fecal incontinence, dysuria, pneumonia, myocardial infarction and pulmonary emboli. Complications correlate with tumours located laterally and more than 8 cm from the anal verge. Overall surgical complications did not correlate with the number of TEM procedures performed, suggesting a short learning curve for the procedure in surgeons with previous experience in minimally invasive surgery.

Pelvic sepsis, which occurs in about 3% of cases, is more common in lesions within 2 cm of the dentate line. Regarding peritoneal perforation, although it was once thought to represent a complication requiring conversion

to laparotomy or even a stoma, in experienced hands this can usually be salvaged by TES[36,37]. A multicenter study performed from a database of 888 TEM procedures, found 22 perforations into the peritoneal cavity. They reported no association with major short-term complications or adverse long-term oncological outcomes[38]. Postoperative complications may be greater after neoadjuvant chemoradiation and include pain and wound dehiscence, but the majority seems to be minor and can be treated conservatively[25,39-43].

OUTCOMES

Functional outcomes

Anorectal function after TEM has been addressed in several studies[26,43,44]. The evidence available suggests that the TEM procedure seems to have no permanent deleterious effect on fecal continence. Although TEM can cause manometric alterations, it does not affect clinical continence scores[45]. Short-term functional results of TAMIS are also excellent and comparable to functional results using the TEM equipment[46]. In many patients with pre-existent impairment of anorectal function, their functional outcome after TES is significantly improved, probably secondary to excision of a mucous producing lesion[47]. Some patients will develop anorectal dysfunction but this is associated with excision of large lesions with changes in rectal capacity and compliance[48].

Sexual or urinary disorders are very rare. When circumferential lesions are resected, particularly carpet adenomas, there can be a higher rate of rectal stenosis. Stenosis will normally respond to surgical or endoscopic balloon dilatations[49,50].

Adenomas

TEM has been used primarily for resection of large adenomas of the rectum[25,51,52]. The evidence supports TEM as the preferred approach to rectal adenoma resection when endoscopic removal is not possible with safety or without fragmentation, with excellent results, low recurrence rates and a favorable complication profile compared with TAE or radical resection[25,53,54]. It would be of interest to report and evaluate the results with more TAMIS series including margin status, specimen fragmentation, and complications associated with the technique in a similar way to the TEM-TEO series. Since a LR rate is higher after excision of adenomas larger than 5 cm, a strict follow-up is recommended. In cases of recurrent adenoma, TEM has been shown to be an important therapeutic option with no increased morbidity[55].

There are still some limitations in the pre-operative diagnosis of large rectal adenomas. Even though ERUS appears to be the most accurate pre-operative diagnostic tool for investigating tumour invasion, the rate of incidental carcinoma in lesions with benign appearance is significant even with multimodal pre-operative assessment. Serra-Aracil *et al*[56], found 52 out of 277 lesions (18.8%) with preoperative diagnosis of adenoma to be invasive carcinomas. Dash *et al*[57] found that 13% of 167 benign lesions (with non full-thickness excision) were unexpected cancers. This is not related to the type of lesion, although exophytic lesions may be harder to assess and classify by ERUS[57]. The rate of occult carcinoma may be as high as 40%, depending on pre-operative imaging assessment[43]. Higher frequency scanning probes and coupling gels seem to have shown better accuracy for early stage cancer[58]. Real-time elastography has been used to assess adenomas and early cancers, and has shown promising discrimination between them[59]. Some recent studies have investigated the role of ERUS, compared with MRI, for the staging of large rectal adenomas, reporting similar rates of over-staging, but MRI might be more appropriate in case of proximal tumours that cannot be reached by the ERUS probe[43].

Carcinomas

Radical surgery with TME is still the cornerstone for the treatment of rectal cancer, offering patients the best results in terms of LR and disease-free survival[60]; however it is associated with significant mortality and morbidity rates[61]. According to the accumulated experience over the last years, TEM-TEO procedures have been accepted as effective treatments in selected patients with early rectal cancer, with similar oncologic outcomes as radical surgery and better functional results[62,63]. Recently, TAMIS has been proposed as an alternative with the same indications, but there is still limited experience in rectal cancer using this approach because of its short follow-up[27,15,31,43,64-67].

Appropriate patient and tumour selection is the main challenge and preoperative staging is of paramount importance for decision-making. Tumour biopsy offers a low accuracy, with a histological discrepancy of up to 20% or even higher[68]. Despite improvement in imaging techniques, there are patients who are not accurately staged[43]. ERUS appears to be the most accurate diagnostic tool for assessing tumour invasion of the rectal wall, especially for small lesions. MRI is more accurate detecting mesorectal invasion and the relation of the tumour with puborectalis muscle and anal sphincter in low cancers, and is preferred for N-staging because it allows for the evaluation of the whole mesorectum[25,33,40,61,63,69-71].

Unfavourable histological characteristics related to a high incidence of positive lymph nodes (N+) and recurrences are: Tumours larger than 3 cm or involving more than one third of the rectal lumen, rectal wall invasion more than T1 sm1, positive resection margins (< 1 mm), poorly differentiated adenocarcinomas, presence of lymphatic, venous or perineural invasion, mucinous component and tumour budding[25,31,40,63,72-76]. High-risk T1 tumours are more likely to be N+

compared to low-risk T2 tumours. Due to a lack of accuracy in the preoperative staging, full-thickness resection with a macroscopic margin of 10 mm is generally recommended[33,40,54,61,62,77]. Some authors[78] remove the perirectal fat to reach the mesorectal fascia, but there are some concerns about the possible major interference in case of completion surgery.

As mentioned above, TEM allows local excision to be performed with a lower positive margin rate compared to conventional TAE, less fragmented specimens and better oncologic outcomes[28,29]. In a multivariate analysis, TAE was an independent predictor of LR when it was compared to TEM[31]. The rate of reported involved margin in the surgical specimen in TAMIS procedures is 4.4%-6%[27,15,66], figures similar to those obtained with TEM, and seems to be related with the T stage[25,54,79]. Some studies have compared TEM with radical TME resection in early rectal cancer, finding similar results in terms of LR and survival[77,80]. In the meta-analysis performed by Winde *et al*[81], the rate of LR was higher with TEM (12% *vs* 0.5%) but no difference in survival was found. Similar conclusions were reported by others[25,64,80,82]. We have to take into account that TES is more frequently used in distal tumours, which have poorer prognosis when they are compared with upper rectal lesions.

TES seems to be a reasonable alternative to radical resection in patients with low-risk T1N0 rectal cancer[25,33,40,61,62,64,80,83] with LR rates ranging from 0%-39%. These wide differences can be explained by the heterogeneity of cases, different selection criteria, risk characteristics, and surgical techniques, but the majority are under 10%[25,30,68,77,78,81,84-87]. The level of submucosal invasion (sm level) has been demonstrated to be a strong predictor of recurrence, with sm1 lesions showing lowest levels of LR and sm2-3 lesions with LR rates similar to T2[66,72]. 5-year survival is consistently high in pT1, ranging 80%-100%, depending on the number of patients with high-risk tumours[25,68,78,81,83,85,87,88].

TES alone is not suitable treatment for fit patients with staged T2 or worse tumours, considering that the risk of LR varies between 9.5% and 47%[25,54,61,64,77,79,80,83-89]. But even in these cases there are considerable differences between low and high-risk cancers[90]. TES might be offered to patients with high-risk T1 or T2-3 tumours with poor life expectancy and multiple co-morbidity, unfit for major surgery, offering a reasonable chance of success, or simply as palliative treatment in case of disseminated disease[40,61,80].

Completion surgery

When pathological evaluation of the TES specimen reveals tumour invasion beyond pT1 sm1 or high-risk features, immediate radical surgery with TME is recommended[25,27,40,43,54,65,85,87,91]. As a matter for concern, Hompes *et al*[92] reported that they had found the completion surgery procedure difficult in 53% of cases. The quality of mesorectum was moderate or poor in 36% of cases in the pathological exam; all of them were in the difficult group, associated with previous full-thickness resection and low tumours. The resected tumours with a good TME specimen had a significantly better 5-year disease-free survival compared to inferior specimens (100% *vs* 51%).

Surgery for recurrence

Salvage surgery for recurrence after TES offers disappointing oncologic outcomes, the stage is usually more advanced than in primary tumours and may require multivisceral resection and an ostomy in up to 43% of cases. Survival is seriously compromised, with a 5-year survival ranging 43%-68%, dropping to 29% in patients with unfavourable histology[40,84,93].

TES and adjuvant therapy

In patients with high-risk pT1 or pT2 after local resection, adjuvant radiotherapy could be an option for selected patients who decline completion surgery or are too frail for radical surgery. Even though radiotherapy appears to have some benefits added to TES, it is not equivalent to radical surgery[62,64]. There are some promising studies, such as Duek *et al*[94], with no LR at 3-year follow-up in 12 patients with T2 tumours treated with local resection and adjuvant radiotherapy. Ramirez *et al*[95] reported 28 pT1 high-risk and pT2 low-risk patients treated with local excision and radiotherapy, with a LR rate of 10.7% and a 5-year cancer-specific survival rate of 93%. Borschitz *et al*[90] had a LR rate 16% in low-risk pT2. But other groups showed worse results, with LR rates over 30%[79,86].

Neoadjuvant therapy and TES

Tumour response to chemoradiation therapy (CRT) may define a subset of patients with a particularly good prognosis, who could benefit from a rectal sparing approach. Patients with early stage rectal cancer seem to respond better. Complete pathological response can be achieved usually in 10%-25%, but can even reach 45%[42,96]. Reliable assessment of the rectal wall and nodal status of mesorectum after CRT remains challenging, because of the induced edema, inflammation and fibrosis. Furthermore, endoscopic biopsies are unreliable to rule out residual cancer cells in rectal wall[42,96]. TES may play a role as a diagnostic procedure in selected patients with complete clinical response to rule out tumour persistence[85,97].

There are several studies which have shown similar oncological outcomes for T2 rectal cancers comparing neoadjuvant therapy and TEM with radical resection[40,79,98]. Other authors confirmed these good results in T2, with no LR in patients who had a significant response to CRT[54,78]. The best candidates for TES following CRT are those with complete pathological response[41]. Partial tumour response to

CRT has increased risk of recurrence after TES, being ypT stage the strongest prognostic factor[99-101]. CRT followed by TES may be a promising way to treat the best responding patients with distal rectal cancer who may require an abdomino-perineal resection or a coloanal anastomosis, but only in well selected cases and in the setting of a controlled clinical trial. It may also be an option as palliative treatment, for patients who refuse a permanent ostomy or are unfit for major surgery.

ALTERNATIVES

Conventional endoscopic mucosal resection (EMR) cannot provide en bloc resection in cases of large lesions. Barendse *et al*[102] have published a systematic review on safety and effectiveness of EMR *vs* TEM for large rectal adenomas. The study has shown the safety of EMR with a lower rate of morbidity but a higher recurrence rate. We are waiting for the results of an ongoing prospective randomized trial by a multicenter collaboration group of Dutch endoscopists and surgeons (TREND study) that compares the cost-effectiveness of EMR and TEM for the resection of large (> 3 cm) rectal adenomas[103].

In recent years, the endoscopic submucosal dissection (ESD) technique was introduced to allow more en-bloc resections, especially in lesions larger than 20 mm. However, ESD has not gained wide acceptance in western countries because it is technically challenging and time consuming, requiring a steep learning curve, while it is affected by a consistent rate of complications (29.2%) and allows a rate of R0 resections of no more than 72.9% of cases[104].

FUTURE PERSPECTIVES

The standard care of rectal cancer is changing. In recent years, the impact of screening is increasing the diagnosis of early cancer and its management is becoming bespoke and has not yet been defined. Molecular markers associated with tumour progression or response to neoadjuvant therapies may help in stratifying patients at high or low risk for local therapies[105,106]. The role of organ-sparing approaches including neoadjuvant therapies followed by TES should be formally assessed by randomized controlled trials. Improvements in preoperative discrimination of benign and malignant rectal lesions are also needed. The management of early rectal cancer should always be based on a multidisciplinary approach without jeopardizing survival[65]. Currently, two controlled trials are examining this. The CARTS study (CRT for rectal cancer in the distal rectum followed by organ-sparing TEM) has been designed to assess the adequacy of TEM following neoadjuvant radiotherapy. Patients with a clinical T1-3N0M0 rectal cancer below 10 cm from the anal verge will receive CRT followed by TEM 8-10 wk later. The UK TREC trial (TEM and Radiotherapy in Early Rectal Cancer) is offered for patients with early rectal cancer (T1-2N0). Patients are randomized between radical TME surgery and short-course neoadjuvant radiotherapy with delayed local excision at 8-10 wk[43].

There are several new techniques and approaches under investigation, which are still preclinical or experimental, such as transanal natural orifice transluminal endoscopic surgery (NOTES), transanal TME, and robotic-TES[25,43,107]. TES platforms seem to be safe for both transanal NOTES and TME procedures[108,109]. Robotic technology can lower the difficulty inherent in the TES platforms for performing such procedures[110]. Clinical trials are necessary for full evaluation of these techniques.

CONCLUSION

TES has improved significantly since its introduction in the 1980s. In recent years, the spread of these techniques has allowed the application of conservative rectal procedures in both benign diseases and selected cases of early rectal cancer. For more advanced rectal cancers it should be considered palliative or, in some controlled trials, experimental. The role of newer endoscopic techniques available has not yet been defined. TES may offer new strategies in the treatment of rectal pathology, like transanal NOTES or TME.

REFERENCES

1 **Serra-Aracil X**, Mora-Lopez L, Alcantara-Moral M, Corredera-Cantarin C, Gomez-Diaz C, Navarro-Soto S. Atypical indications for transanal endoscopic microsurgery to avoid major surgery. *Tech Coloproctol* 2014; **18**: 157-164 [PMID: 23813055 DOI: 10.1007/s10151-013-1040-9]

2 **Atallah S**, Albert M, Debeche-Adams T, Larach S. Transanal minimally invasive surgery (TAMIS): applications beyond local excision. *Tech Coloproctol* 2013; **17**: 239-243 [PMID: 23207713 DOI: 10.1007/s10151-012-0945-z]

3 **Sylla P**, Rattner DW, Delgado S, Lacy AM. NOTES transanal rectal cancer resection using transanal endoscopic microsurgery and laparoscopic assistance. *Surg Endosc* 2010; **24**: 1205-1210 [PMID: 20186432 DOI: 10.1007/s00464-010-0965-6]

4 **Buess G**, Theiss R, Günther M, Hutterer F, Pichlmaier H. Endoscopic surgery in the rectum. *Endoscopy* 1985; **17**: 31-35 [PMID: 3971938]

5 **Nieuwenhuis DH**, Draaisma WA, Verberne GH, van Overbeeke AJ, Consten EC. Transanal endoscopic operation for rectal lesions using two-dimensional visualization and standard endoscopic instruments: a prospective cohort study and comparison with the literature. *Surg Endosc* 2009; **23**: 80-86 [PMID: 18443874 DOI: 10.1007/s00464-008-9918-8]

6 **Serra-Aracil X**, Mora-Lopez L, Alcantara-Moral M, Caro-Tarrago A, Navarro-Soto S. Transanal endoscopic microsurgery with 3-D (TEM) or high-definition 2-D transanal endoscopic operation (TEO) for rectal tumors. A prospective, randomized clinical trial. *Int J Colorectal Dis* 2014; **29**: 605-610 [PMID: 24676506 DOI: 10.1007/s00384-014-1849-3]

7 **Atallah S**, Albert M, Larach S. Transanal minimally invasive surgery: a giant leap forward. *Surg Endosc* 2010; **24**: 2200-2205 [PMID: 20174935 DOI: 10.1007/s00464-010-0927-z]

8 **McLemore EC**, Coker A, Jacobsen G, Talamini MA, Horgan S. eTAMIS: endoscopic visualization for transanal minimally invasive

surgery. *Surg Endosc* 2013; **27**: 1842-1845 [PMID: 23179071 DOI: 10.1007/s00464-012-2652-2]
9 **Barendse RM**, Doornebosch PG, Bemelman WA, Fockens P, Dekker E, de Graaf EJ. Transanal employment of single access ports is feasible for rectal surgery. *Ann Surg* 2012; **256**: 1030-1033 [PMID: 22504282 DOI: 10.1097/SLA.0b013e3182523b31]
10 **Michalik M**, Bobowicz M, Orlowski M. Transanal endoscopic microsurgery via TriPort Access System with no general anesthesia and without sphincter damage. *Surg Laparosc Endosc Percutan Tech* 2011; **21**: e308-e310 [PMID: 22146178 DOI: 10.1097/SLE.0b013e31823cd06b]
11 **Alessandro C**, Daniela M, Michele M, Andrea T, Gianmarco G, Massimo S, Orazio Z, Fabio G, Giuseppe T. Glove port technique for transanal endoscopic microsurgery. *Int J Surg Oncol* 2012; **2012**: 383025 [PMID: 22701788 DOI: 10.1155/2012/383025]
12 **Mclemore EC**, Coker A, Leland H, Yu PT, Devaraj B, Jacobsen G, Talamini MA, Horgan S, Ramamoorthy S. New disposable transanal endoscopic surgery platform: longer channel, longer reach. *Glob J Gastroenterol Hepatol* 2013; **1**: 46-50 [DOI: 10.12970/2308-6483.2013.01.01.7]
13 **Esposito C**, Escolino M, Ascione G, Settimi A. Single trocar transanal endoscopy in a child. *APSP J Case Rep* 2013; **4**: 22 [PMID: 24040600]
14 **Bridoux V**, Schwarz L, Suaud L, Dazza M, Michot F, Tuech JJ. Transanal minimal invasive surgery with the Endorec(TM) trocar: a low cost but effective technique. *Int J Colorectal Dis* 2014; **29**: 177-181 [PMID: 24196874 DOI: 10.1007/s00384-013-1789-3]
15 **Martin-Perez B**, Andrade-Ribeiro GD, Hunter L, Atallah S. A systematic review of transanal minimally invasive surgery (TAMIS) from 2010 to 2013. *Tech Coloproctol* 2014; **18**: 775-788 [PMID: 24848524 DOI: 10.1007/s10151-014-1148-6]
16 **Atallah S**, Parra-Davila E, DeBeche-Adams T, Albert M, Larach S. Excision of a rectal neoplasm using robotic transanal surgery (RTS): a description of the technique. *Tech Coloproctol* 2012; **16**: 389-392 [PMID: 22584407 DOI: 10.1007/s10151-012-0833-6]
17 **Bardakcioglu O**. Robotic transanal access surgery. *Surg Endosc* 2013; **27**: 1407-1409 [PMID: 23239290 DOI: 10.1007/s00464-012-2581-0]
18 **Hompes R**, Rauh SM, Ris F, Tuynman JB, Mortensen NJ. Robotic transanal minimally invasive surgery for local excision of rectal neoplasms. *Br J Surg* 2014; **101**: 578-581 [PMID: 24633833 DOI: 10.1002/bjs.9454]
19 **Lee TG**, Lee SJ. Transanal single-port microsurgery for rectal tumors: minimal invasive surgery under spinal anesthesia. *Surg Endosc* 2014; **28**: 271-280 [PMID: 24061623 DOI: 10.1007/s00464-013-3184-0]
20 **Bislenghi G**, Wolthuis AM, de Buck van Overstraeten A, D'Hoore A. AirSeal system insufflator to maintain a stable pneumorectum during TAMIS. *Tech Coloproctol* 2015; **19**: 43-45 [PMID: 25421704 DOI: 10.1007/s10151-014-1244-7]
21 **Ragupathi M**, Vande Maele D, Nieto J, Pickron TB, Haas EM. Transanal endoscopic video-assisted (TEVA) excision. *Surg Endosc* 2012; **26**: 3528-3535 [PMID: 22729706 DOI: 10.1007/s00464-012-2399-9]
22 **Wilhelm P**, Storz P, Axt S, Falch C, Kirschniak A, Muller S. Use of self-retaining barbed suture for rectal wall closure in transanal endoscopic microsurgery. *Tech Coloproctol* 2014; **18**: 813-816 [PMID: 24667989 DOI: 10.1007/s10151-014-1138-8]
23 **Ramirez JM**, Aguilella V, Arribas D, Martinez M. Transanal full-thickness excision of rectal tumours: should the defect be sutured? a randomized controlled trial. *Colorectal Dis* 2002; **4**: 51-55 [PMID: 12780656]
24 **Paganini AM**, Balla A, Quaresima S, D'Ambrosio G, Bruzzone P, Lezoche E. Tricks to decrease the suture line dehiscence rate during endoluminal loco-regional resection (ELRR) by transanal endoscopic microsurgery (TEM). *Surg Endosc* 2015; **29**: 1045-1050 [PMID: 25154889 DOI: 10.1007/s00464-014-3776-3]
25 **Heidary B**, Phang TP, Raval MJ, Brown CJ. Transanal endoscopic microsurgery: a review. *Can J Surg* 2014; **57**: 127-138 [PMID: 24666451]
26 **Wright CJ**, Tutton M. Early discharge following transanal endoscopic microsurgery is safe. *J Laparoendosc Adv Surg Tech A* 2014; **24**: 399-402 [PMID: 24720502 DOI: 10.1089/lap.2013.0258]
27 **McLemore EC**, Weston LA, Coker AM, Jacobsen GR, Talamini MA, Horgan S, Ramamoorthy SL. Transanal minimally invasive surgery for benign and malignant rectal neoplasia. *Am J Surg* 2014; **208**: 372-381 [PMID: 24832238 DOI: 10.1016/j.amjsurg.2014.01.006]
28 **Moore JS**, Cataldo PA, Osler T, Hyman NH. Transanal endoscopic microsurgery is more effective than traditional transanal excision for resection of rectal masses. *Dis Colon Rectum* 2008; **51**: 1026-1030; discussion 1030-1031 [PMID: 18481147 DOI: 10.1007/s10350-008-9337-x]
29 **Clancy C**, Burke JP, Albert MR, O'Connell PR, Winter DC. Transanal endoscopic microsurgery versus standard transanal excision for the removal of rectal neoplasms: a systematic review and meta-analysis. *Dis Colon Rectum* 2015; **58**: 254-261 [PMID: 25585086 DOI: 10.1097/DCR.0000000000000309]
30 **Sgourakis G**, Lanitis S, Gockel I, Kontovounisios C, Karaliotas C, Tsiftsi K, Tsiamis A, Karaliotas CC. Transanal endoscopic microsurgery for T1 and T2 rectal cancers: a meta-analysis and meta-regression analysis of outcomes. *Am Surg* 2011; **77**: 761-772 [PMID: 21679648]
31 **Elmessiry MM**, Van Koughnett JA, Maya A, DaSilva G, Wexner SD, Bejarano P, Berho M. Local excision of T1 and T2 rectal cancer: proceed with caution. *Colorectal Dis* 2014; **16**: 703-709 [PMID: 24787457 DOI: 10.1111/codi.12657]
32 **Bignell MB**, Ramwell A, Evans JR, Dastur N, Simson JN. Complications of transanal endoscopic microsurgery (TEMS): a prospective audit. *Colorectal Dis* 2010; **12**: e99-103 [PMID: 19843114 DOI: 10.1111/j.1463-1318.2009.02071.x]
33 **Lartigau C**, Lebreton G, Alves A. Local resection for small rectal cancer. *J Visc Surg* 2013; **150**: 325-331 [PMID: 24016715 DOI: 10.1016/j.jviscsurg.2013.08.004]
34 **Kumar AS**, Coralic J, Kelleher DC, Sidani S, Kolli K, Smith LE. Complications of transanal endoscopic microsurgery are rare and minor: a single institution's analysis and comparison to existing data. *Dis Colon Rectum* 2013; **56**: 295-300 [PMID: 23392142 DOI: 10.1097/DCR.0b013e31827163f7]
35 **Kreissler-Haag D**, Schuld J, Lindemann W, König J, Hildebrandt U, Schilling M. Complications after transanal endoscopic microsurgical resection correlate with location of rectal neoplasms. *Surg Endosc* 2008; **22**: 612-616 [PMID: 18095021]
36 **Marks JH**, Frenkel JL, Greenleaf CE, D'Andrea AP. Transanal endoscopic microsurgery with entrance into the peritoneal cavity: is it safe? *Dis Colon Rectum* 2014; **57**: 1176-1182 [PMID: 25203373 DOI: 10.1097/DCR.0000000000000208]
37 **Morino M**, Allaix ME, Famiglietti F, Caldart M, Arezzo A. Does peritoneal perforation affect short- and long-term outcomes after transanal endoscopic microsurgery? *Surg Endosc* 2013; **27**: 181-188 [PMID: 22717799 DOI: 10.1007/s00464-012-2418-x]
38 **Baatrup G**, Borschitz T, Cunningham C, Qvist N. Perforation into the peritoneal cavity during transanal endoscopic microsurgery for rectal cancer is not associated with major complications or oncological compromise. *Surg Endosc* 2009; **23**: 2680-2683 [PMID: 19172355]
39 **Marks JH**, Valsdottir EB, DeNittis A, Yarandi SS, Newman DA, Nweze I, Mohiuddin M, Marks GJ. Transanal endoscopic microsurgery for the treatment of rectal cancer: comparison of wound complication rates with and without neoadjuvant radiation therapy. *Surg Endosc* 2009; **23**: 1081-1087 [PMID: 19263164 DOI: 10.1007/s00464-009-0326-5]
40 **Heafner TA**, Glasgow SC. A critical review of the role of local excision in the treatment of early (T1 and T2) rectal tumors. *J Gastrointest Oncol* 2014; **5**: 345-352 [PMID: 25276407 DOI: 10.3978/j.issn.2078-6891.2014.066]
41 **Stipa F**, Picchio M, Burza A, Soricelli E, Vitelli CE. Long-term outcome of local excision after preoperative chemoradiation for ypT0 rectal cancer. *Dis Colon Rectum* 2014; **57**: 1245-1252 [PMID: 25285690 DOI: 10.1097/DCR.0000000000000221]

42 **Garcia-Aguilar J**, Shi Q, Thomas CR, Chan E, Cataldo P, Marcet J, Medich D, Pigazzi A, Oommen S, Posner MC. A phase II trial of neoadjuvant chemoradiation and local excision for T2N0 rectal cancer: preliminary results of the ACOSOG Z6041 trial. *Ann Surg Oncol* 2012; **19**: 384-391 [PMID: 21755378 DOI: 10.1245/s10434-011-1933-7]

43 **Smart CJ**, Cunningham C, Bach SP. Transanal endoscopic microsurgery. *Best Pract Res Clin Gastroenterol* 2014; **28**: 143-157 [PMID: 24485262 DOI: 10.1016/j.bpg.2013.11.005]

44 **Planting A**, Phang PT, Raval MJ, Brown CJ. Transanal endoscopic microsurgery: impact on fecal incontinence and quality of life. *Can J Surg* 2013; **56**: 243-248 [PMID: 23883494]

45 **Mora López L**, Serra Aracil J, Rebasa Cladera P, Puig Divi V, Hermoso Bosch J, Bombardo Junca J, Alcántara Moral M, Hernando Tavira R, Ayguavives Garnica I, Navarro Soto S. [Anorectal disorders in the immediate and late postoperative period after transanal endoscopic microsurgery]. *Cir Esp* 2007; **82**: 285-289 [PMID: 18021627]

46 **Schiphorst AH**, Langenhoff BS, Maring J, Pronk A, Zimmerman DD. Transanal minimally invasive surgery: initial experience and short-term functional results. *Dis Colon Rectum* 2014; **57**: 927-932 [PMID: 25003287 DOI: 10.1097/DCR.0000000000000170]

47 **Doornebosch PG**, Gosselink MP, Neijenhuis PA, Schouten WR, Tollenaar RA, de Graaf EJ. Impact of transanal endoscopic microsurgery on functional outcome and quality of life. *Int J Colorectal Dis* 2008; **23**: 709-713 [PMID: 18379797 DOI: 10.1007/s00384-008-0442-z]

48 **Herman RM**, Richter P, Walega P, Popiela T. Anorectal sphincter function and rectal barostat study in patients following transanal endoscopic microsurgery. *Int J Colorectal Dis* 2001; **16**: 370-376 [PMID: 11760898]

49 **Barker JA**, Hill J. Incidence, treatment and outcome of rectal stenosis following transanal endoscopic microsurgery. *Tech Coloproctol* 2011; **15**: 281-284 [PMID: 21710207 DOI: 10.1007/s10151-011-0703-7]

50 **Flexer SM**, Durham-Hall AC, Steward MA, Robinson JM. TEMS: results of a specialist centre. *Surg Endosc* 2014; **28**: 1874-1878 [PMID: 24414462 DOI: 10.1007/s00464-013-3407-4]

51 **Steele RJ**, Hershman MJ, Mortensen NJ, Armitage NC, Scholefield JH. Transanal endoscopic microsurgery--initial experience from three centres in the United Kingdom. *Br J Surg* 1996; **83**: 207-210 [PMID: 8689164]

52 **Cocilovo C**, Smith LE, Stahl T, Douglas J. Transanal endoscopic excision of rectal adenomas. *Surg Endosc* 2003; **17**: 1461-1463 [PMID: 12739115]

53 **Langer C**, Liersch T, Süss M, Siemer A, Markus P, Ghadimi BM, Füzesi L, Becker H. Surgical cure for early rectal carcinoma and large adenoma: transanal endoscopic microsurgery (using ultrasound or electrosurgery) compared to conventional local and radical resection. *Int J Colorectal Dis* 2003; **18**: 222-229 [PMID: 12673487]

54 **Morino M**, Allaix ME. Transanal endoscopic microsurgery: what indications in 2013? *Gastroenterol Rep* (Oxf) 2013; **1**: 75-84 [PMID: 24759812 DOI: 10.1093/gastro/got012]

55 **Allaix ME**, Arezzo A, Cassoni P, Famiglietti F, Morino M. Recurrence after transanal endoscopic microsurgery for large rectal adenomas. *Surg Endosc* 2012; **26**: 2594-2600 [PMID: 22476837 DOI: 10.1007/s00464-012-2238-z]

56 **Serra-Aracil X**, Caro-Tarrago A, Mora-López L, Casalots A, Rebasa P, Navarro-Soto S. Transanal endoscopic surgery with total wall excision is required with rectal adenomas due to the high frequency of adenocarcinoma. *Dis Colon Rectum* 2014; **57**: 823-829 [PMID: 24901682 DOI: 10.1097/DCR.0000000000000139]

57 **Dash I**, Walter CJ, Wheeler JM, Borley NR. Does the incidence of unexpected malignancy in 'benign' rectal neoplasms undergoing trans-anal endoscopic microsurgery vary according to lesion morphology? *Colorectal Dis* 2013; **15**: 183-186 [PMID: 22686137 DOI: 10.1111/j.1463-1318.2012.03126.x]

58 **Wang Y**, Zhou CW, Hao YZ, Li L, Liu SM, Feng XL, Zhou ZX, Leung VY. Improvement in T-staging of rectal carcinoma: using a novel endorectal ultrasonography technique with sterile coupling gel filling the rectum. *Ultrasound Med Biol* 2012; **38**: 574-579 [PMID: 22305079 DOI: 10.1016/j.ultrasmedbio.2011.12.020]

59 **Waage JE**, Havre RF, Odegaard S, Leh S, Eide GE, Baatrup G. Endorectal elastography in the evaluation of rectal tumours. *Colorectal Dis* 2011; **13**: 1130-1137 [PMID: 21040360 DOI: 10.1111/j.1463-1318.2010.02440.x]

60 **Peeters KC**, Marijnen CA, Nagtegaal ID, Kranenbarg EK, Putter H, Wiggers T, Rutten H, Pahlman L, Glimelius B, Leer JW, van de Velde CJ. The TME trial after a median follow-up of 6 years: increased local control but no survival benefit in irradiated patients with resectable rectal carcinoma. *Ann Surg* 2007; **246**: 693-701 [PMID: 17968156]

61 **Lezoche G**, Paganini AM, Campagnacci R, Ghiselli R, Pelloni M, Rombini A, Guerrieri M. Treatment of rectal cancer by transanal endoscopic microsurgery: review of the literature. *Minerva Chir* 2013; **68**: 1-9 [PMID: 23584262]

62 **Monson JR**, Weiser MR, Buie WD, Chang GJ, Rafferty JF, Buie WD, Rafferty J. Practice parameters for the management of rectal cancer (revised). *Dis Colon Rectum* 2013; **56**: 535-550 [PMID: 23575392 DOI: 10.1097/DCR.0b013e31828cb66c]

63 **Morino M**, Risio M, Bach S, Beets-Tan R, Bujko K, Panis Y, Quirke P, Rembacken B, Rullier E, Saito Y, Young-Fadok T, Allaix ME. Early rectal cancer: the European Association for Endoscopic Surgery (EAES) clinical consensus conference. *Surg Endosc* 2015; **29**: 755-773 [PMID: 25609317 DOI: 10.1007/s00464-015-4067-3]

64 **Kidane B**, Chadi SA, Kanters S, Colquhoun PH, Ott MC. Local resection compared with radical resection in the treatment of T1N0M0 rectal adenocarcinoma: a systematic review and meta-analysis. *Dis Colon Rectum* 2015; **58**: 122-140 [PMID: 25489704 DOI: 10.1097/DCR.0000000000000293]

65 **Morino M**, Arezzo A, Allaix ME. Transanal endoscopic microsurgery. *Tech Coloproctol* 2013; **17** Suppl 1: S55-S61 [PMID: 23314951 DOI: 10.1007/s10151-012-0936-0]

66 **Albert MR**, Atallah SB, deBeche-Adams TC, Izfar S, Larach SW. Transanal minimally invasive surgery (TAMIS) for local excision of benign neoplasms and early-stage rectal cancer: efficacy and outcomes in the first 50 patients. *Dis Colon Rectum* 2013; **56**: 301-307 [PMID: 23392143 DOI: 10.1097/DCR.0b013e31827ca313]

67 **Lim SB**, Seo SI, Lee JL, Kwak JY, Jang TY, Kim CW, Yoon YS, Yu CS, Kim JC. Feasibility of transanal minimally invasive surgery for mid-rectal lesions. *Surg Endosc* 2012; **26**: 3127-3132 [PMID: 22543995 DOI: 10.1007/s00464-012-2303-7]

68 **Morino M**, Allaix ME, Caldart M, Scozzari G, Arezzo A. Risk factors for recurrence after transanal endoscopic microsurgery for rectal malignant neoplasm. *Surg Endosc* 2011; **25**: 3683-3690 [PMID: 21647814 DOI: 10.1007/s00464-011-1777-z]

69 **Bipat S**, Glas AS, Slors FJ, Zwinderman AH, Bossuyt PM, Stoker J. Rectal cancer: local staging and assessment of lymph node involvement with endoluminal US, CT, and MR imaging--a meta-analysis. *Radiology* 2004; **232**: 773-783 [PMID: 15273331]

70 **Puli SR**, Reddy JB, Bechtold ML, Choudhary A, Antillon MR, Brugge WR. Accuracy of endoscopic ultrasound to diagnose nodal invasion by rectal cancers: a meta-analysis and systematic review. *Ann Surg Oncol* 2009; **16**: 1255-1265 [PMID: 19219506 DOI: 10.1245/s10434-009-0337-4]

71 **Ashraf S**, Hompes R, Slater A, Lindsey I, Bach S, Mortensen NJ, Cunningham C. A critical appraisal of endorectal ultrasound and transanal endoscopic microsurgery and decision-making in early rectal cancer. *Colorectal Dis* 2012; **14**: 821-826 [PMID: 21920011 DOI: 10.1111/j.1463-1318.2011.02830.x]

72 **Bach SP**, Hill J, Monson JR, Simson JN, Lane L, Merrie A, Warren B, Mortensen NJ. A predictive model for local recurrence after transanal endoscopic microsurgery for rectal cancer. *Br J Surg* 2009; **96**: 280-290 [PMID: 19224520 DOI: 10.1002/bjs.6456]

73 **Saraste D**, Gunnarsson U, Janson M. Predicting lymph node metastases in early rectal cancer. *Eur J Cancer* 2013; **49**: 1104-1108 [PMID: 23122785 DOI: 10.1016/j.ejca.2012.10.005]

74 **Beaton C**, Twine CP, Williams GL, Radcliffe AG. Systematic review and meta-analysis of histopathological factors influencing

the risk of lymph node metastasis in early colorectal cancer. *Colorectal Dis* 2013; **15**: 788-797 [PMID: 23331927 DOI: 10.1111/codi.12129]
75 **Yamamoto S**, Watanabe M, Hasegawa H, Baba H, Yoshinare K, Shiraishi J, Kitajima M. The risk of lymph node metastasis in T1 colorectal carcinoma. *Hepatogastroenterology* 2004; **51**: 998-1000 [PMID: 15239233]
76 **Bosch SL**, Teerenstra S, de Wilt JH, Cunningham C, Nagtegaal ID. Predicting lymph node metastasis in pT1 colorectal cancer: a systematic review of risk factors providing rationale for therapy decisions. *Endoscopy* 2013; **45**: 827-834 [PMID: 23884793 DOI: 10.1055/s-0033-1344238]
77 **Hershman MJ**, Mohammad H, Hussain A, Ahmed A. Local excision of rectal tumours by minimally invasive transanal surgery. *Br J Hosp Med* (Lond) 2013; **74**: 387-390 [PMID: 24159640]
78 **Guerrieri M**, Gesuita R, Ghiselli R, Lezoche G, Budassi A, Baldarelli M. Treatment of rectal cancer by transanal endoscopic microsurgery: experience with 425 patients. *World J Gastroenterol* 2014; **20**: 9556-9563 [PMID: 25071352 DOI: 10.3748/wjg.vi28.9556]
79 **Allaix ME**, Arezzo A, Giraudo G, Morino M. Transanal endoscopic microsurgery vs. laparoscopic total mesorectal excision for T2N0 rectal cancer. *J Gastrointest Surg* 2012; **16**: 2280-2287 [PMID: 23070621 DOI: 10.1007/s11605-012-2046-8]
80 **Sajid MS**, Farag S, Leung P, Sains P, Miles WF, Baig MK. Systematic review and meta-analysis of published trials comparing the effectiveness of transanal endoscopic microsurgery and radical resection in the management of early rectal cancer. *Colorectal Dis* 2014; **16**: 2-14 [PMID: 24330432 DOI: 10.1111/codi.12474]
81 **Winde G**, Nottberg H, Keller R, Schmid KW, Bünte H. Surgical cure for early rectal carcinomas (T1). Transanal endoscopic microsurgery vs. anterior resection. *Dis Colon Rectum* 1996; **39**: 969-976 [PMID: 8797643]
82 **Heintz A**, Mörschel M, Junginger T. Comparison of results after transanal endoscopic microsurgery and radical resection for T1 carcinoma of the rectum. *Surg Endosc* 1998; **12**: 1145-1148 [PMID: 9716769]
83 **Damin DC**, Lazzaron AR. Evolving treatment strategies for colorectal cancer: a critical review of current therapeutic options. *World J Gastroenterol* 2014; **20**: 877-887 [PMID: 24574762 DOI: 10.3748/wjg.v20.i4.877]
84 **Doornebosch PG**, Ferenschild FT, de Wilt JH, Dawson I, Tetteroo GW, de Graaf EJ. Treatment of recurrence after transanal endoscopic microsurgery (TEM) for T1 rectal cancer. *Dis Colon Rectum* 2010; **53**: 1234-1239 [PMID: 20706065 DOI: 10.1007/DCR.0b013e3181e73f33]
85 **Dias AR**, Nahas CS, Marques CF, Nahas SC, Cecconello I. Transanal endoscopic microsurgery: indications, results and controversies. *Tech Coloproctol* 2009; **13**: 105-111 [PMID: 19484350 DOI: 10.1007/s10151-009-0466-6]
86 **Serra-Aracil X**, Mora-Lopez L, Alcantara-Moral M, Caro-Tarrago A, Gomez-Diaz CJ, Navarro-Soto S. Transanal endoscopic surgery in rectal cancer. *World J Gastroenterol* 2014; **20**: 11538-11545 [PMID: 25206260 DOI: 10.3748/wjg.v20.i33.11538]
87 **Borschitz T**, Heintz A, Junginger T. The influence of histopathologic criteria on the long-term prognosis of locally excised pT1 rectal carcinomas: results of local excision (transanal endoscopic microsurgery) and immediate reoperation. *Dis Colon Rectum* 2006; **49**: 1492-1506; discussion 1500-1505 [PMID: 16897336]
88 **Stipa F**, Burza A, Lucandri G, Ferri M, Pigazzi A, Ziparo V, Casula G, Stipa S. Outcomes for early rectal cancer managed with transanal endoscopic microsurgery: a 5-year follow-up study. *Surg Endosc* 2006; **20**: 541-545 [PMID: 16508812]
89 **Bhangu A**, Brown G, Nicholls RJ, Wong J, Darzi A, Tekkis P. Survival outcome of local excision versus radical resection of colon or rectal carcinoma: a Surveillance, Epidemiology, and End Results (SEER) population-based study. *Ann Surg* 2013; **258**: 563-569; discussion 569-571 [PMID: 23979270 DOI: 10.1097/SLA.0b013e3182a4e85a]
90 **Borschitz T**, Kneist W, Gockel I, Junginger T. Local excision for more advanced rectal tumors. *Acta Oncol* 2008; **47**: 1140-1147 [PMID: 18607868 DOI: 10.1080/02841860701829653]
91 **Levic K**, Bulut O, Hesselfeldt P, Bülow S. The outcome of rectal cancer after early salvage TME following TEM compared with primary TME: a case-matched study. *Tech Coloproctol* 2013; **17**: 397-403 [PMID: 23192705 DOI: 10.1007/s10151-012-0950-2]
92 **Hompes R**, McDonald R, Buskens C, Lindsey I, Armitage N, Hill J, Scott A, Mortensen NJ, Cunningham C. Completion surgery following transanal endoscopic microsurgery: assessment of quality and short- and long-term outcome. *Colorectal Dis* 2013; **15**: e576-e581 [PMID: 24635913 DOI: 10.1111/codi.12381]
93 **Bikhchandani J**, Ong GK, Dozois EJ, Mathis KL. Outcomes of salvage surgery for cure in patients with locally recurrent disease after local excision of rectal cancer. *Dis Colon Rectum* 2015; **58**: 283-287 [PMID: 25664705 DOI: 10.1097/DCR.0000000000000283]
94 **Duek SD**, Issa N, Hershko DD, Krausz MM. Outcome of transanal endoscopic microsurgery and adjuvant radiotherapy in patients with T2 rectal cancer. *Dis Colon Rectum* 2008; **51**: 379-384; discussion 384 [PMID: 18236108 DOI: 10.1007/s10350-007-9164-5]
95 **Ramirez JM**, Aguilella V, Valencia J, Ortego J, Gracia JA, Escudero P, Esco R, Martinez M. Transanal endoscopic microsurgery for rectal cancer. Long-term oncologic results. *Int J Colorectal Dis* 2011; **26**: 437-443 [PMID: 21271346 DOI: 10.1007/s00384-011-1132-9]
96 **Smith FM**, Chang KH, Sheahan K, Hyland J, O'Connell PR, Winter DC. The surgical significance of residual mucosal abnormalities in rectal cancer following neoadjuvant chemoradiotherapy. *Br J Surg* 2012; **99**: 993-1001 [PMID: 22351592 DOI: 10.1002/bjs.8700]
97 **Garcia-Aguilar J**. Transanal endoscopic microsurgery following neoadjuvant chemoradiation therapy in rectal cancer: a word of caution about patient selection? *Dis Colon Rectum* 2013; **56**: 1-3 [PMID: 23222272 DOI: 10.1097/DCR.0b013e318273f58c]
98 **Lezoche E**, Baldarelli M, Lezoche G, Paganini AM, Gesuita R, Guerrieri M. Randomized clinical trial of endoluminal locoregional resection versus laparoscopic total mesorectal excision for T2 rectal cancer after neoadjuvant therapy. *Br J Surg* 2012; **99**: 1211-1218 [PMID: 22864880 DOI: 10.1002/bjs.8821]
99 **Borschitz T**, Wachtlin D, Möhler M, Schmidberger H, Junginger T. Neoadjuvant chemoradiation and local excision for T2-3 rectal cancer. *Ann Surg Oncol* 2008; **15**: 712-720 [PMID: 18163173]
100 **Pucciarelli S**, De Paoli A, Guerrieri M, La Torre G, Maretto I, De Marchi F, Mantello G, Gambacorta MA, Canzonieri V, Nitti D, Valentini V, Coco C. Local excision after preoperative chemoradiotherapy for rectal cancer: results of a multicenter phase II clinical trial. *Dis Colon Rectum* 2013; **56**: 1349-1356 [PMID: 24201388 DOI: 10.1097/DCR.0b013e3182a2303e]
101 **Perez RO**, Habr-Gama A, São Julião GP, Proscurshim I, Scanavini Neto A, Gama-Rodrigues J. Transanal endoscopic microsurgery for residual rectal cancer after neoadjuvant chemoradiation therapy is associated with significant immediate pain and hospital readmission rates. *Dis Colon Rectum* 2011; **54**: 545-551 [PMID: 21471754 DOI: 10.1007/DCR.0b013e3182083b84]
102 **Barendse RM**, van den Broek FJ, Dekker E, Bemelman WA, de Graaf EJ, Fockens P, Reitsma JB. Systematic review of endoscopic mucosal resection versus transanal endoscopic microsurgery for large rectal adenomas. *Endoscopy* 2011; **43**: 941-949 [PMID: 21971923 DOI: 10.1055/s-0030-1256765]
103 **van den Broek FJ**, de Graaf EJ, Dijkgraaf MG, Reitsma JB, Haringsma J, Timmer R, Weusten BL, Gerhards MF, Consten EC, Schwartz MP, Boom MJ, Derksen EJ, Bijnen AB, Davids PH, Hoff C, van Dullemen HM, Heine GD, van der Linde K, Jansen JM, Mallant-Hent RC, Breumelhof R, Geldof H, Hardwick JC, Doornebosch PG, Depla AC, Ernst MF, van Munster IP, de Hingh IH, Schoon EJ, Bemelman WA, Fockens P, Dekker E. Transanal endoscopic microsurgery versus endoscopic mucosal resection for large rectal adenomas (TREND-study). *BMC Surg* 2009; **9**: 4 [PMID: 19284647 DOI: 10.1186/1471-2482-9-4]
104 **Farhat S**, Chaussade S, Ponchon T, Coumaros D, Charachon A, Barrioz T, Koch S, Houcke P, Cellier C, Heresbach D, Lepilliez V, Napoleon B, Bauret P, Coron E, Le Rhun M, Bichard P, Vaillant E, Calazel A, Bensoussan E, Bellon S, Mangialavori L, Robin F, Prat F. Endoscopic submucosal dissection in a European setting. A multi-

institutional report of a technique in development. *Endoscopy* 2011; **43**: 664-670 [PMID: 21623560 DOI: 10.1055/s-0030-1256413]
105 **Leong KJ**, Wei W, Tannahill LA, Caldwell GM, Jones CE, Morton DG, Matthews GM, Bach SP. Methylation profiling of rectal cancer identifies novel markers of early-stage disease. *Br J Surg* 2011; **98**: 724-734 [PMID: 21360524 DOI: 10.1002/bjs.7422]
106 **García-Flórez LJ**, Gómez-Álvarez G, Frunza AM, Barneo-Serra L, Martínez-Alonso C, Fresno-Forcelledo MF. Predictive markers of response to neoadjuvant therapy in rectal cancer. *J Surg Res* 2015; **194**: 120-126 [PMID: 25481527 DOI: 10.1016/j.jss.2014.10.005]
107 **Aly EH**. SILS TEM: The new armamentarium in transanal endoscopic surgery. *J Minim Access Surg* 2014; **10**: 102-103 [PMID: 24761089]
108 **Wolthuis AM**, de Buck van Overstraeten A, D'Hoore A. Laparoscopic natural orifice specimen extraction-colectomy: a systematic review. *World J Gastroenterol* 2014; **20**: 12981-12992 [PMID: 25278692 DOI: 10.3748/wjg.v20.i36.12981]
109 **Araujo SE**, Crawshaw B, Mendes CR, Delaney CP. Transanal total mesorectal excision: a systematic review of the experimental and clinical evidence. *Tech Coloproctol* 2015; **19**: 69-82 [PMID: 25380741 DOI: 10.1007/s10151-014-1233-x]
110 **Gómez Ruiz M**, Palazuelos CM, Martín Parra JI, Alonso Martín J, Cagigas Fernández C, del Castillo Diego J, Gómez Fleitas M. New technique of transanal proctectomy with completely robotic total mesorrectal excision for rectal cancer. *Cir Esp* 2014; **92**: 356-361 [PMID: 24589418 DOI: 10.1016/j.ciresp.2013.12.007]

P- Reviewer: Coffey JC, Furka A **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9297

World J Gastroenterol 2015 August 21; 21(31): 9297-9316
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Perspectives in the treatment of pancreatic adenocarcinoma

Angel Cid-Arregui, Victoria Juarez

Angel Cid-Arregui, German Cancer Research Center, Translational Immunology, Tumor Immunology Program, 69120 Heidelberg, Germany

Victoria Juarez, AsteriaPharma GmbH, 68723 Oftersheim, Germany

Author contributions: Cid-Arregui A analyzed the data, organized, designed and wrote the paper; Juarez V analyzed and organized the data and wrote the paper.

Conflict-of-interest statement: No conflict of interest exists regarding this paper. VJ is CEO of AsteriaPharma.

Correspondence to: Angel Cid-Arregui, MD, PhD, German Cancer Research Center, Translational Immunology, Tumor Immunology Program, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany. a.cid@dkfz.de
Telephone: +49-622-1423714

Received: February 3, 2015
Peer-review started: February 5, 2015
First decision: April 24, 2015
Revised: May 12, 2015
Accepted: July 18, 2015
Article in press: July 18, 2015
Published online: August 21, 2015

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is an incurable lethal disease whose incidence rate is growing. There is no effective screening for detection of early stage tumors and, in most cases, PDAC is diagnosed at advanced disease stages, when radical pancreatic resection is not possible. The aggressive nature of pancreatic tumor cells lies in the complex genetic mechanisms behind their uncontrolled capability to grow and metastasize, which involve essential adaptive changes in cellular metabolism, signaling, adhesion and immunoediting. In addition, PDAC cells promote a dense functional stroma that facilitates tumor resistance to chemotherapy and radiation. During the last two decades, gemcitabine has been the reference for the systemic treatment of PDAC. However, recently, a regimen combining fluorouracil, irinotecan, oxaliplatin, and leucovorin (FOLFIRINOX) and another combining albumin-bound paclitaxel with gemcitabine have shown clear therapeutic advantage in advanced PDAC, with survival outcomes of 11.3 and 8.5 mo on phase Ⅲ trials, respectively, over single-agent gemcitabine. With the pending issue of their higher toxicities, these regimens set the reference for ongoing and future clinical studies in advanced PDAC. In addition, the efficacy of oral fluoropyrimidine (S-1) has been well documented in Asiatic PDAC patients. The development of therapeutic approaches other than cytotoxic drugs has proven difficult in the past, with only one drug (erlotinib) approved to date. Besides, a number of agents targeting signaling pathways in tumor or stroma cells are being investigated. Likewise, immunotherapies that target PDAC in various ways are the subject of a number of clinical trials. The search for reliable biomarkers with diagnostic and prognostic value using genomics and mass spectrometry methods may facilitate monitoring and refinement of therapies. This review focuses on current understanding of the pathogenesis of PDAC and the latest developments in the treatment of advanced PDAC.

Key words: Pancreatic adenocarcinoma; Tumor surveillance; Biomarkers; Immune surveillance; Chemotherapy; Immunotherapy; Antibody therapy; Adoptive T cell therapy; Vaccines; Stroma; Inhibitors

Core tip: Pancreatic adenocarcinoma is a life threa-

tening, fast evolving disease for which there is no cure. Recently, new chemotherapy regimens have shown significant improvement in survival in patients with advanced disease, opening a way for further progress. New therapeutic strategies based on targeted inhibitors or immunotherapy approaches, in particular antibody and adoptive T cell therapies, are getting growing attention as they are proving beneficial in pre-clinical and early phase clinical studies in combination with chemotherapy. Progress in understanding pancreatic tumor genetics, epigenetics and metabolism is providing new biomarkers that may be of value in early detection and progression assessments.

Cid-Arregui A, Juarez V. Perspectives in the treatment of pancreatic adenocarcinoma. *World J Gastroenterol* 2015; 21(31): 9297-9316 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9297.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9297

INTRODUCTION

Over 95% of pancreatic cancers develop in the exocrine pancreas. Of these, about 95% are adenocarcinomas originating in the ducts of the pancreas. Pancreatic ductal adenocarcinoma (PDAC) is, together with renal cancer, the twelfth most frequent cancer worldwide, representing the eighth and ninth leading cause of death by cancer in men and women, respectively. In the year 2012 about 338000 new cases of PDAC were reported in the world[1]. The incidence varies across countries, ranging from 1 to 10 cases per 100000 (age standardized rate)[1]. According to the United States National Cancer Institute, the 5-year relative survival rate is about 25% for localized PDAC (stages Ⅰ and ⅡA), 9.9% for cases with regional lymph node involvement (stages ⅡB and Ⅲ) and 2.3% for metastasized PDAC (stage Ⅳ)[2]. However, only 9% of cases are diagnosed at the local stages, while 27% are detected at the regional and 53% at the distant stages, with 11% of cases unstaged[2]. Hence, the overall 5-year survival rate is in the range of 6%-10%, what makes PDAC the most lethal cancer type. Approximately 70% of deaths follow widespread metastasis while the rest have limited metastasis but extensive primary tumors. Currently, there is no effective screening for detection of early stage tumors.

ANATOMY AND HISTOPATHOLOGY

The pancreas is a 12-15-cm (6 inches) long, lobulated, retroperitoneal gland, which lies transversally behind the stomach, across the lumbar spine (L1-L2) (Figure 1), in close contact with the duodenum where the bile and pancreatic ducts drain through the major papilla (ampulla of Vater) and the pancreatic accessory duct through the minor papilla[3,4]. The wider end of the pancreas, close to the duodenum, is referred to as the head, the middle portion is called the body and the rest, called tail, extends to the hilum of the spleen. The exocrine pancreas produces the digestive enzymes and represents more than 95% of the pancreatic mass. The endocrine part (the islets of Langerhans) comprises only 1%-2% of pancreatic mass. A ductal system drains the enzymes produced by the acinar cells in a bicarbonate-rich medium secreted by the ductal cells.

At the histopathologic level, PDAC develops in a stepwise progression from low grade to high grade dysplastic lesions known as pancreatic intraepithelial neoplasia (PanIN) types 1, 2 and 3. In addition, intraductal papillary mucinous neoplasms (IPMN) are considered precursor to invasive pancreatic cancer. These tumors transform the stroma into a dense connective tissue called desmoplastic reaction[5,6], a microenvironment consisting of extracellular matrix proteins (hyaluronic acid, type Ⅰ and Ⅲ collagens and fibronectin), fibroblasts, pancreatic stellate cells (myofibroblast-like cells with tissue maintenance function), inflammatory immune cells, endothelial cells, pericytes and nerve fibers. PDAC cells stimulate the stroma and induce the desmoplastic reaction, whereas stroma cells release factors that stimulate tumor cell proliferation, escape from immune surveillance, invasiveness and resistance to therapy[7]. However, several lines of evidence coming from studies in mouse models suggest that stromal cells may also detain tumor growth directly or indirectly[8-10].

BIOLOGY OF PDAC

Molecular pathology and genomics studies have shown accumulating genetic changes, usually mutations in oncogenes and tumor suppressor genes[11]. Over 90% of PanIN of all grades and 40%-65% of IPMN carry KRAS mutations[12,13], being KRAS G12D the most common mutation[14,15]. KRAS activation is essential for pancreatic cancer cell survival[16]. Activated mutant KRAS signals primarily through the PI3K, p38, JNK and FAK signaling pathways. It also involves the epidermal growth factor receptor (EGFR), BCL-XL and the nuclear factor κB[17-20].

KRAS is thought to play a key role in reprogramming the metabolism of hypoxic pancreatic adenocarcinoma cells through activation of glucose uptake and glycolysis to yield pyruvate, which instead of being processed *via* the tricarboxylic acid cycle is converted into lactic acid[21]. Excess of lactic acid released by hypoxic cells causes local acidosis, which facilitates extracellular matrix breakdown and hence tumor invasiveness[22]. In addition, the neighboring normoxic cancer cells use the released lactate to fulfill the increased metabolic needs due to their higher proliferation rates. Indeed, these cells show increased expression of MCT1, a proton-linked monocarboxylate transporter that catalyzes the rapid transport of

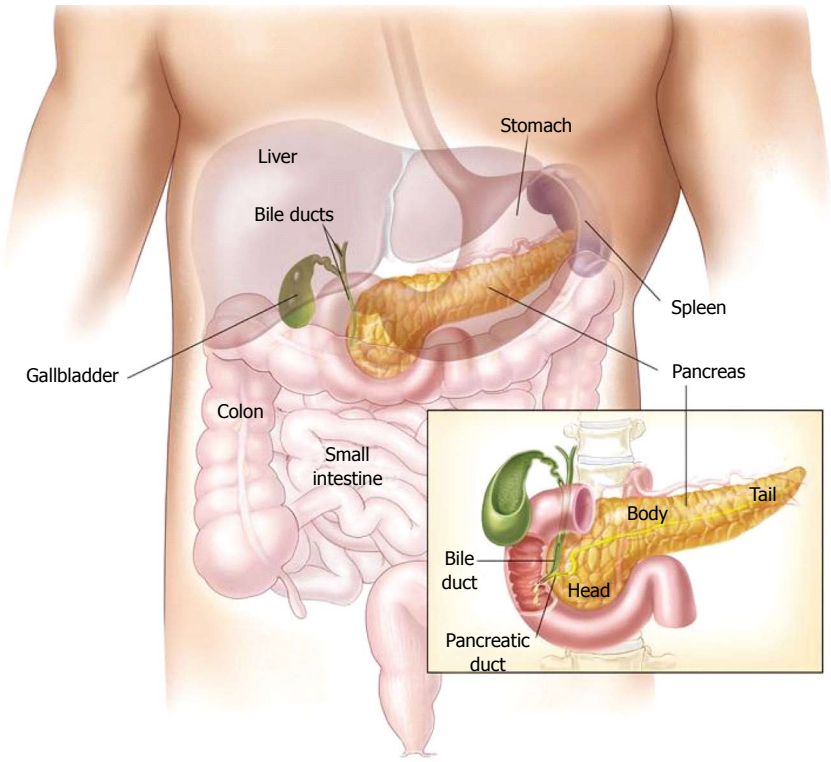


Figure 1 Anatomy of the pancreas and its location in the abdominal cavity. The inset shows the different regions the pancreas is divided into (with permission from Terese Winslow LLC Medical Illustration, for the National Cancer Institute © 2009 Terese Winslow US Gov. has certain rights).

lactate, pyruvate and other monocarboxylates across the plasma membrane[23]. Moreover, KRAS activates glutamine metabolism to yield glutamate and α-ketoglutarate, thus enhancing citrate synthesis and the tricarboxylic acid cycle, *i.e.*, a glucose-independent metabolic pathway; generates NADPH, a cofactor in anabolic reactions and an antioxidant[24], and promotes *de novo* lipogenesis through the isocitrate dehydrogenase (IDH1 and 2)[25,26].

Besides KRAS activation, mutations inactivating tumor suppressor genes accumulate during progression from PanIN1 to PanIN3. Mutational inactivation of p53 is detected in 60%-70% of PDAC, and mutations in CDKN2A (involved in G1 cell cycle arrest) and in members of the TGF-β signaling pathway (most frequently SMAD4, TGF-β1 and TGF-β2) in about 50% of cases[27]. In 10%-15% of cases, exome sequencing has revealed loss-of-function mutations in genes involved in nucleosome remodeling (ARID1A, ARID1B, SMARCA1), responses to DNA damage (ATM, BRCA2) and histone methylation (MLL2, MLL3, KDM6A). It has been estimated that genetic predisposition is present in 5%-10% of PDAC cases (familial PDAC) and several susceptibility genes have been identified. For example, inherited mutations in the gene STK11 cause the Peutz-Jeghers syndrome, and these patients have 130-fold increased risk of PDAC; germline mutations in the *p16/CDKN2A* gene cause the familial atypical multiple mole melanoma (FAMMM) syndrome, which is associated with a 13 to 37-fold increased risk of PDAC; mutations in BCRA2 cause familial breast cancer and increase the risk of PDAC 3.5-fold (reviewed by Hruban *et al*[28]). In addition, as a consequence of genetic changes, cytology studies have shown frequent chromosomal alterations in PDAC such as deletions and rearrangements leading to aneuploidy. For instance, the gene CLPTM1L, which is overexpressed in PDAC as compared with normal pancreatic tissue and has been identified by GWAS (Genome-Wide Association Studies) among the PDAC susceptibility alleles on chromosome 5p15.33, has been shown to interfere with normal cytokinesis and induce aneuploidy *in vitro*[29]. Furthermore, an extensive multistage GWAS of 7683 patients diagnosed with pancreatic cancer and 14397 control individuals identified multiple loci associated with pancreatic cancer, which harbor genes associated with cancer, such as *LINK-PIN, BCAR1/CTRB1/CTRB2, PDX1, ZNRF1, TERT and PVT1*[30]. These studies illustrate a more accurate concept of genetic risk of PDAC.

TUMOR IMMUNE SURVEILLANCE

Inflammatory cells (mainly lymphocytes, plasma cells, macrophages and mast cells) are components of the desmoplastic reaction that forms the microenvironment of PDAC. However, rather than fighting the tumor these cells seem to promote an inflammatory microenvironment that helps tumor cells escape from immune surveillance *via* paracrine cross-talk mechanisms[31]. Indeed, studies have shown that chronic pancreatitis increases the risk of developing

Table 1 Controlled trials in adjuvant chemoradiotherapy for pancreatic ductal adenocarcinoma

Study name	No. of patients enrolled	Regimen	Survival rate	*P* value	Ref.
EORTC	207	5-FU + radiotherapy	34% (2 yr)	0.099	[72]
		Observation	26% (2 yr)		
ESPAC-1	289	5-FU + radiotherapy	10% (5 yr)	-	[73]
		Observation or 5-FU only	20% (5 yr)		
RTOG 9704	451	Gemcitabine + radiotherapy	18% (5 yr)	0.120	[147]
		5-FU + radiotherapy	22% (5 yr)		

EORTC: European Organization for Research and Treatment of Cancer; ESPAC-1: European Study Group for Pancreatic Cancer 1; RTOG 9704: Radiation Therapy Oncology Group 9704; 5-FU: 5-fluoruracil.

pancreatic adenocarcinoma, specially in smokers[32], and that subjects with hereditary pancreatitis caused by mutations in the gene PRSS1 have a significantly increased relative and absolute risk of developing PDAC[33]. Escape from antitumor immunity seems to be linked to KRAS activation, since it has been shown that already in early PanIN stages KRAS G12D induces production and release of GM-CSF[34], which attracts $Gr1^{+}CD11b^{+}$ myeloid suppressor cells to the tumor stroma[34] as well as immunosuppressive regulatory T cells[35,36]. Furthermore, the serum levels of some proinflammatory cytokines, such as IL-6, IL-8, IL-10 and IL-1 receptor antagonist (IL-1RA) are increased in PDAC patients and correlate with tumor aggressiveness[37]. IL-6 signals through the signal transducer and activator of transcription 3 (STAT3), which plays an essential role in the development of most PDAC cases[38]. Studies in mice have shown that tumor infiltrating macrophages release IL-6 in the stroma activating STAT3 and promoting progression from PanIN to PDAC[39]. In yet another mouse model of human PDAC it was shown that a subset of stromal fibroblasts expressing fibroblast activation protein (FAP) release the chemokine CXC-motif ligand 12 (CXCL12), which may facilitate immunosuppression by impeding the contact of T cells with the adenocarcinoma cells. T cell accumulation nearby tumor cells could be restored using an antagonist of CXCR4[40], a cognate receptor for CXCL12, which acted synergistically with the anti-programmed cell dead 1 ligand 1 (PD-L1) antibody[41] to induce tumor regression in a mouse model of PDAC[42].

BIOMARKERS AND TUMOR SURVEILLANCE

To date there is no effective preventive screening for detection of early stage tumors. Further, there is no consensus on the suitability of current biomarkers to predict tumor progression or recurrence. Carbohydrate antigen 19-9 (CA19-9), a sialylated Lewis antigen identified in a colon cancer cell line[43], is the biomarker currently used in the surveillance of PDAC. Its expression depends on the Lewis blood antigens; therefore, 5%-10% of Caucasian patients that are Lewis negative (*a*-/*b*-) do not express CA19-9. Because its expression is not limited to PDAC, CA19-9 cannot be used as screening marker. However, it has been used as prognostic marker after surgical resection[44,45], and some studies suggest that it may be used to predict resectability and outcome after adjuvant chemotherapy[46,47]. Looking for biomarkers linked with survival or response, in the RTOG 9704 study (Table 1), an adjuvant therapy trial comparing 5-FU with gemcitabine chemotherapy administered before and after 5-FU-based chemoradiation in patients with resected PDAC, a probe panel of antibodies was used to detect and quantify 42 proteins. As already shown in previous studies, lower levels of CEA (carcinoembryonic antigen) and CA19-9 were associated with improved overall survival in all patients, whereas low levels of matrix metalloproteinases (MMP)-7 were linked to improved overall survival in the adjuvant gemcitabine arm, but not in the 5-FU arm, suggesting that PDAC patients with low MMP-7 expression levels benefit from gemcitabine rather than 5-FU adjuvant therapy[48].

The protein SMAD4 has been also suggested as prognostic marker. Immunohistochemistry characterization of the expression of SMAD4 in a series of 249 tumors from PDAC patients who underwent pancreaticoduodenectomy showed that cases with SMAD4 expression had significantly longer survival: median survival of 19.2 mo compared with 14.7 mo in patients whose tumors did not express SMAD4 ($P = 0.03$)[49]. Moreover, analysis of SMAD4 (DPC4) expression in a series of rapid autopsies in patients with documented PDAC showed loss of SMAD4 expression in 41 of 65 tumors analyzed, indicating a rate of inactivation of 63%[50]. Interestingly, loss of SMAD4 expression was seen in only 2 (22%) of 9 locally advanced carcinomas, but in 16 (73%) of 22 metastatic carcinomas ($P = 0.007$)[50]. In a prospective phase Ⅱ trial enrolling 69 patients with locally advanced carcinoma who were treated with gemcitabine/oxaliplatin and cetuximab followed by chemoradiotherapy plus cetuximab, 11 (73.3%) of 15 patients with intact SMAD4 expression had a local dominant pattern of progression, whereas 10 (71.4%) of 14 patients with SMAD4 loss had distant dominant pattern of spread ($P = 0.016$)[51], indicating that SMAD4 loss significantly correlated with distant

Table 2 Controlled trials in adjuvant chemotherapy for pancreatic ductal adenocarcinoma

Study name	No. of patients enrolled	Regimen	Survival rate	*P* value	Ref.
CONKO-001	368	Gemcitabine	20.7% (5 yr)	0.01	[145]
		Observation	10.4% (5 yr)		
ESPAC-3	1088	Gemcitabine	23.6 mo	0.39	[146]
		5-FU + Leucovorin	23.0 mo		
JASPAC 01	385	Gemcitabine	53%	0.0001	[64]
		S-1	70%		

CONKO-001: Charité Onkologie 001; ESPAC-3: European Study Group for Pancreatic Cancer 3; JASPAC 01: Japan Adjuvant Study Group of Pancreatic Cancer 01; 5-FU: 5-fluoruracil.

rather than local dominant pattern of spread. In yet another study, retrospective analysis of 471 who had resected PDAC showed that loss of SMAD4 expression did not correlate with recurrence but was predictive for adjuvant chemotherapy benefit ($P = 0.002$)[52]. This study also showed that high expression of the CXCR4 chemokine receptor was significantly associated with worse outcome ($P < 0.0001$) as well as with metastatic recurrence ($P < 0.001$)[52].

Another biomarker that was expected to have prognostic relevance is the human equilibrative nucleoside transporter-1 (hENT1). Gemcitabine requires hENT1 to cross the cell membrane. Therefore, low expression of hENT1 might result in gemcitabine resistance in PDAC. This hypothesis was tested in a randomized clinical trial comparing gemcitabine with gemcitabine elaidate (CO-101), an unsaturated fatty acid ester derivative of gemcitabine, which was designed to enter the cells by diffusion independently of hENT1[53]. This study enrolled 367 patients. The expression of hENT1 was measured in 253, of whom 232 (64.8%) were classified as low-hENT1. It was found no difference in median overall survival between the low and high hENT1 subgroups, indicating that hENT1 status is not relevant and cannot be used to predict gemcitabine outcome.

A recent study has shown that elevated levels of branched-chain amino acids (BCAA) in plasma are associated with an increased risk (> 2.0) of developing PDAC[54]. The highest association was observed in subjects whose samples were collected 2-5 years before PDAC diagnosis, suggesting that early-stage subclinical disease was already present. Such increase in plasma BCAA seems due to breakdown of tissue proteins that would occur early in the development of PDAC[54]. Interestingly, in a mouse model of PDAC, elevated BCAA levels were detected also in mice with early stage pancreatic tumors harboring mutant KRAS, but not in mice with KRAS-driven tumors in other tissues.

The predictive value of other biomarkers, such as ULBP2, a ligand of the natural killer activating receptor NKG2D, and the macrophage inhibitory cytokine-1 (MIC-1) are currently being evaluated in case-control studies[55]. Non-mutated, overexpressed proteins, such as CLPTM1L[29] and DKK-1[56], if validated in terms of prognostic value could serve also as biomarkers. Furthermore, the prognostic value of detection of circulating tumor cells is being evaluated in a prospective study with 79 patients with locally advanced PDAC[57].

MANAGEMENT

A widely used staging system for PDAC is that of the American Joint Committee of Cancer and the Union for International Cancer Control, which is based on the TNM classification[58]. From the management perspective PDAC is divided into three categories: (1) localized surgically resectable tumors; (2) unresectable locally advanced tumors; and (3) metastatic tumors. In between the first and second groups are tumors called borderline resectable, which need to be carefully evaluated as candidates for surgery according to the involvement of adjacent organs and vessels (celiac artery, hepatic artery, portal vein, superior mesenteric artery and vein)[59]. The most frequent location of PDAC is the head of the pancreas (60%-70%), which requires pancreaticoduodenectomy (Whipple procedure). Extensive surgery does not provide better outcomes[60,61]. Tumors of the tail of the pancreas are nowadays resected laparoscopically. Overall, only 15%-20% of newly diagnosed patients are eligible for surgical resection. The five-year survival rates after pancreaticoduodenectomy range from 25%-30% for lymph node-negative and 10% for node-positive cases[62].

Stages Ⅰ and Ⅱ surgically resectable PDAC

In PDAC stage Ⅰ the tumor is restricted to the pancreas and does not involve neither adjacent organs or vessels nor regional lymph nodes. The treatment of choice is surgical removal of all recognizable tumor tissue. However, tumor recurrence occurs in 60%-70% of stage Ⅰ patients due to micrometastases during or after surgical resection. Systemic therapy (chemotherapy, chemoradiation) administered after surgery (called adjuvant therapy) improves survival rates and, eventually, the chances of cure. In some cases chemotherapy, chemoradiation or combination of therapies is applied before surgery (the so-called neoadjuvant therapy) to shrink the tumor and prevent

post-surgical micrometastases and relapse.

Adjuvant therapy in localized resectable PDAC: A number of randomized trials enrolling patients with T1-4 N0-1 M0 PDAC have demonstrated that, following surgical resection of the tumor mass, adjuvant chemotherapy for 6 mo either with gemcitabine or 5-fluoruracil (5-FU) increases overall survival significantly compared with observation (Table 2, see also Goodman *et al*[63], 2014). These studies showed an improvement in the 5-year survival rate from approximately 10% (observation) to approximately 20% (adjuvant therapy) with no significant difference between gemcitabine and 5-FU (Table 2). However, the patients treated with gemcitabine experienced significantly less toxicity and had improved clinical benefit. More recently, in a multicenter, randomized phase Ⅲ study in Japan (JASPAC 01) gemcitabine was compared with S-1 in the adjuvant treatment of patients after curative resection[64]. S-1 is an oral fluoropyrimidine prodrug (tegafur) that is converted by the enzyme dihydropyrimidine dehydrogenase (DPD) into 5-FU[65] (see also Stage Ⅳ below), The study enrolled 385 patients with ECOG performance status 0-1 and with compensated organ functions; of these, 378 were included in the final analysis. The 2-year overall survival rates were 53% for gemcitabine and 70% for S-1 ($P < 0.0001$). Also, the quality of life was significantly higher in the S-1 arm ($P < 0.0001$). The results of the JASPAC-01 study suggest that S-1 may be an effective alternative to the adjuvant chemotherapy with gemcitabine in resected PDAC. However, these studies have been conducted in Asian patients, mostly Japanese and it is uncertain whether S-1 would be as effective in Western patients. Some important aspects remain to be investigated. For instance, the expression levels of cytochrome P450 A26, the enzyme that converts tegafur into 5-FU in the liver, seem to be higher in Caucasian than in Japanese subjects[66]. In addition, gastrointestinal toxicities of grades 3/4 are more common in Caucasians than in Asians[67]. An ongoing multicenter, randomized phase Ⅲ study, the European Study Group for Pancreatic Cancer trial 4 (ESPAC-4), will compare the combination gemcitabine plus capecitabine (another fluoropyrimidine prodrug that is converted into 5-FU by DPD, the same enzyme that converts tegafur) with gemcitabine alone when used as adjuvant therapy after PDAC resection[68]. This study will serve to determine the utility of the addition of a fluoropyrimidine to gemcitabine in the post-resection adjuvant therapy in non-Asian PDAC patients. Nevertheless, previous randomized trials in Western metastatic PDAC patients have not shown superiority for such combination using gemcitabine combined with a variety of oral and bolus fluoropyrimidine regimens[69-71]. Two ongoing clinical studies (CONKO 005 and RTOG 0848) should determine the benefit of combining erlotinib (EGFR inhibitor) with gemcitabine.

The efficacy of adjuvant chemoradiation therapy is still subject of controversy, since two European studies showed no benefit in adding radiation to the adjuvant therapy[72] or even showed detrimental effects[73]. Such studies (Table 1) have been questioned for different reasons, the EORTC because it included patients with pancreatic head carcinomas as well as periampullary tumors (with possible better prognosis), and the ESPAC-1 because of the complexity of its design. Further studies should clarify if adjuvant chemoradiation may be beneficial. Thus, a phase Ⅱ-R/Ⅲ randomized trial ongoing in the United States (NCT01013649), which should be completed in the year 2020. Nevertheless, the impact of chemoradiation therapy on overall survival after pancreaticoduodenectomy was evaluated in a multicenter retrospective study reviewing 955 patients (classified as T1-4; N0-1; M0) who underwent complete resection (R0-1) and showed macroscopically negative margins[74]. Of these, 623 received postoperative radiation, 575 received concurrent chemotherapy, and 462 received adjuvant chemotherapy. Median follow-up was 21.0 mo. Median overall survival was 39.9 mo for patients treated with chemoradiation compared with 24.8 mo for those not receiving chemoradiation ($P < 0.001$), and 27.8 mo for patients treated only with adjuvant chemotherapy ($P < 0.001$). In the population treated with adjuvant chemoradiation (with or without chemotherapy) 5-year overall survival was 41.2% compared with 25.7% in patients treated with adjuvant chemotherapy alone[74]. Therefore, according to this retrospective study, adjuvant chemoradiation was beneficial in terms of overall survival.

Neoadjuvant therapy in borderline resectable PDAC: The high frequency of disease recurrence and the low survival rates associated with surgical resection of pancreatic adenocarcinomas, usually attributed to residual tumor cells left at the surgical margins and to involvement of lymph nodes, led to the evaluation of neoadjuvant (preoperative) chemotherapy with or without radiotherapy. Several studies evaluating different neoadjuvant therapy protocols have evidenced the limited success of this approach as compared with the outcomes of patients with resectable tumors treated with adjuvant (postoperative) therapy[75-77]. Nevertheless, to date there are no controlled, prospective studies comparing neoadjuvant and surgery-first approaches.

Currently, there is consensus that neoadjuvant therapy does not benefit patients with resectable PDAC, and that it is beneficial in tumors with borderline resectability to improve the probability of tumor-free resection margins and in locally advanced, non-resectable tumors to reduce their extension and make them resectable[78,79]. Further, neoadjuvant

chemotherapy applied to borderline resectable patients may help identify a subset of patients that would not benefit from surgery. In a study enrolling 160 borderline resectable patients selected out of 2454 PDAC cases, 125 (78%) completed neoadjuvant therapy (chemotherapy, radiotherapy or both) and restaging. Of these 66 (41%) underwent surgery. Median survival was 40 mo for the 66 patients who completed all therapy and 13 mo for the 94 patients who did not undergo surgery[80]. Consistent and objective definitions of borderline resectable and unresectable PDAC are needed for ongoing and future studies to be sufficiently powered, so that the efficacy of neoadjuvant therapy can be clearly established[79].

In the past, combinations of gemcitabine and chemoradiation with 5-FU have had limited efficacy. In a large meta-analysis including retrospective and prospective studies, Gillen *et al*[76] analyzed 111 trials (n = 4394) showed that in the group of initially resectable tumor patients, approximately 81% of patients receiving monotherapy underwent resection, in contrast, among those receiving combination chemotherapy the number of resections was significantly lower (approximately 66%). However, a comparison of tumor response frequencies in patients treated with mono chemotherapy (n = 44) *vs* combination chemotherapy (n = 48) showed complete and partial responses of 2.2% and 25.8% *vs* 5.3% and 34.7%, respectively[76].

Although at present there is no optimal protocol for neoadjuvant chemotherapy, there is hope in multidrug chemotherapy approaches such as nab-paclitaxel (albumin bound paclitaxel) followed by gemcitabine, or the multiagent regimen FOLFIRINOX (leucovorin, 5-FU, irinotecan, oxaliplatin), which has been shown in a retrospective study to induce conversion to resectability in > 20% of locally advanced PDAC patients[81]. Nevertheless, the toxicity associated with these regimens and the relatively elevated recurrence rate observed after R0 resection[81] make necessary more prospective studies to establish approaches that may be beneficial in the neoadjuvant setting. There are at least two ongoing phase Ⅱ trials using fluorouracil, irinotecan, oxaliplatin, and leucovorin (FOLFIRINOX) plus radiation therapy in borderline resectable PDAC patients (NCT01560949 and NCT01591733).

Stage Ⅲ locally advanced, unresectable PDAC

Approximately 30%-40% of newly diagnosed PDAC cases are classified as stage Ⅲ: locally advanced, non-resectable, non-metastatic, with involvement of major blood vessels and regional lymph nodes. Because of the poor response rates observed with the different therapeutic approaches, management of these patients remains controversial. A frequent option has been upfront chemotherapy with 2-3 cycles of gemcitabine followed by restaging and, in favorable cases, chemoradiation. In the meta-analysis by Gillen *et al*[76], 107 of the 111 selected studies applied chemoradiation to non-resectable locally advanced PDAC, in most cases (54%) with 5-FU, and less frequently with gemcitabine (22%). About 33% of patients with primarily unresectable, locally advanced PDAC turned into resectable cases and the overall survival was 20.5 mo (median), comparable to that of patients with primarily resectable tumors and in contrast to 10.5 mo in those with non-resectable tumors. Although other meta-analyses drew comparable results, the general feeling is that chemoradiation has more toxicity than gemcitabine alone and increases the rate of perioperative risk[82,83]. A recent study compared chemoradiation and chemotherapy after four months of gemcitabine (with or without erlotinib, an EGFR inhibitor) in locally advanced PDAC patients (LAP 07 study, NCT00634725). The study conclusion was that chemoradiation after induction chemotherapy is not superior to continuing chemotherapy in patients with controlled locally advanced PDAC. Median follow-up was 36 mo and overall survival was 16.5 mo for the patients randomized to continue chemotherapy compared with 15.3 mo for patients receiving chemoradiation (P = 0.83)[84].

The improvements in overall survival observed in patients with metastatic PDAC treated with FOLFIRINOX or gemcitabine/nab-paclitaxel multidrug regimens (see below) has led to investigate them in locally advanced PDAC. Their efficacy and toxicities in locally advanced PDAC remain to be determined[81,85]. In their retrospective institutional study, Faris *et al*[81] reported 22 patients with locally advanced PDAC who received treatment with FOLFIRINOX. Median progression free survival was 11.7 mo. Five patients (23%) underwent R0 resection following neoadjuvant FOLFIRINOX and chemoradiation. Of these, three suffered distant recurrence within six months. The high rate of recurrence and the toxicities (non-neutropenic fever, dehydration), observed in 32% of the patients, demonstrate the complexity to find an adequate therapy for locally advanced PDAC patients.

Stage Ⅳ metastatic PDAC

In PDAC stage Ⅳ metastasis have spread to adjacent (stage Ⅳa) or distant organs (stage Ⅳb), such as liver, stomach, spleen or lungs. Surgical removal is not possible, although palliative surgery may be an option. Gemcitabine has been the standard chemotherapy agent since 1997, when it was shown to improve overall survival of advanced PDAC patients compared to 5-FU, with survival rates at 12 mo of 18% and 2%, respectively[86]. Ever since, different gemcitabine-based combinations, for example with irinotecan[87], oxaliplatin[88] or bevacizumab[89], have been investigated in randomized trials in comparison with standard gemcitabine. Most of them failed to improve overall survival rates, yet at least three combinations have shown beneficial effects: gemcitabine plus

erlotinib, gemcitabine plus S-1 and gemcitabine plus nab-paclitaxel. In addition, a new non-gemcitabine-based multidrug regimen (FOLFIRINOX) has revealed as a clear improvement in the therapy of metastatic PDAC.

Gemcitabine plus erlotinib: A regimen of gemcitabine plus erlotinib, a reversible tyrosine kinase inhibitor of EGFR, demonstrated certain benefit in a phase Ⅲ trial (NCI PA.3) enrolling 569 patients[90]. The overall survival rate at 1-year was 23% in the gemcitabine plus erlotinib arm and 17% in the standard gemcitabine arm (P = 0.023). Median overall survival was 6.2 and 5.9 mo, respectively (P = 0.038). However, this slight improvement with the erlotinib regimen was accompanied with higher toxicities (rash, infections, diarrhea, *etc*.) and even 6 deaths, all in the erlotinib arm. The FDA approved this protocol in the year 2005.

Gemcitabine plus S-1: The synergistic cytotoxic effects of gemcitabine and 5-FU against pancreatic adenocarcinoma cells described previously[91,92] led to investigate the combination of gemcitabine and S-1 in clinical trials. S-1 is an oral multiagent formulation with three components: tegafur (a 5-FU prodrug), gimeracil and oteracil at 1:04:1 molar ratio[93]. Gimeracil is a reversible inhibitor of dihydropyrimidine dehydrogenase, a major 5-FU catabolizing enzyme. Oteracil inhibits the phosphoribosyltransferase that phosphorylates 5-FU, and it is intended to reduce the gastrointestinal toxicity of 5-FU. Several randomized phase Ⅲ studies in gastrointestinal cancer patients have shown non-inferiority of S-1 *vs* standard 5-FU infusion regimens[94-96]. Several phase Ⅱ studies in patients with metastatic PDAC treated with gemcitabine/S-1 combinations showed median overall survival rates ranging from 7.89 to 12.5 mo[65]. The combination of gemcitabine plus S-1 was compared with S-1 alone and gemcitabine alone in a recent randomized phase Ⅲ study (GEST) enrolling 834 patients with locally advanced or metastatic PDAC in Japan and Taiwan[97]. The GEST study showed a median overall survival of 8.8, 9.7 and 10.1 mo in the gemcitabine, S-1 and gemcitabine/S-1 arms, respectively. The study did not demonstrate superiority of gemcitabine/S-1 to gemcitabine alone (P = 0.15), but showed non-inferiority (P < 0.001). However, as mentioned above, S-1 trials have been performed only in Asian patients[65,98] and, therefore, further studies are required to determine whether S-1 has the same efficacy in Western PDAC patients. Although previous studies in Western patients with metastatic PDAC did not show superiority of combinations of gemcitabine plus fluoropyrimidines administered in various forms[69-71], it cannot be excluded that S-1 does.

Gemcitabine plus nab-paclitaxel: The first clinical trial with nab-paclitaxel and gemcitabine was a multicenter open label phase Ⅰ/Ⅱ study enrolling 67 patients with PDAC, of whom 44 received the maximum tolerated dose. In this group the 1-year survival rate was 48%, and the median overall survival was 12.2 mo[92]. In preclinical studies, Von Hoff *et al*[92] showed also that, in a mouse xenograft model of PDAC, the intratumoral concentration of gemcitabine was 2.8-fold increased in the mice treated with nab-paclitaxel plus gemcitabine compared with mice treated only with gemcitabine, suggestive of a synergistic effect of these two drugs. nab-Paclitaxel is an albumin-bound formulation of paclitaxel in the form of 130 nm particles, which is administered intravenously as a colloidal suspension. The combination of nab-paclitaxel with gemcitabine in the treatment of advanced PDAC was based on the finding that these tumors overexpress the secreted protein acidic and rich in cysteine (SPARC), an albumin-binding protein, and the fact that nab-paclitaxel had shown efficacy in tumors overexpressing SPARC, such as breast[99,100], melanoma[101] and lung[102] tumors. Further, it has been shown in cultured cells and in a mouse model of PDAC that paclitaxel reduces the levels of cytidine deaminase (major gemcitabine inactivating enzyme) in tumor cells[103] what might explain the higher levels of intratumoral gemcitabine. In addition, it was shown that while nab-paclitaxel accumulation in the murine tumors was dependent on SPARC at low doses, at therapeutic doses it was SPARC independent[104]. On the basis of their previous study, Von Hoff *et al*[92] carried out a multicenter, open label, randomized phase Ⅲ trial enrolling 861 patients with metastatic PDAC to compare nab-paclitaxel plus gemcitabine (431 patients) with standard gemcitabine (430 patients). Median overall survival was 8.5 mo in the nab-paclitaxel/gemcitabine group and 6.7 mo in the gemcitabine group (P < 0.001). The 1-year survival rate was 35% and 22%, respectively, and the 2-year survival was 9% and 4%, respectively. Adverse events of grade 3 or higher, such as myelosuppression and peripheral neuropathy were increased in the combined nab-paclitaxel plus gemcitabine arm.

FOLFIRINOX: Besides the gemcitabine-based chemotherapies, in the last years a multiagent chemotherapy regimen (FOLFIRINOX) has emerged as an effective strategy with significantly higher efficiency compared to standard single-agent gemcitabine in a randomized, multicenter phase Ⅱ/Ⅲ study[105]. The FOLFIRINOX regimen consists of a combination of four intravenously (iv) administered drugs: oxaliplatin 85 mg/m^2 2 h infusion, followed by leucovorin (calcium folinate) 400 mg/m^2 2 h infusion, irinotecan 180 mg/m^2 90 min. infusion, followed by 5-FU mg/m^2 bolus, followed by 5-FU 2400 mg/m^2 infusion over 46 hours, every two weeks. A six-month treatment was recommended for responding patients[105]. This study

included 342 patients with metastatic PDAC that had not been treated with chemotherapy. The median overall survival was 11.1 mo in the FOLFIRINOX group and 6.8 mo in the gemcitabine group ($P < 0.001$). Overall survival rates at 6, 12 and 18 mo were 75.9%, 48.4% and 18.6% in the FOLFIRINOX group and 57.6%, 20.6% and 6.0% in the gemcitabine group. The median progression-free survival was 6.4 mo in the FOLFIRINOX group and 3.3 mo in the gemcitabine group ($P < 0.001$). Definitive deterioration in the quality of life at 6 mo was observed in 31% of patients in the FOLFIRINOX and in 66% in the gemcitabine group.

A relevant concern of this therapy is its increased toxicity, which has been subject of some controversy. Several reports informed of substantial toxicities, such as grade 3 or 4 neutropenia, febrile neutropenia (cause of one treatment-related dead), thrombocytopenia, diarrhea and sensory neuropathy, which were significantly more frequent than with single-agent gemcitabine therapy[105]. In a multicenter study in 61 PDAC patients, 21 (34.4%) were hospitalized as a result of therapy and 23 (37.7%) had discontinued therapy due to adverse events[106]. In a retrospective study including 22 patients, it was reported that toxicities required hospitalization in 7 cases (32%)[81]. Nevertheless, a retrospective study reviewing toxicity and efficacy in 35 patients (16 with locally advanced and 19 with metastatic PDAC), of whom 29 received a modified FOLFIRINOX regimen (attenuation of irinotecan and 5-FU bolus) showed that such regimen improved tolerability with no significant reduction in efficacy[107]. Furthermore, in an attempt to ameliorate the toxicities, a prospective study was conducted using a modified FOLFIRINOX regimen (no bolus 5-FU and addition of hematopoietic growth factor)[108]. This study enrolled a total of 60 PDAC patients, 24 (40%) with non-metastatic and 36 (60%) with metastatic PDAC. It was found that the modified FOLFIRINOX regimen maintained efficacy, whereas the safety profile was improved with significantly less grade 3-4 toxicities. Additional studies should help improve safety and efficacy of FOLFIRINOX by further refinement of regimens.

Another important issue is related to the eligibility criteria for FOLFIRINOX therapy. In a retrospective study reviewing 100 consecutive cases of metastatic PDAC it was found that only 26 patients fulfilled the ACCORD study eligibility criteria, being the most frequent reasons for FOLFIRINOX exclusion ECOG score of 2 or greater (64%), age (≥ 76 years) (22%) and liver and/or renal dysfunction (28%)[109].

EMERGENT THERAPIES

Treatment of PDAC has been restricted for the most part to chemotherapy (cytotoxic) drugs. A number of agents targeting specific proteins in tumor or stroma cells to interfere with their function have shown promise in preclinical studies over the last decade. Of these, only erlotinib (EGFR inhibitor) reached regulatory approval on the basis of some benefit shown for its combination with gemcitabine in a phase Ⅲ trial (see above under *Management*). Reasons for the slow progress in this field might be the complex genetic mechanisms taking place in the pancreatic tumor cells, which favor resistance to cytotoxic as well as targeted agents, and the intricate tumor microenvironment that seems to protect the tumor through a discrete vessel network and a hypoxic milieu. Likewise, immunotherapy approaches may find difficulty in entering the stroma and reaching the tumor cells.

Inhibitors

PARP inhibitors: PDAC tumors harboring germline mutations in the *BRCA1 or BRCA2* genes are highly sensitive to Poly[ADP-ribose] polymerase (PARP) inhibitors[110]. Several PARP inhibitors, such as olaparib, are being tested in clinical trials. A recent multicenter phase Ⅱ study enrolled 298 patients with recurrent ovarian, breast, pancreatic or prostate cancer, harboring a germline BRCA1/2 mutation, who were treated with olaparib (400 mg twice per day)[111]. The overall response rate was 26.2% (78 of 298) and in the subgroup of PDAC patients (treated previously with gemcitabine) the response rate was 21.7 (5 of 23) and stable disease for 8 or more weeks was observed in 35% of the PDAC patients. Adverse events of grade 3 or higher were reported for 35% of patients, the most frequent being anemia (17%). Nine patients died as a result of adverse events. This was a single arm study and, therefore, it is not possible to compare with other therapies. Ongoing studies will determine whether treatment with olaparib or other PARP inhibitors may be an alternative to FOLFIRINOX in patients with germline BRCA1/2 mutations.

Inhibitors of MMP: Tumor growth and metastasis involve the breakdown of tissue stroma. MMPs are a family of about 30 zinc-dependent proteinases that for the most part degrade the extracellular matrix, thereby facilitating tumor growth and metastasis. This was the rationale to investigate the efficacy of MMP inhibitors in cancer therapy. An oral MMP inhibitor, marimastat, was compared with gemcitabine in a randomized study enrolling 414 patients with unresectable pancreatic cancer[112]. The survival rate for patients receiving marimastat 25 mg was similar to that of patients receiving gemcitabine. In a parallel study, 239 patients with unresectable pancreatic cancer were randomized to receive gemcitabine plus either marimastat 10 mg b.i.d or placebo[113]. There was no significant difference in overall survival between ge mcitabine plus marimastat and gemcitabine plus placebo ($P = 0.95$). These studies

provided little support to the utility of MMP inhibitors in therapy of advanced PDAC and, therefore, were discontinued.

Inhibitors of VEGF: Several studies demonstrated a close correlation between vascular endothelial growth factor (VEGF) expression and microvessel density (MDV) in PDAC[114,115]. In addition, VEGF appeared to have predictive value for liver metastasis and poor prognosis[115], and also for early recurrence after curative resection[114]. An oral inhibitor of VEGF receptors, axitinib, was tested in a randomized, placebo-controlled phase Ⅱ study enrolling 103 patients with unresectable or metastatic PDAC. The patients were divided into two groups for treatment with gemcitabine with or without axitinib. Median overall survival was 6.9 and 5.6 mo, respectively[116]. The study was continued with a phase Ⅲ trial including 632 patients[117]. However, an interim analysis concluded that the study was unsuccessful and it was terminated abruptly.

Hedgehog inhibitors: The hedgehog (Hh) pathway is thought to contribute to the growth of a number of tumors of endodermal origin, including PDAC. In a mouse model of PDAC the use of saridegib, an inhibitor of the Hh pathway, in combination with gemcitabine resulted in depletion of desmoplastic stroma, increased delivery of gemcitabine to tumor cells and a statistically significant survival improvement of tumor-bearing mice[118]. A randomized, double-blind, placebo controlled phase Ⅱ trial showed worse median survival for the saridegib plus gemcitabine arm compared to the gemcitabine plus placebo arm, and the study was discontinued[119]. In a randomized, placebo controlled, phase Ⅰb/Ⅱ trial vismodegib, another Hh pathway inhibitor, administered with gemcitabine failed to improve progression-free or overall survival rates compared to gemcitabine alone[120]. At least five more trials are ongoing.

Other inhibitors: Masitinib, an inhibitor of c-kit and platelet-derived growth factor receptor (PDGFR) kinases, was tested in combination with gemcitabine in a phase Ⅱ trial enrolling 22 patients with unresectable locally advanced or metastatic PDAC. The median overall survival was 8.4 and 6.8 mo for locally advanced or metastatic patients, respectively[121]. A phase Ⅲ of this study comparing gemcitabine plus masitinib with gemcitabine plus placebo, showed no improvement in overall survival. Rigosertib, a phosphatidylinositol-3-kinase (PI3K) inhibitor, showed certain activity in a phase Ⅰ trial and is under study in a multicenter, randomized phase Ⅱ trial (NCT01360853).

Immunotherapies

Advances in the understanding of the mechanisms regulating cellular immune responses and immunosurveillance are leading to improved immunotherapy approaches applicable to cancer treatment. Immunotherapy approaches have the potential to assist the patient's immune system to eliminate metastatic tumor cells and residual tumor after pancreatic resection. Some of these have shown success in early clinical trials in PDAC patients. A representative list of immunotherapy approaches currently under study in clinical trials is summarized in Table 3 distributed into the following major groups: monoclonal antibodies (as checkpoint immunomodulators, inhibitors of signaling pathways or as cytotoxicity inducers), adoptive T cell therapy, vaccines, cytokines and adjuvants.

Monoclonal antibodies as checkpoint immunomodulators, signaling pathway inhibitors or cytotoxicity inducers: The incorporation of monoclonal antibodies to the therapeutic regimens of certain types of cancer has become established over the past two decades. The therapeutic activity of monoclonal antibodies can result from: (1) their ability to activate cellular immune responses against tumor antigens; (2) through agonist or antagonist effects on their target proteins; or (3) conjugated to cytotoxic agents, killing selectively tumor cells[122].

Activation, proliferation and differentiation of T cells in response to antigens is regulated by co-stimulatory and co-inhibitory receptors and their ligands, which modulate the signaling pathways triggered by the interaction of T cell receptors with the major histocompatibility complex (MHC)[123]. The immune system uses co-inhibitory signals to maintain self-tolerance and impair deleterious immune reactions by inducing T cell exhaustion or apoptosis. Some co-inhibitory molecules of particular relevance are programmed cell death protein-1 (PD-1), PD-1 ligands 1 and 2 (PD-L1 and 2), cytotoxic T lymphocyte antigen-4 (CTLA-4) and lymphocyte-activation gene 3 (LAG-3). It has become apparent that tumors develop mechanisms to interfere with some immune checkpoint pathways and thus escape from cytotoxic T cell responses triggered by tumor antigens[124]. Monoclonal antibodies targeting PD-1, PD-L1 and CTLA-4 (so called checkpoint blockade, reviewed by Postow *et al*[125]) have been shown in recent clinical trials to promote endogenous antitumor immune activity[126-128]. Table 3 summarizes ongoing clinical trials including patients with advanced PDAC to test PD-1, PD-L1, CTLA-4 and LAG-3. Some of these studies investigate the potential synergistic effects of combining immune checkpoint inhibitors among them and with other therapeutic agents (Table 3). An additional study targets 4-1BB/CD137, a member of the TNF receptor superfamily, which is a potent $CD8^+$ T cell co-stimulatory molecule. There is compelling evidence indicating that anti-4-1BB monoclonal antibodies have antitumor properties[129].

Monoclonal antibodies targeting ERBB family members (*e.g.*, EGFR) and VEGF (vascular endothelial

Table 3 Summary of major ongoing clinical trials evaluating immunotherapy approaches in patients with solid tumors including advanced or metastatic pancreatic adenocarcinoma

Study ID	Sponsor(s)	Therapeutic products	Phase	ClinicalTrials.gov Identifier
Monoclonal antibodies as checkpoint immunomodulators				
CD-ON-MEDI4736-1108	MedImmune LLC	MEDI4736 (monoclonal antibody anti-B7 homolog1; PD-L1)	Ⅰ / Ⅱ	NCT01693562
GO27831	Genentech Inc.	MPDL3280A (human, Fc optimized monoclonal anti-PD-L1 antibody)	Ⅰ	NCT01375842
3475-028	Merck Sharp and Dohme Corp	Pembrolizumab (humanized monoclonal anti-PD-1 antibody)	Ⅰ B	NCT02054806
GP28328	Genentech, Inc.	MPDL3280A (human, Fc optimized monoclonal anti-PD-L1 antibody) plus bevacizumab (anti-VEGF antibody) and/or chemotherapy	Ⅰ	NCT01633970
11-C-0100	Georgia Regents University Cancer Center	CT-011 (pidilizumab, humanized monoclonal antibody anti-PD-1) plus gemcitabine	Ⅱ	NCT01313416
CA209-032	Bristol-Myers Squibb	Nivolumab (fully human monoclonal anti-PD-1 antibody) plus ipilimumab (anti-CTLA-4 monoclonal antibody)	Ⅰ / Ⅱ	NCT01928394
CA223-001	Bristol-Myers Squibb	Lirilumab (fully humanized monoclonal anti-KIR2DL1/2L3 antibody) plus nivolumab (anti-PD-1 antibody)	Ⅰ	NCT01714739
NU 10I02	Northwestern University and Robert H. Lurie Cancer Center	Ipilimumab (anti-CTLA-4 monoclonal antibody) plus gemcitabine	Ⅰ	NCT01473940
NCI-2013-00030	M.D. Anderson Cancer Center	Ipilimumab (anti-CTLA-4 monoclonal antibody) plus imatinib	Ⅰ	NCT01738139
CA224-020	Bristol-Myers Squibb	BMS-986016 (anti-LAG-3 antibody) with or without nivolumab (anti-PD-1 antibody)	Ⅰ	NCT01968109
CA186-011	Bristol-Myers Squibb	Urelumab (BMS-663513, humanized agonistic monoclonal anti-4-1BB/CD137)	Ⅰ	NCT01471210
B1641001	Pfizer	PF-05082566 (4-1BB humanized agonist monoclonal antibody) plus rituximab (anti-CD20)	Ⅰ	NCT01307267
Monoclonal antibodies as signaling pathway inhibitors				
59R5-002	OncoMed Pharmaceuticals, Inc.	OMP-59R5 (anti-Notch2/3) plus chemotherapy	Ⅰ / Ⅱ	NCT01647828
52M51-002	OncoMed Pharmaceuticals, Inc. and GlaxoSmithKline	OMP-52M51 (anti-Notch 1 monoclonal antibody)	Ⅰ	NCT01778439
MORAb-066-001	Morphotek and SCRI Development Innovations, LLC	MORAb-066: anti-human TF (tissue factor, CD142) monoclonal antibody	Ⅰ	NCT01761240
NCI-2012-01702	Morphotek and National Cancer Institute (NCI)	Ontuxizumab (MORAb-004): monoclonal antibody anti-endosialin/TEM1 (CD248)	Ⅰ	NCT01748721
R1400-ST-1113	Regeneron Pharmaceuticals	REGN1400 (anti-ErbB3) with or without erlotinib or cetuximab	Ⅰ	NCT01727869
MM-151-01-01-01	Merrimack Pharmaceuticals	MM-151 [oligoclonal antibody composed of three fully human monoclonal antibodies anti-EGFR (ErbB1)] alone and with irinotecan	Ⅰ	NCT01520389
M12-375	AbbVie (prior sponsor, Abbott)	ABT-700 [monoclonal antibody anti-c-Met human growth factor receptor (HGFR) as monotherapy, or with chemotherapy (FOLFIR/cetuximab) or with erlotinib	Ⅰ	NCT01472016
UPCC-04206	University of Pennsylvania and NCI	Gemcitabine, oxaliplatin and bevacizumab followed by 5-fluorouracil, oxaliplatin, bevacizumab and radiotherapy in patients with locally advanced pancreatic cancer	Ⅱ	NCT00602602
Monoclonal antibodies as cytotoxicity inducers				
Neogenix 0901	Precision Biologics, Inc	Ensituximab (NPC-1C/NEO-102) (anti-MUC5AC-related antigen)	Ⅰ / Ⅱ	NCT01040000
CEP-37250/KHK2804-001	Kyowa Hakko Kirin Pharma, Inc.	CEP-37250/KHK2804 (monoclonal antibody targeting glycolipids on the surface of tumor cells)	Ⅰ	NCT01447732
IMMU-107-04	Immunomedics, Inc.	IMMU-107: radioimmunoconjugate of the humanized monoclonal antibody HuPAM4 (anti-MUC1), plus a chelating agent (DOTA) and radiolabeled with Yttrium Y90	Ⅲ	NCT01956812
Therapeutic vaccines				
NLG0505	NewLink Genetics Corporation	Algenpantucel-L Immunotherapy: 2 pancreatic cancer cell lines (HAPa-1 and HAPa-2) expressing murine alpha-gal carbohydrates on cell surface molecules, in combination with standard therapy, compared with standard therapy.	Ⅲ	NCT01836432
11-C-0148	NCI	Epigenetically modified autologous tumor cells with ISCOMATRIX Adjuvant plus chemotherapy	Ⅰ	NCT01341496
ADU-CL-04	Aduro BioTech, Inc.	GVAX (allogeneic GM-CSF transfected pancreatic tumor vaccine) plus CRS-207 (attenuated *L. monocytogenes* expressing mesothelin)	Ⅱ	NCT02004262
J13108	Sidney Kimmel CCC	GVAX (allogeneic GM-CSF transfected pancreatic tumor vaccine) plus ipilimumab compared with FOLFIRINOX	Ⅱ	NCT01896869
JHOC-J0810	Sidney Kimmel CCC and NCI	GVAX (allogeneic GM-CSF transfected pancreatic tumor vaccine) plus cyclophosphamide	NP	NCT00727441
NCI-2012-01548	Jonsson Comprehensive Cancer Center	NY-ESO-1 (cancer-testis antigen) reactive TCR retroviral vector transduced autologous PBL	Ⅱ	NCT01697527

NWBio 050012	Northwest Biotherapeutics	DCVax-Direct (autologous activated dendritic cells)	I / II	NCT01882946
ONT-10-001	Oncothyreon Inc.	ONT-10 (liposomal MUC1 cancer vaccine)	I	NCT01556789
J1179	Sidney Kimmel Comprehensive Cancer Center	PANC 10.05 pcDNA-1/GM-Neo and PANC 6.03 pcDNA-1/GM-Neo vaccine, plus cyclophosphamide followed by SBRT (fractionated stereotactic body radiation therapy) and FOLFIRINOX chemotherapy	NP	NCT01595321
101778	Medical University of South Carolina	Poly-ICLC (ligand for toll like receptor) and dendritic cells, plus standard chemotherapy	0	NCT01677962
I 191511	Roswell Park Cancer Institute	DEC-205-NY-ESO-1 (cancer-testis antigen) fusion protein vaccine	I	NCT01522820
Adoptive T cell therapy				
UPCC 08212	Abramson Cancer Center of the University of Pennsylvania	Autologous T cells transfected with chimeric anti-mesothelin immunoreceptor SS1, plus chemotherapy	I	NCT01897415
UPCC 31213	Abramson Cancer Center of the University of Pennsylvania	CART-meso (autologous T cells lentivirally transduced with chimeric anti-mesothelin immunoreceptor SS1 fused to the 4-1BB and CD3ζ signaling domains)	I	NCT02159716
12-C-0111	NCI	Anti-mesothelin CAR plus chemotherapy and aldesleukin	I / II	NCT01583686
10-C-0166	NCI	Young tumor infiltrating lymphocytes (TILs), plus chemotherapy and aldesleukin	II	NCT01174121
13-C-0214	NCI	Anti-NY ESO-1 mTCR PBL (autologous white blood cells genetically modified with a retrovirus expressing the gene for anti-ESO-1), plus chemotherapy and aldesleukin	II	NCT01967823
14-C-0052	NCI	Anti-MAGE-A3-DP4 TCR (autologous T cells genetically modified with a retrovirus expressing the gene for anti-MAGE-A3-DP0401/0402), plus chemotherapy and aldesleukin	I / II	NCT02111850
11-C-0013	NCI	Anti-VEGFR2 CAR: Autologous $CD8^+$ T cells genetically modified with a retrovirus expressing the gene for anti-VEGFR2, plus chemotherapy and aldesleukin	I / II	NCT01218867
RWH 111-32	Roger Williams Medical Center	EGFRBi-armed autologous activated T cells, loaded with a bispecific antibody produced by heteroconjugation of anti-CD3 and anti-EGFR monoclonal antibodies	I	NCT01081808
Adjuvant immunotherapies and cytokines				
NCI-2011-03565	Roswell Park Cancer Institute, NCI and Cleveland BioLabs Inc.	Entolimod (CBLB502, recombinant Toll-like receptor 5 agonist)	I	NCT01527136
AM0010-001	ARMO BioSciences	AM0010 (pegylated recombinant human interleukin-10) in combination with chemotherapy	I	NCT02009449

growth factor) and VEGFR (VEGF receptor) have been most successful in patients with solid tumors. However, the application of some of these antibodies to patients with advanced PDAC has been disappointing. In a phase Ⅲ study enrolling 745 patients with advanced or metastatic PDAC, cetuximab, a monoclonal antibody against the ligand-binding domain of EGFR, was administered combined with gemcitabine in comparison with single-agent gemcitabine. No significant difference in median overall survival was observed between both arms (6.3 mo *vs* 5.9 mo, respectively)[130]. Likewise, the combination of cetuximab with gemcitabine in the adjuvant treatment of 73 patients with resected (R0-R1) PDAC was reported recently not to improve overall survival[131]. A study in patients with advanced solid tumors including PDAC is currently recruiting patients for testing a combination of three anti-EGFR (ERBB1) monoclonal antibodies, and another trial with an anti-ERBB2 antibody combined with cetuximab is also open (Table 3).

Bevacizumab (anti-VEGF) was tested in 52 patients with previously untreated advanced PDAC in a single arm, multicenter phase Ⅱ trial. The antibody was administered after gemcitabine treatment. Median overall survival was 8.8 mo and partial responses were observed in 21% of cases[132]. Although the results were not significantly better than those previously reported for gemcitabine, the study moved on to a phase Ⅲ trial of gemcitabine/bevacizumab *vs* gemcitabine/placebo in 535 advanced pancreatic cancer patients. No difference in median overall survival (5.8 and 5.9 mo) was observed[89]. These results were congruent with the lack of effect reported for axitinib (see above *Inhibitors*).

In addition, new studies have been initiated to test monoclonal antibodies against Notch, tissue factor (TF, CD142), tumor endothelial marker 1 (TEM1, endosialin) and human growth factor receptor (HGFR), which also include PDAC patients (Table 3). Furthermore, ongoing studies will determine the benefit in advanced PDAC patients of antibodies against MUC5AC, which has been shown to inhibit TRAIL-induced apoptosis in PDAC cells[133], MUC1, overexpressed in over 60% of PDAC and inducer of drug resistance in PDAC cells[134].

Therapeutic vaccines: Therapeutic vaccines are designed to stimulate the immune system to react against tumor-specific antigens, essentially by inducing specific cytotoxic T cells. These vaccines may be made of whole cells, proteins, peptides or DNA encoding tumor antigens. Several vaccines have been tested in

early clinical studies during the last years and some of them have shown discrete improvement in survival. Clinical trials with vaccines currently in progress are summarized in Table 3.

Algenpantucel-L is a vaccine designed to treat PDAC that has reached phase Ⅲ. It consists of 2 irradiated allogeneic pancreatic cancer cell lines (HAPa-1 and HAPa-2) transfected to express murine α-1,3-galactosyltransferase. These cells carry α-1,3-galactosyl (α-gal) carbohydrates on cell surface glycoproteins and glycolipids, which trigger a rejection of the vaccine cell allograft, to which also contributes the fact that they are recognized by pre-existing anti-α-gal antibodies (naturally occurring against gut flora), resulting in opsonization and lysis of the vaccine cells and hence processing and presentation of tumor antigens by host antigen presenting cells. It follows a T cell response against endogenous tumor cells. This vaccine was tested in a phase Ⅱ trial (multicenter, open-label) enrolling 70 patients with resected (R0-1) PDAC who received Algenpantucel-L in addition to standard adjuvant gemcitabine chemotherapy and chemoradiation[135]. Median follow-up was 21 mo, 1-year median overall survival was 86% and disease-free survival was 62% in the same period. It was concluded that the vaccine added to the adjuvant setting may improve survival and a phase Ⅲ trial is ongoing (Table 3).

Another whole-cell vaccine consisting of irradiated cells stably transfected to express GM-CSF (granulocyte-macrophage colony-stimulating factor) was tested in a phase Ⅱ trial in 60 patients with resected PDAC in combination with 5-FU-based chemoradiation therapy, with four additional immunizations[136]. The vaccine was well tolerated, median disease-free survival was 17.3 mo and 1-year overall survival was 85%, close to published data for resected PDAC treated with standard adjuvant therapy. The vaccine induced mesothelin-specific $CD8^+$ T cells in HLA-A1 and HLA-A2 patients, correlating with longer disease-free survival. The GVAX-pancreas vaccine (GM-CSF-secreting allogenic pancreatic tumor cells) was tested recently in 90 patients with metastatic PDAC. GVAX was administered with low-dose cyclophosphamide (Cy/GVAX) to inhibit regulatory T cells and was combined or not with CRS-207, a live-attenuated Listeria monocytogenes expressing mesothelin, in a prime/boost vaccination setting: Cy/GVAX followed by CRS-207 (arm A) compared with Cy/GVAX alone (arm B)[137]. Overall survival was 6.1 mo in arm A and 3.9 mo in arm B (P = 0.02). Patients who received 3 or more doses of vaccine survived longer (9.7 and 4.6 mo for arms A and B, respectively). Higher mesothelin-specific $CD8^+$ T-cell responses were associated with longer overall survival.

G17DT is an immunogen that induces neutralizing antibodies against the hormone gastrin-17. It was first tested in a randomized, double-blind, placebo controlled multicenter trial in 154 patients with advanced PDAC unsuitable for or unwilling to take chemotherapy[138]. The patients received five doses of the vaccine. The primary end point was survival. Patients who developed anti-G17DT survived longer (median survival 151 d) than non-responders (63 d) or those on placebo (83 d) (P = 0.03). The studies with this vaccine in PDAC patients were discontinued, although a phase Ⅲ trial was registered (NCT02118077), and a recent phase Ⅱ study in colorectal cancer patients has been published[139].

Peptide vaccines, although well tolerated, appear to be less promising. A peptide vaccine against mutant KRAS (codon 12) was tested in 24 positive of 62 patients with resected PDAC analyzed for mutations in codon 12 of KRAS[140]. Only 9 patients were evaluable for immunologic responses, and of these only one showed specific response to the patient's KRAS mutation (assessed by delayed-type hypersensitivity). Median recurrence free survival time was 8.6 mo. No relationship was observed between immune response and clinical outcome. A personalized peptide vaccine administered to advanced PDAC patients in combination with standard gemcitabine[141] induced IgG responses in 14 of 36 patients. Median overall survival was 7.9 mo and 1-year overall survival rate was 26.8%. These results show a low benefit of the vaccine.

Adoptive T cell therapies: Adoptive cell transfer (ACT) therapies are designed to provide the patient with a highly amplified number of autologous tumor-specific cytotoxic T cells. To this end, tumor-specific T lymphocytes are isolated from the patient, expanded *ex vivo* and infused back into the patient's bloodstream. There are several forms of anti-tumor ACT. One is based on the isolation and culture of tumor infiltrating lymphocytes (TIL) followed by selection of tumor-specific T cell clones and their expansion to obtain large numbers of cells that are infused back to the patient together with interleukin-2 (IL-2) (Aldesleukin) to stimulate their proliferation. Because the immune system and the tumor microenvironment contain regulatory $CD4^+$ T cells that may prevent the transferred cells from functioning effectively, the patients are treated with chemotherapy agents, frequently with cyclophosphamide. The method can be improved by isolating T cells from the blood of patients that have received previously a cancer vaccine, which facilitates the expansion of tumor-reacting T cells.

However, in general the number of tumor-specific T cells in the TIL population results insufficient for therapeutic purposes. One strategy to overcome this problem involves transducing TIL isolated from a patient's tumor with a retroviral vector to express a tumor-targeting T cell receptor (TCR) and expanding them in culture before re-infusing the cells back to the patient. This strategy has been shown to shrink tumors in patients with melanoma and synovial cell sarcoma

in patients refractory to standard treatment[142]. The pre-condition for the treatment is that the TCR must be genetically matched to the patient's immune type.

Another approach is based on chimeric antigen receptors (CAR)[143]. A CAR is an artificial molecule engineered to contain three pieces: an extracellular antibody-derived domain that binds a tumor surface antigen, the intracellular domain of the CD3 ζ chain (signal transmitter of endogenous TCR) and, linked to it, one or more stimulatory domains. The main advantage of this approach is the high affinity interaction of the CAR with the tumor antigen, which is independent of the MHC[144].

Table 3 shows a representative list of ongoing clinical studies applying ACT therapies using TIL and CAR approaches designed for advanced solid tumors including PDAC. In most cases, ACT is combined with chemotherapy, except in the RWH111-32 study, which uses only a bispecific antibody (anti-CD-3 and anti-EGFR).

CONCLUSION

In spite of the large number of preclinical and clinical studies focused on the improvement of existing therapies and the development of new therapeutic strategies, pancreatic adenocarcinoma remains an incurable lethal disease. After two decades of gemcitabine as standard reference, recent improvements in the chemotherapy of advanced disease have set a new standard, which is being evaluated also in patients with resectable and locally advanced tumors. In parallel, new developments in the immunotherapy field, in particular those based on antibodies or adoptive cell transfer, are already showing promising results in early phase clinical trials, in general in combination with chemotherapy. Furthermore, improvement in the understanding of the genetic and epigenetic changes taking place in tumor cells and stroma during progression to advanced PDAC, and their effects on tumor metabolism and immunoediting, may be of great value to identify better biomarkers that help in earlier detection of tumors and also in therapeutic decisions, in particular in adjuvant and neoadjuvant treatments. New developments in the fields of inhibitors, monoclonal antibodies and adoptive T cell transfer are expected to have a high impact in the treatment of pancreatic adenocarcinoma in coming years.

ACKNOWLEDGMENTS

We thank R. Eritja (PhD) and A. Aviño (PhD) for stimulating discussions.

REFERENCES

1 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

2 Surveillance Research Program, NCI. SEER Stat Fact Sheets: Pancreas Cancer. Available from: URL: http://seer.cancer.gov/statfacts/html/pancreas.html 2015

3 **Bockman DE**. Anatomy of the Pancreas. In: Go V, DiMagno E, Gardner J, Lebenthal E, Reber H, Scheele G, editors. The Pancreas: Biology, Pathobiology, and Disease, 2nd Ed. New York: Raven Press Ltd., 1993: 1-8

4 **Longnecker DS**. Anatomy and Histology of the Pancreas. The Pancreapedia. Exocrine Pancreas Knowledge Base. Longnecker: Daniel, 2014

5 **Feig C**, Gopinathan A, Neesse A, Chan DS, Cook N, Tuveson DA. The pancreas cancer microenvironment. *Clin Cancer Res* 2012; **18**: 4266-4276 [PMID: 22896693 DOI: 10.1158/1078-0432.CCR-11-3114]

6 **Rasheed ZA**, Matsui W, Maitra A. Pathology of pancreatic stroma in PDAC. In: Grippo PJ, Munshi HG, editors. Pancreatic Cancer and Tumor Microenvironment. Trivandrum (India): Transworld Research Network, 2012

7 **Neesse A**, Frese KK, Bapiro TE, Nakagawa T, Sternlicht MD, Seeley TW, Pilarsky C, Jodrell DI, Spong SM, Tuveson DA. CTGF antagonism with mAb FG-3019 enhances chemotherapy response without increasing drug delivery in murine ductal pancreas cancer. *Proc Natl Acad Sci USA* 2013; **110**: 12325-12330 [PMID: 23836645 DOI: 10.1073/pnas.1300415110]

8 **Lee JJ**, Perera RM, Wang H, Wu DC, Liu XS, Han S, Fitamant J, Jones PD, Ghanta KS, Kawano S, Nagle JM, Deshpande V, Boucher Y, Kato T, Chen JK, Willmann JK, Bardeesy N, Beachy PA. Stromal response to Hedgehog signaling restrains pancreatic cancer progression. *Proc Natl Acad Sci USA* 2014; **111**: E3091-E3100 [PMID: 25024225 DOI: 10.1073/pnas.1411679111]

9 **Özdemir BC**, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, Simpson TR, Laklai H, Sugimoto H, Kahlert C, Novitskiy SV, De Jesus-Acosta A, Sharma P, Heidari P, Mahmood U, Chin L, Moses HL, Weaver VM, Maitra A, Allison JP, LeBleu VS, Kalluri R. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell* 2014; **25**: 719-734 [PMID: 24856586 DOI: 10.1016/j.ccr.2014.04.005]

10 **Rhim AD**, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, Sastra SA, Dekleva EN, Saunders T, Becerra CP, Tattersall IW, Westphalen CB, Kitajewski J, Fernandez-Barrena MG, Fernandez-Zapico ME, Iacobuzio-Donahue C, Olive KP, Stanger BZ. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell* 2014; **25**: 735-747 [PMID: 24856585 DOI: 10.1016/j.ccr.2014.04.021]

11 **Jones S**, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; **321**: 1801-1806 [PMID: 18772397 DOI: 10.1126/science.1164368]

12 **Kanda M**, Matthaei H, Wu J, Hong SM, Yu J, Borges M, Hruban RH, Maitra A, Kinzler K, Vogelstein B, Goggins M. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. *Gastroenterology* 2012; **142**: 730-733.e9 [PMID: 22226782 DOI: 10.1053/j.gastro.2011.12.042]

13 **Wu J**, Matthaei H, Maitra A, Dal Molin M, Wood LD, Eshleman JR, Goggins M, Canto MI, Schulick RD, Edil BH, Wolfgang CL, Klein AP, Diaz LA, Allen PJ, Schmidt CM, Kinzler KW, Papadopoulos N, Hruban RH, Vogelstein B. Recurrent GNAS mutations define an unexpected pathway for pancreatic cyst development. *Sci Transl Med* 2011; **3**: 92ra66 [PMID: 21775669 DOI: 10.1126/scitranslmed.3002543]

14 **Fernández-Medarde A**, Santos E. Ras in cancer and developmental diseases. *Genes Cancer* 2011; **2**: 344-358 [PMID: 21779504 DOI: 10.1177/1947601911411084]

15 **Kim ST**, Lim do H, Jang KT, Lim T, Lee J, Choi YL, Jang HL, Yi JH, Baek KK, Park SH, Park YS, Lim HY, Kang WK, Park JO. Impact of KRAS mutations on clinical outcomes in pancreatic cancer patients treated with first-line gemcitabine-based chemotherapy. *Mol Cancer Ther* 2011; **10**: 1993-1999 [PMID: 21862683 DOI: 10.1158/1535-7163.MCT-11-0269]

16 **Eser S**, Schnieke A, Schneider G, Saur D. Oncogenic KRAS signalling in pancreatic cancer. *Br J Cancer* 2014; **111**: 817-822 [PMID: 24755884 DOI: 10.1038/bjc.2014.215]

17 **Ardito CM**, Grüner BM, Takeuchi KK, Lubeseder-Martellato C, Teichmann N, Mazur PK, Delgiorno KE, Carpenter ES, Halbrook CJ, Hall JC, Pal D, Briel T, Herner A, Trajkovic-Arsic M, Sipos B, Liou GY, Storz P, Murray NR, Threadgill DW, Sibilia M, Washington MK, Wilson CL, Schmid RM, Raines EW, Crawford HC, Siveke JT. EGF receptor is required for KRAS-induced pancreatic tumorigenesis. *Cancer Cell* 2012; **22**: 304-317 [PMID: 22975374 DOI: 10.1016/j.ccr.2012.07.024]

18 **Corcoran RB**, Cheng KA, Hata AN, Faber AC, Ebi H, Coffee EM, Greninger P, Brown RD, Godfrey JT, Cohoon TJ, Song Y, Lifshits E, Hung KE, Shioda T, Dias-Santagata D, Singh A, Settleman J, Benes CH, Mino-Kenudson M, Wong KK, Engelman JA. Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. *Cancer Cell* 2013; **23**: 121-128 [PMID: 23245996 DOI: 10.1016/j.ccr.2012.11.007]

19 **Ling J**, Kang Y, Zhao R, Xia Q, Lee DF, Chang Z, Li J, Peng B, Fleming JB, Wang H, Liu J, Lemischka IR, Hung MC, Chiao PJ. KrasG12D-induced IKK2/β/NF-κB activation by IL-1α and p62 feedforward loops is required for development of pancreatic ductal adenocarcinoma. *Cancer Cell* 2012; **21**: 105-120 [PMID: 22264792 DOI: 10.1016/j.ccr.2011.12.006]

20 **Navas C**, Hernández-Porras I, Schuhmacher AJ, Sibilia M, Guerra C, Barbacid M. EGF receptor signaling is essential for k-ras oncogene-driven pancreatic ductal adenocarcinoma. *Cancer Cell* 2012; **22**: 318-330 [PMID: 22975375 DOI: 10.1016/j.ccr.2012.08.001]

21 **Ying H**, Kimmelman AC, Lyssiotis CA, Hua S, Chu GC, Fletcher-Sananikone E, Locasale JW, Son J, Zhang H, Coloff JL, Yan H, Wang W, Chen S, Viale A, Zheng H, Paik JH, Lim C, Guimaraes AR, Martin ES, Chang J, Hezel AF, Perry SR, Hu J, Gan B, Xiao Y, Asara JM, Weissleder R, Wang YA, Chin L, Cantley LC, DePinho RA. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell* 2012; **149**: 656-670 [PMID: 22541435 DOI: 10.1016/j.cell.2012.01.058]

22 **Chiche J**, Brahimi-Horn MC, Pouysségur J. Tumour hypoxia induces a metabolic shift causing acidosis: a common feature in cancer. *J Cell Mol Med* 2010; **14**: 771-794 [PMID: 20015196 DOI: 10.1111/j.1582-4934.2009.00994.x]

23 **Guillaumond F**, Leca J, Olivares O, Lavaut MN, Vidal N, Berthezène P, Dusetti NJ, Loncle C, Calvo E, Turrini O, Iovanna JL, Tomasini R, Vasseur S. Strengthened glycolysis under hypoxia supports tumor symbiosis and hexosamine biosynthesis in pancreatic adenocarcinoma. *Proc Natl Acad Sci USA* 2013; **110**: 3919-3924 [PMID: 23407165 DOI: 10.1073/pnas.1219555110]

24 **Son J**, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M, Perera RM, Ferrone CR, Mullarky E, Shyh-Chang N, Kang Y, Fleming JB, Bardeesy N, Asara JM, Haigis MC, DePinho RA, Cantley LC, Kimmelman AC. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* 2013; **496**: 101-105 [PMID: 23535601 DOI: 10.1038/nature12040]

25 **Metallo CM**, Gameiro PA, Bell EL, Mattaini KR, Yang J, Hiller K, Jewell CM, Johnson ZR, Irvine DJ, Guarente L, Kelleher JK, Vander Heiden MG, Iliopoulos O, Stephanopoulos G. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* 2012; **481**: 380-384 [PMID: 22101433 DOI: 10.1038/nature10602]

26 **Wise DR**, Ward PS, Shay JE, Cross JR, Gruber JJ, Sachdeva UM, Platt JM, DeMatteo RG, Simon MC, Thompson CB. Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α-ketoglutarate to citrate to support cell growth and viability. *Proc Natl Acad Sci USA* 2011; **108**: 19611-19616 [PMID: 22106302 DOI: 10.1073/pnas.1117773108]

27 **Hustinx SR**, Leoni LM, Yeo CJ, Brown PN, Goggins M, Kern SE, Hruban RH, Maitra A. Concordant loss of MTAP and p16/CDKN2A expression in pancreatic intraepithelial neoplasia: evidence of homozygous deletion in a noninvasive precursor lesion. *Mod Pathol* 2005; **18**: 959-963 [PMID: 15832197 DOI: 10.1038/modpathol.3800377]

28 **Hruban RH**, Canto MI, Goggins M, Schulick R, Klein AP. Update on familial pancreatic cancer. *Adv Surg* 2010; **44**: 293-311 [PMID: 20919528]

29 **Jia J**, Bosley AD, Thompson A, Hoskins JW, Cheuk A, Collins I, Parikh H, Xiao Z, Ylaya K, Dzyadyk M, Cozen W, Hernandez BY, Lynch CF, Loncarek J, Altekruse SF, Zhang L, Westlake CJ, Factor VM, Thorgeirsson S, Bamlet WR, Hewitt SM, Petersen GM, Andresson T, Amundadottir LT. CLPTM1L promotes growth and enhances aneuploidy in pancreatic cancer cells. *Cancer Res* 2014; **74**: 2785-2795 [PMID: 24648346 DOI: 10.1158/0008-5472.CAN-13-3176]

30 **Wolpin BM**, Rizzato C, Kraft P, Kooperberg C, Petersen GM, Wang Z, Arslan AA, Beane-Freeman L, Bracci PM, Buring J, Canzian F, Duell EJ, Gallinger S, Giles GG, Goodman GE, Goodman PJ, Jacobs EJ, Kamineni A, Klein AP, Kolonel LN, Kulke MH, Li D, Malats N, Olson SH, Risch HA, Sesso HD, Visvanathan K, White E, Zheng W, Abnet CC, Albanes D, Andreotti G, Austin MA, Barfield R, Basso D, Berndt SI, Boutron-Ruault MC, Brotzman M, Büchler MW, Bueno-de-Mesquita HB, Bugert P, Burdette L, Campa D, Caporaso NE, Capurso G, Chung C, Cotterchio M, Costello E, Elena J, Funel N, Gaziano JM, Giese NA, Giovannucci EL, Goggins M, Gorman MJ, Gross M, Haiman CA, Hassan M, Helzlsouer KJ, Henderson BE, Holly EA, Hu N, Hunter DJ, Innocenti F, Jenab M, Kaaks R, Key TJ, Khaw KT, Klein EA, Kogevinas M, Krogh V, Kupcinskas J, Kurtz RC, LaCroix A, Landi MT, Landi S, Le Marchand L, Mambrini A, Mannisto S, Milne RL, Nakamura Y, Oberg AL, Owzar K, Patel AV, Peeters PH, Peters U, Pezzilli R, Piepoli A, Porta M, Real FX, Riboli E, Rothman N, Scarpa A, Shu XO, Silverman DT, Soucek P, Sund M, Talar-Wojnarowska R, Taylor PR, Theodoropoulos GE, Thornquist M, Tjønneland A, Tobias GS, Trichopoulos D, Vodicka P, Wactawski-Wende J, Wentzensen N, Wu C, Yu H, Yu K, Zeleniuch-Jacquotte A, Hoover R, Hartge P, Fuchs C, Chanock SJ, Stolzenberg-Solomon RS, Amundadottir LT. Genome-wide association study identifies multiple susceptibility loci for pancreatic cancer. *Nat Genet* 2014; **46**: 994-1000 [PMID: 25086665 DOI: 10.1038/ng.3052]

31 **Vonderheide RH**, Bayne LJ. Inflammatory networks and immune surveillance of pancreatic carcinoma. *Curr Opin Immunol* 2013; **25**: 200-205 [PMID: 23422836 DOI: 10.1016/j.coi.2013.01.006]

32 **Lowenfels AB**, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, Dimagno EP, Andrén-Sandberg A, Domellöf L. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med* 1993; **328**: 1433-1437 [PMID: 8479461 DOI: 10.1056/nejm199305203282001]

33 **Rebours V**, Boutron-Ruault MC, Schnee M, Férec C, Maire F, Hammel P, Ruszniewski P, Lévy P. Risk of pancreatic adenocarcinoma in patients with hereditary pancreatitis: a national exhaustive series. *Am J Gastroenterol* 2008; **103**: 111-119 [PMID: 18184119 DOI: 10.1111/j.1572-0241.2007.01597.x]

34 **Pylayeva-Gupta Y**, Lee KE, Hajdu CH, Miller G, Bar-Sagi D. Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. *Cancer Cell* 2012; **21**: 836-847 [PMID: 22698407 DOI: 10.1016/j.ccr.2012.04.024]

35 **De Monte L**, Reni M, Tassi E, Clavenna D, Papa I, Recalde H, Braga M, Di Carlo V, Doglioni C, Protti MP. Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer. *J Exp Med* 2011; **208**: 469-478 [PMID:

21339327 DOI: 10.1084/jem.20101876]
36 **Tang Y**, Xu X, Guo S, Zhang C, Tang Y, Tian Y, Ni B, Lu B, Wang H. An increased abundance of tumor-infiltrating regulatory T cells is correlated with the progression and prognosis of pancreatic ductal adenocarcinoma. *PLoS One* 2014; **9**: e91551 [PMID: 24637664 DOI: 10.1371/journal.pone.0091551]
37 **Ebrahimi B**, Tucker SL, Li D, Abbruzzese JL, Kurzrock R. Cytokines in pancreatic carcinoma: correlation with phenotypic characteristics and prognosis. *Cancer* 2004; **101**: 2727-2736 [PMID: 15526319 DOI: 10.1002/cncr.20672]
38 **Corcoran RB**, Contino G, Deshpande V, Tzatsos A, Conrad C, Benes CH, Levy DE, Settleman J, Engelman JA, Bardeesy N. STAT3 plays a critical role in KRAS-induced pancreatic tumorigenesis. *Cancer Res* 2011; **71**: 5020-5029 [PMID: 21586612 DOI: 10.1158/0008-5472.CAN-11-0908]
39 **Lesina M**, Kurkowski MU, Ludes K, Rose-John S, Treiber M, Klöppel G, Yoshimura A, Reindl W, Sipos B, Akira S, Schmid RM, Algül H. Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. *Cancer Cell* 2011; **19**: 456-469 [PMID: 21481788 DOI: 10.1016/j.ccr.2011.03.009]
40 **Burger JA**, Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. *Blood* 2006; **107**: 1761-1767 [PMID: 16269611 DOI: 10.1182/blood-2005-08-3182]
41 **Brahmer JR**, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; **366**: 2455-2465 [PMID: 22658128 DOI: 10.1056/NEJMoa1200694]
42 **Feig C**, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, Connell CM, Roberts EW, Zhao Q, Caballero OL, Teichmann SA, Janowitz T, Jodrell DI, Tuveson DA, Fearon DT. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc Natl Acad Sci USA* 2013; **110**: 20212-20217 [PMID: 24277834 DOI: 10.1073/pnas.1320318110]
43 **Koprowski H**, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer P. Colorectal carcinoma antigens detected by hybridoma antibodies. *Somatic Cell Genet* 1979; **5**: 957-971 [PMID: 94699]
44 **Kang CM**, Kim JY, Choi GH, Kim KS, Choi JS, Lee WJ, Kim BR. The use of adjusted preoperative CA 19-9 to predict the recurrence of resectable pancreatic cancer. *J Surg Res* 2007; **140**: 31-35 [PMID: 17418869 DOI: 10.1016/j.jss.2006.10.007]
45 **Montgomery RC**, Hoffman JP, Riley LB, Rogatko A, Ridge JA, Eisenberg BL. Prediction of recurrence and survival by post-resection CA 19-9 values in patients with adenocarcinoma of the pancreas. *Ann Surg Oncol* 1997; **4**: 551-556 [PMID: 9367020]
46 **Hartwig W**, Strobel O, Hinz U, Fritz S, Hackert T, Roth C, Büchler MW, Werner J. CA19-9 in potentially resectable pancreatic cancer: perspective to adjust surgical and perioperative therapy. *Ann Surg Oncol* 2013; **20**: 2188-2196 [PMID: 23247983 DOI: 10.1245/s10434-012-2809-1]
47 **Humphris JL**, Chang DK, Johns AL, Scarlett CJ, Pajic M, Jones MD, Colvin EK, Nagrial A, Chin VT, Chantrill LA, Samra JS, Gill AJ, Kench JG, Merrett ND, Das A, Musgrove EA, Sutherland RL, Biankin AV. The prognostic and predictive value of serum CA19.9 in pancreatic cancer. *Ann Oncol* 2012; **23**: 1713-1722 [PMID: 22241899 DOI: 10.1093/annonc/mdr561]
48 **Heestand GM**, Murphy J, Moughan J, Regine W, Luo J, Graber M, Kunz P, Fisher G, Guha C, Lin B, Mowat R, Gaur R, Buyyounouski M, Chen Y, Chang D, Koong A. A novel biomarker panel examining response to adjuvant pancreatic cancer therapy in RTOG 9704. *J Clin Oncol* 2014; **32** (suppl 3): abstr 176
49 **Tascilar M**, Skinner HG, Rosty C, Sohn T, Wilentz RE, Offerhaus GJ, Adsay V, Abrams RA, Cameron JL, Kern SE, Yeo CJ, Hruban RH, Goggins M. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2001; **7**: 4115-4121 [PMID: 11751510]
50 **Iacobuzio-Donahue CA**, Fu B, Yachida S, Luo M, Abe H, Henderson CM, Vilardell F, Wang Z, Keller JW, Banerjee P, Herman JM, Cameron JL, Yeo CJ, Halushka MK, Eshleman JR, Raben M, Klein AP, Hruban RH, Hidalgo M, Laheru D. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol* 2009; **27**: 1806-1813 [PMID: 19273710 DOI: 10.1200/JCO.2008.17.7188]
51 **Crane CH**, Varadhachary GR, Yordy JS, Staerkel GA, Javle MM, Safran H, Haque W, Hobbs BD, Krishnan S, Fleming JB, Das P, Lee JE, Abbruzzese JL, Wolff RA. Phase II trial of cetuximab, gemcitabine, and oxaliplatin followed by chemoradiation with cetuximab for locally advanced (T4) pancreatic adenocarcinoma: correlation of Smad4(Dpc4) immunostaining with pattern of disease progression. *J Clin Oncol* 2011; **29**: 3037-3043 [PMID: 21709185 DOI: 10.1200/jco.2010.33.8038]
52 **Bachet JB**, Maréchal R, Demetter P, Bonnetain F, Couvelard A, Svrcek M, Bardier-Dupas A, Hammel P, Sauvanet A, Louvet C, Paye F, Rougier P, Penna C, Vaillant JC, André T, Closset J, Salmon I, Emile JF, Van Laethem JL. Contribution of CXCR4 and SMAD4 in predicting disease progression pattern and benefit from adjuvant chemotherapy in resected pancreatic adenocarcinoma. *Ann Oncol* 2012; **23**: 2327-2335 [PMID: 22377565 DOI: 10.1093/annonc/mdr617]
53 **Poplin E**, Wasan H, Rolfe L, Raponi M, Ikdahl T, Bondarenko I, Davidenko I, Bondar V, Garin A, Boeck S, Ormanns S, Heinemann V, Bassi C, Evans TR, Andersson R, Hahn H, Picozzi V, Dicker A, Mann E, Voong C, Kaur P, Isaacson J, Allen A. Randomized, multicenter, phase II study of CO-101 versus gemcitabine in patients with metastatic pancreatic ductal adenocarcinoma: including a prospective evaluation of the role of hENT1 in gemcitabine or CO-101 sensitivity. *J Clin Oncol* 2013; **31**: 4453-4461 [PMID: 24220555 DOI: 10.1200/JCO.2013.51.0826]
54 **Mayers JR**, Wu C, Clish CB, Kraft P, Torrence ME, Fiske BP, Yuan C, Bao Y, Townsend MK, Tworoger SS, Davidson SM, Papagiannakopoulos T, Yang A, Dayton TL, Ogino S, Stampfer MJ, Giovannucci EL, Qian ZR, Rubinson DA, Ma J, Sesso HD, Gaziano JM, Cochrane BB, Liu S, Wactawski-Wende J, Manson JE, Pollak MN, Kimmelman AC, Souza A, Pierce K, Wang TJ, Gerszten RE, Fuchs CS, Vander Heiden MG, Wolpin BM. Elevation of circulating branched-chain amino acids is an early event in human pancreatic adenocarcinoma development. *Nat Med* 2014; **20**: 1193-1198 [PMID: 25261994 DOI: 10.1038/nm.3686]
55 **Zhou YF**, Xu LX, Huang LY, Guo F, Zhang F, He XY, Yuan YZ, Yao WY. Combined detection of serum UL16-binding protein 2 and macrophage inhibitory cytokine-1 improves early diagnosis and prognostic prediction of pancreatic cancer. *Oncol Lett* 2014; **8**: 2096-2102 [PMID: 25295097 DOI: 10.3892/ol.2014.2429]
56 **Takahashi N**, Fukushima T, Yorita K, Tanaka H, Chijiiwa K, Kataoka H. Dickkopf-1 is overexpressed in human pancreatic ductal adenocarcinoma cells and is involved in invasive growth. *Int J Cancer* 2010; **126**: 1611-1620 [PMID: 19711349 DOI: 10.1002/ijc.24865]
57 **Bidard FC**, Huguet F, Louvet C, Mineur L, Bouché O, Chibaudel B, Artru P, Desseigne F, Bachet JB, Mathiot C, Pierga JY, Hammel P. Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial. *Ann Oncol* 2013; **24**: 2057-2061 [PMID: 23676420 DOI: 10.1093/annonc/mdt176]
58 **Cascinu S**, Falconi M, Valentini V, Jelic S. Pancreatic cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2010; **21** Suppl 5: v55-v58 [PMID: 20555103 DOI: 10.1093/annonc/mdq165]
59 **Vauthey JN**, Dixon E. AHPBA/SSO/SSAT Consensus Conference on Resectable and Borderline Resectable Pancreatic Cancer: rationale and overview of the conference. *Ann Surg Oncol* 2009; **16**: 1725-1726 [PMID: 19396495 DOI: 10.1245/s10434-009-0409-5]
60 **Martin RC**, Scoggins CR, Egnatashvili V, Staley CA, McMasters KM, Kooby DA. Arterial and venous resection for

pancreatic adenocarcinoma: operative and long-term outcomes. *Arch Surg* 2009; **144**: 154-159 [PMID: 19221327 DOI: 10.1001/archsurg.2008.547]

61 **Speer AG**, Thursfield VJ, Torn-Broers Y, Jefford M. Pancreatic cancer: surgical management and outcomes after 6 years of follow-up. *Med J Aust* 2012; **196**: 511-515 [PMID: 22571308]

62 **Lim JE**, Chien MW, Earle CC. Prognostic factors following curative resection for pancreatic adenocarcinoma: a population-based, linked database analysis of 396 patients. *Ann Surg* 2003; **237**: 74-85 [PMID: 12496533 DOI: 10.1097/01.SLA.0000041266.10047.38]

63 **Goodman MD**, Saif MW. Adjuvant therapy for pancreatic cancer. *JOP* 2014; **15**: 87-90 [PMID: 24618424]

64 **Fukutomi A**, Uesaka K, Boku N, Kanemoto H, Konishi M, Matsumoto I, Kaneoka Y, Shimizu Y, Nakamori S, Sakamoto H, Morinaga S, Kainuma O, Imai K, Sata N, Hishinuma S, Nakamura T, Kanai M, Hirano S, Yoshikawa Y, Ohashi Y. JASPAC 01: Randomized phase III trial of adjuvant chemotherapy with gemcitabine versus S-1 for patients with resected pancreatic cancer. J Clin Oncol 2013; **31** (suppl): abstr 4008

65 **Sudo K**, Nakamura K, Yamaguchi T. S-1 in the treatment of pancreatic cancer. *World J Gastroenterol* 2014; **20**: 15110-15118 [PMID: 25386059 DOI: 10.3748/wjg.v20.i41.15110]

66 **Shimada T**, Yamazaki H, Guengerich FP. Ethnic-related differences in coumarin 7-hydroxylation activities catalyzed by cytochrome P4502A6 in liver microsomes of Japanese and Caucasian populations. *Xenobiotica* 1996; **26**: 395-403 [PMID: 9173680]

67 **Chuah B**, Goh BC, Lee SC, Soong R, Lau F, Mulay M, Dinolfo M, Lim SE, Soo R, Furuie T, Saito K, Zergebel C, Rosen LS. Comparison of the pharmacokinetics and pharmacodynamics of S-1 between Caucasian and East Asian patients. *Cancer Sci* 2011; **102**: 478-483 [PMID: 21143703 DOI: 10.1111/j.1349-7006.2010.01793.x]

68 ESPAC-4. European Study Group for Pancreatic Ccancer Trial 4. Combination versus single agent chemotherapy in resectable pancreatic ductal and peri-ampullary cancers. UK Clinical Research Network Study Portfolio. Available from: URL: http://public.ukcrn.org.uk/search/StudyDetail.aspx?StudyID=4307.2015

69 **Berlin JD**, Catalano P, Thomas JP, Kugler JW, Haller DG, Benson AB. Phase III study of gemcitabine in combination with fluorouracil versus gemcitabine alone in patients with advanced pancreatic carcinoma: Eastern Cooperative Oncology Group Trial E2297. *J Clin Oncol* 2002; **20**: 3270-3275 [PMID: 12149301]

70 **Boeck S**, Hoehler T, Seipelt G, Mahlberg R, Wein A, Hochhaus A, Boeck HP, Schmid B, Kettner E, Stauch M, Lordick F, Ko Y, Geissler M, Schoppmeyer K, Kojouharoff G, Golf A, Neugebauer S, Heinemann V. Capecitabine plus oxaliplatin (CapOx) versus capecitabine plus gemcitabine (CapGem) versus gemcitabine plus oxaliplatin (mGemOx): final results of a multicenter randomized phase II trial in advanced pancreatic cancer. *Ann Oncol* 2008; **19**: 340-347 [PMID: 17962204 DOI: 10.1093/annonc/mdm467]

71 **Cunningham D**, Chau I, Stocken DD, Valle JW, Smith D, Steward W, Harper PG, Dunn J, Tudur-Smith C, West J, Falk S, Crellin A, Adab F, Thompson J, Leonard P, Ostrowski J, Eatock M, Scheithauer W, Herrmann R, Neoptolemos JP. Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. *J Clin Oncol* 2009; **27**: 5513-5518 [PMID: 19858379 DOI: 10.1200/JCO.2009.24.2446]

72 **Klinkenbijl JH**, Jeekel J, Sahmoud T, van Pel R, Couvreur ML, Veenhof CH, Arnaud JP, Gonzalez DG, de Wit LT, Hennipman A, Wils J. Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region: phase III trial of the EORTC gastrointestinal tract cancer cooperative group. *Ann Surg* 1999; **230**: 776-782; discussion 782-784 [PMID: 10615932]

73 **Neoptolemos JP**, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, Beger H, Fernandez-Cruz L, Dervenis C, Lacaine F, Falconi M, Pederzoli P, Pap A, Spooner D, Kerr DJ, Büchler MW. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. *N Engl J Med* 2004; **350**: 1200-1210 [PMID: 15028824 DOI: 10.1056/NEJMoa032295]

74 **Morganti AG**, Falconi M, van Stiphout RG, Mattiucci GC, Alfieri S, Calvo FA, Dubois JB, Fastner G, Herman JM, Maidment BW, Miller RC, Regine WF, Reni M, Sharma NK, Ippolito E, Valentini V. Multi-institutional pooled analysis on adjuvant chemoradiation in pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2014; **90**: 911-917 [PMID: 25220717 DOI: 10.1016/j.ijrobp.2014.07.024]

75 **Andriulli A**, Festa V, Botteri E, Valvano MR, Koch M, Bassi C, Maisonneuve P, Sebastiano PD. Neoadjuvant/preoperative gemcitabine for patients with localized pancreatic cancer: a meta-analysis of prospective studies. *Ann Surg Oncol* 2012; **19**: 1644-1662 [PMID: 22012027 DOI: 10.1245/s10434-011-2110-8]

76 **Gillen S**, Schuster T, Meyer Zum Büschenfelde C, Friess H, Kleeff J. Preoperative/neoadjuvant therapy in pancreatic cancer: a systematic review and meta-analysis of response and resection percentages. *PLoS Med* 2010; **7**: e1000267 [PMID: 20422030 DOI: 10.1371/journal.pmed.1000267]

77 **Tsvetkova EV**, Asmis TR. Role of neoadjuvant therapy in the management of pancreatic cancer: is the era of biomarker-directed therapy here? *Curr Oncol* 2014; **21**: e650-e657 [PMID: 25089113 DOI: 10.3747/co.21.2006]

78 **Alamo JM**, Marín LM, Suarez G, Bernal C, Serrano J, Barrera L, Gómez MA, Muntané J, Padillo FJ. Improving outcomes in pancreatic cancer: key points in perioperative management. *World J Gastroenterol* 2014; **20**: 14237-14245 [PMID: 25339810 DOI: 10.3748/wjg.v20.i39.14237]

79 **Heinemann V**, Haas M, Boeck S. Neoadjuvant treatment of borderline resectable and non-resectable pancreatic cancer. *Ann Oncol* 2013; **24**: 2484-2492 [PMID: 23852311 DOI: 10.1093/annonc/mdt239]

80 **Katz MH**, Pisters PW, Evans DB, Sun CC, Lee JE, Fleming JB, Vauthey JN, Abdalla EK, Crane CH, Wolff RA, Varadhachary GR, Hwang RF. Borderline resectable pancreatic cancer: the importance of this emerging stage of disease. *J Am Coll Surg* 2008; **206**: 833-846; discussion 846-848 [PMID: 18471707 DOI: 10.1016/j.jamcollsurg.2007.12.020]

81 **Faris JE**, Blaszkowsky LS, McDermott S, Guimaraes AR, Szymonifka J, Huynh MA, Ferrone CR, Wargo JA, Allen JN, Dias LE, Kwak EL, Lillemoe KD, Thayer SP, Murphy JE, Zhu AX, Sahani DV, Wo JY, Clark JW, Fernandez-del Castillo C, Ryan DP, Hong TS. FOLFIRINOX in locally advanced pancreatic cancer: the Massachusetts General Hospital Cancer Center experience. *Oncologist* 2013; **18**: 543-548 [PMID: 23657686 DOI: 10.1634/theoncologist.2012-0435]

82 **Chauffert B**, Mornex F, Bonnetain F, Rougier P, Mariette C, Bouché O, Bosset JF, Aparicio T, Mineur L, Azzedine A, Hammel P, Butel J, Stremsdoerfer N, Maingon P, Bedenne L. Phase III trial comparing intensive induction chemoradiotherapy (60 Gy, infusional 5-FU and intermittent cisplatin) followed by maintenance gemcitabine with gemcitabine alone for locally advanced unresectable pancreatic cancer. Definitive results of the 2000-01 FFCD/SFRO study. *Ann Oncol* 2008; **19**: 1592-1599 [PMID: 18467316 DOI: 10.1093/annonc/mdn281]

83 **Laurence JM**, Tran PD, Morarji K, Eslick GD, Lam VW, Sandroussi C. A systematic review and meta-analysis of survival and surgical outcomes following neoadjuvant chemoradiotherapy for pancreatic cancer. *J Gastrointest Surg* 2011; **15**: 2059-2069 [PMID: 21913045 DOI: 10.1007/s11605-011-1659-7]

84 **Hammel P**, Huguet F, Van Laethem J-L, Goldstein D, Glimelius B, Artru P, Borbath I, Bouche O, Shannon J, André T, Mineur L, Chibaudel B, Bonnetain F, Christophe L. Comparison of chemoradiotherapy (CRT) and chemotherapy (CT) in patients with a locally advanced pancreatic cancer (LAPC) controlled after 4 months of gemcitabine with or without erlotinib: Final results of the international phase III LAP 07 study. *J Clin Onco* 2013; **l31** (suppl): abstr LBA4003

85 **Hosein PJ**, Macintyre J, Kawamura C, Maldonado JC, Ernani V, Loaiza-Bonilla A, Narayanan G, Ribeiro A, Portelance L, Merchan JR, Levi JU, Rocha-Lima CM. A retrospective study of

neoadjuvant FOLFIRINOX in unresectable or borderline-resectable locally advanced pancreatic adenocarcinoma. *BMC Cancer* 2012; **12**: 199 [PMID: 22642850 DOI: 10.1186/1471-2407-12-199]

86 **Burris HA**, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403-2413 [PMID: 9196156]

87 **Rocha Lima CM**, Green MR, Rotche R, Miller WH, Jeffrey GM, Cisar LA, Morganti A, Orlando N, Gruia G, Miller LL. Irinotecan plus gemcitabine results in no survival advantage compared with gemcitabine monotherapy in patients with locally advanced or metastatic pancreatic cancer despite increased tumor response rate. *J Clin Oncol* 2004; **22**: 3776-3783 [PMID: 15365074 DOI: 10.1200/jco.2004.12.082]

88 **Poplin E**, Feng Y, Berlin J, Rothenberg ML, Hochster H, Mitchell E, Alberts S, O'Dwyer P, Haller D, Catalano P, Cella D, Benson AB. Phase III, randomized study of gemcitabine and oxaliplatin versus gemcitabine (fixed-dose rate infusion) compared with gemcitabine (30-minute infusion) in patients with pancreatic carcinoma E6201: a trial of the Eastern Cooperative Oncology Group. *J Clin Oncol* 2009; **27**: 3778-3785 [PMID: 19581537 DOI: 10.1200/jco.2008.20.9007]

89 **Kindler HL**, Niedzwiecki D, Hollis D, Sutherland S, Schrag D, Hurwitz H, Innocenti F, Mulcahy MF, O'Reilly E, Wozniak TF, Picus J, Bhargava P, Mayer RJ, Schilsky RL, Goldberg RM. Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). *J Clin Oncol* 2010; **28**: 3617-3622 [PMID: 20606091 DOI: 10.1200/JCO.2010.28.1386]

90 **Moore MJ**, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960-1966 [PMID: 17452677 DOI: 10.1200/jco.2006.07.9525]

91 **Rauchwerger DR**, Firby PS, Hedley DW, Moore MJ. Equilibrative-sensitive nucleoside transporter and its role in gemcitabine sensitivity. *Cancer Res* 2000; **60**: 6075-6079 [PMID: 11085530]

92 **Von Hoff DD**, Ramanathan RK, Borad MJ, Laheru DA, Smith LS, Wood TE, Korn RL, Desai N, Trieu V, Iglesias JL, Zhang H, Soon-Shiong P, Shi T, Rajeshkumar NV, Maitra A, Hidalgo M. Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. *J Clin Oncol* 2011; **29**: 4548-4554 [PMID: 21969517 DOI: 10.1200/jco.2011.36.5742]

93 **Shirasaka T**, Shimamato Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K, Fukushima M. Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. *Anticancer Drugs* 1996; **7**: 548-557 [PMID: 8862723]

94 **Boku N**, Yamamoto S, Fukuda H, Shirao K, Doi T, Sawaki A, Koizumi W, Saito H, Yamaguchi K, Takiuchi H, Nasu J, Ohtsu A. Fluorouracil versus combination of irinotecan plus cisplatin versus S-1 in metastatic gastric cancer: a randomised phase 3 study. *Lancet Oncol* 2009; **10**: 1063-1069 [PMID: 19818685 DOI: 10.1016/s1470-2045(09)70259-1]

95 **Muro K**, Boku N, Shimada Y, Tsuji A, Sameshima S, Baba H, Satoh T, Denda T, Ina K, Nishina T, Yamaguchi K, Takiuchi H, Esaki T, Tokunaga S, Kuwano H, Komatsu Y, Watanabe M, Hyodo I, Morita S, Sugihara K. Irinotecan plus S-1 (IRIS) versus fluorouracil and folinic acid plus irinotecan (FOLFIRI) as second-line chemotherapy for metastatic colorectal cancer: a randomised phase 2/3 non-inferiority study (FIRIS study). *Lancet Oncol* 2010; **11**: 853-860 [PMID: 20708966 DOI: 10.1016/s1470-2045(10)70181-9]

96 **Yamada Y**, Takahari D, Matsumoto H, Baba H, Nakamura M, Yoshida K, Yoshida M, Iwamoto S, Shimada K, Komatsu Y, Sasaki Y, Satoh T, Takahashi K, Mishima H, Muro K, Watanabe M, Sakata Y, Morita S, Shimada Y, Sugihara K. Leucovorin, fluorouracil, and oxaliplatin plus bevacizumab versus S-1 and oxaliplatin plus bevacizumab in patients with metastatic colorectal cancer (SOFT): an open-label, non-inferiority, randomised phase 3 trial. *Lancet Oncol* 2013; **14**: 1278-1286 [PMID: 24225157 DOI: 10.1016/s1470-2045(13)70490-x]

97 **Ueno H**, Ioka T, Ikeda M, Ohkawa S, Yanagimoto H, Boku N, Fukutomi A, Sugimori K, Baba H, Yamao K, Shimamura T, Sho M, Kitano M, Cheng AL, Mizumoto K, Chen JS, Furuse J, Funakoshi A, Hatori T, Yamaguchi T, Egawa S, Sato A, Ohashi Y, Okusaka T, Tanaka M. Randomized phase III study of gemcitabine plus S-1, S-1 alone, or gemcitabine alone in patients with locally advanced and metastatic pancreatic cancer in Japan and Taiwan: GEST study. *J Clin Oncol* 2013; **31**: 1640-1648 [PMID: 23547081 DOI: 10.1200/jco.2012.43.3680]

98 **O'Reilly EM**. Evolving panorama of treatment for metastatic pancreas adenocarcinoma. *J Clin Oncol* 2013; **31**: 1621-1623 [PMID: 23547071 DOI: 10.1200/JCO.2013.48.7660]

99 **Gradishar WJ**, Tjulandin S, Davidson N, Shaw H, Desai N, Bhar P, Hawkins M, O'Shaughnessy J. Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. *J Clin Oncol* 2005; **23**: 7794-7803 [PMID: 16172456 DOI: 10.1200/jco.2005.04.937]

100 **Lobo C**, Lopes G, Silva O, Gluck S. Paclitaxel albumin-bound particles (abraxane) in combination with bevacizumab with or without gemcitabine: early experience at the University of Miami/Braman Family Breast Cancer Institute. *Biomed Pharmacother* 2007; **61**: 531-533 [PMID: 17913443 DOI: 10.1016/j.biopha.2007.08.008]

101 **Hersh EM**, O'Day SJ, Ribas A, Samlowski WE, Gordon MS, Shechter DE, Clawson AA, Gonzalez R. A phase 2 clinical trial of nab-paclitaxel in previously treated and chemotherapy-naive patients with metastatic melanoma. *Cancer* 2010; **116**: 155-163 [PMID: 19877111 DOI: 10.1002/cncr.24720]

102 **Socinski MA**, Manikhas GM, Stroyakovsky DL, Makhson AN, Cheporov SV, Orlov SV, Yablonsky PK, Bhar P, Iglesias J. A dose finding study of weekly and every-3-week nab-Paclitaxel followed by carboplatin as first-line therapy in patients with advanced non-small cell lung cancer. *J Thorac Oncol* 2010; **5**: 852-861 [PMID: 20521351]

103 **Frese KK**, Neesse A, Cook N, Bapiro TE, Lolkema MP, Jodrell DI, Tuveson DA. nab-Paclitaxel potentiates gemcitabine activity by reducing cytidine deaminase levels in a mouse model of pancreatic cancer. *Cancer Discov* 2012; **2**: 260-269 [PMID: 22585996 DOI: 10.1158/2159-8290.cd-11-0242]

104 **Neesse A**, Frese KK, Chan DS, Bapiro TE, Howat WJ, Richards FM, Ellenrieder V, Jodrell DI, Tuveson DA. SPARC independent drug delivery and antitumour effects of nab-paclitaxel in genetically engineered mice. *Gut* 2014; **63**: 974-983 [PMID: 24067278 DOI: 10.1136/gutjnl-2013-305559]

105 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]

106 **Peddi PF**, Lubner S, McWilliams R, Tan BR, Picus J, Sorscher SM, Suresh R, Lockhart AC, Wang J, Menias C, Gao F, Linehan D, Wang-Gillam A. Multi-institutional experience with FOLFIRINOX in pancreatic adenocarcinoma. *JOP* 2012; **13**: 497-501 [PMID: 22964956 DOI: 10.6092/1590-8577/913]

107 **Gunturu KS**, Yao X, Cong X, Thumar JR, Hochster HS, Stein SM, Lacy J. FOLFIRINOX for locally advanced and metastatic pancreatic cancer: single institution retrospective review of efficacy and toxicity. *Med Oncol* 2013; **30**: 361 [PMID: 23271209 DOI:

10.1007/s12032-012-0361-2]
108 **Mahaseth H**, Brutcher E, Kauh J, Hawk N, Kim S, Chen Z, Kooby DA, Maithel SK, Landry J, El-Rayes BF. Modified FOLFIRINOX regimen with improved safety and maintained efficacy in pancreatic adenocarcinoma. *Pancreas* 2013; **42**: 1311-1315 [PMID: 24152956 DOI: 10.1097/MPA.0b013e31829e2006]
109 **Gill S**, Ho MI, Kennecke HF, Renouf DJ, Cheung WI, Lim HJ. Defining eligibility of FOLFIRINOX for first-line metastatic pancreatic adenocarcinoma (MPC) in the province of British Columbia: A population-based retrospective study. Proceedings of the ASCO: Annual Meeting, 2012
110 **Bryant HE**, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 2005; **434**: 913-917 [PMID: 15829966 DOI: 10.1038/nature03443]
111 **Kaufman B**, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, Balmaña J, Mitchell G, Fried G, Stemmer SM, Hubert A, Rosengarten O, Steiner M, Loman N, Bowen K, Fielding A, Domchek SM. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. *J Clin Oncol* 2015; **33**: 244-250 [PMID: 25366685 DOI: 10.1200/JCO.2014.56.2728]
112 **Bramhall SR**, Rosemurgy A, Brown PD, Bowry C, Buckels JA; Marimastat Pancreatic Cancer Study Group. Marimastat as first-line therapy for patients with unresectable pancreatic cancer: a randomized trial. *J Clin Oncol* 2001; **19**: 3447-3455 [PMID: 11481349]
113 **Bramhall SR**, Schulz J, Nemunaitis J, Brown PD, Baillet M, Buckels JA. A double-blind placebo-controlled, randomised study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer. *Br J Cancer* 2002; **87**: 161-167 [PMID: 12107836 DOI: 10.1038/sj.bjc.6600446]
114 **Niedergethmann M**, Hildenbrand R, Wostbrock B, Hartel M, Sturm JW, Richter A, Post S. High expression of vascular endothelial growth factor predicts early recurrence and poor prognosis after curative resection for ductal adenocarcinoma of the pancreas. *Pancreas* 2002; **25**: 122-129 [PMID: 12142733]
115 **Seo Y**, Baba H, Fukuda T, Takashima M, Sugimachi K. High expression of vascular endothelial growth factor is associated with liver metastasis and a poor prognosis for patients with ductal pancreatic adenocarcinoma. *Cancer* 2000; **88**: 2239-2245 [PMID: 10820344]
116 **Spano JP**, Chodkiewicz C, Maurel J, Wong R, Wasan H, Barone C, Létourneau R, Bajetta E, Pithavala Y, Bycott P, Trask P, Liau K, Ricart AD, Kim S, Rixe O. Efficacy of gemcitabine plus axitinib compared with gemcitabine alone in patients with advanced pancreatic cancer: an open-label randomised phase II study. *Lancet* 2008; **371**: 2101-2108 [PMID: 18514303 DOI: 10.1016/S0140-6736(08)60661-3]
117 **Kindler HL**, Ioka T, Richel DJ, Bennouna J, Létourneau R, Okusaka T, Funakoshi A, Furuse J, Park YS, Ohkawa S, Springett GM, Wasan HS, Trask PC, Bycott P, Ricart AD, Kim S, Van Cutsem E. Axitinib plus gemcitabine versus placebo plus gemcitabine in patients with advanced pancreatic adenocarcinoma: a double-blind randomised phase 3 study. *Lancet Oncol* 2011; **12**: 256-262 [PMID: 21306953 DOI: 10.1016/S1470-2045(11)70004-3]
118 **Olive KP**, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Rückert F, Grützmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 2009; **324**: 1457-1461 [PMID: 19460966 DOI: 10.1126/science.1171362]
119 **Madden JI**. Infinity Reports Update from Phase 2 Study of Saridegib Plus Gemcitabine in Patients with Metastatic Pancreatic Cancer. Available from: URL: http://phx.corporate-ir.net/phoenix.zhtml?c=121941&p=irol-newsArticle&ID=1653550&highlight=.2012
120 **Catenacci DVT**, Bahary N, Nattam SR, Marsh RW, Wallace JA, Rajdev L, Cohen DJ, Sleckman BG, Lenz HJ, Stiff PJ, Thomas SP, Xu P, Henderson L, Horiba MN, Vannier M, Karrison T, W.M. S, Kindler HL. Final analysis of a phase IB/randomized phase II study of gemcitabine (G) plus placebo (P) or vismodegib (V), a hedgehog (Hh) pathway inhibitor, in patients (pts) with metastatic pancreatic cancer (PC): A University of Chicago phase II consortium study. *J Clin Oncol* 2013; **31**: abstr 4012
121 **Mitry E**, Hammel P, Deplanque G, Mornex F, Levy P, Seitz JF, Moussy A, Kinet JP, Hermine O, Rougier P, Raymond E. Safety and activity of masitinib in combination with gemcitabine in patients with advanced pancreatic cancer. *Cancer Chemother Pharmacol* 2010; **66**: 395-403 [PMID: 20364428 DOI: 10.1007/s00280-010-1299-8]
122 **Scott AM**, Wolchok JD, Old LJ. Antibody therapy of cancer. *Nat Rev Cancer* 2012; **12**: 278-287 [PMID: 22437872 DOI: 10.1038/nrc3236]
123 **Chen L**, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol* 2013; **13**: 227-242 [PMID: 23470321 DOI: 10.1038/nri3405]
124 **Pardoll DM**. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; **12**: 252-264 [PMID: 22437870 DOI: 10.1038/nrc3239]
125 **Postow MA**, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. *J Clin Oncol* 2015; **33**: 1974-1982 [PMID: 25605845 DOI: 10.1200/JCO.2014.59.4358]
126 **Page DB**, Postow MA, Callahan MK, Allison JP, Wolchok JD. Immune modulation in cancer with antibodies. *Annu Rev Med* 2014; **65**: 185-202 [PMID: 24188664 DOI: 10.1146/annurev-med-092012-112807]
127 **Powles T**, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, Bellmunt J, Burris HA, Petrylak DP, Teng SL, Shen X, Boyd Z, Hegde PS, Chen DS, Vogelzang NJ. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature* 2014; **515**: 558-562 [PMID: 25428503 DOI: 10.1038/nature13904]
128 **Tumeh PC**, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; **515**: 568-571 [PMID: 25428505 DOI: 10.1038/nature13954]
129 **Vinay DS**, Kwon BS. Immunotherapy of cancer with 4-1BB. *Mol Cancer Ther* 2012; **11**: 1062-1070 [PMID: 22532596 DOI: 10.1158/1535-7163.MCT-11-0677]
130 **Philip PA**, Benedetti J, Corless CL, Wong R, O'Reilly EM, Flynn PJ, Rowland KM, Atkins JN, Mirtsching BC, Rivkin SE, Khorana AA, Goldman B, Fenoglio-Preiser CM, Abbruzzese JL, Blanke CD. Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group-directed intergroup trial S0205. *J Clin Oncol* 2010; **28**: 3605-3610 [PMID: 20606093 DOI: 10.1200/JCO.2009.25.7550]
131 **Fensterer H**, Schade-Brittinger C, Müller HH, Tebbe S, Fass J, Lindig U, Settmacher U, Schmidt WE, Märten A, Ebert MP, Kornmann M, Hofheinz R, Endlicher E, Brendel C, Barth PJ, Bartsch DK, Michl P, Gress TM. Multicenter phase II trial to investigate safety and efficacy of gemcitabine combined with cetuximab as adjuvant therapy in pancreatic cancer (ATIP). *Ann Oncol* 2013; **24**: 2576-2581 [PMID: 23897705 DOI: 10.1093/annonc/mdt270]
132 **Kindler HL**, Friberg G, Singh DA, Locker G, Nattam S, Kozloff M, Taber DA, Karrison T, Dachman A, Stadler WM, Vokes EE. Phase II trial of bevacizumab plus gemcitabine in patients with advanced pancreatic cancer. *J Clin Oncol* 2005; **23**: 8033-8040 [PMID: 16258101 DOI: 10.1200/JCO.2005.01.9661]

133 **Hoshi H**, Sawada T, Uchida M, Iijima H, Kimura K, Hirakawa K, Wanibuchi H. MUC5AC protects pancreatic cancer cells from TRAIL-induced death pathways. *Int J Oncol* 2013; **42**: 887-893 [PMID: 23292004 DOI: 10.3892/ijo.2013.1760]

134 **Nath S**, Daneshvar K, Roy LD, Grover P, Kidiyoor A, Mosley L, Sahraei M, Mukherjee P. MUC1 induces drug resistance in pancreatic cancer cells via upregulation of multidrug resistance genes. *Oncogenesis* 2013; **2**: e51 [PMID: 23774063 DOI: 10.1038/oncsis.2013.16]

135 **Hardacre JM**, Mulcahy M, Small W, Talamonti M, Obel J, Krishnamurthi S, Rocha-Lima CS, Safran H, Lenz HJ, Chiorean EG. Addition of algenpantucel-L immunotherapy to standard adjuvant therapy for pancreatic cancer: a phase 2 study. *J Gastrointest Surg* 2013; **17**: 94-100; discussion 100-101 [PMID: 23229886 DOI: 10.1007/s11605-012-2064-6]

136 **Lutz E**, Yeo CJ, Lillemoe KD, Biedrzycki B, Kobrin B, Herman J, Sugar E, Piantadosi S, Cameron JL, Solt S, Onners B, Tartakovsky I, Choi M, Sharma R, Illei PB, Hruban RH, Abrams RA, Le D, Jaffee E, Laheru D. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma. A Phase II trial of safety, efficacy, and immune activation. *Ann Surg* 2011; **253**: 328-335 [PMID: 21217520 DOI: 10.1097/SLA.0b013e3181fd271c]

137 **Le DT**, Wang-Gillam A, Picozzi V, Greten TF, Crocenzi T, Springett G, Morse M, Zeh H, Cohen D, Fine RL, Onners B, Uram JN, Laheru DA, Lutz ER, Solt S, Murphy AL, Skoble J, Lemmens E, Grous J, Dubensky T, Brockstedt DG, Jaffee EM. Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. *J Clin Oncol* 2015; **33**: 1325-1333 [PMID: 25584002 DOI: 10.1200/jco.2014.57.4244]

138 **Gilliam AD**, Broome P, Topuzov EG, Garin AM, Pulay I, Humphreys J, Whitehead A, Takhar A, Rowlands BJ, Beckingham IJ. An international multicenter randomized controlled trial of G17DT in patients with pancreatic cancer. *Pancreas* 2012; **41**: 374-379 [PMID: 22228104 DOI: 10.1097/MPA.0b013e31822ade7e]

139 **Rocha-Lima CM**, de Queiroz Marques Junior E, Bayraktar S, Broome P, Weissman C, Nowacki M, Leslie M, Susnerwala S. A multicenter phase II study of G17DT immunogen plus irinotecan in pretreated metastatic colorectal cancer progressing on irinotecan. *Cancer Chemother Pharmacol* 2014; **74**: 479-486 [PMID: 25030089 DOI: 10.1007/s00280-014-2520-y]

140 **Abou-Alfa GK**, Chapman PB, Feilchenfeldt J, Brennan MF, Capanu M, Gansukh B, Jacobs G, Levin A, Neville D, Kelsen DP, O'Reilly EM. Targeting mutated K-ras in pancreatic adenocarcinoma using an adjuvant vaccine. *Am J Clin Oncol* 2011; **34**: 321-325 [PMID: 20686403 DOI: 10.1097/COC.0b013e3181e84b1f]

141 **Yutani S**, Komatsu N, Yoshitomi M, Matsueda S, Yonemoto K, Mine T, Noguchi M, Ishihara Y, Yamada A, Itoh K, Sasada T. A phase II study of a personalized peptide vaccination for chemotherapy-resistant advanced pancreatic cancer patients. *Oncol Rep* 2013; **30**: 1094-1100 [PMID: 23784011 DOI: 10.3892/or.2013.2556]

142 **Robbins PF**, Kassim SH, Tran TL, Crystal JS, Morgan RA, Feldman SA, Yang JC, Dudley ME, Wunderlich JR, Sherry RM, Kammula US, Hughes MS, Restifo NP, Raffeld M, Lee CC, Li YF, El-Gamil M, Rosenberg SA. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. *Clin Cancer Res* 2015; **21**: 1019-1027 [PMID: 25538264 DOI: 10.1158/1078-0432.ccr-14-2708]

143 **Kershaw MH**, Westwood JA, Darcy PK. Gene-engineered T cells for cancer therapy. *Nat Rev Cancer* 2013; **13**: 525-541 [PMID: 23880905 DOI: 10.1038/nrc3565]

144 **Slaney CY**, Kershaw MH, Darcy PK. Trafficking of T cells into tumors. *Cancer Res* 2014; **74**: 7168-7174 [PMID: 25477332 DOI: 10.1158/0008-5472.can-14-2458]

145 **Oettle H**, Neuhaus P, Hochhaus A, Hartmann JT, Gellert K, Ridwelski K, Niedergethmann M, Zülke C, Fahlke J, Arning MB, Sinn M, Hinke A, Riess H. Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: the CONKO-001 randomized trial. *JAMA* 2013; **310**: 1473-1481 [PMID: 24104372 DOI: 10.1001/jama.2013.279201]

146 **Neoptolemos JP**, Stocken DD, Bassi C, Ghaneh P, Cunningham D, Goldstein D, Padbury R, Moore MJ, Gallinger S, Mariette C, Wente MN, Izbicki JR, Friess H, Lerch MM, Dervenis C, Oláh A, Butturini G, Doi R, Lind PA, Smith D, Valle JW, Palmer DH, Buckels JA, Thompson J, McKay CJ, Rawcliffe CL, Büchler MW. Adjuvant chemotherapy with fluorouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial. *JAMA* 2010; **304**: 1073-1081 [PMID: 20823433 DOI: 10.1001/jama.2010.1275]

147 **Regine WF**, Winter KA, Abrams R, Safran H, Hoffman JP, Konski A, Benson AB, Macdonald JS, Rich TA, Willett CG. Fluorouracil-based chemoradiation with either gemcitabine or fluorouracil chemotherapy after resection of pancreatic adenocarcinoma: 5-year analysis of the U.S. Intergroup/RTOG 9704 phase III trial. *Ann Surg Oncol* 2011; **18**: 1319-1326 [PMID: 21499862 DOI: 10.1245/s10434-011-1630-6]

P- Reviewer: Hill NJ, Peracaula R **S- Editor**: Ma YJ **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9317

World J Gastroenterol 2015 August 21; 21(31): 9317-9327
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Reciprocal impact of host factors and *Helicobacter pylori* genotypes on gastric diseases

Sahar Honarmand-Jahromy, Farideh Siavoshi, Reza Malekzadeh, Taher Nejad Sattari, Saeid Latifi-Navid

Sahar Honarmand-Jahromy, Taher Nejad Sattari, Department of Biology, Tehran Science and Research Branch, Islamic Azad University, Tehran 1477893855, Iran

Farideh Siavoshi, Department of Microbiology, School of Biology, University College of Sciences, University of Tehran, Tehran 1417614411, Iran

Reza Malekzadeh, Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran 1411713135, Iran

Saeid Latifi-Navid, Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil 5619911367, Iran

Author contributions: Siavoshi F and Latifi-Navid S designed the study; Honarmand-Jahromy S performed the research; Malekzadeh R provided the gastric biopsy specimens; Nejad Sattari T analyzed the data; and Siavoshi F wrote the paper.

Supported by Research Council of the University of Tehran.

Institutional review board statement: The study was approved by the research Ethics Committee of Tehran University of Medical Sciences. All patients signed written informed consent.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: Parts of the study have been presented at the XXVII[th] International Workshop on *Helicobacter* and Microbiota in Chronic Digestive Inflammation and Gastric Cancer.

Correspondence to: Farideh Siavoshi, PhD, Department of Microbiology, School of Biology, University College of Sciences, University of Tehran, Enghelab Avenue, Tehran 1417614411, Iran. siavoshi@khayam.ut.ac.ir
Telephone: +98-21-61112460
Fax: +98-21-66492992

Received: January 3, 2015
Peer-review started: January 4, 2015
First decision: February 10, 2015
Revised: March 22, 2015
Accepted: May 7, 2015
Article in press: May 7, 2015
Published online: August 21, 2015

Abstract

AIM: To assess the impact of *Helicobacter pylori* (*H. pylori*) genotypes and patient age and sex on the development of gastric diseases.

METHODS: *H. pylori*-infected patients (n = 233) referred to the endoscopy unit at Tehran University of Medical Sciences (Tehran, Iran) were diagnosed with chronic gastritis (CG), gastric ulcer (GU), or duodenal ulcer (DU). Brucella blood agar was used for biopsy cultures and *H. pylori* isolation under microaerobic conditions. *H. pylori* isolates were confirmed with biochemical tests and through amplification of the *16S* rRNA gene. DNA was extracted from fresh cultures of the *H. pylori* isolates and used for amplification of *vacA* alleles and the *cagA* gene. Statistical analysis was performed to determine the association between *H. pylori* genotypes, age (< 40 years *vs* > 40 years) and sex of the patient, and gastric diseases.

RESULTS: CG was the most prevalent gastric disease (113/233; 48.5%), compared to GU (64/233; 27.5%)

and DU (56/233; 24%). More patients were male, and gastric diseases were more frequent in patients > 40 years ($P < 0.05$). The percentage of CG and GU patients that were male and female did not show a significant difference; however DU was more common in males ($P < 0.05$). Interestingly, a diagnosis of CG in patients > 40 years was more common in females (18.5%) than males (11.6%) ($P = 0.05$), whereas a diagnosis of GU or DU in patients > 40 years was more frequent in males (14.6% *vs* 10.7% and 12.4% *vs* 4.3%, respectively). Overall, genotyping of the *H. pylori* isolates revealed that the *vacA* s1 (82%), *vacA* m2 (70%), and *cagA*$^+$ (72.5%) alleles were more frequent than *vacA* s2 (18%), *vacA* m1 (29.2%), and *cagA*$^-$ (all $P < 0.05$). The *vacA* s1m2*cagA*$^+$ genotype was the most prevalent within the three disease groups. *vacA* s1m2 frequency was 56.2% with a similar occurrence in all diagnoses, while *vacA* s1m1 appeared more often in DU patients (33.9%). A genotype of *vacA* s2m2 occurred in 15% of isolates and was more common in CG patients (21.2%); *vacA* s2m1 was the least common genotype (3%). The *vacA* s1 allele was found to be a risk factor for DU, *vacA* s2 for CG, and *vacA* s1 and *vacA* s2 for GU (all $P < 0.05$). The *vacA* s2m2 genotype was associated with the development of CG and GU compared to DU ($P < 0.05$). No correlation was found between *vacA* m or *cagA* and gastric diseases.

CONCLUSION: The outcome of *H. pylori* infection is the result of interaction between bacterial genotypes and the age and sex of infected individuals.

Key words: Age; Gastric disease; Gender; Genotype; *Helicobacter pylori*

Core tip: *Helicobacter pylori* (*H. pylori*) genotype and host and environmental factors have emerged as the risk factors of *H. pylori*-associated diseases. However, controversies exist regarding the reciprocal interaction between these factors. Results of this study demonstrate that increased age is an important risk factor for gastric ulcers in both males and females, for chronic gastritis in females, and for duodenal ulcers in males. Genotypes *vacA* s1 and *vacA* s2m2 emerged as significant risk factors for duodenal ulcers, and chronic gastritis and gastric ulcers, respectively. No correlation was found between *vacA* m or *cagA* and gastric diseases.

Honarmand-Jahromy S, Siavoshi F, Malekzadeh R, Nejad Sattari T, Latifi-Navid S. Reciprocal impact of host factors and *Helicobacter pylori* genotypes on gastric diseases. *World J Gastroenterol* 2015; 21(31): 9317-9327 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9317.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9317

INTRODUCTION

Analysis of the genetic composition of *Helicobacter pylori* (*H. pylori*) has revealed a remarkable heterogeneity in gene content and sequence[1]. This versatile gene reservoir appears to serve as a powerful tool for bacterial adaptation when encountering new conditions in different human hosts[2]. Establishment of *H. pylori* in gastric epithelium is associated with a persistent induction of inflammatory responses and tissue damage that could lead to development of more critical clinical diagnoses, including chronic gastritis (CG), peptic ulcers (PUs), or gastric cancer[3,4]. An interaction between *H. pylori* virulence factors, host genetics, and environmental factors is currently thought to determine the extent of tissue damage[5,6]. In this regard, the longevity of *H. pylori* infection and sex of infected individuals have been investigated as important factors in the development of gastric diseases[7-11]. Many investigators have studied *H. pylori* virulence factors and proposed several candidate proteins, including vacuolating cytotoxin A (VacA) and cytotoxin-associated gene A (CagA)[12]. In contrast, few studies have focused on host genetics, eating habits, and lifestyle, and the results remain controversial[13-19].

VacA, which occurs in all strains of *H. pylori*, is regarded as a multifunctional toxin with the potential to insert into the endosomal membranes of epithelial cells, inducing the formation of large vacuoles, and inhibition of antigen presentation[20]. VacA also inserts into the mitochondrial membrane and causes apoptosis[21]. Cellular tight junctions are loosened by VacA, releasing nutrients, such as iron, nickel, sugars, and amino acids, needed for the establishment of *H. pylori*[22]. Furthermore, VacA inhibits the proliferation of T cells, which helps the bacterium to evade an immune response and establish a chronic infection[23,24]. Although VacA is crucial for colonization of all *H. pylori* strains, its toxicity is determined by the presence of different allelic types of the signal sequence (s1 and s2) and middle region (m1 and m2). It has been proposed that *H. pylori* strains carrying *vacA* s1m1 are highly toxigenic, increasing the risk of PUs or gastric cancer, those with *vacA* s1m2 less toxigenic, and *vacA* s2m2 nontoxigenic, while a genotype of *vacA* s2m1 rarely occurs[25,26]. CagA binds to epithelial cells and causes perturbation of tight junctions, cell polarity, and differentiation[27]. Interaction of CagA with E-cadherin and β-catenin causes interruption of the adhesion of epithelial cells, as well as formation of junctions and growth. Furthermore, CagA induces the production of interleukin-8, which leads to an inflammatory response and tissue damage[28]. These interactions of CagA with epithelial cells lead to destabilization and damage of gastric epithelium, and thus, contribute to *H. pylori* pathogenesis[22].

Studies have shown that *cagA*$^+$ strains are often associated with a higher risk of PUs or gastric cancer,

compared to *cagA*$^-$ strains[29,30]. It has been suggested that the combination of an active VacA toxin with CagA constitutes an efficient system for generating an appropriate niche for long-term colonization of *H. pylori* in gastric epithelium. CagA contributes to changes in the gastric epithelium in several ways. It has been demonstrated that CagA protects epithelial cells against apoptotic events induced by VacA, but by inducing proinflammatory and antiapoptotic activities, also causes severe tissue damage, leading to a PU and even gastric cancer[31]. Furthermore, the antiapoptotic activity of CagA has been shown to reduce the rate of turnover of epithelial cells[32], whereas VacA decreases CagA-induced cell scattering and motility[33].

Cure of CG[34], gastric ulcer (GU), and duodenal ulcer (DU)[35] with antimicrobial therapy against *H. pylori* demonstrates that the bacterium is an important risk factor for dyspeptic diseases. However, several studies have observed a correlation between *H. pylori*-associated gastric atrophy and smoking[13], intake of salt[14], alcohol[15], low levels of dietary beta-carotene, and consumption of soybean products[16]. Furthermore, acid-suppression due to GU[17] and consumption of acid-suppressing drugs[36] have been found to be associated with corpus atrophy in *H. pylori*-infected patients. In contrast, no correlation between *H. pylori*-associated gastritis and sex, age, smoking, and coffee intake or between atrophy or intestinal metaplasia and smoking or drinking alcohol was observed in other reports[19]. Furthermore, it is well known that the incidence of *H. pylori* infection increases with age[7,8], and that aging is an important risk factor for dyspeptic diseases[9,10]. However, there are discrepancies about the role of the sex of the patient in *H. pylori*-associated dyspeptic diseases[11]. Although reports indicate a reduced incidence of *H. pylori* infection in some regions of the world due to antimicrobial therapies against *H. pylori* or other infections[10,37-40], a considerable number of patients in Iran are still referred for endoscopy, seeking relief from dyspeptic diseases. The reported frequency of *H. pylori* in the general population of Iran is approximately 69%[41], but reaches up to 89% in the northwestern province of Ardabil[42], where 90% of individuals over 40 years-old suffer from *H. pylori*-associated CG[43]. In this study, *H. pylori* isolates from 233 patients with CG, GU, or DU were genotyped for *vacA* alleles and *cagA* gene. The reciprocal impact of *H. pylori* genotypes and host age and sex on the development of dyspeptic diseases was assessed.

MATERIALS AND METHODS

Patients

The recruited patients (n = 233; 129 men and 104 women) were randomly selected *H. pylori*-positive referrals to the endoscopy unit of Shariati Hospital (Tehran University of Medical Sciences, Tehran, Iran) due to complaint of dyspepsia. Patients were stratified based on diagnosis and age: CG, GU, and DU, and < 40 years and > 40 years, respectively.

H. pylori isolation and cultivation

Two antral biopsies were taken from each patient for a rapid urease test and *H. pylori* cultivation. Biopsies were cultured on selective Brucella agar (Pronadisa, Madrid, Spain) containing 5% defibrinated sheep blood and 10 mg/L vancomycin, 5 mg/L trimethoprim, and 2.5 IU/L polymyxin B (all from MP Biomedical, Santa Ana, CA, United States). Cultures were incubated at 37 ℃ under microaerobic conditions for 3-5 d. Bacterial isolates were identified as *H. pylori* on the basis of Gram-stained morphology, positive urease, catalase, and oxidase tests, and amplification of the *H. pylori*-specific *16S* rRNA gene. The purified bacterial isolates were harvested in phosphate-buffered saline (PBS) and stored at -20 ℃ until further use.

Genotyping of H. pylori isolates

DNA was extracted from fresh cultures of *H. pylori* isolates with phenol/chloroform as previously described[44]. Genotyping of *H. pylori* isolates was performed by polymerase chain reaction (PCR) amplification of the *cagA* gene, *vacA* signal sequences (s1 or s2), and middle regions (m1 or m2). The primers for amplification are listed in Table 1. *Escherichia coli* (DSM 0498) and previously PCR-confirmed *H. pylori* isolates were used as negative and positive controls, respectively. Amplification was carried out in a total volume of 25 μL containing 2.5 μL of 10 × PCR buffer (Sinaclon, Karaj, Iran), 1.5 mmol/L $MgCl_2$, 125 μmol/L of each dNTP (Sinaclon), 1U of Taq DNA polymerase (Sinaclon), 0.5 μmol/L of each primer, and 25 ng of bacterial DNA. Cycling parameters were 94 ℃ (1 min), optimized annealing temperature for each genes/alleles (1 min), and 72 ℃ (1 min) for 33 cycles with a final extension at 72 ℃ (7 min). PCR products were electrophoresed and visualized with a UV transilluminator (UVP, Upland, CA, United States). Amplified fragments of all genes/alleles from the five isolates were purified and sequenced with both forward and reverse primers using BigDye technology, and sequencing reactions were run on an AB13700XL DNA sequencer (Life Technologies of Thermo Fisher Scientific, Waltham, MA, United States). The BLAST program (http://www.ncbi.nlm.nih.gov) was used to match the nucleotide sequences with published sequences in Genbank (data not shown). The size of the PCR products of all genes was similar to those generated from the control *H. pylori* strains, and sequences showed 99%-100% similarity with the corresponding sequences of the reference *H. pylori* strains in Genbank (Table 1).

Statistical analysis

Statistical analysis was performed using Pearson's χ^2 and Fisher's exact probability tests. Kendell's Tau *b*

Table 1 Oligonucleotide primers used for polymerase chain reaction

Gene	Primer	Sequence (5'→3')	PCR products (bp)	Annealing temperature (℃)	Ref.
16S rRNA	HP1	GCAATCAGCGTCAGTAATGTT C	519	58.5	[85]
	HP2	GCTAAGAGATCAGCCTATGTCC			
vacA (s1, s2)	VA1F	ATGGAAATACAAGAAACACACC	s1: 259	56.0	[25]
	VA1R	CTGCTTGAATGCGCCAAACTTTAATC	s2: 286		
vacA (m1, m2)	VAG-F	CAATCTGTCCAATCAAGCGAG	m1: 570	58.5	[25]
	VAG-R	GCGTCAAAATAATTCCAAGG	m2: 645		
cagA	D008	ATAATGCTAAATTAGACAACTTGAGCGA	298	58.5	[86]
	R008	TTAGAATAATCAACAAACATCACGCCAT			

PCR: Polymerase chain reaction.

Table 2 Distribution of genotypes in *Helicobacter pylori* isolates *n* (%)

Characteristic		CG	GU	DU	Total
No. of cases		113 (48.5)	64 (27.5)	56 (24.0)	233 (100)
Sex	Female	62 (54.9)	27 (42.2)	15 (26.8)	104 (44.6)
	Male	51 (45.1)	37 (57.8)	41 (73.2)	129 (55.4)
Age	< 40 yr	43 (38.1)	5 (7.8)	17 (30.4)	65 (27.9)
	> 40 yr	70 (61.9)	59 (92.2)	39 (69.6)	168 (72.1)
vacA	s1	83 (73.5)	53 (82.8)	55 (98.2)	191 (82.0)
	s2	30 (26.5)	11 (17.2)	1 (1.8)	42 (18.0)
	m1	32 (28.3)	16 (25)	20 (35.7)	68 (29.2)
	m2	81 (71.7)	48 (75)	34 (60.7)	163 (70.0)
	s1m1	24 (21.2)	15 (23.4)	19 (33.9)	58 (24.9)
	s1m2	59 (52.2)	38 (59.4)	34 (60.7)	131 (56.2)
	s2m1	6 (5.3)	1 (1.6)	0	7 (3.0)
	s2m2	24 (21.2)	10 (15.6)	1 (1.8)	35 (15.0)
cagA	+	82 (72.6)	50 (78.1)	37 (66.1)	169 (72.5)
	-	31 (27.4)	14 (21.9)	19 (33.9)	64 (27.5)
s1m1*cagA*	+	17 (15.0)	10 (15.6)	10 (17.9)	37 (15.9)
	-	7 (6.2)	5 (7.8)	9 (16.1)	21 (9.0)
s1m2*cagA*	+	41 (36.3)	30 (46.9)	24 (42.9)	95 (40.8)
	-	18 (15.9)	8 (12.5)	10 (17.9)	36 (15.5)
s2m1*cagA*	+	5 (4.4)	1 (1.6)	0	6 (2.6)
	-	1 (0.9)	0	0	1 (0.4)
s2m2*cagA*	+	19 (16.8)	9 (14.1)	1 (1.8)	29 (12.4)
	-	5 (4.4)	1 (1.6)	0	6 (2.6)
s1m1m2*cagA*	+	0	0	2 (3.6)	2 (0.8)
	-	0	0	0	0

CG: Chronic gastritis; DU: Duodenal ulcer; GU: Gastric ulcer.

correlation coefficient was used to measure the strength of dependence between *H. pylori* genotypes, and age or sex and gastric disease. Logistic regression analysis was used to predict the outcome of gastric diseases based on age, sex, or *H. pylori* genotype (SPSS version 20, IBM Corp., Armonk, NY, United States). Statistical significance was defined as $P \leqslant 0.05$.

RESULTS

Classification of patients according to age, sex, and gastric disease

All patients (n = 233) were *H. pylori* positive, but were diagnosed with one of three diseases, CG, DU, or GU. CG was the most prevalent gastric disease (113/233; 48.5%), compared to GU (64/233; 27.5%) and DU (56/233; 24%). The distribution of patients according to sex, age, and *H. pylori* genotype are presented in Table 2. A greater percentage of patients were > 40 years of age (168/233; 72.1%) and male (129/233; 55.4%). More patients were male in both age groups: < 40 years, 16.7% (39/233) *vs* 11.2% (26/233) females, and > 40 years, 38.6% (90/233) were male *vs* 33.5% (78/233) female. For all diagnoses, more patients were > 40 years (Figure 1, Table 2).

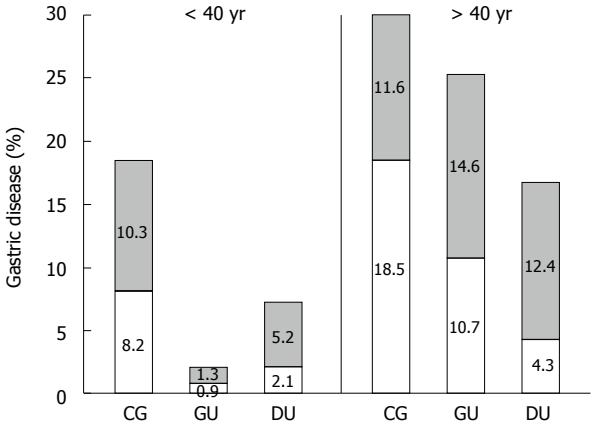


Figure 1 Gastric disease cases are more prevalent in patients > 40 years of age. The percentages of the 233 *Helicobacter pylori*-positive patients are plotted according to disease diagnosis (CG: Chronic gastritis: GU: Gastric ulcer; and DU: Duodenal ulcer), age (< 40 years and > 40 years), and sex (male: White bars; female: Shaded bars).

Genotype frequencies within H. pylori isolates from CG, GU, and DU patients

In order to determine whether *H. pylori* isolates genetically differed among patients and/or disease, *vacA* and *cagA* genes were amplified from 233 *H. pylori* DNAs and sequenced. The most frequently detected alleles were *vacA* s1, *vacA* m2, and *cagA*$^+$, at 82.0% (191/233), 70.0% (163/233), and 72.5% (169/233), respectively (Table 2). All *vacA* s/m genotypes were detected, with *vacA* s1m2 (131/233; 56.2%) as the most common and *vacA* s2m1 (7/233; 3.0%) the least common. Moreover, *vacA* s1m2 was equally prevalent among the three disease groups. For the remaining *vacA* s/m genotypes, *vacA* s1m1 was observed in 24.9% (58/233) of cases and most often associated with a diagnosis of DU (33.9%),

whereas *vacA* s2m2 was detected in 15% (35/233) of cases overall, but most often in CG patients (24/233; 21.2%). For all the alleles detected, *vacA* s1m2 *cagA*$^+$ (95/233; 40.8%) was the most common genotype observed in the cohort.

Sex, age, and H. pylori genotypes of CG, GU, and DU patients

Statistical analysis was first performed on clinical characteristics of the patients, such as sex of the patient and age. In this part of statistical analysis, each individual disease group was considered separately. Whereas CG and GU were not associated with sex of the patient, In contrast, the increased proportion of male relative to female patients in the DU group was statistically significant (73.2% *vs* 26.8%, $P = 0.001$). Increased age (> 40 years) was clearly associated with all diseases ($P = 0.011$, 0.000, and 0.003 for CG, GU, and DU, respectively).

The frequencies of specific virulence alleles were examined based on gastric disease diagnosis. In all diagnoses, the frequencies of the *vacA* s1 allele compared to *vacA* s2 and *vacA* m2 relative to *vacA* m1 were higher. The frequency of the *vacA* s1 allele was higher than *vacA* s2 (73.5% *vs* 26.5% and 82.8% *vs* 17.2%, $P = 0.000$), and *vacA* m2 was higher than *vacA* m1 (71.7% *vs* 28.3% and 75.0% *vs* 25.0%, $P = 0.000$) in CG and GU patients, respectively. In addition, in DU patients, the frequency of the *vacA* s1 allele was significantly higher than *vacA* s2 (98.2% *vs* 1.8%, $P = 0.000$), and the *vacA* m2 allele was at a higher frequency than *vacA* m1 (60.7% *vs* 35.7%, $P = 0.000$). Finally, the *vacA* s1m1m2 genotype was only detected in 2/56 (3.6%) female DU patients. *cagA* was detected in significantly more *H. pylori* strains derived from CG (72.6%; $P = 0.000$) and DU (66.1%; $P = 0.016$) patients. *H. pylori* isolates with *vacA* s1m2*cagA*± genotypes exhibited the highest frequency (56.3%) overall with similar prevalence among CG, GU, and DU patients (Table 2, Figure 2).

Reciprocal impact of host age and sex and H. pylori genotypes on the development of gastric diseases

Clinical characteristics of patients were analyzed for associations with disease development. Increased age clearly emerged as a risk factor for all dyspeptic disease diagnoses. As indicated above, the number of patients in the three disease groups was significantly greater in patients > 40 years than those < 40 years. Increased age appeared as an important risk factor for GU in both males and females, compared to CG and DU ($P = 0.000$). Furthermore, being male was correlated with DU and female with CG ($P = 0.000$), whereas no significant correlation was found between the development of GU and being male or female.

A strong correlation was found between specific *vacA* s genotypes and gastric disease diagnosis. The *vacA* s1 genotype was a risk factor for development of DU and *vacA* s2 for CG ($P = 0.000$); however, both of these genotypes were equally associated with the development of GU. There was no correlation between *vacA* m or *cagA*$^+$ alleles and gastric disease. For the combination of *vacA* s, *vacA* m, and *cagA* genotypes in gastric disease, only *vacA* s2m2 was found to have an association with the development of CG and GU compared to DU ($P = 0.004$). The frequency of *vacA* s1m2*cagA*$^+$ and *vacA* s1m2*cagA*$^-$ strains was higher in male patients with DU compared to female patients ($P = 0.004$ and 0.011, respectively). Statistically significant differences were not observed in CG and GU patients (Figures 1 and 2).

DISCUSSION

Understanding the etiology of the development of gastric diseases will help to develop strategies for prevention and treatment. This study addressed age and sex of the patient and *H. pylori* bacterial genotype as major factors contributing to disease development in a cohort of Iranian patients. Among 233 patients, 48.5% were diagnosed with CG, 27.5% GU, and 24.0% DU. All three diseases were more common in patients > 40 years (72.1%). The most significant difference between patients < 40 and > 40 years was observed in the GU group. In addition, CG was found to be more frequent in females and DU in males; however, GU was similarly prevalent in males and females. Finally, genotyping of the *H. pylori* isolates indicated that the *vacA* s1 allele in combination with being male was a significant risk factor for DU, and that *vacA* s2m2 and being female were risk factors for CG and GU. However, no correlation was found between alleles of *vacA* m or *cagA* and dyspeptic diseases.

In developing countries, the prevalence of *H. pylori* infection reaches up to 80% before the age of 50 years and in developed countries, 50% of individuals older than 60 years are infected[45]. The reported *H. pylori*-infection rate in the adult population of Brazil ranged from 35.3%[46] to 97.9%[47]. In another study performed in Brazil, the incidence of *H. pylori*-related gastritis increased with age in women in their 50s and men in their 70s. Furthermore, the frequency of dyspepsia in patients over 70 years was twofold greater than in young adults, and two thirds of dyspeptic patients were women[10]. In Japan, the prevalence of *H. pylori* was considerably high (85%) and increased with age, from 26% in subjects 16-20 years up to 61% in those 50-64 years[48]. In Africa, an increased prevalence of *H. pylori* infection was detected in older patients[49]. In a study on 1391 Albanian subjects, *H. pylori* seropositivity was more prevalent in females > 40 years[50]. A cross-sectional study in the United Kingdom demonstrated a significant association between *H. pylori* seropositivity and males, shorter height, tobacco consumption, and lower socioeconomic

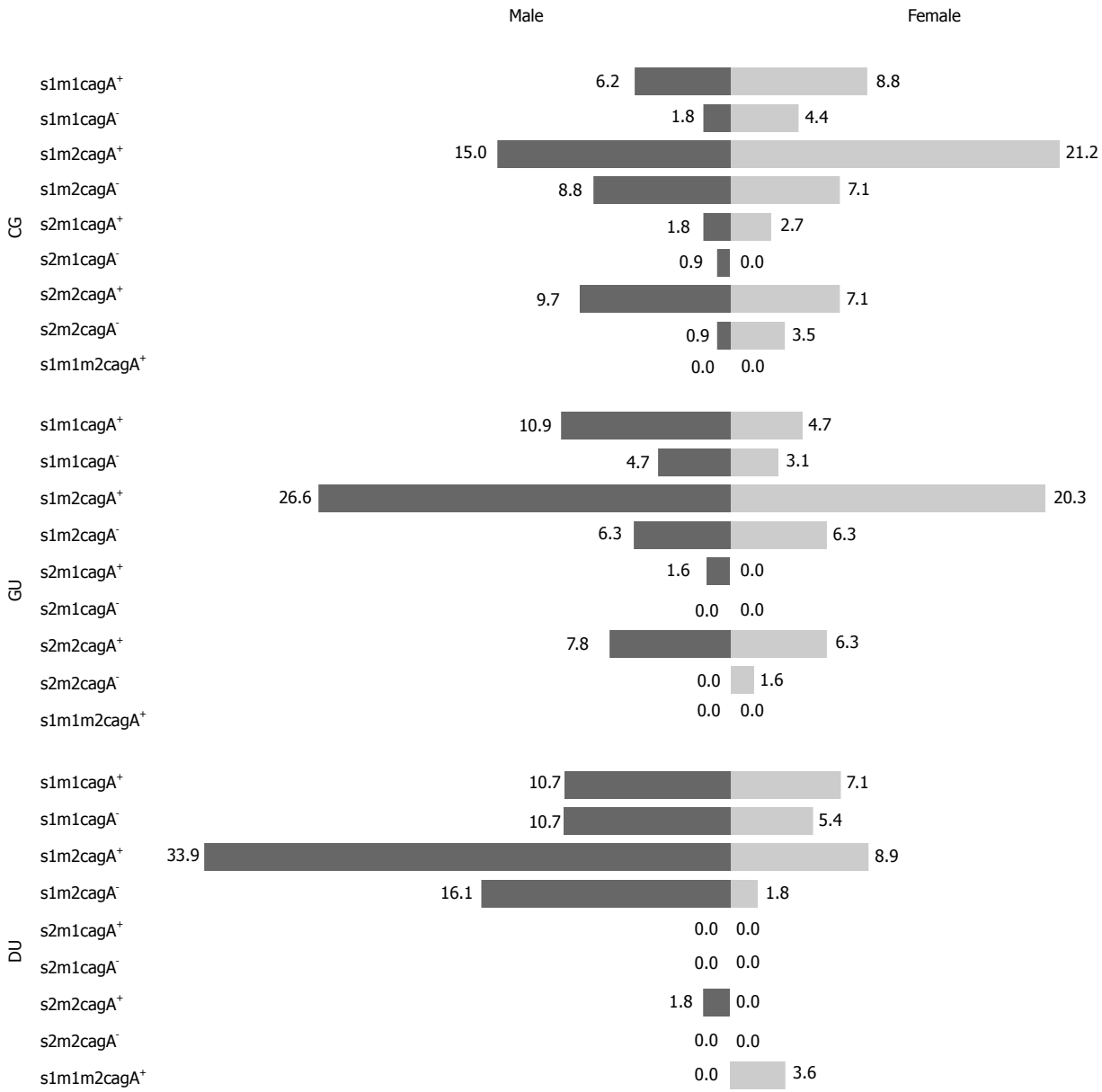


Figure 2 Distribution of *Helicobacter pylori* genotypes according to sex. *vacA* s1m2 *cagA*$^+$ was the most common genotype in both males (dark bars) and females (light bars) of all three gastric diseases. CG: Chronic gastritis; GU: Gastric ulcer; DU: Duodenal ulcer.

status[45]. In a study from Brazil, the most prevalent gastric disease was CG (72.3%), with GU at 5.1% and DU at 6%. Gastroesophageal alterations were detected in 16.7% of these cases. Sex and age played no role in the development of CG; however, being male and older age were associated with GU, whereas being male alone was linked to the development of DU[51]. A similar prevalence of GU and DU and an association with being male was also observed in another study performed in Southern Brazil[11]. However, in a third study in Brazil, GU and DU were significantly more frequent in women[52].

Reports indicate that males and females become similarly infected with *H. pylori*[53]. However, the clinical outcome depends on the longevity and severity of the inflammatory response to *H. pylori* infection in each individual[54]. An increasing body of evidence indicates that the consequences of *H. pylori* infection are more severe in males; however, the contributing factors are currently unknown. Although the prevalence of *H. pylori* in males and females was found to be similar, as determined by the rapid urease test and stained biopsy smear examination, higher levels of IgG were observed in males[55]. Furthermore, being male, having polymorphism at the interleukin-1β promoter, and overexpression of interleukin-1β have all been associated with increasing the risk of atrophic gastritis and gastric adenocarcinoma in *H. pylori*-infected patients[56,57]. It has been demonstrated that gastrin, a hormone which stimulates the proliferation of epithelial cells[58], can lead to gastric cancer if overexpressed, especially in the context of *H. pylori* infection[59]. In

Sweden, higher levels of antibodies against VacA and CagA in *H. pylori*-infected patients were associated with increased risk of the development of gastric cancer by twofold when compared with control patients without *H. pylori* infection[60]. *H. pylori* and aging have also been found to be strongly associated with an increased risk of atrophy and the development of intestinal metaplasia in gastric mucosa[19,61]. In Japan, intestinal metaplasia was evident in a considerable number of males (90%) over the age of 50 years compared to females in the same age group or younger individuals overall[62]. In the United States, the incidence of gastric cancer in males has been reported to be five times higher than in females[63].

The frequencies of *vacA* s1 (82%), *vacA* s2 (18%), *vacA* m1 (29.2%), *vacA* m2 (70.8%), and *cagA* (72.5%) were within ranges reported by other studies performed on patients in Iran: *vacA* s1, 68%-80%; *vacA* s2, 20%-32%; *vacA* m1, 30%-70%; *vacA* m2, 27%-70%[64,65]; and *cagA*, 44%[66] to 91%[67]. *vacA* s1 was detected in 73.5%, 82.8%, and 98.2% of CG, GU, and DU patients, respectively, whereas *vacA* s2 was detected in 26.5%, 17.2%, and 1.8%. *vacA* m1 was found in 28.3% of CG, 25% of GU, and 35.7% of DU patients, and *vacA* m2 in 71.7% CG, 75% GU, and 60.7% of DU patients. The *cagA* gene was detected in most *H. pylori* isolates (66.1%-78.1%). The most frequent genotype among the 233 isolates was *vacA* s1m2*cagA*$^+$ (40.8%) followed by *vacA* s1m1*cagA*$^+$ (15.9%), and *vacA* s1m2*cagA*$^-$ (15.5%), with a similar distribution among gastric disease diagnoses. The frequency of *vacA* s2m2*cagA*$^+$ genotype was lower, at 12.4%. Reports indicate that the s1 genotype is very common in East Asian countries, but with no relationship to the clinical outcomes of infection, whereas *vacA* m1 is more frequent in North East Asia and *vacA* m2 in South Asia[30,68]. Several studies in Western countries have shown that individuals infected with *H. pylori* strains carrying *vacA* s1 or m1 alleles are at a higher risk of PU or gastric cancer when compared to those infected with *vacA* s2 or *vacA* m2-carrying strains[69,70]. In this cohort, genotypes *vacA* s1m1 and *vacA* s2m2 were detected at a high frequency. The *H. pylori vacA* s1m1 genotype is in fact common worldwide, ranging from 42% to 84%[71] around the globe, whereas *vacA* s2m2 varies from 0% to 57%[71,72]. The frequencies of the *vacA* s1m1 genotype within the isolates of this study exhibited no significant difference among gastric disease diagnoses (21.2%, 23.4%, and 33.9% for CG, GU, and DU patients, respectively). However, the frequencies of the *vacA*s2m2 genotype were significantly higher in CG and GU patients compared to DU patients (21.2%, 15.6%, and 1.8%, respectively). In a study from Japan, *H. pylori* strains with the *vacA* s1m1 genotype were isolated from 59.2%, 79.2%, and 87.5% of CG, GU, and gastric cancer patients, respectively[73]. Furthermore, the *vacA* s1m2 genotype was found in 17.3%, 7.9%, and 27.2% of isolates from CG, GU, and DU patients, respectively. The *vacA* s2m2 genotype was more common in *H. pylori* isolates from CG (22.4%) than GU (11.9%), DU (10.5%), and gastric cancer (4.2%) patients.

Although the frequency of *cagA* was high (72.5%) in our cohort, an association of *cagA*$^+$ genotypes with the development of CG, GU, and DU was not observed. The frequency of *cagA* has been reported to range from 50% in some Middle Eastern countries[74] to 88% in Europe and North America[75,76] and 99% in many East Asian countries[77,78]. Studies in Western countries have revealed a significant association of *cagA*$^+$ *H. pylori* strains with severe gastritis, PU, and gastric cancer[29,30,79]. However, such a relationship was not found between *cagA*$^+$ strains and PU, gastric cancer, and non-ulcer dyspepsia in Far Eastern countries[80]. In a study from Italy, 72% (132/193) of *H. pylori* isolates were *cagA*$^+$, and *cagA* positivity was associated with PU and gastric cancer but not gastritis[81]. It has been proposed that the v*acA* s1m1 genotype is often linked to the presence of *cagA* and the *vacA* s2m2 genotype with its absence[25,82]. In Alaska, *cagA* was detected in 85% of *H. pylori* isolates; however, no correlation was found between the *cagA*$^+$ or *cagA*$^-$ genotype and development of gastric diseases. In the same study, 66% of *vacA* s2m2-carrying *H. pylori* strains contained the *cagA* gene[12].

Results of this study demonstrate that gastric diseases are significantly more frequent in patients > 40 years. Being male and the *vacA* s1 genotype played an important role in the development of DU. Aging and the *vacA* s2m2 genotype were associated with a diagnosis of GU, and being female and the *vacA* s2m2 genotype with CG. However, no correlation was found between *vacA* m or *cagA* and gastric diseases. A large body of evidence indicates that the heterogeneity of *H. pylori* underlies the diversity of gastric diseases observed. This bacterial genetic diversity appears to be the result of recombination processes that evolved for the purpose of long-term colonization in humans, despite eliciting chronic inflammatory responses[83]. In this regard, investigators believe that VacA and CagA act together to stimulate signals in epithelial cells, affecting cell structure, differentiation and behavior[27], and are balanced with the damage needed for long-term colonization[31,84]. Results of this study indicate that VacA and CagA are mainly involved in the colonization of *H. pylori* in the human stomach. However, the interplay between *H. pylori* genotypes and age and sex of the human hosts is likely to determine the severity of the gastric disease diagnosis.

COMMENTS

Background

Helicobacter pylori (*H. pylori*) infection has been regarded as a risk factor for gastric diseases, ranging from chronic gastritis to more severe outcomes, such as peptic ulcers, gastric cancer, and mucosa-associated lymphoid tissue lymphoma. *H. pylori* has a remarkable heterogeneous genetic reservoir which may enable efficient bacterial adaptation to the gastric niche in different

patients. Disease development is potentially the result of the interaction between *H. pylori* virulence factors, VacA, and CagA and the host, which leads to inflammation and tissue damage. Thus, underlying the clinical outcome of *H. pylori* infection may be the interplay between virulence factors, host genetics, and environmental factors. However, despite extensive research on *H. pylori*-related diseases, the impact of risk factors alone or in concert has not been thoroughly evaluated. Therefore, it remains possible that the age and sex of infected individuals play important roles in determining the outcome of *H. pylori* infection.

Research frontiers

Reports on the risk factors involved in development of *H. pylori*-associated gastric diseases are controversial. *H. pylori*-associated gastric atrophy has been correlated to smoking, intake of salt, alcohol, or low beta-carotene, consumption of soybean products, and even acid-suppressing drugs. No correlation with age, sex, smoking, or coffee intake, however, has been observed in other studies. Currently, the relationship between bacterial, host, and environmental factors has only been examined in a few studies with larger numbers of patients. The incidence of *H. pylori* infection is considerably high in Iran (69%-80%); correspondingly, the frequency of referrals to endoscopy rooms due to complaint of dyspepsia is also high. Therefore, knowledge of the risk factors may contribute to the management and/or prevention of the more severe consequences of *H. pylori* infection in high-risk patients.

Innovations and breakthroughs

The focus of this study was to assess the potential impact of individual factors, including host age and sex and *H. pylori* genotypes, on the development of *H. pylori*-associated chronic gastritis (CG), gastric ulcer (GU), and duodenal ulcer (DU). Results indicated that age and sex were associated with the development of gastric disease in the context of *H. pylori* infection, and specific *H. pylori* genotypes were differentially associated with the diagnosis of CG, GU, and DU.

Applications

Increased age, being female, and the *vacA* s2m2 genotype were risk factors for CG, increased age in males and females and *vacA* s2m2 for GU, and increased age and *vacA* s1 for DU. Accordingly, for prevention and control of *H. pylori*-associated gastric diseases, results of this study might help to identify high-risk patients, particularly in the Iranian population.

Terminology

GU is a defect in gastric mucosa that penetrates deep into the muscularis mucosa. The sensation of indigestion is described as burning and can be relieved by antacid. DU, the duodenal deformity caused by acid and pepsin from the duodenal mucosa, is often associated with pain in the upper stomach, vomiting, bleeding, perforation, and obstruction, and is also relieved by taking antacids. CG is the inflammation of gastric mucosa, mainly caused by *H. pylori* infection. CG usually has no definite symptoms, but the patient is susceptible to the development of GU.

Peer-review

The relationship between *H. pylori* genotypes and host age and sex on the development of *H. pylori*-associated gastric diseases was investigated. Increased age (> 40 years) was found to be a risk factor for CG, GU, and DU. Furthermore, being female and *vacA* s2m2 were risk factors for CG, *vacA* s2m2 for GU, and *vacA* s1 for DU in males. No correlation between *H. pylori* alleles *vacA* m or *cagA* and gastric diseases was observed. Therefore, the disease outcome of *H. pylori* infection may be a direct result of the interaction of specific bacterial genotypes with the age and sex of infected individuals.

REFERENCES

1 **Alm RA**, Ling LS, Moir DT, King BL, Brown ED, Doig PC, Smith DR, Noonan B, Guild BC, deJonge BL, Carmel G, Tummino PJ, Caruso A, Uria-Nickelsen M, Mills DM, Ives C, Gibson R, Merberg D, Mills SD, Jiang Q, Taylor DE, Vovis GF, Trust TJ. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori. *Nature* 1999; **397**: 176-180 [PMID: 9923682 DOI: 10.1038/16495]

2 **Baltrus DA**, Amieva MR, Covacci A, Lowe TM, Merrell DS, Ottemann KM, Stein M, Salama NR, Guillemin K. The complete genome sequence of Helicobacter pylori strain G27. *J Bacteriol* 2009; **191**: 447-448 [PMID: 18952803 DOI: 10.1128/JB.01416-08]

3 **Nomura A**, Stemmermann GN, Chyou PH, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and the risk for duodenal and gastric ulceration. *Ann Intern Med* 1994; **120**: 977-981 [PMID: 7741826]

4 **Algood HM**, Cover TL. Helicobacter pylori persistence: an overview of interactions between H. pylori and host immune defenses. *Clin Microbiol Rev* 2006; **19**: 597-613 [PMID: 17041136 DOI: 10.1128/CMR.00006-06]

5 **Akopyanz N**, Bukanov NO, Westblom TU, Kresovich S, Berg DE. DNA diversity among clinical isolates of Helicobacter pylori detected by PCR-based RAPD fingerprinting. *Nucleic Acids Res* 1992; **20**: 5137-5142 [PMID: 1408828]

6 **Marshall DG**, Coleman DC, Sullivan DJ, Xia H, O'Moráin CA, Smyth CJ. Genomic DNA fingerprinting of clinical isolates of Helicobacter pylori using short oligonucleotide probes containing repetitive sequences. *J Appl Bacteriol* 1996; **81**: 509-517 [PMID: 8939029]

7 **Iijima K**, Ohara S, Koike T, Sekine H, Shimosegawa T. Gastric acid secretion of normal Japanese subjects in relation to Helicobacter pylori infection, aging, and gender. *Scand J Gastroenterol* 2004; **39**: 709-716 [PMID: 15513353 DOI: 10.1080/00365520410005911]

8 **Sasidharan S**, Lachumy SJ, Ravichandran M, Latha LY, Gegu SR. Epidemiology of Helicobacter pylori among multiracial community in Northern Peninsular, Malaysia: effect of age across race and gender. *Asian Pac J Trop Med* 2011; **4**: 72-75 [PMID: 21771421 DOI: 10.1016/S1995-7645(11)60037-0]

9 **El-Serag HB**, Talley NJ. Systemic review: the prevalence and clinical course of functional dyspepsia. *Aliment Pharmacol Ther* 2004; **19**: 643-654 [PMID: 15023166 DOI: 10.1111/j.1365-2036.2004.01897.x]

10 **Mapel D**, Roberts M, Overhiser A, Mason A. The epidemiology, diagnosis, and cost of dyspepsia and Helicobacter pylori gastritis: a case-control analysis in the Southwestern United States. *Helicobacter* 2013; **18**: 54-65 [PMID: 23067108 DOI: 10.1111/j.1523-5378.2012.00988.x]

11 **Saul C**, Teixeira CR, Pereira-Lima JC, Torresini RJ. [Prevalence reduction of duodenal ulcer: a Brazilian study. (retrospective analysis in the last decade: 1996-2005)]. *Arq Gastroenterol* 2007; **44**: 320-324 [PMID: 18317651]

12 **Miernyk K**, Morris J, Bruden D, McMahon B, Hurlburt D, Sacco F, Parkinson A, Hennessy T, Bruce M. Characterization of Helicobacter pylori cagA and vacA genotypes among Alaskans and their correlation with clinical disease. *J Clin Microbiol* 2011; **49**: 3114-3121 [PMID: 21752979 DOI: 10.1128/JCM.00469-11]

13 **Kneller RW**, You WC, Chang YS, Liu WD, Zhang L, Zhao L, Xu GW, Fraumeni JF, Blot WJ. Cigarette smoking and other risk factors for progression of precancerous stomach lesions. *J Natl Cancer Inst* 1992; **84**: 1261-1266 [PMID: 1640486]

14 **Correa P**. Chronic gastritis: a clinico-pathological classification. *Am J Gastroenterol* 1988; **83**: 504-509 [PMID: 3364410]

15 **Dixon MF**. Campylobacter pylori and chronic gastritis. In: Rathbone BJ, Heatlly RV, editors. Campylobacter pylori and Gastroduodenal Disease. Oxford: Blackwell Scientific, 1989: 106-116

16 **Tsugane S**, Kabuto M, Imai H, Gey F, Tei Y, Hanaoka T, Sugano K, Watanabe S. Helicobacter pylori, dietary factors, and atrophic gastritis in five Japanese populations with different gastric cancer mortality. *Cancer Causes Control* 1993; **4**: 297-305 [PMID: 8347778]

17 **Maaroos HI**, Salupere V, Uibo R, Kekki M, Sipponen P. Seven-year follow-up study of chronic gastritis in gastric ulcer patients. *Scand J Gastroenterol* 1985; **20**: 198-204 [PMID: 3992177]

18 **Kuipers EJ**, Klinkenberg-Knol EC, Vandenbroucke-Grauls CM, Appelmelk BJ, Schenk BE, Meuwissen SG. Role of

Helicobacter pylori in the pathogenesis of atrophic gastritis. *Scand J Gastroenterol Suppl* 1997; **223**: 28-34 [PMID: 9200303]
19 **Ohkuma K**, Okada M, Murayama H, Seo M, Maeda K, Kanda M, Okabe N. Association of Helicobacter pylori infection with atrophic gastritis and intestinal metaplasia. *J Gastroenterol Hepatol* 2000; **15**: 1105-1112 [PMID: 11106088]
20 **Molinari M**, Salio M, Galli C, Norais N, Rappuoli R, Lanzavecchia A, Montecucco C. Selective inhibition of Iidependent antigen presentation by Helicobacter pylori toxin VacA. *J Exp Med* 1998; **187**: 135-140 [PMID: 9419220]
21 **Willhite DC**, Blanke SR. Helicobacter pylori vacuolating cytotoxin enters cells, localizes to the mitochondria, and induces mitochondrial membrane permeability changes correlated to toxin channel activity. *Cell Microbiol* 2004; **6**: 143-154 [PMID: 14706100]
22 **Papini E**, Satin B, Norais N, de Bernard M, Telford JL, Rappuoli R, Montecucco C. Selective increase of the permeability of polarized epithelial cell monolayers by Helicobacter pylori vacuolating toxin. *J Clin Invest* 1998; **102**: 813-820 [PMID: 9710450 DOI: 10.1172/JCI2764]
23 **Ji X**, Fernandez T, Burroni D, Pagliaccia C, Atherton JC, Reyrat JM, Rappuoli R, Telford JL. Cell specificity of Helicobacter pylori cytotoxin is determined by a short region in the polymorphic midregion. *Infect Immun* 2000; **68**: 3754-3757 [PMID: 10816542]
24 **Gebert B**, Fischer W, Weiss E, Hoffmann R, Haas R. Helicobacter pylori vacuolating cytotoxin inhibits T lymphocyte activation. *Science* 2003; **301**: 1099-1102 [PMID: 12934009 DOI: 10.1126/science.1086871]
25 **Atherton JC**, Cao P, Peek RM, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995; **270**: 17771-17777 [PMID: 7629077]
26 **Letley DP**, Lastovica A, Louw JA, Hawkey CJ, Atherton JC. Allelic diversity of the Helicobacter pylori vacuolating cytotoxin gene in South Africa: rarity of the vacA s1a genotype and natural occurrence of an s2/m1 allele. *J Clin Microbiol* 1999; **37**: 1203-1205 [PMID: 10074554]
27 **Amieva MR**, El-Omar EM. Host-bacterial interactions in Helicobacter pylori infection. *Gastroenterology* 2008; **134**: 306-323 [PMID: 18166359 DOI: 10.1053/j.gastro.2007.11.009]
28 **Brandt S**, Kwok T, Hartig R, König W, Backert S. NF-kappaB activation and potentiation of proinflammatory responses by the Helicobacter pylori CagA protein. *Proc Natl Acad Sci USA* 2005; **102**: 9300-9305 [PMID: 15972330 DOI: 10.1073/pnas.0409873102]
29 **van Doorn LJ**, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, de Boer W, Quint W. Clinical relevance of the cagA, vacA, and iceA status of Helicobacter pylori. *Gastroenterology* 1998; **115**: 58-66 [PMID: 9649459]
30 **Yamaoka Y**, Kikuchi S, el-Zimaity HM, Gutierrez O, Osato MS, Graham DY. Importance of Helicobacter pylori oipA in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. *Gastroenterology* 2002; **123**: 414-424 [PMID: 12145793]
31 **Oldani A**, Cormont M, Hofman V, Chiozzi V, Oregioni O, Canonici A, Sciullo A, Sommi P, Fabbri A, Ricci V, Boquet P. Helicobacter pylori counteracts the apoptotic action of its VacA toxin by injecting the CagA protein into gastric epithelial cells. *PLoS Pathog* 2009; **5**: e1000603 [PMID: 19798427 DOI: 10.1371/journal.ppat.1000603]
32 **Mimuro H**, Suzuki T, Nagai S, Rieder G, Suzuki M, Nagai T, Fujita Y, Nagamatsu K, Ishijima N, Koyasu S, Haas R, Sasakawa C. Helicobacter pylori dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach. *Cell Host Microbe* 2007; **2**: 250-263 [PMID: 18005743 DOI: 10.1016/j.chom.2007.09.005]
33 **Tegtmeyer N**, Zabler D, Schmidt D, Hartig R, Brandt S, Backert S. Importance of EGF receptor, HER2/Neu and Erk1/2 kinase signalling for host cell elongation and scattering induced by the Helicobacter pylori CagA protein: antagonistic effects of the vacuolating cytotoxin VacA. *Cell Microbiol* 2009; **11**: 488-505 [PMID: 19046339 DOI: 10.1111/j.1462-5822.2008.01269.x]
34 **Mazzoleni LE**, Sander GB, Francesconi CF, Mazzoleni F, Uchoa DM, De Bona LR, Milbradt TC, Von Reisswitz PS, Berwanger O, Bressel M, Edelweiss MI, Marini SS, Molina CG, Folador L, Lunkes RP, Heck R, Birkhan OA, Spindler BM, Katz N, Colombo Bda S, Guerrieri PP, Renck LB, Grando E, Hocevar de Moura B, Dahmer FD, Rauber J, Prolla JC. Helicobacter pylori eradication in functional dyspepsia: HEROES trial. *Arch Intern Med* 2011; **171**: 1929-1936 [PMID: 22123802 DOI: 10.1001/archinternmed.2011.533]
35 **Leodolter A**, Kulig M, Brasch H, Meyer-Sabellek W, Willich SN, Malfertheiner P. A meta-analysis comparing eradication, healing and relapse rates in patients with Helicobacter pylori-associated gastric or duodenal ulcer. *Aliment Pharmacol Ther* 2001; **15**: 1949-1958 [PMID: 11736726]
36 **Kuipers EJ**, Lundell L, Klinkenberg-Knol EC, Havu N, Festen HP, Liedman B, Lamers CB, Jansen JB, Dalenback J, Snel P, Nelis GF, Meuwissen SG. Atrophic gastritis and Helicobacter pylori infection in patients with reflux esophagitis treated with omeprazole or fundoplication. *N Engl J Med* 1996; **334**: 1018-1022 [PMID: 8598839 DOI: 10.1056/NEJM199604183341603]
37 **Yim JY**, Kim N, Choi SH, Kim YS, Cho KR, Kim SS, Seo GS, Kim HU, Baik GH, Sin CS, Cho SH, Oh BH. Seroprevalence of Helicobacter pylori in South Korea. *Helicobacter* 2007; **12**: 333-340 [PMID: 17669107 DOI: 10.1111/j.1523-5378.2007.00504.x]
38 **Chen J**, Bu XL, Wang QY, Hu PJ, Chen MH. Decreasing seroprevalence of Helicobacter pylori infection during 1993-2003 in Guangzhou, southern China. *Helicobacter* 2007; **12**: 164-169 [PMID: 17309754 DOI: 10.1111/j.1523-5378.2007.00487.x]
39 **Fialho AM**, Braga AB, Braga Neto MB, Carneiro JG, Rocha AM, Rodrigues MN, Queiroz DM, Braga LL. Younger siblings play a major role in Helicobacter pylori transmission among children from a low-income community in the Northeast of Brazil. *Helicobacter* 2010; **15**: 491-496 [PMID: 21073604 DOI: 10.1111/j.1523-5378.2010.00791.x]
40 **Nakajima S**, Nishiyama Y, Yamaoka M, Yasuoka T, Cho E. Changes in the prevalence of Helicobacter pylori infection and gastrointestinal diseases in the past 17 years. *J Gastroenterol Hepatol* 2010; **25** Suppl 1: S99-S110 [PMID: 20586876 DOI: 10.1111/j.1440-1746.2009.06214.x]
41 **Nouraie M**, Latifi-Navid S, Rezvan H, Radmard AR, Maghsudlu M, Zaer-Rezaii H, Amini S, Siavoshi F, Malekzadeh R. Childhood hygienic practice and family education status determine the prevalence of Helicobacter pylori infection in Iran. *Helicobacter* 2009; **14**: 40-46 [PMID: 19191895 DOI: 10.1111/j.1523-5378.2009.00657.x]
42 **Malekzadeh R**, Sotoudeh M, Derakhshan MH, Mikaeli J, Yazdanbod A, Merat S, Yoonessi A, Tavangar M, Abedi BA, Sotoudehmanesh R, Pourshams A, Asgari AA, Doulatshahi S, Alizadeh BZ, Arshi S, Madjidpoor A, Mir Moomen S, Fleischer DE. Prevalence of gastric precancerous lesions in Ardabil, a high incidence province for gastric adenocarcinoma in the northwest of Iran. *J Clin Pathol* 2004; **57**: 37-42 [PMID: 14693833]
43 **Sadjadi A**, Malekzadeh R, Derakhshan MH, Sepehr A, Nouraie M, Sotoudeh M, Yazdanbod A, Shokoohi B, Mashayekhi A, Arshi S, Majidpour A, Babaei M, Mosavi A, Mohagheghi MA, Alimohammadian M. Cancer occurrence in Ardabil: results of a population-based cancer registry from Iran. *Int J Cancer* 2003; **107**: 113-118 [PMID: 12925965 DOI: 10.1002/ijc.11359]
44 **Sambrook J**, Russell DW. Molecular cloning: A Laboratory manual. New York: Cold Spring Harbor Laboratory Press, 2001
45 **Jackson L**, Britton J, Lewis SA, McKeever TM, Atherton J, Fullerton D, Fogarty AW. A population-based epidemiologic study of Helicobacter pylori infection and its association with systemic inflammation. *Helicobacter* 2009; **14**: 108-113 [PMID: 19751435 DOI: 10.1111/j.1523-5378.2009.00711.x]
46 **Ito LS**, Oba SM, Hamajima N, Marie SK, Uno M, Shinjo SK, Kino A, Lavilla F, Inoue M, Tajima K, Tominaga S. Helicobacter

pylori seropositivity among 963 Japanese Brazilians according to sex, age, generation, and lifestyle factors. *Jpn J Cancer Res* 2001; **92**: 1150-1156 [PMID: 11714438]
47 **Almeida Cunha RP**, Alves FP, Rocha AM, Rocha GA, Camargo LM, Nogueira PO, Camargo EP, Queiroz DM. Prevalence and risk factors associated with Helicobacter pylori infection in native populations from Brazilian Western Amazon. *Trans R Soc Trop Med Hyg* 2003; **97**: 382-386 [PMID: 15259462]
48 **Kimura K**. Gastritis and gastric cancer. Asia. *Gastroenterol Clin North Am* 2000; **29**: 609-621 [PMID: 11030076]
49 **Dube C**, Nkosi TC, Clarke AM, Mkwetshana N, Green E, Ndip RN. Helicobacter pylori antigenemia in an asymptomatic population of Eastern Cape Province, South Africa: public health implications. *Rev Environ Health* 2009; **24**: 249-255 [PMID: 19891122]
50 **Monno R**, Volpe A, Basho M, Fumarola L, Trerotoli P, Kondili LA, Bino S, Schinaia N, Dentico P. Helicobacter pylori seroprevalence in selected groups of Albanian volunteers. *Infection* 2008; **36**: 345-350 [PMID: 18574555 DOI: 10.1007/s15010-008-6338-6]
51 **Suzuki RB**, Cola RF, Cola LT, Ferrari CG, Ellinger F, Therezo AL, Silva LC, Eterovic A, Sperança MA. Different risk factors influence peptic ulcer disease development in a Brazilian population. *World J Gastroenterol* 2012; **18**: 5404-5411 [PMID: 23082057 DOI: 10.3748/wjg.v18.i38.5404]
52 **Mattar R**, dos Santos AF, Eisig JN, Rodrigues TN, Silva FM, Lupinacci RM, Iriya K, Carrilho FJ. No correlation of babA2 with vacA and cagA genotypes of Helicobacter pylori and grading of gastritis from peptic ulcer disease patients in Brazil. *Helicobacter* 2005; **10**: 601-608 [PMID: 16302986 DOI: 10.1111/j.1523-5378.2005.00360.x]
53 **Brown LM**. Helicobacter pylori: epidemiology and routes of transmission. *Epidemiol Rev* 2000; **22**: 283-297 [PMID: 11218379]
54 **McColl KE**. What remaining questions regarding Helicobacter pylori and associated diseases should be addressed by future research? View from Europe. *Gastroenterology* 1997; **113**: S158-S162 [PMID: 9394779]
55 **Harris P**, Perez-Perez G, Zylberberg A, Rollán A, Serrano C, Riera F, Einisman H, García D, Viviani P. Relevance of adjusted cut-off values in commercial serological immunoassays for Helicobacter pylori infection in children. *Dig Dis Sci* 2005; **50**: 2103-2109 [PMID: 16240223 DOI: 10.1007/s10620-005-3015-9]
56 **Sipponen P**, Marshall BJ. Gastritis and gastric cancer. Western countries. *Gastroenterol Clin North Am* 2000; **29**: 579-592, v-vi [PMID: 11030074]
57 **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; **404**: 398-402 [PMID: 10746728 DOI: 10.1038/35006081]
58 **Iwase K**, Evers BM, Hellmich MR, Guo YS, Higashide S, Kim HJ, Townsend CM. Regulation of growth of human gastric cancer by gastrin and glycine-extended progastrin. *Gastroenterology* 1997; **113**: 782-790 [PMID: 9287969]
59 **Fox JG**, Wang TC, Rogers AB, Poutahidis T, Ge Z, Taylor N, Dangler CA, Israel DA, Krishna U, Gaus K, Peek RM. Host and microbial constituents influence Helicobacter pylori-induced cancer in a murine model of hypergastrinemia. *Gastroenterology* 2003; **124**: 1879-1890 [PMID: 12806621]
60 **Enroth H**, Kraaz W, Engstrand L, Nyrén O, Rohan T. Helicobacter pylori strain types and risk of gastric cancer: a case-control study. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 981-985 [PMID: 11008919]
61 **Fontham ET**, Ruiz B, Perez A, Hunter F, Correa P. Determinants of Helicobacter pylori infection and chronic gastritis. *Am J Gastroenterol* 1995; **90**: 1094-1101 [PMID: 7611204]
62 **Imai T**, Murayama H. Time trend in the prevalence of intestinal metaplasia in Japan. *Cancer* 1983; **52**: 353-361 [PMID: 6861078]
63 **El-Serag HB**, Mason AC, Petersen N, Key CR. Epidemiological differences between adenocarcinoma of the oesophagus and adenocarcinoma of the gastric cardia in the USA. *Gut* 2002; **50**: 368-372 [PMID: 11839716]
64 **Dabiri H**, Maleknejad P, Yamaoka Y, Feizabadi MM, Jafari F, Rezadehbashi M, Nakhjavani FA, Mirsalehian A, Zali MR. Distribution of Helicobacter pylori cagA, cagE, oipA and vacA in different major ethnic groups in Tehran, Iran. *J Gastroenterol Hepatol* 2009; **24**: 1380-1386 [PMID: 19702906 DOI: 10.1111/j.1440-1746.2009.05876.x]
65 **Doosti A**, Ghasemi-Dehkordi P. Helicobacter pylori vacA genotypes in Shahrekordian (Iran) H. pylori-positive patients. *Res J Biol Sci* 2009; **4**: 11-15
66 **Siavoshi F**, Malekzadeh R, Daneshmand M, Ashktorab H. Helicobacter pylori endemic and gastric disease. *Dig Dis Sci* 2005; **50**: 2075-2080 [PMID: 16240218 DOI: 10.1007/s10620-005-3010-1]
67 **Talebkhan Y**, Mohammadi M, Mohagheghi MA, Vaziri HR, Eshagh Hosseini M, Mohajerani N, Oghalaei A, Esmaeili M, Zamaninia L. cagA gene and protein status among Iranian Helicobacter pylori strains. *Dig Dis Sci* 2008; **53**: 925-932 [PMID: 17939043 DOI: 10.1007/s10620-007-9978-y]
68 **Yamaoka Y**. Mechanisms of disease: Helicobacter pylori virulence factors. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 629-641 [PMID: 20938460 DOI: 10.1038/nrgastro.2010.154]
69 **Cover TL**, Halter SA, Blaser MJ. Characterization of HeLa cell vacuoles induced by Helicobacter pylori broth culture supernatant. *Hum Pathol* 1992; **23**: 1004-1010 [PMID: 1381332]
70 **Sugimoto M**, Yamaoka Y. The association of vacA genotype and Helicobacter pylori-related disease in Latin American and African populations. *Clin Microbiol Infect* 2009; **15**: 835-842 [PMID: 19392900 DOI: 10.1111/j.1469-0691.2009.02769.x]
71 **Van Doorn LJ**, Figueiredo C, Mégraud F, Pena S, Midolo P, Queiroz DM, Carneiro F, Vanderborght B, Pegado MD, Sanna R, De Boer W, Schneeberger PM, Correa P, Ng EK, Atherton J, Blaser MJ, Quint WG. Geographic distribution of vacA allelic types of Helicobacter pylori. *Gastroenterology* 1999; **116**: 823-830 [PMID: 10092304]
72 **Kim SY**, Woo CW, Lee YM, Son BR, Kim JW, Chae HB, Youn SJ, Park SM. Genotyping CagA, VacA subtype, IceA1, and BabA of Helicobacter pylori isolates from Korean patients, and their association with gastroduodenal diseases. *J Korean Med Sci* 2001; **16**: 579-584 [PMID: 11641526]
73 **Matsunari O**, Shiota S, Suzuki R, Watada M, Kinjo N, Murakami K, Fujioka T, Kinjo F, Yamaoka Y. Association between Helicobacter pylori virulence factors and gastroduodenal diseases in Okinawa, Japan. *J Clin Microbiol* 2012; **50**: 876-883 [PMID: 22189111 DOI: 10.1128/JCM.05562-11]
74 **Al Qabandi A**, Mustafa AS, Siddique I, Khajah AK, Madda JP, Junaid TA. Distribution of vacA and cagA genotypes of Helicobacter pylori in Kuwait. *Acta Trop* 2005; **93**: 283-288 [PMID: 15715995 DOI: 10.1016/j.actatropica.2005.01.004]
75 **Miehlke S**, Kibler K, Kim JG, Figura N, Small SM, Graham DY, Go MF. Allelic variation in the cagA gene of Helicobacter pylori obtained from Korea compared to the United States. *Am J Gastroenterol* 1996; **91**: 1322-1325 [PMID: 8677987]
76 **Yamaoka Y**, Kodama T, Gutierrez O, Kim JG, Kashima K, Graham DY. Relationship between Helicobacter pylori iceA, cagA, and vacA status and clinical outcome: studies in four different countries. *J Clin Microbiol* 1999; **37**: 2274-2279 [PMID: 10364597]
77 **Chomvarin C**, Namwat W, Chaicumpar K, Mairiang P, Sangchan A, Sripa B, Tor-Udom S, Vilaichone RK. Prevalence of Helicobacter pylori vacA, cagA, cagE, iceA and babA2 genotypes in Thai dyspeptic patients. *Int J Infect Dis* 2008; **12**: 30-36 [PMID: 17548220 DOI: 10.1016/j.ijid.2007.03.012]
78 **Lai CH**, Kuo CH, Chen YC, Chao FY, Poon SK, Chang CS, Wang WC. High prevalence of cagA- and babA2-positive Helicobacter pylori clinical isolates in Taiwan. *J Clin Microbiol* 2002; **40**: 3860-3862 [PMID: 12354901]
79 **Sezikli M**, Guliter S, Apan TZ, Aksoy A, Keles H, Ozkurt ZN. Frequencies of serum antibodies to Helicobacter pylori CagA and VacA in a Turkish population with various gastroduodenal

diseases. *Int J Clin Pract* 2006; **60**: 1239-1243 [PMID: 16669834 DOI: 10.1111/j.1742-1241.2005.00778.x]

80 **Palli D**, Menegatti M, Masala G, Ricci C, Saieva C, Holton J, Gatta L, Miglioli M, Vaira D. Helicobacter pylori infection, anti-cagA antibodies and peptic ulcer: a case-control study in Italy. *Aliment Pharmacol Ther* 2002; **16**: 1015-1020 [PMID: 11966512]

81 **Basso D**, Zambon CF, Letley DP, Stranges A, Marchet A, Rhead JL, Schiavon S, Guariso G, Ceroti M, Nitti D, Rugge M, Plebani M, Atherton JC. Clinical relevance of Helicobacter pylori cagA and vacA gene polymorphisms. *Gastroenterology* 2008; **135**: 91-99 [PMID: 18474244 DOI: 10.1053/j.gastro.2008.03.041]

82 **Cover TL**, Blaser MJ. Helicobacter pylori in health and disease. *Gastroenterology* 2009; **136**: 1863-1873 [PMID: 19457415 DOI: 10.1053/j.gastro.2009.01.073]

83 **Mueller A**, Falkow S, Amieva MR. Helicobacter pylori and gastric cancer: what can be learned by studying the response of gastric epithelial cells to the infection? *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 1859-1864 [PMID: 16103426 DOI: 10.1158/1055-9965.EPI-04-0820]

84 **Gangwer KA**, Shaffer CL, Suerbaum S, Lacy DB, Cover TL, Bordenstein SR. Molecular evolution of the Helicobacter pylori vacuolating toxin gene vacA. *J Bacteriol* 2010; **192**: 6126-6135 [PMID: 20870762 DOI: 10.1128/JB.01081-10]

85 **Lu Y**, Redlinger TE, Avitia R, Galindo A, Goodman K. Isolation and genotyping of Helicobacter pylori from untreated municipal wastewater. *Appl Environ Microbiol* 2002; **68**: 1436-1439 [PMID: 11872498]

86 **Covacci A**, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z, Figura N. Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* 1993; **90**: 5791-5795 [PMID: 8516329]

P- Reviewer: Chmiela M **S- Editor**: Ma YJ **L- Editor**: A
E- Editor: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9328

World J Gastroenterol 2015 August 21; 21(31): 9328-9336
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Influence of the *hTERT* rs2736100 polymorphism on telomere length in gastric cancer

Byung Joon Choi, Jung Hwan Yoon, Olga Kim, Won Suk Choi, Suk Woo Nam, Jung Young Lee, Won Sang Park

Byung Joon Choi, Department of Pediatrics, College of Medicine, The Catholic University of Korea, Seoul 137-701, South Korea

Jung Hwan Yoon, Olga Kim, Won Suk Choi, Suk Woo Nam, Jung Young Lee, Won Sang Park, Department of Pathology, College of Medicine, The Catholic University of Korea, Seoul 137-701, South Korea

Suk Woo Nam, Jung Young Lee, Won Sang Park, Functional Rnomics Research Center, College of Medicine, The Catholic University of Korea, Seoul 137-701, South Korea

Author contributions: Park WS designed the research; Choi BJ, Yoon JH, Kim O and Choi WS performed research; Choi BJ, Yoon JH, Park WS analyzed the data; Nam SW and Lee JY contributed new reagents/analytic tools; Choi BJ and Park WS wrote the paper.

Supported by Basic Science Research Programs (2012R1A2A-2A01002531) through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology.

Institutional review board statement: This study was approved by the Institutional Review Board of the Catholic University of Korea, College of Medicine (CUMC09U089).

Conflict-of-interest statement: The authors disclose no potential conflicts of interest.

Correspondence to: Won Sang Park, MD, PhD, Professor of Medicine, Department of Pathology, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul 137-701, South Korea. wonsang@catholic.ac.kr

Telephone: +82-2-22587310
Fax: +82-2-5376586

Received: March 27, 2015
Peer-review started: March 28, 2015
First decision: June 2, 2015
Revised: June 22, 2015
Accepted: July 3, 2015
Article in press: July 3, 2015
Published online: August 21, 2015

Abstract

AIM: To investigate the functional consequences of rs2736100 polymorphism in telomere length and examine its link to gastric cancer risk.

METHODS: Telomere length and human telomerase reverse transcriptase (*hTERT*) mRNA expression were measured in 35 gastric cancer tissues and 5 cell lines and correlated to rs2736100 polymorphism. The relationship between rs2736100 polymorphism and the risk of gastric cancer were examined in 243 gastric cancer patients and 246 healthy individuals.

RESULTS: The rs2736100 A allele carrier is closely associated with reduced *hTERT* mRNA expression and shortened telomere length in gastric cancer tissue and cell lines. When gastric cancers were stratified by histological subtype, telomere length and *hTERT* mRNA levels were significantly increased in those with the C/C genotype in intestinal-type gastric cancer, but not in diffuse-type gastric cancer. Interestingly, there was no significant difference in the genotype and allele frequencies of the rs2736100 polymorphism between the patients with gastric cancer and healthy controls.

CONCLUSION: The rs2736100 polymorphism of the *hTERT* gene is involved in the regulation of *hTERT* expression and telomere length, but not in the risk of

gastric cancer.

Key words: Human telomerase reverse transcriptase; Telomere; Gastric cancer; Polymorphism; Risk

Core tip: The rs2736100 polymorphism is closely associated with reduced human telomerase reverse transcriptase (*hTERT*) mRNA expression and shortened telomere length in gastric cancer tissue and cell lines. Additionally, telomere length and *hTERT* mRNA levels were significantly increased in those with the C/C genotype in intestinal-type gastric cancer. Notably, there was no significant difference in the genotype and allele frequencies of the rs2736100 polymorphism between the patients with gastric cancer and healthy controls. These results suggest that the rs2736100 polymorphism of the *hTERT* gene is involved in the regulation of *hTERT* expression and telomere length, but not in the risk of gastric cancer.

Choi BJ, Yoon JH, Kim O, Choi WS, Nam SW, Lee JY, Park WS. Influence of the *hTERT* rs2736100 polymorphism on telomere length in gastric cancer. *World J Gastroenterol* 2015; 21(31): 9328-9336 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9328.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9328

INTRODUCTION

Gastric cancer remains one of the malignancies with the highest incidence and mortality rates worldwide, accounting for approximately 10% of all newly diagnosed cancers[1-3]. Gastric mucosal inflammation is generally believed to be caused by chronic *Helicobacter pylori* (*H. pylori*) infection and atrophic gastritis, while intestinal metaplasia and dysplasia represent different stages of the gastric carcinogenesis cascade[4,5]. Thus, gastric carcinogenesis is a multistep process involving genetic and epigenetic changes in oncogenes, tumor-suppressor genes, cell adhesion molecules and DNA repair genes. There are two main types of gastric adenocarcinoma according to the Lauren classification defined as intestinal-type and diffuse-type. Of these, intestinal-type carcinomas showed obvious glandular differentiation, whereas diffuse-type carcinomas are typically poorly differentiated. The phenotype variation has led to substantial research interest on the regulation of genes. Although numerous advances in the understanding of gastric cancer have been made, the precise molecular mechanism underlying gastric carcinogenesis is not yet fully understood.

Telomere length in healthy cells is highly regulated in a tissue- and cell type-specific manner and is dependent on mitotic turnover rate, telomerase activity, and telomerase-associated factors[6]. In most normal human somatic tissues, telomerase activity is undetectable, whereas it is frequently detectable in almost all types of human cancers, suggesting the importance of telomerase in the development of cancer[7]. Defects in telomere maintenance contribute to the initiation of genomic instability during carcinogenesis, including gastric cancer[8]. The telomerase reverse transcriptase (*TERT*) gene is located in chromosome 5p15.33 and a variant rs2736100 localized in the second intron of the gene. The rs2736100 C allele is one of the eight variants associated with long telomeres in white blood cells[9]. We previously reported that telomere length in 35 gastric cancer tissues was shortened significantly, compared with the corresponding non-cancerous gastric mucosae, and was positively correlated with human telomerase reverse transcriptase (*hTERT*) expression[10]. This led us to hypothesize that the rs2736100 polymorphism is closely associated with telomere length and gastric cancer risk.

In the present study, we investigated whether the rs2736100 single-nucleotide polymorphism (SNP) of the *hTERT* gene is associated with *hTERT* mRNA expression level and telomere length, and examined the ability of rs2736100 to predict gastric cancer risk. Overall, we found that rs2736100 A allele carrier is closely associated with shortened telomere length and reduced *hTERT* mRNA expression in gastric cancer, especially in intestinal-type. However, genotype and allele frequencies of rs2736100 polymorphism were not associated with an increased risk for gastric cancer in this population.

MATERIALS AND METHODS

Samples

In this study, archival non-cancerous gastric mucosa specimens from 243 gastric cancer patients who underwent subtotal or total gastrectomy at the Catholic University of Korea, College of Medicine in Seoul, were enrolled. All gastric cancers specimens were pathologically confirmed as gastric adenocarcinoma. None of the patients received chemotherapy or radiotherapy before surgery. The healthy control subjects (n = 246) were collected from residents of the same racial and geographic area without personal or familial history of malignancy and other major disease. To exclude the effects of ethnic differences, only Koreans were included in this study. Informed consents were obtained according to the Helsinki Declaration. This study was approved by the institutional review board of the Catholic University of Korea, College of Medicine (CUMC09U089).

AGS, MKN-1, MKN-45, SNU-638, and Kato-Ⅲ gastric cancer cell lines were obtained from the American Type Culture Collection. These cells were cultured at 37 ℃ in 5% CO_2 in RPMI-1640 medium (Lonza, Basel, Switzerland) supplemented with 10%

Table 1 The Primer sequences used in this study

Gene	Primer sequences
hTERT rs2736100	F: 5′-GGT GCC TCC AGA AAA GCA G-3′ R: 5′-GAC ACG GAT CCA GGA CCT C-3′
Telomere standard	5′-TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG-3′
36B4 standard	5′-CAG CAA GTG GGA AGG TGT AAT CCG TCT CCA CAG ACA AGG CCA GGA CTC GTT TGT ACC CGT TGA TGA TAG AAT GGG-3′
Telomere	F: 5′-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3′ R: 5′-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3′
36B4	F: 5′-CAG CAA GTG GGA AGG TGT AAT CC-3′ R: 5′-CCC ATT CTA TCA TCA ACG GGT ACA A-3′
hTERT	F: 5′-ATG CGA CAG TTC GTG GCT CA-3′ R: 5′-ATC CCC TGG CAC TGG ACG TA-3′
GAPDH	F: 5′- AAA TCA AGT GGG GCG ATG CTG-3′ R: 5′- GCA GAG ATG ATG ACC CTT TTG-3′

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; hTERT: Human telomerase reverse transcriptase.

heat-inactivated fetal bovine serum.

DNA and RNA extraction

Non-neoplastic cells from specimens of gastric cancer patients were obtained from frozen cancer-free gastric mucosa. For healthy control population, a leukocyte cell pellet from each blood sample was obtained from the Buffy coat by centrifugation of 2 mL of whole blood. The cell pellet was used for DNA extraction. The Qiagen DNA Blood Mini Kit (Qiagen, Valencia, CA, United States) was used according to the manufacturer's instructions to obtain genomic DNA. The DNA purity and concentration were determined by Nanodrop® ND-1000 spectrophotometer (Nanodrop technologies, Wilmingon, DE, United States).

After quantification of mRNA extracted from frozen gastric mucosae, cDNA was synthesized using the reverse transcription kit from Roche Molecular System (Roche, Mannheim, Germany), according to the manufacturer's protocol.

Measurement of telomere length and hTERT expression in gastric cancer tissues and cell lines

Previously, we examined telomere length in 35 gastric cancer tissues by real-time quantitative polymerase chain reaction (qPCR)[10]. Briefly, after quantifying the genomic DNAs extracted from each sample, real-time SYBR Green qPCR was performed on a Stratagene Mx 3000P qPCR system (Stratagene, La Jolla, CA, United States). Sequences of the primers are described in Table 1. All samples were subjected to PCR amplification with specific oligonucleotide primers for the constitutively expressed single gene copy number (36B4) and normalized. The ratio of telomere repeat copy number to single-copy gene number (T/S ratio) was determined using the comparative Ct method. Sample T/S ratios were then divided with the T/S ratio of a reference DNA included in each plate, generating relative telomere length values. Each sample was loaded in triplicate, and all PCR-plates included a standard curve for PCR efficiency calculations.

In addition, *hTERT* mRNA transcript expression was examined in 5 gastric cancer cell lines and 35 gastric cancer tissues by real time reverse transcription PCR. After cDNA synthesis, 50 ng cDNA was amplified using Fullvelocity SYBR Green QPCR Master Mix (Stratagene) and 20 pmol/μL each of the forward and reverse primers on the Stratagene Mx 3000P QPCR system. To ensure the fidelity of mRNA extraction and reverse transcription, all samples were subjected to PCR amplification with oligonucleotide primers specific for the constitutively expressed gene, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and normalized. Sequences of the primers are described in Table 1. The standard curve method was used for quantification of the relative amounts of gene expression products. All samples were tested in duplicate, and the average values were used for quantification.

Since gastrokine 1 (GKN1) induces senescence and apoptosis in gastric cancer cells by regulating telomere length and hTERT expression[10], *hTERT* mRNA expression were measured in 5 gastric cancer cell lines after treatment with recombinant GKN1 (10 nmol/L, A&R therapeutics, Daejeon, South Korea) and correlated to rs2736100 polymorphism. To identify the effect of GKN1 on *hTERT* expression, non-treated gastric cells were used as a control. The effects of GKN1 were presented as the fold changes in *hTERT* expression relative to control.

PCR-restriction fragment length polymorphism analysis for rs2736100 polymorphism

Genomic DNAs were amplified with primers covering the rs2736100 polymorphism in the human *hTERT* gene. For PCR, the primer sequences were described in Table 1. The PCR procedure was performed under standard conditions in a 20 μL reaction mixture containing 2 μL of template DNA, 0.5 μmol/L of each primer, 0.2 μmol/L of each deoxynucleotide triphosphate, 1.5 mmol/L $MgCl_2$, 0.4 unit of Ampli Taq gold polymerase (Perkin-Elmer, Foster City, CA, United States), and 2 μL of 10 × buffer. The reaction mixture was denatured for 12 min at 95 ℃ and then incubated for 40 cycles (denaturing for 30 s at 95 ℃, annealing for 30 s at 55.4 ℃ and extension for 30 s at 72 ℃). A final extension step was performed for 5 min at 72 ℃. After amplification, the PCR products for rs2736100 polymorphism of *hTERT* were digested with 5 U of restriction enzyme SfcI at 37 ℃ for 4 h. DNA fragments then were electrophoresed on a 3% agarose gel. To ensure the reliability of the restriction fragment length polymorphism results, 50 (20.6%) PCR products of 243 gastric cancer cases were sequenced using the fluorescent dideoxy chain termination method with on ABI 3730XL Analyzer (Applied Biosystems, Foster city,

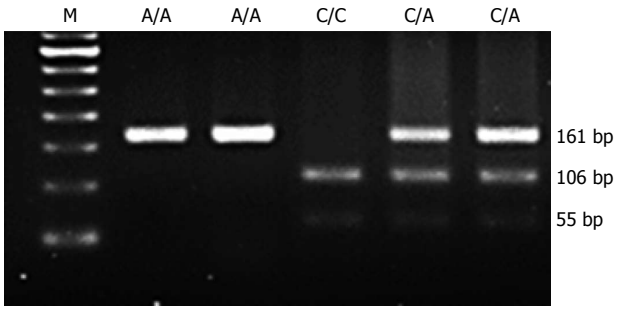


Figure 1 Genotypes of rs2736100 by polymerase chain reaction-restriction fragment length polymorphism. SfcI restriction enzyme recognizes and cuts the polymerase chain reaction (PCR) product of C-type allele. Lane M: 50 base pair ladder; Lane 1 and 2: SfcI digested PCR product is homozygous (A/A); Lane 3: SfcI digested PCR product is homozygous (C/C); Lane 4 and 5: SfcI digested PCR product is heterozygous (C/A).

CA, United States).

Statistical analysis

We firstly examined the association of *hTERT* polymorphism (rs2736100) with *hTERT* mRNA expression and telomere length in gastric cancer cell lines and tissues by student *t*-test and χ^2 test. Next, we conducted a two-tailed *t*-test to determine the differences in the percentages of genotypes and alleles between patients and healthy controls, and between the two histological types. The strength of association between allele frequencies and the stomach cancer was estimated by calculating the OR and 95%CI by logistic regression analysis using genotype or the number of allele as a regressor. An adjusted analysis was also performed by logistic regression analysis after adjustment for gender and age. A P values < 0.05 were considered to be statistically significant. The statistical methods of this study were reviewed by Professor Yong Gyu Park from The Catholic University of Korea.

RESULTS

Characterization of the study population

A total of 243 patients and 246 healthy individuals were included in this study. The 243 cases included 174 men (71.6%) and 69 women (28.4%) with a median age of 62.5 (22-83) years at initial diagnosis. Histologically, 145 cases were of intestinal-type (59.6%) and 98 were of diffuse-type (40.4%) gastric cancers. The average age was 63 ± 12 in cancer patients and 44 ± 8 in control group. Age of 50 was used for stratification and χ^2 test showed that age distribution had no statistical difference between two groups (P = 0.5721 and P = 0.6110, respectively).

Influence of rs2736100 polymorphism on telomere length and hTERT mRNA expression

A 161-bp PCR fragment containing the *hTERT* SfcI polymorphic site was amplified. The A allele has no restriction site and the C allele has one restriction site. After digestion with SfcI, three genotypes were demonstrated; C/C homozygote, yielding 106- and 55-bp fragments; C/A heterozygote, yielding 161-, 106-, and 55-bp fragments; and A/A homozygote as, 161-bp fragment (Figure 1).

Next, to investigate whether the genotype of rs2736100 polymorphism contributes to hTERT expression, the genotypes of 5 gastric cancer cell lines were compared with *hTERT* mRNA expression. As shown in Figure 2A, the genotype of AGS and MKN1cells was C/C homozygote, MKN-45 and SNU-638 were heterozygote, and KATO-Ⅲ was A/A homozygote. Interestingly, *hTERT* mRNA expression was markedly increased in AGS and MKN1 cells with C/C homozygote and reduced in KATO-Ⅲ cells with A/A homozygote, compared the cells with C/A heterozygote (Figure 2B). Consistent with our previous results[10], GKN1 treatment significantly down-regulated *hTERT* mRNA expression in all gastric cancer cell lines, especially in MKN-45 and SNU-638 cells with C/A heterozygote and KATO-Ⅲ cell with A/A homozygote (Figure 2C), suggesting that *hTERT* mRNA expression level is closely associated with the genotype of the rs2736100 polymorphism.

Previously, telomere length and *hTERT* mRNA levels in gastric cancer were measured in 35 subjects[10]. As shown in Figure 3A, the genotype of rs2736100 polymorphism of the *hTERT* gene was closely associated with *hTERT* mRNA and telomere length in gastric cancers (P = 0.0026 and P = 0.0199, respectively). When we compared the genotype of rs2736100 polymorphism with telomere length and *hTERT* mRNA levels, subjects carrying the A allele (A/A and C/A genotypes) showed lower *hTERT* mRNA levels and shortened telomere length, compared with those who had C/C homozygote genotype (P = 0.0016 and P = 0.0098, respectively) (Figure 3B).

When we stratified the gastric cancers by the Lauren classification, the telomere length was shortened in diffuse-type gastric cancer (P = 0.0251). However, there was no difference in *hTERT* mRNA expression between intestinal- and diffuse-type gastric cancers (P = 0.1485) (Figure 4A). When we combined C/A and A/A genotypes, telomere length and *hTERT* mRNA levels were significantly increased in those with the C/C genotype in intestinal-type gastric cancer (P = 0.0069 and P = 0.0204, respectively) (Figure 4B). However, the genotype of rs2736100 polymorphism was not associated with telomere length and *hTERT* expression in those with diffuse-type gastric cancer (P = 0.1414 and P = 0.2298, respectively) (Figure 4C).

Association of rs2736100 polymorphism with risk of gastric cancer

Next, to determine whether the rs2736100 polymorphism is associated with risk of gastric cancer in the Korean population, we investigated the genotype and allele frequencies of rs2736100 polymorphism in 243 gastric cancer patients and 246 healthy individuals.

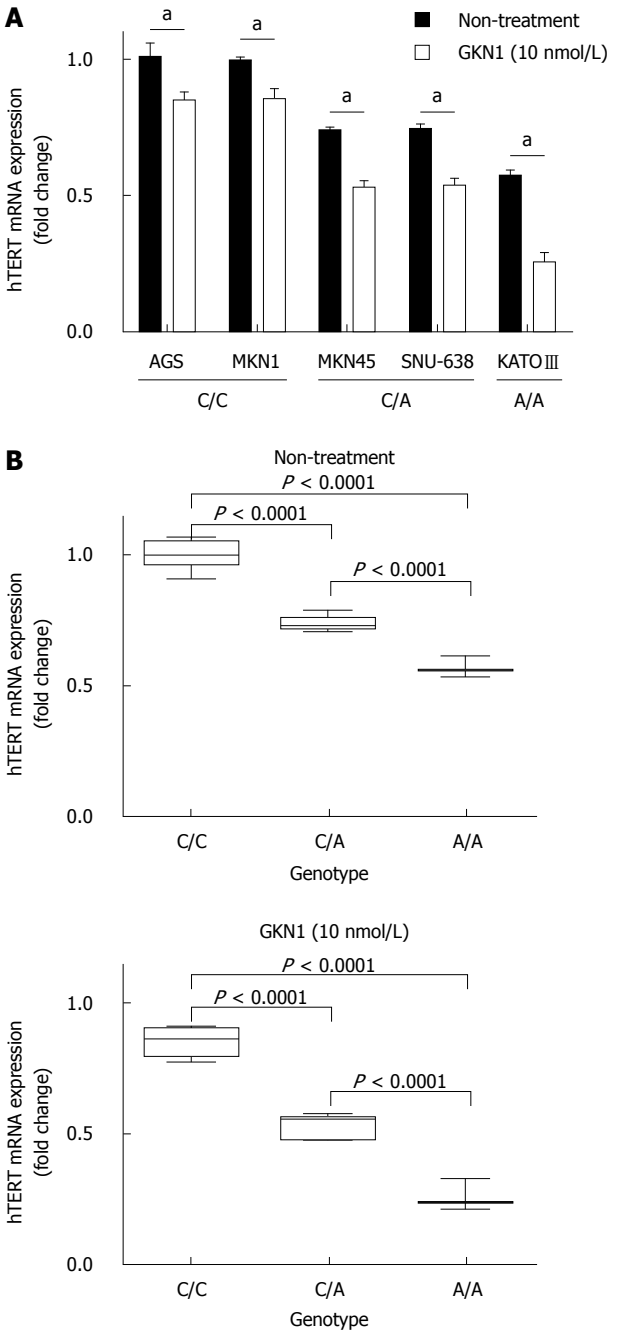


Figure 2 Association of the rs2736100 polymorphism with *hTERT* mRNA expression in 5 gastric cancer cell lines. A: The genotype of rs2736100 polymorphism of the *hTERT* gene was determined by polymerase chain reaction-restriction fragment length polymorphism. The genotype of AGS and MKN1 cells was C/C homozygote, MKN45 and SNU-638 was heterozygote, and KATO-Ⅲ was A/A homozygote. GKN1 treatment significantly reduced the expression of *hTERT* gene in 5 gastric cancer cell lines ($^aP < 0.05$); B: The genotype of rs2736100 polymorphism of the *hTERT* gene was closely associated with *hTERT* mRNA expression in non-treated gastric cancer cells; C: Fold changes of *hTERT* mRNA expression in GKN1-treated compared to non-treated gastric cancer cells were assessed by real time QPCR. GKN1 treatment markedly down-regulated *hTERT* mRNA expression, especially in MKN-45 and SNU-638 cells with C/A heterozygote and KATO-Ⅲ cell with A/A homozygote. hTERT: Human telomerase reverse transcriptase.

The distribution of genotypes in cancer group and control group was conformed to be in Hardy-Weinberg equilibrium, indicating that genotype distribution of both groups was a representative of the general population. The frequencies of the rs2736100 C/C, C/A, and A/A genotypes were 42.0% (102/243), 44.0% (107/243), and 14.0% (34/243), respectively, in gastric cancer patients and 35.0% (86/246), 49.6% (122/246) and 15.4% (38/246), respectively, in the healthy controls (Table 2). Statistically, no differences in the genotype and allele frequency of *hTERT* rs2736100 were observed between the healthy controls and gastric cancer patients ($P = 0.2797$ and $P = 0.1727$, respectively).

When the data were stratified according to histological subtype of gastric cancer, there was no significant difference in the risk of gastric cancer between intestinal- and diffuse-type ($P = 0.5448$) (Table 3). Compared with genotypes of healthy controls, no significant difference in the risk of intestinal- and diffuse-type gastric cancers was found in the carriers with an A allele (A/C or A/A genotypes) and those with the C/C genotype ($P = 0.0528$ and $P = 0.6256$) (Table 3). These findings suggest that rs2736100 polymorphism of the *hTERT* gene may not be associated with susceptibility to the development and differentiation of gastric cancer in Korean population.

DISCUSSION

Normal cells divide for a limited number of times, whereas cancer cells usually have the ability to proliferate indefinitely[11]. In healthy cells, telomere length is highly regulated in a tissue- and cell type-specific manner and is dependent on mitotic turnover rate, telomerase activity, and telomerase-associated factors[12]. Telomerase, which adds TTAGGG sequences to the 3' ends of telomeric DNA and promotes genomic stability, is a critical determinant of telomere length[13]. Telomeres play an important role in the maintenance of genomic stability and defects in telomere maintenance contribute to the initiation of genomic instability during carcinogenesis, including gastric cancer[8,14,15]. Interestingly, telomere shortening and dysfunction have been suggested to contribute to cancer susceptibility by increasing the risk of chromosomal aberrations caused by the breakage-fusion-bridge cycle[16]. Since cells with sufficiently shortened telomere enter an irreversible growth arrest called cellular senescence, it is necessary to elucidate mechanisms of telomere length regulation for the development of novel anti-cancer agents.

Human telomerase is composed of two subunits: telomerase RNA (hTERC) which serves as the template for telomere elongation and telomerase reverse transcriptase (hTERT) which possesses the catalytic activity to synthesize DNA from the RNA template[17-19]. The SNP rs2736100 is one of the most common variants of the *hTERT* gene associated with cancer risk[20-23]. In particular, it has been reported that the C allele of rs2736100 was significantly associated with longer telomere length of germ-cells[9,24]. Since telomerase activity was presently increased in most

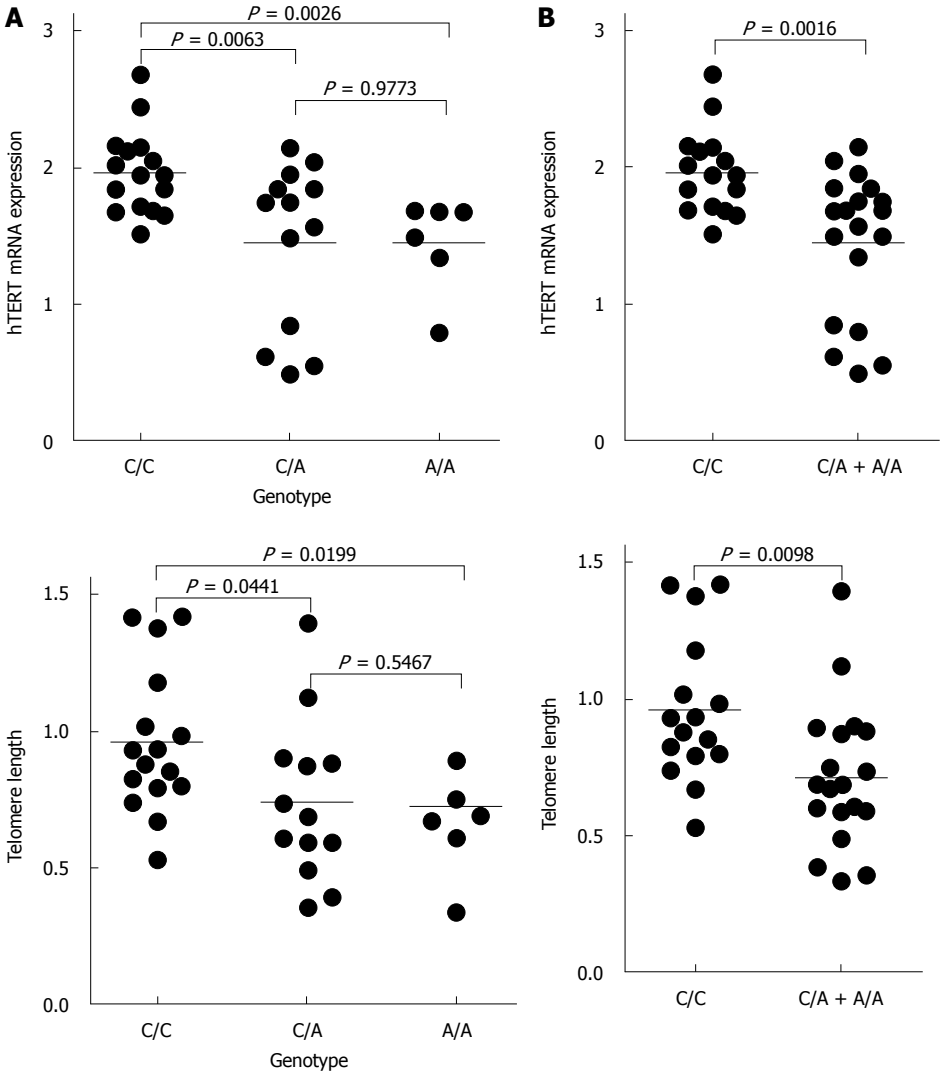


Figure 3 Association of the rs2736100 polymorphism with *hTERT* mRNA expression and telomere length in 35 gastric cancers. A: The genotype of rs2736100 polymorphism of the *hTERT* gene was closely associated with *hTERT* mRNA expression and telomere length ($P = 0.0026$ and $P = 0.0199$, respectively); B: When we combined C/A and A/A genotypes, the cases carrying the A allele (A/A and C/A genotypes) showed lower *hTERT* mRNA expression levels and shortened telomere length, compared with those who had C/C homozygote genotype ($P = 0.0016$ and $P = 0.0098$, respectively). hTERT: Human telomerase reverse transcriptase.

cancer cells, it is possible that the C allele is closely associated with increased *hTERT* expression and a higher telomerase activity, suggesting that this SNP acts on the *hTERT* gene encoding the reverse transcriptase of the telomerase complex, essential for maintaining the telomere length. In this study, we also found that *hTERT* mRNA expression level markedly increased in the gastric cancer cells with C/C homozygote and was reduced in cells with A/A homozygote, compared the cells with C/A heterozygote (Figure 2). Furthermore, the rs2736100 C allele specifically influences the cellular response to anti-mitotic agents[25]. Our previous study indicated that GKN1 may inhibit the telomere elongation by suppressing c-myc-induced hTERT expression[10]. Thus, we investigated whether the effect of GKN1 on hTERT expression is associated with the rs2736100 polymorphism in 5 gastric cancer cell lines. Expectedly, GKN1 treatment significantly down-regulated *hTERT* mRNA expression in MKN-45 and SNU-638 cells with C/A heterozygote and KATO-Ⅲ cells with A/A homozygote (Figure 2C). Taken together, these results suggest that *hTERT* mRNA expression level is closely associated with rs2736100 polymorphism. However, further studies are strongly needed to identify the molecular mechanisms by which GKN1 regulates the rs2736100 polymorphism.

Next, we further confirmed the effect of rs2736100 polymorphism on *hTERT* mRNA expression and telomere length in gastric cancer tissues (Figure 3A and B). As shown in Figure 3B, the cases carrying the A allele (A/A and C/A genotypes) showed lower *hTERT* mRNA levels and shortened telomere length, compared with those who had C/C homozygote genotype ($P = 0.0016$ and $P = 0.0098$, respectively). Although the sample size ($n = 35$) was limited, it is likely that genotype of the rs2736100 polymorphism

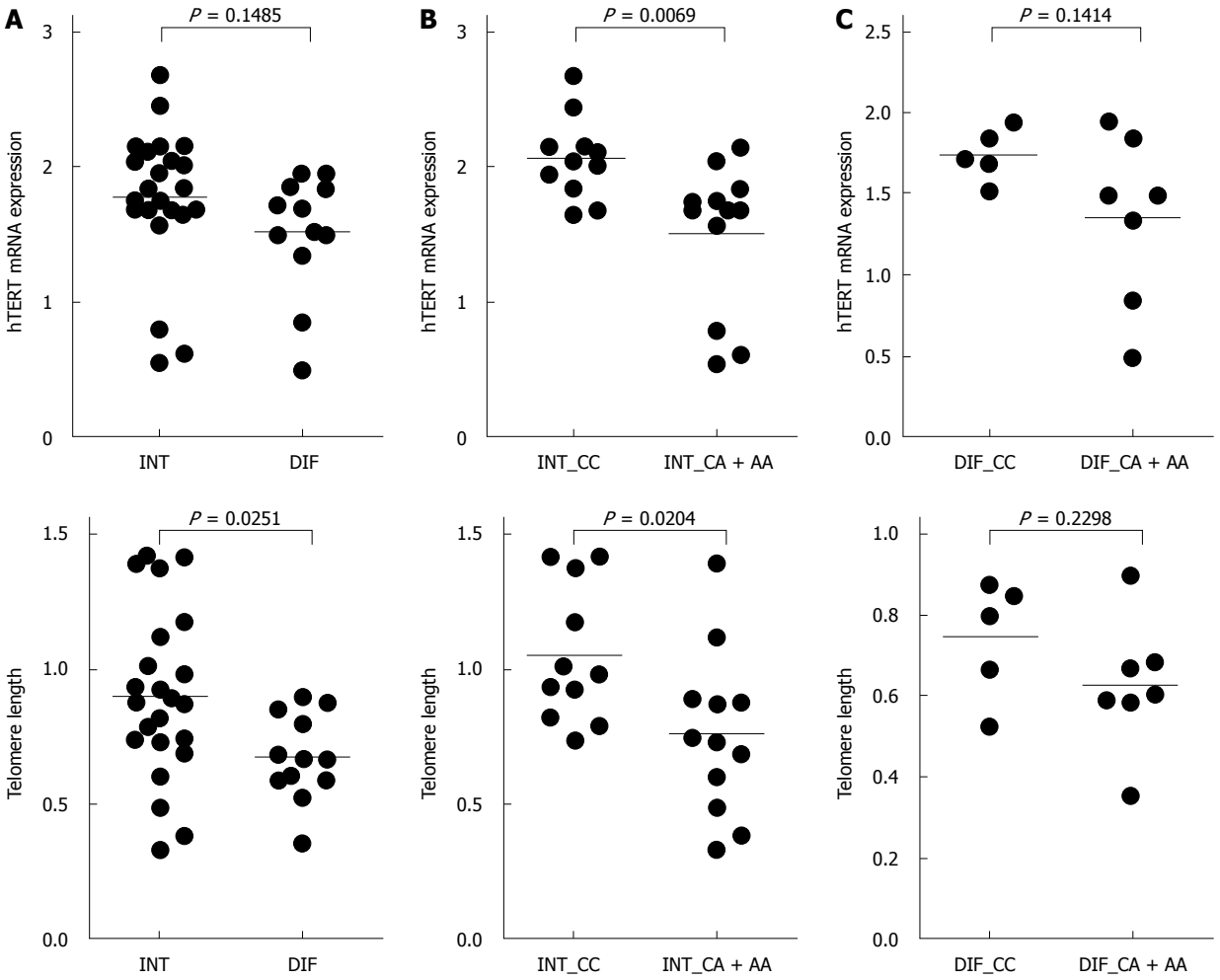


Figure 4 Association of the rs2736100 polymorphism with *hTERT* mRNA expression and telomere length in intestinal- and diffuse-type gastric cancers. A: The telomere length shortened in diffuse-type gastric cancer (P = 0.0251), but *hTERT* mRNA expression was not associated with histologic differentiation of gastric cancers (P = 0.1485); B: When we combined C/A and A/A genotypes, telomere length and *hTERT* mRNA levels were markedly increased in those with the C/C genotype in intestinal-type gastric cancer (P = 0.0069 and P = 0.0204, respectively); C: The rs2736100 polymorphism was not associated with *hTERT* expression and telomere length in diffuse-type gastric cancers (P = 0.1414 and P = 0.2298, respectively). hTERT: Human telomerase reverse transcriptase; INT: Intestinal-type gastric cancer, DIF: Diffuse-type gastric cancer.

Table 2 Genotype and allele distribution of rs2736100 gene polymorphism in gastric cancer patients and controls

	Patients (n = 243), n (%)	Controls (n = 246), n (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI)
CC	102 (42.0)	86 (35.0)	1.00	1.00
CA	107 (44.0)	122 (49.6)	0.739 (0.502-1.089)	0.741 (0.447-1.228)
AA	34 (14.0)	38 (15.4)	0.754 (0.438-1.300)	0.811 (0.373-1.760)
C:A allele frequency[2]	311:175 (0.640)	294:198 (0.598)		
Trend test[3]			P = 0.1501	P = 0.3454

[1]Adjusted for age (in year) and sex; [2]Two-sided χ^2 test: for allele frequencies, P = 0.1727; for genotype distribution, P = 0.2797; [3]Calculated in the logistic regression model using the number of A alleles in the genotypes as a continuous variable.

may be closely associated with *hTERT* expression and telomere length in gastric cancers.

When the gastric cancers were stratified by histologic type, the telomere length was significantly shortened in the diffuse-type (P = 0.0251), but not the intestinal-type (Figure 4A). When we combined C/A and A/A genotypes, telomere length and *hTERT* mRNA levels were markedly increased in intestinal-type gastric cancers with the C/C genotype (P = 0.0069 and P = 0.0204, respectively) (Figure 4B). However, these effects of the rs2736100 polymorphism on *hTERT* expression and telomere length were not detected in diffuse-type gastric cancers (P = 0.1414 and P = 0.2298, respectively) (Figure 4C). Therefore, our findings suggest that the rs2736100 polymorphism may be involved in telomere length maintenance of intestinal-type gastric cancer cells. In the future, to determine the significance of rs2736100 polymorphism on the differentiation of gastric cancer cells, further studies on large-scale should be performed.

To identify that rs2736100 polymorphism contribute to the risk of gastric cancer in the Korean population,

Table 3 Genotype and allele distribution of rs2736100 gene polymorphism in gastric cancer patients according to histopathology

	Patients (n = 243)			Controls (n = 246)			Adjusted[1] OR (95%CI)	Adjusted[1] OR (95%CI)
	CC	CA	AA	CC	CA	AA	CA *vs* CC	AA *vs* CC
Age (yr)								
≤ 50	14	20	1	72	98	33	1.053 (0.498-2.226)	0.229 (0.039-1.351)
> 50	88	87	33	14	24	5	0.568 (0.272-1.184)	1.029 (0.342-3.101)
Gender								
M	72	77	25	49	63	23	0.871 (0.452-1.679)	1.070 (0.392-2.923)
F	30	30	9	37	59	15	0.582 (0.262-1.293)	0.574 (0.162-2.031)
Lauren's								
Diffuse	37	46	15		P = 0.5448			
Intestinal	65	61	19		(χ^2 test)			

[1]Adjusted for the other covariates [age (in years) as a continuous variable] presented in this table in a logistic regression model for each stratum.

we investigated the genotype and allele frequencies of rs2736100 polymorphism in tissue specimens from 243 gastric cancer patients and 246 healthy individuals. Statistically, no differences in the genotype and allele frequencies of *hTERT* rs2736100 polymorphism were observed between the healthy controls and gastric cancer patients (P = 0.2797 and P = 0.1727, respectively) (Table 2). When gastric cancers were stratified according to histological subtype, there was no significant difference in the risk of intestinal- and diffuse-type gastric cancer between the carriers with an A allele (A/C or A/A genotypes) and those with the C/C genotype (P = 0.0528 and P = 0.6256) (Table 3), compared with genotypes of healthy controls. Thus, we conclude that rs2736100 polymorphism of the *hTERT* gene may not be associated with susceptibility to the development and differentiation of gastric cancer in Korean population.

Although our study had limited statistical power probably due to their small sample size, while limited, we showed that the rs2736100 polymorphism of *hTERT* significantly affect telomere length and *hTERT* mRNA expression in gastric cancer cell lines and tissues. Unexpectedly, genotype and allele frequencies of the polymorphism were not associated with susceptibility to the development and the differentiation of gastric cancer in the Korean population. Further studies on large population are strongly necessary to clarify the biological significances and the exact effects of these polymorphisms in regulating hTERT expression.

COMMENTS

Background

Telomere length is dependent on mitotic turnover rate, telomerase activity, and telomerase-associated factors. Cells with sufficiently shortened telomere enter an irreversible growth arrest called cellular senescence. Telomerase activity is frequently detected in almost all types of human cancers, suggesting the importance of telomerase in cancer development. Single nucleotide polymorphism (SNP) in the telomerase reverse transcriptase (*TERT*) gene may influence *hTERT* expression and telomere length, thereby modulating the susceptibility to some cancers.

Research frontiers

It is necessary to elucidate mechanisms of telomere length regulation for the development of novel anti-cancer agents. SNP in the second intron of the *hTERT* gene, rs2736100, may affect either the expression or telomerase activity and therefore may be associated with gastric cancer risk. It has been reported that telomere length in gastric cancers is positively correlated with *hTERT* expression and the rs2736100 C allele is associated with long telomere. The current research hotspot is to identify the significance of the rs2736100 polymorphism in telomere length regulation and genetic susceptibility of an individual to gastric cancer.

Innovations and breakthroughs

Telomerase activity was frequently increased in most cancer cells. The rs2736100 polymorphism was closely associated with the *hTERT* expression and telomere length in gastric cancer cells and tissues. Treatment with Gastrokine 1 (GKN1), an inhibitor of telomere elongation, significantly decreased *hTERT* mRNA expression. However, rs2736100 polymorphism of the *hTERT* gene was not associated with susceptibility to the development and differentiation of gastric cancer in the Korean population.

Applications

These results suggest that the rs2736100 polymorphism of *hTERT* can be used as a potential biomarker for the development of anti-cancer agents regulating telomere length in gastric cancer cells.

Peer-review

This is a good study in which the authors analyzed the influence of rs2736100 polymorphism on telomere length and gastric cancer risk in the Koreans. The results are interesting and suggest that the rs2736100 polymorphism of *hTERT* could be used as a potential biomarker of telomere length and *hTERT* mRNA expression in gastric cancer cells.

REFERENCES

1 **Cunningham SC**, Kamangar F, Kim MP, Hammoud S, Haque R, Iacobuzio-Donahue CA, Maitra A, Ashfaq R, Hustinx S, Heitmiller RE, Choti MA, Lillemoe KD, Cameron JL, Yeo CJ, Schulick RD, Montgomery E. Claudin-4, mitogen-activated protein kinase kinase 4, and stratifin are markers of gastric adenocarcinoma precursor lesions. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 281-287 [PMID: 16492916]

2 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

3 **Jung KH**, Kim SM, Choi MG, Lee JH, Noh JH, Sohn TS, Bae JM, Kim S. Preoperative smoking cessation can reduce postoperative complications in gastric cancer surgery. *Gastric Cancer* 2014; Epub ahead of print [PMID: 25139298]

4 **Correa P**. A human model of gastric carcinogenesis. *Cancer Res* 1988; **48**: 3554-3560 [PMID: 3288329]

5 **Stemmermann GN**. Intestinal metaplasia of the stomach. A status report. *Cancer* 1994; **74**: 556-564 [PMID: 8033033]

6 **Donate LE**, Blasco MA. Telomeres in cancer and ageing. *Philos Trans R Soc Lond B Biol Sci* 2011; **366**: 76-84 [PMID: 21115533 DOI: 10.1098/rstb.2010.0291]

7 **Dhaene K**, Van Marck E, Parwaresch R. Telomeres, telomerase and cancer: an up-date. *Virchows Arch* 2000; **437**: 1-16 [PMID: 10963374]

8 **Yoo J**, Park SY, Kang SJ, Kim BK, Shim SI, Kang CS. Expression of telomerase activity, human telomerase RNA, and telomerase reverse transcriptase in gastric adenocarcinomas. *Mod Pathol* 2003; **16**: 700-707 [PMID: 12861067]

9 **Codd V**, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, Hottenga JJ, Fischer K, Esko T, Surakka I, Broer L, Nyholt DR, Mateo Leach I, Salo P, Hägg S, Matthews MK, Palmen J, Norata GD, O'Reilly PF, Saleheen D, Amin N, Balmforth AJ, Beekman M, de Boer RA, Böhringer S, Braund PS, Burton PR, de Craen AJ, Denniff M, Dong Y, Douroudis K, Dubinina E, Eriksson JG, Garlaschelli K, Guo D, Hartikainen AL, Henders AK, Houwing-Duistermaat JJ, Kananen L, Karssen LC, Kettunen J, Klopp N, Lagou V, van Leeuwen EM, Madden PA, Mägi R, Magnusson PK, Männistö S, McCarthy MI, Medland SE, Mihailov E, Montgomery GW, Oostra BA, Palotie A, Peters A, Pollard H, Pouta A, Prokopenko I, Ripatti S, Salomaa V, Suchiman HE, Valdes AM, Verweij N, Viñuela A, Wang X, Wichmann HE, Widen E, Willemsen G, Wright MJ, Xia K, Xiao X, van Veldhuisen DJ, Catapano AL, Tobin MD, Hall AS, Blakemore AI, van Gilst WH, Zhu H, Consortium C, Erdmann J, Reilly MP, Kathiresan S, Schunkert H, Talmud PJ, Pedersen NL, Perola M, Ouwehand W, Kaprio J, Martin NG, van Duijn CM, Hovatta I, Gieger C, Metspalu A, Boomsma DI, Jarvelin MR, Slagboom PE, Thompson JR, Spector TD, van der Harst P, Samani NJ. Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet* 2013; **45**: 422-427, 427e1-e2 [PMID: 23535734 DOI: 10.1038/ng.2528]

10 **Yoon JH**, Seo HS, Choi WS, Kim O, Nam SW, Lee JY, Park WS. Gastrokine 1 induces senescence and apoptosis through regulating telomere length in gastric cancer. *Oncotarget* 2014; **5**: 11695-11708 [PMID: 25344918]

11 **Shay JW**, Zou Y, Hiyama E, Wright WE. Telomerase and cancer. *Hum Mol Genet* 2001; **10**: 677-685 [PMID: 11257099]

12 **Collado M**, Blasco MA, Serrano M. Cellular senescence in cancer and aging. *Cell* 2007; **130**: 223-233 [PMID: 17662938]

13 **Blackburn EH**, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nat Med* 2006; **12**: 1133-1138 [PMID: 17024208]

14 **Fang DC**, Yang SM, Zhou XD, Wang DX, Luo YH. Telomere erosion is independent of microsatellite instability but related to loss of heterozygosity in gastric cancer. *World J Gastroenterol* 2001; **7**: 522-526 [PMID: 11819821]

15 **Omori Y**, Nakayama F, Li D, Kanemitsu K, Semba S, Ito A, Yokozaki H. Alternative lengthening of telomeres frequently occurs in mismatch repair system-deficient gastric carcinoma. *Cancer Sci* 2009; **100**: 413-418 [PMID: 19154407 DOI: 10.1111/j.1349-7006.2008.01063.x]

16 **DePinho RA**. The age of cancer. *Nature* 2000; **408**: 248-254 [PMID: 11089982]

17 **Newbold RF**. The significance of telomerase activation and cellular immortalization in human cancer. *Mutagenesis* 2002; **17**: 539-550 [PMID: 12435851]

18 **Shay JW**, Wright WE. Senescence and immortalization: role of telomeres and telomerase. *Carcinogenesis* 2005; **26**: 867-874 [PMID: 15471900]

19 **Artandi SE**, DePinho RA. Telomeres and telomerase in cancer. *Carcinogenesis* 2010; **31**: 9-18 [PMID: 19887512 DOI: 10.1093/carcin/bgp268]

20 **Zou P**, Gu A, Ji G, Zhao L, Zhao P, Lu A. The TERT rs2736100 polymorphism and cancer risk: a meta-analysis based on 25 case-control studies. *BMC Cancer* 2012; **12**: 7 [PMID: 22221621 DOI: 10.1186/1471-2407-12-7]

21 **Mocellin S**, Verdi D, Pooley KA, Landi MT, Egan KM, Baird DM, Prescott J, De Vivo I, Nitti D. Telomerase reverse transcriptase locus polymorphisms and cancer risk: a field synopsis and meta-analysis. *J Natl Cancer Inst* 2012; **104**: 840-854 [PMID: 22523397 DOI: 10.1093/jnci/djs222]

22 **Kinnersley B**, Migliorini G, Broderick P, Whiffin N, Dobbins SE, Casey G, Hopper J, Sieber O, Lipton L, Kerr DJ, Dunlop MG, Tomlinson IP, Houlston RS. The TERT variant rs2736100 is associated with colorectal cancer risk. *Br J Cancer* 2012; **107**: 1001-1008 [PMID: 22878375 DOI: 10.1038/bjc.2012.329]

23 **Hu Z**, Wu C, Shi Y, Guo H, Zhao X, Yin Z, Yang L, Dai J, Hu L, Tan W, Li Z, Deng Q, Wang J, Wu W, Jin G, Jiang Y, Yu D, Zhou G, Chen H, Guan P, Chen Y, Shu Y, Xu L, Liu X, Liu L, Xu P, Han B, Bai C, Zhao Y, Zhang H, Yan Y, Ma H, Chen J, Chu M, Lu F, Zhang Z, Chen F, Wang X, Jin L, Lu J, Zhou B, Lu D, Wu T, Lin D, Shen H. A genome-wide association study identifies two new lung cancer susceptibility loci at 13q12.12 and 22q12.2 in Han Chinese. *Nat Genet* 2011; **43**: 792-796 [PMID: 21725308 DOI: 10.1038/ng.875]

24 **Melin BS**, Nordfjäll K, Andersson U, Roos G. hTERT cancer risk genotypes are associated with telomere length. *Genet Epidemiol* 2012; **36**: 368-372 [PMID: 22539396 DOI: 10.1002/gepi.21630]

25 **Kim J**, Jones-Hall YL, Wei R, Myers J, Qi Y, Knipp GT, Liu W. Association between hTERT rs2736100 polymorphism and sensitivity to anti-cancer agents. *Front Genet* 2013; **4**: 162 [PMID: 23986774 DOI: 10.3389/fgene.2013.00162]

P- Reviewer: Boardman LA, Silva AE, G Shen GM, Yang SM
S- Editor: Yu J **L- Editor**: A **E- Editor**: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9337

World J Gastroenterol 2015 August 21; 21(31): 9337-9347
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

MiR-30b suppresses tumor migration and invasion by targeting EIF5A2 in gastric cancer

Shu-Bo Tian, Jian-Chun Yu, Yu-Qin Liu, Wei-Ming Kang, Zhi-Qiang Ma, Xin Ye, Chao Yan

Shu-Bo Tian, Jian-Chun Yu, Wei-Ming Kang, Zhi-Qiang Ma, Xin Ye, Chao Yan, Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China

Yu-Qin Liu, Cell Centre, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing 100730, China

Author contributions: Tian SB and Yu JC designed the research; Tian SB, Ye X and Yan C performed the experiments; Liu YQ, Kang WM and Ma ZQ analyzed the data; Tian SB and Yan C wrote the paper.

Supported by Beijing Municipal Natural Science Foundation of China, No. 7132209; and The Capital Health Research and Development of Special, No. 2014-3-4014.

Institutional review board statement: The study was reviewed and approved by the Ethics Committee of Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Data sharing statement: No additional data are available.

Correspondence to: Jian-Chun Yu, Professor, Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 1 Shuaifuyuan, Dongcheng District, Beijing 100730, China. yujch_pumch@126.com
Telephone: +86-10-69152215
Fax: +86-10-69152215

Received: March 11, 2015
Peer-review started: March 12, 2015
First decision: March 26, 2015
Revised: June 17, 2015
Accepted: July 8, 2015
Article in press: July 8, 2015
Published online: August 21, 2015

Abstract

AIM: To elucidate the potential biological role of miR-30b in gastric cancer and investigate the underlying molecular mechanisms of miR-30b to inhibit metastasis of gastric cancer cells.

METHODS: The expression of miR-30b was detected in gastric cancer cell lines and samples by reverse transcription-polymerase chain reaction. CCK-8 assays were conducted to explore the impact of miR-30b overexpression on the proliferation of gastric cancer cells. Flow cytometry was used to examine the effect of miR-30b on the apoptosis. Transwell test was used for the migration and invasion assays. Luciferase reporter assays and Western blot were employed to validate regulation of putative target of miR-30b.

RESULTS: The results showed that miR-30b was downregulated in gastric cancer tissues and cancer cell lines and functioned as a tumor suppressor. Over-expression of miR-30b promoted cell apoptosis, and suppressed proliferation, migration and invasion of the gastric cancer cell lines AGS and MGC803. Bioinformatic analysis identified the 3′-untranslated region of eukaryotic translation initiation factor 5A2 (EIF5A2) as a putative binding site of miR-30b. Luciferase reporter assays and Western blot analysis confirmed the EIF5A2 gene as a target of miR-30b. Moreover, expression levels of the

EIF5A2 targets E-cadherin and Vimentin were altered following transfection of miR-30b mimics.

CONCLUSION: Our findings describe a link between miR-30b and EIF5A2, which plays an important role in mediating epithelial-mesenchymal transition.

Key words: miR-30b; Gastric cancer; EIF5A2; Migration; Invasion

Core tip: In this study, we found that miR-30b expression was reduced in gastric cancer cell lines and in gastric cancer tissues. Moreover, we found that miR-30b inhibited gastric cancer cell proliferation, migration, invasion and promoted apoptosis by targeting EIF5A2. Restoration of miR-30b expression could enhance E-cadherin and β-catenin expression and suppress Vimentin expression by targeting EIF5A2 and eventually inhibit the epithelial-to-mesenchymal transition (EMT) process in gastric cancer cells, whereas knockdown of miR-30b promoted cell invasion and EMT in cancer cells.

Tian SB, Yu JC, Liu YQ, Kang WM, Ma ZQ, Ye X, Yan C. MiR-30b suppresses tumor migration and invasion by targeting EIF5A2 in gastric cancer. *World J Gastroenterol* 2015; 21(31): 9337-9347 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9337.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9337

INTRODUCTION

Metastasis to distant sites is the primary cause of death in patients with gastric cancer. Patients with advanced disease frequently develop recurrence and metastasis, even in early gastric cancer, the incidence of lymph node metastasis exceeds 10%[1]. However, the underlying molecular mechanism of metastasis is not entirely clear. Epithelial-to-mesenchymal transition (EMT) is a key molecular step during progression of gastric cancer to metastasis[2], and is associated with poor prognosis[3]. In this process, epithelial cancer cells in primary tumors lose cell-cell adhesion following E-cadherin repression and acquire a mesenchymal phenotype. This enhances the ability of cancer cells to metastasize and invade distant locations.

MicroRNAs (miRNAs) are small non-coding RNAs which negatively regulate gene expression. Various studies have described functional roles for miRNAs as oncogenes or tumor-suppressor genes. For example, miR-199a was found significantly upregulated in gastric cancer where it mediated an increase in cell proliferation and suppressed apoptosis[4]. miR-7 and miR-9 are important tumor suppressors which target various genes in gastric cancer[5,6]. Additionally, the miRNA expression profile in plasma from gastric cancer patients is different to that from normal individuals and may represent an early diagnostic biomarker for gastric cancer[7]. Furthermore, miR-15b and miR-16 could modulate the sensitivity of gastric cancer cells to chemotherapeutic drugs by regulating BCL2 expression[8]. All these suggest that miRNAs could serve as potential diagnostic biomarkers and therapeutic tools.

Accumulating evidence describes vital roles for many miRNAs in tumor initiation and metastasis. For example, miR-205 and the miR-200 family influence the EMT process during cancer metastasis[9]. Additionally, miR-7 can inhibit the EMT process in gastric cancer through targeting *IGF1R* expression[5]. In colorectal carcinoma, miR-30b directly targets the EMT-related gene *SIX1* to impair metastasis of colorectal cancer cells[10]. Our current study adds to this knowledge by describing a role for miR-30b in the repression of gastric cancer cell metastasis.

The mechanisms underlying action of miR-30b on gastric cancer cell regulation have not yet been characterized. EIF5A2 functions as an oncogenic protein in many human cancers[11], and we have identified an miR-30b target site in the 3'-untranslated region (UTR) of *EIF5A2* mRNA. Overexpression of miR-30b reduces levels of EIF5A2 mRNA and protein, affecting expression of downstream targets of EIF5A2. To the best of our knowledge, this is the first report of miR-30b directly targeting EIF5A2 to promote cellular apoptosis, and suppress proliferation, invasion, and metastasis of gastric cancer cells.

MATERIALS AND METHODS

Gastric cancer tissue specimens

Gastric cancer and corresponding non-tumorous gastric tissue specimens were collected from patients who underwent surgical resection at Peking Union Medical College Hospital (Beijing, China). No patients underwent chemotherapy or radiotherapy before surgery. A pathological diagnosis of gastric cancer was verified by at least two pathologists. All samples were frozen in liquid nitrogen and stored at -80 ℃ until use.

Cell culture and reagents

The human gastric cancer cell lines MKN45, MKN28, HGC27, and SGC7901, and human embryonic kidney (HEK) 293T cells were provided by the Cell Center of the Chinese Academy of Medical Sciences. The gastric cancer cell lines MGC803, N87, and AGS, and immortalized gastric mucosa GES-1 cells were from stores in our institute. HEK 293T cells were cultured in Dulbecco's modified Eagle's medium (Hyclone, Logan Utah, United States) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, CA, United States). All other cell lines were grown routinely in RPMI-1640 medium with 10% FBS. All cells were

cultured at 37 ℃ in a humidified incubator with 5% CO_2.

SYBR green quantitative RT-PCR analysis

Total RNA from tissues and cell lines was extracted using Trizol Reagent (Invitrogen) according to the manufacturer's instructions. RNA was reverse-transcribed into cDNA with miRNA PrimeScript RT Enzyme (Takara, Dalian, China). Real-time RT-PCR was performed using SYBR Premix Ex Taq Ⅱ (Takara), using U6 as the internal reference. PCR reactions were conducted using a 7300 Real-Time PCR system (ABI, United States) under the following conditions: 95 ℃ for 30 s followed by 40 cycles of 95 ℃ for 5 s, and 60 ℃ for 34 s. DNA primers specific for miR-30b and U6 small nuclear RNA were purchased from RiboBio (Guangzhou, China). The $2^{-\Delta\Delta Ct}$ method was used to quantify relative miRNA expression. Experiments were performed in triplicate.

Transient transfection with miRNA mimic and inhibitor

Ectopic expression of miR-30b was performed by transfection with an miR-30b mimic or inhibitor (RiboBio) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. MiR-30b mimic control and inhibitor control were also synthesized by RiboBio. The sequences are as follows: miR-30b mimic, 5'-UGUAAACAUCCUACACUCAGCU-3' (sense), and 3'-ACAUUUGUAGGAUGUGAGUCGA-5' (antisense); miR-30b inhibitor, 5'-AGCUGAGUGUAGGAUGUUUACA-3'; miR-30b mimic control, 5'-UCACAACCUCCUAGAAA-GAGUAGA-3'; miR-30b inhibitor control, 5'-UCACAACCU-CCUAGAAAGAGUAGA-3'.

Cellular proliferation assays

Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) (Dojindo) according to manufacturer's instructions. Twenty-four hours after transient transfection of miRNA mimic or inhibitor, cells were harvested and seeded into 96-well plates at a density of 2×10^3 cells/well. Following incubation of cells for 24, 48, 72, or 96 h, the CCK-8 reagent (10 μL/well) was added to each well 1 h before the assay. The number of viable cells was assessed by measurement of OD450 values.

Apoptosis analysis

Quantification of apoptosis was conducted using an Annexin V-FITC Apoptosis Detection Kit (NeoBioscience, China). Cells were transfected with 50 nmol/L miR-30b mimic upon reaching 60% confluence in 6-well plates. Cells were then analyzed using a flow cytometer (BD Accuri C6).

Cell migration and invasion

Analyses of tumor cell migration and invasion were carried out using transwell chambers (8 μm Corning, United States). Forty-eight hours after transfection with miR-30b mimic or inhibitor, 2×10^5 AGS or MGC803 cells in serum-free medium were collected and seeded in an upper chamber containing a non-Matrigel coated membrane. Next, 500 μL medium with 20% FBS was added to the lower chamber. For the invasion assay, chambers were coated with extracellular Matrigel (BD Biosciences, United States). Cells were cultured at 37 ℃ in a humidified incubator with 5% CO_2. Non-migrating or non-invading cells in the upper chamber were removed with a cotton swab and cells that migrated or invaded to the bottom chamber were fixed and stained with 0.1% crystal violet. Nine fields at × 100 magnification were randomly selected and cell numbers counted. The results were averaged among three independent experiments.

Plasmid construction and luciferase activity assay

The 751-bp fragment of wild-type (wt) EIF5A2-3'-UTR containing the putative miR-30b binding site was synthesized by PCR with the primers 5'-GCGCTCGAGTATTGTAGTCTGTTGGTGCC-3' (forward) and 5'-AATGCGGCCGCTTTTCTTAAATCTTTGTTGC-3' (reverse). This fragment was then inserted between the XhoI and NotI sites of the luciferase reporter vector pmiR-RB-REPORT™ (RiboBio). Mutations to the miR-30b seed sequence within the EIF5A2 3'-UTR were also generated. Constructs were validated by DNA sequencing. HEK293T cells were grown in 6-well plates and transiently co-transfected with 2 μg reporter plasmid and 50 nmol/L miRNA using Lipofectamine 2000. Twenty-four hours after transfection, luciferase activity was measured using the Dual-luciferase Reporter Assay System (Promega, United States). Firefly luciferase activity was normalized against the Renilla luciferase activity. Three independent experiments were performed in triplicate.

Western blot analysis

Cells were seeded in 6-well plates and transfected with miR-30b mimic or inhibitor for 72 h. Cells were then lysed in RIPA buffer (Genstar, Beijing, China) with 1% phenylmethylsulfonyl fluoride, and protein concentrations were determined by BCA assay. Samples were then denatured and 80 μg total proteins from each sample separated on a 10% SDS-PAGE gel and transferred onto PVDF membranes. Membranes were then blocked in 5% non-fat milk in Tris-buffered saline with 0.1% Tween-20 and incubated with primary antibody (rabbit anti-EIF5A2 monoclonal antibody, 1:1000; mouse anti-β-actin monoclonal antibody, 1:1000, Abcam, United States) overnight at 4 ℃. The next day, membranes were washed and incubated with appropriate horseradish peroxidase-conjugated secondary antibodies. Signals were visualized using enhanced chemiluminescence reagent (Thermo) according to the manufacturer's instructions.

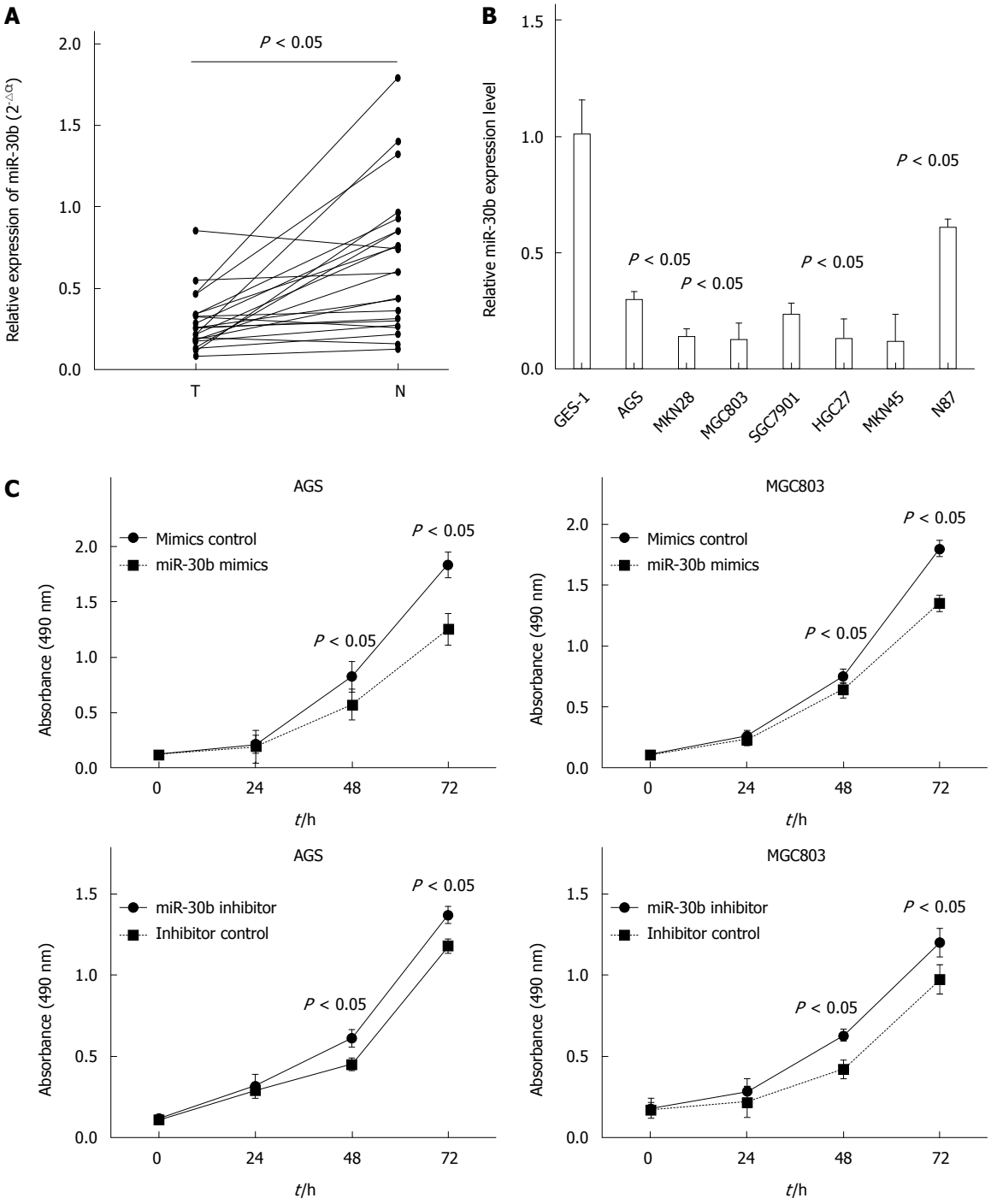


Figure 1 Expression levels of miR-30b in gastric tissue samples and gastric cell lines. A: MiR-30b expression was determined in 23 pairs of gastric cancer tissues compared with corresponding normal tissues by quantitative RT-PCR. Each sample was analyzed in triplicate and normalized to U6. T: tumor tissues; N: adjacent normal tissues; B: Lower miR-30b expression was observed in gastric cancer cell lines compared to that in GES-1; C: AGS and MGC803 cell proliferation was determined by the CCK-8 assay. Upregulation of miR-30b by transfection with mimic suppressed cell proliferation. Downregulation of miR-30b by transfection with inhibitor promoted cell proliferation. Data are displayed as mean ± SD.

Statistical analysis

To identify potential target genes of miR-30b, bioinformatics analysis was performed using an online miRNA target prediction database (Targetscan and miRNA.org).

Quantitative data were analyzed using SPSS 18.0 software (SPSS Inc., Chicago, IL, United States). Experimental data are presented as mean ± SD. Differences between two groups were compared using a Student's *t*-test and comparisons amongst three or more groups were made by analysis of variance. Differences were considered statistically significant

Table 1 Relationship between clinicopathological parameters and miR-30b expression

Clinicopathologic parameter	Number of cases	$2^{-\triangle\triangle CT}$(mean)	*P* value
Age (yr)			0.621
≥ 60	8	0.2078 ± 0.0285	
< 60	15	0.2165 ± 0.0143	
Gender			0.427
Male	16	0.1951 ± 0.0198	
Female	7	0.2083 ± 0.0239	
Lauren type			0.371
Intestinal type	11	0.2148 ± 0.0316	
Diffuse type	12	0.1932 ± 0.0257	
Lymph node metastasis			0.021
No	9	0.2693 ± 0.0381	
Yes	14	0.1651 ± 0.0259	

when $P < 0.05$.

RESULTS

MiR-30b is downregulated in gastric cancer tissues

We used real-time PCR to examine miR-30b expression in human gastric adenocarcinoma and adjacent normal tissues. Expression of miR-30b was significantly decreased in gastric cancer tissue when compared with paired normal tissue in all 23 samples examined ($P = 0.0016$) (Figure 1A). Examining the relationship between clinicopathological factors and the expression of miR-30b showed that only lymph node metastasis was associated with low miR-30 expression ($P = 0.021$). No association was found between miR-30b expression and age, gender or Lauren type (Table 1). Furthermore, we identified reduced miR-30b expression in seven gastric cancer cell lines compared with that in human immortalized gastric mucosa cell line GES-1 (Figure 1B).

High expression of miR-30b suppresses gastric cancer cell proliferation

We next investigated the effects of miR-30b overexpression on cell growth using CCK-8 assay and synthetic miR-30b mimic or inhibitor that were transfected into AGS and MGC803 cell lines. Overexpression of miR-30b suppressed AGS and MGC803 cell growth, whereas miR-30b inhibitor enhanced gastric cancer cell proliferation (Figure 1C).

MiR-30b overexpression can induce gastric cancer cell apoptosis

We used flow cytometry to identify increased apoptosis in AGS and MGC803 cancer cells transfected with miR-30b mimic compared with control cells ($P < 0.05$, Figure 2). This suggests that apoptosis contributed to the growth-inhibitory properties of miR-30b.

Re-expression of miR-30b suppresses gastric cancer cell migration and invasion

AGS and MGC803 cells transfected with 50 nmol/L miR-30b underwent reduced migration and invasion compared with control cells. Conversely, we detected increased migration and invasion in AGS and MGC803 cells transfected with an antisense oligonucleotide inhibitor of miR-30b (Figure 3). These results indicate that miR-30b attenuated gastric cancer cell migration and invasion *in vitro*.

EIF5A2 is a candidate target gene of miR-30b

We conducted a bioinformatics analysis to identify potential targets of miR-30b using the online tools miRanda and TargetScan. *EIF5A2* mRNA was found to contain a 3′-UTR element complementary to miR-30b, and the binding site of miR-30b in the 3′-UTR of EIF5A2 is highly conserved across species (Figure 4A and B). Therefore, we cloned the region of the EIF5A2 3′-UTR containing this complementary site into a luciferase reporter vector. Luciferase activity levels in HEK293T cells transfected with this construct and miR-30b mimic were significantly decreased compared with control. However, luciferase activity in cells transfected with reporter constructs harboring mutations at the suspected miR-30b target site was unaffected by co-transfection with miR-30b (Figure 4C). These results indicate that the 3′-UTR of EIF5A2 was targeted by miR-30b.

Downregulation of EIF5A2 by miR-30b promotes EMT

Western blot analysis identified significantly higher expression of EIF5A2 in all seven gastric cancer cell lines examined compared with GES-1 cells (Figure 4D).

We next examined the influence of miR-30b by measuring EIF5A2 protein levels following transfection of AGS and MGC803 cells with miR-30b mimic or inhibitor. Cells transfected with miR-30b mimic had significantly lower EIF5A2 protein levels compared with control (Figure 4E). Furthermore, transfection of miR-30b mimic led to increased expression of the epithelial marker E-cadherin and β-catenin and reduced expression of the mesenchymal marker Vimentin, whereas silencing miR-30b suppressed E-cadherin and β-catenin expression, and induced vimentin expression in cancer cells (Figure 4F). In addition, transfection with miR-30b mimic or inhibitor had no effect on the MMP-9 and TIMP-1, indicating that miR-30b suppressed cancer cell metastasis via downregulation of EIF5A2. These results suggest that miR-30b enhances E-cadherin and β-catenin

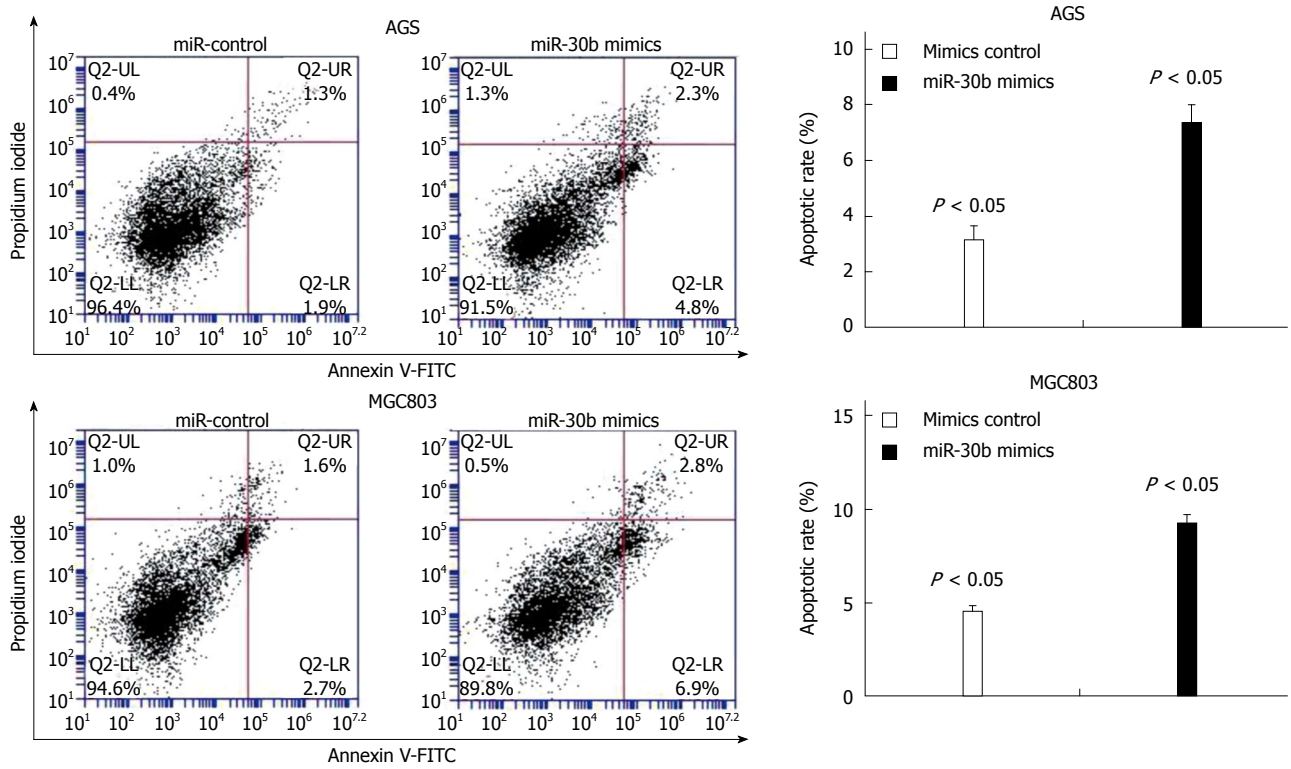
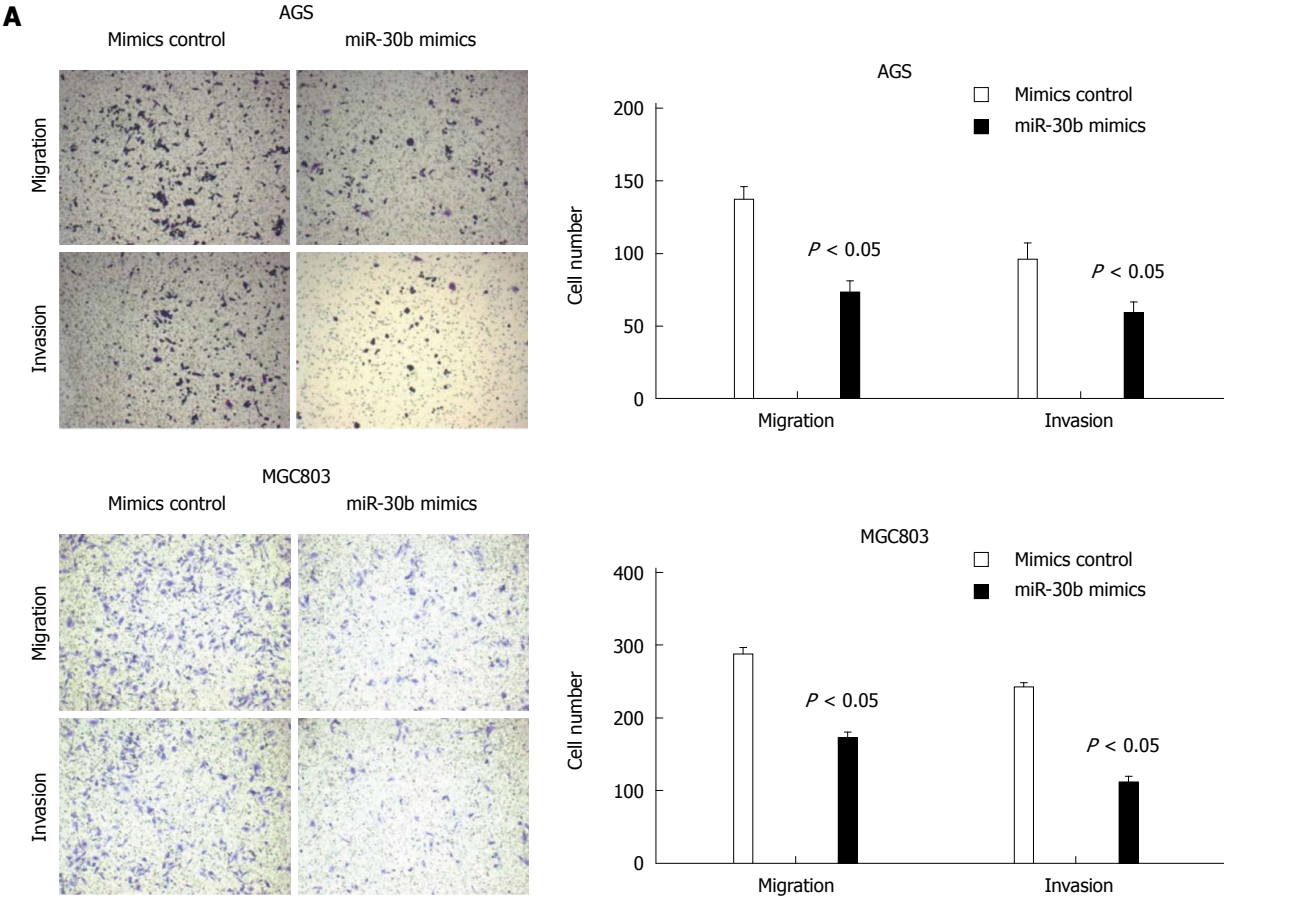


Figure 2 Effect of miR-30b on cell apoptosis. The histograms depict apoptosis of AGS cells and MGC803 cells transiently transfected with miR-30 mimic or control.



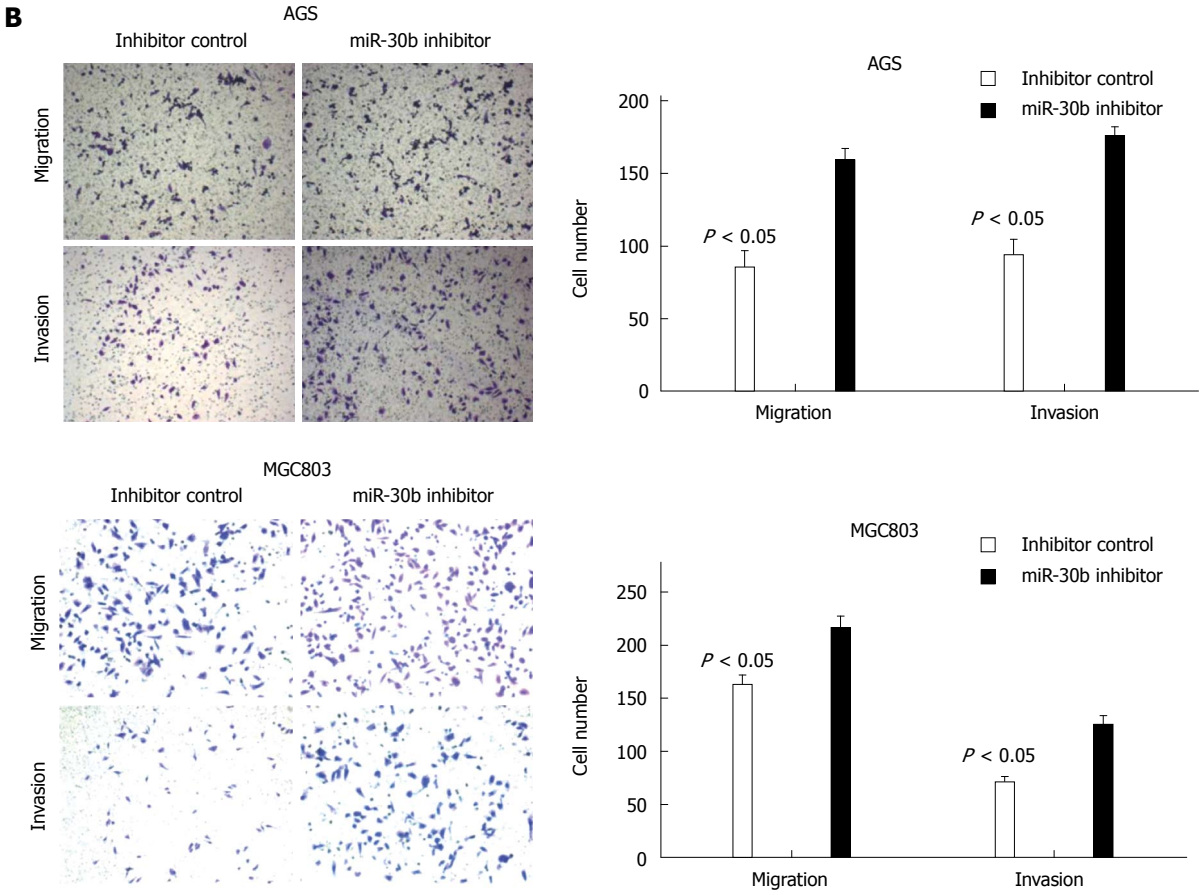
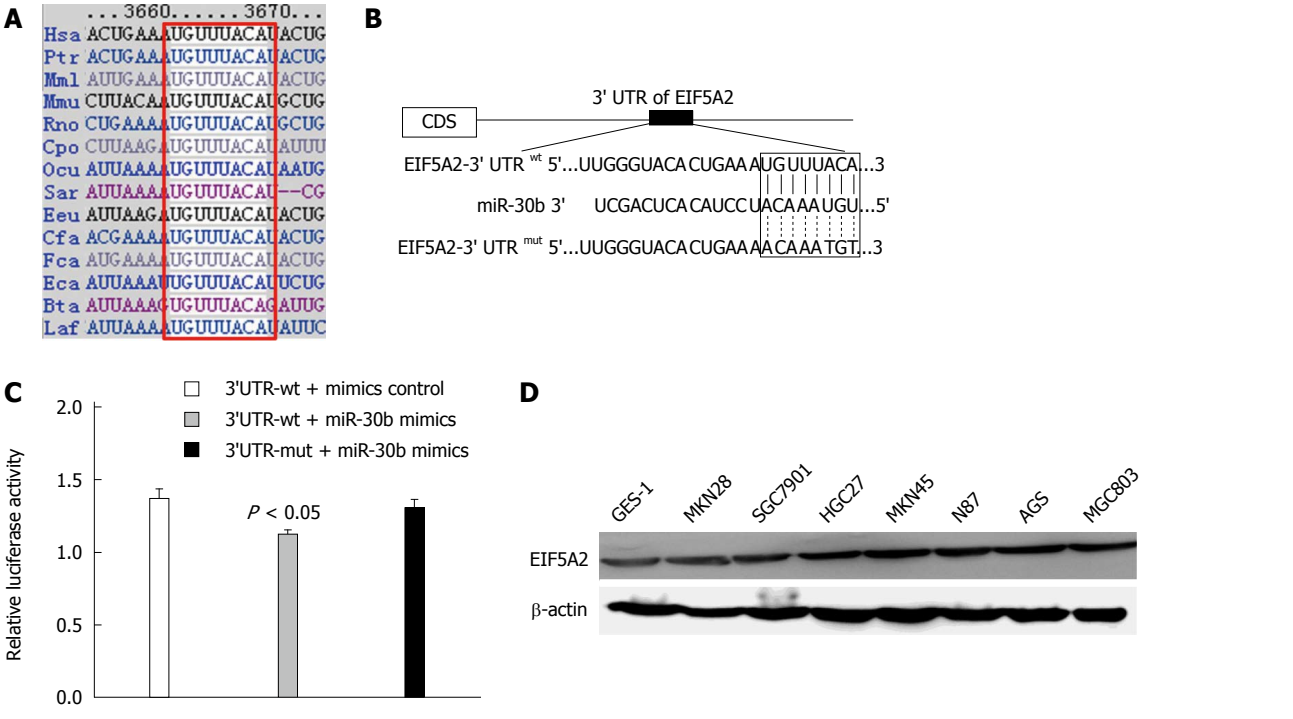


Figure 3 Effect of miR-30b on the migration and invasion of AGS and MGC803 cells in a transwell assay. A: Overexpression of miR-30b notably inhibited the migration and invasion of AGS and MGC803 cells; B: The migration and invasion abilities of AGS and MGC803 cells were dramatically increased after miR-30b inhibitor treatment. The bar shows the average ± SD of three independent experiments.



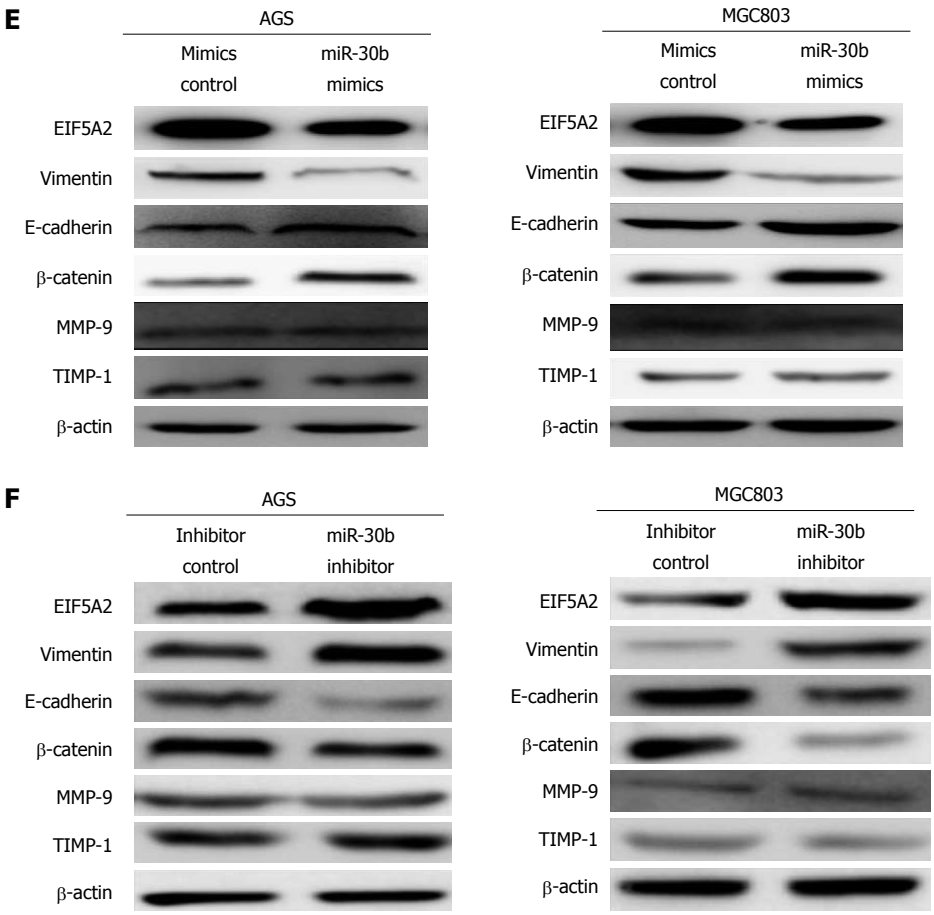


Figure 4 miR-30b decreases eukaryotic translation initiation factor 5A2 expression by targeting its 3'-UTR. A: The binding site of miR-30b in the 3'-UTR of eukaryotic translation initiation factor 5A2 (EIF5A2) is highly conserved across species; B: The putative binding sites for miR-30b was found in the 3'-UTR of EIF5A2 at 3664-3671bp; C: miR-30b mimic downregulated luciferase activities controlled by wild-type 3'-UTR of EIF5A2, but did not affect luciferase activity controlled by mutant 3'-UTR of EIF5A2; D: The expression levels of EIF5A2 protein in different gastric cell lines; E: The expression levels of EIF5A2 and of its downstream genes were detected by Western blot analysis in AGS and MGC803 cells transfected with the miR-30b mimic or control for 48 h. β-actin was used as an internal control; F: Western blot analysis of EIF5A2 and its downstream genes transfection of miR-30b inhibitor in AGS and MGC803 cells.

expression by targeting EIF5A2 and eventually inhibits the EMT process in gastric cancer cells.

DISCUSSION

Increased expression of miR-30b has been identified in multiple malignancies including parathyroid carcinoma[12], medulloblastoma[13], and oral squamous cell cancer[14]. These findings support a role for miR-30b as an oncogene in these tumors. However, miR-30b may also function as a tumor suppressor. Reduced expression of miR-30b has been found in various human cancers, including colorectal cancer[10,15], non-small cell lung cancer[16], and prostate cancer[17]. These studies found that miR-30b could inhibit cancer cell proliferation and/or suppress cancer cell invasion and migration. Additionally, Ueda *et al*[18] found that miR-30b was significantly downregulated in 184 gastric cancers compared with 169 non-tumor mucosa samples. Moreover, restoration of miR-30b expression can inhibit gastric cancer cell migration and increase gastric cancer cell apoptosis[19,20]. This decreased expression of miR-30b in gastric cancer may result from miR-30b promoter methylation[20]. Taken together, these findings indicate that miR-30b can act as either an oncogene or a tumor suppressor depending on the circumstance.

We have identified low expression of miR-30b in gastric cancer specimens compared with adjacent non-cancerous tissues using real-time PCR-based miRNA assays. At the cellular level, miR-30b overexpression inhibited cancer cell proliferation, promoted cellular apoptosis, and decreased cancer cell migration and invasion. Additionally, gastric cancer cells transfected with miR-30b inhibitor exhibited increased growth, migration, and invasion compared with controls. Taken together, these data suggest that miR-30b plays a tumor suppressor role in gastric cancer.

A multiple-to-multiple relationship exists between miRNAs and their targets in gastric cancer, suggesting that their regulation is complex[21]. Members of the miR-30 family exert various effects in tumors from different tissues. In multiple myeloma, miR-30 family members are downregulated, which results in enhanced expression of BCL9 and subsequent promotion of tumor cell proliferation and migration[22].

Recently, two studies investigating the role of miR-30b in colorectal carcinoma development have identified that the oncogenes KRAS, PIK3CD, BCL9, and the EMT-related gene SIX1 are all targets of miR-30b[10,15]. Moreover, miR-30b expression in clinical samples was inversely correlated with the above genes. Additionally, Zhong *et al*[16] have reported that miR-30b is involved in non-small cell lung cancer carcinogenesis through downregulating the Ras superfamily member Rab18. Last, miR-30b inhibits expression of plasminogen activator inhibitor (PAI-1) in gastric cancer, thereby suppressing tumor growth[19]. So, more targets of miR-30b should be validated to investigate its function in gastric cancer.

The loss of miR-30b expression influences gastric cancer metastasis through altered regulation of miR-30b target gene expression. We employed a bioinformatics approach and luciferase reporter assay to identify EIF5A2 as a critical novel target of miR-30b. Western blot analysis revealed that miR-30b overexpression resulted in upregulation of E-cadherin and β-catenin and downregulation of Vimentin, demonstrating that miR-30b could suppress EMT in gastric cancer. However, no change was observed of MMP-9 and TIMP-1 levels after transfection with miR-30b mimic or inhibitor in cancer cells. These results indicated that miR-30b inhibits gastric cancer cell invasion and migration not through the MMP-9 or TIMP-1.

EFI5A2 is a potentially important tumor promoting molecule. Several studies have described an oncogenic role for EIF5A2 in multiple tumor types, including esophageal squamous cell carcinoma[23], hepatocellular carcinoma[24], bladder carcinoma[25], ovarian carcinoma[26], and colorectal carcinoma[27]. Additionally, EIF5A2 overexpression can initiate tumor formation, promote cancer cell growth, and contribute to cancer cell metastasis. Furthermore, high levels of EIF5A2 indicate a more advanced clinical stage in ovarian[26] and hepatocellular carcinomas[28]. Tang *et al*[24] found that EIF5A2 could induce EMT in hepatocellular carcinoma by activating RhoA/Rac1 and downregulating epithelial markers including E-cadherin and β-catenin. Overexpression of EIF5A2 can also promote EMT by regulating MTA1 through c-myc in human colorectal carcinoma[29]. In our previous study, we found that EIF5A2 was overexpressed in gastric cancer compared with matched adjacent non-tumor mucosal tissues[30]. Meanwhile, knockdown of EIF5A2 can suppress MKN28 and HGC27 cell proliferation, migration, and invasion by inhibiting EMT, and E-cadherin levels were upregulated and vimentin levels were downregulated after transfection with EIF5A2 siRNA[30,31]. Together, these findings support an oncogenic role for EIF5A2.

To the best of our knowledge, this is the first report that miR-30b directly regulates EIF5A2. miR-30b has a functional role in suppressing gastric cancer metastasis by impairing cellular migration and invasion. Downregulation of EIF5A2 by miR-30b could increase E-cadherin and β-catenin levels and reduce Vimentin expression. However, it should be noted that this study is based on a small number of samples and no experiments in relevant animal models were conducted. Therefore, future studies are necessary to elaborate upon our current findings. Overall, this report sheds new light on the role of miR-30b in gastric cancer development, and supports the targeting of miR-30b as a potentially effective therapeutic strategy for gastric cancer in the future.

COMMENTS

Background

Gastric cancer is one of the leading causes of cancer-related death worldwide. MicroRNAs (miRNAs) play an important role in gastric cancer carcinogenesis and tumor progression by negatively regulating oncogenes and tumor suppressors. However, the precise biological role of miRNAs in mediating metastasis remains relatively unexplored.

Research frontiers

MicroRNAs (miRNAs) are small non-coding RNAs which negatively regulate gene expression. Various studies have described functional roles for miRNAs as oncogenes or tumor-suppressor genes. Accumulating evidence describes vital roles for many miRNAs in tumor initiation and metastasis. For example, miR-7 can inhibit the epithelial-mesenchymal transition (EMT) process in gastric cancer through targeting IGF1R expression. In colorectal carcinoma, miR-30b directly targets the EMT-related gene SIX1 to impair metastasis of colorectal cancer cells. However, the role of miR-30b in gastric cancer progression and metastasis is still largely unknown and the molecular mechanism needs further exploration.

Innovations and breakthroughs

The authors found that miR-30b is downregulated in gastric cancer tissues and cancer cell lines and functions as a tumor suppressor. Overexpression of miR-30b can promote cell apoptosis, and suppress proliferation, migration, and invasion of the gastric cancer cell lines AGS and MGC803. Luciferase reporter assays and Western blot analysis confirmed the EIF5A2 gene as a target of miR-30b. Moreover, miR-30b enhances E-cadherin and β-catenin expression by targeting EIF5A2 and eventually inhibits the EMT process in gastric cancer cells, thus providing a valuable target for cancer therapy.

Applications

In this study, the authors found that miR-30b is significantly downregulated in gastric cancer tissues and cell lines. Increased miR-30b expression reduced cancer cell migration and invasion, and promoted cell apoptosis. They also found that suppression of EIF5A2 by miR-30b increased E-cadherin expression and partially reversed the EMT. All these provide insight into the specific role of miR-30b in EMT and tumor metastasis. The authors propose that miR-30b may be a novel target for the treatment of gastric cancer.

Terminology

MicroRNAs (miRNAs) are a novel class of small, non-coding endogenous RNAs that regulate gene expression by directing their target mRNAs for degradation or translational repression. EMT is a biological process during tumor development by which epithelial cells acquire mesenchymal, fibroblast-like properties and show reduced intercellular adhesion and increased motility.

Peer-review

The authors have described a putative role of miR-30b in regulating EIFA2 expression and function. The article is well organized and well written. The methods used are well studied and appropriate for the experimental design.

REFERENCES

1 **Akagi T**, Shiraishi N, Kitano S. Lymph node metastasis of gastric cancer. *Cancers* (Basel) 2011; **3**: 2141-2159 [PMID: 24212800 DOI: 10.3390/cancers3022141]

2 **Yang J**, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell* 2008; **14**: 818-829 [PMID: 18539112 DOI: 10.1016/j.devcel.2008.05.009]

3 **Katoh M**. Epithelial-mesenchymal transition in gastric cancer (Review). *Int J Oncol* 2005; **27**: 1677-1683 [PMID: 16273224]

4 **Wang Z**, Ma X, Cai Q, Wang X, Yu B, Cai Q, liu B, Zhu Z, Li C. MiR-199a-3p promotes gastric cancer progression by targeting ZHX1. *FEBS Lett* 2014; **588**: 4504-4512 [PMID: 25448600 DOI: 10.1016/j.febslet.2014.09.047]

5 **Zhao X**, Dou W, He L, Liang S, Tie J, Liu C, Li T, Lu Y, Mo P, Shi Y, Wu K, Nie Y, Fan D. MicroRNA-7 functions as an anti-metastatic microRNA in gastric cancer by targeting insulin-like growth factor-1 receptor. *Oncogene* 2013; **32**: 1363-1372 [PMID: 22614005 DOI: 10.1038/onc.2012.156]

6 **Rotkrua P**, Akiyama Y, Hashimoto Y, Otsubo T, Yuasa Y. MiR-9 downregulates CDX2 expression in gastric cancer cells. *Int J Cancer* 2011; **129**: 2611-2620 [PMID: 21225631 DOI: 10.1002/ijc.25923]

7 **Liu R**, Zhang C, Hu Z, Li G, Wang C, Yang C, Huang D, Chen X, Zhang H, Zhuang R, Deng T, Liu H, Yin J, Wang S, Zen K, Ba Y, Zhang CY. A five-microRNA signature identified from genome-wide serum microRNA expression profiling serves as a fingerprint for gastric cancer diagnosis. *Eur J Cancer* 2011; **47**: 784-791 [PMID: 21112772 DOI: 10.1016/j.ejca.2010.10.025]

8 **Xia L**, Zhang D, Du R, Pan Y, Zhao L, Sun S, Hong L, Liu J, Fan D. miR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells. *Int J Cancer* 2008; **123**: 372-379 [PMID: 18449891 DOI: 10.1002/ijc.23501]

9 **Gregory PA**, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 2008; **10**: 593-601 [PMID: 18376396 DOI: 10.1038/ncb1722]

10 **Zhao H**, Xu Z, Qin H, Gao Z, Gao L. miR-30b regulates migration and invasion of human colorectal cancer via SIX1. *Biochem J* 2014; **460**: 117-125 [PMID: 24593661 DOI: 10.1042/BJ20131535]

11 **Wang FW**, Guan XY, Xie D. Roles of eukaryotic initiation factor 5A2 in human cancer. *Int J Biol Sci* 2013; **9**: 1013-1020 [PMID: 24250246 DOI: 10.7150/ijbs.7191]

12 **Rahbari R**, Holloway AK, He M, Khanafshar E, Clark OH, Kebebew E. Identification of differentially expressed microRNA in parathyroid tumors. *Ann Surg Oncol* 2011; **18**: 1158-1165 [PMID: 21086055 DOI: 10.1245/s10434-010-1359-7]

13 **Lu Y**, Ryan SL, Elliott DJ, Bignell GR, Futreal PA, Ellison DW, Bailey S, Clifford SC. Amplification and overexpression of Hsa-miR-30b, Hsa-miR-30d and KHDRBS3 at 8q24.22-q24.23 in medulloblastoma. *PLoS One* 2009; **4**: e6159 [PMID: 19584924 DOI: 10.1371/journal.pone.0006159]

14 **Shao C**, Yu Y, Yu L, Pei Y, Feng Q, Chu F, Fang Z, Zhou Y. Amplification and up-regulation of microRNA-30b in oral squamous cell cancers. *Arch Oral Biol* 2012; **57**: 1012-1017 [PMID: 22542163 DOI: 10.1016/j.archoralbio.2012.04.002]

15 **Liao WT**, Ye YP, Zhang NJ, Li TT, Wang SY, Cui YM, Qi L, Wu P, Jiao HL, Xie YJ, Zhang C, Wang JX, Ding YQ. MicroRNA-30b functions as a tumour suppressor in human colorectal cancer by targeting KRAS, PIK3CD and BCL2. *J Pathol* 2014; **232**: 415-427 [PMID: 24293274 DOI: 10.1002/path.4309]

16 **Zhong K**, Chen K, Han L, Li B. MicroRNA-30b/c inhibits non-small cell lung cancer cell proliferation by targeting Rab18. *BMC Cancer* 2014; **14**: 703 [PMID: 25249344 DOI: 10.1186/1471-2407-14-703]

17 **Kao CJ**, Martiniez A, Shi XB, Yang J, Evans CP, Dobi A, deVere White RW, Kung HJ. miR-30 as a tumor suppressor connects EGF/Src signal to ERG and EMT. *Oncogene* 2014; **33**: 2495-2503 [PMID: 23728339 DOI: 10.1038/onc.2013.200]

18 **Ueda T**, Volinia S, Okumura H, Shimizu M, Taccioli C, Rossi S, Alder H, Liu CG, Oue N, Yasui W, Yoshida K, Sasaki H, Nomura S, Seto Y, Kaminishi M, Calin GA, Croce CM. Relation between microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis. *Lancet Oncol* 2010; **11**: 136-146 [PMID: 20022810 DOI: 10.1016/S1470-2045(09)70343-2]

19 **Zhu ED**, Li N, Li BS, Li W, Zhang WJ, Mao XH, Guo G, Zou QM, Xiao B. miR-30b, down-regulated in gastric cancer, promotes apoptosis and suppresses tumor growth by targeting plasminogen activator inhibitor-1. *PLoS One* 2014; **9**: e106049 [PMID: 25170877 DOI: 10.1371/journal.pone.0106049]

20 **Qiao F**, Zhang K, Gong P, Wang L, Hu J, Lu S, Fan H. Decreased miR-30b-5p expression by DNMT1 methylation regulation involved in gastric cancer metastasis. *Mol Biol Rep* 2014; **41**: 5693-5700 [PMID: 24913034 DOI: 10.1007/s11033-014-3439-4]

21 **Hashimoto Y**, Akiyama Y, Yuasa Y. Multiple-to-multiple relationships between microRNAs and target genes in gastric cancer. *PLoS One* 2013; **8**: e62589 [PMID: 23667495 DOI: 10.1371/journal.pone.0062589]

22 **Zhao JJ**, Lin J, Zhu D, Wang X, Brooks D, Chen M, Chu ZB, Takada K, Ciccarelli B, Admin S, Tao J, Tai YT, Treon S, Pinkus G, Kuo WP, Hideshima T, Bouxsein M, Munshi N, Anderson K, Carrasco R. miR-30-5p functions as a tumor suppressor and novel therapeutic tool by targeting the oncogenic Wnt/β-catenin/BCL9 pathway. *Cancer Res* 2014; **74**: 1801-1813 [PMID: 24599134 DOI: 10.1158/0008-5472.CAN-13-3311-T]

23 **Li Y**, Fu L, Li JB, Qin Y, Zeng TT, Zhou J, Zeng ZL, Chen J, Cao TT, Ban X, Qian C, Cai Z, Xie D, Huang P, Guan XY. Increased expression of EIF5A2, via hypoxia or gene amplification, contributes to metastasis and angiogenesis of esophageal squamous cell carcinoma. *Gastroenterology* 2014; **146**: 1701-1713.e9 [PMID: 24561231 DOI: 10.1053/j.gastro.2014.02.029]

24 **Tang DJ**, Dong SS, Ma NF, Xie D, Chen L, Fu L, Lau SH, Li Y, Li Y, Guan XY. Overexpression of eukaryotic initiation factor 5A2 enhances cell motility and promotes tumor metastasis in hepatocellular carcinoma. *Hepatology* 2010; **51**: 1255-1263 [PMID: 20112425 DOI: 10.1002/hep.23451]

25 **Wei JH**, Cao JZ, Zhang D, Liao B, Zhong WM, Lu J, Zhao HW, Zhang JX, Tong ZT, Fan S, Liang CZ, Liao YB, Pang J, Wu RH, Fang Y, Chen ZH, Li B, Xie D, Chen W, Luo JH. EIF5A2 predicts outcome in localised invasive bladder cancer and promotes bladder cancer cell aggressiveness in vitro and in vivo. *Br J Cancer* 2014; **110**: 1767-1777 [PMID: 24504366 DOI: 10.1038/bjc.2014.52]

26 **Yang GF**, Xie D, Liu JH, Luo JH, Li LJ, Hua WF, Wu HM, Kung HF, Zeng YX, Guan XY. Expression and amplification of eIF-5A2 in human epithelial ovarian tumors and overexpression of EIF-5A2 is a new independent predictor of outcome in patients with ovarian carcinoma. *Gynecol Oncol* 2009; **112**: 314-318 [PMID: 19054548 DOI: 10.1016/j.ygyno.2008.10.024]

27 **Xie D**, Ma NF, Pan ZZ, Wu HX, Liu YD, Wu GQ, Kung HF, Guan XY. Overexpression of EIF-5A2 is associated with metastasis of human colorectal carcinoma. *Hum Pathol* 2008; **39**: 80-86 [PMID: 17949776 DOI: 10.1016/j.humpath.2007.05.011]

28 **Lee NP**, Tsang FH, Shek FH, Mao M, Dai H, Zhang C, Dong S, Guan XY, Poon RT, Luk JM. Prognostic significance and therapeutic potential of eukaryotic translation initiation factor 5A (eIF5A) in hepatocellular carcinoma. *Int J Cancer* 2010; **127**: 968-976 [PMID: 19998337 DOI: 10.1002/ijc.25100]

29 **Zhu W**, Cai MY, Tong ZT, Dong SS, Mai SJ, Liao YJ, Bian XW, Lin MC, Kung HF, Zeng YX, Guan XY, Xie D. Overexpression of EIF5A2 promotes colorectal carcinoma cell aggressiveness by upregulating MTA1 through C-myc to induce epithelial-mesenchymaltransition. *Gut* 2012; **61**: 562-575 [PMID: 21813470 DOI: 10.1136/gutjnl-2011-300207]

30 **Meng QB**, Kang WM, Yu JC, Liu YQ, Ma ZQ, Zhou L, Cui QC, Zhou WX. Overexpression of eukaryotic translation initiation factor 5A2 (EIF5A2) correlates with cell aggressiveness and poor survival in gastric cancer. *PLoS One* 2015; **10**: e0119229 [PMID: 25793713 DOI: 10.1371/journal.pone.0119229]

31 **Meng QB**, Yu JC, Kang WM, Ma ZQ, Zhou L, Ye X, Cao ZJ, Tian

SB. [Effects of eukaryotic translation initiation factor 5A2 down-regulation by small interfering RNA on aggressiveness of MKN28 human]. *Zhongguo Yixue Kexueyuan Xuebao* 2014; **36**: 482-487 [PMID: 25360644 DOI: 10.3881/j.issn.1000-503X.2014.05.005]

P- Reviewer: Klempner SJ, Li Y, Zhang J
S- Editor: Ma YJ **L- Editor**: Wang TQ **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9348

World J Gastroenterol 2015 August 21; 21(31): 9348-9357
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Linc00675 is a novel marker of short survival and recurrence in patients with pancreatic ductal adenocarcinoma

Dou-Dou Li, Zhi-Qiang Fu, Qing Lin, Yu Zhou, Quan-Bo Zhou, Zhi-Hua Li, Lang-Ping Tan, Ru-Fu Chen, Yi-Min Liu

Dou-Dou Li, Yi-Min Liu, Department of Radiotherapy, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, Guangdong Province, China

Zhi-Qiang Fu, Qing Lin, Yu Zhou, Quan-Bo Zhou, Lang-Ping Tan, Ru-Fu Chen, Department of Pancreaticobiliary and Hepatobiliary Surgery, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, Guangdong Province, China

Zhi-Hua Li, Department of Medical Oncology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, Guangdong Province, China

Author contributions: Li DD and Fu ZQ contributed equally to this study; Li DD and Fu ZQ performed the majority of experiments; Lin Q conducted part of the experiments, and contributed to the revision of this paper; Zhou Y and Zhou QB conceived the experiments, analyzed and interpreted the data, and wrote the manuscript; Li ZH and Tan LP participated in the experiments and helped to analyze the data; all authors read and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Ethics Committee of Sun Yat-sen Memorial Hospital.

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors disclose no conflicts.

Data sharing statement: The technical appendix, statistical code, and dataset are available from the corresponding author at liuyimin_sysu@163.com. Consent was not obtained but the presented data are anonymized and the risk of identification is low.

Correspondence to: Dr. Yi-Min Liu, Department of Radiotherapy, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, 107 Yan-Jiang Xi Road, Guangzhou 510120, Guangdong Province, China. liuyimin_sysu@163.com
Telephone: +86-20-81332480
Fax: +86-20-81332480

Received: January 28, 2015
Peer-review started: January 29, 2015
First decision: March 10, 2015
Revised: April 7, 2015
Accepted: June 16, 2015
Article in press: June 16, 2015
Published online: August 21, 2015

Abstract

AIM: To detect linc00675 expression in pancreatic ductal adenocarcinoma (PDAC), to analyze the relationship between the expression level of linc00675 and the clinical pathological characteristics, to explore the biological functions of linc00675, and to determine whether linc00675 has independent prognostic value in PDAC.

METHODS: We studied linc00675 expression among eight histologically confirmed PDAC tissue samples and four chronic pancreatitis tissue samples through microarray screening. RT-qPCR was conducted to further investigate linc00675 expression in PDAC cell lines as well as archived tissues from a large cohort of PDAC patients. The correlations between the level of lnc00675 and clinicopathological characteristics and survival in patients with pancreatic cancer were evaluated using Correlation analysis. Univariate and

multivariate analyses were conducted to predict whether lnc00675 expression is an independent prognostic and recurrence factor in patients with pancreatic cancer. After downregulating the expression of linc00675 through siRNA, MTT assay, flow cytometry, transwell assay and Western blot were used to explore the biological function of linc00675 in proliferation, invasion, and cell cycle progression of pancreatic cancer cells. The relative molecular expression levels of epithelial-mesenchymal transition were determined by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot.

RESULTS: The expression of Linc00675 in PDAC tissue samples was shown to be 672 times that in chronic pancreatitis tissue samples by microarray screening ($P = 3.69 \times 10^{-5}$). This finding was confirmed in tumor tissues from 90 patients with PDAC compared with adjacent normal tissue samples by quantitative RT-PCR. We found that linc00675 overexpression positively correlated with lymph node metastasis ($P = 0.005$), perineural invasion ($P = 0.006$), and poor survival ($P < 0.001$). Univariate and multivariate analyses showed that linc00675 expression served as an independent predictor of overall survival ($P = 0.009$). Additionally, receiver operating characteristic curve analysis showed that high linc00675 might serve as a predictor of tumor progression within 6 mo to a year after surgery. *In vitro* functional analysis demonstrated that knockdown of linc00675 attenuated pancreatic cancer cell proliferation and invasion as well as induced S phase arrest. Suppression of linc00675 in pancreatic cancer cells resulted can reverse the progress of epithelial-mesenchymal transition.

CONCLUSION: Linc00675 may function as an oncogene during PDAC development, and its expression is an independent predictor of unfavorable prognosis in patients with PDAC.

Key words: Linc00675; Long noncoding RNAs; Prognosis biomarker; Pancreatic cancer; Epithelial-mesenchymal transition

Core tip: This is the first study to report that linc00675 is more highly expressed in pancreatic ductal adenocarcinoma (PDAC) tissues than in adjacent normal tissues. Overexpression of linc00675 in PDAC tissues positively correlated with short survival and tumor progression. The prominent finding in this study is that linc00675 is an independent prognostic marker for predicting the survival of PDAC patients after surgery.

Li DD, Fu ZQ, Lin Q, Zhou Y, Zhou QB, Li ZH, Tan LP, Chen RF, Liu YM. Linc00675 is a novel marker of short survival and recurrence in patients with pancreatic ductal adenocarcinoma. *World J Gastroenterol* 2015; 21(31): 9348-9357 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9348.htm
DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9348

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) that originates in the glandular epithelium accounts for approximately 90% of all pancreatic tumors and exhibits a high grade of malignancy[1]. PDAC patients have an extremely poor prognosis, with a 5-year survival rate of approximately 6%. Even in patients who undergo surgical resection, the disease commonly recurs and the 5-year survival rate remains low at 15%-25%[2]. This dismal prognosis is due to the aggressive nature of this disease, and its resistance to traditional therapeutic strategies. Therefore, the development of an effective treatment for PDAC requires further research to reveal the molecular mechanisms underlying its aggressive pathogenesis.

Long noncoding RNAs (lncRNAs) are RNA molecules over 200 nucleotides in length with little protein-coding potential. Long intergenic noncoding RNAs (lincRNAs) have transcription loci that fall between two protein-coding genes and function to regulate gene expression at various levels, including transcription, epigenetic regulation and post-transcriptional processing[3-7]. Accumulating evidence suggests that deregulation of lincRNAs may contribute to many types of human diseases, including cancer[8,9]. Moreover, they play critical roles in cancer initiation, progression and metastasis[10-12]. LincRNA expression signatures have been associated with patient survival and may be useful in the patient management and the design of anticancer treatments[13]. Several lincRNAs have been implicated in tumorigenesis. However, the biological functions and prognostic value of lincRNAs in pancreatic cancer remain largely unexplored. Thus, there is an urgent need to identify the etiology and biological function of lincRNAs that may serve as markers of diagnosis and prognosis in PDAC to improve survival in this disease.

In the present study, based on microarray analysis, we focused on a long intergenic noncoding RNA named linc00675 that showed 672-fold upregulation in PDAC compared with normal pancreatic tissues. Building on this finding, we determined the significance of linc00675 in PDAC by investigating the relationship between aberrantly expressed linc00675 and patients' clinicopathological features, as well as performing further *in vitro* study of PDAC cell lines. We found that upregulation of linc00675 was associated with short survival. In addition to affecting the cell cycle, overexpression of linc00675 could therefore promote cancer cell proliferation, migration and invasion. Thus, our study revealed that linc00675 is a promising prognostic biomarker in pancreatic cancer, and could be useful in pancreatic cancer risk assessment and

Table 1 Correlation between linc00675 expression and clinical characteristics

Factor	Linc00675 expression		*P* value[1]
	High (n = 45)	Low (n = 45)	
Age (yr)			
< 60	22	23	0.833
⩾ 60	23	22	
Sex			
Male	30	27	0.512
Female	15	18	
Differentiation			
Well	16	17	0.304
Moderate	15	20	
Poor	14	8	
UICC stage			
p Ⅰ	9	15	0.153
p Ⅱ	36	30	
T stage			
T1	6	9	0.697
T2	16	15	
T3	23	21	
N stage			
N0	12	25	0.005
N1	33	20	
Perineural invasion			
Negative	17	30	0.006
Positive	28	15	

[1]Pearson χ^2 test.

future therapeutic targeting.

MATERIALS AND METHODS

Patients and tissue samples

Samples of fresh frozen cancer tissues, together with normal adjacent tissues, were obtained during surgical resection from Sun Yat-sen Memorial Hospital of Sun Yat-sen University. Informed consent was obtained from the patients before sample collection, and approved by the hospital's Ethics Review Committee. All samples were confirmed by pathological examination.

Cell culture

The human pancreatic cancer cell lines PANC1, Capan2, BXPC-3, Mia PaCa2, SW1990, and immortalized human pancreatic ductal epithelial cells (HPDE6) were purchased from the American Type Culture Collection and grown in complete growth medium with 10% FBS and 1% penicillin/streptomycin as recommended by the manufacturer. All the cells were cultured in a humidified 5% CO_2 incubator at 37 ℃.

RNA isolation, microarrays, and quantitative reverse transcription-PCR

Total mRNA was extracted, purified using the mRNA-ONLY™ Eukaryotic mRNA Isolation Kit (Epicentre, Madison, CA). Total RNA was fragmented and then labeled (One-Color, Cy3, Agilent). After purification, the labeled RNA was hybridized to probes on the Hybridization Chamber gasket slides (Agilent). After being washed, the slides were scanned using an Agilent Microarray Scanner. The raw data were extracted with the Feature Extraction software (Agilent Technology). This software utilizes the robust multiarray average algorithm to adjust the background signals. Normalized data were obtained using the quantile method of intra-microarray normalization and median method of baseline transformation between the microarrays. Differentially expressed genes with a raw expression level of over 400 in more than 4 of the 12 samples used for profiling were extracted. Then they were ordered by *P* value. The 10 most significantly de-regulated genes (those with the smallest *P* values) were selected for validation. We also computed the maximum false discovery rate based on a single gene-probe *P* value threshold of 0.05. We considered as significant signatures with a false discovery rate ⩽ 0.1. The microarray platform and data were submitted to the Gene Expression Omnibus public database at the National Center for Biotechnology Information (accession number: GSE61166, http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE61166).

Real-time quantitative PCR (RT-qPCR) was performed for linc00675 and EMT marker (E-cadherin, N-cadherin, and Vimentin) mRNAs, with β-actin as an internal control. The total RNA was then converted to cDNA by reverse-transcription using oligodT primers and SuperScript Ⅱ reverse transcriptase (Invitrogen). Quantitative PCR was performed with SYBR green master mix (Roche). Relative expression values were calculated (ΔΔCT method) using β-actin as a normalizer. The primer sequences used in the study are listed in Supplementary Table 1.

RNA interference

siRNA oligos targeting linc00675 (CTGATGGAGG-AGAATCAATT, GTCCGAGAATGGCT GTGATT, and GTTCCAGACTCCATCACAATT), and nontargeting siRNAs (UUCUCCGAACGUG UCACGUTT) were purchased from Sigma Aldrich. siRNA transfections were done with 80 nmol/L siRNA and Lipofectamine 2000 (Life Technologies) following the manufacturer's instructions.

Cell growth assay, cell cycle analysis, and invasion assay

After transfection, 2 × 10^3 cells (SW1990 or Mia PaCa-2) were plated in 96-well plates. A cell proliferation reagent kit (Roche) was used to assess cell proliferation. Transfected cells were assessed every 24 h according to the manufacturer's instructions. For cell cycle analysis, transfected cells were collected, washed in PBS, stained with propidium oxide using the Cell Cycle Analysis Kit (Beyotime, Haimen, China), and then subjected to FACS analysis. *In vitro* cell invasion assay was performed using the BD BioCoat™ Matrigel ™ Invasion Chamber (Becton Dickinson) according to manufacturer's instructions, with 3 × 10^4 cells

seeded in the upper chamber. At least three biological replicates of the experiments were performed.

Western blot analysis

Cells were washed in PBS and lysed with RIPA buffer (Invitrogen, Carlsbad, CA, United States), and a bicinchoninic acid protein assay kit (Pierce) was used to calculate the protein concentration of each sample. Equivalent amounts of proteins were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes for immunoblotting. The membranes were blocked in 5% fat-free milk for 1 h at room temperature, then incubated with the following primary antibodies: anti-CyclinA, anti-CyclinE, anti-Cyclin D1, anti-CDK2 and anti-β-actin (Abcam, Cambridge, MA); anti-Vimentin, Anti-E-cadherin, anti-N-cadherin, and anti-GAPDH (Pro-teinTech Group, Chicago, IL, United States). GAPDH was used as a loading control. Horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology) and an ECL chemiluminescence kit (Pierce) were used to detect bound antibody.

Statistical analysis

Statistical analyses were performed using SPSS Statistics 17.0 (SPSS Inc). All *in vitro* experiment quantitative data are presented as the mean ± SD from at least three independent experiments, unless otherwise noted. The differences between two groups were analyzed using a Student's *t*-test. The correlation between linc00675 and clinical and pathological characteristics was assessed using Pearson's χ^2 test. Survival was evaluated using the Kaplan-Meier method. Cox proportional hazard analysis was performed to calculate the hazard ratio and 95% confidence interval (CI) to evaluate the association between linc00675 and other clinicopathologic factors and survival. All tests performed were two-sided. Differences were considered statistically significant if $P < 0.05$.

RESULTS

Linc00675 is aberrantly overexpressed in human PDAC cell lines and cancerous tissues

We conducted an analysis of tissues from eight PDAC cases and four cases of chronic pancreatitis (CP) using a microarray targeting 7419 lncRNAs (Agilent). We discovered that the expression of the long intergenic noncoding RNA linc00675 (LOC100289255) in PDAC tissues was 672 times that in CP tissues ($P = 3.69 \times 10^{-5}$, Figure 1A). The hybridization signals of another three long noncoding RNAs, HULC, MALAT1, and HOTAIR, which have previously been reported to be upregulated in pancreatic cancer, are also shown in Figure 1A. The expression of linc00675 had the most obvious difference. Next, we investigated whether linc00675 was upregulated in PDAC cell lines and a large cohort of PDAC tissues. As shown in Figure 1B and 1C, RT-qPCR revealed that expression of linc00675 was significantly higher in tumor tissues compared with matched adjacent non-tumor tissues ($P < 0.001$). We also found that the expression of linc00675 in each PDAC cell line was significantly higher than in the HPDE6 cell line (Figure 1D).

Association between linc00675 expression and overall survival of PDAC patients

We assessed the correlation between linc00675 expression and clinical characteristics using expression levels obtained from qRT-PCR data of a cohort of 90 patients (Figure 1B). We found that linc00675 expression level was significantly associated with both lymph node metastasis ($P = 0.005$) and perineural invasion ($P = 0.006$) (Table 1). Log-rank analysis demonstrated that overall survival was significantly worse in patients with higher linc00675 expression ($P < 0.001$) (Figure 2A and B). Further multivariate analysis confirmed that linc00675 expression level was an independent prognostic indicator for overall survival of patients with PDAC ($P = 0.009$) (Table 2).

Linc00675 is a potential biomarker for predicting recurrence in PDAC patients

Because linc00675 showed a significant correlation with lymph node metastasis and perineural invasion, we went on to assess the value of linc00675 in predicting tumor progression after surgery and conducted a ROC (Receiver Operating Characteristic) curve analysis. Results showed that for predicting tumor progression within one year, the area under the ROC curve was 0.893 ($P < 0.0001$, Figure 2C); and for predicting progression within six months, the area under the ROC curve was 0.928 ($P < 0.0001$, Figure 2D). These findings suggested that linc00675 has potential diagnostic value in predicting recurrence in PDAC patients after radical surgical resection.

Linc00675 regulates proliferation of pancreatic cancer cells

To further examine whether linc00675 functions in pancreatic cancer progression, *in vitro* studies were conducted. We knocked down linc00675 expression in SW1990 and MIA PaCa-2 cells using small interfering RNAs; the most effective siRNA that showed more than 70% knockout efficiency was selected for the following test (Figure 3A). Linc00675 depletion resulted in decreased tumor cell proliferation in both the pancreatic cancer cell lines SW1990 and MIA PaCa-2, as determined by MTT assay (Figure 3B). Further flow cytometry analysis showed that linc00675 knockdown induced S phase arrest in both SW1990 and MIA PaCa-2 cells (Figure 3C and D). The expression of Cyclin E, CyclinA, Cyclin D1 and CDK2, which are markers of S phase arrest, was analyzed by Western blot. In both linc00675 knockdown-treated

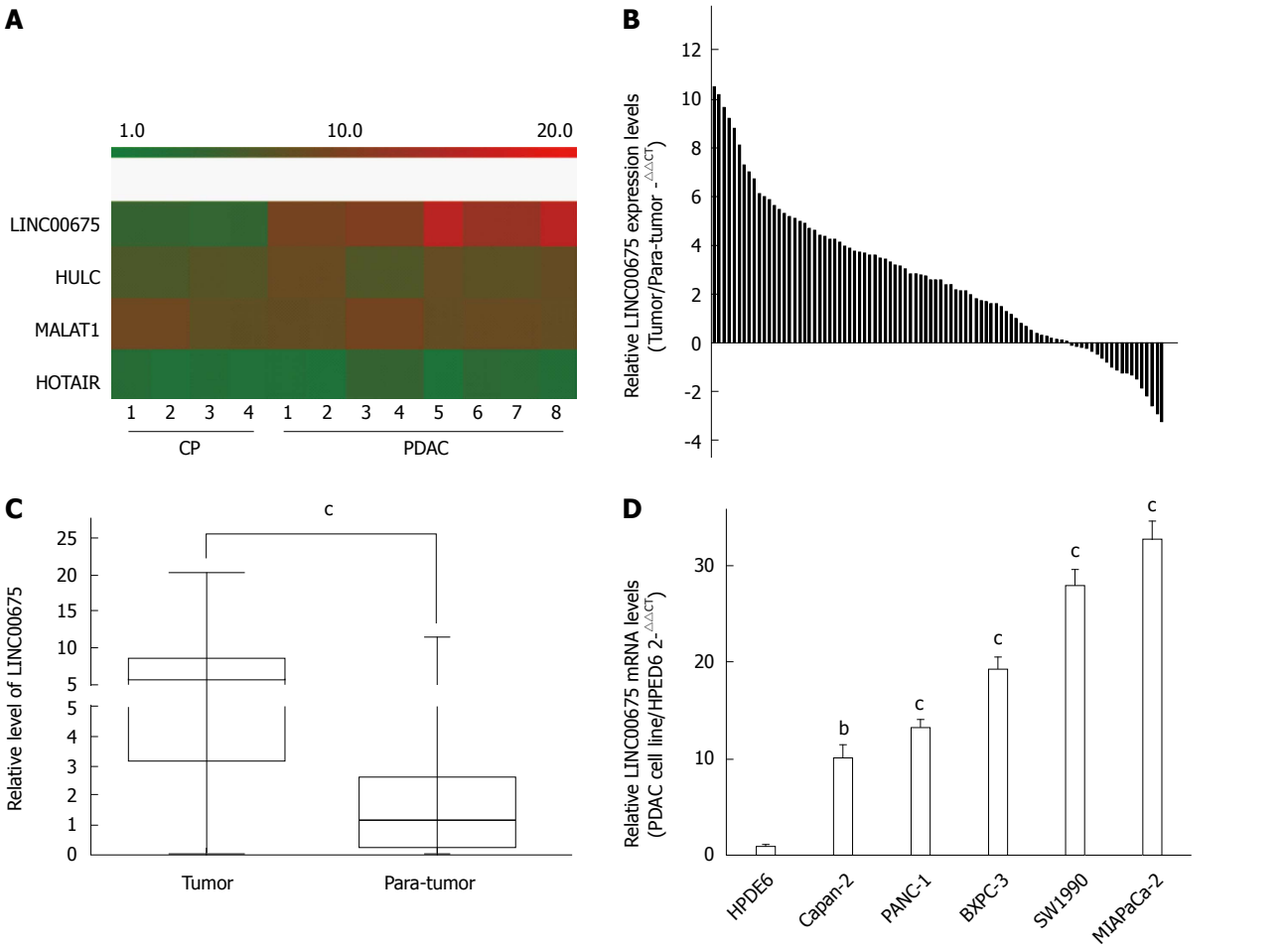


Figure 1 Expression of linc00675 in human pancreatic ductal adenocarcinoma cell lines and cancerous tissues. A: Heat map showing linc00675, HULC, MALAT1, and HOTAIR expression in the microarray analysis. The rectangular indicates the hybridization signal (replicate probes) of linc00675 in pancreatic ductal adenocarcinoma (PDAC) and chronic pancreatitis (CP) tissues; B: Quantitative real time-polymerase chain reaction analysis of linc00675 expression level in 90 cases of PDAC tissues. GAPDH was used as an internal control; C: The boxes represent the distribution of linc00675 expression from the 25th to 75th percentiles of all patient samples with the horizontal lines showing the median levels of linc00675, $^{c}P < 0.001$, tumor *vs* para-tumor, Student's *t*-test; D: Relative abundance of linc00675 in multiple pancreatic cancer cell lines. Data are represented as the mean ± SD from three independent experiments, $^{b}P < 0.01$, $^{c}P < 0.001$, the corresponding pancreatic cancer cell line *vs* HPDE6, Student's *t*-test.

Table 2 Multivariate analysis of clinicopathological factors for overall survival

Variable	Univariate		Multivariate	
	P value	HR	95%CI	*P* value
T stage	0.031	1.812	1.008-3.258	0.047
N stage	0.026	2.016	1.112-3.657	0.021
Perineural invasion	0.016	2.611	1.246-5.471	0.011
Linc00675 expression	0.013	4.620	1.233-4.336	0.009

HR: Hazard ratio.

SW1990 and Miapaca-2 cells, Cyclin E and Cyclin A, which are responsible for G1/S transition and S phase progression, respectively, were significantly upregulated, whereas the levels of Cyclin D1 and CDK2, which are suppressed in the S phase, were found to be significantly reduced (Figure 3E). The latter findings are consistent with S phase arrest *via* reduced expression of linc00675 in PDAC cell lines.

Linc00675 regulates invasion ability and expression of epithelial-mesenchymal transition-related genes in pancreatic cancer cells

Cell invasiveness is closely correlated with cancer metastasis. We therefore examined whether linc00675 knockdown affects invasiveness of pancreatic cancer cells. A Matrigel invasion assay showed that linc00675 knockdown significantly inhibited invasiveness of SW1990 and MIA PaCa-2 cells (Figure 4A). Since EMT is closely related to cell invasiveness, we also examined whether the suppression of linc00675 can affect the expression of epithelial-mesenchymal transition (EMT)-related genes. Both PCR (Figure 4B) and Western blot analyses (Figure 4C) showed that suppression of linc00675 in pancreatic cells resulted in decreased expression of mesenchymal markers

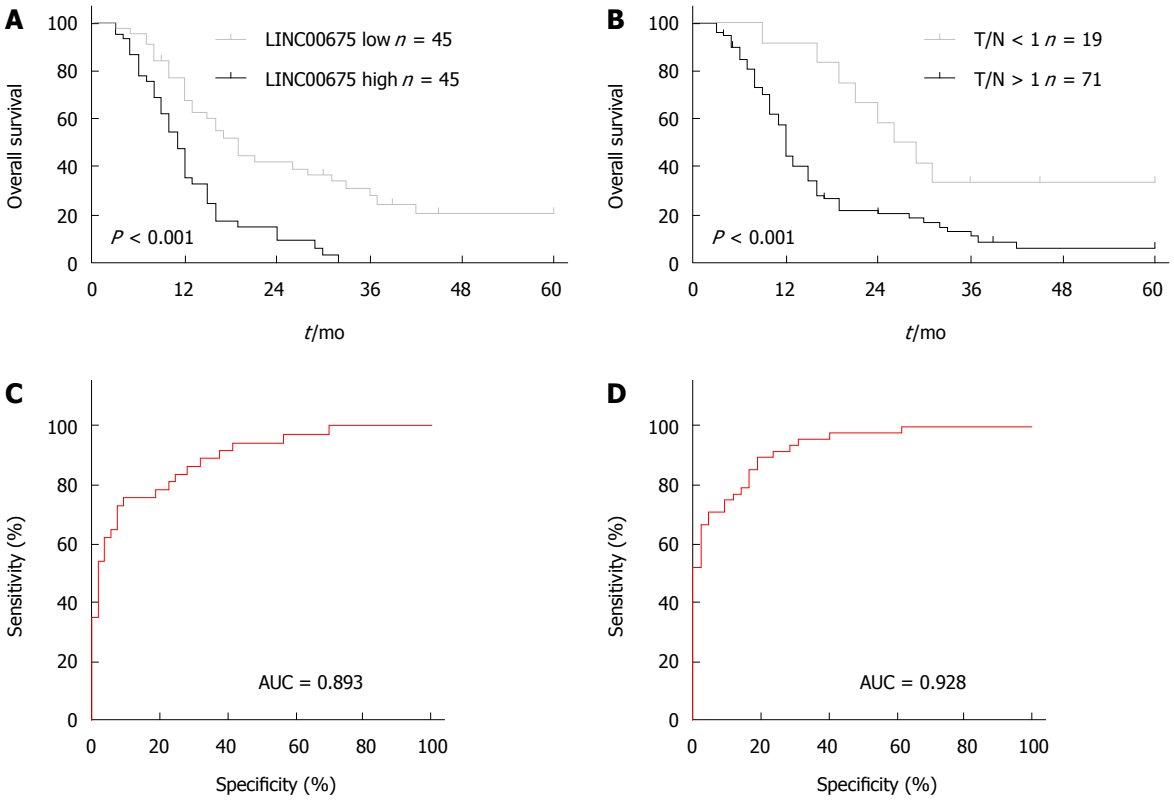
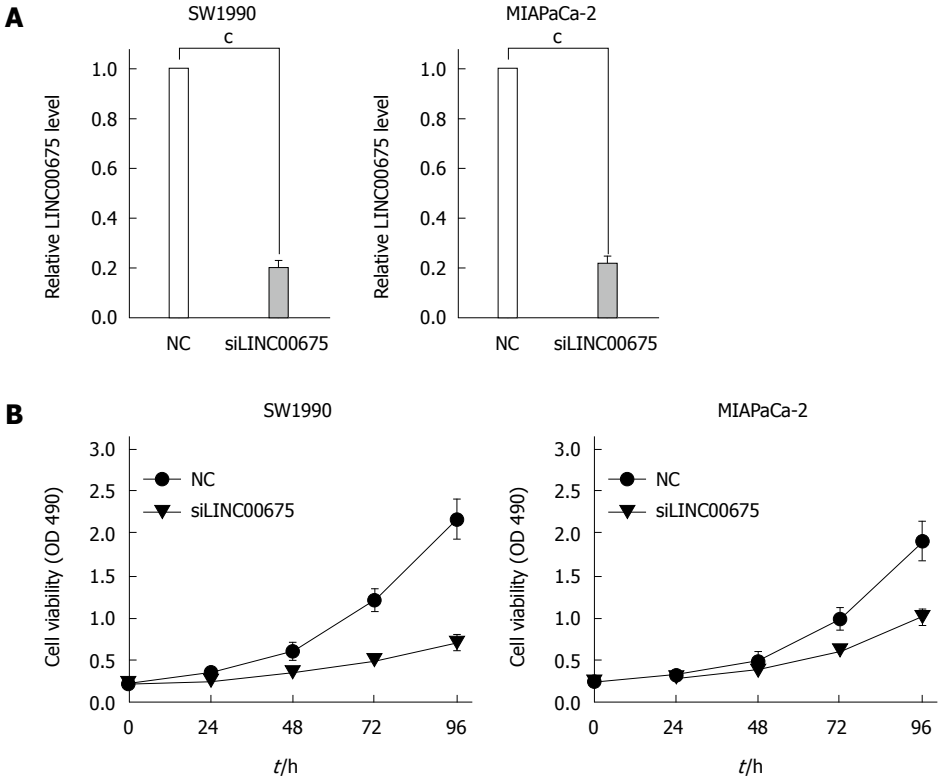


Figure 2 Overall survival of patients with pancreatic ductal adenocarcinoma based on linc00675 expression status and ROC curves of pancreatic ductal adenocarcinoma patients based on linc00675 for predicting recurrence. A: Pancreatic ductal adenocarcinoma (PDAC) patients were equally divided into two groups based on linc00675 mRNA levels, and then Kaplan-Meier survival curves were employed for comparing overall survival between two groups; B: Overall survival of patients with PDAC was evaluated *via* Kaplan-Meier survival curves based on whether linc00675 was increased in tumor tissues compared with paired non-cancerous tissue; C: ROC curves of PDAC patients based on linc00675 for predicting recurrence within one year; D: ROC curves of PDAC patients based on linc00675 for predicting recurrence within 6 mo.



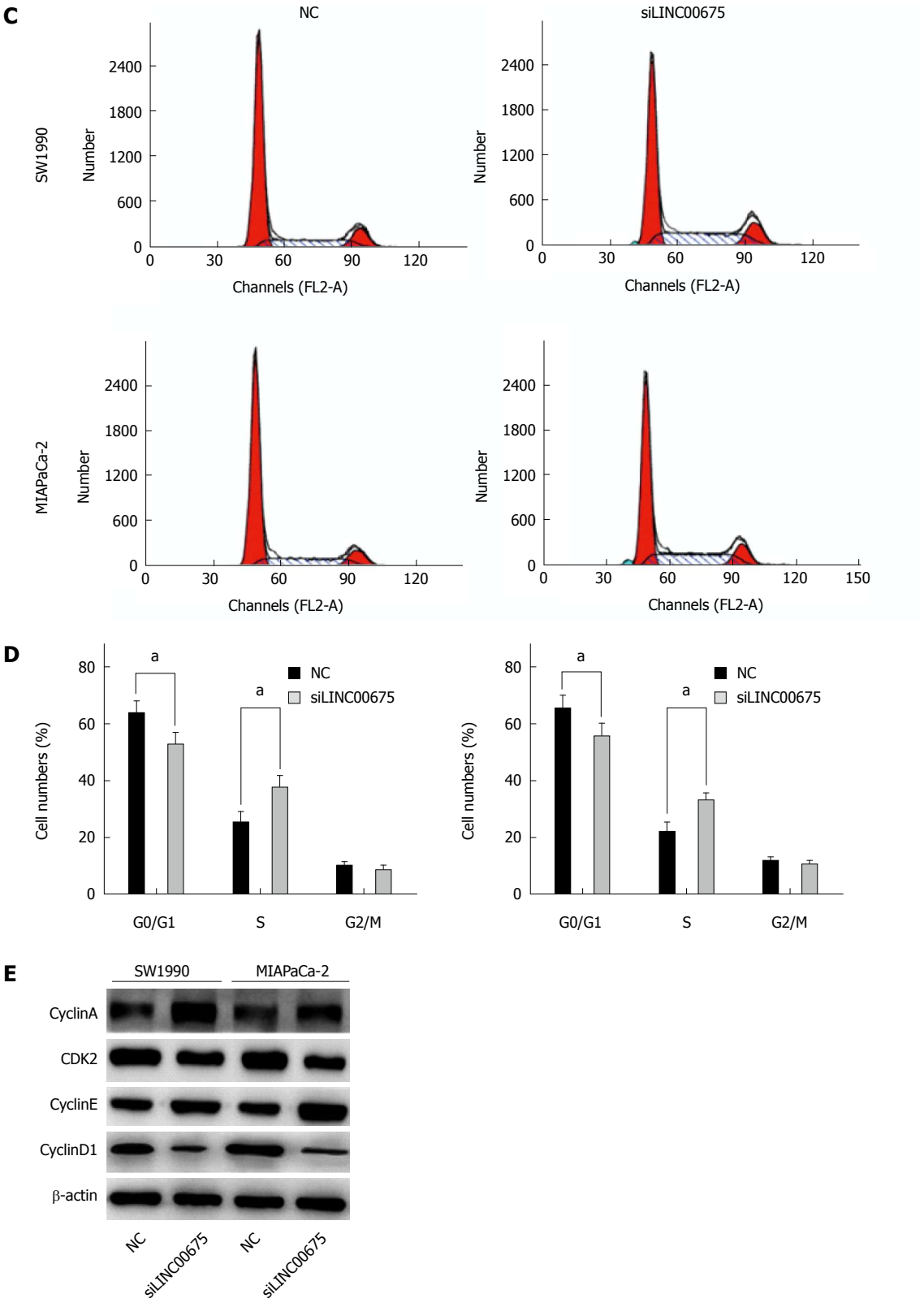


Figure 3 Effect of linc00675 knockdown on pancreatic ductal adenocarcinoma growth *in vitro*. A: Knockout efficiency of siRNA targeting Linc00675 was confirmed by quantitative real time-polymerase chain reaction in SW1990 and MIA PaCa-2 cell lines, $^{c}P < 0.001$, NC *vs* siLINC00675, Student's *t*-test; B: Effects of knockdown of linc00675 on the proliferation of SW1990 and MIA PaCa-2 cells were assessed with MTT assay; C: Cell cycle of SW1990 and MIA PaCa-2 was analyzed by flow cytometry 48 h after transfection; D: Effect of knockdown of linc00675 on percentage of cells in G1-G0, S, and G2-M phase was examined quantitatively, $^{a}P < 0.05$, NC *vs* siLINC00675, Student's *t*-test; E: Cells were untreated, or transfected with linc00675, then the expression of CyclinA, CDK2, CyclinE and CyclinD1 was detected by Western blot. Data are represented as the mean ± SD from three independent experiments.

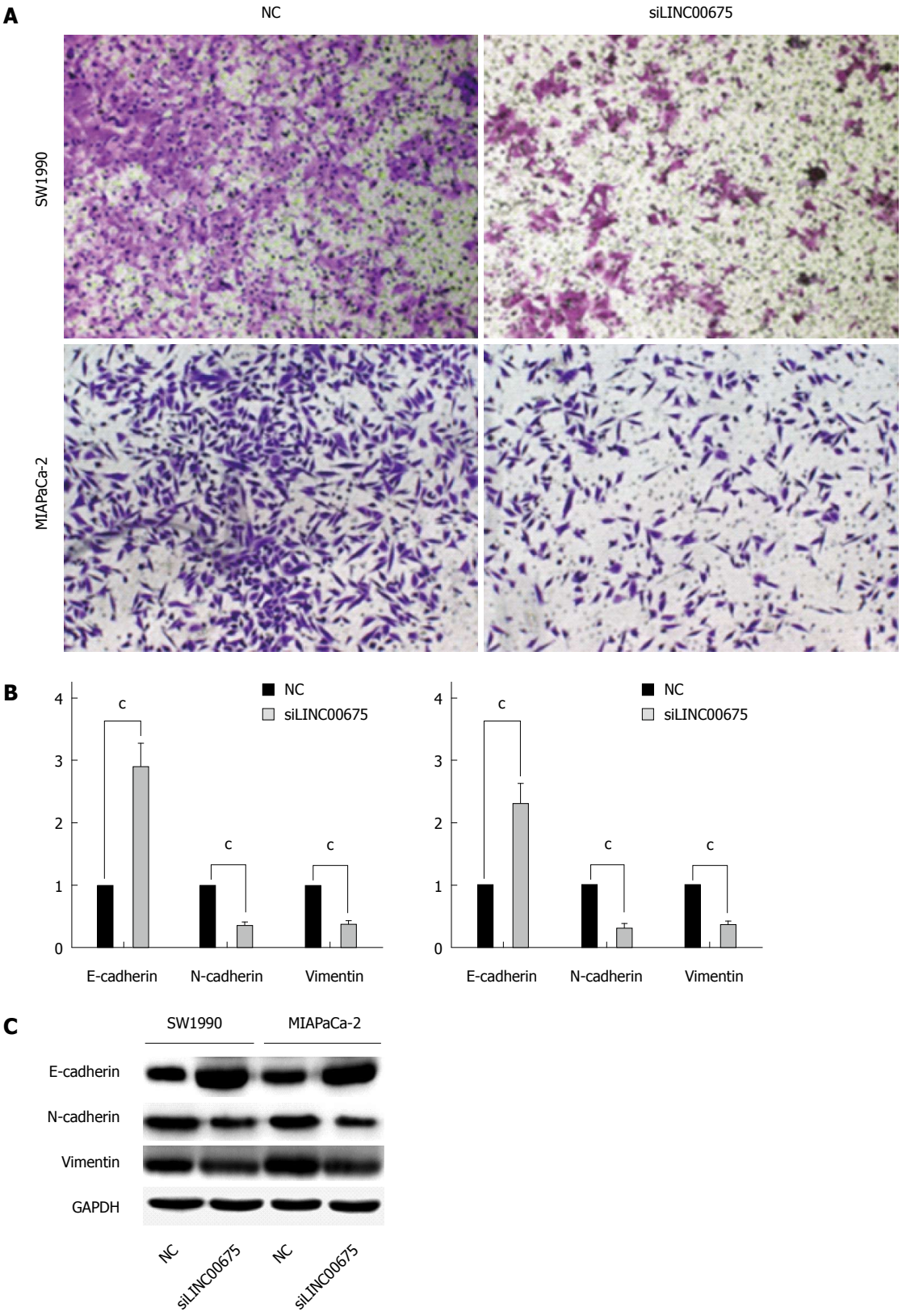


Figure 4 Effect of linc00675 knockdown on pancreatic ductal adenocarcinoma cell invasiveness *in vitro*. A: Representative images of transwell assay after linc00675 knockdown in pancreatic cancer cell line SW1990 and MIA PaCa-2; B: Quantitative real time-polymerase chain reaction analysis of E-cadherin, N-cadherin and Vimentin was performed in SW1990 and MIA PaCa-2 cells at 72 h after transfection; $^{c}P < 0.001$, NC *vs* siLINC00675, Student's *t*-test; C: Western blot analysis of E-cadherin, N-cadherin and Vimentin was performed in SW1990 and MIA PaCa-2 cells at 72 h after transfection. Data are represented as the mean ± SD from three independent experiments.

N-cadherin and vimentin, and upregulation of epithelial marker E-cadherin.

DISCUSSION

To our knowledge, the findings of the present study provide the first evidence of the potential clinical utility of linc00675 expression as a prognostic factor in PDAC, and support a role for this lncRNA in cancer cell behavior. We showed that linc00675 is frequently highly expressed in PDAC tissues as well as in PDAC cell lines, and its overexpression positively correlates with lymph node metastasis, perineural invasion and poor prognosis in PDAC patients, suggesting that linc00675 might to be a useful biomarker. Supporting this observation, functional studies revealed a strong correlation between linc00675 level and malignant behavior of pancreatic carcinoma cells.

LncRNAs are non-protein coding transcripts longer than 200 nucleotides which can regulate gene transcription indirectly through the targeting and recruitment of chromatin-modifying complexes as well as directly at the transcriptional or posttranscriptional levels[14,15]. An increasing number of studies have revealed that lncRNAs have important functions and implicated them in a wide range of diseases. Moreover, lncRNA biology is attracting great attention in cancer research because dysregulated lncRNAs occur in a variety of cancers, including pancreatic cancer[10,16]. Recently, several lncRNAs have been identified as oncogenes or tumor suppressors during cancer progression. In pancreatic cancer, the lncRNA HOTAIR[17], MALAT1[18] and HULC[19] have been found to be associated with either clinical characteristics of PDAC patients or cancer cell behavior. Abnormally expressed lncRNAs may play a causal role in pancreatic cancer initiation, development and progression by regulating cell proliferation, migration, invasion, and EMT[20-22]. However, research investigating functional lncRNAs in PDAC is still limited. In this study, based on microarray screening, we found that linc00675 expression in PDAC tissues was 672 times that in CP tissues. Notably, the level of linc00675 in pancreatic cancer tissues was markedly higher than other lncRNAs already found to recurrently upregulated and of potential prognostic use, further supporting the value of linc00675 as a biomarker and therapeutic target.

Identification of biomarkers that accurately predict survival, disease recurrence and response to chemotherapy would help to assess individual risk and treatment selection. Besides protein coding genes, noncoding RNAs have also been shown to possess potential diagnostic and prognostic value. Efforts have been made to identify molecular predictive factors for lymph node metastasis, perineural invasion, and the survival of cancer patients[23,24]. Here we found that linc00675 expression correlated with the prognosis of PDAC patients. PDAC patients with a high linc00675 level in tumor tissue had a very poor outcome. Importantly, this subset of patients showed a higher recurrence rate with increased linc00675 expression. By ROC curve analysis, the AUC using linc00675 as a predictor for tumor recurrence was around 0.9, which is higher than that reported for most prognostic tumor markers, such as CA19-9. Linc00675 may therefore prove to be a more useful prognostic factor in PDAC patients than biomarkers reported to date.

The prognostic value of linc00675 in patients with PDAC is supported by functional experiments. We modulated its expression in SW1990 and Miacapa-2 cell lines and found that suppression of linc00675 could reduce cell proliferation and invasion ability, which was consistent with clinical findings. Interestingly, we found that knockdown of linc00675 resulted in S phase arrest in pancreatic cancer cells. Gemcitabine, a chemotherapy agent, exerts its cytotoxic effect mainly by targeting tumor cells in S phase, which remains a standard therapy in PDAC patients. Some tumor suppressor genes and molecules were identified to increase gemcitabine sensitivity in pancreatic cancer cells through S-phase arrest[25]. Because the patients analyzed in this study were receiving gemcitabine-based chemotherapy after surgery, and linc00675 showed S phase arrest in pancreatic cancer cells, it will be interesting to explore whether linc00675 contributes to increased gemcitabine sensitivity.

In summary, we found strong expression of linc00675 in patients with PDAC, and suggest that linc00675 may serve as an oncogenic lincRNA that promotes pancreatic cancer cell growth and progression. Further study is required to completely define the function of linc00675, its utility in guiding patient management and its potential as a therapeutic target.

COMMENTS

Background

Pancreatic ductal adenocarcinoma (PDAC) is a highly malignant digestive tumor with extremely poor prognosis. Long intergenic noncoding RNAs (lincRNAs) have key roles in the regulation of multiple biological processes, including development, differentiation and carcinogenesis. There is, therefore, a need to explore the potential of lincRNAs as markers of diagnosis and prognosis and to investigate their biological functions to improve the outcome of PDAC patients.

Research frontiers

Recently, lincRNAs have been found to play critical roles in cancer initiation, progression and metastasis. LincRNA expression has been associated with patient survival and may be useful in outcome prediction and the design of anticancer treatments. Several lincRNAs have been implicated in pancreatic tumorigenesis; however, the role of linc00675 in PDAC is still unknown. In this study, the authors demonstrate that linc00675 was highly expressed in PDAC tissues compared with adjacent normal tissues. Increased expression of linc00675 in PDAC tissues positively correlated with poor survival and tumor progression. These results indicate that linc00675 could be a potential prognostic factor for PDAC patients.

Innovations and breakthroughs

This is the first study to report that linc00675 is overexpressed in PDAC tissue

compared with adjacent normal tissue. The overexpression of linc00675 positively correlates with poor survival and short-term recurrence in patients with PDAC and in functional experiments was shown to promote pancreatic cancer cell growth and progression.

Applications

This study showed that the linc00675 expression level may be useful as a predictor of prognosis in pancreatic cancer.

Terminology

Linc00675 serves as an oncogenic lincRNA that promotes pancreatic cancer cell growth and progression. Since linc00675 is associated with the malignancy phenotypes of pancreatic cancer, further study is required to determine the potential roles of linc00675 as a candidate therapeutic target.

Peer-review

This is an interesting study with valuable information regarding the expression and clinical impact of linc00675 in pancreatic ductal adenocarcinoma.

REFERENCES

1 **Reichert M**, Rustgi AK. Pancreatic ductal cells in development, regeneration, and neoplasia. *J Clin Invest* 2011; **121**: 4572-4578 [PMID: 22133881 DOI: 10.1172/JCI57131]

2 **Michl P**, Gress TM. Current concepts and novel targets in advanced pancreatic cancer. *Gut* 2013; **62**: 317-326 [PMID: 23112132 DOI: 10.1136/gutjnl-2012-303588]

3 **Mercer TR**, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet* 2009; **10**: 155-159 [PMID: 19188922 DOI: 10.1038/nrg2521]

4 **Wilusz JE**, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. *Genes Dev* 2009; **23**: 1494-1504 [PMID: 19571179 DOI: 10.1101/gad.1800909]

5 **Ponting CP**, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. *Cell* 2009; **136**: 629-641 [PMID: 19239885 DOI: 10.1016/j.cell.2009.02.006]

6 **Kung JT**, Colognori D, Lee JT. Long noncoding RNAs: past, present, and future. *Genetics* 2013; **193**: 651-669 [PMID: 23463798 DOI: 10.1534/genetics.112.146704]

7 **Cheetham SW**, Gruhl F, Mattick JS, Dinger ME. Long noncoding RNAs and the genetics of cancer. *Br J Cancer* 2013; **108**: 2419-2425 [PMID: 23660942 DOI: 10.1038/bjc.2013.233]

8 **Brunner AL**, Beck AH, Edris B, Sweeney RT, Zhu SX, Li R, Montgomery K, Varma S, Gilks T, Guo X, Foley JW, Witten DM, Giacomini CP, Flynn RA, Pollack JR, Tibshirani R, Chang HY, van de Rijn M, West RB. Transcriptional profiling of long non-coding RNAs and novel transcribed regions across a diverse panel of archived human cancers. *Genome Biol* 2012; **13**: R75 [PMID: 22929540 DOI: 10.1186/gb-2012-13-8-r75]

9 **Wapinski O**, Chang HY. Long noncoding RNAs and human disease. *Trends Cell Biol* 2011; **21**: 354-361 [PMID: 21550244 DOI: 10.1016/j.tcb.2011.04.001]

10 **Prensner JR**, Chinnaiyan AM. The emergence of lncRNAs in cancer biology. *Cancer Discov* 2011; **1**: 391-407 [PMID: 22096659 DOI: 10.1158/2159-8290.CD-11-0209]

11 **Gutschner T**, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. *RNA Biol* 2012; **9**: 703-719 [PMID: 22664915 DOI: 10.4161/rna.20481]

12 **Gupta RA**, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* 2010; **464**: 1071-1076 [PMID: 20393566 DOI: 10.1038/nature08975]

13 **Deng Q**, He B, Gao T, Pan Y, Sun H, Xu Y, Li R, Ying H, Wang F, Liu X, Chen J, Wang S. Up-regulation of 91H promotes tumor metastasis and predicts poor prognosis for patients with colorectal cancer. *PLoS One* 2014; **9**: e103022 [PMID: 25058480 DOI: 10.1371/journal.pone.0103022]

14 **Mattick JS**. The genetic signatures of noncoding RNAs. *PLoS Genet* 2009; **5**: e1000459 [PMID: 19390609 DOI: 10.1371/journal.pgen.1000459]

15 **Mattick JS**, Makunin IV. Non-coding RNA. *Hum Mol Genet* 2006; **15** Spec No 1: R17-R29 [PMID: 16651366]

16 **Gibb EA**, Brown CJ, Lam WL. The functional role of long non-coding RNA in human carcinomas. *Mol Cancer* 2011; **10**: 38 [PMID: 21489289 DOI: 10.1186/1476-4598-10-38]

17 **Kim K**, Jutooru I, Chadalapaka G, Johnson G, Frank J, Burghardt R, Kim S, Safe S. HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. *Oncogene* 2013; **32**: 1616-1625 [PMID: 22614017 DOI: 10.1038/onc.2012.193]

18 **Jiao F**, Hu H, Yuan C, Wang L, Jiang W, Jin Z, Guo Z, Wang L. Elevated expression level of long noncoding RNA MALAT-1 facilitates cell growth, migration and invasion in pancreatic cancer. *Oncol Rep* 2014; **32**: 2485-2492 [PMID: 25269958 DOI: 10.3892/or.2014.3518]

19 **Peng W**, Gao W, Feng J. Long noncoding RNA HULC is a novel biomarker of poor prognosis in patients with pancreatic cancer. *Med Oncol* 2014; **31**: 346 [PMID: 25412939 DOI: 10.1007/s12032-014-0346-4]

20 **Sun YW**, Chen YF, Li J, Huo YM, Liu DJ, Hua R, Zhang JF, Liu W, Yang JY, Fu XL, Yan T, Hong J, Cao H. A novel long non-coding RNA ENST00000480739 suppresses tumour cell invasion by regulating OS-9 and HIF-1α in pancreatic ductal adenocarcinoma. *Br J Cancer* 2014; **111**: 2131-2141 [PMID: 25314054 DOI: 10.1038/bjc.2014.520]

21 **Ma C**, Nong K, Zhu H, Wang W, Huang X, Yuan Z, Ai K. H19 promotes pancreatic cancer metastasis by derepressing let-7's suppression on its target HMGA2-mediated EMT. *Tumour Biol* 2014; **35**: 9163-9169 [PMID: 24920070 DOI: 10.1007/s13277-014-2185-5]

22 **Pang EJ**, Yang R, Fu XB, Liu YF. Overexpression of long non-coding RNA MALAT1 is correlated with clinical progression and unfavorable prognosis in pancreatic cancer. *Tumour Biol* 2015; **36**: 2403-2407 [PMID: 25481511 DOI: 10.1007/s13277-014-2850-8]

23 **Xiao Z**, Luo G, Liu C, Wu C, Liu L, Liu Z, Ni Q, Long J, Yu X. Molecular mechanism underlying lymphatic metastasis in pancreatic cancer. *Biomed Res Int* 2014; **2014**: 925845 [PMID: 24587996 DOI: 10.1155/2014/925845]

24 **Bapat AA**, Hostetter G, Von Hoff DD, Han H. Perineural invasion and associated pain in pancreatic cancer. *Nat Rev Cancer* 2011; **11**: 695-707 [PMID: 21941281 DOI: 10.1038/nrc3131]

25 **Guo Y**, Ziesch A, Hocke S, Kampmann E, Ochs S, De Toni EN, Göke B, Gallmeier E. Overexpression of heat shock protein 27 (HSP27) increases gemcitabine sensitivity in pancreatic cancer cells through S-phase arrest and apoptosis. *J Cell Mol Med* 2015; **19**: 340-350 [PMID: 25331547 DOI: 10.1111/jcmm.12444]

P- Reviewer: Park JY, Ritchie S, Stanojevic GZ, Wongkham S **S- Editor**: Wang JL **L- Editor**: Wang TQ **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9358

World J Gastroenterol 2015 August 21; 21(31): 9358-9366
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Mechanism of aqueous fructus aurantii immaturus extracts in neuroplexus of cathartic colons

Shi-Yi Wang, Yan-Ping Liu, Yi-Hong Fan, Lu Zhang, Li-Jun Cai, Bin Lv

Shi-Yi Wang, Yan-Ping Liu, Yi-Hong Fan, Lu Zhang, Li-Jun Cai, Bin Lv, Department of Gastroenterology, The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou 310006, Zhejiang Province, China

Author contributions: Wang SY and Liu YP performed the experiments, analyzed the data, and wrote the paper; Fan YH and Zhang L designed the research, revised the paper, and contributed equally to this study; Cai LJ and Lv B performed parts of the experiments and provided valuable suggestions for this study; all authors have read and approved the final manuscript.

Supported by National Natural Science Foundation of China, No. 81473506; Natural Science Foundation of Zhejiang Province, No. LY13H030011; State Administration of Traditional Chinese Medicine of Zhejiang Province, No. 2013ZB050.

Institutional review board statement: The study was reviewed and approved by the Institutional Review Board of Zhejiang Chinese Medical University.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Zhejiang Chinese Medical University.

Conflict-of-interest statement: All the authors declare that they have no conflicts of interest.

Data sharing statement: No additional data are available.

Correspondence to: Yi-Hong Fan, PhD, Associate Professor, Department of Gastroenterology, The First Affiliated Hospital of Zhejiang Chinese Medical University, No. 54, Youdian Road, Hangzhou 310006, Zhejiang Province, China. yhfansjr@163.com
Telephone: +86-571-87608001
Fax: +86-571-87608001

Received: March 9, 2015
Peer-review started: March 10, 2015
First decision: April 23, 2015
Revised: May 21, 2015
Accepted: June 26, 2015
Article in press: June 26, 2015
Published online: August 21, 2015

Abstract

AIM: To examine the effect of aqueous fructus aurantii immaturus (FAI) extracts on the intestinal plexus of cathartic colons.

METHODS: Cathartic colons were induced in rats with dahuang, a laxative used in traditional Chinese medicine. Once the model was established (after approximately 12 wk), rats were administered mosapride (1.54 mg/kg) or various doses of aqueous FAI extracts (1-4 g/kg) for 14 d. Transit function was assessed using an ink propulsion test. Rats were then sacrificed, and the ultramicrostructure of colonic tissue was examined using transmission electron microscopy. The expression of the 5-hydroxytryptamine receptor 4 (5-HTR4) and neurofilament-H was assessed in colon tissues using real-time PCR, Western blot, and immunohistochemistry.

RESULTS: Mosapride and high dose (4 g/kg) of aqueous FAI extracts significantly improved the bowel movement in cathartic colons compared to untreated model colons as measured by the intestinal transit rate (70.06 ± 7.25 and 72.02 ± 8.74, respectively, *vs* 64.12 ± 5.19; $P < 0.05$ for both). Compared to controls, the ultramicrostructure of cathartic colons showed signs

of neural degeneration. Treatment with mosapride and aqueous FAI extracts resulted in recovery of ultrastructural pathology. Treatment with mosapride alone upregulated the gene and protein expression of 5-HTR4 compared to untreated controls ($P < 0.05$ for both). Treatment with aqueous FAI extracts ($\geqslant$ 2 g/kg) increased 5-HTR4 mRNA levels ($P < 0.05$), but no change in protein level was observed by Western blot or immunohistochemistry. The mRNA and protein levels of neurofilament-H were significantly increased with mosapride and $\geqslant$ 2 g/kg aqueous FAI extracts compared to controls ($P < 0.05$ for all).

CONCLUSION: Aqueous FAI extracts and mosapride strengthen bowel movement in cathartic colons *via* increasing the expression of 5-HTR4 and neurofilament-H.

Key words: Fructus aurantii immaturus; Aqueous extracts; Cathartic colon; 5-HTR4; Neurofilament; Myenteric plexus

Core tip: Bowel movements in cathartic colon can be strengthened with mosapride. However, recent studies show that aqueous fructus aurantii immaturus (FAI) extracts, a traditional Chinese medicine, can also strengthen bowel movement, and are widely used to treat gastrointestinal symptoms. The aim of this study was to identify the mechanism by which aqueous FAI extracts exert these effects. In a rat model of cathartic colons, treatment with mosapride and aqueous FAI extracts improved the intestinal transit rate, and increased the expression of 5-hydroxytryptamine receptor 4 and neurofilament-H.

Wang SY, Liu YP, Fan YH, Zhang L, Cai LJ, Lv B. Mechanism of aqueous fructus aurantii immaturus extracts in neuroplexus of cathartic colons. *World J Gastroenterol* 2015; 21(31): 9358-9366 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9358.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9358

INTRODUCTION

Slow transit constipation is a type of intractable constipation of an unknown etiology. The slowed colonic transmission results in functional constipation, thus affecting an individual's quality of life[1]. Chronic use of anthraquinones or traditional Chinese laxatives, such as dahuang and fanxieye, can result in laxative-dependent defecation, also referred to as "cathartic colon". Long-term laxative use causes damage to the intestinal myenteric plexus[2-4], resulting in movement disability within the colon and aggravation of constipation.

Mosapride is an agonist of 5-hydroxytryptamine receptor 4 (5-HTR4), and is widely used to treat constipation. It activates cholinergic neurons in the intestinal myenteric plexus to strengthen the movement of the digestive tract[5]. However, the traditional Chinese medicine fructus aurantii immaturus (FAI) has also been used to treat gastrointestinal diseases. Aqueous extracts of FAI, obtained from *Citrus aurantium* L. or *C. sinensis* Osbeck, have also been shown to strengthen the movement of the gastrointestinal tract[6-8]. The mechanism of action is not understood, but is thought to involve stimulation of substance P secretion from the nerve plexus[9], or activation of muscarinic acetylcholine[6] and histamine[10] receptors. It is not known whether aqueous FAI extracts also act on 5-HTR4.

The aim of the present study was to investigate the effects of aqueous FAI extracts in comparison to mosapride, and to evaluate the mechanism of action. To this end, a cathartic colon rat model was treated with mosapride and various doses of aqueous FAI extracts. The expression levels of 5-HTR4 and the structural protein neurofilament-H (NF-H) were then examined in the myenteric plexus of the intestinal wall.

MATERIALS AND METHODS

Drugs

Mosapride (5 mg; batch No. 2757C) was purchased from Dainippon Sumitomo Pharma Co., Ltd. (Chuo-ku, Osaka, Japan). Dahuang (batch No. 20130302) and FAI (batch No. 20131017) were produced by the Zhejiang Chinese Medical University Medical Pieces Co. Ltd. (Linan, Zhejiang, China). High-performance liquid chromatography analysis of the FAI indicated that it contained 0.79% hesperidin, 0.18% aloe emodin, 0.31% rheic acid, 0.28% rheum emodin, 0.35% chrysophanic acid, and 1.17% physcion.

To prepare the aqueous extracts, FAI was soaked for 30 min in water (8-10 × *V*), and then boiled for 30 min. Next, water was added (3-5 × *V*), and the solution was boiled for an additional 25 min. The decoction was concentrated to 1 g/mL crude drug using rotary evaporators (SENCO R-201; Shanghai Zhicheng Biological Technology Co., Ltd., Shanghai, China) and sterilized.

Animal model of cathartic colon

Eighty-two specific pathogen-free male Sprague-Dawley rats (200 ± 10 g) were purchased from Shanghai Xipuer-bikai Experimental Animal Co., Ltd. (Shanghai, China) and housed for 1 wk under a 12 h light/dark cycle at 22-24 ℃ with 50%-60% humidity and a noise level < 50 db. Prior to experimentation, rats were allowed free access to food and tap water. All the procedures involving animals were conducted in accordance with the ethical principles adopted by

the Animal Experimental Center of Zhejiang Chinese Medical University and were approved by the Ethics Committee on Animal Experiments at Zhejiang Chinese Medical University.

The experimental group of rats (n = 70) received daily oral administration of 15 mL dahuang at an initial dose of 200 mg/kg (13.3 mg/mL). The dose increased by 200 mg/kg each day until 50% of the rats exhibited loose stools. This occurred when the dosage was about 2400 mg/kg per day, which was maintained until the loose stools disappeared in 80% of the rats. In the next stage, the dosage was again increased by 200 mg/kg per day until 50% of the rats exhibited loose stools again. The final dosage of dahuang was 3800 mg/kg per day (at 253.3 mg/mL). The time to establish the laxative-dependent slow transit constipation model was 12 wk. Food and water were not limited during the modeling procedure. Animals in the control group (n = 12) received daily oral administration of 15 mL/kg normal saline.

Drug treatment

Two animals died during establishment of the cathartic colon model; thus, the remaining 68 rats were divided into the following five treatment groups: model (n = 12); mosapride (n = 14); low-dose (1 g/kg) aqueous FAI extract (FAI-L; n = 14); medium-dose (2 g/kg) aqueous FAI extract (FAI-M; n = 14); high-dose (4 g/kg) aqueous FAI extract (FAI-H; n = 14). The treatment consisted of daily oral administration of 15 mL/kg per day for 2 wk; normal saline was administrated to the control and model groups.

The dose of mosapride was 1.54 mg/kg according to the surface area conversion[11], which is equivalent to 6.2 × the human adult dosage used in clinical work. The doses of 1 g/kg, 2 g/kg, and 4 g/kg aqueous FAI extract are equivalent to 6.2 ×, 12.4 ×, and 24.8 × the human adult dosage, respectively, used in clinical work.

Intestinal transit rate testing

To evaluate the intestinal transit rate (ITR), animals received an oral administration of 2 mL carbonic ink. After 40 min, the rats were anesthetized with 350 mg/kg chloral hydrate and the complete intestinal tract, from the pylorus to the terminal rectum, was removed. Without applying tension, the lengths of the whole intestinal tract, small intestine, large intestine, and ink propulsion were measured. The percentage of blackened intestinal tracts was calculated: ITR (%) = pushing length/total length × 100.

Specimen collection

Colonic tissue located 1 cm from the anus was collected from one randomly selected rat in each group, cut into 1 mm^3 portions, and fixed for 24 h in 2.5% buffered glutaraldehyde for transmission electron microscopy (TEM). From all rats, colonic tissue (1 cm) dissected approximately 2 cm from the anus was fixed in 4% buffered neutral formalin and stored at 4 ℃ for subsequent immunohistochemical analyses. Colonic tissue (2 cm) located approximately 4 cm from the anus was rapidly frozen in liquid nitrogen for PCR and Western blot analyses.

TEM

Following fixation in 2.5% buffered glutaraldehyde solution, colonic tissue samples were washed with phosphate buffered saline (PBS) for 30 min, fixed for 1 h in 1% osmic acid, washed in PBS again, and dehydrated through a graded ethanol series. Specimens were embedded in epoxy resin (Epon 812) and stained with methylene blue. Sections were cut using an ultramicrotome (HM335E; Microm GmbH, Waldrof, Germany), and stained with uranyl acetate and lead citrate. Sections were imaged under a transmission electron microscope (H-7650; Hitachi, Ltd., Tokyo, Japan).

Real-time PCR

RNAiso Plus (9108; Takara Bio, Inc., Otsu, Shiga, Japan) was used to extract RNA from frozen tissue samples, and the concentration of RNA was measured using a trace nucleic acid analyzer (Thermo Fisher Scientific, Waltham, MA, United States). RNA (1 μg/μL) was reverse transcribed to cDNA using a PrimeScript RT reverse transcription kit (RR036A; Takara Bio Inc.). Amplification reactions were as follows: 2 μL cDNA, 10 μL SYBR Premix Ex *Taq* Ⅱ, 0.4 μL ROX Ⅱ, 0.8 μL forward and reverse primers (10 μmol/L), and 6.0 μL dH_2O. The two-step amplification method was performed on a real-time PCR system (7500; Applied Biosystems of Thermo Fisher Scientific): initial denaturation at 95 ℃ for 30 s, followed by 40 cycles of denaturation at 95 ℃ for 5 s and annealing and extension at 60 ℃ for 30 s. A final melting curve protocol was performed to confirm the specificity of the primers. Primers were designed and synthesized by Shenggong Biology and Engineering Co., Ltd. (Shanghai, China) (Table 1). GAPDH was used as the normalization control, and the $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression of target genes.

Western blot

Colon tissue samples (50 mg) were lysed and homogenized in 200 μL lysis buffer and centrifuged at 10000 *g* for 10 min at 4 ℃. The concentration of protein in the supernatant was determined using a BCA protein assay kit (P0012; Beyotime Technology Co., Ltd., Jiangsu, China). Proteins were separated by SDS-PAGE using an electrophoresis apparatus (PowerPac 3000; Bio-Rad Laboratories, Inc., Hercules, CA, United States), and then transferred to PVDF membranes. The membranes were blocked in skim milk for 2 h and then incubated overnight with GAPDH (sc-365062; 1:500), 5-HTR4 (sc-32564; 1:200) (Santa Cruz Biotechnology,

Table 1 Primer sequences and amplification length

Gene	Primer sequence	Amplification length (bp)
NF-H	Forward: 5'-GCCCTCACCAAACAGGAAT-3' Reverse: 5'-GCGTTCAGCAATACATCACG-3'	147
5-HTR4	Forward: 5'-GCCTTCTACATCCCGTTTCTC-3' Reverse: 5'-CTTGGCTGCTTTGGTCTCTG-3'	180
GAPDH	Forward: 5'-GGCACAGTCAAGGCTGAGAATG-3' Reverse: 5'-ATGGTGGTGAAGACGCCAGTA-3'	252

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; NF-H: Neurofilament-H; 5-HTR4: 5-hydroxytryptamine receptor 4.

Table 2 Intestinal transit rate

Group	*n*	Pushing length (cm)	Total length (cm)	ITR
Control	12	84.35 ± 10.27	107.82 ± 10.83	78.14 ± 4.26
Model	12	59.51 ± 11.08	92.40 ± 13.14	64.12 ± 5.19[b]
Mosapride	14	82.40 ± 11.46	117.43 ± 11.48	70.06 ± 7.25[b,c]
FAI-L	14	70.50 ± 17.30	101.90 ± 17.04	68.76 ± 7.48[b]
FAI-M	13	75.74 ± 16.39	110.93 ± 13.16	67.78 ± 8.00[b]
FAI-H	14	84.71 ± 13.53	117.73 ± 13.60	72.02 ± 8.74[a,d]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* control; [c]$P < 0.05$, [d]$P < 0.01$ *vs* model. ITR: Intestinal transit rate; FAI: Fructus aurantii immaturus; H: High dose (4 g/kg); L: Low dose (1 g/kg); M: Medium dose (2 g/kg).

Inc., Dallas, TX, United States), and NF-H (#2836, 1:500; Cell Signaling Technology, Inc., Danvers, MA, United States) primary antibodies. Then, the membranes were incubated in horseradish peroxidase-conjugated goat-anti-mouse (sc-2005) or donkey-anti-goat (sc-2020) IgGs (Santa Cruz Biotechnology, Inc.) for 2 h at room temperature. Proteins were visualized with enhanced chemiluminescence (GE Healthcare, Little Chalfont, United Kingdom) and quantified using Quantity One 4.6.2 software (Bio-Rad Laboratories, Inc.). Protein expression was normalized to GAPDH.

Immunohistochemistry

The formalin-fixed colon tissues were embedded in paraffin and sectioned at a thickness of 4 μm. Sections were prepared for immunostaining using a two-step Envision method involving high-pressure antigen retrieval and quenching of endogenous peroxidase activity with hydrogen peroxide. Sections were incubated with primary antibodies (anti-NF-H, 1:80; anti-5-HTR4, 1:30), followed by horseradish peroxidase-conjugated secondary antibodies, and visualized with diaminobenzidine with hematoxylin counterstaining. The slides were imaged using a Nikon Eclipse 80i optical microscope (Nikon Corp., Tokyo, Japan). Positive staining was defined by the presence of brown-colored staining in the cytoplasm. IPP 6.0 color image analysis software (Media Cybernetics, Rockville, MD, United States) was used to identify the mean integrated optical density (IOD) from five randomly selected positive areas using the formula: IOD = ∑ area (positive expression) × density (mean IOD in this area).

Statistical analysis

All analyses were performed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, United States). Comparisons between groups were conducted using repeated measures analysis of variance followed by a least significant difference test in the case of equal variance, otherwise a Dunnett's T3 method was used. All data are expressed as the mean ± SD. $P < 0.05$ was considered statistically significant.

RESULTS

Rats' general condition

Cathartic colons were successfully established in 68/70 animals administered dahuang; two animals died during the 12-wk period. Model animals were thinner than controls, with substantially less defecation and hard stools.

Of the 68 animals with cathartic colons, one rat from the FAI-M group died during the study. All cathartic colon groups had significantly shorter ITR than control animals ($P < 0.05$ for all) (Table 2). Compared to the model group, mosapride treatment and high-dose FAI significantly improved the ITR ($P < 0.05$ for both).

TEM assessment of neurodegeneration in cathartic colons

In the control group, neurons in the colonic tissue had a normal morphology and contained abundant rough endoplasmic reticulum, free ribosomes, mitochondria, and neurofilaments (Figure 1A and B). Cathartic colons in model animals showed signs of neurodegeneration,

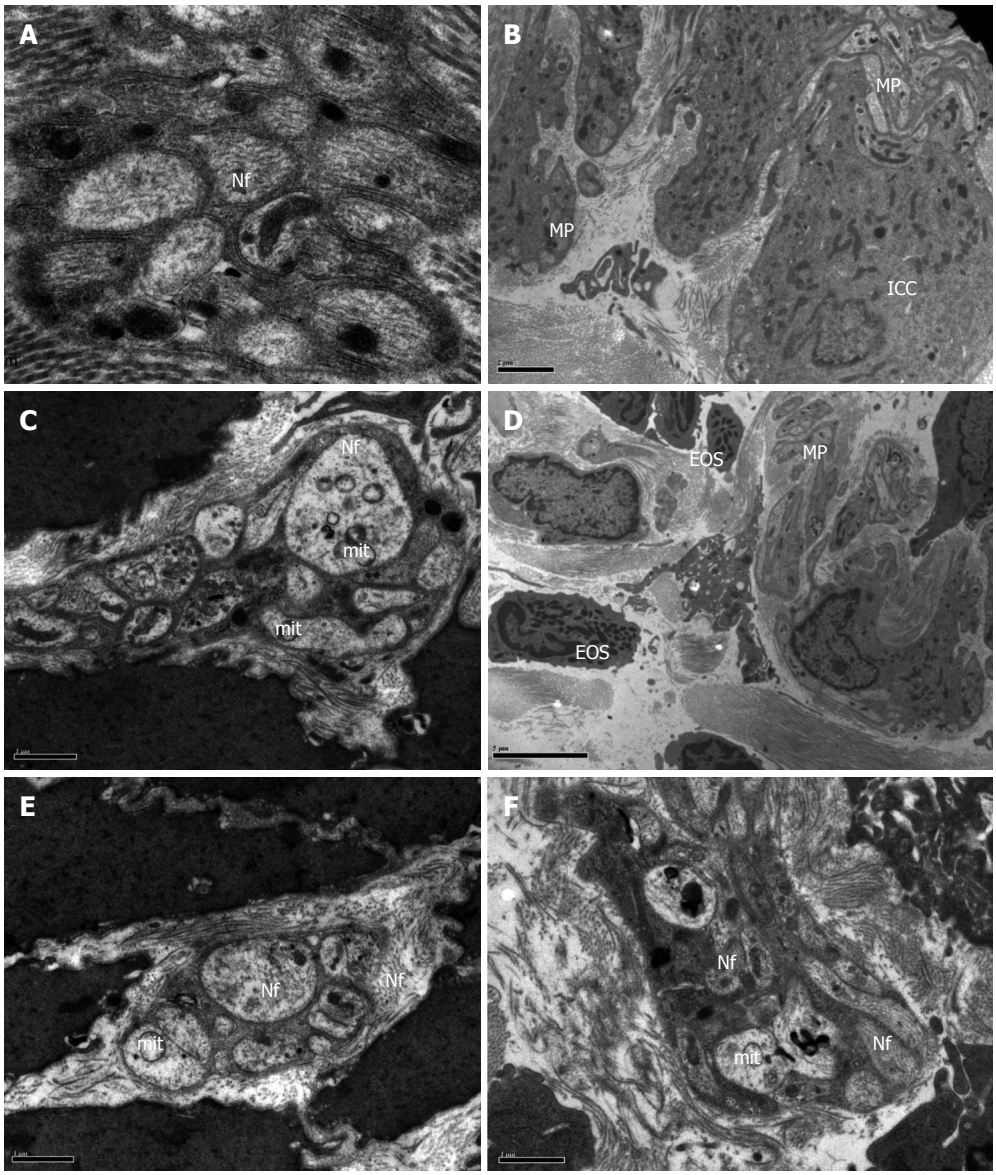


Figure 1 Transmission electron microscopy of cathartic colons. In the colons of control animals, normal A: Nerve fibers (Nf; magnification × 40000); and B: Interstitial cells of Cajal (ICC; magnification × 2500) can be seen. In contrast, cathartic colons in model animals showed C: Vacuole formation in 'mit' and sparse neurofilament (magnification × 11500); D: Eosinophil (EOS) infiltration (magnification × 1750); E (magnification × 5900) and F (magnification × 5900) each for mosapride and FAI showing recovery of damage.

with eosinophil infiltration, fractured collagenous fibers with disordered arrangement, vacuoles in swollen mitochondria, and autophagic vacuoles in the cytoplasm (Figure 1C and D). In addition, sparse neurofilaments and debris of eosinophils, which contained a large amount of lipofuscin, could be seen in the muscular layer and myenteric plexus. Interstitial cells of Cajal appeared shrunken. In contrast, these changes were not observed in animals receiving mosapride treatment or FAI (Figure 1E and F).

Upregulation of 5-HTR4 and NF-H mRNA with FAI and mosapride

Expression of *5-HTR4* and *NF-H* mRNAs was significantly reduced in cathartic colons compared to normal controls (both $P < 0.01$) (Figure 2). However, treatment with mosapride or $\geqslant$ 2 g/kg aqueous FAI extracts (medium- and high-dose groups) significantly upregulated the expression compared to the model group ($P < 0.05$ for all).

Upregulation of 5-HTR4 and NF-H protein with FAI and mosapride

Expression of 5-HTR4 and NF-H proteins was significantly reduced in cathartic colons compared to normal controls, as assessed by Western blot ($P < 0.05$ for both) (Figure 3). Treatment with mosapride significantly upregulated 5-HTR4 and NF-H ($P < 0.01$ for both); however, only NF-H expression was increased significantly in the FAI-M and FAI-H groups (*P*

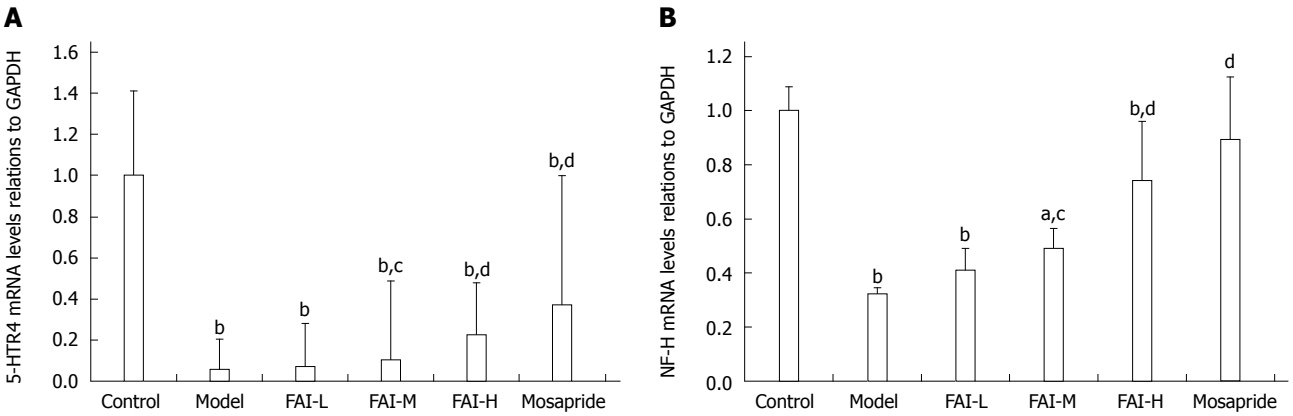


Figure 2 Relative mRNA expression of 5-hydroxytryptamine receptor 4 and neurofilament-H in rat colon tissue. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* model. FAI: Fructus aurantii immaturus; H: High dose (4 g/kg); L: Low dose (1 g/kg); M: Medium dose (2 g/kg); NF-H: Neurofilament-H; 5-HTR4: 5-hydroxytryptamine receptor 4.

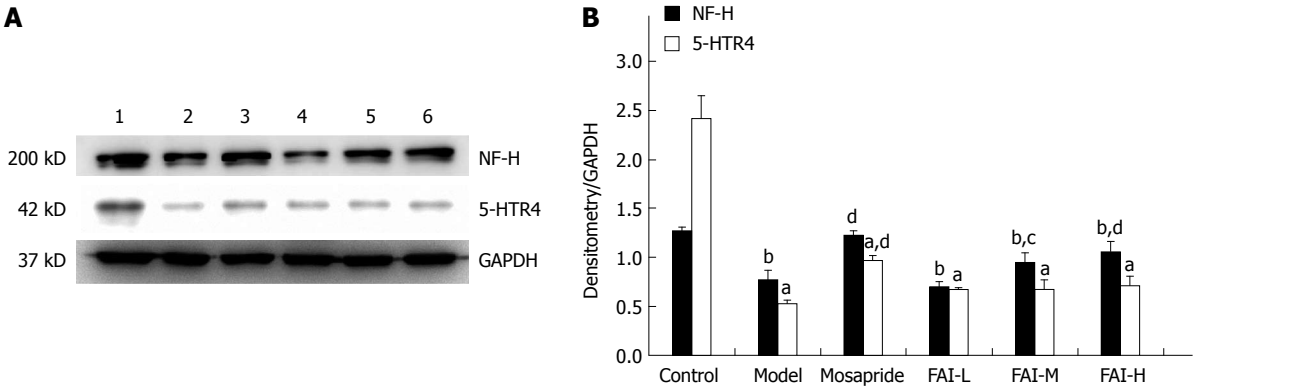


Figure 3 Protein expression of 5-hydroxytryptamine receptor 4 and neurofilament-H in rat colon tissue. A: Representative Western blots showing 5-HTR4 and NF-H protein expression in rat colon tissue: 1 = normal, 2 = model, 3 = mosapride, 4 = FAI-L, 5 = FAI-M, 6 = FAI-H; B: Quantification of protein expression. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* model. FAI: Fructus aurantii immaturus; H: High dose (4 g/kg); L: Low dose (1 g/kg); M: Medium dose (2 g/kg); NF-H: Neurofilament-H; 5-HTR4: 5-hydroxytryptamine receptor 4.

Table 3 Semi-quantitative analysis of 5-hydroxytryptamine receptor 4 and neurofilament-H protein expression intensity in rat colon tissue

Protein	Control	Model	Mosapride	FAI-L	FAI-M	FAI-H
5-HTR4	161.73 ± 67.56	7.85 ± 4.44^{b}	75.13 ± 92.17^{b,c}	19.31 ± 22.41^{b}	26.89 ± 19.04^{b}	23.89 ± 32.09^{b}
NF-H	1064.45 ± 358.96	166.22 ± 230.38^{b}	649.13 ± 306.48^{a,c}	219.42 ± 177.20^{b}	420.89 ± 476.75^{b}	631.78 ± 456.29^{a,c}

$^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control; $^{c}P < 0.05$ *vs* model. FAI: Fructus aurantii immaturus; H: High dose (4 g/kg); L: Low dose (1 g/kg); M: Medium dose (2 g/kg); NF-H: Neurofilament-H; 5-HTR4: 5-hydroxytryptamine receptor 4.

< 0.05).

Immunohistochemical analysis of 5-HTR4 and NF-H expression

5-HTR4-positive cells were observed within the mucous layer, submucosal plexus, muscular layer, and myenteric plexus (Figure 4). Semi-quantitative analysis of expression revealed that 5-HTR4 expression was significantly reduced in cathartic colons compared to control animals ($P < 0.01$ for all) (Table 3). However, only mosapride treatment resulted in a significant increase in expression compared to the model group ($P < 0.05$).

NF-H-positive cells were found primarily within the myenteric plexus (Figure 5). Compared with the control group, semi-quantitative analysis showed significantly decreased NF-H protein expression in the cathartic colons ($P < 0.05$ for all) (Table 3). Treatment with mosapride and high-dose FAI significantly increased NF-H expression compared to model animals ($P < 0.05$ for both).

DISCUSSION

Pathologic changes in the enteric nervous system are responsible for slow transit constipation[12]. Together,

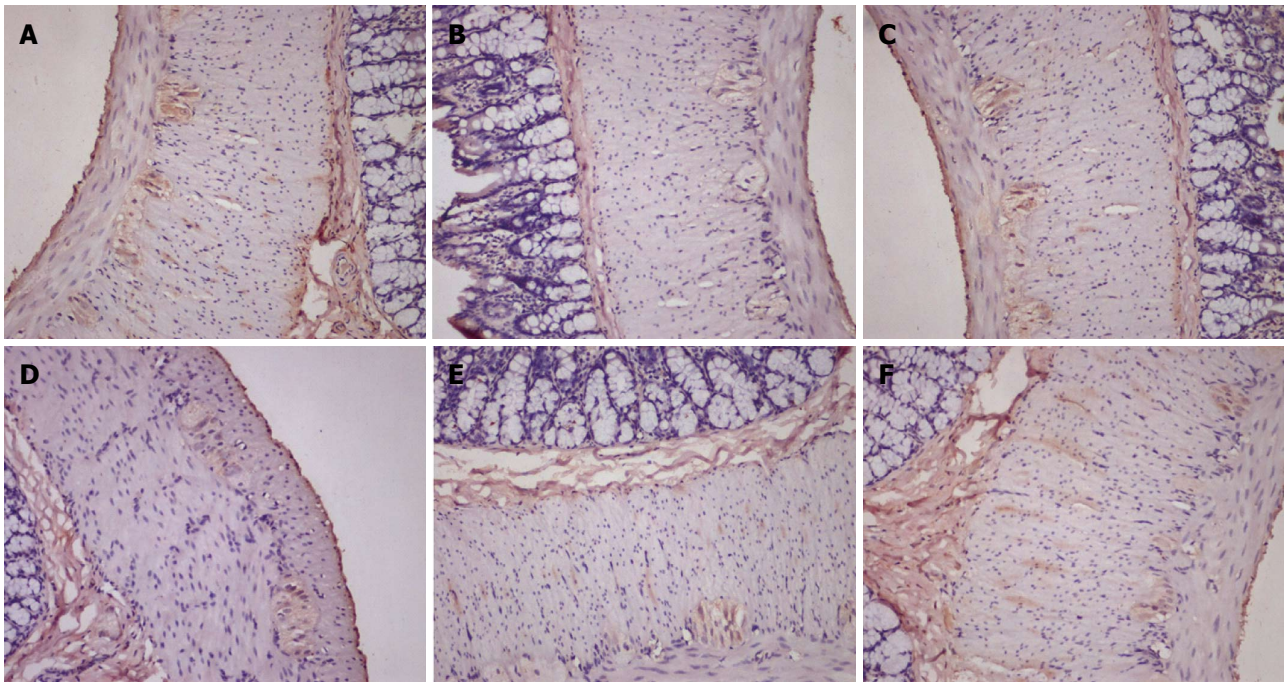


Figure 4 Immunohistochemical staining for 5-hydroxytryptamine receptor 4 in rat colon tissue. A: Control group; B: Model group; C: Mosapride group; D: Low dose (1 g/kg) aqueous fructus aurantii immaturus (FAI) group; E: Medium dose (2 g/kg) aqueous FAI group; F: High dose (4 g/kg) aqueous FAI group (magnification × 200).

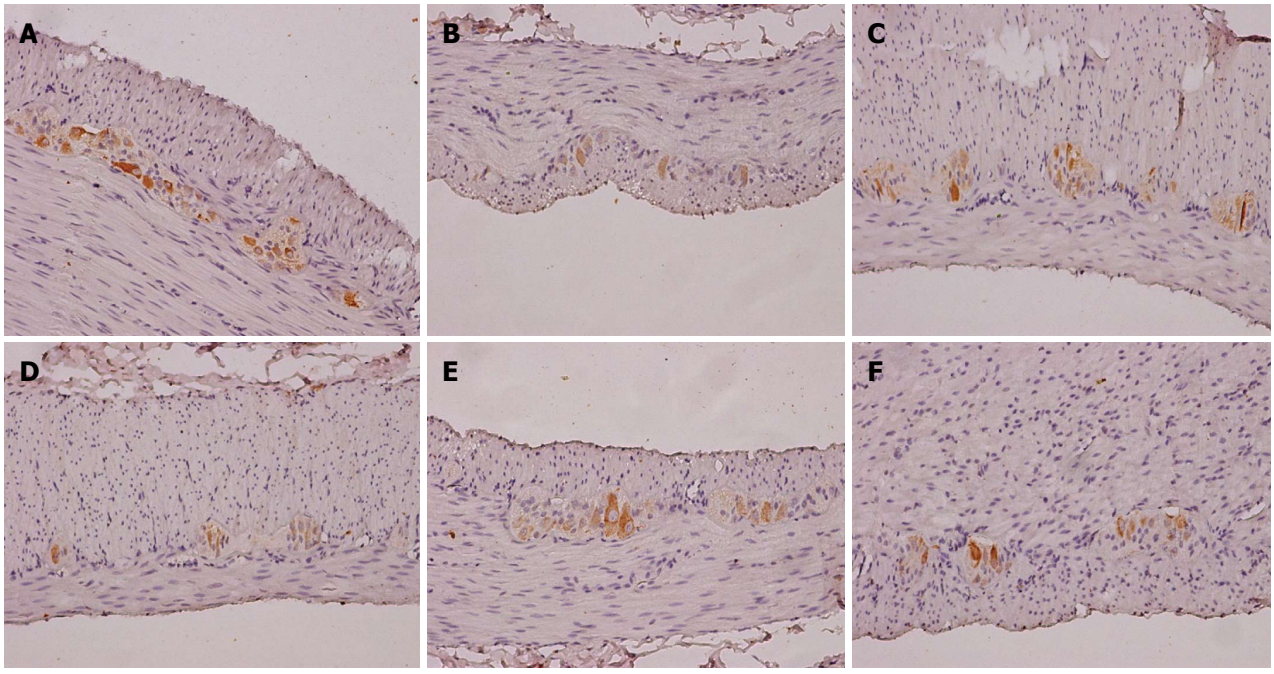


Figure 5 Immunohistochemical staining for neurofilament-H in rat colon tissue. A: Control group; B: Model group; C: Mosapride group; D: Low dose (1 g/kg) aqueous fructus aurantii immaturus (FAI) group; E: Medium dose (2 g/kg) aqueous FAI group; F: High dose (4 g/kg) aqueous FAI group (magnification × 200).

the myenteric plexus, innervating the smooth muscle, and the submucosal plexus, innervating the intestinal mucosa, regulate gastrointestinal function[13]. Clinical evidence indicates that degeneration of the myenteric plexus is the primary pathologic finding[2-4], possibly due to increased neuronal apoptosis. Thus, slow transit constipation is not simply a functional disease, but may also represent an enteric neuropathy[14,15].

In the present study, a rat model of slow transit constipation was induced by chronic administration of dahuang, demonstrated by the reduced ITR in ink propulsion tests. Ultrastructural examination revealed that a possible mechanism for this effect was loss of ganglion in the myenteric plexus and a decrease in

neurofilaments, which is consistent with our previous research[16] and reports of others[17]. Indeed, a previous study showed that the expression of the neurotrophin receptor p75 is increased in the intestinal wall of cathartic colon[18], which is known to mediate neuronal apoptosis[19]. Furthermore, the presence of eosinophil infiltration suggests that an additional inflammatory component may contribute to the observed reduction in intestinal function.

Gastrointestinal motility is enhanced with mosapride treatment, as shown in clinical studies[20-24] and in animal models[25-27]. The findings of the present study are consistent with this, as mosapride treatment significantly increased the ITR in animals with cathartic colons. Moreover, this recovery of function was accompanied by increased expression of 5-HTR4 and NF-H, which is consistent with previous studies[5,28]. This study shows that functional recovery with aqueous FAI extracts may occur *via* a similar mechanism, as treatment with a high dose significantly increased transcription of both 5-HTR4 and NF-H. The upregulation of NF-H expression by mosapride and aqueous FAI extracts is indicative of neuronal repair within the intestinal wall of cathartic colons. However, whereas mosapride increased both 5-HTR4 and NF-H protein expression, FAI led to upregulation of only NF-H protein. As aqueous FAI extracts increased the transcription of 5-HTR4, it is also possible that they affected related functional RNAs, such as microRNAs or long noncoding RNAs, to regulate 5-HTR4 at the post-transcriptional level[29].

It has been demonstrated that 5-HTR4 agonists play an important role in the development and survival of intestinal neurons[30-32]. Therefore, these agonists may represent a new therapeutic tool to treat enteric nervous system-deficiency diseases[33]. The results presented here indicate that aqueous FAI extracts could also be therapeutic in these cases. However, further studies are needed to establish an exact mechanism for the observed recovery of intestinal function. Importantly, the composition of the aqueous FAI extracts is complex[34]. Therefore, the active component(s) have yet to be identified.

CONCLUSION

Slow transit constipation can be alleviated by treatment with mosapride and aqueous FAI extracts; both of which promote repair of the myenteric plexus and upregulate transcription of 5-HTR4 and NF-H. Therefore, patients with laxative-dependent constipation may benefit from administration of either mosapride or aqueous FAI extracts.

ACKNOWLEDGMENTS

The authors would like to thank Professor Wang Li from the Electron Microscope Center at the Medical College of Zhejiang University for assistance with TEM experiments.

COMMENTS

Background

In China, fructus aurantii immaturus (FAI) is widely used to treat various kinds of gastrointestinal diseases. Aqueous FAI extracts promote movement of the gastrointestinal tract, though the mechanism remains unknown.

Research frontiers

Evidence suggests that irritant laxatives can damage the enteric nervous system, resulting in laxative-dependent constipation, also known as cathartic colon. This type of slow transit constipation can be treated with agonists of the 5-hydroxytryptamine receptor 4 (5-HTR4). Aqueous FAI extracts have a similar effect, though the mechanism is not known.

Innovations and breakthroughs

This study shows that mosapride and aqueous FAI extracts strengthen bowel movement in rat cathartic colon and promote myenteric plexus repair. Whereas mosapride increases expression of 5-HTR4 and neurofilament-H (NF-H) protein, aqueous FAI extracts only result in an increase of NF-H protein.

Applications

New therapeutic agents for the treatment of slow transit constipation are needed to counteract the increased use of laxatives in China, which can cause myenteric plexus damage. The findings of this study indicate that aqueous FAI extracts, a traditional Chinese medicine, may be effective for recovery of intestinal functional and enteric nervous system damage.

Terminology

Cathartic colon is a condition resulting from long-term use of stimulant/irritant weight-control agents (*e.g.*, phenolphthalein, cascara, castor oil, and senna extract). Prolonged misuse causes neuromuscular disruption in the intestine resulting in stimulant-dependent constipation.

Peer-review

This article describes the effect of aqueous FAI extracts on cathartic colons in rats. The results show that treatment increases the intestinal transit rate, promotes myenteric plexus recovery, and upregulates transcription of 5-HTR4 and NF-H, similar to what is observed with mosapride. In contrast, aqueous FAI extracts increase protein expression of NF-H only, whereas mosapride increases expression of both 5-HTR4 and NF-H protein.

REFERENCES

1 **Shahid S**, Ramzan Z, Maurer AH, Parkman HP, Fisher RS. Chronic idiopathic constipation: more than a simple colonic transit disorder. *J Clin Gastroenterol* 2012; **46**: 150-154 [PMID: 22011587 DOI: 10.1097/MCG.0b013e318231fc64]

2 **Krishnamurthy S**, Schuffler MD, Rohrmann CA, Pope CE. Severe idiopathic constipation is associated with a distinctive abnormality of the colonic myenteric plexus. *Gastroenterology* 1985; **88**: 26-34 [PMID: 3964770 DOI: 10.1016/S0016-5085]

3 **Schouten WR**, ten Kate FJ, de Graaf EJ, Gilberts EC, Simons JL, Klück P. Visceral neuropathy in slow transit constipation: an immunohistochemical investigation with monoclonal antibodies against neurofilament. *Dis Colon Rectum* 1993; **36**: 1112-1117 [PMID: 8253006 DOI: 10.1007/BF02052258]

4 **Wedel T**, Roblick UJ, Ott V, Eggers R, Schiedeck TH, Krammer HJ, Bruch HP. Oligoneuronal hypoganglionosis in patients with idiopathic slow-transit constipation. *Dis Colon Rectum* 2002; **45**: 54-62 [PMID: 11786765]

5 **Kawahara I**, Kuniyasu H, Matsuyoshi H, Goto K, Obata K, Misawa H, Fujii H, Takaki M. Comparison of effects of a selective 5-HT reuptake inhibitor versus a 5-HT4 receptor agonist on in vivo neurogenesis at the rectal anastomosis in rats. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G588-G597 [PMID: 22194416

DOI: 10.1152/ajpgi.00284.2011]
6 **Huang ZH**, Yang DZ, Wei YQ. Effect of atropine on the enhancing action of Fructus Aurantii Immaturus on the myoelectric activity of small intestine in dogs. *Zhongguo Zhongxiyi Jiehe Zazhi* 1996; **16**: 292-294
7 **Li XW**, Ren JG. Experimental study on the coordinative application of Immature Bitter Orange and Bighead Atractylodes. *Zhongguo Chuantong Yiyao Yanjiu* 2002; **15**: 23-23
8 **Lee HT**, Seo EK, Chung SJ, Shim CK. Effect of an aqueous extract of dried immature fruit of Poncirus trifoliata (L.) Raf. on intestinal transit in rodents with experimental gastrointestinal motility dysfunctions. *J Ethnopharmacol* 2005; **102**: 302-306 [PMID: 16169174 DOI: 10.1016/j.jep.2005.08.015]
9 **Wang CF**, Yang DZ. Study on the effect of fructus aurantii immaturus on myoelectric of gastrointestinal tract in rats. *Dongnan Daxue Xuebao* 2001; **20**: 153-154
10 **Liu LJ**, Wei YQ. The regulation of histamine receptor antagonist in the effect of fructus aurantii immaturus on the motor activity of small intestine in mice. *Dongnan Daxue Xuebao* 2001; **20**: 144-146
11 **Reagan-Shaw S**, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J* 2008; **22**: 659-661 [PMID: 17942826 DOI: 10.1096/fj.07-9574LSF]
12 **Bassotti G**, De Giorgio R, Stanghellini V, Tonini M, Barbara G, Salvioli B, Fiorella S, Corinaldesi R. Constipation: a common problem in patients with neurological abnormalities. *Ital J Gastroenterol Hepatol* 1998; **30**: 542-548 [PMID: 9836115]
13 **Furness JB**. The enteric nervous system and neurogastroenterology. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 286-294 [PMID: 22392290 DOI: 10.1038/nrgastro.2012.32]
14 **Bassotti G**, Villanacci V. Slow transit constipation: a functional disorder becomes an enteric neuropathy. *World J Gastroenterol* 2006; **12**: 4609-4613 [PMID: 16937428 DOI: 10.3748/wjg.v12.i29.4609]
15 **Bassotti G**, Villanacci V, Crețoiu D, Crețoiu SM, Becheanu G. Cellular and molecular basis of chronic constipation: taking the functional/idiopathic label out. *World J Gastroenterol* 2013; **19**: 4099-4105 [PMID: 23864772 DOI: 10.3748/wjg.v19.i26.4099]
16 **Fan YH**, Zhou Y, Li YL, LV B, Zhang L, Huang Z. The effect of glial cell line derived neurotrophic factor (GDNF) to colonic myenteric plexus ultrastructural changes in slow transit constipation rats. *Zhonghua Xiaohua Zazhi* 2009; (**12**): 845-846
17 **Zhang Y**, Li HY. Colonic ultrastructural changes in rats with constipation. *Beijing Zhongyiyao Daxue Xuebao* 2005; **28**: 63-65
18 **Fan YH**, Lu B, Wang M, Ni GB, Chen MT, Xu Y. Expression and significance of nerve growth factor receptor p75 in rats' cathartic colonic wall. *Zhonghua Xiaohua Zazhi* 2006; **7**: 225-229
19 **Bamji SX**, Majdan M, Pozniak CD, Belliveau DJ, Aloyz R, Kohn J, Causing CG, Miller FD. The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. *J Cell Biol* 1998; **140**: 911-923 [PMID: 9472042 DOI: 10.1083/jcb.140.4.911]
20 **Mine Y**, Yoshikawa T, Oku S, Nagai R, Yoshida N, Hosoki K. Comparison of effect of mosapride citrate and existing 5-HT4 receptor agonists on gastrointestinal motility in vivo and in vitro. *J Pharmacol Exp Ther* 1997; **283**: 1000-1008 [PMID: 9399969]
21 **Liu Z**, Sakakibara R, Odaka T, Uchiyama T, Uchiyama T, Yamamoto T, Ito T, Asahina M, Yamaguchi K, Yamaguchi T, Hattori T. Mosapride citrate, a novel 5-HT4 agonist and partial 5-HT3 antagonist, ameliorates constipation in parkinsonian patients. *Mov Disord* 2005; **20**: 680-686 [PMID: 15719424 DOI: 10.1002/mds.20387]
22 **Ueno N**, Inui A, Satoh Y. The effect of mosapride citrate on constipation in patients with diabetes. *Diabetes Res Clin Pract* 2010; **87**: 27-32 [PMID: 19889470 DOI: 10.1016/j.diabres.2009.09.024]
23 **Odaka T**, Suzuki T, Seza A, Yamaguchi T, Saisho H. [Serotonin 5-HT4 receptor agonist (mosapride citrate)]. *Nihon Rinsho* 2006; **64**: 1491-1494 [PMID: 16898619]
24 **Kanazawa M**, Watanabe S, Tana C, Komuro H, Aoki M, Fukudo S. Effect of 5-HT4 receptor agonist mosapride citrate on rectosigmoid sensorimotor function in patients with irritable bowel syndrome. *Neurogastroenterol Motil* 2011; **23**: 754-e332 [PMID: 21615623 DOI: 10.1111/j.1365-2982.2011.01732.x]
25 **Inui A**, Yoshikawa T, Nagai R, Yoshida N, Ito T. Effects of mosapride citrate, a 5-HT4 receptor agonist, on colonic motility in conscious guinea pigs. *Jpn J Pharmacol* 2002; **90**: 313-320 [PMID: 12501007 DOI: 10.1254/jjp.90.313]
26 **Makimoto N**, Sakurai-Yamashita Y, Furuichi A, Kawakami S, Enjoji A, Kanematsu T, Taniyam K. In vivo assessment of acceleration of motor activity associated with acetylcholine release via 5-hydroxytryptamine4 receptor in dog intestine. *Jpn J Pharmacol* 2002; **90**: 28-35 [PMID: 12396025 DOI: 10.1254/jjp.90.28]
27 **Okamura K**, Sasaki N, Yamada M, Yamada H, Inokuma H. Effects of mosapride citrate, metoclopramide hydrochloride, lidocaine hydrochloride, and cisapride citrate on equine gastric emptying, small intestinal and caecal motility. *Res Vet Sci* 2009; **86**: 302-308 [PMID: 18723200 DOI: 10.1016/j.rvsc.2008.07.008]
28 **Matsuyoshi H**, Kuniyasu H, Okumura M, Misawa H, Katsui R, Zhang GX, Obata K, Takaki M. A 5-HT(4)-receptor activation-induced neural plasticity enhances in vivo reconstructs of enteric nerve circuit insult. *Neurogastroenterol Motil* 2010; **22**: 806-813, e226 [PMID: 20146727 DOI: 10.1111/j.1365-2982.2010.01474.x]
29 **Bai M**, Zhu XZ, Zhang Y, Zhang S, Zhang L, Xue L, Zhong M, Zhang X. Anhedonia was associated with the dysregulation of hippocampal HTR4 and microRNA Let-7a in rats. *Physiol Behav* 2014; **129**: 135-141 [PMID: 24582667 DOI: 10.1016/j.physbeh.2014.02]
30 **Liu MT**, Kuan YH, Wang J, Hen R, Gershon MD. 5-HT4 receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. *J Neurosci* 2009; **29**: 9683-9699 [PMID: 19657021 DOI: 10.1523/JNEUROSCI.1145-09.2009]
31 **Liu M**, Gershon MD. Neuroprotective/trophic effects of 5-HT4 receptor stimulation on enteric neurons of mice. *Neurogastroenterol Motil* 2006; **18**: 780-781
32 **Gershon MD**, Liu MT. Serotonin and neuroprotection in functional bowel disorders. *Neurogastroenterol Motil* 2007; **19** Suppl 2: 19-24 [PMID: 17620084 DOI: 10.1111/j1365-2982.2007.00962.x]
33 **Takaki M**, Goto K, Kawahara I. The 5-hydroxytryptamine 4 Receptor Agonist-induced Actions and Enteric Neurogenesis in the Gut. *J Neurogastroenterol Motil* 2014; **20**: 17-30 [PMID: 24466442 DOI: 10.5056/jnm.2014.20.1.17]
34 **Starkenmann C**, Niclass Y, Escher S. Volatile organic sulfur-containing constituents in Poncirus trifoliata (L.) Raf. (Rutaceae). *J Agric Food Chem* 2007; **55**: 4511-4517 [PMID: 17488024 DOI: 10.1021/jf063453h]

P- Reviewer: Harmanci O, Luo HS, Pescatori M, Raju J, Shehata MMM, Tsai KW
S- Editor: Ma YJ **L- Editor**: Wang TQ **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9367

World J Gastroenterol 2015 August 21; 21(31): 9367-9372
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Fluid resuscitation in acute pancreatitis: Normal saline or lactated Ringer's solution?

Michal Lipinski, Alicja Rydzewska-Rosolowska, Andrzej Rydzewski, Grazyna Rydzewska

Michal Lipinski, Grazyna Rydzewska, Department of Gastroenterology, Central Clinical Hospital of The Ministry of Interior, 02-507 Warsaw, Poland

Alicja Rydzewska-Rosolowska, Department of Nephrology and Transplantation, Medical University of Bialystok, 15-540 Bialystok, Poland

Andrzej Rydzewski, Department of Internal Medicine and Nephrology, Central Clinical Hospital of The Ministry of Interior, 02-507 Warsaw, Poland

Andrzej Rydzewski, Grazyna Rydzewska, The Faculty of Health Sciences, UJK, 25-369 Kielce, Poland

Author contributions: Lipinski M and Rydzewska G designed the study; Lipinski M performed the research; Lipinski M, Rydzewska-Rosolowska A and Rydzewski A contributed new reagents or analytic tools; Lipinski M analysed the data; Lipinski M wrote the paper; and all authors have read and approved the final version to be published.

Conflict-of-interest statement: Authors declare no conflict of interest.

Data sharing statement: The technical appendix, statistical code, and dataset are available from the corresponding author at michal7lipinski@yahoo.com. Consent was not obtained, but the presented data are anonymized, and the risk of identification is low. No additional data are available.

Correspondence to: Michal Lipinski, MD, PhD, Department of Gastroenterology, Central Clinical Hospital of The Ministry of Interior, 137 Woloska Street, 02-507 Warsaw, Poland. michal7lipinski@yahoo.com

Telephone: +48-22-5081240
Fax: +48-22-5081040

Received: December 5, 2014
Peer-review started: December 5, 2014
First decision: January 22, 2015
Revised: February 28, 2015
Accepted: April 28, 2015
Article in press: April 28, 2015
Published online: August 21, 2015

Abstract

AIM: To investigate whether administration of Ringer's solution (RL) could have an impact on the outcome of acute pancreatitis (AP).

METHODS: We conducted a retrospective study on 103 patients [68 men and 35 women, mean age 51.2 years (range, 19-92 years)] hospitalized between 2011 and 2012. All patients admitted to the Department of Gastroenterology of the Central Clinical Hospital of the Ministry of Interior (Poland) with a diagnosis of AP who had disease onset within 48 h of presentation were included in this study. Based on the presence of persistent organ failure (longer than 48 h) as a criterion for the diagnosis of severe AP (SAP) and the presence of local complications [diagnosis of moderately severe AP (MSAP)], patients were classified into 3 groups: mild AP (MAP), MSAP and SAP. Data were compared between the groups in terms of severity (using the revised Atlanta criteria) and outcome. Patients were stratified into 2 groups based on the type of fluid resuscitation: the 1-RL group who underwent standard fluid resuscitation with a RL 1000 mL solution or the 2-NS group who underwent standard fluid resuscitation with 1000 mL normal saline (NS). All patients from both groups received an additional 5% glucose solution (1000-1500 mL) and a multi-electrolyte solution (500-1000 mL).

RESULTS: We observed 64 (62.1%) patients with MAP, 26 (25.24%) patients with MSAP and 13 (12.62%) patients with SAP. No significant difference in the distribution of AP severity between the two groups was found. In the 1-RL group, we identified 22 (55.5%) MAP, 10 (25.5%) MSAP and 8 (20.0%) SAP patients, compared with 42 (66.7%) MAP, 16 (24.4%) MSAP and 5 (7.9%) SAP cases in the 2-NS group (P = 0.187). The volumes of fluid administered during the initial 72-h period of hospitalization were similar among the patients from both the 1-RL and 2-NS groups (mean 3400 mL *vs* 3000 mL, respectively). No significant differences between the 1-RL and 2-NS groups were found in confirmed pancreatic necrosis [10 patients (25%) *vs* 12 patients (19%), respectively, P = 0.637]. There were no statistically significant differences between the 1-RL and 2-NS groups in the percentage of patients who required enteral nutrition (23 patients *vs* 17 patients, respectively, P = 0.534). Logistic regression analysis confirmed these findings (OR = 1.344, 95%CI: 0.595-3.035, P = 0.477). There were no significant differences between the 1-RL and 2-NS groups in mortality and the duration of hospital stay (median of 9 d for both groups, P = 0.776).

CONCLUSION: Our study failed to find any evidence that the administration of RL in the first days of AP leads to improved clinical outcomes.

Key words: Acute pancreatitis; Fuid therapy; Lactated Ringer's solution; Treatment; Normal saline

Core tip: To date, only a handful of studies have focused on the effect of Ringer's solution in the treatment of acute pancreatitis (AP). We believe that our findings could be of interest to the readers because they may allow for a more reliable review of a complex area of fluid resuscitation in the setting of AP compared with existing studies. These results were mainly achieved by applying the modified Atlanta score in our study, which includes "end points" focused on the final AP treatment outcome rather than only on changes in a single laboratory parameter and clinical signs.

Lipinski M, Rydzewska-Rosolowska A, Rydzewski A, Rydzewska G. Fluid resuscitation in acute pancreatitis: Normal saline or lactated Ringer's solution? *World J Gastroenterol* 2015; 21(31): 9367-9372 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9367.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9367

INTRODUCTION

Fluid therapy is a crucial aspect of the management of patients with acute pancreatitis (AP), especially in the early hours of the disease. Crystalloids are currently recommended as the initial resuscitation fluids in patients with AP. Nevertheless, the optimal type of fluid therapy remains unclear. The composition, volume and rate of fluid administration that is most appropriate for the treatment of AP is currently being debated[1].

Isotonic crystalloids, particularly normal saline (NS), are commonly used as the preferred first-line fluid treatment for patients with AP[2,3]. NS is an isotonic solution (osmolality 308 mOsm/L) with a nominal pH of 5.5 (4.5-7.0). However, balanced crystalloid lactated Ringer's solution (RL) may be more beneficial than NS[4] as it reduces the risk of hyperchloremic acidosis associated with impaired renal function.

Experimental studies show that zymogens may be activated by low pH. Furthermore, low pH may also adversely impact acinar cells and make them more vulnerable to injury, thereby contributing to the increase in severity of AP[5]. From that perspective, RL with its pH (normal range, 6.0-7.5) may potentially provide for the protective effects on tissue and improve the outcome of AP.

In AP, hypovolemia is associated with an increased risk of tissue hypoperfusion and the development of organ failure[6]. There is a hypothesis based on the analysis of a study on the experimental model of AP, which assumes that intensive fluid resuscitation can reduce the extent of hypoperfusion[7]. Fluid replacement in the setting of SAP is intended not only to replace the deficiency in blood volume but also to stabilize capillary permeability, maintain the function of the intestinal barrier[8] and modulate the inflammatory response[9].

In the assessment of AP prognosis, there is a tendency to consider factors that reflect the intravascular volume depletion [serum creatinine, eGFR, BUN, urine neutrophil gelatinase-associated lipocalin (uNGAL)], which often present during the first hours of AP[10-14].

Hypovolemia is one of the major pathological symptoms associated with early stage AP; however, there is no conclusive research that would suggest the use of a specific fluid therapy in AP. The type of solution recommended as the initial fluid therapy has been a subject of ongoing debate for many years. The current recommendations do not specify the precise strategy for fluid therapy in AP.

Therefore, this study was designed to investigate the effect of type of fluid therapy on SAP outcome.

MATERIALS AND METHODS

We conducted a retrospective study on 103 patients [68 men and 35 women, mean age 51.2 years (range, 19-92 years)] hospitalized between 2011 and 2012. All patients admitted to the Department of Gastroenterology of the Central Clinical Hospital of the Ministry of Interior (Poland) with a diagnosis of AP who

Table 1 Demographic data of patients in the Ringer's solution and normal saline groups *n* (%)

	1-RL group (*n* = 40)	2-NS group (*n* = 63)
Age (yr), mean ± SD	49.2 ± 18.0	52.5 ± 17.6
Male	30 (75)	38 (60.3)
Female	10 (25)	25 (39.7)
BMI (kg/m^2), mean ± SD	27.6 ± 5.0	26.3 ± 5.4

The 1-Ringer's solution (1-RL) group with standard fluid resuscitation with lactated Ringer's 1000 mL solution; The 2-normal saline (2-NS) group with standard fluid resuscitation with 1000 mL normal saline. BMI: Body mass index.

Table 2 Prediction of severe acute pancreatitis in the Ringer's solution and normal saline groups *n* (%)

	1-RL	2-NS	*P* value
BISAP ⩾ 3	8 (20.0)	8 (12.7)	0.405
uNGAL ⩾ 73 ng/mL	15 (37.5)	11 (17.5)	0.035

The 1-Ringer's solution (1-RL) group with standard fluid resuscitation with lactated Ringer's 1000 mL solution; The 2-normal saline (2-NS) group with standard fluid resuscitation with 1000 mL normal saline. BISAP: Bedside index of severity in acute pancreatitis; uNGAL: Urinary neutrophil gelatinase-associated lipocalin.

had disease onset within 48 h of presentation were included in this study. Transferred patients or those patients with symptoms lasting more than 48 h were excluded. A total of 103 patients were divided into 2 groups based on whether or not they received early RL (1000 mL/24 h, initiated within 24 h of admission for a duration of 3 d): 40 patients received early RL (1-RL group), while 63 patients did not (2-NS group). There were no significant differences between the groups with respect to demographic data including age, sex and BMI (Table 1).

The aetiology of AP in the 1-RL group was biliary in 14 patients (35%), alcoholic in 23 patients (57.5%), and other (post-ERCP, idiopathic, hereditary, *etc*.) in 3 patients (7.5%). In the 2-NS group, the aetiology of AP was biliary in 29 patients (46.1%), alcoholic in 16 patients (25.5%), and other in 18 patients (28.4%).

Patients were divided into 2 groups based on the type of fluid resuscitation: the 1-RL group who underwent standard fluid resuscitation with RL 1000 mL solution or the 2-NS group who underwent standard fluid resuscitation with 1000 mL NS. All patients from both groups received additional 5% glucose solution (1000-1500 mL) and a multi-electrolyte solution (500-1000 mL). The use of RL or NS was dictated only by experience and conviction of the physicians prescribing fluid for the particular patient. No additional clinical or other types of criteria were applied. No specific protocol indicating the need for a specific fluid therapy was applied. In case when intravenous hydration was still necessary after 72 h - patient consistently received NS or RL with additional crystalloids previously described.

Because the study involved the assessment of fluid therapy in the first 3 d of AP, the prognostic methods used in predicting the course of pancreatitis had to be already applied in the first stage of the disease. Therefore, it was decided that the BISAP score and uNGAL values be used. The BISAP score was determined in all patients within the first 24 h of admission. Urine samples obtained from 24-h urine collections were gathered for determination of the urinary level of neutrophil gelatinase-associated lipocalin from the first day. We did not exclude geriatric patients (over 65 years of age) due to the negative impact of such a decision on the credibility of the BISAP scale prediction.

The crystalloids used in the study (NS, RL, multi-electrolyte solution) were commercially-available products (manufactured by Frescenius Kabi Polska) and were provided from a hospital pharmacy.

Based on the presence of persistent organ failure (more than 48 h) as a criterion for the diagnosis of severe AP (SAP) and the presence of local complications [diagnosis of moderately severe AP (MSAP)], patients were classified into 3 groups: mild AP (MAP), MSAP and SAP. Organ failure was identified using the Modified Marshall Scoring System. Data were compared between the groups in terms of severity (using the revised Atlanta criteria) and outcome. Primary endpoints of the study were the distribution of AP severity, mortality and pancreatic necrosis, while secondary endpoints were the percentage of patients requiring enteral nutrition and the duration of hospital stay.

The χ^2 test and Fisher exact test were used to compare the distribution of patient characteristics. The risk score was developed using a logistic regression model. A value of $P < 0.05$ was considered statistically significant. Overall survival of these patients was examined using log-rank tests.

RESULTS

There were no statistically significant differences in the proportion of patients with predicted SAP in the 1-RL and 2-NS groups using the BISAP score (Table 2). SAP was predicted using the BISAP score in 8 patients (20%) from the 1-RL group and in 8 patients (12.7%) from the 2-NS group ($P = 0.405$).

Using the concentration of NGAL in the urine as a prognostic parameter, SAP was predicted in 15 patients (37.5%) from the 1-RL group and 11 patients (17.5%) from the 2-NS group ($P = 0.035$).

We observed 64 (62.1%) patients with MAP, 26 (25.24%) patients with MSAP and 13 (12.62%) patients with SAP. No significant difference in the distribution of AP severity between the two groups was found. In the 1-RL group, we identified 22 (55.5%) MAP, 10 (25.5%) MSAP and 8 (20.0%) SAP patients, compared with 42 (66.7%) MAP, 16 (24.4%) MSAP and 5 (7.9%) SAP cases in the 2-NS group ($P = 0.187$,

Table 3 The distribution of acute pancreatitis severity between the two groups *n* (%)

P = 0.187	Total (*n* = 103)	1-RL group (*n* = 40)	2-NS group (*n* = 63)
Mild AP	64 (62.1)	22 (55.5)	42 (66.7)
Moderate AP	26 (25.24)	10 (25.5)	16 (24.4)
Severe AP	13 (12.62)	8 (20.0)	5 (7.9)

The 1-Ringer's solution (1-RL) group with standard fluid resuscitation with lactated Ringer's 1000 mL solution; The 2-normal saline (2-NS) group with standard fluid resuscitation with 1000 mL normal saline. AP: Acute pancreatitis.

Table 4 Clinical data of patients in the Ringer's solution and normal saline groups *n* (%)

	1-RL	2-NS	*P* value
Pancreatic necrosis	10 (25.0)	12 (19)	0.637
Enteral nutrition	23 (57.5)	17 (27)	0.534
Hospital stay	9 d	9 d	0.776
Fatal AP	5 (12.5)	3 (4.7)	0.256

The 1-Ringer's solution (1-RL) group with standard fluid resuscitation with lactated Ringer's 1000 mL solution; The 2-normal saline (2-NS) group with standard fluid resuscitation with 1000 mL normal saline. AP: Acute pancreatitis.

Table 3).

The volumes of fluid administered during the first 24 and 72 h of hospitalization were similar among patients from the 1-RL and 2-NS groups (mean 3400 and 10000 *vs* 3000 and 9000 mL, respectively).

Clinical data for both groups are shown in Table 4. No significant differences between the 1-RL and 2-NS groups were found in confirmed pancreatic necrosis [10 patients (25%) *vs* 12 patients (19%), respectively, *P* = 0.637].

There were no statistically significant differences between the 1-RL and 2-NS groups in the percentage of patients requiring enteral nutrition (23 patients *vs* 17 patients, respectively, *P* = 0.534). Logistic regression analysis also confirmed this calculation (OR = 1.344, 95%CI: 0.595-3.035, *P* = 0.477).

There were no significant differences between the 1-RL and 2-NS groups in the duration of hospital stay (median of 9 d for both groups, *P* = 0.776) (Figure 1) and mortality (fatal AP in 5 patients *vs* 3 patients, *P* = 0.256; OR = 2.857, 95%CI: 0.643-12.687, *P* = 0.168).

DISCUSSION

There is still a debate concerning the optimal fluid therapy for the early phase of AP. Administered solutions should not only improve hemodynamic parameters but also exert positive effects on the microcirculation and metabolic processes. The type of fluid, crystalloid and/or colloid, preferred in the early hours of AP remains under discussion.

NS may contribute to the development of metabolic

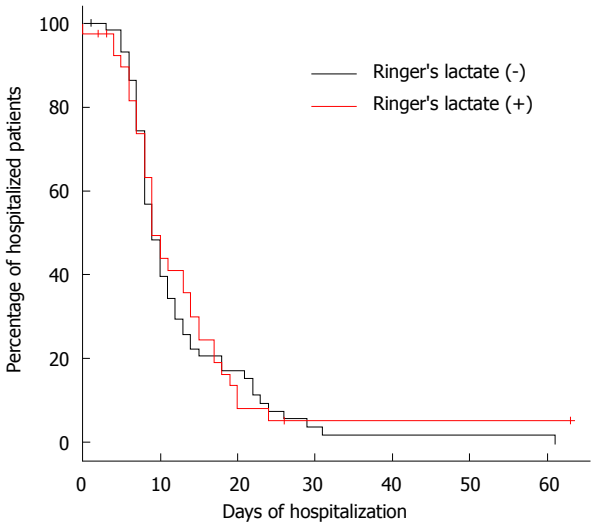


Figure 1 Duration of hospitalization in the Ringer's solution (red line) and normal saline groups (black line).

acidosis when administered in large volumes; in contrast, RL does not produce a similar effect. Taking into account the above findings, it is important to remember that SAP is often associated with coexisting metabolic acidosis[11,12]. To date, only a handful of studies have focused on the effect of RL for the treatment of AP[3,13]. Interestingly, most of these studies evaluated only the effects of selected fluid therapy on single prognostic parameters or symptoms and did not assess the impact on the results of the treatment.

The main objective of our study was to investigate the effects of a NS-based *vs* a RL-based fluid therapy on outcomes in AP.

No statistically significant difference was demonstrated between the 1-RL and 2-NS groups for AP severity. Most of the studies concerning the prognosis of AP conducted to date have not utilized the new criteria to classify disease severity. In this study, patients were retrospectively analysed from the time of hospital admission until discharge or death, as required by the revised Atlanta criteria, which includes persistent organ failure as an essential component[14]. Evaluation of patients based on the revised Atlanta criteria is associated with a lower percentage of patients with a diagnosis of SAP (indicating persistent organ failure). For this reason, the proportion of patients with SAP is less than the observations conducted by 2012. Moreover, the mortality rate in this group is higher because the eligibility criteria for this group indicate a high risk of death.

Differences in the proportion of patients with predicted SAP in the 1-RL and 2-NS groups also were not statistically significant. Our study did not confirm that the administration of RL had more favourable effects than NS with respect to the treatment and outcomes of AP. Our results indicate that, in terms of confirmed pancreatic necrosis, there were no

statistically significant differences between the two study groups. These findings are particularly interesting in view of a major role of hypoperfusion in the pathogenesis of not only pancreatic necrosis but also organ failure[6,15,16].

Also notable is the result suggesting that RL does not significantly impact the need for enteral nutrition, when necessary[17]. Therefore, the outcome of the study does not confirm that RL may effectively modulate therapeutic management with regard to enteral nutrition.

It was difficult to demonstrate the beneficial effects of RL on the reduction of mortality in our analysis. We decided to calculate the influence of intravenous hydration on the risk of metabolic acidosis, which is directly related to the risk of death[18,19]. Using this approach, our study failed to find any benefits of using RL solution, compared to 0.9% sodium chloride solution, on the duration of hospital stay and mortality.

It is important to emphasize that the volumes of fluid administered in both groups during the first 72 h of hospitalization were similar. This finding excludes the possibility of differences in the Ⅳ fluid volume administered having an impact on the outcome of the study. Our study was retrospective and was not associated with specific recommendations for the hydration of patients that considered their body weight. It is worth noting that intensive fluid resuscitation may lead to tissue oedema and result in organ failure[20,21]. Furthermore, the study groups were different in terms of percentage of patients with prognosed SAP, with the 1-RL group having a higher proportion.

Although the arterial pH of patients from either group was not controlled during their stay at the hospital, we assume that potential changes in pH and acidosis coexisting with AP (expected particularly in the 2-NS group) were not significant. If present, these changes may have been temporary and did not impact the results in either group.

Answers to the question of what is the optimal type of fluid resuscitation in AP and if crystalloid or colloid solutions are more beneficial are still under debate. Recently, a controversy concerning the specific fluid resuscitation protocol in AP was raised by studies using hydroxyethyl starch, which may be associated with increased mortality in critically ill patients[22]. Indeed, the initial fluid resuscitation protocol can have more direct impact on necrosis than mortality. On the other hand, studies exist that support the effectiveness of a mixed ratio of crystalloid-colloid fluid resuscitation in improving the prognosis of patients with SAP[23] and reducing the risk of threatening complications (intra-abdominal hypertension) caused by hydroxyethyl starch[14].

Our study has several limitations. First, it was a retrospective study and our data came from one centre with a limited number of patients. Second, we did not control the arterial pH in our study groups due to retrospective nature of our study (arterial pH was not controlled for all patients in this cohort). It is possible that the volume of RL (1000 mL) used was not sufficient to achieve the target of modulating local pH or alleviating the acidosis in AP.

Also notable is the result achieved using the concentration of NGAL in the urine as a prognostic parameter of SAP. We found statistically significant differences in the proportion of patients with predicted SAP in the 1-RL and 2-NS groups (15 patients *vs* 11 patients, $P = 0.035$). A greater proportion of patients with predicted SAP (classified using NGAL but not the BISAP score) in the 1-RL group could also make it more difficult to demonstrate the benefits of RL. Moreover, a greater proportion of patients in the 1-RL group with an alcoholic aetiology (57.5% *vs* 25.5%) complicates the interpretation of the pancreatic necrosis results. Including a larger number of patients would probably produce more conclusive results.

Nevertheless, our results allow for a more reliable review of a complex area of fluid resuscitation in the setting of AP compared with existing studies. This improvement was mainly achieved by applying the modified Atlanta score in our study, which includes "end points" focused on the final AP treatment outcome rather than only on changes of single laboratory parameters and clinical signs.

Additional studies are needed to provide further data on the benefits and risks of specific fluid regimens in patients with AP. Until the outcomes of such studies are published, the management of AP patients should probably focus more on ensuring that sufficient fluid volume is provided to maintain perfusion rather than on which type of fluid (crystalloids or colloids) is used to achieve it.

COMMENTS

Background

Fluid therapy is a crucial aspect of the management of patients with acute pancreatitis (AP), especially in the early hours of the disease. Crystalloids are currently recommended as initial resuscitation fluids for patients with AP. Nevertheless, the optimal type of fluid therapy remains unclear. Therefore, this study was designed to investigate the effect of the type of fluid therapy on SAP outcome.

Research frontiers

Hypovolemia is one of the major pathological symptoms associated with the early stages of AP; however, there is no conclusive research that would suggest the use of a specific fluid therapy in AP.

Innovations and breakthroughs

To date, only a handful of studies have focused on the effect of Ringer's solution in the treatment of AP. Interestingly, most of these studies evaluated only the effects of selected fluid therapy on individual prognostic parameters or symptoms and did not assess the impact on the results of the treatment. In this study, data were compared between groups receiving different treatments in terms of severity (revised Atlanta criteria) and outcomes.

Applications

Current results allow for a more reliable review of a complex area of fluid resuscitation in the setting of AP compared with existing studies. Additional

studies are needed to provide further data on the benefits and risks of specific fluid regimens in patients with AP.

Terminology

The composition, volume and rate of fluid administration that is most appropriate for the treatment of AP is currently being debated, particularly for the early phase of AP. Administered solution should not only improve the hemodynamic parameters but also exert positive effects on the microcirculation and metabolic processes.

Peer-review

This study was designed to investigate the effect of the type of fluid therapy on SAP outcomes. This paper addresses an important gap in the literature regarding fluid therapy for AP.

REFERENCES

1 **Haydock MD**, Mittal A, Wilms HR, Phillips A, Petrov MS, Windsor JA. Fluid therapy in acute pancreatitis: anybody's guess. *Ann Surg* 2013; **257**: 182-188 [PMID: 23207241 DOI: 10.1097/SLA.0b013e31827773ff]

2 **Aggarwal A**, Manrai M, Kochhar R. Fluid resuscitation in acute pancreatitis. *World J Gastroenterol* 2014; **20**: 18092-18103 [PMID: 25561779 DOI: 10.3748/wjg.v20.i48.18092]

3 **Trikudanathan G**, Navaneethan U, Vege SS. Current controversies in fluid resuscitation in acute pancreatitis: a systematic review. *Pancreas* 2012; **41**: 827-834 [PMID: 22781906 DOI: 10.1097/MPA.0b013e31824c1598]

4 **Wu BU**, Hwang JQ, Gardner TH, Repas K, Delee R, Yu S, Smith B, Banks PA, Conwell DL. Lactated Ringer's solution reduces systemic inflammation compared with saline in patients with acute pancreatitis. *Clin Gastroenterol Hepatol* 2011; **9**: 710-717.e1 [PMID: 21645639 DOI: 10.1016/j.cgh.2011.04.026]

5 **Bhoomagoud M**, Jung T, Atladottir J, Kolodecik TR, Shugrue C, Chaudhuri A, Thrower EC, Gorelick FS. Reducing extracellular pH sensitizes the acinar cell to secretagogue-induced pancreatitis responses in rats. *Gastroenterology* 2009; **137**: 1083-1092 [PMID: 19454288 DOI: 10.1053/j.gastro.2009.05.041]

6 **Petejova N**, Martinek A. Acute kidney injury following acute pancreatitis: A review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2013; **157**: 105-113 [PMID: 23774848 DOI: 10.5507/bp.2013.048]

7 **Klar E**, Messmer K, Warshaw AL, Herfarth C. Pancreatic ischaemia in experimental acute pancreatitis: mechanism, significance and therapy. *Br J Surg* 1990; **77**: 1205-1210 [PMID: 2252994]

8 **Capurso G**, Zerboni G, Signoretti M, Valente R, Stigliano S, Piciucchi M, Delle Fave G. Role of the gut barrier in acute pancreatitis. *J Clin Gastroenterol* 2012; **46** Suppl: S46-S51 [PMID: 22955357 DOI: 10.1097/MCG.0b013e3182652096]

9 **Zhao G**, Zhang JG, Wu HS, Tao J, Qin Q, Deng SC, Liu Y, Liu L, Wang B, Tian K, Li X, Zhu S, Wang CY. Effects of different resuscitation fluid on severe acute pancreatitis. *World J Gastroenterol* 2013; **19**: 2044-2052 [PMID: 23599623 DOI: 10.3748/wjg.v19.i13.2044]

10 **Lipinski M**, Rydzewski A, Rydzewska G. Early changes in serum creatinine level and estimated glomerular filtration rate predict pancreatic necrosis and mortality in acute pancreatitis: Creatinine and eGFR in acute pancreatitis. *Pancreatology* 2013; **13**: 207-211 [PMID: 23719589 DOI: 10.1016/j.pan.2013.02.002]

11 **Sharma V**, Shanti Devi T, Sharma R, Chhabra P, Gupta R, Rana SS, Bhasin DK. Arterial pH, bicarbonate levels and base deficit at presentation as markers of predicting mortality in acute pancreatitis: a single-centre prospective study. *Gastroenterol Rep* (Oxf) 2014; **2**: 226-231 [PMID: 24994834 DOI: 10.1093/gastro/gou037]

12 **Pant N**, Kadaria D, Murillo LC, Yataco JC, Headley AS, Freire AX. Abdominal pathology in patients with diabetes ketoacidosis. *Am J Med Sci* 2012; **344**: 341-344 [PMID: 23095441 DOI: 10.1097/MAJ.0b013e3182648740]

13 **Du XJ**, Hu WM, Xia Q, Huang ZW, Chen GY, Jin XD, Xue P, Lu HM, Ke NW, Zhang ZD, Li QS. Hydroxyethyl starch resuscitation reduces the risk of intra-abdominal hypertension in severe acute pancreatitis. *Pancreas* 2011; **40**: 1220-1225 [PMID: 21775917 DOI: 10.1097/MPA.0b013e3182217f17]

14 **Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]

15 **Koziel D**, Kozłowska M, Deneka J, Matykiewicz J, Głuszek S. Retrospective analysis of clinical problems concerning acute pancreatitis in one treatment center. *Prz Gastroenterol* 2013; **8**: 320-326 [PMID: 24868277 DOI: 10.5114/pg.2013.38736]

16 **Patschan D**, Müller GA. Acute kidney injury. *J Inj Violence Res* 2015; **7**: 19-26 [PMID: 25618438 DOI: 10.5249/jivr.v7i1.604]

17 **Kosel J**, Kosciuczuk U, Siemiatkowski A. Wpływ leczenia zywieniowego na funkcjonowanie układu immunologicznego. *Prz Gastroenterol* 2013; **8**: 147-155

18 **Jung B**, Rimmele T, Le Goff C, Chanques G, Corne P, Jonquet O, Muller L, Lefrant JY, Guervilly C, Papazian L, Allaouchiche B, Jaber S. Severe metabolic or mixed acidemia on intensive care unit admission: incidence, prognosis and administration of buffer therapy. A prospective, multiple-center study. *Crit Care* 2011; **15**: R238 [PMID: 21995879 DOI: 10.1186/cc10487]

19 **Adeva-Andany MM**, Fernández-Fernández C, Mouriño-Bayolo D, Castro-Quintela E, Domínguez-Montero A. Sodium bicarbonate therapy in patients with metabolic acidosis. *ScientificWorldJournal* 2014; **2014**: 627673 [PMID: 25405229 DOI: 10.1155/2014/627673]

20 **Kuwabara K**, Matsuda S, Fushimi K, Ishikawa KB, Horiguchi H, Fujimori K. Early crystalloid fluid volume management in acute pancreatitis: association with mortality and organ failure. *Pancreatology* 2011; **11**: 351-361 [PMID: 21757973 DOI: 10.1159/000328965]

21 **de-Madaria E**, Soler-Sala G, Sánchez-Payá J, Lopez-Font I, Martínez J, Gómez-Escolar L, Sempere L, Sánchez-Fortún C, Pérez-Mateo M. Influence of fluid therapy on the prognosis of acute pancreatitis: a prospective cohort study. *Am J Gastroenterol* 2011; **106**: 1843-1850 [PMID: 21876561 DOI: 10.1038/ajg.2011.236]

22 **Perel P**, Roberts I, Ker K. Colloids versus crystalloids for fluid resuscitation in critically ill patients. *Cochrane Database Syst Rev* 2013; **2**: CD000567 [PMID: 23450531 DOI: 10.1002/14651858.CD000567.pub6]

23 **Chang YS**, Fu HQ, Zou SB, Yu BT, Liu JC, Xia L, Lv NH. [The impact of initial fluid resuscitation with different ratio of crystalloid-colloid on prognosis of patients with severe acute pancreatitis]. *Zhonghua Weizhongbing Jijiu Yixue* 2013; **25**: 48-51 [PMID: 23611098 DOI: 10.3760/cma.j.issn.2095-4352.2013.01.013]

P- Reviewer: Berger Z, Rakonczay Z, Zhao QH **S- Editor**: Yu J
L- Editor: A **E- Editor**: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9373

World J Gastroenterol 2015 August 21; 21(31): 9373-9379
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Colorectal stenting for palliation and as a bridge to surgery: A 5-year follow-up study

Baris Bayraktar, Ibrahim Ali Ozemir, Umut Kefeli, Gokhan Demiral, Julide Sagiroğlu, Onur Bayraktar, Gupse Adali, Alp Ozcelik, Osman Baran Tortum

Baris Bayraktar, Alp Ozcelik, General Surgery Department, Yalova Hospital, Yalova 77100, Turkey

Ibrahim Ali Ozemir, General Surgery Department, Goztepe Training and Research Hospital, Medeniyet University, Istanbul 34100, Turkey

Umut Kefeli, Medical Oncology Department, Kocaeli University Medical Faculty, Kocaeli 41112, Turkey

Gokhan Demiral, General Surgery Department, Rize Public Hospitals Association, Rize 53100, Turkey

Julide Sagiroğlu, General Surgery Department, Goztepe Training and Research Hospital, Medeniyet University, Istanbul 34100, Turkey

Onur Bayraktar, General Surgery Department, Acibadem Atakent Hospital, Istanbul 34210, Turkey

Gupse Adali, Gastroenterology Department, Corum Research and Training Hospital, Hitit University, Corum 19110, Turkey

Osman Baran Tortum, General Surgery Department, Istanbul University Cerrahpasa Medical Faculty, Istanbul 34200, Turkey

Author contributions: All authors contributed equally in performing the literature search and analyzing results; Kefeli U, Bayraktar B and Demiral G wrote the manuscript.

Institutional review board statement: This study was reviewed and approved by the Medeniyet University Institutional Review Board.

Informed consent statement: All patients gave their verbal consent for reporting these cases.

Conflict-of-interest statement: All authors declare no conflicts of interest (including commercial, personal, political, intellectual, or religious interests).

Data sharing statement: No unpublished data were available.

Correspondence to: Baris Bayraktar, MD, General Surgery Department, Yalova Hospital, Yalova 77100, Turkey. ofbeabim@yahoo.com
Telephone: +90-533-4419046
Fax: +90-226-8137324

Received: March 10, 2015
Peer-review started: March 11, 2015
First decision: March 26, 2015
Revised: April 15, 2015
Accepted: June 10, 2015
Article in press: June 10, 2015
Published online: August 21, 2015

Abstract

AIM: To evaluate the long-term effectiveness of colonic stents in colorectal tumors causing large bowel obstruction.

METHODS: We retrospectively analyzed data from 49 patients with colorectal cancer who had undergone colorectal stent placement between January 2008 and January 2013. Patients' symptoms, characteristics and clinicopathological data were obtained by reviewing medical records. The obstruction was diagnosed clinically and radiologically. Histopathological diagnosis was achieved endoscopically. Technical success rate (TSR)

was defined as the ratio of patients with correctly placed SEMS upon stent deployment across the entire stricture length to total number of patients. Clinical success rate (CSR) was defined as the ratio of patients with technical success and successful maintenance of stent function before elective surgery (regardless of number of SEMS deployed) to total number of patients. The surgical success rate (SSR) of colorectal stent as a bridge to surgery was defined as the ratio of patients with successful surgical procedures. Unsuccessful surgical outcomes were defined as being due to insufficient colonic decompression. The technical, clinical, surgical success rates and complications after stenting were assessed.

RESULTS: The median age of patients was 64 (36 to 89). 44.9% of patients were male and 55.1% were female. Eighteen patients had the obstruction located in the rectum, 15 patients in the rectosigmoid region, 10 patients in the sigmoid region, and 6 patients had a tumor causing obstruction in the proximal colon. Each patient was categorized pathologically as stage 2 (32.7%, 16 patients) or stage 3 (42.9%, 21 patients) and 12 patients (24.4%) had metastatic disease. None of the patients received chemotherapy before stenting. Stenting was undertaken in 37 patients as a bridge to surgery, and in 12 patients stents were used for palliation. Median time to surgery after stenting was 30 ± 91.9 d. All surgery was completed in one single operation and thus no colostomy with stoma was needed. The median overall survival rate of patients with stage 2-3 colorectal cancer was 53.1 mo and stage 4 was 37.1 mo (P = 0.04). Metastatic colorectal patients who were treated palliatively with stents had backbone chemotherapy with oxaliplatin and/or irinotecan-based regimens plus antiangiogenic therapies, especially bevacizumab. Resolution of the obstruction and clinical improvement was achieved in all patients. The technical, clinical and surgical success rates were 95.9%, 100% and 94.6%, respectively.

CONCLUSION: The efficacy and safety of colonic stents was demonstrated both as a bridge to surgery and for palliative decompression. In addition, results emphasize the importance of the skills of the endoscopist in colonic stenting.

Key words: Large bowel obstruction; Colonic decompression; Colorectal tumors; Metallic stent; Palliative therapy

Core tip: Colorectal stents can be used for two indications in colorectal malignancies; palliative dilatation of advanced disease, and preoperative decompression as a bridge to surgery. In both indications, colonic stents prevent colostomy with stoma. Decompression of the bowel gives time for surgeons to stabilize the patient, stage the disease with imaging techniques, and take a biopsy. Thus, it allows one-stage surgery with primary anastomosis. Palliative colorectal stenting was shown to be as effective and acceptable as palliative surgery. Colonic stents showed long-term efficacy comparable to that of surgery.

Bayraktar B, Ozemir IA, Kefeli U, Demiral G, Sagiroğlu J, Bayraktar O, Adali G, Ozcelik A, Tortum OB. Colorectal stenting for palliation and as a bridge to surgery: A 5-year follow-up study. *World J Gastroenterol* 2015; 21(31): 9373-9379 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9373.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9373

INTRODUCTION

Colorectal cancer alone is expected to account for 8.2% (136830) of all new cancer cases and it is estimated that about 50310 (8.3%) deaths from colorectal cancer will occur in the United States in 2014[1]. The rate of colorectal tumors causing large bowel obstruction is between 15% and 20%[2,3]. Obstruction requires immediate treatment and the mortality rate of emergency surgery is high. However, colonic stents are being safely used in malignant colorectal obstruction. Colorectal stents can be successfully placed in the majority of cases with good clinical results[4,5]. They are used for two indications in colorectal malignancies; palliative dilatation of advanced disease, and preoperative decompression as a bridge to surgery[6]. In both indications, colonic stents prevent colostomy with stoma[7]. Colonic stents are well-tolerated and have low rates of morbidity and mortality[4,8]. These stents have therefore attracted wide attention. In the light of these facts, we here report a long-term (5 year) follow-up assessment of the management of malignant colorectal obstructions using colonic stents, both as a bridge to surgery and as palliation.

MATERIALS AND METHODS

Patients and follow-up

Forty-nine patients with colorectal cancer who had undergone colorectal stent placement at two hospitals in Istanbul (Istanbul University Cerrahpasa School of Medicine and Medeniyet University Education and Research Hospital) were reviewed retrospectively over a 5-year period from January 2008 to January 2013. The obstruction was diagnosed clinically and radiologically. Histopathological diagnosis was achieved endoscopically. Stages of the disease for each patient were determined with pathological (if the patient underwent surgery) and clinical findings. Patients' symptoms, characteristics and clinicopathological data were obtained by reviewing medical records. All patients were enrolled after informed consent. All patients were staged according to the American Joint Committee on Cancer (7th edition) tumor node

metastasis (TNM) staging manual[9]. Clinical follow-up included history-taking, physical examination, laboratory tests, and radiological imaging tests if indicated for detection of relapse.

Endoscopic stenting procedure

Briefly, all self-expanding metallic stent (SEMS) placement procedures were performed by experienced endoscopists using colonoscopes (CV-40; Olympus, Tokyo, Japan and EPX-4400HD; Fujinon, Tokyo, Japan) with fluoroscopic guidance. Before the procedure was carried out, sedation with midazolam 2-5 mg was applied in the presence of an anesthesiologist. Water-soluble contrast material was injected through the catheter to visualize the stricture. The luminal diameters of the Over-the-Wire (OTW) and Through-the-Stent (TTS) stents were 30 mm (body of stent) and 36 mm (both ends) respectively, and the lengths varied from 8 to 12 cm. The length of the stent was at least an additional 3 cm on each side of the stricture preference. Uncovered SEMS (Micro-Tech Europe GmbH, Düsseldorf, Germany) were placed in all patients.

Routine checks with direct radiography were performed after the procedure had been completed. All patients were allowed to take liquid 6 h after the procedure and were discharged after 12 to 24 h. A liquid and semi-solid diet was recommended to patients for the first week. Thereafter, on the seventh post-procedure day, direct control radiography was performed.

Here the technical success rate (TSR) was defined as the ratio of patients with a correctly placed SEMS upon stent deployment across the entire stricture length to the total number of patients. The clinical success rate (CSR) was defined as the ratio of patients with technical success and successful maintenance of stent function before elective surgery (regardless of number of SEMS deployed) to the total number of patients. The surgical success rate (SSR) of colorectal stents as a bridge to surgery was defined as the ratio of patients with successful surgical procedures. Unsuccessful surgical outcomes were defined as being due to insufficient colonic decompression.

Statistical analysis

Statistical analyses were conducted using SPSS 20.0 (SPSS Inc., Chicago, IL) software. Survival analysis and curves were established according to the Kaplan-Meier method and compared using the log-rank test. Median follow-up time was calculated as the median observation interval for all patients, being the time from diagnosis or colorectal stenting for obstruction to the last follow-up or death. Disease-free survival (DFS) was defined as the time since diagnosis or stenting to the first evidence of relapse in stages 2-3 of the disease. Progression-free survival (PFS) was described as the period following diagnosis or stenting to the first evidence of relapse in stage 4 of the disease. In addition, overall survival (OS) was described as the time from diagnosis to the date of the patient's death or last known contact. Prognostic factors analyzed by univariate analysis were also evaluated with multivariate analysis using the Cox proportional hazards model to predict the risk factors for relapse. Multivariate *P* values were used to characterize the independence of these factors. A 95% confidence interval (CI) was used to quantify the relationship between survival time and each independent factor. All *P* values were 2-sided in tests and *P* values $\leqslant$ 0.05 were considered significant.

RESULTS

A total of 49 colorectal cancer patients were evaluated. The median age of patients was 64, ranging from 36 to 89 years. 44.9% of patients were male and 55.1% were female. Each patient's disease was categorized pathologically as stage 2 (32.7%, 16 patients) or stage 3 (42.9%, 21 patients). Twelve patients (24.4%) had metastatic disease. Eighteen patients had an obstruction located in the rectum, 15 patients in the rectosigmoid region, 10 patients in the sigmoid region, and 6 patients had a tumor causing obstruction in the proximal colon. None of the patients received chemotherapy before stenting. Clinicopathological characteristics are shown in Table 1.

Only in two patients was stenting done at the second attempt. Except for these, in all patients only one colorectal stent was used. In all patients, resolution of the obstruction and clinical improvement was achieved. Thus, the TSR and CSR were 95.9% and 100%, respectively. Stenting was undertaken in 37 patients as a bridge to surgery, and in 12 patients stents were used for palliation. Median duration of the stenting procedure was 18 (± 4.48) min for the entire group.

Bridge to surgery group

In the bridge to surgery group, abdominal pain occurred in one patient (2.7%) as an early complication (< 7 d), while one patient (2.7%) experienced tenesmus. Three patients suffered tenesmus (8.1%), in two patients stent migration occurred (5.4%), and one patient (2.7%) had a fecal obstruction as a late complication (> 7 d) that was solved endoscopically. Patients defined with tenesmus who experienced stent migration after the stenting procedure had rectal cancer. Thus, decompression caused complications for 41.6% of patients with rectal cancer, who were in stages 2 and 3 of the disease.

Palliation group

As early complications in the palliative stage 4 group, two patients suffered abdominal pain (16.6%), one patient (8.3%) had tenesmus, and one patient

Table 1 Clinical characteristics of patients *n* (%)

Characteristics	Patients (*n* = 49)
Gender	
Men	22 (44.9)
Women	27 (55.1)
Age	64.0 (36-89)
Men	64.1 (34-89)
Women	64.6 (42-87)
Stage	
Stage 2	16 (32.7)
Stage 3	21 (42.9)
Stage 4	12 (24.4)
Obstruction	
Proximal colon	6 (12.2)
Sigmoid colon	10 (20.4)
Rectosigmoid	15 (30.6)
Rectum	8 (36.8)

Table 2 Early and late complications after colorectal stenting in bridge to surgery and palliation groups *n* (%)

Groups	Complications	
	Early complications	Late complications
Bridge to surgery group	Abdominal pain 1 (2.7) Tenesmus 1 (2.7)	Stent migration 2 (5.4) Tenesmus 3 (8.1) Obstruction with feces 1 (2.7)
Palliation group	Abdominal pain 2 (16.6) Tenesmus 1 (8.3) Fever 1 (8.3)	Stent migration 1 (8.3) Tumor migration 2 (16.6)

Table 3 Multivariate analysis of clinicopathological characteristics comparing patient group and overall survival

Variables	OS		
	HR	95%CI	*P* value
Surgery	0.069	0.06-0.96	0.044
Stage	0.082	0.59-10.29	0.214

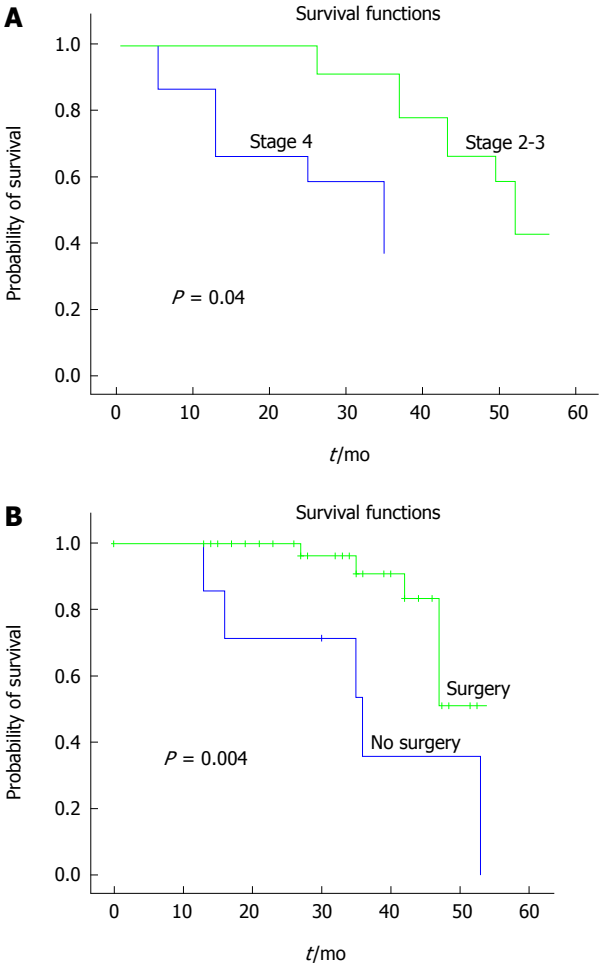


Figure 1 Overall survival. A: Overall survival of patients according to stage of diseases; B: Overall survival of patients able to undergo surgery and those without surgery.

(8.3%) experienced fever. As late complications, stent migration and obstruction occurred in one patient (8.3%) and two patients had an obstruction due to tumor migration (16.6%). Each complication was recorded in a different tumor region.

For the entire group, early complication rates totaled 13.1%; three patients with abdominal pain (6.2%), two patients with tenesmus (4.2%) and one patient with fever (2.1%). Late complications totaled 18.7%; three patients with tenesmus (6.2%), one patient with fecal obstruction (2.1%), three patients with stent migration (6.2%), and two patients with tumor migration and obstruction (4.2%) (Table 2). For the entire group, the median duration of hospitalization was 4 ± 1.17 d. The median time to surgery after stenting was 30 ± 91.9 d. All surgery was done in one single operation; therefore no colostomy with stoma was needed. All patients who had rectal cancer received neoadjuvant chemoradiation and patients with stage 2 and 3 disease received adjuvant chemotherapy. Only two patients with stage 2 and 3 disease failed to undergo surgery due to cardiac problems. Thus, SSR was 94.6%. Metastatic colorectal patients who were managed palliatively with stents had backbone chemotherapy with oxaliplatin and/or irinotecan-based regimens plus antiangiogenic therapies, especially bevacizumab. Colorectal stent and patient characteristics are shown in Table 1.

At a median follow-up of 33.0 mo, median OS was 53.0 (95%CI: 46.57-59.42) mo. Median OS of the entire group was 47.0 mo (95%CI: 21.71-72.28). Median OS of stage 2-3 patients was 53.1 mo and stage 4 patients was 37.1 mo (P = 0.04). Patients who underwent surgery had an OS of 49.1 mo, while patients who were unable to undergo surgery had an OS of 37.2 mo (P = 0.004) (Figure 1). There was no statistically significant relationship between OS, age, location of obstruction and gender (P > 0.05). In multivariate analysis, only surgery was an independent prognostic factor for OS (Table 3). Median PFS time was not reached. The two and three-year PFS rate for stage 2 disease was 100%; and the rates were

94.0% and 84.7% for stage 3 disease ($P > 0.05$), respectively. The two-year DFS rate was 91.3% and three-year DFS rate was 87.4% in stage 4 patients. There was no statistically significant relationship between PFS, age, gender, location of obstruction and surgery ($P > 0.05$).

DISCUSSION

Colonic obstruction occurs in 15%-20% of colorectal cancers[2,3]. Colonic stenting is recommended only for those patients with both obstructive symptoms and radiological or endoscopic findings suspicious of malignant large-bowel obstruction[6]. Experience with stenting has generally been performed for left-sided lesions and only one randomized trial was carried out in the case of malignant obstruction of rectal cancer. Rectal stenting is often avoided because of presumed association with complications such as pain, tenesmus, incontinence, and stent migration. For this reason, guidelines suggest that stenting only be applied for malignant colonic obstruction[6,10]. In our study, no difficulty was encountered in applying the stenting procedure, except for one patient who had a tumor in the colonic region. However, the stenting process caused complications both in the early and late phases of patients with obstructed rectal cancer. Tenesmus (8.1% overall, 25.0% for rectal cancer) and stent migration (5.4% overall, 16.6% for rectal cancer) occurred in two patients with stage 2 and 3 of the disease. Thus, we found that the stenting procedure for cancers in the rectal region caused relatively more complications compared with proximal tumors.

Our TSR was 95.9% and CSR was 100%. In the literature, TSR varies between 70%-100% and CSR from 85% to 96%[11-14]. In a trial where most of the endoscopists were from a non-university setting, TSR was reported as 70%. Factors associated with technical failure included the severity of obstruction, extra-colonic origin of tumor, proximal colonic obstruction, and presence of carcinomatosis[14]. In our study, a low technical failure rate (only two patients) did not allow us to determine the factors associated with technical failure. Our total early and late complication rates for the entire group were 13.1% and 18.7%, respectively. Early complications were three patients with abdominal pain (6.2%), two patients with tenesmus (4.2%), and one patient with fever (2.1%). Late complications were three patients with tenesmus (6.2%), one patient with fecal obstruction (2.1%), three patients with stent migration (6.2%), and two patients with tumor migration and obstruction (4.2%). These complication rates are much lower than previous studies[15,16]. In this study, all endoscopic stent placements were handled by the same general surgeon in a university setting. Therefore, higher success rates and lower complication rates with no perforation can be attributable to the circumstances in the particular treatment facility and experience of well-trained endoscopists.

The use of stents for acute colorectal obstruction due to malignancy is controversial. van Hooft *et al*[6] found high perforation rates related to the use of colorectal stenting in malignant obstructions[2,3]. A decision analysis concluded that colonic stent insertion followed by elective surgery was more effective and less costly than emergency surgery, but results of randomized trials and meta-analyses are contradictory[17-19]. Decompression of the bowel gives time for surgeons to stabilize the patient, stage the disease with imaging techniques, and take a biopsy. Thus, it allows one-stage surgery with primary anastomosis. In our study, except for two patients who had cardiac problems, all patients with stage 2 and 3 disease were able to undergo one-stage surgery without stoma. In the group overall, patients who underwent surgery had longer OS. Based on our results, we oppose the belief in favor of emergency surgery rather than stenting in situations of malignant colonic obstruction, based on high perforation rates[14].

Palliative colorectal stenting was shown to be as effective and acceptable as palliative surgery in one retrospective study, where colonic stents showed long-term efficacy comparable to that of surgery[20]. Published follow-up data are limited because of poor survival rates of the patient population in the palliative group. Stefanidis *et al*[21] reported one-year effectiveness and the patency of colonic stents used for palliation. Bevacizumab is an antiangiogenic agent used in treating metastatic colorectal cancer[22]. Bevacizumab was found to be associated with high complication rates in patients who had palliative stents for malignant obstruction[23]. In our study, at a median follow-up of 33 mo, we found no significant complications due to the addition of bevacizumab.

In the bridge to surgery group, the surgical success rate, *i.e.*, the ratio of patients that had stent placement who underwent elective primary anastomosis surgery, was 95.6%. This ratio is very high compared to other studies, which were between 55.3%-77.9%[24-26]. This might be explained by low TSR and CSR in these studies *vs* high rates in our study. Previous studies have reported that the factors associated with technical failure included severity of obstruction, extra-colonic origin of tumor, proximal colonic obstruction, and presence of carcinomatosis.

In conclusion, we demonstrated in this study the efficacy and safety of colonic stents both as a bridge to surgery and for palliative decompression. Surgery was found to be an independent prognostic factor in patients with malignant colorectal obstruction. Although technical and clinical success rates were high, rectal stents had greater complication rates, consistent with the literature. Palliative stenting with a median follow-up of 33 mo did not add any severe extra complications in the era of bevacizumab. In addition, results highlight the importance of the skill of

the endoscopist when carrying out colonic stenting.

COMMENTS

Background

Colorectal tumors may cause large bowel obstruction and in such cases they require immediate treatment. The mortality rate of emergency surgery is high. However, colonic stents are being safely used in malignant colorectal obstruction in the majority of cases with good clinical results. They are used for two indications in colorectal malignancies; palliative dilatation of advanced disease, and preoperative decompression as a bridge to surgery. In both indications, colonic stents prevent colostomy with stoma.

Research frontiers

Colorectal stents can be used for two indications in colorectal malignancies; palliative dilatation of advanced disease, and preoperative decompression as a bridge to surgery. In both indications, colonic stents prevent colostomy with stoma.

Innovations and breakthroughs

The authors proved the efficacy and safety of colonic stents in both bridge to surgery and for palliative decompression. In addition, the importance of the skills of the endoscopist in colonic stenting was also exposed. Decompression of the bowel gives time for surgeons to stabilize and prepare the patient for further treatment approaches.

Applications

Colorectal stents are used increasingly as a nonsurgical alternative for the palliation of luminal gastrointestinal neoplasms. They also have an emerging role in the treatment of obstruction in gastrointestinal segments.

Terminology

A colostomy is a surgically created opening on the abdomen which allows stools to exit the body.

Peer-review

The manuscript describes the evaluation of colonic stents. The manuscript is well presented and tackles an important aspect in clinical colon cancer biology. A significant group of nearly 50 patients have been studied. Statistical analysis is well carried out.

REFERENCES

1 **Siegel R**, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 9-29 [PMID: 24399786 DOI: 10.3322/caac.21208]

2 **Phillips RK**, Hittinger R, Fry JS, Fielding LP. Malignant large bowel obstruction. *Br J Surg* 1985; **72**: 296-302 [PMID: 3986481]

3 **Mella J**, Biffin A, Radcliffe AG, Stamatakis JD, Steele RJ. Population-based audit of colorectal cancer management in two UK health regions. Colorectal Cancer Working Group, Royal College of Surgeons of England Clinical Epidemiology and Audit Unit. *Br J Surg* 1997; **84**: 1731-1736 [PMID: 9448628]

4 **Khot UP**, Lang AW, Murali K, Parker MC. Systematic review of the efficacy and safety of colorectal stents. *Br J Surg* 2002; **89**: 1096-1102 [PMID: 12190673]

5 **Sebastian S**, Johnston S, Geoghegan T, Torreggiani W, Buckley M. Pooled analysis of the efficacy and safety of self-expanding metal stenting in malignant colorectal obstruction. *Am J Gastroenterol* 2004; **99**: 2051-2057 [PMID: 15447772]

6 **van Hooft JE**, van Halsema EE, Vanbiervliet G, Beets-Tan RG, DeWitt JM, Donnellan F, Dumonceau JM, Glynne-Jones RG, Hassan C, Jiménez-Perez J, Meisner S, Muthusamy VR, Parker MC, Regimbeau JM, Sabbagh C, Sagar J, Tanis PJ, Vandervoort J, Webster GJ, Manes G, Barthet MA, Repici A. Self-expandable metal stents for obstructing colonic and extracolonic cancer: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2014; **46**: 990-1053 [PMID: 25325682 DOI: 10.1055/s-0034-1390700]

7 **Karoui M**, Charachon A, Delbaldo C, Loriau J, Laurent A, Sobhani I, Tran Van Nhieu J, Delchier JC, Fagniez PL, Piedbois P, Cherqui D. Stents for palliation of obstructive metastatic colon cancer: impact on management and chemotherapy administration. *Arch Surg* 2007; **142**: 619-623; discussion 623 [PMID: 17638798]

8 **Faragher IG**, Chaitowitz IM, Stupart DA. Long-term results of palliative stenting or surgery for incurable obstructing colon cancer. *Colorectal Dis* 2008; **10**: 668-672 [PMID: 18266885 DOI: 10.1111/j.1463-1318.2007.01446.x]

9 **Edge SB**, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. AJCC Cancer Staging Manual. 7th ed. New York, NY: Springer, 2010: 347-376

10 **Fiori E**, Lamazza A, De Cesare A, Bononi M, Volpino P, Schillaci A, Cavallaro A, Cangemi V. Palliative management of malignant rectosigmoidal obstruction. Colostomy vs. endoscopic stenting. A randomized prospective trial. *Anticancer Res* 2004; **24**: 265-268 [PMID: 15015606]

11 **Baron TH**, Dean PA, Yates MR, Canon C, Koehler RE. Expandable metal stents for the treatment of colonic obstruction: techniques and outcomes. *Gastrointest Endosc* 1998; **47**: 277-286 [PMID: 9540883]

12 **Mainar A**, De Gregorio Ariza MA, Tejero E, Tobío R, Alfonso E, Pinto I, Herrera M, Fernández JA. Acute colorectal obstruction: treatment with self-expandable metallic stents before scheduled surgery--results of a multicenter study. *Radiology* 1999; **210**: 65-69 [PMID: 9885588]

13 **de Gregorio MA**, Mainar A, Tejero E, Tobío R, Alfonso E, Pinto I, Fernández R, Herrera M, Fernández JA. Acute colorectal obstruction: stent placement for palliative treatment--results of a multicenter study. *Radiology* 1998; **209**: 117-120 [PMID: 9769821]

14 **van Hooft JE**, Bemelman WA, Oldenburg B, Marinelli AW, Lutke Holzik MF, Grubben MJ, Sprangers MA, Dijkgraaf MG, Fockens P. Colonic stenting versus emergency surgery for acute left-sided malignant colonic obstruction: a multicentre randomised trial. *Lancet Oncol* 2011; **12**: 344-352 [PMID: 21398178 DOI: 10.1016/S1470-2045(11)70035-3]

15 **Jost RS**, Jost R, Schoch E, Brunner B, Decurtins M, Zollikofer CL. Colorectal stenting: an effective therapy for preoperative and palliative treatment. *Cardiovasc Intervent Radiol* 2007; **30**: 433-440 [PMID: 17225973]

16 **Watt AM**, Faragher IG, Griffin TT, Rieger NA, Maddern GJ. Self-expanding metallic stents for relieving malignant colorectal obstruction: a systematic review. *Ann Surg* 2007; **246**: 24-30 [PMID: 17592286]

17 **Targownik LE**, Spiegel BM, Sack J, Hines OJ, Dulai GS, Gralnek IM, Farrell JJ. Colonic stent vs. emergency surgery for management of acute left-sided malignant colonic obstruction: a decision analysis. *Gastrointest Endosc* 2004; **60**: 865-874 [PMID: 15604999]

18 **Sagar J**. Colorectal stents for the management of malignant colonic obstructions. *Cochrane Database Syst Rev* 2011; (**11**): CD007378 [PMID: 22071835 DOI: 10.1002/14651858]

19 **Zhang Y**, Shi J, Shi B, Song CY, Xie WF, Chen YX. Self-expanding metallic stent as a bridge to surgery versus emergency surgery for obstructive colorectal cancer: a meta-analysis. *Surg Endosc* 2012; **26**: 110-119 [PMID: 21789642 DOI: 10.1007/s00464-011-1835-6]

20 **Lee HJ**, Hong SP, Cheon JH, Kim TI, Min BS, Kim NK, Kim WH. Long-term outcome of palliative therapy for malignant colorectal obstruction in patients with unresectable metastatic colorectal cancers: endoscopic stenting versus surgery. *Gastrointest Endosc* 2011; **73**: 535-542 [PMID: 21257165 DOI: 10.1016/j.gie.2010.10.052]

21 **Stefanidis D**, Brown K, Nazario H, Trevino HH, Ferral H, Brady CE, Gross GW, Postoak DW, Chadhury R, Rousseau DL, Kahlenberg MS. Safety and efficacy of metallic stents in the management of colorectal obstruction. *JSLS* 2005; **9**: 454-459 [PMID: 16381366]

22 **Bendell JC**, Bekaii-Saab TS, Cohn AL, Hurwitz HI, Kozloff M, Tezcan H, Roach N, Mun Y, Fish S, Flick ED, Dalal D, Grothey A. Treatment patterns and clinical outcomes in patients with metastatic colorectal cancer initially treated with FOLFOX-bevacizumab or FOLFIRI-bevacizumab: results from ARIES, a bevacizumab observational cohort study. *Oncologist* 2012; **17**: 1486-1495 [PMID: 23015662 DOI: 10.1634/theoncologist.2012-0190]

23 **Small AJ**, Coelho-Prabhu N, Baron TH. Endoscopic placement of self-expandable metal stents for malignant colonic obstruction: long-term outcomes and complication factors. *Gastrointest Endosc* 2010; **71**: 560-572 [PMID: 20189515 DOI: 10.1016/j.gie.2009.10.012]

24 **Ye GY**, Cui Z, Chen L, Zhong M. Colonic stenting vs emergent surgery for acute left-sided malignant colonic obstruction: a systematic review and meta-analysis. *World J Gastroenterol* 2012; **18**: 5608-5615 [PMID: 23112555 DOI: 10.3748/wjg.v18.i39.5608]

25 **Cirocchi R**, Farinella E, Trastulli S, Desiderio J, Listorti C, Boselli C, Parisi A, Noya G, Sagar J. Safety and efficacy of endoscopic colonic stenting as a bridge to surgery in the management of intestinal obstruction due to left colon and rectal cancer: a systematic review and meta-analysis. *Surg Oncol* 2013; **22**: 14-21 [PMID: 23183301 DOI: 10.1016/j.suronc.2012.10.003]

26 **Kim JH**, Kwon KA, Lee JJ, Lee WS, Baek JH, Kim YJ, Chung JW, Kim KO, Park DK, Kim JH. Surgical failure after colonic stenting as a bridge to surgery. *World J Gastroenterol* 2014; **20**: 11826-11834 [PMID: 25206288 DOI: 10.3748/wjg.v20.i33.11826]

P- Reviewer: Braet F, Lakatos PL **S- Editor**: Ma YJ
L- Editor: O'Neill M **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9380

World J Gastroenterol 2015 August 21; 21(31): 9380-9386
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Simple colonoscopy reporting system checking the detection rate of colon polyps

Jae Hyun Kim, Youn Jung Choi, Hye Jung Kwon, Seun Ja Park, Moo In Park, Won Moon, Sung Eun Kim

Jae Hyun Kim, Youn Jung Choi, Hye Jung Kwon, Seun Ja Park, Moo In Park, Won Moon, Sung Eun Kim, Department of Internal Medicine, Kosin University College of Medicine, Busan 602-702, South Korea

Author contributions: Kim JH, Choi YJ, Kwon HJ and Park SJ designed study; Kim JH wrote the paper; Park MI, Moon W and Kim SE critically reviewed the manuscript for important intellectual content; and Park SJ approved the manuscript.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: Technical appendix and dataset available from the corresponding author at parksj6406@daum.net.

Correspondence to: Seun Ja Park, MD, Department of Internal Medicine, Kosin University College of Medicine, 34 Amnam-dong, Seo-gu, Busan 602-702, South Korea. parksj6406@daum.net
Telephone: +82-51-9905061
Fax: +82-51-9905055

Received: January 20, 2015
Peer-review started: January 21, 2015
First decision: March 10, 2015
Revised: March 20, 2015
Accepted: April 28, 2015
Article in press: April 28, 2015
Published online: August 21, 2015

Abstract

AIM: To present a simple colonoscopy reporting system that can be checked easily the detection rate of colon polyps.

METHODS: A simple colonoscopy reporting system Kosin Gastroenterology (KG quality reporting system) was developed. The polyp detection rate (PDR), adenoma detection rate (ADR), serrated polyp detection rate (SDR), and advanced adenoma detection rate (AADR) are easily calculated to use this system.

RESULTS: In our gastroenterology center, the PDR, ADR, SDR, and AADR test results from each gastroenterologist were updated, every month. Between June 2014, when the program was started, and December 2014, the overall PDR and ADR in our center were 62.5% and 41.4%, respectively. And the overall SDR and AADR were 7.5% and 12.1%, respectively.

CONCLUSION: We envision that KG quality reporting system can be applied to develop a comprehensive system to check colon polyp detection rates in other gastroenterology centers.

Key words: Colon polyp; Detection rate; Reporting system

Core tip: Detecting the rate of colon polyps, such as the adenoma detection rate is an important quality indicator during colonoscopy exams. However, reporting the detection rate in daily practice is not easy because manual reporting requires a lot of time and effort. To our knowledge, reporting systems for detecting the

rate of colon polyps are rare. We developed a simple colonoscopy reporting system that can be checked easily the detection rate of colon polyps. We envision our system can be applied to develop an optimal system for assessing the detection rate of colon polyps in other gastroenterology centers.

Kim JH, Choi YJ, Kwon HJ, Park SJ, Park MI, Moon W, Kim SE. Simple colonoscopy reporting system checking the detection rate of colon polyps. *World J Gastroenterol* 2015; 21(31): 9380-9386 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9380.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9380

INTRODUCTION

Colorectal cancer is ranked as the third leading cause of cancer-related death in the world[1-3]. It is well known that most colorectal cancers arise from adenomatous polyps and patients with adenomatous polyps have greater risks of future development of advanced neoplasia[4-8]. A colonoscopy is a useful tool to detect and remove colorectal polyps. However, a number of polyps could be missed clinically during colonoscopic examination, which can increase the incidence of interval cancer[9-12]. A large tandem colonoscopy study that assessed variable detection of adenomas, demonstrated miss rates ranged from 17% to 48%[13]. To reduce the missing rate of colon polyps, several quality indicators including adenoma detection, bowel preparation, cecal intubation, withdrawal time, patient's discomfort, and complications, such as perforation and postpolypectomy bleeding have been suggested, all of which aim to measure colonoscopists' performance and to target quality improvement[14,15]. Of such indicators, the adenoma detection rate (ADR) is currently considered the most important quality indicator during colonoscopy[16-18]. According to data from the study by Boroff *et al*[19], the polyp detection rate (PDR) may be a valid surrogate marker of ADR in the proximal but not the distal colon. In daily practice however, reporting for the PDR and ADR is not easy, because manual reporting requires a lot of time and effort. Recently, van Doorn *et al*[20] developed a new colonoscopy reporting system that enables automatic quality assessment, and reported that 94% of colonoscopies that are performed overall were reported completely and that ADR was used as the measurement unit in 35.4% of the system reports. However, in practice it is not easy to introduce this system to other gastroenterology centers, because additional costs will be incurred as this system was developed in collaboration with the Olympus Corporation.

To our knowledge, reporting systems for detecting the rate of colon polyps are rare. Our gastroenterology center also had not checked the PDR and ADR until May 2014, because we thought that this process was complicated. The "KG (Kosin gastroenterology) quality reporting system" was produced because we wanted to identify an easier and more accessible way to check PDR and ADR on routine colonoscopic examination. We tried to develop a simple colonoscopy reporting system which can possibly check the PDR and ADR, as well as the serrated polyp detection rate (SDR) and advanced adenoma detection rate (AADR). In this study, we describe a simple colonoscopy reporting system that was applied in our gastroenterology center to check the PDR, ADR, SDR, and AADR.

MATERIALS AND METHODS

KG quality reporting system production

The "KG Quality reporting system" platform used our hospital's computer program system. In previous colonoscopic input program systems that were used in our gastroenterology center, we manually entered some data including the insertion time, withdrawal time, bowel preparation, and cecal intubation. Based on the current literature and knowledge[15,20], we developed a colonoscopic input program system list that included established quality indicators, such as "reason for colonoscopy, insertion time, withdrawal time, bowel preparation, cecal intubation, patient's discomfort status, and polyp detection (including whether polyp size are larger or smaller than 1 cm)". Two indicators, including "last examination date and anticoagulants", were also added (Figure 1). We used the Aronchick bowel preparation scale as the bowel preparation index, and the Gloucester Comfort Scale[21] as the patient's discomfort scale index. All indicators could be entered with one click, except for insertion time and withdrawal time which are entered as the number of minutes and seconds. If one or more polyps were located during the colonoscopic examination, the gastroenterologist would check "yes" for the polyp detection indicator (violet colored box on Figure 1). The polyp size which is measure by using open biopsy forceps, could be indicated by selecting whether "Size $\geqslant$ 1 cm" or not.

We made a patients' list searchable so each gastroenterologist could find the examined patients. In this step, several items including "Polyp", "Adenoma", "Serrated polyp", "HGD (high grade dysplasia)", "Villous comp (component)", and "Advanced adenoma" are presented on a new page (Figure 2). Serrated polyps are defined as hyperplastic polyps (HP) which excluded small lesions (< 10 mm) of HP limited to the rectum and sigmoid, sessile serrated adenoma/polyp (SSA/P), or traditional serrated adenoma (TSA)[22]. Advanced adenoma is defined as adenoma that was 1 cm or greater, or with HGD, or with villous component (tubulovillous or villous)[23,24]. The "Polyp" column (green colored box on Figure 2) is automatically presented as "Yes" or "No" which is linked with a click of "Yes" or "No" to the polyp detection indicator shown in Figure 1. For other items including "Adenoma",

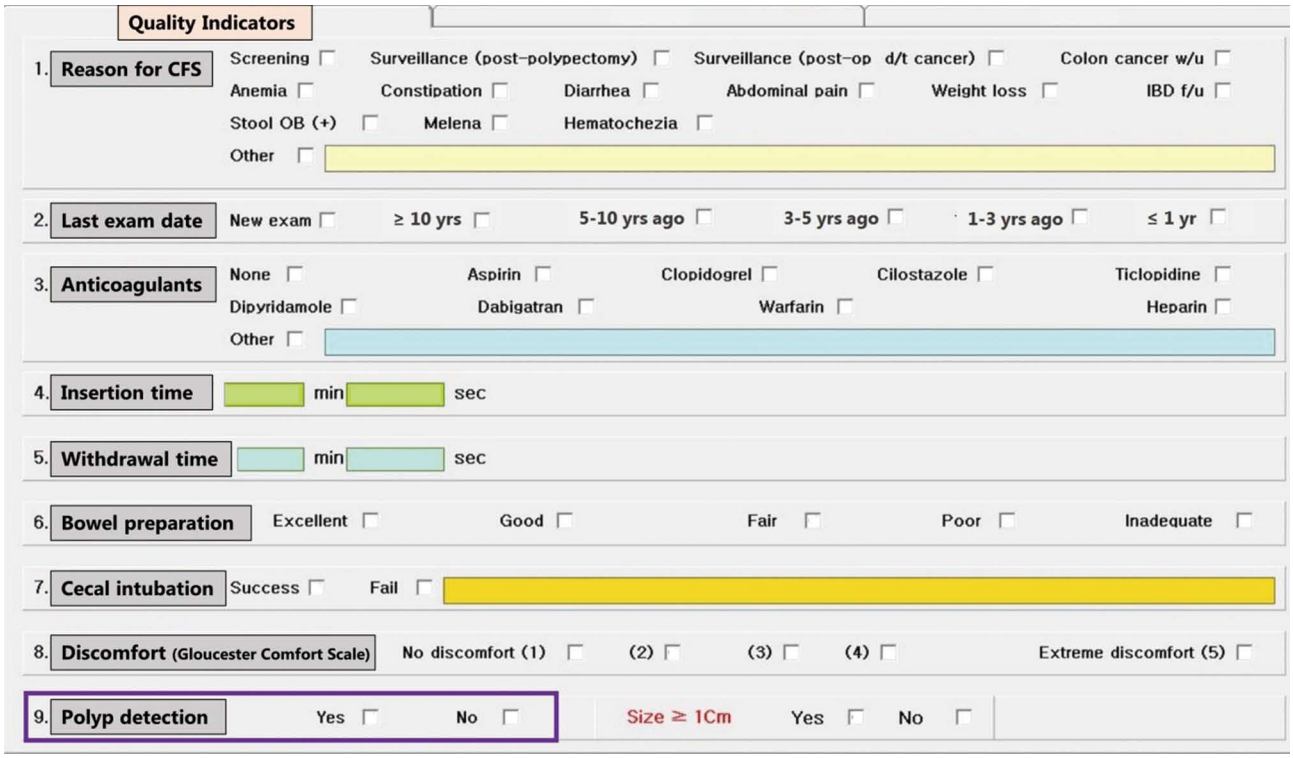
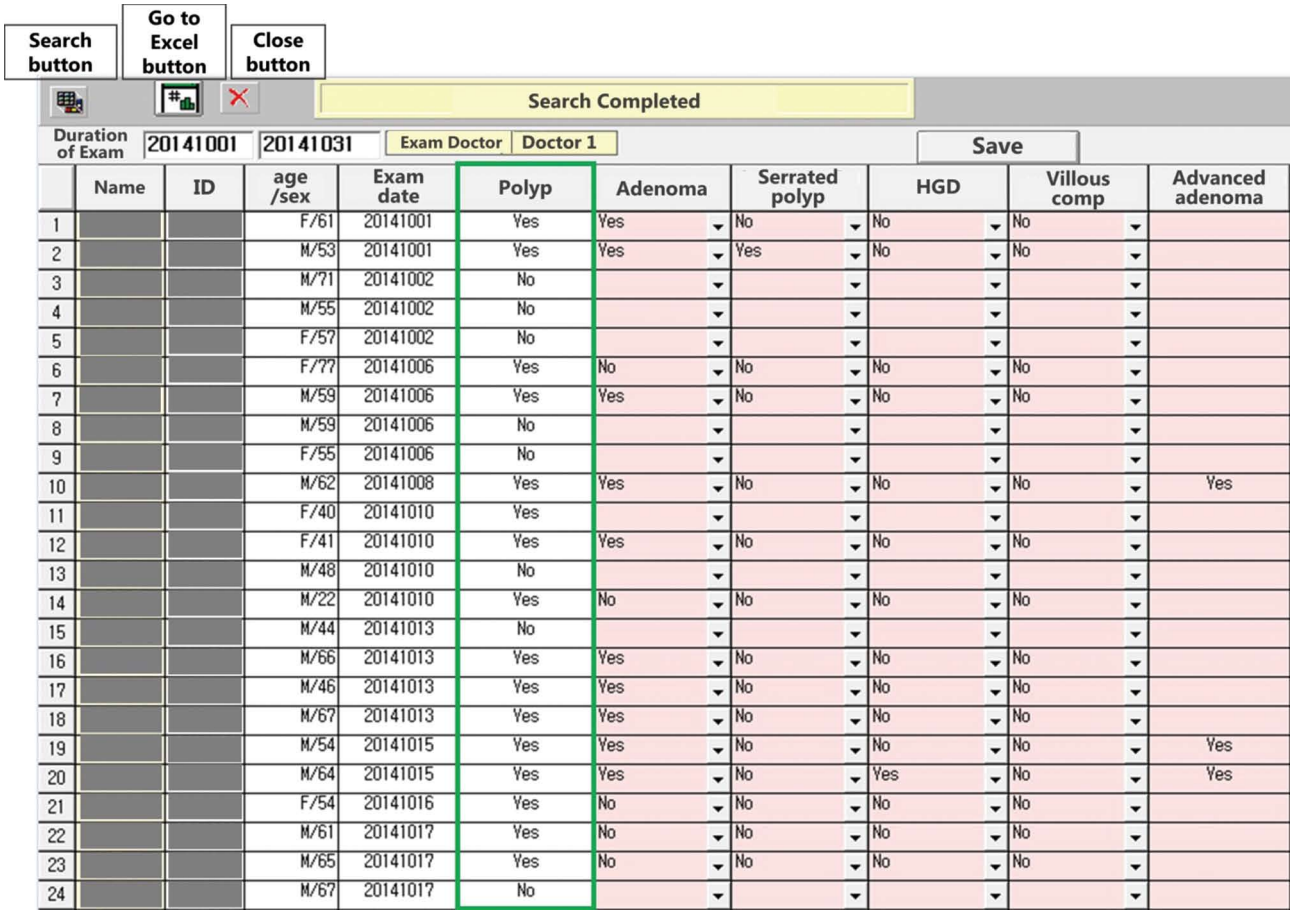


Figure 1 Quality indicators in the Kosin Gastroenterology quality reporting system.



	Name	ID	age /sex	Exam date	Polyp	Adenoma	Serrated polyp	HGD	Villous comp	Advanced adenoma
1			F/61	20141001	Yes	Yes	No	No	No	
2			M/53	20141001	Yes	Yes	Yes	No	No	
3			M/71	20141002	No					
4			M/55	20141002	No					
5			F/57	20141002	No					
6			F/77	20141006	Yes	No	No	No	No	
7			M/59	20141006	Yes	Yes	No	No	No	
8			M/59	20141006	No					
9			F/55	20141006	No					
10			M/62	20141008	Yes	Yes	No	No	No	Yes
11			F/40	20141010	Yes					
12			F/41	20141010	Yes	Yes	No	No	No	
13			M/48	20141010	No					
14			M/22	20141010	Yes	No	No	No	No	
15			M/44	20141013	No					
16			M/66	20141013	Yes	Yes	No	No	No	
17			M/46	20141013	Yes	Yes	No	No	No	
18			M/67	20141013	Yes	Yes	No	No	No	
19			M/54	20141015	Yes	Yes	No	No	No	Yes
20			M/64	20141015	Yes	Yes	No	Yes	No	Yes
21			F/54	20141016	Yes	No	No	No	No	
22			M/61	20141017	Yes	No	No	No	No	
23			M/65	20141017	Yes	No	No	No	No	
24			M/67	20141017	No					

Figure 2 Search results for the patient lists by each examining gastroenterologist and the input-system for pathologic data in the Kosin Gastroenterology quality reporting system.

Table 1 Overall detection rate of colon polyps between June 2014 and December 2014 in our gastroenterology center

Month	6	7	8	9	10	11	12	Overall (male *vs* female)
PDR	64.6%	62.4%	64.3%	61.0%	59.6%	63.7%	64.2%	62.5% (66.0% *vs* 55.8%)
ADR	40.4%	36.1%	44.3%	38.2%	40.7%	41.0%	47.2%	41.4% (45.5% *vs* 36.3%)
SDR	13.5%	8.2%	11.2%	8.0%	5.8%	6.6%	8.7%	7.5% (7.7% *vs* 7.4%)
AADR	6.8%	13.5%	16.0%	11.2%	13.3%	11.2%	12.8%	12.1% (13.6% *vs* 9.7%)

PDR: Polyp detection rate; ADR: Adenoma detection rate; SDR: Serrated polyp detection rate; AADR: Advanced adenoma detection rate.

"Serrated polyp", "HGD", "Villous comp", and "Advanced adenoma", we tried to link automatically with the pathological data, however, in practice, this approach was not easy. We modified this approach and adjusted the program so that data such as "Adenoma", "Serrated polyp", "HGD", and "Villous comp" could be entered manually. Each gastroenterologist was asked to input this specific data. For example, if the histology of a resected polyp was adenoma, the examining gastroenterologist was asked to enter "Yes" in the "Adenoma" space. Similarly, if the histology of a resected polyp was serrated polyp, the examining gastroenterologist entered "Yes" in the 'Serrated polyp' space. In cases of adenoma with HGD or villous components, the gastroenterologist input "Yes" in the "HGD" or "Villous comp" spaces, respectively. If any one of the three components including "HGD", "Villous comp" (shown in Figure 2), and "Size $\geqslant$ 1 cm (shown in Figure 1)" were entered as "Yes", this information was automatically presented as "Yes" in the "Advanced adenoma" space. For cases with multiple resected polyps, additional work is needed to identify whether the resected polyp was an adenoma larger than 1 cm in size. Although this process could be tedious, entering one month of data only requires about 15 min. Additionally, this process can help to identify gastroenterologist's own pathological data for resected polyps. These inputted data can be saved by selecting the "Save" button, and can be transferred to the Excel program (Microsoft Corporation, Washington, United States) (Figure 2).

PDR, ADR, SDR, and AADR calculations

Calculating PDR, ADR, SDR, and AADR is easily accomplished using the Microsoft Excel program. This process was performed by the investigator. PDR is calculated as the number of colonoscopies in which one or more polyps were detected, divided by the total number of colonoscopies performed by the colonoscopist. ADR is calculated as the number of colonoscopies in which one or more adenomas were detected, divided by the total number of colonoscopies performed by the colonoscopist. SDR is calculated as the number of colonoscopies in which one or more serrated polyps were identified, divided by the total number of colonoscopies performed by the colonoscopist. AADR is calculated as the number of colonoscopies in which one or more advanced adenomas were detected, divided by the total number of colonoscopies performed by the colonoscopist.

RESULTS

In our gastroenterology center there are three experts and eleven training fellows; a monthly average of 60 colonoscopic examinations are performed per gastroenterologist. By using our system, we calculated the PDR, ADR, SDR, and AADR of each gastroenterologist in our clinic. Based on these data, we analyzed the flow of data of each gastroenterologist, and estimated the overall PDR, ADR, SDR, and AADR of all gastroenterologists in our clinic. At the beginning of every month, the results of the last month, which consisted of tables and figures were updated and sent to all gastroenterologists in our clinic by e-mail. Between June 2014, when the program was started, and December 2014, the overall PDR of gastroenterologists in our clinic was 62.5% (66.0% in male patients, and 55.8% in female patients). The overall ADR was 41.4% (45.5% in male patients, and 36.3% in female patients), the overall SDR was 7.5% (7.7% in male patients, and 7.4% in female patients), and the overall AADR was 12.1% (13.6% in male patients, and 9.7% in female patients). These data are summarized in Table 1. The flow of each gastroenterologist's monthly data was plotted with a broken line graph. Figure 3A and Figure 3B show examples for the flow of monthly data of two fellows.

DISCUSSION

We worked to develop an easy system to check the PDR, ADR, SDR, and AADR during routine colonscopic examinations. The first step was to search the list of patients that were examined by each gastroenterologist. We allowed the examined patients' list to be searchable by each gastroenterologist in our clinic, and we also made it possible to automatically link between the "Polyp" indicator (violet colored box

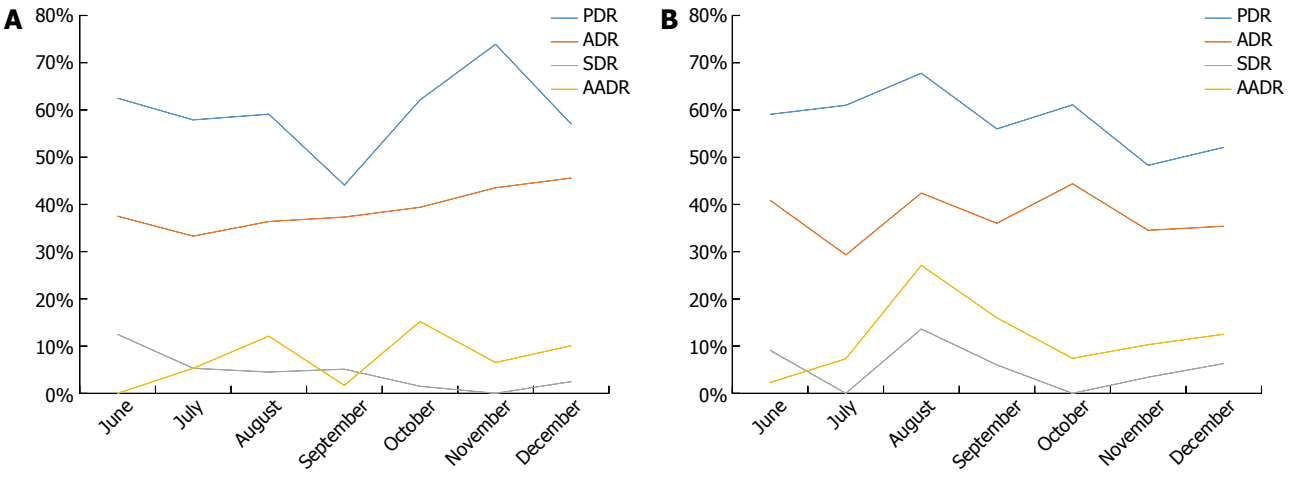


Figure 3 Monthly polyp detection rate data flow for fellow 1 (A) and fellow 2 (B). PDR: Polyp detection rate; ADR: Adenoma detection rate; SDR: Serrated polyp detection rate; AADR: Advanced adenoma detection rate.

on Figure 1) and the results of the "Polyp" column (green colored box on Figure 2). The second step was to input the "Adenoma", "Serrated polyp", "HGD", and "Villous comp" data. This process was performed by each examining gastroenterologist. And then, PDR, ADR, SDR, and AADR were easily calculated as described in the "Methods" section.

Several quality indicators for colonoscopy are used in the KG quality reporting system (Figure 1). Of these indicators, ADR is the quality indicator with the strongest association to post-colonoscopy colorectal cancer or missed colorectal cancer. Among healthy asymptomatic patients that undergo screening colonoscopy, adenomas should detected in $\geqslant$ 25% of men and $\geqslant$ 15% women more than 50 years old[15]. In our gastroenterology center, the overall ADR was 41.4% (45.5% in male patients, and 36.3% in female patients) between June and December 2014. Serrated polyps are classified as HP which excluded small lesions (< 10 mm) of HP limited to the rectum and sigmoid, SSA/P, or TSA[22]. Both SSA/P and TSA are pre-cancerous lesions, and SSA/P located in the proximal colon is considered as a cause of interval cancer[25]. A recent article by these investigators showed that the SDR correlated with the ADR in their routine colonoscopic examinations[26]. In our gastroenterology center, the overall SDR was 7.5% (7.7% in male patients, and 7.4% in female patients) between June and December 2014. According to the current literature and knowledge, about one-third of all polyps larger than 10 mm in size have advanced histology, but diminutive polyps ($\leqslant$ 5 mm in size) rarely have advanced pathology[27,28]. A polyp larger than 10 mm in size is categorized as advanced adenoma, which also includes adenoma with high-grade dysplasia (HGD), or with villous components (tubulovillous or villous)[23,24]. Recently, Greenspan *et al*[29] reported that the overall AADR was 7.97% for 14 colonoscopists who performed a total of 1944 colonoscopies. Additionally, Lee *et al*[30] showed that the overall AADR was 4.46% for 18 colonoscopists who performed a total of 561 colonoscopies. In our gastroenterology center, the overall AADR was 12.1% (13.6% in male patients, and 9.7% in female patients) between June and December 2014.

There are some limitations to the system we developed. First, the detection rate of colon polyps is not categorized by the reason for colonoscopy. Our data included patients with prior polypectomy as well as screening colonoscopy, so the detection rate of colon polyps in our gastroenterology center could be overestimated. In the next step, we will develop this system to categorize the detection rate of colon polyps by the reason for colonoscopy. Second, the input of pathologic data is performed manually in our system. Currently, each examining gastroenterologist enters the pathologic data of resected polyps, which it can cause manual data entry errors. The next step is to develop an automatic linkage between our system and pathologic data.

In addition to the practical application of our system which checks the detection rate of colon polyps, other possible associations between various quality indicators and the detection rate of colon polyps can be evaluated. The data acquired from our program can be used as a basis for performing colonoscopy research. Furthermore, identifying gastroenterologists' own data for polyp detection rates can facilitate colonoscopic examination quality improvements. In the next step, we will develop our system to possible the statistical analysis for each quality indicator. Although the KG quality reporting system is still being developed, we hope that this system can be applied to develop an optimal system for assessing the detection rate of colon polyps in other gastroenterology centers.

COMMENTS

Background

Detecting the rate of colon polyps, such as the adenoma detection rate (ADR)

is an important quality indicator during colonoscopy exams. However, reporting the detection rate in daily practice is not easy because manual reporting is time consuming and requires effort. To our knowledge, reporting systems for detecting the rate of colon polyps are rare.

Research frontiers

This study presents a simple colonoscopy reporting system (KG quality reporting system) that can be checked easily the detection rate of colon polyps.

Innovations and breakthroughs

The KG quality reporting system is focused on the detection rate of colon polyps, such as polyp detection rate, ADR, serrated polyp detection rate, and advanced adenoma detection rate.

Applications

This system can be applied to develop an optimal system for assessing the detection rate of colon polyps in other gastroenterology centers.

Terminology

Serrated polyps are defined as hyperplastic polyps which excluded small lesions (< 10 mm) of hyperplastic polyps limited to the rectum and sigmoid, sessile serrated adenoma/polyp, or traditional serrated adenoma. Advanced adenoma is defined as adenoma that was 1 cm or greater, or with high grade dysplasia, or with villous component (tubulovillous or villous).

Peer-review

This paper deals with a simple colonoscopy reporting system that can facilitate the automatic analysis of colonoscopy quality indicators. This system may be useful for performing colonoscopy research and improving the quality of colonoscopy.

REFERENCES

1 **Edwards BK**, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, Jemal A, Schymura MJ, Lansdorp-Vogelaar I, Seeff LC, van Ballegooijen M, Goede SL, Ries LA. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. *Cancer* 2010; **116**: 544-573 [PMID: 19998273 DOI: 10.1002/cncr.24760]

2 **Ferlay J**, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. *Ann Oncol* 2007; **18**: 581-592 [PMID: 17287242 DOI: 10.1093/annonc/mdl498]

3 **Joseph DA**, King JB, Miller JW, Richardson LC. Prevalence of colorectal cancer screening among adults--Behavioral Risk Factor Surveillance System, United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012; **61** Suppl: 51-56 [PMID: 22695464]

4 **Cottet V**, Jooste V, Fournel I, Bouvier AM, Faivre J, Bonithon-Kopp C. Long-term risk of colorectal cancer after adenoma removal: a population-based cohort study. *Gut* 2012; **61**: 1180-1186 [PMID: 22110052 DOI: 10.1136/gutjnl-2011-300295]

5 **Loeve F**, van Ballegooijen M, Boer R, Kuipers EJ, Habbema JD. Colorectal cancer risk in adenoma patients: a nation-wide study. *Int J Cancer* 2004; **111**: 147-151 [PMID: 15185356 DOI: 10.1002/ijc.20241]

6 **Robertson DJ**, Greenberg ER, Beach M, Sandler RS, Ahnen D, Haile RW, Burke CA, Snover DC, Bresalier RS, McKeown-Eyssen G, Mandel JS, Bond JH, Van Stolk RU, Summers RW, Rothstein R, Church TR, Cole BF, Byers T, Mott L, Baron JA. Colorectal cancer in patients under close colonoscopic surveillance. *Gastroenterology* 2005; **129**: 34-41 [PMID: 16012932]

7 **Yamaji Y**, Mitsushima T, Ikuma H, Watabe H, Okamoto M, Kawabe T, Wada R, Doi H, Omata M. Incidence and recurrence rates of colorectal adenomas estimated by annually repeated colonoscopies on asymptomatic Japanese. *Gut* 2004; **53**: 568-572 [PMID: 15016753]

8 **Muto T**, Bussey HJ, Morson BC. The evolution of cancer of the colon and rectum. *Cancer* 1975; **36**: 2251-2270 [PMID: 1203876]

9 **Ananthakrishnan AN**, Chan AT. Missed polyps, missed opportunities. *Gastrointest Endosc* 2011; **74**: 262-265 [PMID: 21802584 DOI: 10.1016/j.gie.2011.05.019]

10 **Corley DA**, Jensen CD, Marks AR, Zhao WK, Lee JK, Doubeni CA, Zauber AG, de Boer J, Fireman BH, Schottinger JE, Quinn VP, Ghai NR, Levin TR, Quesenberry CP. Adenoma detection rate and risk of colorectal cancer and death. *N Engl J Med* 2014; **370**: 1298-1306 [PMID: 24693890 DOI: 10.1056/NEJMoa1309086]

11 **le Clercq CM**, Winkens B, Bakker CM, Keulen ET, Beets GL, Masclee AA, Sanduleanu S. Metachronous colorectal cancers result from missed lesions and non-compliance with surveillance. *Gastrointest Endosc* 2015; **82**: 325-333.e2 [PMID: 25843613 DOI: 10.1016/j.gie.2014.12.052]

12 **Samadder NJ**, Curtin K, Tuohy TM, Pappas L, Boucher K, Provenzale D, Rowe KG, Mineau GP, Smith K, Pimentel R, Kirchhoff AC, Burt RW. Characteristics of missed or interval colorectal cancer and patient survival: a population-based study. *Gastroenterology* 2014; **146**: 950-960 [PMID: 24417818 DOI: 10.1053/j.gastro.2014.01.013]

13 **Rex DK**, Cutler CS, Lemmel GT, Rahmani EY, Clark DW, Helper DJ, Lehman GA, Mark DG. Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. *Gastroenterology* 1997; **112**: 24-28 [PMID: 8978338]

14 **Kaminski MF**, Regula J, Kraszewska E, Polkowski M, Wojciechowska U, Didkowska J, Zwierko M, Rupinski M, Nowacki MP, Butruk E. Quality indicators for colonoscopy and the risk of interval cancer. *N Engl J Med* 2010; **362**: 1795-1803 [PMID: 20463339 DOI: 10.1056/NEJMoa0907667]

15 **Rex DK**, Petrini JL, Baron TH, Chak A, Cohen J, Deal SE, Hoffman B, Jacobson BC, Mergener K, Petersen BT, Safdi MA, Faigel DO, Pike IM. Quality indicators for colonoscopy. *Am J Gastroenterol* 2006; **101**: 873-885 [PMID: 16635231 DOI: 10.1111/j.1572-0241.2006.00673.x]

16 **Anderson JC**, Butterly LF. Colonoscopy: quality indicators. *Clin Transl Gastroenterol* 2015; **6**: e77 [PMID: 25716302 DOI: 10.1038/ctg.2015.5]

17 **Leung FW**. PDR or ADR as a quality indicator for colonoscopy. *Am J Gastroenterol* 2013; **108**: 1000-1002 [PMID: 23735919 DOI: 10.1038/ajg.2013.99]

18 **Rex DK**, Kahi C, O'Brien M, Levin TR, Pohl H, Rastogi A, Burgart L, Imperiale T, Ladabaum U, Cohen J, Lieberman DA. The American Society for Gastrointestinal Endoscopy PIVI (Preservation and Incorporation of Valuable Endoscopic Innovations) on real-time endoscopic assessment of the histology of diminutive colorectal polyps. *Gastrointest Endosc* 2011; **73**: 419-422 [PMID: 21353837 DOI: 10.1016/j.gie.2011.01.023]

19 **Boroff ES**, Gurudu SR, Hentz JG, Leighton JA, Ramirez FC. Polyp and adenoma detection rates in the proximal and distal colon. *Am J Gastroenterol* 2013; **108**: 993-999 [PMID: 23567353 DOI: 10.1038/ajg.2013.68]

20 **van Doorn SC**, van Vliet J, Fockens P, Dekker E. A novel colonoscopy reporting system enabling quality assurance. *Endoscopy* 2014; **46**: 181-187 [PMID: 24500977 DOI: 10.1055/s-0034-1364877]

21 **National Health Service Bowel Cancer Screening Programme Endoscopy Quality Assurance Group**. Quality assurance guidelines for colonoscopy publication no. 6. National Health Service; 2011. Available from: URL: http://www.cancerscreening.nhs.uk/bowel/publications/nhsbcsp06.pdf

22 **Rex DK**, Ahnen DJ, Baron JA, Batts KP, Burke CA, Burt RW, Goldblum JR, Guillem JG, Kahi CJ, Kalady MF, O'Brien MJ, Odze RD, Ogino S, Parry S, Snover DC, Torlakovic EE, Wise PE, Young J, Church J. Serrated lesions of the colorectum: review and recommendations from an expert panel. *Am J Gastroenterol* 2012; **107**: 1315-1329; quiz 1314, 1330 [PMID: 22710576 DOI: 10.1038/ajg.2012.161]

23 **Martínez ME**, Sampliner R, Marshall JR, Bhattacharyya AK, Reid ME, Alberts DS. Adenoma characteristics as risk

factors for recurrence of advanced adenomas. *Gastroenterology* 2001; **120**: 1077-1083 [PMID: 11266371 DOI: 10.1053/gast.2001.0050101083]
24 **Saini SD**, Kim HM, Schoenfeld P. Incidence of advanced adenomas at surveillance colonoscopy in patients with a personal history of colon adenomas: a meta-analysis and systematic review. *Gastrointest Endosc* 2006; **64**: 614-626 [PMID: 16996358 DOI: 10.1016/j.gie.2006.06.057]
25 **Arain MA**, Sawhney M, Sheikh S, Anway R, Thyagarajan B, Bond JH, Shaukat A. CIMP status of interval colon cancers: another piece to the puzzle. *Am J Gastroenterol* 2010; **105**: 1189-1195 [PMID: 20010923 DOI: 10.1038/ajg.2009.699]
26 **Kahi CJ**, Li X, Eckert GJ, Rex DK. High colonoscopic prevalence of proximal colon serrated polyps in average-risk men and women. *Gastrointest Endosc* 2012; **75**: 515-520 [PMID: 22018551 DOI: 10.1016/j.gie.2011.08.021]
27 **Lieberman D**, Moravec M, Holub J, Michaels L, Eisen G. Polyp size and advanced histology in patients undergoing colonoscopy screening: implications for CT colonography. *Gastroenterology* 2008; **135**: 1100-1105 [PMID: 18691580 DOI: 10.1053/j.gastro.2008.06.083]
28 **Pickhardt PJ**. The natural history of colorectal polyps and masses: rediscovered truths from the barium enema era. *AJR Am J Roentgenol* 2007; **188**: 619-621 [PMID: 17312044 DOI: 10.2214/ajr.06.0731]
29 **Greenspan M**, Rajan KB, Baig A, Beck T, Mobarhan S, Melson J. Advanced adenoma detection rate is independent of nonadvanced adenoma detection rate. *Am J Gastroenterol* 2013; **108**: 1286-1292 [PMID: 23711625 DOI: 10.1038/ajg.2013.149]
30 **Lee SY**, Kim NH, Chae HB, Han KJ, Lee TH, Jang CM, Yoo KM, Jung YS, Park JH, Kim HJ, Cho YK, Sohn CI, Jeon WK, Kim BI, Park DI. [Correlation between adenoma detection rate and advanced adenoma detection rate]. *Korean J Gastroenterol* 2014; **64**: 18-23 [PMID: 25073667]

P- Reviewer: Sakata Y, Trevisani L **S- Editor**: Ma YJ **L- Editor**: A
E- Editor: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9387

World J Gastroenterol 2015 August 21; 21(31): 9387-9393
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Advantage of endoscopic mucosal resection with a cap for rectal neuroendocrine tumors

Su Bum Park, Hyung Wook Kim, Dae Hwan Kang, Cheol Woong Choi, Su Jin Kim, Hyeong Seok Nam

Su Bum Park, Hyung Wook Kim, Dae Hwan Kang, Cheol Woong Choi, Su Jin Kim, Hyeong Seok Nam, Department of Internal Medicine, Pusan National University School of Medicine and Research institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan-si, Gyeongsangnam-do 626-770, South Korea

Author contributions: Kim HW designed the study; Park SB, Kim HW and Choi CW carried out the procedures; Kim SJ helped data collection; Nam HS and Kang DH analyzed the data and performed the statistical analysis; Park SB wrote the paper; Kang DH and Kim HW revised the article; all authors approved the final version of the paper.

Institutional review board statement: The study was reviewed and approved by the Pusan National University Yangsan Hospital Institutional Review Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors have no conflicts of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at mdkhwook@gmail.com Participants gave informed consent for data sharing.

Correspondence to: Hyung Wook Kim, MD, PhD, Department of Internal Medicine, Pusan National University School of Medicine and Research institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Beomeo-ri, Mulgeum-eup, Yangsan-si, Gyeongsangnam-do 626-770, South Korea. mdkhwook@gmail.com
Telephone: +82-55-360-1535
Fax: +82-55-360-1536

Received: January 22, 2015
Peer-review started: January 23, 2015
First decision: March 10, 2015
Revised: March 30, 2015
Accepted: May 7, 2015
Article in press: May 7, 2015
Published online: August 21, 2015

Abstract

AIM: To compare the outcomes of endoscopic mucosal resection with a cap (EMR-C) with those of endoscopic submucosal dissection (ESD) for the resection of rectal neuroendocrine tumors.

METHODS: One hundred and sixteen lesions in 114 patients with rectal neuroendocrine tumor (NET) resected with EMR-C or ESD were included in the study. This study was performed at Pusan National University Yangsan Hospital between July 2009 and August 2014. We analyzed endoscopic complete resection rate, pathologic complete resection rate, procedure time, and adverse events in the EMR-C (n = 65) and ESD (n = 51) groups. We also performed a subgroup analysis by tumor size.

RESULTS: Mean tumor size was 4.62 ± 1.66 mm in the EMR-C group and 7.73 ± 3.14 mm in the ESD group (P < 0.001). Endoscopic complete resection rate was 100% in both groups. Histologic complete resection rate was significantly greater in the EMR-C group (92.3%) than in the ESD group (78.4%) (P = 0.042). Mean procedure time was significantly longer in the ESD group (14.43 ± 7.26 min) than in the

EMR-C group (3.83 ± 1.17 min) (P < 0.001). Rates of histologic complete resection without complication were similar for tumor diameter ⩽ 5 mm (EMR-C, 96%; ESD, 100%, P = 0.472) as well as in cases of 5 mm < tumor diameter ⩽ 10 mm (EMR-C, 80%; ESD, 71.0%, P = 0.524).

CONCLUSION: EMR-C may be simple, faster, and more effective than ESD in removing rectal NETs and may be preferable for resection of small rectal NETs.

Key words: Neuroendocrine tumor; Endoscopic mucosal resection with cap; Endoscopic submucosal dissection; Complete resection; Complication

Core tip: This study suggests that rates of endoscopic and histologic complete resection without adverse events were high in endoscopic mucosal resection with a cap (EMR-C) for treating rectal neuroendocrine tumors (NETs). EMR-C seems to be a safe, easy, and effective method for the resection of rectal NETs because it is technically easier and less time-consuming than endoscopic submucosal dissection.

Park SB, Kim HW, Kang DH, Choi CW, Kim SJ, Nam HS. Advantage of endoscopic mucosal resection with a cap for rectal neuroendocrine tumors. *World J Gastroenterol* 2015; 21(31): 9387-9393 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9387.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9387

INTRODUCTION

Rectal neuroendocrine tumor (NET) is a slow growing tumor that originates in the cells of the neuroendocrine system. Rectal NETs comprise 8% to 30% of all gastro-intestinal (GI) NETs[1]. The frequency of rectal NETs is higher in Asian countries compared to Western countries[2]. The incidence of rectal NETs has recently increased more in South Korea; this is related to the increasing number of screening colonoscopies, improved detection through endoscopic developments, and a deeper understanding of rectal NETs. Rectal NETs usually appear as sessile submucosal lesions covered with yellowish mucosa[3,4]. Clinical course of tumors less than 10 mm in diameter is relatively good. The risk of metastases has been reported to be 0%-10% for tumors < 10 mm and 4%-30%, for tumors 10-19 mm in diameter[5], and 57%-80% for tumors ⩾ 20 mm in diameter[6]. Standard treatment for rectal NETs < 10 mm is endoscopic resection, and the appropriate therapies for rectal NETs 10-19 mm in diameter are endoscopic resection, transanal resection [or transanal endoscopic microsurgery (TEM)], or radical rectal resection[7]. Endoscopic treatment is acceptable for small rectal NETs, but there is still controversy regarding which type of endoscopic resection is best. Numerous treatment strategies for rectal NETs have been reported. Conventional endoscopic mucosal resection (EMR) is simple, but there is some risk of incomplete resection. Endoscopic submucosal dissection (ESD) has several advantages over conventional EMR, including a higher *en bloc* resection rate, lower local recurrence rate, and more accurate pathological evaluation[8]. However, ESD requires great skill and experience and more time, and carries a risk of adverse events such as perforation and bleeding[9]. EMR with a cap (EMR-C) is an endoscopic procedure for cutting the submucosal layer by lifting the mucosa with saline injection, followed by aspirating the lesion into a transparent cap. EMR-C has advantages over both EMR and ESD. An advantage of EMR-C is that it is simpler and less time-consuming. Previous studies comparing EMR-C with ESD showed inconsistent results; Zhou *et al*[9] reported a complete-resection rate of only 52.5% for EMR-C, but Zhao *et al*[10] reported the rate to be 100%. This study compares the efficacy of EMR-C with that of ESD for the resection of rectal NETs.

MATERIALS AND METHODS

Between July 2009 and August 2014, EMR-C and ESD were performed for 116 lesions in 114 patients with rectal NETs at Pusan National University Yangsan Hospital. Sixty-five lesions were resected with EMR-C and 51 with ESD. We performed endoscopic ultrasonography (EUS) using a UM-DP20-25R, 20-MHz (Olympus Medical Systems Corp., Tokyo, Japan) to estimate the size and the depth of invasion of rectal NETs in all patients before resection. Tumors invading the muscularis propria layer were treated surgically, tumors measuring 10-19 mm on EUS were treated with ESD, and EMR-C and ESD were alternated, if possible, for tumors ⩽ 10 mm in diameter. The procedures were performed by three endoscopists (authors KHW, CCW, and PSB). Data were obtained retrospectively from a database. All resection specimens were measured and vertical and lateral resection margins evaluated. We analyzed the rate of endoscopic and histologic complete resection, procedure time, and adverse events. We also performed a subgroup analysis of EMR-C and ESD outcomes by tumor size. Tumors ⩽ 10 mm in diameter were divided into two groups, ⩽ 5 mm and 5-10 mm. Procedure time was defined as the time from submucosal injection to completion of resection, and adverse events were defined as bleeding (including immediate and delayed bleeding) and perforation (including microperforation and frank perforation).

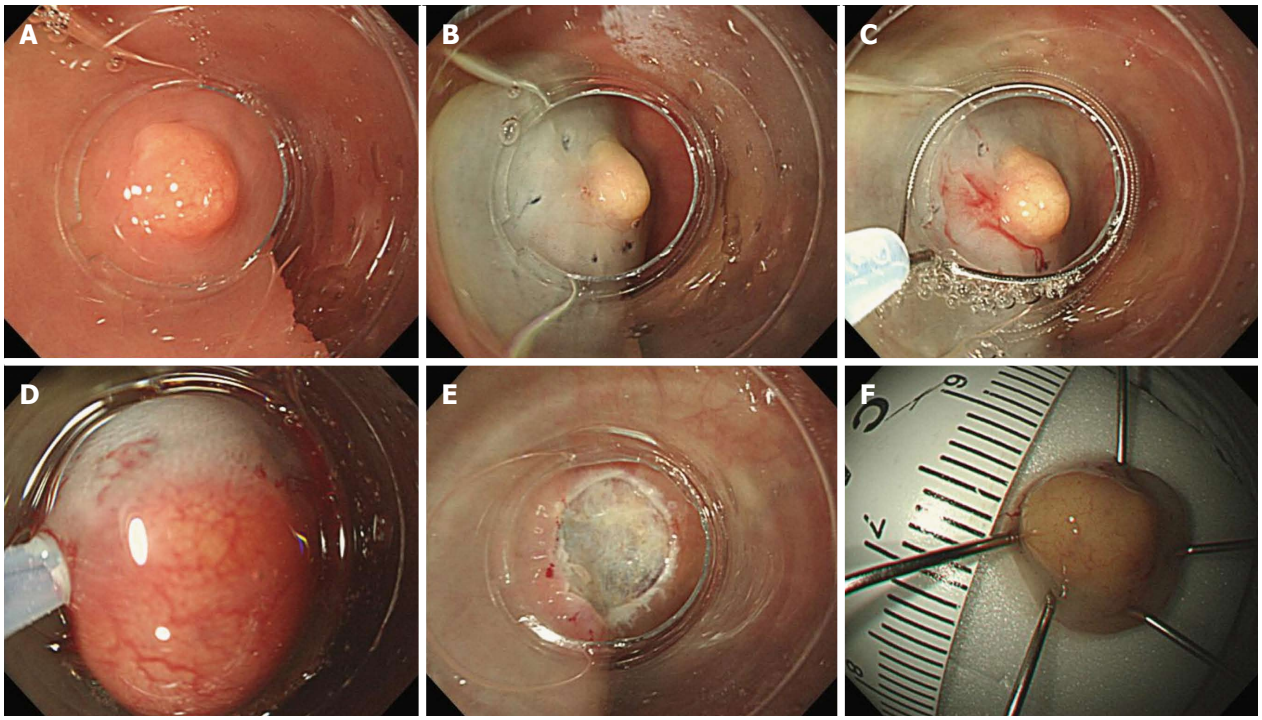


Figure 1 Endoscopic mucosal resection with a cap. A: Transparent cap is attached to the distal end of the scope; B: Saline solution with indigo-carmine solution is injected submucosally beneath the tumor; C: A crescent-shaped snare is positioned on the internal circumferential ridge; D: The submucosal layer is suctioned and dissected with the snare; E: A clear resection surface is observed; F: The resection specimen is retrieved and measured. EMR-C: Endoscopic mucosal resection with a cap.

Technique

EMR-C: We used a single-channel scope (GIF-H260, Olympus Medical Systems Corp.) with an oblique distal attachment (MAJ-290, Olympus Medical Systems Corp.) and a 25-mm single-use crescent electrosurgical snare (SD-221L-25, Olympus Medical Systems Corp.). First, submucosal injection was used to lift the tumor. After attaching the oblique cap to the distal end of the endoscope, the crescent-shaped snare was positioned on the internal circumferential ridge at the tip of the cap. The lesion was drawn into the cap using the suction function, then snared and resected using an electrosurgical unit (ERBE VIO 300 D, ERBE Elektromedizin GmbH, Tübingen, Germany) (Figure 1).

ESD: ESD was carried out with a single-channel scope (GIF-H260, Olympus Medical Systems Corp.) and an electrosurgical unit (ERBE VIO 300 D, ERBE Elektromedizin GmbH). After lifting the tumor with submucosal injection, a circumferential incision was made around the lesion and submucosal dissection carried out with a DualKnife Electrosurgical Knife (Olympus Medical Systems Corp.).

Histopathologic evaluation: Each resected rectal NET was measured. All specimens were fixed in 10% formalin and stained with hematoxylin-eosin. Depth of invasion and margin infiltration were evaluated. Histologic complete resection (R0) was defined as histopathologically tumor-free lateral and vertical margins. Indeterminate margin (Rx) was defined as margin that could not be evaluated for reasons such as electrocautery artifact and inappropriate orientation.

Statistical analysis

Statistical analysis was performed using PASW for Windows, Version 18.0 (SPSS Inc., Chicago, IL, United States). Quantitative results are expressed as medians and ranges. Statistical comparisons between the two groups were performed using the χ^2 statistic and Fisher's exact test. A P value < 0.05 was considered to be significant.

RESULTS

Of 116 lesions in 114 patients, 65 lesions were treated with EMR-C and 51 with ESD. The baseline characteristics and clinical outcomes of the EMR-C and ESD groups are shown in Tables 1 and 2, respectively.

Outcomes of EMR-C and ESD

As seen in Table 1, mean patient age did not differ significantly between the EMR-C and ESD groups (P = 0.063). The size of the resected specimen was significantly larger in the ESD group than in the EMR-C group (P < 0.001). The size of the tumor was also significantly larger in the ESD group (P < 0.001). Table 2 shows that resection time was significantly longer in the ESD group (P < 0.001). Endoscopic complete resection rates were 100% in both groups, and histologic complete resection rate was significantly higher in the EMR-C group than in the ESD group (P

Table 1 Baseline patient characteristics *n* (%)

	EMR-C (*n* = 65)	ESD (*n* = 51)	*P* value
Age (yr), mean ± SD	52.31 ± 9.83	48.47 ± 12.23	0.063
Male gender	43 (66.2)	33 (64.7)	0.872
Follow up period (d), mean ± SD	689.58 ± 468.94	760.84 ± 458.91	0.414
Specimen size (mm), mean ± SD (range)	10.15 ± 2.21 (6.0-15.0)	13.10 ± 3.99 (8.0-25.0)	< 0.001
Tumor size (mm), mean ± SD (range)	4.62 ± 1.66 (1.0-10.0)	7.73 ± 3.14 (3.0-18.0)	< 0.001
EUS measured size (mm), mean ± SD (range)	4.72 ± 1.51 (1.0-8.0)	7.27 ± 2.54 (2.7-17.0)	< 0.001
Tumor size (mm)			
0 < tumor size ≤ 5	50	13	
5 < tumor size ≤ 10	15	31	
> 10	0	7	

EMR-C: Endoscopic mucosal resection with a cap; ESD: Endoscopic submucosal dissection.

= 0.042). Vertical, lateral, and vertical and lateral resection margin involvement did not differ between groups (P = 0.864, P = 0.710, and P = 0.159, respectively; Table 2). Indeterminate margin resection rate did not differ between groups (P = 0.178). No local recurrence or distant metastasis occurred during the follow-up periods of cases of incomplete resection. Rates of adverse events are shown in Table 2. All bleeding was successfully controlled using coagulation. There were significantly more adverse events in the ESD group than in the EMR-C group (P = 0.044). Lymphovascular invasion occurred in one case in the ESD group.

Outcomes of EMR-C/ESD by tumor size

A subgroup analysis was performed for patients who had small rectal NETs (≤ 10 mm in diameter). Of 116 lesions, 109 were ≤ 10 mm in diameter. Tumors ≤ 10 mm in diameter were further divided into two groups: ≤ 5 mm and 5-10 mm in diameter.

Tumor size ≤ 5 mm

Table 3 presents the results of subgroup analysis of baseline characteristics for both groups by tumor size ≤ 5 mm. Mean diameters were similar between groups (P = 0.158), but resection time was significantly longer in the ESD group than in the EMR-C group (P < 0.001). Rates of endoscopic complete resection were 100% in both groups. Histologic complete resection rate did not differ significantly between groups (P = 0.472). Margin involvement for both groups is also given in Table 3. Adverse events occurred more frequently in the ESD group, but there was not a significant difference between groups [two cases of bleeding (15.4%) in the ESD group; no adverse events in the EMR-C group (P = 0.165)].

Table 2 Clinical outcomes by endoscopic treatment modality *n* (%)

	EMR-C (*n* = 65)	ESD (*n* = 51)	*P* value
Procedure time (min), mean ± SD,	3.83 ± 1.17	14.43 ± 7.26	< 0.001
Complication	0 (0.0)	4 (7.8)	0.044
Bleeding	0	4 (7.8)	
Perforation	0	0	
Endoscopic complete resection	65/65 (100)	51/51 (100)	
Histologic complete resection	60/65 (92.3)	40/51 (78.4)	0.042
Vertical margin involvement	1 (1.5)	1 (2.0)	0.864
Lateral margin involvement	1 (1.5)	2 (3.9)	0.710
Vertical and Lateral margin involvement	0 (0.0)	2 (3.9)	0.159
Indeterminate margin	3 (4.6)	6 (11.8)	0.178
Vertical:Lateral:Vertical and Lateral, *n*	2:1:0	1:3:2	
Lymphovascular invasion	0	1	0.322

EMR-C: Endoscopic mucosal resection with a cap; ESD: Endoscopic submucosal dissection.

Tumor sizes > 5 mm and ≤ 10 mm

Results of subgroup analysis of clinical outcomes for both groups by tumor size > 5 mm and ≤ 10 mm are shown in Table 4. Patients in the EMR-C group were significantly older than those in the ESD group (P = 0.011). Although the size of the resected specimen was significantly larger in the ESD group (P = 0.004), tumor size was similar between groups (P = 0.051). Resection time was significantly longer in the ESD group (P < 0.001), and rates of endoscopic complete resection were 100% in both groups. There was not a significant difference in histologic complete resection rate between groups (P = 0.524). Margin involvement for both groups for this range of tumor sizes is shown in Table 4. Vertical margin involvement was 0% for both groups. Between-group differences in lateral, vertical and lateral, and indeterminate margin involvement were not significant (P = 0.979, P = 0.161 and P = 0.810, respectively; Table 4). Adverse events occurred more frequently in the ESD group, but there was not a significant difference between groups [two cases of bleeding (6.5%) in the ESD group; no adverse events in the EMR-C group (P = 0.161)].

DISCUSSION

The incidence of rectal NETs is increasing with the number of screening colonoscopies, advances in endoscope technology, and deeper understanding of rectal NETs. Rectal NETs commonly appear as submucosal lesions with yellowish mucosa[3,4]. There is still controversy about the treatment of rectal NETs, and numerous treatment strategies have been reported[7]. The standard treatment for rectal

Table 3 Baseline characteristics by tumor size *n* (%)

NET size	Size ≤ 5 mm (*n* = 63)			5 mm < size ≤ 10 mm (*n* = 46)		
	EMR-C (*n* = 50)	ESD (*n* = 13)	*P* value	EMR-C (*n* = 15)	ESD (*n* = 31)	*P* value
Age (yr), mean ± SD	50.78 ± 9.45	45.85 ± 15.46	0.151	57.4 ± 9.63	48.48 ± 11.04	0.011
Male gender	33 (66.0)	7 (53.8)	0.417	10 (66.7)	21 (67.7)	0.942
Follow up period (d), mean ± SD	714.1 ± 489.29	806.31 ± 604.66	0.567	607.87 ± 397.56	710.9 ± 403.00	0.419
Specimen size (mm), mean ± SD	10.05 ± 2.21	10.83 ± 2.78	0.287	10.47 ± 2.26	13.14 ± 3.68	0.004
Tumor size (mm), mean ± SD	3.9 ± 0.95	4.31 ± 0.75	0.158	7.00 ± 1.25	7.87 ± 1.43	0.051
EUS measured size (mm), mean ± SD	4.44 ± 1.31	5.77 ± 2.01	0.005	5.80 ± 1.47	7.03 ± 5.80	0.019

EMR-C: Endoscopic mucosal resection with a cap; ESD: Endoscopic submucosal dissection; EUS: Endoscopic ultrasound; NET: Neuroendocrine tumor.

Table 4 Clinical outcomes by tumor size *n* (%)

	size ≤ 5 mm (*n* = 63)			5 mm < size ≤ 10 mm (*n* = 46)		
	EMR-C (*n* = 50)	ESD (*n* = 13)	*P* value	EMR-C (*n* = 15)	ESD (*n* = 31)	*P* value
Procedure time (min), mean ± SD	3.94 ± 1.27	12.52 ± 3.42	< 0.001	3.45 ± 0.60	14.96 ± 8.85	< 0.001
Complication	0	2 (15.4)	0.165	0	2 (6.5)	0.161
Bleeding	0	2 (15.4)		0	2 (6.5)	
Perforation	0	0		0	0	
Endoscopic complete resection	50 (100)	13 (100)		15 (100)	31 (100)	
Histologic complete resection	48 (96.0)	13 (100)	0.472	12 (80.0)	22 (71.0)	0.524
Vertical margin involvement	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Lateral margin involvement	0 (0.0)	0 (0.0)		1 (6.7)	2 (6.5)	0.979
Vertical and Lateral margin involvement	1 (2.0)	0 (0.0)	0.614	0	2 (6.5)	0.161
Indeterminate margin	1 (2.0)	0 (0.0)	0.614	2 (13.3)	5 (16.1)	0.810
Vertical:lateral: Vertical and Lateral	1:0:0	0:0:0		1:1:0	1:2:2	

EMR-C: Endoscopic mucosal resection with a cap; ESD: Endoscopic submucosal dissection.

NETs < 10 mm in diameter is endoscopic resection; appropriate therapies for lesions 10-19 mm in diameter are endoscopic local resection, transanal resection, or radical rectal resection[7]. The European Neuroendocrine Tumors Society has published consensus guidelines for the management of patients with colorectal neuroendocrine neoplasms[11]. EUS can be used to evaluate tumor size and depth of invasion, and its accuracy in determination of depth of invasion is high (91%-100%)[12-14].

EMR has been widely used because of its simplicity and low rate of adverse events[15-17]. However, rectal NETs are located in the deep mucosal layer toward the submucosal layer, so conventional EMR can result in incomplete resection or local recurrence after EMR[18,19]. Compared with conventional EMR, the advantage of EMR-C is its low rate of positive tumor resection margin[20,21]. ESD has advantages of higher *en bloc* resection and more accurate pathologic evaluation, but ESD requires great skill and experience and longer time, and carries a risk of adverse events such as perforation and bleeding[9]. Compared with ESD, the advantage of EMR-C is that it is simpler and less time-consuming. EMR-C has several advantages over both EMR and ESD. The tumor is elevated into the cap using suction, and the procedure can be safely and easily performed in a short time and does not require special endoscopic skill. This study demonstrated that EMR-C was a simple and less time consuming endoscopic procedure than ESD.

Previous studies of EMR-C reported complete resection rates from 52.5% to 100%, but those studies contained small numbers of patients[9,10,20-25]. In our study, EMR-C was performed on 65 lesions, with a complete resection rate of 92.3%. Interestingly, the histologic complete resection rate was significantly higher in the EMR-C group (92.3%) than in the ESD group (78.4%) (P = 0.042). The rate of indeterminate margin was higher in the ESD group (six cases, 11.8%) than in the EMR-C group (three cases, 4.6%). Positive resection margins do not necessarily indicate remnant tumor; rather, they may indicate cautery damage during endoscopic resection[12,26]. The circular resected margin of a tumor obtained using EMR-C can remain undamaged, but during ESD, the use of electrocautery during incision and dissection may damage the tumor margins and explains the high rate of pathologic incomplete resection in the ESD group. More important is the endoscopic complete resection rate. We followed up patients every six months for

two years, and then yearly thereafter. We checked general condition, computed tomography scans and colonoscopy (or sigmoidoscopy) results. There was no tumor recurrence in either group, including in patients who had positive or indeterminate margins, during a mean follow-up period of 720.91 d.

Lymphovascular invasion was found in one patient with a 6-mm tumor treated with ESD. Although metastatic involvement of tumors < 10 mm occurs in about 0%-10% of cases[1,5,6,26,27], endoscopic treatment is acceptable for small rectal NETs. Konishi *et al*[28] reported an incidence of lymph node metastasis as high as 7% in rectal NETs ≤ 10 mm treated with radical resection. Our study demonstrated a rate of metastasis of 0.92% (one of 109 patients). This patient with lymphovascular invasion required additional surgical therapy.

In cases of tumors ≤ 5 mm in size, endoscopic and histologic complete resections were 100% and 96.0%, respectively, for the EMR-C group, and there were no adverse events. In cases of 5 < tumor diameter ≤ 10 mm, endoscopic and histologic complete resections were 100% and 80.0%, respectively, for the EMR-C group, without adverse events. These results are similar to those of ESD, suggesting that EMR-C is not inferior to ESD for resecting small NETs. There is a theoretical possibility that perforation due to insufficient submucosal injection or too much suction could cause adverse events in EMR-C. However, because appropriate suction was used in our study, no perforation occurred.

This study has some limitations. Firstly, this is a retrospective, single-center study. Secondly, the study was not randomized. EUS was performed before resection, so the procedure was performed according to the endoscopist's preference. Large prospective, randomized, controlled studies will be necessary to validate our results.

In conclusion, EMR-C may be feasible for the resection of rectal NETs because it is technically easier and less time-consuming than ESD. This study also suggests that rates of endoscopic and histologic complete resection without adverse events were high in EMR-C for treating rectal NETs.

COMMENTS

Background

Endoscopic mucosal resection with a cap (EMR-C) and endoscopic submucosal dissection (ESD) have been used to treat neuroendocrine tumors (NETs) for decades and the reported results have been variable. Until now, there has been no consensus about which modality is superior. The advantages of ESD include more accurate pathological evaluation, but the authors experienced some indeterminate margin pathologic results after ESD. So, they compared the efficacy of EMR-C with that of ESD for the resection of rectal NETs.

Research frontiers

Many previous studies have evaluated the outcomes of endoscopic procedures for rectal NETs. The authors evaluated a somewhat larger number of patients undergoing EMR-C and performed a subgroup analysis by tumor size.

Innovations and breakthroughs

Endoscopic complete resection rate was 100% in both groups. Histologic complete resection rate was significantly greater in the EMR-C group than in the ESD group. The study demonstrates the rate of histologic complete resection of EMR-C to be superior to that of ESD because the circumferential tumor-resection margin treated in EMR-C will be undamaged.

Applications

EMR-C is feasible for the resection of rectal NETs because it is technically easier and less time-consuming than ESD. These results will provide important information to select a treatment strategy for patients with small rectal NETs.

Terminology

EMR-C is an endoscopic procedure for cutting the submucosal layer by lifting the mucosa with saline injection, followed by aspirating the lesion into a transparent cap.

Peer-review

The authors concluded that EMR-C may be simple, faster, and more effective than ESD in removing rectal NETs and may be preferable for resection of small rectal NETs. The paper is well written and brings forward some new information. The experience the authors shared in this manuscript is beneficial for further study in this field.

REFERENCES

1 **Gleeson FC**, Levy MJ, Dozois EJ, Larson DW, Wong Kee Song LM, Boardman LA. Endoscopically identified well-differentiated rectal carcinoid tumors: impact of tumor size on the natural history and outcomes. *Gastrointest Endosc* 2014; **80**: 144-151 [PMID: 24462168 DOI: 10.1016/j.gie.2013.11.031]

2 **Choi HH**, Kim JS, Cheung DY, Cho YS. Which endoscopic treatment is the best for small rectal carcinoid tumors? *World J Gastrointest Endosc* 2013; **5**: 487-494 [PMID: 24147192 DOI: 10.4253/wjge.v5.i10.487]

3 **Jetmore AB**, Ray JE, Gathright JB, McMullen KM, Hicks TC, Timmcke AE. Rectal carcinoids: the most frequent carcinoid tumor. *Dis Colon Rectum* 1992; **35**: 717-725 [PMID: 1643994 DOI: 10.1007/BF02050318]

4 **Matsui K**, Iwase T, Kitagawa M. Small, polypoid-appearing carcinoid tumors of the rectum: clinicopathologic study of 16 cases and effectiveness of endoscopic treatment. *Am J Gastroenterol* 1993; **88**: 1949-1953 [PMID: 8237948]

5 **Fahy BN**, Tang LH, Klimstra D, Wong WD, Guillem JG, Paty PB, Temple LK, Shia J, Weiser MR. Carcinoid of the rectum risk stratification (CaRRs): a strategy for preoperative outcome assessment. *Ann Surg Oncol* 2007; **14**: 1735-1743 [PMID: 17294074 DOI: 10.1245/s10434-006-9311-6]

6 **Soga J**. Early-stage carcinoids of the gastrointestinal tract: an analysis of 1914 reported cases. *Cancer* 2005; **103**: 1587-1595 [PMID: 15742328 DOI: 10.1002/cncr.20939]

7 **de Mestier L**, Brixi H, Gincul R, Ponchon T, Cadiot G. Updating the management of patients with rectal neuroendocrine tumors. *Endoscopy* 2013; **45**: 1039-1046 [PMID: 24163193 DOI: 10.1055/s-0033-1344794]

8 **Lee DS**, Jeon SW, Park SY, Jung MK, Cho CM, Tak WY, Kweon YO, Kim SK. The feasibility of endoscopic submucosal dissection for rectal carcinoid tumors: comparison with endoscopic mucosal resection. *Endoscopy* 2010; **42**: 647-651 [PMID: 20669076 DOI: 10.1055/s-0030-1255591]

9 **Zhou PH**, Yao LQ, Qin XY, Xu MD, Zhong YS, Chen WF, Ma LL, Zhang YQ, Qin WZ, Cai MY, Ji Y. Advantages of endoscopic submucosal dissection with needle-knife over endoscopic mucosal resection for small rectal carcinoid tumors: a retrospective study. *Surg Endosc* 2010; **24**: 2607-2612 [PMID: 20361212 DOI: 10.1007/s00464-010-1016-z]

10 **Zhao ZF**, Zhang N, Ma SR, Yang Z, Han X, Zhao YF, Gao F, Gong ZJ, Yang L. A comparative study on endoscopy treatment in rectal

carcinoid tumors. *Surg Laparosc Endosc Percutan Tech* 2012; **22**: 260-263 [PMID: 22678324 DOI: 10.1097/SLE.0b013e3182512e0f]
11 **Caplin M**, Sundin A, Nillson O, Baum RP, Klose KJ, Kelestimur F, Plöckinger U, Papotti M, Salazar R, Pascher A; Barcelona Consensus Conference participants. ENETS Consensus Guidelines for the management of patients with digestive neuroendocrine neoplasms: colorectal neuroendocrine neoplasms. *Neuroendocrinology* 2012; **95**: 88-97 [PMID: 22261972 DOI: 10.1159/000335594]
12 **Park CH**, Cheon JH, Kim JO, Shin JE, Jang BI, Shin SJ, Jeen YT, Lee SH, Ji JS, Han DS, Jung SA, Park DI, Baek IH, Kim SH, Chang DK. Criteria for decision making after endoscopic resection of well-differentiated rectal carcinoids with regard to potential lymphatic spread. *Endoscopy* 2011; **43**: 790-795 [PMID: 21735371 DOI: 10.1055/s-0030-1256414]
13 **Ishii N**, Horiki N, Itoh T, Maruyama M, Matsuda M, Setoyama T, Suzuki S, Uchida S, Uemura M, Iizuka Y, Fukuda K, Suzuki K, Fujita Y. Endoscopic submucosal dissection and preoperative assessment with endoscopic ultrasonography for the treatment of rectal carcinoid tumors. *Surg Endosc* 2010; **24**: 1413-1419 [PMID: 20033710 DOI: 10.1007/s00464-009-0791-x]
14 **Abe T**, Kakemura T, Fujinuma S, Maetani I. Successful outcomes of EMR-L with 3D-EUS for rectal carcinoids compared with historical controls. *World J Gastroenterol* 2008; **14**: 4054-4058 [PMID: 18609690 DOI: 10.3748/wjg.14.4054]
15 **Puli SR**, Kakugawa Y, Gotoda T, Antillon D, Saito Y, Antillon MR. Meta-analysis and systematic review of colorectal endoscopic mucosal resection. *World J Gastroenterol* 2009; **15**: 4273-4277 [PMID: 19750569 DOI: 10.3748/wjg.15.4273]
16 **Ichikawa J**, Tanabe S, Koizumi W, Kida Y, Imaizumi H, Kida M, Saigenji K, Mitomi H. Endoscopic mucosal resection in the management of gastric carcinoid tumors. *Endoscopy* 2003; **35**: 203-206 [PMID: 12584637 DOI: 10.1055/s-2003-37256]
17 **Kiesslich R**, Neurath MF. Endoscopic mucosal resection: an evolving therapeutic strategy for non-polypoid colorectal neoplasia. *Gut* 2004; **53**: 1222-1224 [PMID: 15306573 DOI: 10.1136/gut.2004.043281]
18 **Oda I**, Saito D, Tada M, Iishi H, Tanabe S, Oyama T, Doi T, Otani Y, Fujisaki J, Ajioka Y, Hamada T, Inoue H, Gotoda T, Yoshida S. A multicenter retrospective study of endoscopic resection for early gastric cancer. *Gastric Cancer* 2006; **9**: 262-270 [PMID: 17235627 DOI: 10.1007/s10120-006-0389-0]
19 **Oka S**, Tanaka S, Kaneko I, Mouri R, Hirata M, Kawamura T, Yoshihara M, Chayama K. Advantage of endoscopic submucosal dissection compared with EMR for early gastric cancer. *Gastrointest Endosc* 2006; **64**: 877-883 [PMID: 17140890 DOI: 10.1016/j.gie.2006.03.932]
20 **Oshitani N**, Hamasaki N, Sawa Y, Hara J, Nakamura S, Matsumoto T, Kitano A, Arakawa T. Endoscopic resection of small rectal carcinoid tumours using an aspiration method with a transparent overcap. *J Int Med Res* 2000; **28**: 241-246 [PMID: 11092235 DOI: 10.1177/147323000002800507]
21 **Kim YJ**, Lee SK, Cheon JH, Kim TI, Lee YC, Kim WH, Chung JB, Yi SW, Park S. [Efficacy of endoscopic resection for small rectal carcinoid: a retrospective study]. *Korean J Gastroenterol* 2008; **51**: 174-180 [PMID: 18451691]
22 **Sohn DK**, Han KS, Hong CW, Chang HJ, Jeong SY, Park JG. Selection of cap size in endoscopic submucosal resection with cap aspiration for rectal carcinoid tumors. *J Laparoendosc Adv Surg Tech A* 2008; **18**: 815-818 [PMID: 19105668 DOI: 10.1089/lap.2008.0210]
23 **Celebi Kobak A**, Zeybel M, Ayhan S, Kara E, Ellidokuz E. Endoscopic submucosal resection of a rectal carcinoid tumor by cap aspiration - snare resection method. *Turk J Gastroenterol* 2006; **17**: 313-315 [PMID: 17205414]
24 **Imada-Shirakata Y**, Sakai M, Kajiyama T, Kin G, Inoue K, Torii A, Kishimoto H, Ueda S, Okuma M. Endoscopic resection of rectal carcinoid tumors using aspiration lumpectomy. *Endoscopy* 1997; **29**: 34-38 [PMID: 9083735 DOI: 10.1055/s-2007-1024058]
25 **Nagai T**, Torishima R, Nakashima H, Ookawara H, Uchida A, Kai S, Sato R, Murakami K, Fujioka T. Saline-assisted endoscopic resection of rectal carcinoids: cap aspiration method versus simple snare resection. *Endoscopy* 2004; **36**: 202-205 [PMID: 14986216]
26 **Kwaan MR**, Goldberg JE, Bleday R. Rectal carcinoid tumors: review of results after endoscopic and surgical therapy. *Arch Surg* 2008; **143**: 471-475 [PMID: 18490556 DOI: 10.1001/archsurg.143.5.471]
27 **Modlin IM**, Oberg K, Chung DC, Jensen RT, de Herder WW, Thakker RV, Caplin M, Delle Fave G, Kaltsas GA, Krenning EP, Moss SF, Nilsson O, Rindi G, Salazar R, Ruszniewski P, Sundin A. Gastroenteropancreatic neuroendocrine tumours. *Lancet Oncol* 2008; **9**: 61-72 [PMID: 18177818 DOI: 10.1016/S1470-2045(07)70410-2]
28 **Konishi T**, Watanabe T, Kishimoto J, Kotake K, Muto T, Nagawa H; Japanese Society for Cancer of the Colon and Rectum. Prognosis and risk factors of metastasis in colorectal carcinoids: results of a nationwide registry over 15 years. *Gut* 2007; **56**: 863-868 [PMID: 17213340]

P- Reviewer: Buanes TA, Guo XZ, Lorenzo-Zuniga V, Paoluzi OA, Tomizawa M
S- Editor: Ma YJ **L- Editor**: Logan S **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9394

World J Gastroenterol 2015 August 21; 21(31): 9394-9402
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Safety validation of decision trees for hepatocellular carcinoma

Xian-Qiang Wang, Zhe Liu, Wen-Ping Lv, Ying Luo, Guang-Yun Yang, Chong-Hui Li, Xiang-Fei Meng, Yang Liu, Ke-Sen Xu, Jia-Hong Dong

Xian-Qiang Wang, Ke-Sen Xu, Jia-Hong Dong, Department of Hepatobiliary Surgery, Qilu Hospital, Shandong University, Jinan 250012, Shandong Province, China

Zhe Liu, Wen-Ping Lv, Ying Luo, Guang-Yun Yang, Chong-Hui Li, Xiang-Fei Meng, Yang Liu, Jia-Hong Dong, Department of Hepatobiliary Surgery, PLA General Hospital, Beijing 100853, China

Author contributions: Dong JH, Lv WP and Xu KS designed the research; Wang XQ and Liu Z performed the research; Li CH and Yang GY contributed new reagents and analytic tools; Liu Y and Meng XF analyzed the data; and Wang XQ and Luo Y wrote the paper.

Supported by Grants from the Project of the National Science and Technology Major Project, No. 2012BAI06B01; and Postdoctoral Science Foundation funded project, No. 201003781.

Institutional review board statement: The study was reviewed and approved by the PLA General Hospital Institutional Review Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors declare no conflicting interest.

Data sharing statement: No additional data are available.

Correspondence to: Jia-Hong Dong, MD, PhD, FACS, Department of Hepatobiliary Surgery, PLA General Hospital, No. 28 Fuxing Road, Beijing 100853, China. dongjh_301@163.com
Telephone: +86-10-66938030
Fax: +86-10-66938030

Received: November 2, 2014
Peer-review started: November 2, 2014
First decision: December 2, 2014
Revised: March 11, 2015
Accepted: April 17, 2015
Article in press: April 17, 2015
Published online: August 21, 2015

Abstract

AIM: To evaluate a different decision tree for safe liver resection and verify its efficiency.

METHODS: A total of 2457 patients underwent hepatic resection between January 2004 and December 2010 at the Chinese PLA General Hospital, and 634 hepatocellular carcinoma (HCC) patients were eligible for the final analyses. Post-hepatectomy liver failure (PHLF) was identified by the association of prothrombin time < 50% and serum bilirubin > 50 μmol/L (the "50-50" criteria), which were assessed at day 5 postoperatively or later. The Swiss-Clavien decision tree, Tokyo University-Makuuchi decision tree, and Chinese consensus decision tree were adopted to divide patients into two groups based on those decision trees in sequence, and the PHLF rates were recorded.

RESULTS: The overall mortality and PHLF rate were 0.16% and 3.0%. A total of 19 patients experienced PHLF. The numbers of patients to whom the Swiss-Clavien, Tokyo University-Makuuchi, and Chinese consensus decision trees were applied were 581, 573, and 622, and the PHLF rates were 2.75%, 2.62%, and 2.73%, respectively. Significantly more cases satisfied the Chinese consensus decision tree than the Swiss-

Clavien decision tree and Tokyo University-Makuuchi decision tree ($P < 0.01$, $P < 0.01$); nevertheless, the latter two shared no difference ($P = 0.147$). The PHLF rate exhibited no significant difference with respect to the three decision trees.

CONCLUSION: The Chinese consensus decision tree expands the indications for hepatic resection for HCC patients and does not increase the PHLF rate compared to the Swiss-Clavien and Tokyo University-Makuuchi decision trees. It would be a safe and effective algorithm for hepatectomy in patients with hepatocellular carcinoma.

Key words: Hepatectomy; Liver failure; Decision tree

Core tip: We have established a decision tree for safe hepatectomy based on four variables: normal or cirrhotic liver, Child-Turcotte-Pugh score, the indocyanine green retention rate at 15 min, and the ratio of reserved functional liver volume to standard liver volume. Post-hepatectomy liver failure (PHLF) has been identified by the "50-50" criteria. The Chinese consensus decision tree expands the indications for hepatic resection for liver tumor and does not increase the PHLF rate compared to the Swiss-Clavien and Tokyo University-Makuuchi decision trees. The Chinese consensus decision tree would be a safe and effective algorithm for hepatectomy in patients with liver tumor.

Wang XQ, Liu Z, Lv WP, Luo Y, Yang GY, Li CH, Meng XF, Liu Y, Xu KS, Dong JH. Safety validation of decision trees for hepatocellular carcinoma. *World J Gastroenterol* 2015; 21(31): 9394-9402 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9394.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9394

INTRODUCTION

Hepatectomy and liver transplantation are two curative treatments for liver tumors. Liver transplantation donations are far fewer than the demand in China[1,2]. Hepatectomy is the preferred approach to treating malignant liver cancer. It is a complicated surgery accompanied by high morbidity and mortality, especially for patients with cirrhosis undergoing major hepatectomy. With improvements in technique and peri-operative management, the mortality and complication rates have significantly decreased. Recent studies indicate that the hospital mortality rate is lower than 1%, even approaching zero mortality[3-8].

However, post-hepatectomy complications, especially hepatic failure, are still dreadful complications for the surgeon. The incidence rate of hepatic failure is approximately 0%-32% and accounts for 50%-75% of post-hepatectomy mortality. As the mortality rate after hepatectomy significantly decreases, it becomes an important indicator for evaluating the effect of hepatectomy. Based on the actual situations of patients, certain high-volume centers have created complete and effective management strategies and decision trees for safe hepatectomy. Professor Makuuchi proposed the University of Tokyo standard for hepatectomy[9], and Professor Clavien established the Swiss criteria for safe liver resection.

Advances in liver surgery have reduced blood loss and have led to a decline in morbidity and mortality. However, the mortality rate in extended liver resection, the rate of curative resection for liver malignancies, and postoperative long-term survival remain far from satisfactory. Therefore, we hope the concept of precision will help propel liver surgery into a brand new era. Precise liver surgery has become an important direction of development[10,11].

Due to limitations based on the characteristics of the local population and the available background information, there is no single decision tree for hepatectomy based on the Chinese population. Thus, based on past experience and data, we propose a Chinese decision tree system for hepatectomy.

This study aims to compare the incidence rate of postoperative hepatic failure according to the aforementioned three decision-making systems for hepatectomy with respect to the patients with hepatocellular carcinoma in a single center to identify the best decision tree for safe hepatectomies.

MATERIALS AND METHODS

From January of 2004 to December of 2010, we selected 2457 cases of hepatectomy with indocyanine green (ICG) records at the People's Liberation Army General Hospital. The inclusion criteria were: (1) complete biochemical data during the perioperative period; (2) availability of preoperative enhanced helical computed tomography (CT) or enhanced magnetic resonance imaging (MRI) data (.dcm format); (3) pathologically confirmed hepatocellular carcinoma; and (4) initial hepatectomy. The exclusion criteria were: (1) INR-negative postoperative blood test; (2) concurrent additional pathological diagnosis; (3) no raw image data (digital format); (4) preoperative bilirubin > 50 μmol/L (2.9 mg/dL); and (5) perioperative period bleeding > 1500 mL. Altogether, 634 cases of hepatectomy were incorporated into this study.

We adopted a standardized procedure for the preoperative patient evaluation and hepatectomy. All patients underwent complete questioning about their medical history and a physical examination. For patients over 65 years old and patients with complications and other surgical risks, we performed a complete evaluation of heart and lung function. Before the surgery, we conducted a complete imaging examination. We reviewed the cases and imaging data

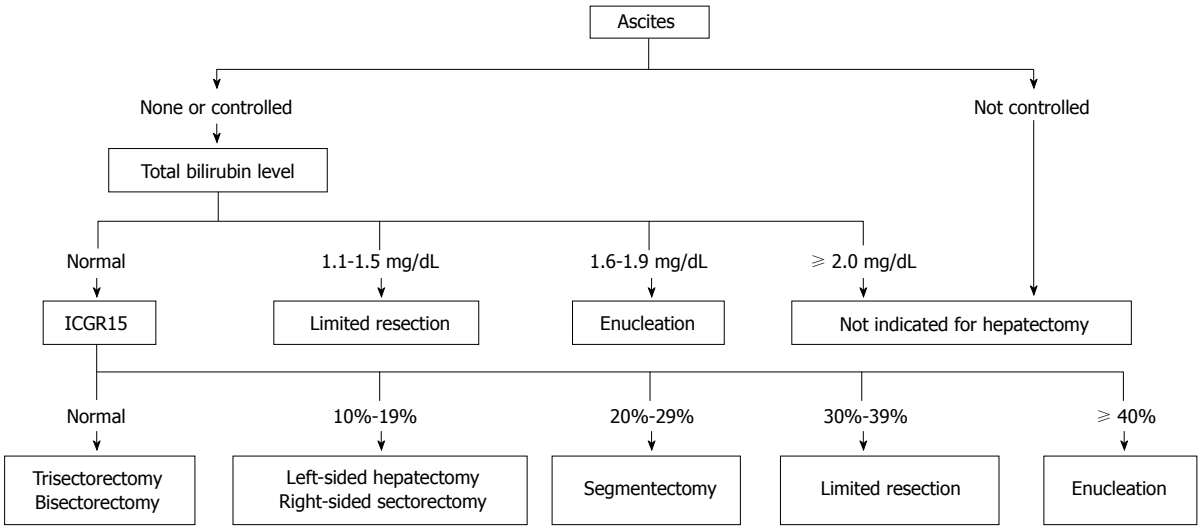


Figure 1 Makuuchi decision tree for a safe hepatectomy.

and found the necessary content, and we re-inspected cases with missing data. Most cases were treated by the surgical approach of anatomical hepatectomy. For cases that required hepatic portal occlusion, we usually adopted the Pringle approach. Since 2008, some cases involved hepatectomy performed under the simple condition of portal vein occlusion. During the hepatectomy, we adopted a low central venous pressure (< 5 mmHg) and the Trendelenburg position, and a conventional abdominal drainage tube was placed.

When reviewing the cases, the examined preoperative factors included gender, age, preoperative comorbidities, esophageal and gastric varices, and routine preoperative examinations of biochemical meridians, blood, and blood clotting. We recorded the 15-min retention rate (ICGR15) of the indocyanine green excretion test, the Child-Turcotte-Pugh score, and the MELD score. The surgical factors included the recorded surgical time, the type of surgery, tumor size, the amount of bleeding, blood transfusion status, and the intra-operative occlusion method and time.

We recorded the postoperative incidence of hepatic failure, postoperative complication, and mortality. Postoperative hepatic failure was defined based on the 50-50 criteria; that is, four days after the surgery, the total bilirubin was > 50 μmol/L (2.9 mg/dL), and the prothrombin ratio was < 50% (INR > 1.7)[12]. Postoperative mortality was defined as mortality during surgery and the hospital stay. Postoperative ascites was diagnosed for patients who, after surgery, experienced more than 2 L of drainage for three consecutive days, needed a puncture drain again after the peritoneal drainage tube was removed, or had a postoperative hospital duration of more than 30 d because of persistent ascites. Intra-abdominal infection was defined as a positive bacteria culture from the drainage. Postoperative bile leakage was defined as a bilirubin level over 85.5 μmol/L in the postoperative drainage for seven consecutive days (5.0 mg/dL)[13].

Calculation of the relevant liver volume with three-dimensional reconstruction

The liver volume was measured using three-dimensional reconstruction software (EDDA Company, United States) to reconstruct three-dimensional liver images based on enhanced thin CT or MRI scanning. Then, the volume of each liver segment was calculated based on the voxel principle, and the segmentation and surgical planning for the scope of surgery was completed. The error was 5%-8%[14-16].

The standardized liver volume is relatively stable in adults in a physiological state, and its size depends on the human's body surface area (BSA), which depends on the height and weight of the human body. The ideal liver volume for the normal human body has sufficient reserve function and compensatory potential in the healthy state.

BSA is calculated according to the DuBois formula: BSA (m^2) = weight (kg) 0.425 × height (cm) 0.725 × 0.007184.

We adopted the adult standard liver volume formula established by Urata *et al*[17] from the University of Tokyo in Japan: standard liver volume (SLV) (m^2) = 706.2 × BSA (m^2) + 2.4.

Whole liver volume = reserved liver volume + prehepatic resection liver volume:

Standardized residual liver volume ratio = reserved liver volume/standard liver volume × 100%.

Decision trees for hepatectomy

In this study, we compared the Makuuchi decision tree (Figure 1), the Clavien decision tree (Figures 2 and 3), and the Chinese consensus decision tree (Figure 4), which are grouped. For each group, we compared the indication for hepatectomy and the incidence rate of hepatic failure. Professor Makuuchi from Japan replaced the Child-Pugh score with two parameters: whether ascites can be controlled and the total plasma bilirubin level, which is used as an index for assessing the hepatic functional reserve[9]. We adopted the

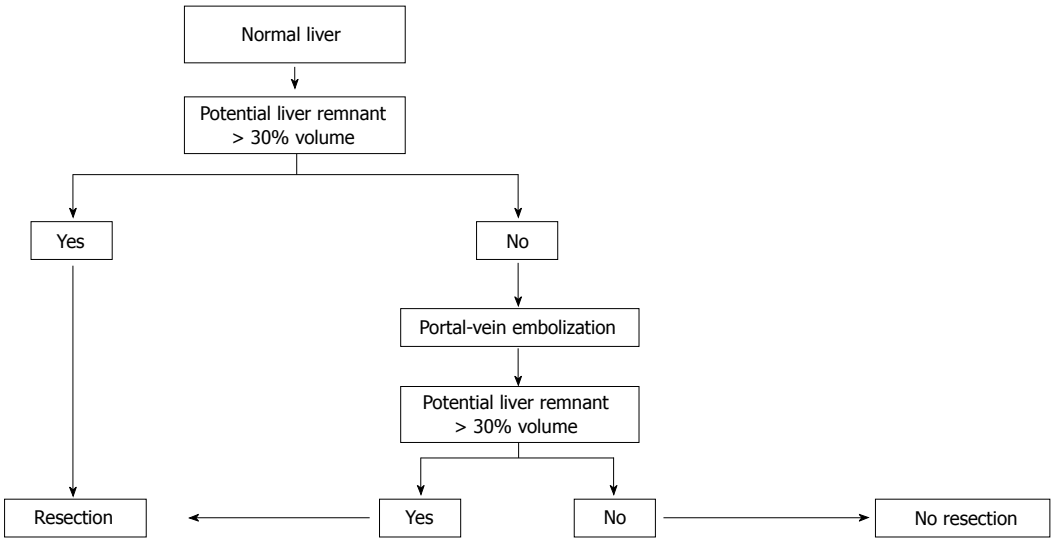


Figure 2 Clavien decision tree for safe hepatectomy in a normal liver.

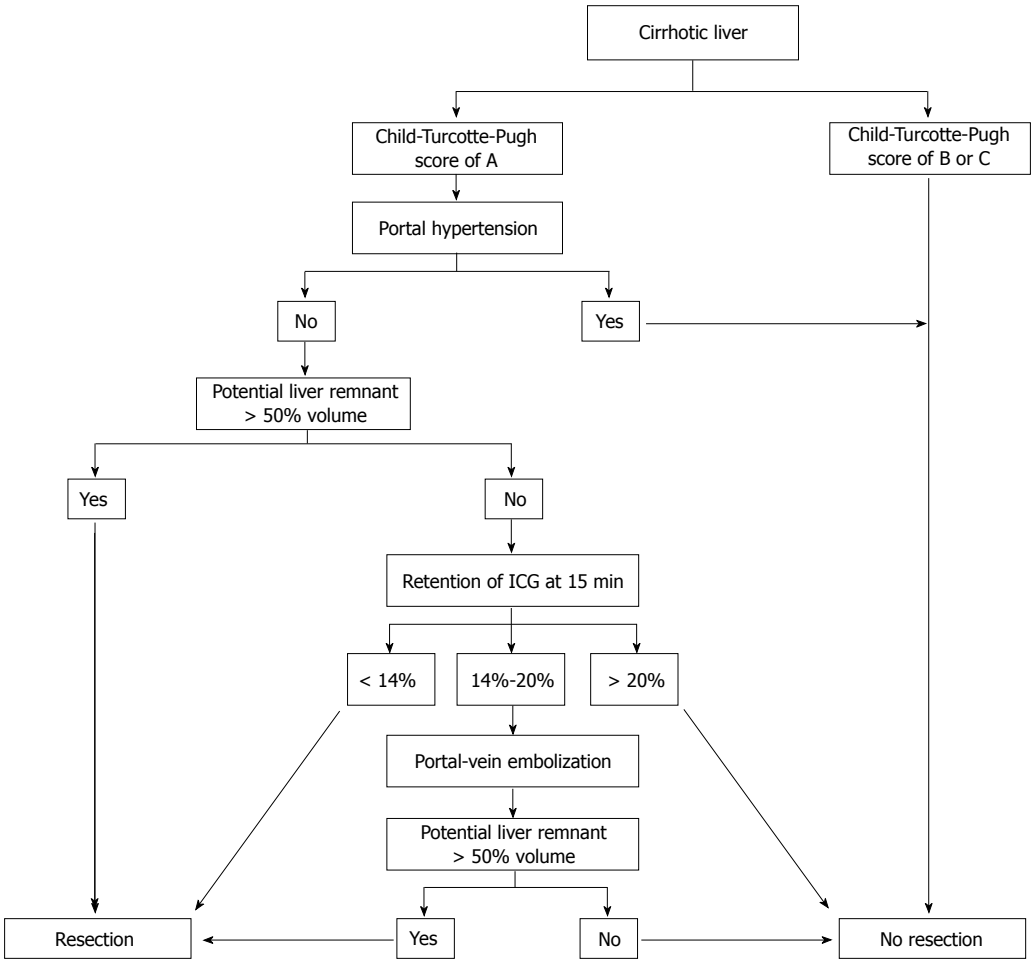
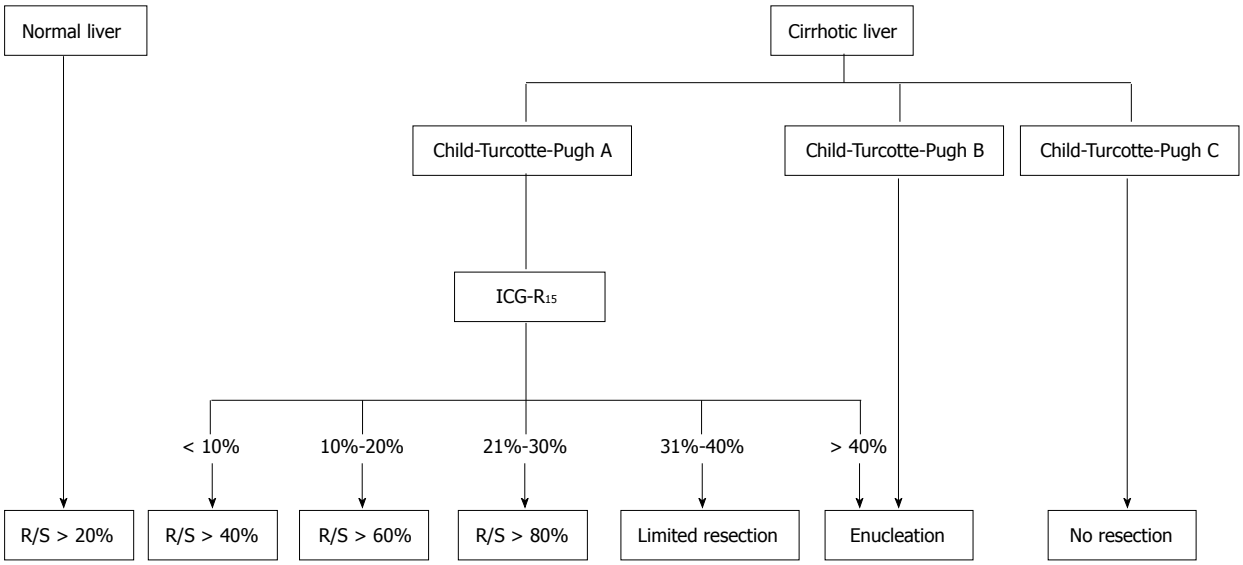


Figure 3 Decision tree from the University of Zurich for the individual evaluation of hepatectomy for a cirrhotic liver.

ICGR15 to determine the specific surgical approach for the hepatectomy.

By combining the parameters of cirrhosis incidence, Child-Pugh score, portal hypertension, and ICG-R15, Professor Clavien of Switzerland determined the corresponding safe extent of hepatectomy[18].

Based on four indexes, namely, cirrhosis, the Child-Pugh score, the 15-min retention rate of indocyanine green (ICGR15), and the standardized residual liver volume rate, we established a decision tree for hepatectomy, termed the Chinese consensus system[10].



R = Reserved functional liver volume (RFLV)
S = Standard liver volume (SLV)

Figure 4 Chinese consensus decision tree for the individual evaluation of the safe extent of hepatectomy.

Table 1 Basic information on the study population

Factors	*n* (%)
Age (yr), mean (SD)	51.8 (12.4)
Age > 65 yr	102 (15.1)
Male	502 (79.2)
ICG R15	
< 10%	545 (86.8)
10%-20%	69 (10.9)
20%-30%	8 (1.3)
30%-40%	6 (0.9)
> 40%	6 (0.9)
Hepatitis serology	
Hepatitis B	452 (71.3)
Hepatitis C	34 (5.4)
Hepatitis B and C	25 (3.9)
Negative or not determined	123 (19.4)
Cirrhosis	479 (75.5)
Portal vein hypertention	78 (12.3)

ICG R15: Indocyanine green retention rate at 15 min.

Statistical analysis

We retrospectively collected and recorded the preoperative, intra-operative, and postoperative data. The classification variables were evaluated using a χ^2 or Fisher's exact test, and the continuous variables were compared using a *t*-test or Mann-Whitney test. We performed logistic regression analysis to evaluate hepatic failure, mortality, or the risk factors of complications after hepatectomy. We defined $P < 0.05$ as indicating a significant difference. We used SPSS 20.0 software for the statistical analysis.

RESULTS

General information

From January 2004 to December 2010, 634 cases of hepatectomy due to hepatocellular carcinoma at the Department of Hepatobiliary Surgery of People's Liberation Army General Hospital were included in the study. There were 502 male patients and 132 female patents; the average age was 51.8 years (12.4) (Table 1). There were 452 cases of hepatitis B, 34 cases of hepatitis C, and 25 cases of concurrent hepatitis B and hepatitis C. Additionally, there were 123 cases without infection, 78 cases with portal hypertension, and 34 cases with preoperative TACE. The cases with ICGR15 < 10%, 10%-20%, 20%-30%, 30%-40%, and > 40% were 545 (86.8%), 69 (10.9%), 8 (1.3%), 6 (0.9%), and 6 (0.9%), respectively. The postoperative pathology confirmed that 419 patients had cirrhosis. There were 629 cases (99.2%) with Child-Pugh grade A and five cases (0.8%) with Child-Pugh grade B, and the mean MELD score was 8.4 ± 1.5 (Table 2).

Among the cases of hepatectomy, there were 119 (18.8%) cases of major hepatectomy (not less than three segments), 90 (14.2%) cases with resection of two segments, 163 cases (25.7%) with resection of one segment, and 262 cases (41.3%) with limited hepatectomy and sub-segmental hepatectomy. The surgery time was 251 ± 52.1 min, and the surgical blood loss was 438 ± 559.7 mL. There were 142 cases (22.4%) with intra-operative blood transfusion. The adopted occlusion method was the Pringle approach, simple portal vein occlusion, and no occlusion in 392 (61.8%), 30 (4.7%), and 202 (33.4%) cases, respectively. The average block time was 20 ± 11.2 min (Table 3).

The postoperative mortality for these patents was 0.16% (1/634). In one case, the patient died of hepatic failure after surgery. The overall complication rate was 44.9% (Table 4). In 19 cases, the patients suffered hepatic failure after surgery. In 40 cases, there was postoperative bile leakage. In seven cases, the patients suffered postoperative bleeding. In four

Table 2 Preoperative biochemical indices and ratings *n* (%)

Serum albumin (g/L)	41.1 (4.0)
Platelet count (10^3/mm^3)	178.6 (75.1)
Serum creatinine (μmol/L)	67.7 (14.0)
Serum total bilirubin (μmol/L)	
Serum total bilirubin (μmol/L)	6.3 (14.2)
Serum sodium (mmol/L)	142.0 (2.8)
INR, mean (SD)	1.04 (0.10)
CTP score	
Class A	629 (98.2)
Class B	5 (0.8)
MELD score, mean (SD)	8.4 (1.5)
Tumor size (cm)	7.4 (4.9)

Tumor size: The largest diameter of tumor, even for multiple lesions; CTP score: Child-Turcotte-Pugh score; MELD score: Model for End-stage Liver Disease score.

Table 3 Relevant surgical indices *n* (%)

Operating time (min)	251 (52)
Porta hepatis clamping time (min)	20 (11.2)
Estimated blood loss (mL)	438 (559.7)
Extent of hepatectomy	
Subsegmentectomy or limited resection	262 (41.3)
Segmentectomy	163 (5.7)
Bisegmentectomy	90 (14.2)
Major hepatectomy (3 or more segments)	119 (18.8)
Intraoperative RBC transfusion	
Yes	142 (22.4)
No	492 (77.6)
Blocking methods	
Portal vein blocking	30 (4.7)
Pringle maneuver	392 (61.8)
No	212 (33.4)

RBC: Red blood cells.

cases, the patients suffered reoperation-related complications. In 62 cases, the patients suffered pleural effusion or thoracic puncture and drainage.

The numbers of cases satisfying the Makuuchi decision tree of the University of Tokyo, the Clavien decision tree of the University of Zurich, and the Chinese consensus decision tree were 573, 581, and 622, respectively. The incidence of hepatic failure in these groups was 2.62%, 2.75%, and 2.73%, respectively. Meanwhile, the incidences of hepatic failure in cases outside the decision tree were 5.66%, 6.56%, and 16.67%, respectively (Table 5).

The multivariate analysis indicates that liver cirrhosis, abdominal infection, major hepatectomy, and total hospital days are related to hepatic failure after hepatectomy (Table 6). Therefore, this analysis demonstrates that liver cirrhosis, abdominal infection, and major hepatectomy are the main factors that affect hepatic failure. The total hospital duration of the patients with hepatic failure is significantly prolonged.

DISCUSSION

Hepatitis B is an epidemic disease in China. Approximately 85% (or more) of patients with hepatocellular carcinoma also have cirrhosis; therefore, reducing the risk of hepatectomy with cirrhosis has always been a focus of hepatectomy. Hepatectomy has always been affected by the range of surgical options due to its complexity, high surgery morbidity, and mortality. In the past several decades, due to increasing improvements in liver surgery techniques and careful treatment during the perioperative period, the hospital mortality rate for hepatectomy has decreased, the safety of the surgery has increased, and the indications for hepatectomy have expanded.

Table 4 Postoperative complications of hepatectomy

Overall morbidity	44.90%
Surgical morbidity	
Posthepatectomy liver failure	19%
Biliary leakage	40%
Posthepatectomy hemorrhage	7%
Perihepatic abscess	22%
Wound infection	42%
Cholangitis	5%
Pleural effusion	62%
Portal vein thrombosis	3%
Medical morbidity	
Respiratory insufficiency/failure	12%
Renal failure	6%
Pneumonia	28%
Cardiac arrhythmia	20%
Pulmonary embolism	2%
Cardiac failure	8%
Myocardial infarction	5%
Relaparotomy	4%

Table 5 Postoperative liver failure rate according to criteria

Criteria	Yes	No
Swiss	2.75%	5.66%
Japan	2.62%	6.56%
Chinese	2.73%	16.67%

In China, hepatectomy is the primary method for treating hepatocellular carcinoma. With improvements in both technology and the treatment capabilities during the perioperative period, the mortality and complication rates of hepatectomy have significantly declined, and the hospital mortality of this study is 0.16%, which is similar to that of our previous studies[19]. The indications for surgery have gradually expanded. However, hepatic failure after hepatectomy still has a relatively high incidence rate and accounts for 18%-75% of postoperative deaths[20].

The decision-making system for hepatectomy consists of decision trees for hepatectomy proposed by individual centers based on scientific theory and practical experience[3,4,9,10,18,21,22]. At present, the primary decision-making systems are based on the following three decision-making systems: the Makuuchi decision-making system of Japan, the Clavien system, and the Chinese consensus decision-making system for hepatectomies.

The Makuuchi decision-making system was gene-

Table 6 Multivariate logistic analysis of hepatic failure

Variables in the equation	Sig.
Liver cirrhosis	0.009
Bile leakage	0.199
Ascites	0.184
Abdominal infection	0.014
Major hepatectomy	0.006
Bleeding	0.878
Height (cm)	0.710
Weight (kg)	0.361
HB (g/dL)	0.819
BSA (m^2)	0.384
BMI (kg/m^2)	0.733
Kmin	0.356
ICGR15	0.510
T12 (min)	0.617
EHBF (L/min)	0.472
Total hospital duration	0.017

rated in the late 1990s, when the mortality rate of hepatectomy was approximately 10%, which is much higher than the current level. At present, the mortality rate is lower than 1%[20,23,24]. Therefore, the safety of hepatectomy was the main problem confronted by hepatobiliary surgery at that time. From the perspective of safety, Professor Makuuchi proposed the decision tree for hepatectomy according to the existing data and clinical experience. In the subsequent 1245 hepatectomies, there were no reported deaths, which confirmed that the safety of this decision tree is very good[4,8]. However, it appears to be too general to assume that patients with ICG-R15 > 40% can still undergo liver surgery. It is not sufficiently accurate to use the type of hepatectomy surgery and the amount of resectable liver segment to characterize the safe limit of hepatectomy. For example, for an individual patient with cirrhosis, right hepatic atrophy, and left liver enlargement as well as good hepatic function reserve, it is safe to perform regular right hemihepatectomy, whereas it is dangerous to perform regular left hemihepatectomy. Moreover, in this standard, whether ascites can be controlled and the Child-Pugh score be replaced with the bilirubin level seem to be crude determinants.

In contrast with Asian experiences, European experts believe that a clinical situation with a Child-Pugh score above grade B, portal hypertension, and ICG-R15 > 20% is a contraindication for hepatectomy. The Zurich decision tree is based on the assumption that for a normal liver, a safe hepatectomy can only be performed if the residual liver volume (potential liver volume) exceeds 30% of the functional liver volume (Total liver volume-Tumor Volume), and it is also safe to achieve this index through preoperative portal vein embolization. However, many cases in which the hepatectomy can be performed safely miss this cut-off[25]. Previous study confirmed that cirrhosis patients have the same preoperative index, evolution process during the perioperative period, incidence rate of postoperative hepatic failure, complication rate, hospital time, and survival rate regardless of whether they also have portal hypertension[26-30]. Italian scholars also suggested that the clinical situation in which the cirrhosis patients have portal hypertension should be viewed as an absolute contraindication for hepatectomy; for patients with Child-Pugh grade A, the short-term and long-term postoperative effects are similar to the effects in patients with normal portal vein pressure.

Based on the characteristics of hepatectomy for the Chinese population, hepatocellular carcinoma, and the fact that the theory and practice of other decision trees are not suitable for China and its surgical conditions, we established the Chinese consensus decision tree. The safe resection extent for hepatectomy is established according to the proportion of reserved liver volume relative to the liver volume of the patient. Among patients with liver disease, the liver volume differs considerably, and the function of different parts of the liver also differs. Therefore, it is inaccurate to set the safe amount of reserved liver volume according to the proportion of diseased liver volume. Moreover, it is also very difficult to generally set the safe limit of resection with pen and paper using the same percentage of reserved liver volume without considering the degree to which the liver function is impaired. There is no specification regarding the extent of hepatectomy when less than 50% of the liver will remain.

Applying the minimum residual liver volume or the necessary functional liver volume and SLV is a relatively reliable method for setting the safe limit of hepatectomy. We selected liver parenchymal disease, Child-Pugh score, and ICGR-15 as the assessment standards to classify the reserved hepatic function. The ratio (F/S) between EFLV and SLV is used to set the safe extent of hepatectomy and to establish a decision tree for the individual evaluation of the safe limit of hepatectomy.

Liver volume analysis is the most important determinant factor for whether hepatectomy or liver transplantation is selected to treat liver cancer. The maximum resectable volume of liver depends on the liver function. In the People's Liberation Army General Hospital, conventionally, the preoperative liver volume is calculated using enhanced CT, and liver function is estimated through the indocyanine green retention test before every hepatectomy. The equilibrium between the range of hepatectomy and liver function is closely related to postoperative complications and mortality[25,31]. The range of hepatectomy can usually be evaluated through the CT volume analysis of the relevant liver segment. This detailed information is helpful for making the most reasonable decision about the hepatectomy. The CT volume analysis can be implemented in 2D or 3D. Our 3D analysis adopts the principle of basin analysis[32]. According to each portal vein or hepatic venous system, we accurately analyze the range of each segment and then calculate the liver

volume.

Because the 50-50 criteria are indices for evaluating hepatic failure after hepatectomy for patients with a normal bilirubin range, this study excluded patients with hepatocellular carcinoma and preoperative jaundice (with total bilirubin > 50 mmol/L). Blood transfusions during the perioperative period will certainly affect the bilirubin level. In the original experiment, we excluded patients with an intra-operative blood transfusion when evaluating the 50-50 criteria[33]. However, according to previous studies and our experience, we believe that the likelihood that a blood transfusion in the perioperative period affects postoperative bilirubin and coagulation is very small; therefore, in this study, we did not exclude the relevant cases with blood transfusion.

Our studies indicate that the numbers of cases that satisfy the Makuuchi decision tree of the University of Tokyo, the Clavien decision tree of the University of Zurich, and the Chinese consensus decision tree were 573, 581, and 622, respectively, and the corresponding incidence of hepatic failure was 2.62%, 2.75%, and 2.73%, respectively. The incidence of hepatic failure in cases that did not satisfy the decision tree was 5.66%, 6.56%, and 16.67%, respectively. In comparison with the Makuuchi decision system of Japan and the Clavien system, the Chinese consensus decision tree expands the indications for hepatectomy without significantly increasing the incidence of hepatic failure after hepatectomy and is a safe and effective decision tree.

The multivariate analysis indicates that liver cirrhosis, abdominal infection, major hepatectomy, and total hospital duration are related to hepatic failure after hepatectomy and thus indicates that liver cirrhosis, abdominal infection, and major hepatectomy are the main factors that affect hepatic failure. The total hospital duration for patients with hepatic failure is significantly prolonged, which is consistent with previous research findings.

In summary, this study indicates that, in comparison with the Makuuchi decision tree of the University of Tokyo and the Clavien decision tree of the University of Zurich, the Chinese consensus decision tree can expand the scope of hepatectomy without significantly increasing the incidence of postoperative hepatic failure and that it is a safe and effective decision tree. Liver cirrhosis, abdominal infection, and major hepatectomy are the main factors that affect hepatic failure after hepatectomy.

COMMENTS

Background

Although many criteria have been established for safe hepatic resection, the evaluation of post-hepatectomy liver failure (PHLF) associated with several competing decision trees remains unknown.

Research frontiers

This article aims to compare a new embracing algorithm with two widely accepted criteria for safe liver resection and verify its efficiency in a large Chinese cohort.

Innovations and breakthroughs

In this study, the authors established a decision tree for safe hepatectomy based on four variables: normal or cirrhotic liver, the Child-Turcotte-Pugh score, the indocyanine green retention rate at 15 min, and the ratio of reserved functional liver volume to standard liver volume. They proved that the Chinese consensus decision tree expands the indications for hepatic resection for HCC patients and does not increase the PHLF rate compared to the Swiss-Clavien and Tokyo University-Makuuchi decision trees.

Applications

The Chinese consensus decision tree seems to be a safe and effective guideline for hepatectomy in patients with hepatocellular carcinoma.

Peer-review

PHLF is the most dreadful complication and it has many different definitions. In future, it may need to test those in a prospective study. In this large population, retrospective study, they proved the efficiency of the new criteria in elective patients. It is beneficial for elective hepatocellular carcinoma patients.

REFERENCES

1 **Makuuchi M**, Tamura S, Sugawara Y. New national policy for deceased organ donation in China. *Hepatobiliary Surg Nutr* 2013; **2**: 307-308 [PMID: 24570968 DOI: 10.3978/j.issn.2304-3881.2013.10.05]

2 **Huang J**, Wang H, Fan ST, Zhao B, Zhang Z, Hao L, Huo F, Liu Y. The national program for deceased organ donation in China. *Transplantation* 2013; **96**: 5-9 [PMID: 23743728 DOI: 10.1097/TP.0b013e3182985491]

3 **Fan ST**, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Hepatectomy for hepatocellular carcinoma: toward zero hospital deaths. *Ann Surg* 1999; **229**: 322-330 [PMID: 10077043]

4 **Imamura H**, Seyama Y, Kokudo N, Maema A, Sugawara Y, Sano K, Takayama T, Makuuchi M. One thousand fifty-six hepatectomies without mortality in 8 years. *Arch Surg* 2003; **138**: 1198-1206; discussion 1206 [PMID: 14609867 DOI: 10.1001/archsurg.138.11.1198]

5 **Jarnagin WR**, Gonen M, Fong Y, DeMatteo RP, Ben-Porat L, Little S, Corvera C, Weber S, Blumgart LH. Improvement in perioperative outcome after hepatic resection: analysis of 1,803 consecutive cases over the past decade. *Ann Surg* 2002; **236**: 397-406; discussion 406-407 [PMID: 12368667 DOI: 10.1097/01.sla.0000029003.66466.b3]

6 **Kamiyama T**, Nakanishi K, Yokoo H, Kamachi H, Tahara M, Yamashita K, Taniguchi M, Shimamura T, Matsushita M, Todo S. Perioperative management of hepatic resection toward zero mortality and morbidity: analysis of 793 consecutive cases in a single institution. *J Am Coll Surg* 2010; **211**: 443-449 [PMID: 20822741 DOI: 10.1016/j.jamcollsurg.2010.06.005]

7 **Seyama Y**, Kubota K, Sano K, Noie T, Takayama T, Kosuge T, Makuuchi M. Long-term outcome of extended hemihepatectomy for hilar bile duct cancer with no mortality and high survival rate. *Ann Surg* 2003; **238**: 73-83 [PMID: 12832968 DOI: 10.1097/01.sla.0000074960.55004.72]

8 **Torzilli G**, Makuuchi M, Inoue K, Takayama T, Sakamoto Y, Sugawara Y, Kubota K, Zucchi A. No-mortality liver resection for hepatocellular carcinoma in cirrhotic and noncirrhotic patients: is there a way? A prospective analysis of our approach. *Arch Surg* 1999; **134**: 984-992 [PMID: 10487594]

9 **Miyagawa S**, Makuuchi M, Kawasaki S, Kakazu T. Criteria for safe hepatic resection. *Am J Surg* 1995; **169**: 589-594 [PMID: 7771622]

10 **Dong J**, Yang S, Zeng J, Cai S, Ji W, Duan W, Zhang A, Ren W, Xu Y, Tan J, Bu X, Zhang N, Wang X, Wang X, Meng X, Jiang K, Gu W, Huang Z. Precision in liver surgery. *Semin Liver Dis* 2013; **33**: 189-203 [PMID: 23943100 DOI: 10.1055/s-0033-1351781]

11 **Qian NS**, Liao YH, Cai SW, Raut V, Dong JH. Comprehensive

application of modern technologies in precise liver resection. *Hepatobiliary Pancreat Dis Int* 2013; **12**: 244-250 [PMID: 23742768]
12 **Balzan S**, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D, Durand F. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**: 824-828, discussion 828-829 [PMID: 16327492]
13 **Koch M**, Garden OJ, Padbury R, Rahbari NN, Adam R, Capussotti L, Fan ST, Yokoyama Y, Crawford M, Makuuchi M, Christophi C, Banting S, Brooke-Smith M, Usatoff V, Nagino M, Maddern G, Hugh TJ, Vauthey JN, Greig P, Rees M, Nimura Y, Figueras J, DeMatteo RP, Büchler MW, Weitz J. Bile leakage after hepatobiliary and pancreatic surgery: a definition and grading of severity by the International Study Group of Liver Surgery. *Surgery* 2011; **149**: 680-688 [PMID: 21316725 DOI: 10.1016/j.surg.2010.12.002]
14 **Dello SA**, Stoot JH, van Stiphout RS, Bloemen JG, Wigmore SJ, Dejong CH, van Dam RM. Prospective volumetric assessment of the liver on a personal computer by nonradiologists prior to partial hepatectomy. *World J Surg* 2011; **35**: 386-392 [PMID: 21136056 DOI: 10.1007/s00268-010-0877-6]
15 **DuBray BJ**, Levy RV, Balachandran P, Conzen KD, Upadhya GA, Anderson CD, Chapman WC. Novel three-dimensional imaging technique improves the accuracy of hepatic volumetric assessment. *HPB* (Oxford) 2011; **13**: 670-674 [PMID: 21843269 DOI: 10.1111/j.1477-2574.2011.00350.x]
16 **Takamoto T**, Hashimoto T, Ogata S, Inoue K, Maruyama Y, Miyazaki A, Makuuchi M. Planning of anatomical liver segmentectomy and subsegmentectomy with 3-dimensional simulation software. *Am J Surg* 2013; **206**: 530-538 [PMID: 23809675 DOI: 10.1016/j.amjsurg.2013.01.041]
17 **Urata K**, Kawasaki S, Matsunami H, Hashikura Y, Ikegami T, Ishizone S, Momose Y, Komiyama A, Makuuchi M. Calculation of child and adult standard liver volume for liver transplantation. *Hepatology* 1995; **21**: 1317-1321 [PMID: 7737637 DOI: 10.1002/hep.1840210515]
18 **Clavien PA**, Petrowsky H, DeOliveira ML, Graf R. Strategies for safer liver surgery and partial liver transplantation. *N Engl J Med* 2007; **356**: 1545-1559 [PMID: 17429086 DOI: 10.1056/NEJMra065156]
19 **Huang ZQ**, Xu LN, Yang T, Zhang WZ, Huang XQ, Liu R, Cai SW, Zhang AQ, Feng YQ, Zhou NX, Dong JH. [Liver resection: single center experiences of 2008 consecutive resections in 20 years]. *Zhonghua Waike Zazhi* 2008; **46**: 1314-1321 [PMID: 19094562]
20 **Rahbari NN**, Garden OJ, Padbury R, Brooke-Smith M, Crawford M, Adam R, Koch M, Makuuchi M, Dematteo RP, Christophi C, Banting S, Usatoff V, Nagino M, Maddern G, Hugh TJ, Vauthey JN, Greig P, Rees M, Yokoyama Y, Fan ST, Nimura Y, Figueras J, Capussotti L, Büchler MW, Weitz J. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). *Surgery* 2011; **149**: 713-724 [PMID: 21236455 DOI: 10.1016/j.surg.2010.10.001]
21 **Yamanaka N**, Okamoto E, Kuwata K, Tanaka N. A multiple regression equation for prediction of posthepatectomy liver failure. *Ann Surg* 1984; **200**: 658-663 [PMID: 6486915]
22 **Yamanaka N**, Okamoto E, Oriyama T, Fujimoto J, Furukawa K, Kawamura E, Tanaka T, Tomoda F. A prediction scoring system to select the surgical treatment of liver cancer. Further refinement based on 10 years of use. *Ann Surg* 1994; **219**: 342-346 [PMID: 8161258]
23 **Makuuchi M**, Sano K. Surgical treatment of HCC: update topics. *Updates Surg* 2011; **63**: 67-68 [PMID: 21505905 DOI: 10.1007/s13304-011-0069-4]
24 **Dokmak S**, Ftériche FS, Borscheid R, Cauchy F, Farges O, Belghiti J. 2012 Liver resections in the 21st century: we are far from zero mortality. *HPB* (Oxford) 2013; **15**: 908-915 [PMID: 23461811 DOI: 10.1111/hpb.12069]
25 **Breitenstein S**, Apestegui C, Petrowsky H, Clavien PA. "State of the art" in liver resection and living donor liver transplantation: a worldwide survey of 100 liver centers. *World J Surg* 2009; **33**: 797-803 [PMID: 19172348 DOI: 10.1007/s00268-008-9878-0]
26 **Capussotti L**, Ferrero A, Viganò L, Muratore A, Polastri R, Bouzari H. Portal hypertension: contraindication to liver surgery? *World J Surg* 2006; **30**: 992-999 [PMID: 16736327 DOI: 10.1007/s00268-005-0524-9]
27 **Ishizawa T**, Hasegawa K, Aoki T, Takahashi M, Inoue Y, Sano K, Imamura H, Sugawara Y, Kokudo N, Makuuchi M. Neither multiple tumors nor portal hypertension are surgical contraindications for hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1908-1916 [PMID: 18549877 DOI: 10.1053/j.gastro.2008.02.091]
28 **Cucchetti A**, Ercolani G, Vivarelli M, Cescon M, Ravaioli M, Ramacciato G, Grazi GL, Pinna AD. Is portal hypertension a contraindication to hepatic resection? *Ann Surg* 2009; **250**: 922-928 [PMID: 19855258 DOI: 10.1097/SLA.0b013e3181b977a5]
29 **Ruzzenente A**, Valdegamberi A, Campagnaro T, Conci S, Pachera S, Iacono C, Guglielmi A. Hepatocellular carcinoma in cirrhotic patients with portal hypertension: is liver resection always contraindicated? *World J Gastroenterol* 2011; **17**: 5083-5088 [PMID: 22171142 DOI: 10.3748/wjg.v17.i46.5083]
30 **Santambrogio R**, Kluger MD, Costa M, Belli A, Barabino M, Laurent A, Opocher E, Azoulay D, Cherqui D. Hepatic resection for hepatocellular carcinoma in patients with Child-Pugh's A cirrhosis: is clinical evidence of portal hypertension a contraindication? *HPB* (Oxford) 2013; **15**: 78-84 [PMID: 23216782 DOI: 10.1111/j.1477-2574.2012.00594.x]
31 **Clavien PA**, Oberkofler CE, Raptis DA, Lehmann K, Rickenbacher A, El-Badry AM. What is critical for liver surgery and partial liver transplantation: size or quality? *Hepatology* 2010; **52**: 715-729 [PMID: 20683967 DOI: 10.1002/hep.23713]
32 **D'Onofrio M**, De Robertis R, Demozzi E, Crosara S, Canestrini S, Pozzi Mucelli R. Liver volumetry: Is imaging reliable? Personal experience and review of the literature. *World J Radiol* 2014; **6**: 62-71 [PMID: 24778768 DOI: 10.4329/wjr.v6.i4.62]
33 **Paugam-Burtz C**, Janny S, Delefosse D, Dahmani S, Dondero F, Mantz J, Belghiti J. Prospective validation of the "fifty-fifty" criteria as an early and accurate predictor of death after liver resection in intensive care unit patients. *Ann Surg* 2009; **249**: 124-128 [PMID: 19106687 DOI: 10.1097/SLA.0b013e31819279cd]

P- Reviewer: Ehrenpreis ED, Takeda R **S- Editor**: Yu J
L- Editor: Logan S **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9403

World J Gastroenterol 2015 August 21; 21(31): 9403-9412
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

High expression of CD11c indicates favorable prognosis in patients with gastric cancer

Yuan Wang, Bin Xu, Wen-Wei Hu, Lu-Jun Chen, Chang-Ping Wu, Bin-Feng Lu, Yue-Ping Shen, Jing-Ting Jiang

Yuan Wang, Bin Xu, Wen-Wei Hu, Lu-Jun Chen, Chang-Ping Wu, Jing-Ting Jiang, Department of Tumor Biological Treatment, the Third Affiliated Hospital of Soochow University, Changzhou 213003, Jiangsu Province, China

Yuan Wang, School of Clinical Medicine, North China University of Science and Technology, Tangshan 063000, Hebei Province, China

Bin-Feng Lu, Department of Immunology, University of Pittsburgh, Pittsburgh, PA 15213, United States

Yue-Ping Shen, Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, School of Public Health, Soochow University, Suzhou 215123, Jiangsu Province, China

Author contributions: Wang Y and Xu B contributed equally to this work; Wang Y and Xu B contributed substantially to the conception and design of the study; Wang Y, Hu WW and Chen LJ performed research; Wang Y, Xu B and Shen YP performed analysis and interpretation of all data and drafted the article; Wu CP and Lu BF critically read the manuscript; Jiang JT and Shen YP supervised the study and revised the manuscript.

Supported by National Natural Science Foundation of China, No. 81171653, No. 81301960 and No. 31428005; and Natural Science Foundation of Jiangsu Province, China, No. BK2011246 and No. BK2011247.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest in this study.

Data sharing statement: No additional data are available.

Correspondence to: Jing-Ting Jiang, MD, PhD, Department of Tumor Biological Treatment, the Third Affiliated Hospital of Soochow University, No. 185 Juqian Road, Changzhou 213003, Jiangsu Province, China. jiangjingting@suda.edu.cn
Telephone: +86-519-68870884
Fax: +86-519-68870978

Received: April 7, 2015
Peer-review started: April 8, 2015
First decision: April 23, 2015
Revised: May 1, 2015
Accepted: June 10, 2015
Article in press: June 10, 2015
Published online: August 21, 2015

Abstract

AIM: To determine the relationship between CD11c expression level and prognosis in patients with gastric cancer (GC).

METHODS: This retrospective survival study was performed from July 31, 2008 to June 30, 2014. Our study inclusion criteria included all the patients with GC who underwent surgical resection between January 1998 and December 2009 in the Third Affiliated Hospital of Soochow University. CD11c expression levels in 140 patients with GC at different UICC stages were evaluated using immunohistochemistry, and GC tissues from 16 cases were further verified by qRT-PCR. The χ^2 test was used to compare the patient- and disease-related factors between the low CD11c expression group and the high expression group. Univariate probabilities of overall survival (OS) and disease-free survival (DFS) were assessed using the Kaplan-Meier method. The log rank test was used to compare survival curves. Different multivariate COX models were used to estimate the association between CD11c expression and both death and recurrence risk

in GC patients.

RESULTS: The average CD11c expression level was 5.1 ± 1.8/high power field (HPF) in 10 gastritis samples, 4.5 ± 2.3/HPF in 10 gastric polyp samples and 9.7 ± 6.3/HPF in 140 gastric cancer samples, respectively. The CD11c expression level was significantly decreased from UICC stage Ⅰ to stage Ⅳ (stage Ⅰ: 16.0 ± 7.4, stage Ⅱ: 10.4 ± 5.5, stage Ⅲ: 9.4 ± 6.1, stage Ⅳ: 5.3 ± 3.2, P < 0.001). Patients in the high CD11c expression group had a greater 3- and 5-year OS probability and longer median survival time compared with the low CD11c expression group, (67.7% *vs* 39.2%; 51.4% *vs* 29.0%; 67.0 mo *vs* 28.0 mo; χ^2 = 6.80, P = 0.009), and had a greater 3- and 5-year DFS probability and longer median DFS time (63.7% *vs* 24.0%; 49.1% *vs* 11.9%; 64.0 mo *vs* 18.0 mo; χ^2 = 15.39, P < 0.001). Patients with high CD11c high expression had a reduced risk of death (HR = 0.56, 95%CI: 0.33-0.98, P < 0.05) and relapse (HR = 0.39, 95%CI: 0.23-0.67, P < 0.01) compared with patients with low CD11c expression after adjustment of potential confounders, with the exception of tumor size. However, the protective effect related to death (HR = 0.90, 95%CI: 0.49-1.67, P = 0.749) and relapse (HR = 0.65, 95%CI: 0.36-1.19, P = 0.160) disappeared when tumor size was incorporated into the model.

CONCLUSION: High expression of CD11c decreased the risk of death and relapse, and may be regarded as an alternative indicator of favorable prognosis in patients with GC.

Key words: CD11c; Gastric cancer; Dendritic cell; Tumor microenvironment

Core tip: The progression of gastric cancer is closely related to the tumor microenvironment. In the present study, we found that CD11c expression level significantly decreased from UICC stage Ⅰ to stage Ⅳ, and patients with high CD11c expression had a reduced risk of death and relapse compared with patients with low CD11c expression after adjustment of potential confounders, with the exception of tumor size. Based on our research, we suggest that high expression of CD11c decreased the risk of death and relapse and may act as an indicator of favorable prognosis in patients with gastric cancer.

Wang Y, Xu B, Hu WW, Chen LJ, Wu CP, Lu BF, Shen YP, Jiang JT. High expression of CD11c indicates favorable prognosis in patients with gastric cancer. *World J Gastroenterol* 2015; 21(31): 9403-9412 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9403.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9403

INTRODUCTION

Throughout the history of human civilization, cancer has been a major health problem. As one of the most common malignancies, gastric cancer (GC) remains the second leading cause of cancer-related death worldwide. Over 70% of new cases and deaths are observed in developing countries[1]. In China, GC has the second highest incidence among commonly diagnosed cancers[2]. Currently, the prevalence of GC is high and a large number of patients are first diagnosed in the advanced stages. Despite the availability of several anticancer drugs and treatments, GC cannot be cured, especially in the late stages, without exhibiting side effects, and the prognosis of these patients in the late stages is poor[3,4].

Dendritic cells (DCs) exhibit a strong capacity to receive and integrate innate and adaptive immune signals. In general, DCs are divided into myeloid-, plasmacytoid-, and monocyte-associated DCs[5-7]. Several studies have determined the relationship between immune cell infiltration and tumor progression in various cancers, and tumor-infiltrating mature DCs are associated with a favorable prognosis, but immature DCs are not associated with a favorable prognosis[8,9]. Therefore, they are regarded as key antigen-presenting cells (APCs), which are crucial in the enhancement and regulation of cell-mediated immune responses, and DCs play an important role in most cancers[10,11]. As a member of the adhesion molecule integrin family β2, CD11c is over-expressed in myeloid- and monocyte-associated DCs, and the expression of CD11c has been observed in NK cells, macrophages and even some activated B and T cells[12]. Currently, there are few clinical data available on the impact of CD11c expression level in the tumor microenvironment on the prognosis of patients with GC. In the present study, we aimed to determine the relationship between CD11c expression in the tumor and the progression of GC.

MATERIALS AND METHODS

Patients

The study inclusion criteria included all the patients with GC who underwent surgical resection between January 1998 and December 2009 in the Third Affiliated Hospital of Soochow University, Changzhou, China. None of the subjects had received chemotherapy or radiotherapy before surgery. A total of 202 patients were enrolled; however, 62 patients were excluded due to lack of pathological specimen, failed assessment of CD11c scores or loss to follow-up at the early stage of the study, resulting in 140 participants in the final study population. The tumor stages were determined using the International Union against Cancer Staging System[13].

Table 1 Primer sequences used in this study

Gene	Forward primer sequence (5'→3')	Reverse primer sequence (5'→3')
CD11c	GGGATGCCGCCAAAATTCTC	ATTGCATAGCGGATGATGCCT
GAPDH	GGAAGGTGAAGGTCGGAGTC	CGTTCTCAGCCTTGACGGT

Data collection

Demographics and clinical data were obtained by reviewing the medical records. All the patients were followed up yearly from July 31, 2008 to June 30, 2014.

Study endpoints

Primary endpoints were overall survival (OS) and disease-free survival (DFS). OS was defined as the time from registration until death from any cause, and DFS was defined as the time from randomization until recurrence of tumor or death from any cause. Surviving patients were censored on June 30, 2014.

Immunohistochemistry

Formalin-fixed and paraffin-embedded tissues were cut into 3-μm-thick sections, de-waxed in xylene and then progressively rehydrated by gradient concentrations of ethanol. Antigens were retrieved by heating the tissue sections at 100 ℃ for 30 min in citrate solution (10 mmol/L, pH 6.0). The sections were cooled and immersed in methanol in the presence of 0.3% hydrogen peroxide for 15 min to block the endogenous peroxidase activity. The sections were subsequently rinsed in PBS for 5 min and then incubated with primary antibody against CD11c (1:150, Epitomics, Burlingame, CA, United States) at 4 ℃ overnight. Sections incubated without the primary antibody were used as negative controls. The sections were then incubated with horseradish peroxidase-labeled goat against mouse/rabbit secondary antibody (Maixin Biotechnology, Fuzhou, China). Diaminobenzene was used as the chromogen, and hematoxylin was used as the nuclear counterstain. Finally, the sections were dehydrated, cleared and mounted.

The stained sections were independently reviewed by two pathologists without knowing the clinical diagnosis. The slides were graded according to the staining intensity. CD11c-positive signals located in the cell membrane were counted according to the brown diaminobenzidine precipitate. The CD11c signals from five visual areas enriched with tumor-infiltrating lymphocytes were examined using a low-magnification lens, and then examined using a high-magnification lens (× 200) to determine the average number of CD11c positive cells. Immunohistochemical expression scores of CD11c were obtained using a Leica microscope. The patients were classified into two groups as follows: low expression [≤ 14/high power field (HPF)] and high expression (> 14/HPF) based on expression scores of CD11c per slide.

RNA isolation and quantitative real time-PCR analysis

Total RNA was extracted from different gastric cancer tissues using TRIzol reagent (Invitrogen Company, St. Louis, MO, United States). The RNA quality was evaluated according to the absorbance at a wavelength of 260/280 nm. Subsequently, purified RNA was reversely transcribed into cDNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, United States). Table 1 lists the sequences of all primers used in the present study. The CD11c expression at the mRNA level was evaluated using a 7500 Real-Time PCR System with SYBR® Green PCR Master Mix (Applied Biosystems). GAPDH was selected as a housekeeping gene. The CD11c expression was normalized to the level of the housekeeping gene and analyzed using the comparative CT method ($2^{-\Delta\Delta}$CT method).

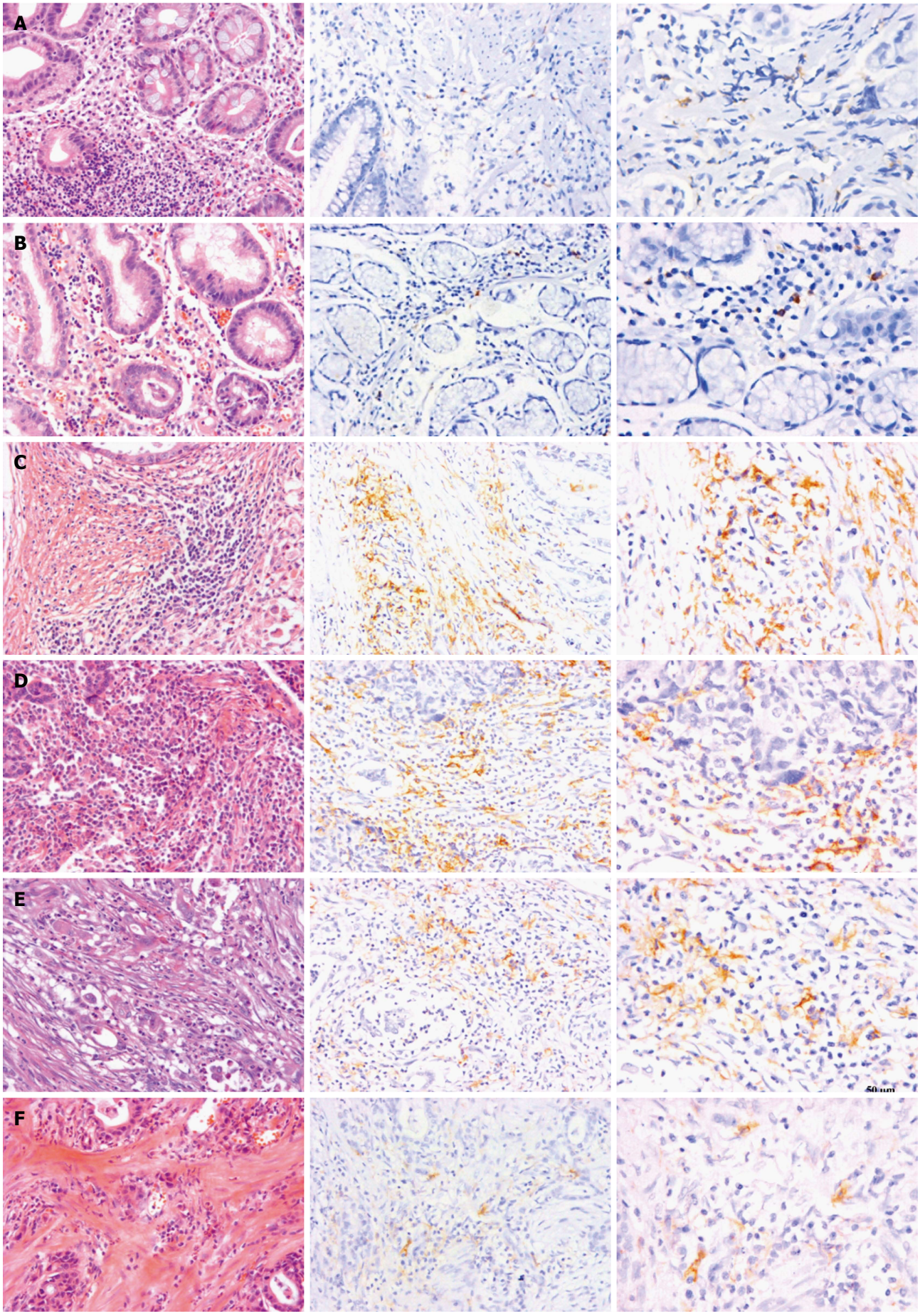
Data analysis

The χ^2 test was used to compare the patient- and disease-related factors between the low CD11c expression group and the high expression group. Univariate probabilities of OS and DFS were assessed using the Kaplan-Meier method. The log rank test was used to compare survival curves. The Cox model, HR with a 95%CI was used to estimate the association between CD11c expression (> 14/HPF = 1; ≤ 14/HPF = 0) and death or relapse of all patients with adjustments of the following potential confounders: age (about 45 = 1; 60 = 2; > 60 = 3), gender (male = 0; female = 1), tumor location: gastric cardia or not (yes = 1; no = 0), gastric body or not (yes = 1; no = 0), gastric antrum or not (yes = 1; no = 0), tumor size (< 5 cm = 0; ≥ 5 cm = 1), histological type (poorly differentiated = 1; differentiated = 0), invasion to muscular layer (yes = 1; no = 0), nodal metastasis (yes = 1; no = 0), recurrence (yes = 1; no = 0), pathological grade (grade 1-2 = 1; grade 3 = 2; grade 4 = 3), and UICC stage (Ⅰ = 1; Ⅱ = 2; Ⅲ = 3; Ⅳ = 4).

RESULTS

CD11c expression in gastric cancer is negatively correlated with UICC stage

Immunohistochemistry (IHC) revealed that the staining intensity of CD11c was differently distributed among various tissues, including gastritis (Figure 1A), gastric polyps (Figure 1B) and gastric cancer tissues (Figure 1C-F). As shown in Figure 1, the average expression levels of CD11c were 5.1 ± 1.8/HPF in 10 gastritis samples, 4.5 ± 2.3/HPF in 10 gastric polyp samples


A
B
C
D
E
F
100 ×
100 ×
50 ×

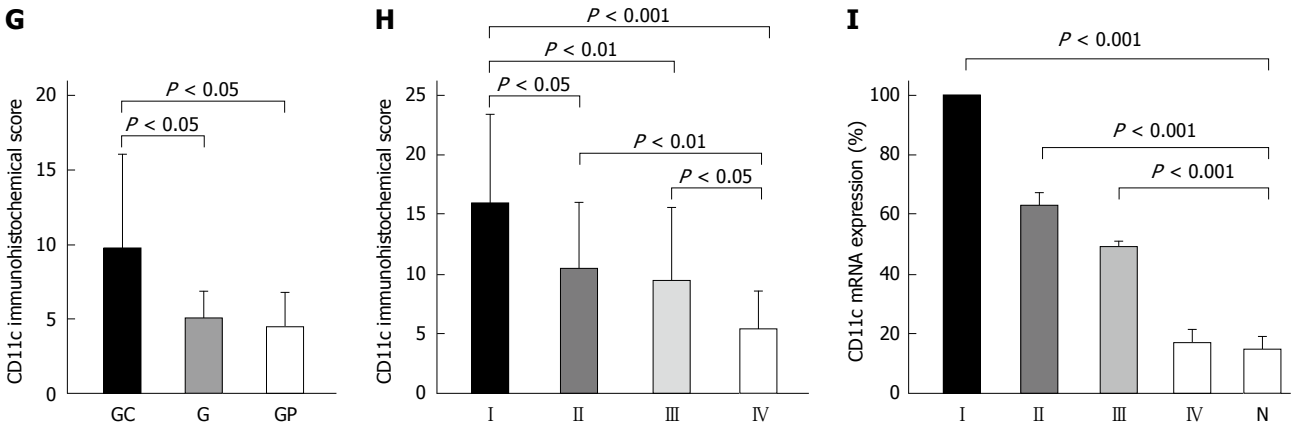


Figure 1 Validation of differentially expressed CD11c by IHC (magnification × 100) and qRT-PCR. Representative IHC for gastritis tissues (A), gastric polyp tissues (B) and gastric cancer tissues at different stages Ⅰ-Ⅳ (C-F) showed that high CD11c expression was present in UICC stage Ⅰ, and the stain density of CD11c decreased progressively from UICC stage Ⅰ to stage Ⅳ. CD11c showed differential expression in gastric cancer, gastritis, and gastric polyp tissues and the stain density of CD11c was significantly increased in gastric cancer tissue compared with gastritis and gastric polyp tissues (G). The expression of CD11c progressively decreased from UICC stage Ⅰ to UICC stage Ⅳ (H). qRT-PCR using mRNA from gastric cancer at UICC stages Ⅰ-Ⅳ and normal gastric tissue was conducted. Using CD11c expression of UICC stage Ⅰ as 100%, CD11c expression levels were significantly different at UICC stages Ⅰ-Ⅲ compared with normal gastric tissue (I). GC: Gastric cancer; G: Gastritis; GP: Gastric polyp; N: Normal gastric tissue.

and 9.7 ± 6.3/HPF in 140 gastric cancer samples, respectively, which suggests that CD11c is involved in the pathological changes in GC. Furthermore, CD11c expression gradually decreased from UICC stage Ⅰ to stage Ⅳ (stage Ⅰ: 16.0 ± 7.4, stage Ⅱ: 10.4 ± 5.5, stage Ⅲ: 9.4 ± 6.1, stage Ⅳ: 5.3 ± 3.2, $P < 0.001$, Figure 1H). Based on these results, qRT-PCR was subsequently performed to further validate these findings. Moreover, as shown in Figure 1I, low expression of CD11c in normal and para-carcinoma tissues was also observed, which indicated that tumor cells recruited more $CD11c^{+}$TILs than inflammation and polyps.

Baseline characteristics

Table 2 shows the patient characteristics. The median age of all 140 participants was 60 years, and the age range was 35 to 86 years. More male patients were included in the study population than females (76.43% *vs* 23.57%). In addition, 73.57% and 85.71% of the 140 patients died or experienced disease progression during the study period, respectively.

Significant differences in tumor size, histological type, invasion depth, nodal metastasis status, pathological grade, UICC stage and DFS outcome were detected between the two CD11c groups (all $P < 0.05$). No significant differences in other clinical variables were observed between the CD11c groups (all $P > 0.05$) (Table 2).

OS and DFS

Table 3 shows that patients in the high CD11c expression group had a greater 3- and 5-year OS probability and longer median survival time compared with the low CD11c expression group, (67.7% *vs* 39.2%; 51.4% *vs* 29.0%; 67.0 mo *vs* 28.0 mo; $\chi^2 = 6.80$, $P = 0.009$), and had a greater 3- and 5-year DFS probability and longer median DFS time (63.7% *vs* 24.0%; 49.1% *vs* 11.9%; 64.0 mo *vs* 18.0 mo; $\chi^2 = 15.39$, $P < 0.001$).

Figure 2 show the OS and DFS curves for the low CD11c expression group and high expression group, respectively. Patients in the high CD11c expression group had a significantly better OS and DFS compared with the low CD11c expression group (log-rank, $P = 0.009$ for OS and $P < 0.001$ for DFS).

The above-mentioned statistical results comprehensively revealed that stage Ⅳ was related to an increased risk of death and relapse.

Cox model analysis

Table 4 shows that the risk of death and relapse in patients with high CD11c expression was significantly decreased by 44% (HR = 0.56, 95%CI: 0.33-0.98, $P = 0.041$) and 61% (HR = 0.39, 95%CI: 0.23-0.67, $P = 0.001$) compared with the reference group (patients with low CD11c expression) after adjustments for gender, age, tumor location, histological type, pathological grade and UICC stage (Table 5, model 1 and 3). When tumor size was incorporated into the Cox model (Table 5, model 2 and 4), the risk of death and relapse in patients with high CD11c expression was increased to 0.90 and 0.65, with a P value of 0.749 and 0.160, respectively.

DISCUSSION

In the present study, we demonstrated that the infiltrating intensity of CD11c positive immune cells varied in different gastric tissues, including gastritis, gastric polyps and gastric cancer tissues. CD11c expression level in patients with GC was significantly higher than that in patients with gastritis or gastric polyps. Furthermore, CD11c expression level gradually

Table 2 Baseline characteristics of the low and high CD11c expression groups (n = 140)

Clinicopathological characteristics	All		CD11c expression				*P* value
			Low		High		
	No.	%	No.	%	No.	%	
Gender							0.113
Male	107	76.43	80	73.39	27	87.10	
Female	33	23.57	29	26.61	4	12.90	
Age (yr)							0.162
About 45	12	8.57	9	8.26	3	9.68	
About 60	66	47.14	56	51.38	10	32.26	
> 60	62	44.29	44	40.37	18	58.06	
Tumor location							
Gastric cardia							0.342
No	87	62.14	70	64.22	17	54.84	
Yes	53	37.86	39	35.78	14	45.16	
Tumor size (cm)[1]							< 0.001
< 5	56	47.46	36	38.71	20	80.00	
⩾ 5	62	52.54	57	61.29	5	20.00	
Histological type[2]							0.042
Differentiated	54	40.60	37	35.92	17	56.67	
Poorly differentiated	79	59.40	66	64.08	13	43.33	
Invasion to muscular layer[3]							0.004
No	14	11.57	7	7.29	7	28.00	
Yes	107	88.43	89	92.71	18	72.00	
Nodal metastasis[4]							0.001
No	33	27.27	19	20.00	14	53.85	
Yes	88	72.73	76	80.00	12	46.15	
Pathological grade							0.047
1-2	17	12.14	13	11.93	4	12.90	
3	70	50.00	49	44.95	21	67.74	
4	53	37.86	47	43.12	6	19.35	
UICC stage							0.002
Ⅰ	11	7.86	4	3.67	7	22.58	
Ⅱ	27	19.29	22	20.18	5	16.13	
Ⅲ	88	62.86	69	63.30	19	61.29	
Ⅳ	14	10.00	14	12.84	0	0.00	
Endpoint: DFS							0.038
No recurrence	20	14.29	12	11.01	8	25.81	
Recurrence	120	85.71	97	88.99	23	74.19	
Endpoint: OS							0.404
Survival	37	26.43	27	24.77	10	32.26	
Died	103	73.57	82	75.23	21	67.74	

[1]Missing 22 cases; [2]Missing 7 cases; [3]Missing 19 cases; [4]Missing 19 cases.

Table 3 Three- and 5-year overall survival and disease-free survival probability and median survival time (mo) in the low and high CD11c expression groups

Expression level of CD11c	3-yr OS (95%CI)	5-yr OS (95%CI)	Median survival time (95%CI)[1]	3-yr DFS (95%CI)	5-yr DFS (95%CI)	Median disease free time (95%CI)[2]
Low	39.2 (30.0-48.4)	29.0 (20.2-37.8)	28.0 (15.1-40.9)	24.0 (19.8-28.2)	11.9 (8.7-15.1)	18.0 (14.6-21.4)
High	67.7 (59.3-76.1)	51.4 (42.4-60.4)	67.0 (53.9-80.1)	63.7 (54.9-72.5)	49.1 (39.8-58.4)	64.0 (42.0-86.0)

[1]Survival curve comparison, χ^2 = 6.801, P = 0.009; [2]Disease-free curve comparison, χ^2 = 15.387, P < 0.001. OS: Overall survival; DFS: Disease-free survival.

decreased from UICC stage Ⅰ to stage Ⅳ, as confirmed by the qRT-PCR. We also found that patients with higher infiltrating intensity of CD11c positive cells had a significantly reduced risk of cancer-related death and relapse compared with patients with lower infiltrating intensity of CD11c positive cells after adjustments for potential confounders, with the exception of tumor size. However, the protective effect related to death and relapse disappeared when tumor size was incorporated into the model. Our findings suggested that low CD11c expression was associated with the risk of death and relapse in patients with GC, but was not an independent risk factor.

Interestingly, our previous findings suggested that a decrease in CD11c in GC tissues compared with normal gastric tissue samples may be related to a local

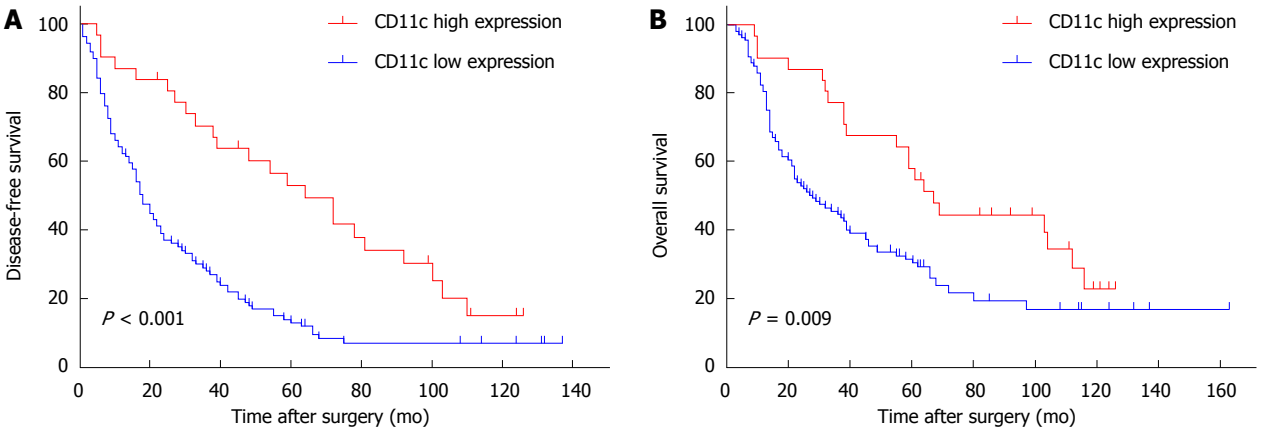


Figure 2 CD11c expression in gastric cancer tissues correlates with survival rate of patients. Based on the minimum *P* value observed, patients were divided into two groups with low or high CD11c expression, respectively. The DFS of patients with low and high CD11c expression was evaluated by Kaplan-Meier survival analysis. Log-rank: $P < 0.001$ (A). The OS of patients with low and high CD11c expression was evaluated by Kaplan-Meier survival analysis. Log-rank: $P = 0.009$ (B).

Table 4 Multivariate Cox model analysis of the association between CD11c expression and the risk of death and relapse *n* (%)

Clinicopathological parameters	Number	OS			DFS		
		HR	95%CI	*P* value	HR	95%CI	*P* value
CD11C expression							
Low	109 (77.86)		1.00 (ref)			1.00 (ref)	
High	31 (22.14)	0.56	0.33-0.98	0.041	0.39	0.23-0.67	0.001
Gender							
Male	107 (76.43)		1.00 (ref)			1.00 (ref)	
Female	33 (23.57)	1.08	0.64-1.83	0.769	0.77	0.46-1.27	0.306
Age groups							
About 45	12 (8.57)		1.00 (ref)			1.00 (ref)	
About 60	66 (47.14)	0.52	0.25-1.08	0.080	0.51	0.26-1.03	0.060
> 60	62 (44.29)	0.82	0.39-1.73	0.608	0.70	0.35-1.41	0.316
Gastric cardia							
No	87 (62.14)		1.00 (ref)			1.00 (ref)	
Yes	53 (37.86)	1.58	1.02-2.43	0.039	1.42	0.95-2.11	0.085
Histological type%							
Differentiated	54 (40.60)		1.00 (ref)			1.00 (ref)	
Poorly differentiated	79 (59.40)	1.26	0.79-2.02	0.333	1.025	0.68-1.56	0.907
Pathological grade							
1-2	17 (12.14)		1.00 (ref)			1.00 (ref)	
3	70 (50.00)	0.79	0.35-1.76	0.564	0.85	0.41-1.75	0.659
4	53 (37.86)	1.08	0.47-2.48	0.849	1.32	0.63-2.79	0.466
UICC stage							
Ⅰ	11 (7.86)		1.00 (ref)			1.00 (ref)	
Ⅱ	27 (19.20)	2.90	0.74-11.32	0.126	1.99	0.65-6.10	0.230
Ⅲ	88 (62.86)	3.36	0.91-12.39	0.069	2.12	0.74-6.10	0.162
Ⅳ	14 (10.00)	9.94	2.46-40.19	0.001	9.20	2.75-30.78	< 0.001

OS: Overall survival; DFS: Disease-free survival.

imbalance in the tumor immune microenvironment[14]. A previous study suggested that tumor-infiltrating $CD11b^+$ DCs are associated with worse prognosis in patients with GC, and that almost all $CD11b^+$ DCs showed CD11c[15]. In addition, it has been reported that $CD11b^+$ DCs are potent inducers of antigen-specific IL-10, producing type 1 regulatory T cells that induce antigen-specific tolerance[16]. Given these varied results, it is possible that the authors did not completely distinguish $CD11b^+$ cells from macrophages and DCs due to the similar characteristics in inducing immune tolerance between $CD11b^+$ DCs and tumor-associated M2 macrophages[17-19].

APCs, such as macrophages and DCs, can directly activate antigen-specific Th1 or CTLs, which can activate the anti-tumor immune response and are associated with favorable prognosis of patients with many types of cancers[8,9,20,21]. Early studies also demonstrated a significant association between tumor-infiltrating $CD1a^+$ DCs and unfavorable prognosis in patients with colorectal cancer and lung cancer[8,9,20]. In contrast, high expression of CD208 or CD86 in DCs has been found to contribute to a better prognosis in colorectal cancer, gastric cancer, and malignant

Table 5 Comparison of HRs and association between CD11c expression and the risk of death and relapse in different Cox models

Cox models	CD11c expression	HR	95%CI	*P* value
OS				
Model 1[1]	Low	1.00 (ref)		
	High	0.56	0.33-0.98	0.041
Model 2[2]	Low	1.00 (ref)		
	High	0.90	0.49-1.67	0.749
DFS				
Model 3[1]	Low	1.00 (ref)		
	High	0.39	0.23-0.67	0.001
Model 4[2]	Low	1.00 (ref)		
	High	0.65	0.36-1.19	0.160

[1]Adjusted for gender, age, tumor location, histological type, pathological grade and UICC stage; [2]Adjusted for gender, age, tumor location, tumor size, histological type, pathological grade and UICC stage. OS: Overall survival; DFS: Disease-free survival.

melanoma[8,22,23]. Based on their polarization, tumor-infiltrating antigen-presenting cells have dual characteristics, which include the capacity to trigger an antitumor or protumor immune response. CD11c is a component of complement receptor 4, which is predominantly expressed on DCs, some macrophages, natural killer (NK) and activated T cells[24-26]. Therefore, the staining scores of CD11c were composed of not only DCs, but also other effector cells in the local tumor microenvironment, such as some macrophages, NK and activated T cells. To the best of our knowledge, a DC-enriched infiltration is necessary for the activation of naive $CD8^+$ T cells *in vivo*. Fahlén-Yrlid *et al*[27] showed that $CD11c^+$DCs can activate $CD4^+$ T cells during the process of mucosal immunization. In addition, CD11c is also expressed on some $CD8^+$ T cells, and these $CD11c^+CD8^+$ T cells sometimes act as immune regulators by suppressing $CD4^+$ T cells and sometimes as immune effectors[28]. Interestingly, their activities are beneficial to the hosts and boost immune potential in the tumor microenvironment in both cases[29].

Our findings suggested that low CD11c expression was associated with the risk of death and relapse in patients with GC, but was not an independent risk factor. This finding may have been caused by the following reasons: Firstly, univariate survival analysis revealed that low CD11c expression in the tumor was significantly associated with a high risk of death and relapse. Secondly, there was a strong correlation between the CD11c score and tumor size, which may have caused multicolinearity in the Cox model, leading to this result. When CD11c expression and tumor size, respectively, were incorporated into the Cox model (adjustments for other variables in the Cox model remained the same: gender, age, tumor location, histological type, pathological grade and UICC stage), high CD11c expression and small tumor size both showed a significant protective effect. However, when these two variables were simultaneously incorporated into the Cox model, the protective effect disappeared. Previous studies have also shown that lower CD11c expression in the tumor is associated with lower infiltration of effector cells[27-29], indicating low control of tumor progression, leading to rapid tumor growth. Consequently, CD11c may be a potential alternative indicator of tumor size. The causal relationship between CD11c expression and tumor progression (tumor size) has not been clarified.

More and more attention is being paid to tumor size, an important characteristic of the tumor, in order to predict tumor burden, prognosis and UICC staging. Some studies suggested that tumor size was closely correlated with the number of metastatic lymph nodes, which also improved the power of UICC staging in predicting 5-year survival in GC, and the change in tumor size at the first follow-up CT was strongly prognostic for DFS and OS in colorectal cancer[30-32]. However, it is difficult to assess tumor size in some relapsed and metastatic patients, and tumor burden is also difficult to evaluate accurately. Thus, CD11c expression might be useful in predicting tumor burden, prognosis and UICC staging, and in helping clinicians to create rational treatment programs.

In summary, low CD11c expression may be a risk factor for relapse and death of patients with GC. In addition, CD11c could act as an alternative index of tumor size or lymph node metastasis in predicting prognosis in patients with GC in special cases.

COMMENTS

Background

CD11c is an antigen receptor predominantly expressed on dendritic cells (DC), to which antigen targeting has been shown to induce robust antigen-specific immune responses. It has been demonstrated that CD11c provided costimulatory signals to activated lymphocytes. The exact roles and regulatory mechanisms of CD11c in the tumor microenvironment have not yet been defined.

Research frontiers

Over the decades, more and more studies have shown that the progression of gastric cancer is closely related to the tumor microenvironment. CD11c is a positive regulator present on $CD11c^+$DCs and activates lymphocytes, leading to amelioration of immune status. Therefore, it is necessary to confirm the influence of C11c on the tumor microenvironment in patients with gastric cancer.

Innovations and breakthroughs

Based on the investigation of clinical characteristics and CD11c expression level in 140 patients with gastric cancer, the authors demonstrated that CD11c expression levels significantly decreased from UICC stage Ⅰ to stage Ⅳ, and patients with high CD11c expression had a reduced risk of death and relapse. These findings suggest that high CD11c expression decreased the risk of death and relapse, and may act as an indicator of favorable prognosis in patients with gastric cancer.

Applications

The results of this study may provide clinicians with information on the influence of CD11c on the tumor microenvironment in patients with gastric cancer, and provide strategies for the treatment of patients with advanced gastric cancer.

CD11c may be regarded as a potential prognostic indicator in patients with gastric cancer.

Terminology

Tumor microenvironment refers to the unique properties of the tissue microenvironment conferred by abnormal interactions between tumor and host cells. The tumor microenvironment is often characterized by hypoxia, nutrient deprivation, acidosis, and aberrant stroma.

Peer-review

This is an interesting study on evaluating prognosis of patients with GC by means of measuring CD11c expression level.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Chen W**, Zheng R, Zhang S, Zhao P, Zeng H, Zou X, He J. Annual report on status of cancer in China, 2010. *Chin J Cancer Res* 2014; **26**: 48-58 [PMID: 24653626 DOI: 10.3978/j.issn.1000-9604.2014.01.08]

3 **Kim HJ**, Karpeh MS. Surgical approaches and outcomes in the treatment of gastric cancer. *Semin Radiat Oncol* 2002; **12**: 162-169 [PMID: 11979417 DOI: 10.1053/srao.2002.30818]

4 **Ali I**, Lone MN, Al-Othman ZA, Al-Warthan A, Sanagi MM. Heterocyclic Scaffolds: Centrality in Anticancer Drug Development. *Curr Drug Targets* 2015; **16**: 711-734 [PMID: 25751009]

5 **Ju X**, Clark G, Hart DN. Review of human DC subtypes. *Methods Mol Biol* 2010; **595**: 3-20 [PMID: 19941102 DOI: 10.1007/978-1-60761-421-0_1]

6 **Steinman RM**, Banchereau J. Taking dendritic cells into medicine. *Nature* 2007; **449**: 419-426 [PMID: 17898760 DOI: 10.1038/nature06175]

7 **Dzionek A**, Sohma Y, Nagafune J, Cella M, Colonna M, Facchetti F, Günther G, Johnston I, Lanzavecchia A, Nagasaka T, Okada T, Vermi W, Winkels G, Yamamoto T, Zysk M, Yamaguchi Y, Schmitz J. BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. *J Exp Med* 2001; **194**: 1823-1834 [PMID: 11748283]

8 **Gulubova MV**, Ananiev JR, Vlaykova TI, Yovchev Y, Tsoneva V, Manolova IM. Role of dendritic cells in progression and clinical outcome of colon cancer. *Int J Colorectal Dis* 2012; **27**: 159-169 [PMID: 22065108 DOI: 10.1007/s00384-011-1334-1]

9 **Sandel MH**, Dadabayev AR, Menon AG, Morreau H, Melief CJ, Offringa R, van der Burg SH, Janssen-van Rhijn CM, Ensink NG, Tollenaar RA, van de Velde CJ, Kuppen PJ. Prognostic value of tumor-infiltrating dendritic cells in colorectal cancer: role of maturation status and intratumoral localization. *Clin Cancer Res* 2005; **11**: 2576-2582 [PMID: 15814636 DOI: 10.1158/1078-0432.CCR-04-1448]

10 **Swartz MA**. Immunomodulatory roles of lymphatic vessels in cancer progression. *Cancer Immunol Res* 2014; **2**: 701-707 [PMID: 25092811 DOI: 10.1158/2326-6066.CIR-14-0115]

11 **Ramanathapuram LV**, Hopkin D, Kurago ZB. Dendritic Cells (DC) Facilitate Detachment of Squamous Carcinoma Cells (SCC), While SCC Promote an Immature CD16(+) DC Phenotype and Control DC Migration. *Cancer Microenviron* 2013; **6**: 41-55 [PMID: 21809059 DOI: 10.1007/s12307-011-0077-4]

12 **Castro FV**, Tutt AL, White AL, Teeling JL, James S, French RR, Glennie MJ. CD11c provides an effective immunotarget for the generation of both CD4 and CD8 T cell responses. *Eur J Immunol* 2008; **38**: 2263-2273 [PMID: 18651710 DOI: 10.1002/eji.200838302]

13 **Tanji Y**, Mizoguchi K, Yoichi M, Morita M, Hori K, Unno H. Fate of coliphage in a wastewater treatment process. *J Biosci Bioeng* 2002; **94**: 172-174 [PMID: 16233288]

14 **Chen J**, Yang J, Jiang J, Zhuang Y, He W. Function and subsets of dendritic cells and natural killer cells were decreased in gastric cancer. *Int J Clin Exp Pathol* 2014; **7**: 8304-8311 [PMID: 25550889]

15 **Okita Y**, Tanaka H, Ohira M, Muguruma K, Kubo N, Watanabe M, Fukushima W, Hirakawa K. Role of tumor-infiltrating CD11b+ antigen-presenting cells in the progression of gastric cancer. *J Surg Res* 2014; **186**: 192-200 [PMID: 24120241 DOI: 10.1016/j.jss.2013.08.024]

16 **Gregori S**, Tomasoni D, Pacciani V, Scirpoli M, Battaglia M, Magnani CF, Hauben E, Roncarolo MG. Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent ILT4/HLA-G pathway. *Blood* 2010; **116**: 935-944 [PMID: 20448110 DOI: 10.1182/blood-2009-07-234872]

17 **Allavena P**, Sica A, Garlanda C, Mantovani A. The Yin-Yang of tumor-associated macrophages in neoplastic progression and immune surveillance. *Immunol Rev* 2008; **222**: 155-161 [PMID: 18364000 DOI: 10.1111/j.1600-065X.2008.00607.x]

18 **Liu YJ**. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. *Cell* 2001; **106**: 259-262 [PMID: 11509173]

19 **Ratzinger G**, Baggers J, de Cos MA, Yuan J, Dao T, Reagan JL, Münz C, Heller G, Young JW. Mature human Langerhans cells derived from CD34+ hematopoietic progenitors stimulate greater cytolytic T lymphocyte activity in the absence of bioactive IL-12p70, by either single peptide presentation or cross-priming, than do dermal-interstitial or monocyte-derived dendritic cells. *J Immunol* 2004; **173**: 2780-2791 [PMID: 15294997]

20 **Al-Shibli K**, Al-Saad S, Donnem T, Persson M, Bremnes RM, Busund LT. The prognostic value of intraepithelial and stromal innate immune system cells in non-small cell lung carcinoma. *Histopathology* 2009; **55**: 301-312 [PMID: 19723145 DOI: 10.1111/j.1365-2559.2009.03379.x]

21 **Mahmoud SM**, Lee AH, Paish EC, Macmillan RD, Ellis IO, Green AR. Tumour-infiltrating macrophages and clinical outcome in breast cancer. *J Clin Pathol* 2012; **65**: 159-163 [PMID: 22049225 DOI: 10.1136/jclinpath-2011-200355]

22 **Ishigami S**, Ueno S, Matsumoto M, Okumura H, Arigami T, Uchikado Y, Setoyama T, Arima H, Sasaki K, Kitazono M, Shinchi H, Kijima Y, Natsugoe S. Prognostic value of CD208-positive cell infiltration in gastric cancer. *Cancer Immunol Immunother* 2010; **59**: 389-395 [PMID: 19760221 DOI: 10.1007/s00262-009-0758-8]

23 **Ladányi A**, Kiss J, Somlai B, Gilde K, Fejos Z, Mohos A, Gaudi I, Tímár J. Density of DC-LAMP(+) mature dendritic cells in combination with activated T lymphocytes infiltrating primary cutaneous melanoma is a strong independent prognostic factor. *Cancer Immunol Immunother* 2007; **56**: 1459-1469 [PMID: 17279413 DOI: 10.1007/s00262-007-0286-3]

24 **Cooney LA**, Gupta M, Thomas S, Mikolajczak S, Choi KY, Gibson C, Jang IK, Danziger S, Aitchison J, Gardner MJ, Kappe SH, Wang R. Short-lived effector CD8 T cells induced by genetically attenuated malaria parasite vaccination express CD11c. *Infect Immun* 2013; **81**: 4171-4181 [PMID: 23980113 DOI: 10.1128/IAI.00871-13]

25 **Chan CW**, Crafton E, Fan HN, Flook J, Yoshimura K, Skarica M, Brockstedt D, Dubensky TW, Stins MF, Lanier LL, Pardoll DM, Housseau F. Interferon-producing killer dendritic cells provide a link between innate and adaptive immunity. *Nat Med* 2006; **12**: 207-213 [PMID: 16444266 DOI: 10.1038/nm1352]

26 **Caminschi I**, Ahmet F, Heger K, Brady J, Nutt SL, Vremec D, Pietersz S, Lahoud MH, Schofield L, Hansen DS, O'Keeffe M, Smyth MJ, Bedoui S, Davey GM, Villadangos JA, Heath WR, Shortman K. Putative IKDCs are functionally and developmentally similar to natural killer cells, but not to dendritic cells. *J Exp Med* 2007; **204**: 2579-2590 [PMID: 17923506 DOI: 10.1084/jem.20071351]

27 **Fahlén-Yrlid L**, Gustafsson T, Westlund J, Holmberg A, Strömbeck A, Blomquist M, MacPherson GG, Holmgren J, Yrlid U. CD11c(high)dendritic cells are essential for activation of CD4+

T cells and generation of specific antibodies following mucosal immunization. *J Immunol* 2009; **183**: 5032-5041 [PMID: 19786541 DOI: 10.4049/jimmunol.0803992]

28 **Choi BK**, Kim YH, Kang WJ, Lee SK, Kim KH, Shin SM, Yokoyama WM, Kim TY, Kwon BS. Mechanisms involved in synergistic anticancer immunity of anti-4-1BB and anti-CD4 therapy. *Cancer Res* 2007; **67**: 8891-8899 [PMID: 17875731 DOI: 10.1158/0008-5472.CAN-07-1056]

29 **Vinay DS**, Kwon BS. CD11c+CD8+ T cells: two-faced adaptive immune regulators. *Cell Immunol* 2010; **264**: 18-22 [PMID: 20620256 DOI: 10.1016/j.cellimm.2010.05.010]

30 **Huang CM**, Xu M, Wang JB, Zheng CH, Li P, Xie JW, Lin JX, Lu J. Is tumor size a predictor of preoperative N staging in T2-T4a stage advanced gastric cancer? *Surg Oncol* 2014; **23**: 5-10 [PMID: 24508061 DOI: 10.1016/j.suronc.2014.01.003]

31 **Lu J**, Huang CM, Zheng CH, Li P, Xie JW, Wang JB, Lin JX. Consideration of tumor size improves the accuracy of TNM predictions in patients with gastric cancer after curative gastrectomy. *Surg Oncol* 2013; **22**: 167-171 [PMID: 23787074 DOI: 10.1016/j.suronc.2013.05.002]

32 **Suzuki C**, Blomqvist L, Sundin A, Jacobsson H, Byström P, Berglund Å, Nygren P, Glimelius B. The initial change in tumor size predicts response and survival in patients with metastatic colorectal cancer treated with combination chemotherapy. *Ann Oncol* 2012; **23**: 948-954 [PMID: 21832285 DOI: 10.1093/annonc/mdr350]

P- Reviewer: Ali I, Merino G, Syed V, Weyemi U **S- Editor**: Ma YJ
L- Editor: O'Neill M **E- Editor**: Ma S

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9413

World J Gastroenterol 2015 August 21; 21(31): 9413-9419
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Presence of c.3956delC mutation in familial adenomatous polyposis patients from Brazil

Caroline Aquino Moreira-Nunes, Diego di Felipe Ávila Alcântara, Sérgio Figueiredo Lima-Júnior, Sandro Roberto de Araújo Cavalléro, Juan Antonio Rey, Giovanny Rebouças Pinto, Paulo Pimentel de Assumpção, Rommel Rodriguez Burbano

Caroline Aquino Moreira-Nunes, Diego di Felipe Ávila Alcântara, Rommel Rodriguez Burbano, Biological Science Institute, Federal University of Pará, Belem 66075110, Brazil

Sérgio Figueiredo Lima-Júnior, Sandro Roberto de Araújo Cavalléro, Paulo Pimentel de Assumpção, Nucleus of Research in Oncology, Federal University of Pará, Belem 66073000, Brazil

Juan Antonio Rey, Molecular Neuro-oncogenetics Laboratory, Research Unit-Unidad de Investigación, Hospital Universitario La Paz, 28046 Madrid, Spain

Giovanny Rebouças Pinto, Genetics and Molecular Biology Laboratory, Federal University of Piaui, Parnaiba 64049-550, Brazil

Author contributions: Moreira-Nunes CA and Alcântara DFA contributed equally to this work; Moreira-Nunes CA, Alcântara DFA, Lima-Júnior SF, Cavalléro SRA, Pinto GR, Rey JA, Assumpção PP and Burbano RR conceived and designed the experiments; Moreira-Nunes CA, Alcântara DFA, Lima-Júnior SF and Cavalléro SRA performed the experiments; Moreira-Nunes CA, Alcântara DFA, Rey JA, Assumpção PP and Burbano RR analyzed the data; Moreira-Nunes CA, Alcântara DFA, Pinto GR and Burbano RR wrote the paper; all authors read and approved the final manuscript.

Supported by Grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (www.cnpq.br), No. 401976/2010-6 and No. 305220/2013-6 (to Burbano RR); and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (www.capes.gov.br), No. PNPD 2810/2011 (to Moreira-Nunes CA).

Institutional review board statement: The study was approved by the Research Ethics Committee of the João de Barros Barreto University Hospital (Belém, Pará, Brazil), approval number: 274/12.

Informed consent statement: All analyzed patients or their guardians signed a consent form, and it was assured that the use of biological material and study participation would not be harmful or negatively influence the patients' treatment.

Conflict-of-interest statement: The authors have no conflicts of interest to declare. The funders of this study had no role in study design, data collection/analysis, publication decisions, or manuscript preparation.

Data sharing statement: No additional data are available.

Correspondence to: Caroline Aquino Moreira-Nunes, PhD, Post-doctoral Research Fellow, Biological Science Institute, Federal University of Pará. Augusto Correa Street 01, Belem 66075110, Brazil. caroline@ufpa.br
Telephone: +559-182285161
Fax: +559-132017102

Received: July 24, 2014
Peer-review started: July 25, 2014
First decision: September 3, 2014
Revised: May 21, 2015
Accepted: June 16, 2015
Article in press: June 16, 2015
Published online: August 21, 2015

Abstract

AIM: To characterize *APC* gene mutations and correlate them with patient phenotypes in individuals diagnosed

with familial adenomatous polyposis (FAP) in northern Brazil.

METHODS: A total of 15 individuals diagnosed with FAP from 5 different families from the north of Brazil were analyzed in this study. In addition to patients with histopathological diagnosis of FAP, family members who had not developed the disease were also tested in order to identify mutations and for possible genetic counseling. All analyzed patients or their guardians signed a consent form approved by the Research Ethics Committee of the João de Barros Barreto University Hospital (Belem, Brazil). DNA extracted from the peripheral blood of a member of each of the affected families was subjected to direct sequencing. The proband of each family was sequenced to identify germline mutations using the Ion Torrent platform. To validate the detected mutations, Sanger sequencing was also performed. The samples from all patients were also tested for the identification of mutations by real-time quantitative polymerase chain reaction using the amplification refractory mutation system.

RESULTS: Through interviews with relatives and a search of medical records, it was possible to construct genograms for three of the five families included in the study. All 15 patients from the five families with FAP exhibited mutations in the *APC* gene, and all mutations were detected in exon 15 of the *APC* gene. In addition to the patients with a histological diagnosis of FAP, family members without disease symptoms showed the mutation in the *APC* gene. In the present study, we detected two of the three most frequent germline mutations in the literature: the mutation at codon 1309 and the mutation at codon 1061. The presence of c.3956delC mutation was found in all families from this study, and suggests that this mutation was introduced in the population of the State of Pará through ancestor immigration (*i.e.*, a *de novo* mutation that arose in one member belonging to this state from Brazil).

CONCLUSION: Regardless of its origin, the c.3956delC mutation is a strong candidate biomarker of this hereditary cancer syndrome in families of northern Brazil.

Key words: Familial adenomatous polyposis; APC; Torrent sequencing; Colorectal cancer

Core tip: In the northern region of Brazil, gastrointestinal tumors are the second most frequent type of cancer among men and the third most frequent among women. These tumors are considered a serious public health problem because they are often diagnosed in advanced stages and have extremely low survival rates. Evaluation of family history to determine the number of relatives affected and genetic screening analysis are important preventive measures to assist in the early diagnosis of patients who have not yet developed the disease, as was the case of some patients analyzed in this study.

Moreira-Nunes CA, Alcântara DFA, Lima-Júnior SF, Cavalléro SRA, Rey JA, Pinto GR, Assumpção PP, Burbano RR. Presence of c.3956delC mutation in familial adenomatous polyposis patients from Brazil. *World J Gastroenterol* 2015; 21(31): 9413-9419 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9413.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9413

INTRODUCTION

Familial adenomatous polyposis (FAP) is a hereditary cancer predisposition syndrome with autosomal dominant inheritance caused by germline mutations, mainly in the adenomatous polyposis coli *(APC)* gene. The main clinical feature of FAP is the development, in the second and third decades of life, of multiple (hundreds to thousands) adenomatous polyps in the colon with the capacity for malignant transformation[1-3].

The tumor suppressor gene *APC*, located on chromosome 5q21, has 16 exons and encodes a 300-kDa protein that participates in the Wnt signaling pathway, which is important in signal transduction and the control of apoptosis[4-6]. *APC* gene inactivation occurs through allelic loss, primarily through mutations, which generally produces a truncated protein lacking the carboxyl-terminal region and loss of function[7,8].

To date, 858 different mutations in the *APC* gene have been recorded in the Human Gene Mutation Database (HGMD). Twenty-three percent of the germline alterations of this gene occur between codons 1055 and 1309. The three most common germline mutations are the deletion of 5 base pairs (bp) from codons 1309 and 1061 and the deletion of 4 bp from codon 1064[9].

The FAP phenotype (number of polyps and disease aggressiveness) can be predicted from the *APC* gene mutation. The FAP phenotypes can be defined as the following: (1) severe/profuse, for mutations located between codons 1250 and 1464; (2) intermediate, for mutations located between codons 158 and 1595, except for mutations located between codons 312 and 412 and for mutations located between codons 1250 and 1464; and (3) attenuated, for mutations located in exon 9 and near the 5′ and 3′ ends of the *APC* gene[10-16].

In the north and northeast regions of Brazil, gastrointestinal tumors are the second most frequent type of cancer among men and the third most frequent among women. These tumors are considered a serious public health problem because they are often diagnosed in advanced stages and have extremely low

Table 1 Patients analyzed in the study

Patients	Gender	Age at diagnosis	Histopathology
Family 1 (FAP1)			
01A	Female	23	FAP
01B	Female	25	FAP
01C	Female	18	FAP
01D	Male	14	FAP
01E	Male	17	FAP
Family 2 (FAP2)			
02F	Female	40	FAP
02G	Male	[1]	FAP
02H	Female	15	FAP
02I	Male	NA[2]	-
Family 3 (FAP3)			
03J	Male	30	FAP
03H	Female	NA	-
03L	Female	NA	-
03M	Female	NA	-
Family 4 (FAP4)			
04N	Male	40	FAP
Family 5 (FAP5)			
05O	Male	25	FAP

[1]Unknown; [2]Patients without disease. FAP: Familial adenomatous polyposis; NA: Not applicable.

survival rates[17].

The aim of this study was to characterize the mutations present in the *APC* gene, correlate them with patient phenotypes, and evaluate genomic alterations in individuals diagnosed with FAP in northern Brazil.

MATERIALS AND METHODS

Ethics statement

The study was approved by the Research Ethics Committee of the João de Barros Barreto University Hospital (Belém, Pará, Brazil; approval number: 274/12). All analyzed patients or their guardians signed a consent form, and it was assured that the use of biological material and study participation would not be harmful or negatively influence the patients' treatment.

Patients

A total of 15 patients belonging to 5 different families were analyzed in this study (Table 1). All patients resided in the State of Pará and were assisted at the Coloproctology Outpatient Clinic of the João de Barros Barreto University Hospital (Belém, Pará, Brazil). Peripheral blood samples were collected from all individuals for analysis.

In addition to patients with histopathological diagnosis of FAP, family members who had not developed the disease were also tested to identify mutations and for possible genetic counseling in the same manner as provided to members of the FAP2 and FAP3 families (Table 1).

DNA extraction

Genomic DNA was extracted from human peripheral blood samples using the QIAamp DNA Blood Kit (Qiagen®), following the manufacturer's instructions.

Sequencing the APC gene

The DNA extracted from the peripheral blood of a member of each of the affected families was subjected to direct sequencing.

Direct sequencing of all exons of the *APC* gene (NM_000038.5) was performed by the next-generation sequencing platform Ion Torrent™ (Life Technologies ™), following the Ion AmpliSeq™ Library Preparation methodology (Life Technologies™).

The primers for detection of changes in the nucleotide sequence of the gene were designed through the Ion Torrent™ platform using the Ion AmpliSeq Designer software (available at: https://www.ampliseq.com).

Analysis of the sequenced data was performed using the analysis software available in the Ion Torrent ™ platform.

Sanger sequencing

To validate the detected mutations, Sanger sequencing was performed using the BigDye Terminator v1.1 Cycle Sequencing Kit. After ethanol purification, the samples were run on an ABI 3730 sequencer. The chromatogram of the Sanger sequencing results was analyzed using Sequencing Analysis *vs* 5.2 Program (Applied Biosystem, United States).

RESULTS

Through interviews with relatives and a search of medical records, it was possible to construct genograms for three of the five families included in the study - FAP1, FAP2, and FAP3 - which, coincidentally, are the families with the largest number of individuals affected by the disease in subsequent generations (Figure 1). The genealogical analysis of the remaining two families was not possible due to a lack of information from patients regarding their relatives.

All 15 patients from the five families with FAP exhibited mutations in the *APC* gene, and all mutations were detected in exon 15 of the *APC* gene. The presence of c.3956delC mutation was found in all families from this study. In addition to the patients with a histological diagnosis of FAP, family members without disease symptoms showed the mutation in the *APC* gene (Table 2).

DISCUSSION

FAP is caused by germline mutations primarily in the *APC* gene and shows autosomal dominant inheritance. The main clinical feature of the disease is the deve-

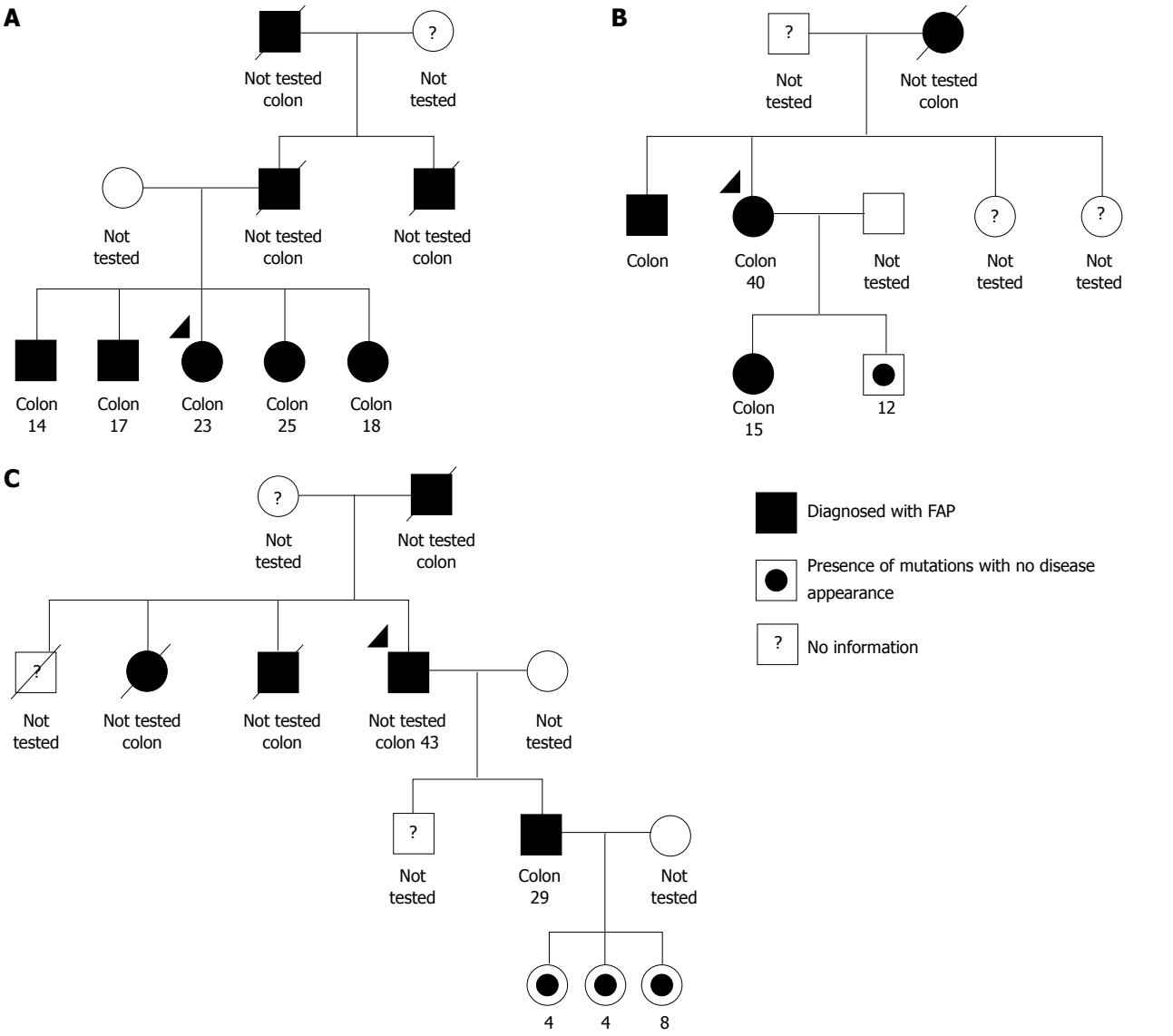


Figure 1 Pedigrees of hereditary diffuse gastric cancer families. A: FAP1 - A family with eight affected members and the presence of *APC* mutations; B: FAP2 - a family with four affected members and the presence of *APC* mutations; C: FAP3 - a family with five affected members with *APC* mutations, and three members with no disease appearance, although with the presence of *APC* mutations. The numbers present under the symbols represent the age at diagnosis. The solid symbols represent the affected members with confirmed adenomatous polyposis diagnoses. Upper left arrows indicate the probands.

lopment, in the second and third decades of life, of multiple (hundreds to thousands) adenomatous polyps in the colon and/or rectum capable of malignant transformation from the fourth decade of life[1-3].

Literature reports reveal that most mutations found in FAP (⩾ 60%) are located in the central region of the protein (between codons 1281-1556), the so-called "mutation cluster region" (MCR). The MCR corresponds to the region of the *APC* gene encoding the protein domain responsible for β-catenin regulation[20,23-25]. Mutations that occur in this region generally produce a truncated protein responsible for an increase in the free pool of β-catenin, which is transported to the nucleus, thus activating the transcription of genes involved in cell proliferation[26]. In this study, all mutations discovered in the families with FAP occurred in the MCR region, confirming this region as a hotspot for mutations in the *APC* gene.

In the present study, we detected two of the three most frequent germline mutations: the mutation at codon 1309 (found in the FAP2 and FAP5 families) and the mutation at codon 1061 (found in the FAP1 family). Half *et al*[2] found an association between the mutation at codon 1309 and 10% of patients with FAP in the literature, which is in agreement with the study by Torrezan *et al*[27], who identified this mutation in 9% of the 23 families with FAP in southeastern Brazil. In the present study, the mutation at codon 1309 occurred in all patients with FAP. This frequency is higher than the global average, and, for this reason, we are currently increasing the sample size of families with FAP to assess the validity of this frequency.

The mutation at codon 1061 affects 5% of patients in the literature, according to the survey performed by

Table 2 Mutations in the *APC* gene: Relationship between genotype and phenotype in patients with familial adenomatous polyposis

Families	Exon	Codon	Mutation	Phenotype[1]	Ref.
			Frameshift mutations		
FAP1	15	1061	c.3183_3187delACAAA	Intermediate	Ficari *et al*[18], Jarry *et al*[19]
FAP2	15	1309	c.3927_3931delAAAGA	Severe	Miyoshi *et al*[20], Jarry *et al*[19]
FAP5	15	1309	c.3921_3925delAAAAG	Severe	Miyaki *et al*[21], Mulkens *et al*[22]
FAP1-FAP5	15	1319	c.3956delC	Severe	Miyaki *et al*[21]

[1]According to Nieuwenhuis *et al*[16].

Half *et al*[2]. This mutation was found in 20% of the five families with FAP from the State of Pará analyzed in this study, although it was not detected in the studies with families from southeastern Brazil performed by Torrezan *et al*[27] and De Queiroz Rossanese *et al*[28]. This result reinforces the difference found between the types of *APC* gene alterations and the geographic region of Brazil to which the FAP patients belong.

FAP is caused by a highly heterogeneous spectrum of mutations, which are shared among patients from different families, as shown in this study[29,30]. In a comprehensive study performed in a Canadian population by Jarry *et al*[19], it was demonstrated that several families with FAP exhibited c.3183_3187delACAAA and c.3927_3931delAAAGA mutations, which were also described in this study.

Through direct sequencing of the *APC* gene, we identified the pathogenic mutation c.3956delC in all families analyzed in this study. This mutation, considered as a frameshift mutation type, and was not found in other Brazilian studies conducted with patients from southeastern Brazil[27,28]. This disparity demonstrates a difference in the spectrum of *APC* gene alterations in families with FAP according to the geographic region of Brazil. This phenomenon most likely occurs because of miscegenation-related ethnic differences between the populations of the south and north of Brazil[31,32].

Thus, the c.3956delC mutation proved to be an important cause of FAP in northern Brazil and results from a founder effect, most likely originating from Japanese communities, where this mutation was first described by Miyaki *et al*[21] in colorectal tumors from three unrelated families. To our knowledge, there are no reports describing the c.3956delC mutation in the germline of families with FAP, as shown in this study.

It is likely that either this germline mutation was introduced into the State of Pará after the 1929 landing of Japanese immigrants in Belém[33] or that c.3956delC is a *de novo* mutation that emerged in a member of the Pará population. Regardless of its origin, the c.3956delC mutation is a strong candidate biomarker for this hereditary cancer syndrome in families of northern Brazil.

According to Crabtree *et al*[34], the presence of a mutation in the MCR region confers to FAP the severe phenotype, regardless of the contribution of mutations characteristic of other phenotypes. In this study, this assumption held because all families with FAP had in common the presence of mutation at codon 1319, in the MCR region.

The genotype-phenotype relationship in FAP is a determining factor for clinical guidance and genetic counseling, as well as for simplifying the search for mutations in patients with FAP and their relatives[14,15,35].

The diagnosis of severe FAP in the study population occurred around the second and third decade of life, while intermediate FAP was diagnosed in the fourth decade of life. This timeline is in accordance with the guidelines for the clinical management of FAP published by Vasen *et al*[36], who demonstrated that the onset of the disease in patients with the severe form of FAP occurred, on average, 10 years before the onset in carriers of intermediate and attenuated forms. As an example, in this study the FAP1 family exhibited the early form of the disease, and one of the members had the syndrome diagnosed at 14 years of age, with the presence of thousands of polyps throughout the intestine. This aggressive phenotype is justified by the presence of the c.3956delC mutation, associated with the severe phenotype. For this reason, and in accordance with the abovementioned guidelines, clinical and diagnostic evaluations should be performed in all FAP family members, starting at the second decade of life.

In conclusion, the presence of the c.3956delC mutation in all families studied leads us to believe that this mutation was introduced in the population of the State of Pará through the immigration of ancestors (*i.e.*, a *de novo* mutation that emerged in a member belonging to this State). Regardless of its origin, the c.3956delC mutation is a strong candidate biomarker of this hereditary cancer syndrome in families of northern Brazil.

It is noteworthy that different germline mutations were found in families of different ethnic groups. Therefore, there is a need to identify the alterations responsible for hereditary cancer syndromes in the Brazilian population, especially in populations of the northern and northeastern regions, where a high incidence of gastrointestinal tumors is observed.

COMMENTS

Background

The main clinical feature of familial adenomatous polyposis (FAP) is the development, in the second and third decades of life, of multiple (hundreds to thousands) adenomatous polyps in the colon with the capacity for malignant transformation. It is diagnosed when a person develops more than 100 adenomatous colon polyps. An adenomatous polyp is an area where normal cells that line the inside of the colon become mucous and form a mass on the inside of the intestinal tract. The average age for polyps to develop in people with FAP is in the mid-teens. More than 95% of individuals with FAP will have multiple colon polyps by 35 years of age.

Research frontiers

The incidence of FAP is associated with mutations in the q21-q22 region of the long arm of chromosome 5 in 80% of patients. From then onwards, large deletions in the *APC* gene germline have been reported in families with FAP from different geographic regions. Screening of family members of patients with FAP should begin by 12 years of age. Genetic testing for germline mutations may eliminate the need for surveillance in some family members. Visualization of more than 100 polyps usually establishes the diagnosis because of the diffuse nature of the polyposis.

Treatment

Cancers of the rectum in patients who have had subtotal colectomy with ileorectal anastomosis have been reported with sulindac and celecoxib therapy. Because of the inability to control polyps medically, eventual rectal resection is usually necessary.

Related reports

The mutations found in this study have been extensively described in patients with colorectal malignancies of different ethnic groups and geographic regions, mainly Asian and European populations. This fact reinforces the role of miscegenation in our study population in the appearance of several germline mutations in patients with FAP.

Term explanation

Next-generation sequencing: refers to non-Sanger-based high-throughput DNA sequencing technologies. Millions or billions of DNA strands can be sequenced in parallel, yielding substantially more throughput and minimizing the need for the fragment-cloning methods that are often used in Sanger sequencing of genomes.

Innovations and breakthroughs

FAP is caused by a highly heterogeneous spectrum of mutations, which are shared among patients from different families, as shown in this study. The identification of alterations responsible for the onset of hereditary gastrointestinal cancer syndrome in patients allows the assessment, through molecular techniques, of whether their relatives are carriers of these alterations and of their risk of developing the syndrome. The genotype-phenotype relationship in FAP is a determining factor for clinical guidance and genetic counseling, as well as for simplifying the search for mutations in patients with FAP and their relatives.

Peer-review

The authors found that the c.3956delC mutation is a strong candidate biomarker of this hereditary cancer syndrome in families of northern Brazil. It is noteworthy that different germline mutations were found in families of different ethnic groups. Therefore, there is a need to identify the alterations responsible for hereditary cancer syndromes in the Brazilian population, especially in populations of the northern and northeastern regions, where a high incidence of gastrointestinal tumors is observed.

REFERENCES

1 **Pineda M**, González S, Lázaro C, Blanco I, Capellá G. Detection of genetic alterations in hereditary colorectal cancer screening. *Mutat Res* 2010; **693**: 19-31 [PMID: 19931546 DOI: 10.1016/j.mrfmmm.2009.11.002]

2 **Half E**, Bercovich D, Rozen P. Familial adenomatous polyposis. *Orphanet J Rare Dis* 2009; **4**: 22 [PMID: 19822006 DOI: 10.1186/1750-1172-4-22]

3 **Hosogi H**, Nagayama S, Kanamoto N, Yoshizawa A, Suzuki T, Nakao K, Sakai Y. Biallelic APC inactivation was responsible for functional adrenocortical adenoma in familial adenomatous polyposis with novel germline mutation of the APC gene: report of a case. *Jpn J Clin Oncol* 2009; **39**: 837-846 [PMID: 19684041 DOI: 10.1093/jjco/hyp093]

4 **Polakis P**. The adenomatous polyposis coli (APC) tumor suppressor. *Biochim Biophys Acta* 1997; **1332**: F127-F147 [PMID: 9196022 DOI: 10.1016/S0304-419X(97)00008-5]

5 **Fearnhead NS**, Britton MP, Bodmer WF. The ABC of APC. *Hum Mol Genet* 2001; **10**: 721-733 [PMID: 11257105 DOI: 10.1093/hmg/10.7.721]

6 **Virmani AK**, Rathi A, Sathyanarayana UG, Padar A, Huang CX, Cunnigham HT, Farinas AJ, Milchgrub S, Euhus DM, Gilcrease M, Herman J, Minna JD, Gazdar AF. Aberrant methylation of the adenomatous polyposis coli (APC) gene promoter 1A in breast and lung carcinomas. *Clin Cancer Res* 2001; **7**: 1998-2004 [PMID: 11448917]

7 **Toyota M**, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, Baylin SB, Issa JP. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Res* 1999; **59**: 5438-5442 [PMID: 10554013]

8 **Lesko AC**, Goss KH, Prosperi JR. Exploiting APC function as a novel cancer therapy. *Curr Drug Targets* 2014; **15**: 90-102 [PMID: 24200292 DOI: 10.2174/1389450114666131108155418]

9 **Plawski A**, Banasiewicz T, Borun P, Kubaszewski L, Krokowicz P, Skrzypczak-Zielinska M, Lubinski J. Familial adenomatous polyposis of the colon. *Hered Cancer Clin Pract* 2013; **11**: 15 [PMID: 24148210 DOI: 10.1186/1897-4287-11-15]

10 **Newton CR**, Graham A, Heptinstall LE, Powell SJ, Summers C, Kalsheker N, Smith JC, Markham AF. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res* 1989; **17**: 2503-2516 [PMID: 2785681 DOI: 10.1093/nar/17.7.2503]

11 **Bunyan DJ**, Shea-Simonds J, Reck AC, Finnis D, Eccles DM. Genotype-phenotype correlations of new causative APC gene mutations in patients with familial adenomatous polyposis. *J Med Genet* 1995; **32**: 728-731 [PMID: 8544194]

12 **Soravia C**, Berk T, Madlensky L, Mitri A, Cheng H, Gallinger S, Cohen Z, Bapat B. Genotype-phenotype correlations in attenuated adenomatous polyposis coli. *Am J Hum Genet* 1998; **62**: 1290-1301 [PMID: 9585611 DOI: 10.1086/301883]

13 **Wallis YL**, Morton DG, McKeown CM, Macdonald F. Molecular analysis of the APC gene in 205 families: extended genotype-phenotype correlations in FAP and evidence for the role of APC amino acid changes in colorectal cancer predisposition. *J Med Genet* 1999; **36**: 14-20 [PMID: 9950360 DOI: 10.1136/jmg.36.1.14]

14 **Groves C**, Lamlum H, Crabtree M, Williamson J, Taylor C, Bass S, Cuthbert-Heavens D, Hodgson S, Phillips R, Tomlinson I. Mutation cluster region, association between germline and somatic mutations and genotype-phenotype correlation in upper gastrointestinal familial adenomatous polyposis. *Am J Pathol* 2002; **160**: 2055-2061 [PMID: 12057910 DOI: 10.1016/S0002-9440(10)61155-8]

15 **Vandrovcová J**, Stekrová J, Kebrdlová V, Kohoutová M. Molecular analysis of the APC and MYH genes in Czech families affected by FAP or multiple adenomas: 13 novel mutations. *Hum Mutat* 2004; **23**: 397 [PMID: 15024739 DOI: 10.1002/humu.9224]

16 **Nieuwenhuis MH**, Vasen HF. Correlations between mutation site in APC and phenotype of familial adenomatous polyposis (FAP): a review of the literature. *Crit Rev Oncol Hematol* 2007; **61**: 153-161 [PMID: 17064931 DOI: 10.1016/j.critrevonc.2006.07.004]

17 **Silva JAG**. Estimate/2012-Cancer Incidence in Brazil. From National Cancer Institute. Rio de Janeiro 2011. Available from: URL: http://www.inca.gov.br/estimativa/2012

18 **Ficari F**, Cama A, Valanzano R, Curia MC, Palmirotta R, Aceto G, Esposito DL, Crognale S, Lombardi A, Messerini L, Mariani-Costantini R, Tonelli F, Battista P. APC gene mutations and colorectal adenomatosis in familial adenomatous polyposis. *Br J Cancer* 2000; **82**: 348-353 [PMID: 10646887 DOI: 10.1054/bjoc.1999.0925]

19 **Jarry J**, Brunet JS, Laframboise R, Drouin R, Latreille J, Richard C, Gekas J, Maranda B, Monczak Y, Wong N, Pouchet C, Zaor S, Kasprzak L, Palma L, Wu MK, Tischkowitz M, Foulkes WD, Chong G. A survey of APC mutations in Quebec. *Fam Cancer* 2011; **10**: 659-665 [PMID: 21779980 DOI: 10.1007/s10689-011-9468-4]

20 **Miyoshi Y**, Ando H, Nagase H, Nishisho I, Horii A, Miki Y, Mori T, Utsunomiya J, Baba S, Petersen G. Germ-line mutations of the APC gene in 53 familial adenomatous polyposis patients. *Proc Natl Acad Sci USA* 1992; **89**: 4452-4456 [PMID: 1316610]

21 **Miyaki M**, Konishi M, Kikuchi-Yanoshita R, Enomoto M, Igari T, Tanaka K, Muraoka M, Takahashi H, Amada Y, Fukayama M. Characteristics of somatic mutation of the adenomatous polyposis coli gene in colorectal tumors. *Cancer Res* 1994; **54**: 3011-3020 [PMID: 8187091]

22 **Mulkens J**, Poncin J, Arends JW, De Goeij AF. APC mutations in human colorectal adenomas: analysis of the mutation cluster region with temperature gradient gel electrophoresis and clinicopathological features. *J Pathol* 1998; **185**: 360-365 [PMID: 9828834 DOI: 10.1002/(SICI)1096-9896(199808)185]

23 **Cheadle JP**, Krawczak M, Thomas MW, Hodges AK, Al-Tassan N, Fleming N, Sampson JR. Different combinations of biallelic APC mutation confer different growth advantages in colorectal tumours. *Cancer Res* 2002; **62**: 363-366 [PMID: 11809680]

24 **De Rosa M**, Scarano MI, Panariello L, Morelli G, Riegler G, Rossi GB, Tempesta A, Romano G, Renda A, Pettinato G, Izzo P. The mutation spectrum of the APC gene in FAP patients from southern Italy: detection of known and four novel mutations. *Hum Mutat* 2003; **21**: 655-656 [PMID: 14961559 DOI: 10.1002/humu.9151]

25 **Christie M**, Jorissen RN, Mouradov D, Sakthianandeswaren A, Li S, Day F, Tsui C, Lipton L, Desai J, Jones IT, McLaughlin S, Ward RL, Hawkins NJ, Ruszkiewicz AR, Moore J, Burgess AW, Busam D, Zhao Q, Strausberg RL, Simpson AJ, Tomlinson IP, Gibbs P, Sieber OM. Different APC genotypes in proximal and distal sporadic colorectal cancers suggest distinct WNT/β-catenin signalling thresholds for tumourigenesis. *Oncogene* 2013; **32**: 4675-4682 [PMID: 23085758 DOI: 10.1038/onc.2012.486]

26 **Minde DP**, Anvarian Z, Rüdiger SG, Maurice MM. Messing up disorder: how do missense mutations in the tumor suppressor protein APC lead to cancer? *Mol Cancer* 2011; **10**: 101 [PMID: 21859464 DOI: 10.1186/1476-4598-10-101]

27 **Torrezan GT**, da Silva FC, Santos EM, Krepischi AC, Achatz MI, Aguiar S, Rossi BM, Carraro DM. Mutational spectrum of the APC and MUTYH genes and genotype-phenotype correlations in Brazilian FAP, AFAP, and MAP patients. *Orphanet J Rare Dis* 2013; **8**: 54 [PMID: 23561487 DOI: 10.1186/1750-1172-8-54]

28 **De Queiroz Rossanese LB**, De Lima Marson FA, Ribeiro JD, Coy CS, Bertuzzo CS. APC germline mutations in families with familial adenomatous polyposis. *Oncol Rep* 2013; **30**: 2081-2088 [PMID: 23970361 DOI: 10.3892/or.2013.2681]

29 **Powell SM**, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, Vogelstein B, Kinzler KW. APC mutations occur early during colorectal tumorigenesis. *Nature* 1992; **359**: 235-237 [PMID: 1528264 DOI: 10.1038/359235a0]

30 **Goss KH**, Groden J. Biology of the adenomatous polyposis coli tumor suppressor. *J Clin Oncol* 2000; **18**: 1967-1979 [PMID: 10784639]

31 **Batista dos Santos SE**, Rodrigues JD, Ribeiro-dos-Santos AK, Zago MA. Differential contribution of indigenous men and women to the formation of an urban population in the Amazon region as revealed by mtDNA and Y-DNA. *Am J Phys Anthropol* 1999; **109**: 175-180 [PMID: 10378456]

32 **Pena SD**, Bastos-Rodrigues L, Pimenta JR, Bydlowski SP. DNA tests probe the genomic ancestry of Brazilians. *Braz J Med Biol Res* 2009; **42**: 870-876 [PMID: 19738982 DOI: 10.1590/S0100-879X2009005000026]

33 **Jordan CF**. Working with Nature: Resource Management for Sustainability. Amsterdam: Harwood Academic Publishers, 1998

34 **Crabtree MD**, Fletcher C, Churchman M, Hodgson SV, Neale K, Phillips RK, Tomlinson IP. Analysis of candidate modifier loci for the severity of colonic familial adenomatous polyposis, with evidence for the importance of the N-acetyl transferases. *Gut* 2004; **53**: 271-276 [PMID: 14724163 DOI: 10.1136/gut.2003.015586]

35 **Munck A**, Gargouri L, Alberti C, Viala J, Peuchmaur M, Lenaerts C, Michaud L, Lamireau T, Mougenot JF, Dabadie A, Maurage C, Lachaux A, Scaillon M, Languepin J, Spyckerelle C, Meyer M, Olschwang S. Evaluation of guidelines for management of familial adenomatous polyposis in a multicenter pediatric cohort. *J Pediatr Gastroenterol Nutr* 2011; **53**: 296-302 [PMID: 21865978 DOI: 10.1097/MPG.0b013e3182198f4d]

36 **Vasen HF**, Möslein G, Alonso A, Aretz S, Bernstein I, Bertario L, Blanco I, Bülow S, Burn J, Capella G, Colas C, Engel C, Frayling I, Friedl W, Hes FJ, Hodgson S, Järvinen H, Mecklin JP, Møller P, Myrhøi T, Nagengast FM, Parc Y, Phillips R, Clark SK, de Leon MP, Renkonen-Sinisalo L, Sampson JR, Stormorken A, Tejpar S, Thomas HJ, Wijnen J. Guidelines for the clinical management of familial adenomatous polyposis (FAP). *Gut* 2008; **57**: 704-713 [PMID: 18194984 DOI: 10.1136/gut.2007.136127]

P- Reviewer: Zhao YS **S- Editor**: Yu J **L- Editor**: Rutherford A
E- Editor: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9420

World J Gastroenterol 2015 August 21; 21(31): 9420-9429
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

SYSTEMATIC REVIEWS

Robotic radiosurgery in pancreatic cancer: A systematic review

Milly Buwenge, Francesco Cellini, Nicola Silvestris, Savino Cilla, Francesco Deodato, Gabriella Macchia, Gian C Mattiucci, Vincenzo Valentini, Alessio G Morganti

Milly Buwenge, Francesco Deodato, Gabriella Macchia, Department of Radiation Oncology, Università Cattolica del Sacro Cuore, 86100 Campobasso, Italy

Francesco Cellini, Radiation Oncology Department, Policlinico Universitario Campus Bio-Medico, 00144 Rome, Italy

Nicola Silvestris, Medical Oncology Unit, National Cancer Research Centre "Giovanni Paolo Ⅱ", 70124 Bari, Italy

Savino Cilla, Department of Medical Physics, Università Cattolica del Sacro Cuore; Fondazione di Ricerca e Cura, 86100 Campobasso, Italy

Gian C Mattiucci, Vincenzo Valentini, Department of Radiation Oncology, Università Cattolica del Sacro Cuore, 00168 Roma, Italy

Alessio G Morganti, Radiation Oncology Unit, Department of Experimental, Diagnostic and Specialty Medicine-DIMES, University of Bologna, S.Orsola-Malpighi Hospital, Bologna 40138, Italy

Author contributions: Buwenge M and Cellini F contributed equally to this work; Morganti AG and Buwenge M designed the research; Buwenge M and Cellini F performed the literature research; Buwenge M, Cellini F, Silvestris N, Cilla S, Deodato F, Macchia G, Mattiucci GC analyzed the data; Buwenge M and Cellini F wrote the paper; Cilla S, Deodato F, Macchia G double-checked the data and analysis; Silvestris N and Mattiucci GC reviewed the paper; Morganti AG and Valentini V supervised the project.

Conflict-of-interest statement: No actual or potential conflicts of interest exist regarding this paper. This article does not contain any studies with human or animal subjects performed by any of the authors.

Data sharing statement: No additional data are available.

Correspondence to: Nicola Silvestris, MD, Medical Oncology Unit, National Cancer Research Centre "Giovanni Paolo Ⅱ", Viale Orazio Flacco, 65, 70124 Bari, Italy. n.silvestris@oncologico.bari.it
Telephone: +39-80-5555419
Fax: +39-80-5555419

Received: September 30, 2014
Peer-review started: October 1, 2014
First decision: November 26, 2014
Revised: December 21, 2014
Accepted: April 3, 2015
Article in press: April 3, 2015
Published online: August 21, 2015

Abstract

AIM: To present a systematic review of techniques and clinical results.

METHODS: A systematic review of published literature was performed. Only studies reporting patient outcome after radiosurgery (single fraction) delivered with robotic devices [*i.e.*, robotic radiosurgery (RRS)] have been analyzed.

RESULTS: A total of 96 patients from 5 studies were included. The studies are characterized by small series and different methods in terms of dose, target definition, combination with chemotherapy and/or standard fractionated radiotherapy and evaluation modalities. Preliminary results are positive in terms of tumor response (ORR = 56%) and local control of the tumor (crude rate of local progressions: 19.5%).

Results for median overall survival (11.4 mo) seem comparable with the ones of prolonged chemoradiation (range: 8.6-13.0 mo). However, gastrointestinal toxicity seems to be the main limitation of RRS, especially at the duodenal level.

CONCLUSION: RRS allows for local treatment in a shortened time (1 fraction) compared to traditional treatments (about 1 mo), providing the possibility for an easy integration with systemic therapies. Preliminary results did not show any outcome differences compared to standard chemoradiation. Thus, further efforts to reduce gastrointestinal toxicity are strongly needed.

Key words: Robotic; Radiosurgery; Pancreatic neoplasms; Systematic review; Review; Stereotactic radiotherapy; Pancreas

Core tip: Robotic radiosurgery, a type of stereotactic body radiotherapy, has been applied in a few experiences as an alternative to long course, conventional radiotherapy. As described in this systematic review, results suggest a good profile of efficacy. Its use in further trials appears justified to treat pancreatic lesions. Particular attention is needed to manage acute and late toxicity. Its potential is highly interesting for the opportunity of integration with chemotherapy and surgery.

Buwenge M, Cellini F, Silvestris N, Cilla S, Deodato F, Macchia G, Mattiucci GC, Valentini V, Morganti AG. Robotic radiosurgery in pancreatic cancer: A systematic review. *World J Gastroenterol* 2015; 21(31): 9420-9429 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9420.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9420

INTRODUCTION

The prognosis of pancreatic cancer is dismal. Even in patients with non-metastatic disease at diagnosis, recurrences after primary therapy are very common both as local relapse/progression and as distant metastases. Local recurrence rates even for patients who have undergone surgery reach percentages of 70%-80%[1,2]. In addition, local disease progression can produce severe symptoms (pain, biliary and/or intestinal obstruction, malnutrition) significantly worsening the quality of life of patients.

Traditionally, radiotherapy (RT) has been used to obtain a local control of the disease. RT, usually associated with concurrent and adjuvant chemotherapy, is potentially useful to improve the resection rate[3], control symptoms in locally advanced carcinomas[4] and to reduce the risk of recurrence in resected patients[5]. The main limitation of RT is the presence of radiosensitive organs in the upper abdomen in close proximity with the pancreas. In fact, due to these anatomic relationships, RT can produce severe side effects especially at the level of the duodenum. Therefore, a strong interest has arisen in the use of RT techniques gaining a higher level of precision with the aim of administering effective doses to the target while reducing the irradiation of surrounding healthy organs.

One of the most promising newer techniques is robotic radiosurgery (RRS). This is a particular technique of stereotactic body radiotherapy (SBRT). Based on American Society of Radiation Oncology definition, SBRT is an external beam technique able to deliver high radiation dose to an extra-cranial body target with high precision in a single or few fractions[6]. RRS is based on the delivery of a single large fraction of radiation using a robotic linear accelerator. The reduced volume of irradiated normal tissue achieved by improving the treatment precision allows the delivery of a single fraction of radiation (with RRS), which can potentially ablate all tissue in the treated area. However, in literature there is currently only limited evidence on SBRT, represented by preliminary studies generally performed on small patient populations[7-16]. Evidence on RRS is even more limited[17-21]. Therefore, the purpose of this analysis is to present a systematic review of the techniques and clinical results of RRS in pancreatic cancer.

MATERIALS AND METHODS

Inclusion criteria

Type of studies: In this review were included all studies (case studies or clinical trials) reporting outcome and toxicity of patients treated with RSS.

Type of participants: Only studies enrolling patients suffering from unresectable and/or locally advanced adenocarcinoma of the pancreas were included in this analysis.

Type of interventions: (1) radiotherapy - eligible interventions were single fractionated radiation therapy performed with a robotic machine; (2) chemotherapy - all systematic treatments based on chemotherapy, regardless of the type of antineoplastic agent and the use of single or combination chemotherapy were eligible; and (3) supportive care - studies were included in the analysis regardless of the type of supportive therapy or other palliative treatments, including blood transfusions, analgesic treatments, bypass palliative interventions or stents placement.

Type of outcome measures: Primary endpoint of the analysis was overall survival after RRS and secondary endpoints were: clinical response, local control, and treatment-related toxicity.

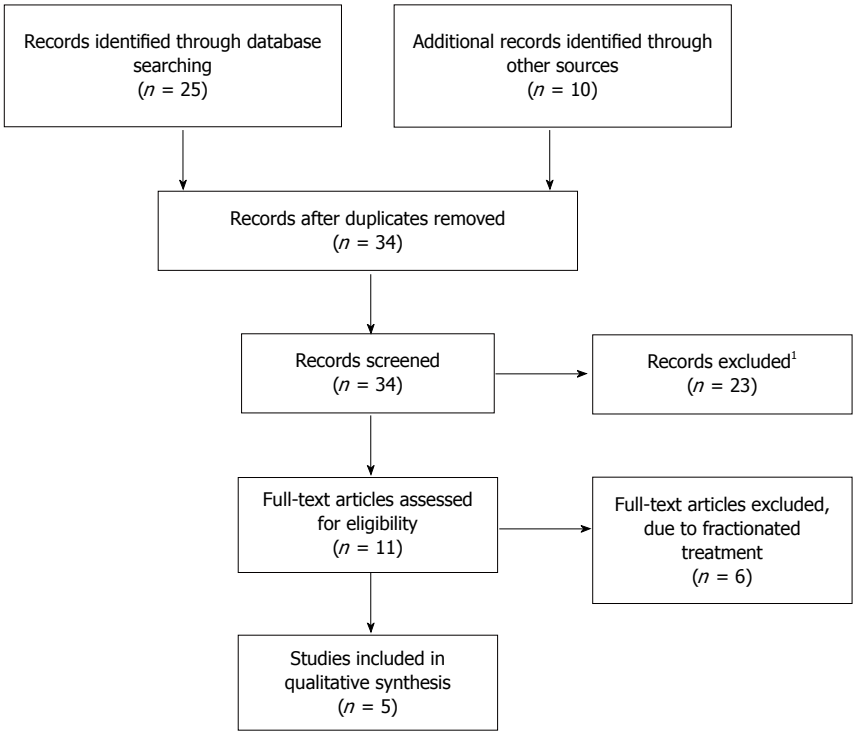


Figure 1 Process of paper selection. Evaluated studies (35); Excluded studies (30); Included studies (5). [1]Studies excluded because they did not individuate all the inclusion criteria after collegial evaluation of the full-text manuscript.

Literature search strategy

A bibliographic research was performed based on the PRISMA methodology[22] using PubMed. In the database, a search was carried out using the Medical Subject Heading (MeSH) database; the search algorithm was "Radiotherapy" [MeSH] AND "Pancreatic" [MeSH] AND ("robotic" OR "cyberknife"). The research in Pubmed was complemented by an additional screening of the references of publication identified through the database. The search was not limited to a particular time interval. It was restricted to English-language peer-reviewed journal publications. The found papers were independently selected and evaluated by two different authors (Buwenge M and Cellini F). Any discrepancies in the selection of papers and data collection were managed by the senior author (AGM). Potentially eligible studies were retrieved and a full-text evaluation was performed as to whether it satisfied both the inclusion and exclusion criteria. Only clinical studies on RRS delivered with robotic devices in patients with pancreatic carcinoma were included in the review process. Studies including patients with metastatic disease were not excluded.

RESULTS

Search results

Through the literature search, performed as previously described, 35 papers were identified. Figure 1 describes the process of paper selection. Five studies fulfilled the inclusion criteria and were included in this review.

Literature review

Koong *et al*[17] designed a phase I trial for patients with locally advanced carcinoma of the pancreas. The purpose of the study was to define the maximum tolerated dose of RRS. The authors enrolled 15 patients at three subsequent dose levels: 15 Gy, 20 Gy, and 25 Gy. Clinical response was evaluated using high resolution CT and acute toxicity was scored with the RTOG scale. Local tumor control was recorded in all 6 patients who received 25 Gy without cases of grade > 3 gastrointestinal toxicity. Given the result in terms of tumor response, the trial was stopped even if no cases of dose-limiting toxicity were recorded. Based on these results, the authors concluded that the recommended dose for the RRS is 25 Gy.

Koong *et al*[18] enrolled 19 patients with locally advanced pancreatic cancer in a subsequent trial. Treatment was based on IMRT (45 Gy with concurrent 5-fluorouracil) followed by RRS boost (25 Gy). Sixteen of 19 patients completed the treatment and only one of these developed local progression of the disease while 2 patients showed grade 3 toxicity. Considering a median survival of only 33 wk, the authors concluded that this treatment regimen although feasible, seems not able to improve survival.

Seo *et al*[20] performed a retrospective analysis of 30 patients with similar stage of disease. These patients underwent conformal radiotherapy (40 Gy) followed by RRS boost (14-17 Gy). Twenty-one

Table 1 Study and treatment characteristics

Ref.	Study design	Inclusion criteria	Simulation	PTV	Image guidance	Dose prescription	Radiotherapy dose, median (range)	% of patients receiving chemotherapy
Koong *et al*[17], 2004	Phase Ⅰ	LA; < 7.5 cm[1]	Pancreatic protocol CT	NR	Fiducials tracking	To isodose surrounding PTV	20 Gy SF RRS	6.6 before RRS
Koong *et al*[18], 2005	Phase Ⅱ	LA	Pancreatic protocol CT	NR	Mid-breath-hold or fiducials tracking	To isodose surrounding PTV	45 Gy in 1.8 Gy/fr (IMRT) +; 25 Gy SF RRS boost	100.0 concurrent to IMRT: 5-FU or CAP
Schellenberg *et al*[19], 2008	Case serie	LA	End-expiration biphasic CT + respiratory gated CT + PET-CT	PTV: GTV + 2-3 mm	Fiducials tracking	To isodose surrounding PTV	25 Gy SF RRS	100.0 1 cycle GEM pre-RRS; 0-8 cycles GEM post-RRS
Seo *et al*[20], 2009	Phase Ⅰ	LA; no duodenal invasion; < 3 N+	CT + PET-CT (restricted respiratory motion)	PTV: GTV + 2 mm, or 4 mm cranio-caudally	Fiducials tracking	To isodose covering 97% of PTV	40 Gy in 2 Gy/fr (3D-CRT) + 16.5 Gy (14-17) SF RRS boost	70.0 (6 before RT; 15 concurrent to 3D-CRT)
Goyal *et al*[21], 2012	Case serie	LA	CT + MRI ± PET-CT	NR	Fiducial tracking	To 70% isodose	14 pts: 25 (20-25) SF RRS 5 pts: 3 fractions (24-30)	68% before RRS (various schedules)

[1]Two patients received previous radiotherapy. 3D-CRT: 3D-conformal radiation therapy; 5-FU: 5-fluorouracil; GEM: Gemcitabine; GTV: Gross tumor volume; IMRT: Intensity modulated radiation therapy; LA: Locally advanced; NR: Not reported; PTV: Planning target volume; RRS: Robotic radiosurgery; SF: Single fraction.

patients had received chemotherapy. One-year local progression-free survival (LPFS) was 70.2% and 1-year overall survival (OS) was 60.0%. Given an incidence of grade 4 toxicity of only 3%, the authors concluded that this regimen is feasible and deserves further evaluation in prospective studies.

Goyal *et al*[21] reported their experience with RRS in patients with unresectable pancreatic cancer. Twenty patients received a RRS dose of 22-30 Gy (median 25 Gy). Chemotherapy was administered in 68% of patients. One-year LPFS was 65% and 1-year OS was 56%. The incidence of grade 3 and grade 4 toxicity was 16% and 0%, respectively. The authors concluded that RRS treatment is tolerable and allows satisfactory local control of the disease.

The details on definition of "locally advanced inoperable lesions" criteria were not specified for all the above previously mentioned reports.

Schellenberg *et al*[19] designed a prospective trial to test a combined modality treatment including RRS. Sixteen patients with locally advanced disease (defined as "> 50% involvement of the superior mesenteric vein/superior mesenteric artery or any involvement of the celiac axis") received 1-9 cycles (median: 4) of gemcitabine-based chemotherapy. Patients underwent RRS (25 Gy) between the 1st and 2nd chemotherapy cycles. Local progression of the disease was observed in 19% of patients and 1-year OS was 50% (median: 11.4 mo). The incidence of grade 3 acute toxicity was only 6%. However, 7 patients showed late toxicity: 5 ulcers, 1 duodenal stenosis, and 1 duodenal perforation. In this group of 7 patients there was a trend toward a greater irradiated volume of the duodenum. The authors concluded that using this regimen, the clinical outcome is similar to standard concurrent chemoradiation but with an alarming incidence of intestinal toxicity.

Analysis of the selected studies

Methods: Table 1 shows study and treatment characteristics of the analyzed series. Of the 5 studies included in this analysis, 2 were phase Ⅰ trials, 2 were case series and 1 was a phase Ⅱ trial. The number of patients enrolled was 15-30 (median: 19). In a study in which RRS was used as a boost, only 16/19 patients (84.2%) received the RRS treatment[18]. In another study, 14 patients (73.7%) received RRS treatment, while another 5 patients received a fractionated (3 fractions) treatment[21]. All studies enrolled patients with locally advanced disease. In one study, patients who had received prior radiotherapy were explicitly excluded[20], while in another 2 studies some patients were previously irradiated in the same site[17,21]. In addition, in one study, patients with tumor diameter > 7.5 cm were excluded[17], while in another study patients with more than 3 metastatic lymph nodes or tumor invasion of the duodenum were excluded[20]. The definition of the target was based on a pancreatic protocol CT in 2 studies[17,18], while in other 3 studies even ^{18}F-FDG-PET was used[19-21]. In 3 studies the margins between tumor and PTV were not described[17,18,21]. In the study of Schellenberg a margin of 2-3 mm between GTV and PTV was used[19], while in the study of Seo the PTV was defined as GTV + 2 mm in radial direction and as GTV + 4 mm in cranio-caudal direction[20]. In all studies, an image-guided technique based on fiducial tracking was used. The dose was prescribed to different points. In 3 studies, the dose was prescribed to the isodose completely surrounding the tumor[17-19]. In 1 study,

Table 2 Patient and tumor characteristics

Ref.	Patients	Stage	Median GTV size (cm^3); mean (range)	Site	Median follow-up (mo), range
Koong *et al*[17], 2004	15	NR	29 (19-72)	H: 66.6%, B: 26.6%, T: 6.6%	5.0
Koong *et al*[18], 2005	19[1]	NR	50 (14-92)	H: 68%, B: 32%	5.4
Schellenberg *et al*[19], 2008	16	NR	PTV: 48 (21-84)	H: 87.5%, B: 12.5%	NR
Seo *et al*[20], 2009	30	T4: 100.0% N1: 30.0%	41 (21-96)	H: 56.7% B/T: 43.3%	14.5
Goyal *et al*[21], 2012	19	M1: 4 pts	57 (10-118)	NR	9 (5.8-23.1)

[1]Sixteen patients received RRS. B: Body of the pancreas; H: Head of the pancreas; NR: Not reported; PTV: Planning target volume; T: Tail of the pancreas.

Table 3 Results

Ref.	Tumor response criteria	Tumor response (%)	Median overall survival (mo)	Local control	Toxicity scale	Grade 3-4 toxicity
Koong *et al*[17], 2004	-	NR	11.0	LP: 20%[1]	RTOG	0%
Koong *et al*[18], 2005	-	NR	7.7; 1-yr: 15%	LP: 6.2%[2]	RTOG	Acute: Gastroparesis:10.5%[3]
Schellenberg *et al*[19], 2008	-	NR	11.4; 1 yr: 50%	LP: 19%	CTC 3.0	Acute: gastric ulcer: 6.2%; Late: duodenal stenosis: 6.2%; Duodenal perforation: 6.2%[4]
Seo *et al*[20], 2009	RECIST	68 (PR: 68)	14.0; 1 yr: 60%	LP: 44% LPFS (1-yr): 70.2 %;	RTOG	Acute: duodenal obstruction: 3.3%; Late: 0%
Goyal *et al*[21], 2012	RECIST	44 (CR: 13, PR: 31)	14.4[5]; 1 yr: 56%	LPFS (1-yr): 65% LPFS (median): 11.4 mo	CTC 3.0	GI ulcer: 16%[6]

[1]Local progressions: 0% in patients receiving 25 Gy; [2]In patients receiving RRS; [3]Unreported rate of late duodenal ulcers; [4]Five duodenal ulcers were classified G2; [5]14.8 mo in M0 patients; [6]Asymptomatic pyloric ulcer: 5.7%. CR: Complete response; GI: Gastrointestinal; LP: Local progression; LPFS: Local progression free survival; NR: Not reported; PR: Partial response.

the dose was prescribed to the isodose that covered at least 97% of the PTV[20], while in the last study it was prescribed to 70% isodose[21]. In the two studies in which RRS was used as a boost, the median prescribed dose to the target was 25 Gy in 1 study[18] and 16.5 Gy in the other study[20]. In the studies based on RRS alone, the median prescribed dose to the target was 20 Gy in one study[17] and 25 Gy in 2 other studies[19,21]. All patients received chemotherapy in 2 studies[18,19], while in the other 3 studies the percentage of patients treated with chemotherapy was variable (6.6%-70.0%)[17,20,21]. In the 2 studies reporting tumor response, the RECIST criteria were used[20,21]. Toxicity evaluation was performed using the RTOG scale in 3 studies[17,18,21] and the CTC scale in the other 2 studies[19,21].

Results: Table 2 reports patient and tumor characteristics of the selected studies. All studies enrolled patients with locally advanced cancer. However, in 3 trials the clinical stage was not reported[17-19], in 1 study all patients had cT4 tumor stage[20], while in the last study even metastatic patients (4/19: 21.1%) were enrolled[21]. Four studies[17-20] reported the GTV size, which varied between 29.0 and 57.2 cm^3 (median: 45.5 cm^3). In 4 studies, the tumor site in the pancreas was reported[17-20] with a percentage of tumors in the head of the pancreas between 56.7% and 87.5% (median: 67.3%). In 4 papers reporting the median follow-up[17,18,20,21], this was between 5 and 14.5 mo (median: 7.2 mo). Table 3 shows the results of the selected studies.

Only 2 studies showed the results in terms of tumor response. In 1 study, a partial response rate of 68% was reported[20], while in another study a partial response rate of 31% and a complete response rate of 13% (overall response rate: 44%) were reported[21]. The crude percentage of local progressions was reported in 4 studies[17-20] with values ranging from 6.2% to 44.0% (median: 19.5%). Two studies[20,21] also reported 1-year local progression-free survival with values of 65.0% and 70.2%, respectively. All studies reported median survival, ranging from 7.7 mo to 14.4 mo (median 11.4 mo)[17-21]. In addition, 4 studies[18-21] presented the results in terms of 1-year survival, with values ranging from 15% to 60% (median: 53%).

In all studies, the only severe toxicity (grade > 3) recorded was gastrointestinal. Three studies reported cases of obstructive damage: 10.5% of acute gastroparesis[18], 12.5% duodenal stenosis[19] and 3.3% of duodenal obstruction[20]. The other type

of gastrointestinal toxicity was ulcerative: 6.2% of gastric ulcer and 6.2% of duodenal perforation[19] and 16% of gastrointestinal ulcer[21]. It should be noted that, in some cases, other cases of ulcerative damages were reported but classified as grade < 3 toxicity. More specifically, Koong *et al*[18] reported an unknown number of late duodenal ulcers, Schellenberg *et al*[19] reported a percentage 31.2% of G2 ulcers and Goyal *et al*[20] also described 1 case (5.7%) of asymptomatic pyloric ulcer.

DISCUSSION

Pancreatic cancer is highly aggressive, and very little space to provide cure for patients is currently available. Significant improvements in diagnostics for staging, and both local (*i.e.*, surgery and radiotherapy) and systemic treatments (*i.e.*, full dose chemotherapy, molecular and immune response targeted therapies) have been reported, however, during recent years[23]. Prognosis is in general dismal, with overall 5-year survival rates inferior to 20% even for favorable presentations[24,25]. Resections with microscopically-free margins (R0) still represent the optimal chance to achieve best survival rates[26], but apart from upfront resectable presentations (generally representing 10%-20% of cases), rates of R0 resection after neoadjuvant treatments are still suboptimal for locally advanced unresectable (LA) and borderline resectable (BR) lesions[23,27,28]. Radiochemotherapy (RTCT) has the potential to convert both LA (in around 23% to 40%)[3,29] and BR lesions (40% to 54%) to resectable[30,31]. The role played by integrating RTCT into the neoadjuvant schedules was questioned by some recent Phase Ⅲ trials, although with non-definitive results[32,33]. The new integration of modern drugs and modern radiotherapy techniques could enhance the efficacy of RTCT[34,35]. For instance, intensity modulated radiotherapy (IMRT) provided better clinical results in terms of both limitations of treatment toxicity[36] and dose escalation[37]. In this frame, the intriguing potential of stereotatic body radiotherapy (SBRT) to deliver a biologically high effective dose to the tumor, in a much shorter interval (1-5 fractions *vs* 25-30 fractions), almost without interference with full dose chemotherapy opens new perspectives in both research and routine clinical activities[11]. SBRT obtained clinical outcome for survival at least comparable to the literature for LA lesions in some preliminary experiments, and excellent rates of local control over 90%[7]. Moreover, SBRT gained resection rates of up to 56% for BR lesions[38].

SBRT requires less time to be delivered and is easily integrated with systemic therapies, therefore it also has great potential in the palliative use of radiotherapy for such tumors[39].

RRS of pancreatic cancer presents several theoretical advantages. From the radiobiological point of view, the extreme concentration of the dose in the short time could improve the antineoplastic effect by avoiding the risk of tumor repopulation during the treatment. From the technical point of view, the use of robotic equipment is able to produce an extreme spatial concentration of the dose potentially able to reduce the risk of side effects. In addition, the brevity of the treatment favors the integration with systemic treatments, currently considered as a standard therapeutic option in pancreatic cancer. In order to analyze the results available in the scientific literature, a systematic review was performed. Only little evidence was found to be available on this topic. In particular, only five studies were retrieved within the last decade. These studies are methodologically heterogeneous in terms of inclusion criteria, target definition, dose prescription, chemotherapy usage and criteria for toxicity assessment.

The various studies also present obvious methodological limitations: missing study design in 2 trials[19,21], lack of justification of the sample size in the phase Ⅱ study[18], no description of the definition of the target in 3 studies[17,18,21], inclusion of patients with metastases in one study[21], inclusion of patients previously irradiated in two studies[17,21], and shortness of the follow-up (< 10 mo) in 3 studies[17,18,21], which severely limits the reliability of the results, especially in terms of late toxicity and rate of local progression. Regarding the latter, it can be observed that the study with higher incidence of local progression (44%) is the one with the longer follow-up period (14.5 mo)[20]. Even the reporting of results shows obvious shortcomings: lack of description of the case series in terms of tumor stage, lymph nodes in 4 studies[17-20] and tumor site in 1 study[21], no description of follow-up observation time in 1 study[19], no description of tumor response in 3 studies[17-19], no description of local control with actuarial analysis in 3 studies[17-19], and no description of the number of cases of duodenal ulceration in 1 study[18].

With these limitations it is not possible to perform some analyses on the studies evaluated in this review. Indeed, the lack of description of target definition prevents assessment of whether this issue affects toxicity and local control. In addition, the lack of description of tumor response or actuarial local control, along with the variable use of chemotherapy, the inhomogeneity of dose prescription methods and of the doses administered within the different case series, prevents analysis of dose response. However, some considerations may be proposed. The few data available on the response rate (ORR = 44%-68%) appear at least comparable to those on standard treatment based on concurrent chemoradiation with conventional fractionation (0%-36%)[40-42]. Even more interesting are the results in terms of survival (median, 11.4 mo) - quite similar to those recorded with standard treatment (range: 8.6%-13.0%)[32,43-50].

The most negative aspect reported in the analyzed studies is represented by gastrointestinal toxicity,

especially in terms of ulcerations. In particular, the frequency of such complications has reached in some cases very high rates, mostly for the trials adding chemotherapy, while previous irradiation (for treatments applying RRS as boost of dose) did not clearly enhance toxicity. For example, in the series by Schellenberg and colleagues, when grouping all cases of ulceration regardless of the degree assigned by the authors, the rate of ulcerations reached a percentage of 43.7% of the patients[19]. In contrast, there have been no reports of severe hematological toxicity, frequently observed in studies of concurrent chemoradiation (G3-4: 30.9%)[32].

The use of a standard radiation treatment before RRS does not appear to produce significant benefits. In fact, of the 2 studies using RRS as a boost, one is the paper showing the absolute worst results in terms of local progression (44%)[20] and the other one is the study reporting the worst survival (median: 7.7 mo)[18]. There is no clear relationship between dose and survival. The study showing the better survival (median, 14.4 mo) employed relatively low doses compared to other studies (20-25 Gy), with some patients receiving fractionated treatment[21]. On the contrary, in the study with worse survival (median 7.7 mo) the highest dose (IMRT + RRS: 70 Gy) was delivered[18]. As previously noted, it is particularly difficult to identify factors predictive of toxicity. For example, in the study using the highest dose[18] the rate of late ulcerations was not reported. However, it may be noted that in another study using RRS as a boost[20], the complication rate was relatively low (3.3%), with no cases of ulceration. This fact might be related to the relatively low dose of RRS (median: 16.5 Gy) or to the exclusion of patients with tumor invasion of the duodenum.

On the other hand, the study with the highest rate of ulcers (43.7%) and the only described case of duodenal perforation was the one in which all patients received a dose of 25 Gy RRS[19]. The results in terms of survival seem to suggest the usefulness of integrating RRS with chemotherapy. In fact, in the 3 studies with best results in terms of survival (median: 11.4-14.4 mo) chemotherapy was prescribed in 68%-100% of patients[19-21] while in the 2 studies with worse results (median: 7.7-11.0 mo) chemotherapy was used only in 6.6% of patients[17] or only as concomitant therapy to IMRT[18]. Furthermore, it is also possible that the imaging methods used in staging and planning affected treatment outcome due to a better patient selection. In fact, it can be noted that in the same 3 studies with improved survival[19-21] RRS simulation and planning were based both on pancreatic protocol CT and on ^{18}F-FDG-PET.

The overall conclusion is that future studies of RRS or SBRT appear justified in cancers of the pancreas, considering the practical benefits and the preliminary outcomes, similar to those of standard radiotherapy regimens. It would be helpful if these studies could report the dose-volume histogram parameters to allow correlation analysis between dosimetric data and toxicity. In addition, it would be useful if these studies were planned as part of combined modality treatments based on homogeneous and standard chemotherapy regimens. Furthermore, treatment techniques should be optimized to reduce the incidence of gastrointestinal toxicity. Possible strategies for this purpose may be based on the technique of simultaneous integrated boost (SIB), with a possible reduction of the dose in sites most at risk of ulceration (duodenal wall).

One of the most interesting perspectives of concomitant chemoradiation is the possibility of improving the resectability rate in locally advanced tumors[3]. Therefore, further studies might be aimed at assessing the role of RRS for the same purpose, possibly in combination with neoadjuvant chemotherapy. In fact, the brevity of the RRS would be a useful element to integrate local radiotherapy with systemic therapy and to reduce the delay of the surgery. Recent reports suggest achievement of R0 resection rates up to 55% and 10% for BR and LA presentations, respectively, by the use of modern regimens like FOLFIRINOX[51]. To explore the potential of these schedules some trials are ongoing testing the integration of stereotatic treatments with new drugs like Nab-Paclitaxel (Identifier: NCT02241551) or multidrug regimens like FOLFIRINOX (Identifier: NCT01992705) to understand if even better results are achievable and how toxic such combinations could be.

Finally, it is well known that the treatment of pancreatic tumors at an advanced stage has in most cases a palliative aim and that most of these patients suffer from abdominal and lumbar pain. Therefore, it would be useful to evaluate the role of this technique in pain control, even considering the advantage of using a very brief treatment in patients with poor prognosis.

ACKNOWLEDGMENTS

We thank Caroline Oakley (Scientific Direction - National Cancer Research Centre "Giovanni Paolo Ⅱ") for the English Language Assistance.

COMMENTS

Background

Pancreatic cancer is one of the most aggressive neoplastic lesions and its prognosis is usually dismal even for more favorable presentations. In addition, local disease progression can produce severe symptoms (pain, biliary and/or intestinal obstruction, malnutrition) significantly worsening the quality of life of patients. Traditionally, radiotherapy (usually associated with concurrent and/or adjuvant chemotherapy) has been used to obtain a local control of the disease. A conventional treatment course of radiotherapy requires on average 1-1.5 mo. This can sometimes be difficult to integrate with full-dose courses of chemotherapy; thus, it is usually required to choose what approach to perform first. One of the most promising and innovative techniques of radiotherapy is robotic radiosurgery. Many technical features of this type of treatment delivery make it appealing in the subset of primary treatments for pancreatic cancers. Clinical evidence is still preliminary, but extremely promising.

Research frontiers

The major impact in the treatment of pancreatic cancers is nowadays represented by any attempt to improve the resection rates providing microscopically-negative margins. That includes both the development of more effective chemotherapeutic schedules and the optimization of more effective, less toxic radiotherapy treatment courses. The introduction of a radiotherapy technique allowing delivery of an efficient treatment in few or even one single application could potentially lead to an ideal integration of radiotherapy with full-dose chemotherapy.

Innovations and breakthroughs

The described radiotherapy technique (*i.e.*, robotic radiosurgery), from a technological point of view represents the most advanced form of radiotherapy treatment delivery. It requires particular treatment facilities, providing the highest level of precision in targeting the lesions to irradiate. It allows the highest levels of treatment conformation to the target, thus reducing the amount of normal tissue involved in the radiotherapy fields. Moreover, it applies the delivery of high radiotherapy doses in a short time that biologically increases the damage to the tumor.

Applications

The increased level of awareness of the potential efficacy of the described technique will lead to further investigation in the near future of what represents one of the most promising and revolutionary treatment approaches, both regarding its intrinsic efficacy and the possibility of widespread use of a most effective regimen of integration with systemic treatments.

Terminology

Stereotactic body radiotherapy (SBRT): this is an external beam radiotherapy technique able to deliver high radiation dose to an extra-cranial body target with high precision in a single or few fractions. Robotic radiosurgery: this is a specific type of SBRT treating patients by the delivery of a single large fraction of radiation using a robotic linear accelerator. Robotic linear accelerator: this is a particular type of linear accelerator (for radiotherapy), characterized by the highest level of precision obtainable in the visualization of the target, image-guidance of the treatment delivery and device precision to conform the dose.

Peer-review

The authors systematically review currently available evidence on robotic radiosurgery in the treatment of locally advanced pancreatic cancer. The topic is interesting and timely and the literature review has been well conducted, providing interesting points for discussion and inspiration for performing prospective clinical trials with such novel RT delivery approaches.

REFERENCES

1 **Hishinuma S**, Ogata Y, Tomikawa M, Ozawa I, Hirabayashi K, Igarashi S. Patterns of recurrence after curative resection of pancreatic cancer, based on autopsy findings. *J Gastrointest Surg* 2006; **10**: 511-518 [PMID: 16627216 DOI: 10.1016/j.gassur.2005.09.016]

2 **Kayahara M**, Nagakawa T, Ueno K, Ohta T, Takeda T, Miyazaki I. An evaluation of radical resection for pancreatic cancer based on the mode of recurrence as determined by autopsy and diagnostic imaging. *Cancer* 1993; **72**: 2118-2123 [PMID: 8104092]

3 **Morganti AG**, Massaccesi M, La Torre G, Caravatta L, Piscopo A, Tambaro R, Sofo L, Sallustio G, Ingrosso M, Macchia G, Deodato F, Picardi V, Ippolito E, Cellini N, Valentini V. A systematic review of resectability and survival after concurrent chemoradiation in primarily unresectable pancreatic cancer. *Ann Surg Oncol* 2010; **17**: 194-205 [PMID: 19856029 DOI: 10.1245/s10434-009-0762-4]

4 **Morganti AG**, Trodella L, Valentini V, Barbi S, Macchia G, Mantini G, Turriziani A, Cellini N. Pain relief with short-term irradiation in locally advanced carcinoma of the pancreas. *J Palliat Care* 2003; **19**: 258-262 [PMID: 14959596]

5 **Valentini V**, Morganti AG, Macchia G, Mantini G, Mattiucci GC, Brizi MG, Alfieri S, Bossola M, Pacelli F, Sofo L, Doglietto G, Cellini N. Intraoperative radiation therapy in resected pancreatic carcinoma: long-term analysis. *Int J Radiat Oncol Biol Phys* 2008; **70**: 1094-1099 [PMID: 18313525 DOI: 10.1016/j.ijrobp.2007.07.2346]

6 **Potters L**, Kavanagh B, Galvin JM, Hevezi JM, Janjan NA, Larson DA, Mehta MP, Ryu S, Steinberg M, Timmerman R, Welsh JS, Rosenthal SA. American Society for Therapeutic Radiology and Oncology (ASTRO) and American College of Radiology (ACR) practice guideline for the performance of stereotactic body radiation therapy. *Int J Radiat Oncol Biol Phys* 2010; **76**: 326-332 [PMID: 20117285 DOI: 10.1016/j.ijrobp.2009.09.042]

7 **Didolkar MS**, Coleman CW, Brenner MJ, Chu KU, Olexa N, Stanwyck E, Yu A, Neerchal N, Rabinowitz S. Image-guided stereotactic radiosurgery for locally advanced pancreatic adenocarcinoma results of first 85 patients. *J Gastrointest Surg* 2010; **14**: 1547-1559 [PMID: 20839073 DOI: 10.1007/s11605-010-1323-7]

8 **Hoyer M**, Roed H, Sengelov L, Traberg A, Ohlhuis L, Pedersen J, Nellemann H, Kiil Berthelsen A, Eberholst F, Engelholm SA, von der Maase H. Phase-II study on stereotactic radiotherapy of locally advanced pancreatic carcinoma. *Radiother Oncol* 2005; **76**: 48-53 [PMID: 15990186 DOI: 10.1016/j.radonc.2004.12.022]

9 **Lominska CE**, Unger K, Nasr NM, Haddad N, Gagnon G. Stereotactic body radiation therapy for reirradiation of localized adenocarcinoma of the pancreas. *Radiat Oncol* 2012; **7**: 74 [PMID: 22607687 DOI: 10.1186/1748-717X-7-74]

10 **Macchia G**, Morganti AG, Cilla S, Ippolito E, Massaccesi M, Picardi V, Mattiucci GC, Bonomo P, Tambaro R, Pacelli F, Piermattei A, De Spirito M, Valentini V, Cellini N, Deodato F. Quality of life and toxicity of stereotactic radiotherapy in pancreatic tumors: a case series. *Cancer Invest* 2012; **30**: 149-155 [PMID: 22250589 DOI: 10.3109/07357907.2011.640649]

11 **Mahadevan A**, Jain S, Goldstein M, Miksad R, Pleskow D, Sawhney M, Brennan D, Callery M, Vollmer C. Stereotactic body radiotherapy and gemcitabine for locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2010; **78**: 735-742 [PMID: 20171803 DOI: 10.1016/j.ijrobp.2009.08.046]

12 **Mahadevan A**, Miksad R, Goldstein M, Sullivan R, Bullock A, Buchbinder E, Pleskow D, Sawhney M, Kent T, Vollmer C, Callery M. Induction gemcitabine and stereotactic body radiotherapy for locally advanced nonmetastatic pancreas cancer. *Int J Radiat Oncol Biol Phys* 2011; **81**: e615-e622 [PMID: 21658854 DOI: 10.1016/j.ijrobp.2011.04.045]

13 **Polistina F**, Costantin G, Casamassima F, Francescon P, Guglielmi R, Panizzoni G, Febbraro A, Ambrosino G. Unresectable locally advanced pancreatic cancer: a multimodal treatment using neoadjuvant chemoradiotherapy (gemcitabine plus stereotactic radiosurgery) and subsequent surgical exploration. *Ann Surg Oncol* 2010; **17**: 2092-2101 [PMID: 20224860 DOI: 10.1245/s10434-010-1019-y]

14 **Rwigema JC**, Parikh SD, Heron DE, Howell M, Zeh H, Moser AJ, Bahary N, Quinn A, Burton SA. Stereotactic body radiotherapy in the treatment of advanced adenocarcinoma of the pancreas. *Am J Clin Oncol* 2011; **34**: 63-69 [PMID: 20308870 DOI: 10.1097/COC.0b013e3181d270b4]

15 **Rwigema JC**, Heron DE, Parikh SD, Zeh HJ, Moser JA, Bahary N, Ashby K, Burton SA. Adjuvant stereotactic body radiotherapy for resected pancreatic adenocarcinoma with close or positive margins. *J Gastrointest Cancer* 2012; **43**: 70-76 [PMID: 20809393 DOI: 10.1007/s12029-010-9203-7]

16 **Shen ZT**, Wu XH, Li B, Wang L, Zhu XX. Preliminary efficacy of CyberKnife radiosurgery for locally advanced pancreatic cancer. *Chin J Cancer* 2010; **29**: 802-809 [PMID: 20800022]

17 **Koong AC**, Le QT, Ho A, Fong B, Fisher G, Cho C, Ford J, Poen J, Gibbs IC, Mehta VK, Kee S, Trueblood W, Yang G, Bastidas JA. Phase I study of stereotactic radiosurgery in patients with locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2004; **58**: 1017-1021 [PMID: 15001240 DOI: 10.1016/j.ijrobp.2003.11.004]

18 **Koong AC**, Christofferson E, Le QT, Goodman KA, Ho A, Kuo T, Ford JM, Fisher GA, Greco R, Norton J, Yang GP. Phase II study

to assess the efficacy of conventionally fractionated radiotherapy followed by a stereotactic radiosurgery boost in patients with locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2005; **63**: 320-323 [PMID: 16168826 DOI: 10.1016/j.ijrobp.2005.07.002]

19 **Schellenberg D**, Goodman KA, Lee F, Chang S, Kuo T, Ford JM, Fisher GA, Quon A, Desser TS, Norton J, Greco R, Yang GP, Koong AC. Gemcitabine chemotherapy and single-fraction stereotactic body radiotherapy for locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2008; **72**: 678-686 [PMID: 18395362 DOI: 10.1016/j.ijrobp.2008.01.051]

20 **Seo Y**, Kim MS, Yoo S, Cho C, Yang K, Yoo H, Choi C, Lee D, Kim J, Kim MS, Kang H, Kim Y. Stereotactic body radiation therapy boost in locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2009; **75**: 1456-1461 [PMID: 19783379 DOI: 10.1016/j.ijrobp.2009.01.042]

21 **Goyal K**, Einstein D, Ibarra RA, Yao M, Kunos C, Ellis R, Brindle J, Singh D, Hardacre J, Zhang Y, Fabians J, Funkhouser G, Machtay M, Sanabria JR. Stereotactic body radiation therapy for nonresectable tumors of the pancreas. *J Surg Res* 2012; **174**: 319-325 [PMID: 21937061 DOI: 10.1016/j.jss.2011.07.044]

22 **Moher D**, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009; **151**: 264-269, W64 [PMID: 19622511]

23 **Ryan DP**, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. *N Engl J Med* 2014; **371**: 1039-1049 [PMID: 25207767 DOI: 10.1056/NEJMra1404198]

24 **Cleary SP**, Gryfe R, Guindi M, Greig P, Smith L, Mackenzie R, Strasberg S, Hanna S, Taylor B, Langer B, Gallinger S. Prognostic factors in resected pancreatic adenocarcinoma: analysis of actual 5-year survivors. *J Am Coll Surg* 2004; **198**: 722-731 [PMID: 15110805 DOI: 10.1016/j.jamcollsurg.2004.01.008]

25 **Conlon KC**, Klimstra DS, Brennan MF. Long-term survival after curative resection for pancreatic ductal adenocarcinoma. Clinicopathologic analysis of 5-year survivors. *Ann Surg* 1996; **223**: 273-279 [PMID: 8604907]

26 **Wagner M**, Redaelli C, Lietz M, Seiler CA, Friess H, Büchler MW. Curative resection is the single most important factor determining outcome in patients with pancreatic adenocarcinoma. *Br J Surg* 2004; **91**: 586-594 [PMID: 15122610 DOI: 10.1002/bjs.4484]

27 **Huguet F**, Mukherjee S, Javle M. Locally advanced pancreatic cancer: the role of definitive chemoradiotherapy. *Clin Oncol (R Coll Radiol)* 2014; **26**: 560-568 [PMID: 25001636 DOI: 10.1016/j.clon.2014.06.002]

28 **Katz MH**, Crane CH, Varadhachary G. Management of borderline resectable pancreatic cancer. *Semin Radiat Oncol* 2014; **24**: 105-112 [PMID: 24635867 DOI: 10.1016/j.semradonc.2013.11.006]

29 **Laurence JM**, Tran PD, Morarji K, Eslick GD, Lam VW, Sandroussi C. A systematic review and meta-analysis of survival and surgical outcomes following neoadjuvant chemoradiotherapy for pancreatic cancer. *J Gastrointest Surg* 2011; **15**: 2059-2069 [PMID: 21913045 DOI: 10.1007/s11605-011-1659-7]

30 **Katz MH**, Pisters PW, Evans DB, Sun CC, Lee JE, Fleming JB, Vauthey JN, Abdalla EK, Crane CH, Wolff RA, Varadhachary GR, Hwang RF. Borderline resectable pancreatic cancer: the importance of this emerging stage of disease. *J Am Coll Surg* 2008; **206**: 833-846; discussion 846-848 [PMID: 18471707 DOI: 10.1016/j.jamcollsurg.2007.12.020]

31 **Takahashi H**, Ohigashi H, Gotoh K, Marubashi S, Yamada T, Murata M, Ioka T, Uehara H, Yano M, Ishikawa O. Preoperative gemcitabine-based chemoradiation therapy for resectable and borderline resectable pancreatic cancer. *Ann Surg* 2013; **258**: 1040-1050 [PMID: 23799421 DOI: 10.1097/SLA.0b013e31829b3ce4]

32 **Chauffert B**, Mornex F, Bonnetain F, Rougier P, Mariette C, Bouché O, Bosset JF, Aparicio T, Mineur L, Azzedine A, Hammel P, Butel J, Stremsdoerfer N, Maingon P, Bedenne L. Phase III trial comparing intensive induction chemoradiotherapy (60 Gy, infusional 5-FU and intermittent cisplatin) followed by maintenance gemcitabine with gemcitabine alone for locally advanced unresectable pancreatic cancer. Definitive results of the 2000-01 FFCD/SFRO study. *Ann Oncol* 2008; **19**: 1592-1599 [PMID: 18467316 DOI: 10.1093/annonc/mdn281]

33 **Hammel P**, Huguet F, Van Laethem JL, Goldstein D, Glimelius B. Comparison of chemoradiotherapy (CRT) and chemotherapy (CT) in patients with a locally advanced pancreatic cancer (LAPC) controlled after 4 months of gemcitabine with or without erlotinib: final results of the international phase III LAP 07 study. *J Clin Oncol* 2013; **31** Suppl: LBA4003

34 **Faris JE**, Blaszkowsky LS, McDermott S, Guimaraes AR, Szymonifka J, Huynh MA, Ferrone CR, Wargo JA, Allen JN, Dias LE, Kwak EL, Lillemoe KD, Thayer SP, Murphy JE, Zhu AX, Sahani DV, Wo JY, Clark JW, Fernandez-del Castillo C, Ryan DP, Hong TS. FOLFIRINOX in locally advanced pancreatic cancer: the Massachusetts General Hospital Cancer Center experience. *Oncologist* 2013; **18**: 543-548 [PMID: 23657686 DOI: 10.1634/theoncologist.2012-0435]

35 **Von Hoff DD**, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691-1703 [PMID: 24131140 DOI: 10.1056/NEJMoa1304369]

36 **Yovino S**, Poppe M, Jabbour S, David V, Garofalo M, Pandya N, Alexander R, Hanna N, Regine WF. Intensity-modulated radiation therapy significantly improves acute gastrointestinal toxicity in pancreatic and ampullary cancers. *Int J Radiat Oncol Biol Phys* 2011; **79**: 158-162 [PMID: 20399035 DOI: 10.1016/j.ijrobp.2009.10.043]

37 **Ben-Josef E**, Schipper M, Francis IR, Hadley S, Ten-Haken R, Lawrence T, Normolle D, Simeone DM, Sonnenday C, Abrams R, Leslie W, Khan G, Zalupski MM. A phase I/II trial of intensity modulated radiation (IMRT) dose escalation with concurrent fixed-dose rate gemcitabine (FDR-G) in patients with unresectable pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2012; **84**: 1166-1171 [PMID: 22543215 DOI: 10.1016/j.ijrobp.2012.02.051]

38 **Chuong MD**, Springett GM, Freilich JM, Park CK, Weber JM, Mellon EA, Hodul PJ, Malafa MP, Meredith KL, Hoffe SE, Shridhar R. Stereotactic body radiation therapy for locally advanced and borderline resectable pancreatic cancer is effective and well tolerated. *Int J Radiat Oncol Biol Phys* 2013; **86**: 516-522 [PMID: 23562768 DOI: 10.1016/j.ijrobp.2013.02.022]

39 **National Comprehensive Cancer Network**. NCCN clinical practice guidelines in oncology: pancreatic adenocarcinoma. Version 2.2014. Available from: URL: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp

40 **Martin JL**, Harvey HA, Lipton A, Martin R. Combined chemoradiotherapy for unresectable pancreatic cancer. *Am J Clin Oncol* 1999; **22**: 309-314 [PMID: 10362344]

41 **Safran H**, Cioffi W, Iannitti D, Mega A, Akerman P. Paclitaxel and concurrent radiation for locally advanced pancreatic carcinoma. *Front Biosci* 1998; **3**: E204-E206 [PMID: 9792903]

42 A multi-institutional comparative trial of radiation therapy alone and in combination with 5-fluorouracil for locally unresectable pancreatic carcinoma. The Gastrointestinal Tumor Study Group. *Ann Surg* 1979; **189**: 205-208 [PMID: 426553]

43 **Ishii H**, Okada S, Tokuuye K, Nose H, Okusaka T, Yoshimori M, Nagahama H, Sumi M, Kagami Y, Ikeda H. Protracted 5-fluorouracil infusion with concurrent radiotherapy as a treatment for locally advanced pancreatic carcinoma. *Cancer* 1997; **79**: 1516-1520 [PMID: 9118032]

44 Treatment of locally unresectable carcinoma of the pancreas: comparison of combined-modality therapy (chemotherapy plus radiotherapy) to chemotherapy alone. Gastrointestinal Tumor Study Group. *J Natl Cancer Inst* 1988; **80**: 751-755 [PMID: 2898536]

45 **Loehrer PJ**, Powell ME, Cardenes HR, Wagner L, Brell JM, Ramanathan RK, Crane CH, Alberts SR, Benson AB. A randomized phase III study of gemcitabine in combination with

radiation therapy versus gemcitabine alone in patients with localized, unresectable pancreatic cancer. *J Clin Oncol* 2008; **26**: 15S

46 **Aristu J**, Cañón R, Pardo F, Martínez-Monge R, Martin-Algarra S, Manuel Ordoñez J, Villafranca E, Moreno M, Cambeiro M, Azinovic I. Surgical resection after preoperative chemoradiotherapy benefits selected patients with unresectable pancreatic cancer. *Am J Clin Oncol* 2003; **26**: 30-36 [PMID: 12576921]

47 **Adhoute X**, Smith D, Vendrely V, Rault A, Sa Cunha A, Legoux JL, Belleannée G, De Lédinghen V, Couzigou P, Masson B. Subsequent resection of locally advanced pancreatic carcinoma after chemoradiotherapy. *Gastroenterol Clin Biol* 2006; **30**: 224-230 [PMID: 16565654]

48 **Wilkowski R**, Thoma M, Schauer R, Wagner A, Heinemann V. Effect of chemoradiotherapy with gemcitabine and cisplatin on locoregional control in patients with primary inoperable pancreatic cancer. *World J Surg* 2004; **28**: 1011-1018 [PMID: 15573257 DOI: 10.1007/s00268-004-7338-z]

49 **Wilkowski R**, Thoma M, Bruns C, Wagner A, Heinemann V. Chemoradiotherapy with gemcitabine and continuous 5-FU in patients with primary inoperable pancreatic cancer. *JOP* 2006; **7**: 349-360 [PMID: 16832132]

50 **Snady H**, Bruckner H, Cooperman A, Paradiso J, Kiefer L. Survival advantage of combined chemoradiotherapy compared with resection as the initial treatment of patients with regional pancreatic carcinoma. An outcomes trial. *Cancer* 2000; **89**: 314-327 [PMID: 10918161]

51 **Boone BA**, Steve J, Krasinskas AM, Zureikat AH, Lembersky BC, Gibson MK, Stoller RG, Zeh HJ, Bahary N. Outcomes with FOLFIRINOX for borderline resectable and locally unresectable pancreatic cancer. *J Surg Oncol* 2013; **108**: 236-241 [PMID: 23955427 DOI: 10.1002/jso.23392]

P- Reviewer: Milella M, Nakano H, Seicean A **S- Editor**: Ma YJ
L- Editor: Logan S **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9430

World J Gastroenterol 2015 August 21; 21(31): 9430-9436
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Helicobacter pylori and gastric mucin expression: A systematic review and meta-analysis

Yaron Niv

Yaron Niv, Department of Gastroenterology, Rabin Medical Center, Tel Aviv University, Petach Tikva 49100, Israel

Author contributions: Niv Y solely contributed to this paper.

Conflict-of-interest statement: There is no conflict of interest.

Data sharing statement: No additional data available.

Correspondence to: Yaron Niv, MD, AGAF, FACG, Professor, Department of Gastroenterology, Rabin Medical Center, Tel Aviv University, 39 Jabotinski Street, Petach Tikva 49100, Israel. yniv@clalit.org.il
Telephone: +972-3-9377237
Fax: +972-3-9210313

Received: March 7, 2015
Peer-review started: March 11, 2015
First decision: April 13, 2015
Revised: April 22, 2015
Accepted: June 16, 2015
Article in press: June 16, 2015
Published online: August 21, 2015

Abstract

AIM: To investigate the relationship between *Helicobacter pylori* (*H. pylori*) and mucin expression in gastric mucosa.

METHODS: English Medical literature searches were conducted for gastric mucin expression in *H. pylori* infected people *vs* uninfected people. Searches were performed up to December 31th 2014, using MEDLINE, PubMed, EMBASE, Scopus, and CENTRAL. Studies comparing mucin expression in the gastric mucosa in patients positive and negative for *H. pylori* infection, were included. Meta-analysis was performed by using Comprehensive meta-analysis software (Version 3, Biostat Inc., Englewood, NJ, United States). Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated compared mucin expression in individual studies by using the random effects model. Heterogeneity between studies was evaluated using the Cochran *Q*-test, and it was considered to be present if the *Q*-test *P* value was less than 0.10. I^2 statistic was used to measure the proportion of inconsistency in individual studies, with I^2 > 50% representing substantial heterogeneity. We also calculated a potential publication bias.

RESULTS: Eleven studies, which represent 53 sub-studies of 15 different kinds of mucin expression, were selected according to the inclusion criteria. Every kind of mucin has been considered as one study. When a specific mucin has been studied in more than one paper, we combined the results in a nested meta-analysis of this particular mucin: MUC2, MUC6, STn, Paradoxical con A, Tn, T, Type 1 chain mucin, LeA, SLeA, LeB, AB-PAS, MUC1, and MUC5AC. The odds ratio of mucin expression in random analysis was 2.33, 95%CI: 1.230-4.411, *P* = 0.009, higher expression in *H. pylori* infected patients. Odds ratio for mucin expression in *H. pylori* positive patients was higher for MUC6 (9.244, 95%CI: 1.567-54.515, *P* = 0.014), and significantly lower for MUC5AC (0.447, 95%CI: 0.211-0.949, *P* = 0.036). Thus, *H. pylori* infection may increase MUC6 expression and decrease MUC5AC expression by 924% and 52%, respectively.

CONCLUSION: *H. pylori* inhibits MUC5AC expression in the gastric epithelium, and facilitates colonization. In contrast, increased MUC6 expression may help inhibiting colonization, using MUC6 antibiotics properties.

Key words: *Helicobacter pylori*; Gastric mucin; Stomach; Secretion

Core tip: In this meta-analysis we looked at studies that investigated the relationship between *Helicobacter pylori* (*H. pylori*) and mucin expression in the human gastric mucosa. English Medical literature searches were conducted for studies comparing mucin expression in the gastric mucosa in patients positive and negative for *H. pylori* infection. Meta-analysis was performed, and pooled odds ratios were calculated compared mucin expression in individual studies. Eleven studies, which represent 53 sub studies of 15 different kinds of mucin, were found. *H. pylori* inhibited MUC5AC expression and facilitated colonization. In contrast, increased MUC6 expression may help inhibiting colonization, using MUC6 antibiotics properties.

Niv Y. *Helicobacter pylori* and gastric mucin expression: A systematic review and meta-analysis. *World J Gastroenterol* 2015; 21(31): 9430-9436 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9430.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9430

INTRODUCTION

Mucins are the main component of the mucus layer attached to the gastric mucosa. These high molecular weight glycoproteins give the mucus unstirred layer the quality of viscosity and protect the mucosa form bacterial invasion or damage of toxic material, pepsin and acid. Mucin and *Helicobacter pylori* (*H. pylori*) have a complicated relationship. On the one hand this specific bacterium adopted to live in the mucin environment, enable moving in the viscous material by liquefying the surrounding mucin using urease and higher pH, and on the other hand mucin has antibiotic effect against the bug that control its proliferation and aggressiveness[1-3].

There are 3 main mucin types expressed in the gastric mucosa: MUC1, a membrane-bound mucin, and MUC5AC and MUC6 that are secreted mucins. MUC5AC is expressed mainly in the superficial epithelium and MUC6 in the glands[4]. Mucins are heavily glycosylated with sugar side-chains, and relatively stable to the active action of peptidases such as pepsin.

In this meta-analysis we looked at studies that investigated the relationship between *H. pylori* and mucin expression in the gastric mucosa. The possible hypothesis that the bug suppresses mucin synthesis, secretion and expression is controversial and some small studies gave confusing results. Mucin secretion could prevent aggressive behavior of *H. pylori* by inhibition of the bug proliferation and movement, but also supplies a preferred environment for the bug survival, protected from acid and pepsin.

We collected all the relevant studies that looked at mucin expression in the gastric mucosa of *H. pylori* infected patients in comparison with healthy controls.

MATERIALS AND METHODS

Search strategy

English Medical literature searches were conducted for gastric mucin expression in *H. pylori* infected *vs* uninfected people. Searches were performed through December 31th 2014, using MEDLINE, PubMed, EMBASE, Scopus, and CENTRAL. Search terms were: "*Helicobacter pylori*" OR "*H. pylori*" OR "*Helicobacter*" AND "mucin". Hand searches of articles were performed after the initial search, and included article bibliography. Only fully published human studies in English were included (Figure 1).

Study selection

Case-control studies comparing mucin expression in the gastric mucosa in patients positive and in those negative for *H. pylori* infection, were included. *H. pylori* infection should be diagnosed with at least one of the following method: histology, urease test, ^{13}C-urea breath test, stool antigen test or *H. pylori* DNA. We selected only studies that used standard immunohistochemistry with antibodies against mucin proteins, and only those that expressed results by percentage of moderate or strong positive staining. Thus, studies where results were expressed with mean ± SD of staining scores were excluded, since meta-analysis could not be performed. We looked at all sorts of mucins that have been studied, in most of the cases more than one in a single study. In some of the studies mucins were separately measured at the superficial epithelium and the deep glands.

Data extraction

Mucin gene expression in the gastric mucosa compared quantitatively between the groups: patients with and without *H. pylori* infection. In the first run we considered every study where more than one mucin compared as a composite of several studies, and calculated all the sub-studies together. Then, in nested calculations, we isolated comparisons of different mucins, combined the sub-studies of different papers.

Statistical analysis

Meta-analysis was performed by using Comprehensive meta-analysis software (Version 3, Biostat Inc., Englewood, NJ, United States). Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated compared mucin expression in individual studies by using the random effects model.

Heterogeneity between studies was evaluated using the Cochran *Q*-test, and it was considered to

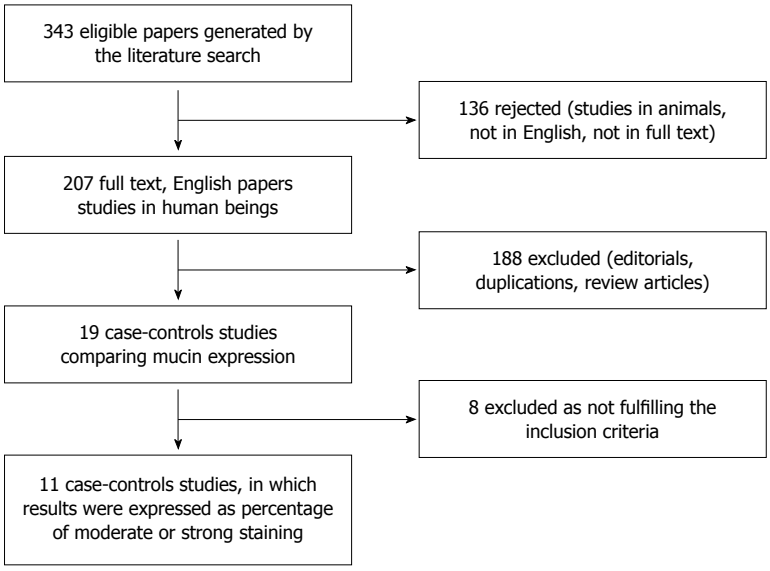


Figure 1 Flow chart of the articles identified in the meta-analysis.

be present if the *Q*-test *P* value was less than 0.10. I^2 statistic was used to measure the proportion of inconsistency in individual studies, with I^2 > 50% representing substantial heterogeneity. We also calculated a potential publication bias.

RESULTS

All together we found 19 studies in human beings that measured gastric mucin staining intensity, using immunohistochemistry, and compared the staining score between positive and negative *H. pylori* patients (Figure 1)[5-21]. Eight studies were excluded for using average score and standard deviation for comparison of *H. pylori* positive and negative states, thus, meta-analysis could not be performed. From the results of these papers we could not retrieve the relative strength of each study as we could for studies where results were expressed as percentage of moderate or strong staining. We were left with 11 studies that fulfilled the inclusion criteria, published between 1997 to 2012 from 10 countries, 1 from United States, 1 from Argentina, 4 from Europe (Italy, Portugal, England and Turkey) and 5 from Asia (2 Japan, 1 Hong Kong, 1 China, 1 Israel).

Each study looked at 1 to 7 different kinds of mucin. All together 15 kinds of mucin were studies: MUC1, MUC2, MUC3, MUC5AC, MUC6, Paradoxical con A (PcA), Tn Ag (Tn), Sialyl Tn Ag (STn), T Ag (T), Type 1 chain mucin (T1), Lewis A Ag (LeA), Sialyl Lewis A Ag (SLeA), Lewis B Ag (LeB), T Ag after treatment with neuroaminidase (TN), AB-PAS positive (Figure 2).

Every kind of mucin has been considered as one study, thus we had 53 studies investigated 15 different mucins (Figure 2A). When a specific mucin has been studied in more than one paper, we combined the results in a nested meta-analysis of this particular mucin: MUC2 (3 papers, 4 studies) (Figure 3A), MUC6 (6 papers, 11 studies) (Figure 3B), STn (4 papers, 5 studies) (Figure 3C), Paradoxical con A, Tn, T, Type 1 chain mucin, LeA, SLeA, LeB, AB-PAS (5 papers, 12 studies) (Figure 3D), MUC1 (4 papers, 4 studies) (Figure 3E), and MUC5AC (5 papers, 7 studies) (Figure 4).

Eleven papers represent 53 sub-studies of 15 different kinds of mucin expression (Figure 2A). The odds ratio of mucin expression in random analysis was 2.33, 95%CI: 1.230-4.411, P = 0.009, higher in *H. pylori* infected patients. Funnel plot denies a significant publication bias (Figure 2B). There was significant heterogeneity in the included studies: Q = 190.6, df (Q) = 43, I^2 = 77.4, P < 0.0001.

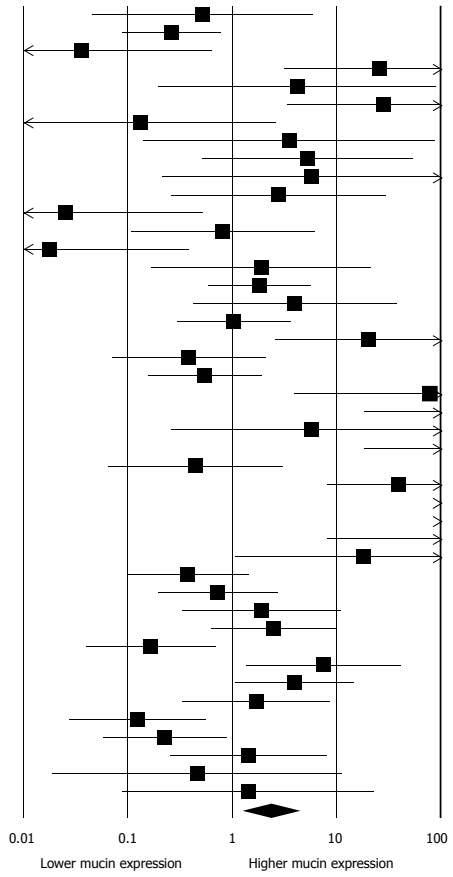
Odds ratio for mucin expression in *H. pylori* positive patients was higher for MUC2 (2.835, 95%CI: 0.890-9.035, P = 0.078; Figure 3A), MUC6 (9.244, 95%CI: 1.567-54.515, P = 0.014; Figure 3B), STn (1.511, 95%CI: 0.106-21.533, P = 0.761; Figure 3C), PcA, Tn, T, T1, LeA, SLeA, LeB, TN, and AB-PAS taken together (2.315, 95%CI: 0.824-6.503, P = 0.111, Figure 3D), and for MUC1 (3.675, 95%CI: 0.208-64.844, P = 0.374; Figure 3E). Odds ratio for mucin expression in *H. pylori* positive patients was lower only for MUC5AC (0.447, 95%CI: 0.211-0.949, P = 0.036; Figure 4). Thus, *H. pylori* infection increased MUC6 expression and decreased MUC5AC expression by 924% and 52%, respectively.

DISCUSSION

Higher mucin expression in the gastric epithelium of *H. pylori* positive patients than in healthy controls was demonstrated. This observation has a limited importance since mucins synthesis in the gastric epithelium is a complex of many processes, and

A

Study name	Subgroup within study	Comparison	Time point	Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Byrd JC	MUC5AC glands	USA	1997	0.519	0.044	6.078	-0.522	0.602
Byrd JC	MUC5AC surface	USA	1997	0.265	0.087	0.802	-2.350	0.019
Byrd JC	MUC6 glands	USA	1997	0.036	0.002	0.660	-2.241	0.025
Byrd JC	MUC6 surface	USA	1997	25.714	3.059	216.134	2.989	0.003
Byrd JC	Paradoxical con A glands	USA	1997	4.245	0.194	93.104	0.918	0.359
Byrd JC	Paradoxical con A surface	USA	1997	28.364	3.276	245.544	3.038	0.002
Byrd JC	Sialyl Tn glands	USA	1997	0.133	0.007	2.697	-1.313	0.189
Byrd JC	STn surface	USA	1997	3.526	0.138	89.981	0.763	0.446
Ota H	Lewis A	Japan	1998	5.250	0.502	54.911	1.384	0.166
Ota H	Lewis B	Japan	1998	5.800	0.210	160.397	1.038	0.299
Ota H	Sia Lewis A	Japan	1998	2.800	0.255	30.703	0.843	0.399
Ota H	STn	Japan	1998	0.025	0.001	0.537	-2.359	0.018
Ota H	T	Japan	1998	0.818	0.106	6.337	-0.192	0.848
Ota H	Tn	Japan	1998	0.018	0.001	0.395	-2.549	0.011
Ota H	Type 1 chain mucin	Japan	1998	1.909	0.164	22.202	0.517	0.605
Barresi G	STn	Italy	2001	1.838	0.576	5.865	1.029	0.304
Barresi G	T Ag	Italy	2001	4.000	0.412	38.844	1.195	0.232
Barresi G	T Ag + neuraminidase	Italy	2001	1.037	0.288	3.736	0.056	0.956
Vinall LE	MUC1	England	2002	20.192	2.505	162.744	2.823	0.005
Vinall LE	MUC5AC	England	2002	0.385	0.069	2.164	-1.083	0.279
Vinall LE	MUC6	England	2002	0.548	0.153	1.966	-0.922	0.356
Cohen M	AB-PAS sulfomucin	Argentina	2003	79.222	3.867	1622.842	2.838	0.005
Cohen M	MUC1	Argentina	2003	961.000	17.909	51566.381	3.380	0.001
Cohen M	MUC2	Argentina	2003	5.741	0.253	130.372	1.097	0.273
Cohen M	STn children	Argentina	2003	961.000	17.909	51566.381	3.380	0.001
Tanaka S	AB-PAS	Japan	2003	0.444	0.063	3.112	-0.817	0.414
Xia HH	MUC6 Antrum Foveola	Hong Kong	2004	39.950	7.935	201.135	4.472	0.000
Xia HH	MUC6 Body Foveola	Hong Kong	2004	5445.000	105.099	282095.366	4.271	0.000
Xia HH	MUC6 Body Glands	Hong Kong	2004	5445.000	105.099	282095.366	4.271	0.000
Xia HH	MUC6 Incisura Foveola	Hong Kong	2004	142.043	7.928	2544.916	3.366	0.001
Xia HH	MUC6 Incisura Glands	Hong Kong	2004	18.333	1.040	323.090	1.987	0.047
Kocer B	MUC1	Turkey	2004	0.375	0.096	1.471	-1.047	0.159
Kocer B	MUC2	Turkey	2004	0.727	0.190	2.790	-0.464	0.642
Marques T	MUC5AC	Portugal	2005	1.900	0.322	11.200	0.709	0.478
Marques T	MUC6	Portugal	2005	2.500	0.609	10.261	1.272	0.203
Wang RQ	MUC1 pericancer mucosa	China	2006	0.167	0.039	0.711	-2.421	0.015
Wang RQ	MUC2 intestinal metaplasia	China	2006	7.600	1.350	42.799	2.300	0.021
Wang RQ	MUC2 pericancer mucosa	China	2006	4.000	1.052	15.207	2.035	0.042
Wang RQ	MUC3 pericancer	China	2006	1.714	0.329	8.943	0.640	0.522
Wang RQ	MUC5AC pericancer	China	2006	0.125	0.027	0.580	-2.657	0.008
Wang RQ	MUC6 pericancer	China	2006	0.227	0.056	0.915	-2.085	0.037
Boltin D	MUC5AC gland	Israel	2012	1.440	0.250	8.292	0.408	0.683
Boltin D	MUC5AC surface	Israel	2012	0.463	0.018	11.678	-0.467	0.640
Boltin D	MUC6 gland	Israel	2012	1.432	0.087	23.637	0.251	0.802
				2.330	1.230	4.411	2.597	0.009



B

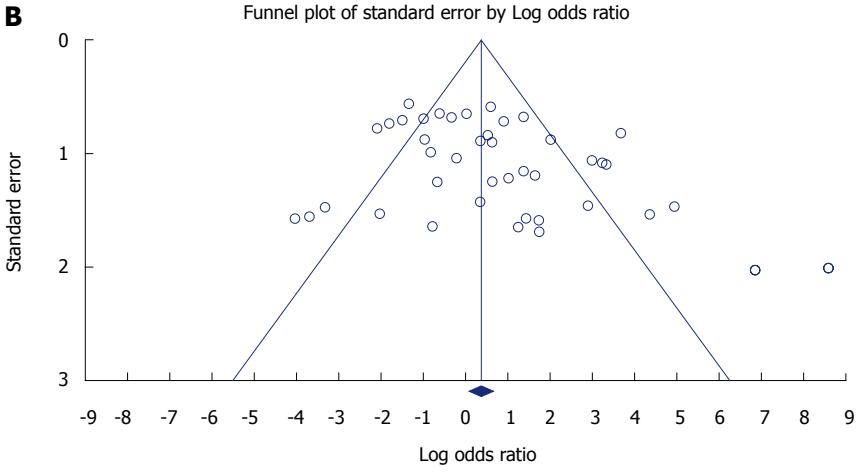
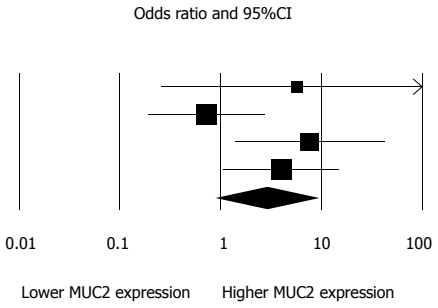


Figure 2 Meta-analysis of studies comparing mucin expression in the gastric epithelium of *Helicobacter pylori* positive and negative patients (A) and funnel plot for publication bias (B). Including 11 papers and 53 studies.

involved different kinds of secreted and membrane-bound mucins. But, nested evaluation of gastric specific mucins, MUC5AC and MUC6 revealed a very interesting observation. The main mucin that protect the gastric surface epithelium is MUC5AC, which also responsible for *H. pylori* adhesion to LeA and LeB antigens[1]. We observed a decrease in MUC5AC expression in *H. pylori* positive patients, explained by the inhibition of galactosyltransferase[2]. The decrease in MUC5AC expression may facilitate *H. pylori* swimming and attaching the epithelium, become closer to the mucosa, and facilitate its nutrition support. On the other hand MUC6 expression increased in *H. pylori* positive patients. MUC6 has an antibiotic effect on *H. pylori*, and may be part of the stomach defensive mechanisms against the bug[3]. Only these changes

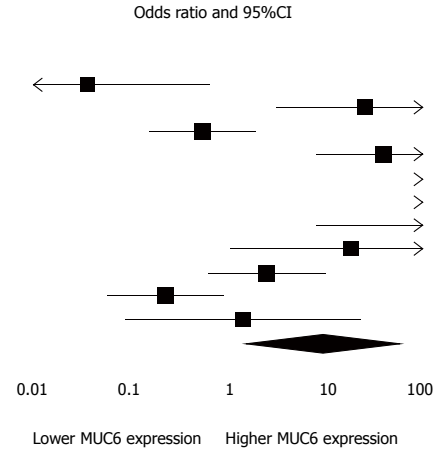
A

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Cohen M	MUC2	Argentina	2003	5.741	0.253	130.372	1.097	0.273
Kocer B	MUC2	Turkey	2004	0.727	0.190	2.790	-0.464	0.642
Wang RQ	MUC2 intestinal metaplasia	China	2006	7.600	1.350	42.799	2.300	0.021
Wang RQ	MUC2 pericancer mucosa	China	2006	4.000	1.052	15.207	2.035	0.042
				2.835	0.890	9.035	1.762	0.078



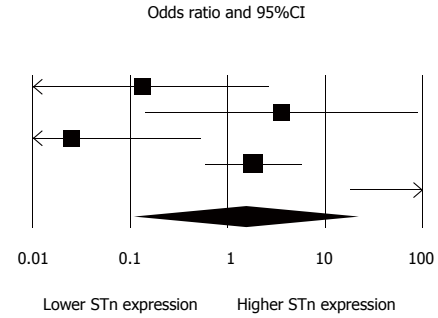
B

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Byrd JC	MUC6 glands	USA	1997	0.04	0.00	0.66	-2.24	0.03
Byrd JC	MUC6 surface	USA	1997	25.71	3.06	216.13	2.99	0.00
Vinall LE	MUC6	England	2002	0.55	0.15	1.97	-0.92	0.36
Xia HH	MUC6 Antrum Foveola	Hong Kong	2004	39.95	7.93	201.13	4.47	0.00
Xia HH	MUC6 Body Foveola	Hong Kong	2004	5445.00	105.10	282095.34	4.27	0.00
Xia HH	MUC6 Body Glands	Hong Kong	2004	5445.00	105.10	282095.34	4.27	0.00
Xia HH	MUC6 Incisura Foveola	Hong Kong	2004	142.04	7.93	2544.92	3.37	0.00
Xia HH	MUC6 Incisura Glands	Hong Kong	2004	18.33	1.04	323.09	1.99	0.05
Marques T	MUC6	Portugal	2005	2.50	0.61	10.26	1.27	0.20
Wang RQ	MUC6 pericancer	China	2006	0.23	0.06	0.92	-2.08	0.04
Boltin D	MUC6 gland	Israel	2012	1.43	0.09	23.64	0.25	0.80
				9.24	1.57	54.52	2.46	0.01



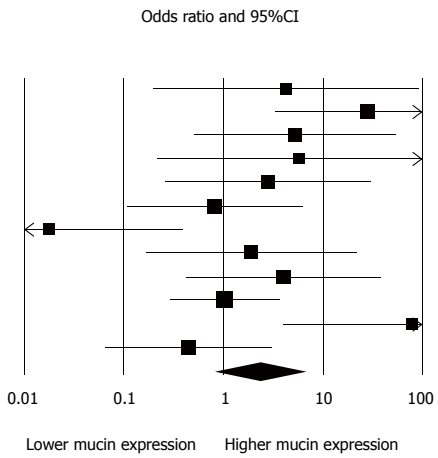
C

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Byrd JC	STn glands	USA	1997	0.133	0.007	2.697	-1.313	0.189
Byrd JC	STn surface	USA	1997	3.526	0.138	89.981	0.763	0.446
Ota H	STn	Japan	1998	0.025	0.001	0.537	-2.359	0.018
Barresi G	STn	Italy	2001	1.838	0.576	5.865	1.029	0.304
Cohen M	STn children	Argentina	2003	961.000	17.909	51566.381	3.380	0.001
				1.511	0.106	21.533	0.304	0.761



D

Study name	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	*Z*-value	*P*-value
Byrd JC	Paradoxical con A glands	USA	1997	4.245	0.194	93.104	0.918	0.359
Byrd JC	Paradoxical con A surface	USA	1997	28.364	3.276	245.544	3.038	0.002
Ota H	Lewis A	Japan	1998	5.250	0.502	54.911	1.384	0.166
Ota H	Lewis B	Japan	1998	5.800	0.210	160.397	1.038	0.299
Ota H	Sia Lewis A	Japan	1998	2.800	0.255	30.703	0.843	0.399
Ota H	T Ag	Japan	1998	0.818	0.106	6.337	-0.192	0.848
Ota H	Tn Ag	Japan	1998	0.018	0.001	0.395	-2.549	0.011
Ota H	Type 1 chain mucin	Japan	1998	1.909	0.164	22.202	0.517	0.605
Barresi G	T Ag	Italy	2001	4.000	0.412	38.844	1.195	0.232
Barresi G	T Ag + neuraminidase	Italy	2001	1.037	0.288	3.736	0.056	0.956
Cohen M	AB-PAS sulfomucin	Argentina	2003	79.222	3.867	1622.842	2.838	0.005
Tanaka S	AB-PAS	Japan	2003	0.444	0.063	3.112	-0.817	0.414
				2.315	0.824	6.503	1.593	0.111



E

Study name	Subgroup within study	Comparison	Time point	Odds ratio	Statistics for each study: Lower limit	Upper limit	*Z*-value	*P*-value
Vinall LE	MUC1	England	2002	20.192	2.505	162.744	2.823	0.005
Cohen M	MUC1	Argentina	2003	961.000	17.909	51566.381	3.380	0.001
Kocer B	MUC1	Turkey	2004	0.375	0.096	1.471	-1.407	0.159
Wang RQ	MUC1 pericancer	China	2006	0.167	0.039	0.711	-2.421	0.015
				3.675	0.208	64.844	0.889	0.374

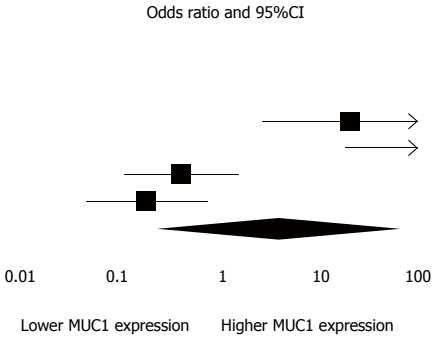


Figure 3 Nested meta-analysis of studies comparing specific mucins in the gastric epithelium demonstrating increased mucin expression in *Helicobacter pylori* positive than in *Helicobacter pylori* negative patients. A: MUC2 (3 papers and 4 studies); B: MUC6 (6 papers and 11 studies); C: STn (4 papers and 5 studies); D: PcA, Tn, T, T1, LeA, SLeA, LeB, TN, AB-PAS (5 papers and 12 studies); E: MUC1 (4 papers, 4 studies).

Study name	Subgroup within study	Comparison	Time point	Odds ratio	Statistics for each study: Lower limit	Upper limit	*Z*-value	*P*-value
Byrd JC	MUC5AC gland	USA	1997	0.519	0.044	6.078	-0.522	0.602
Byrd JC	MUC5AC surface	USA	1997	0.265	0.087	0.802	-2.350	0.019
Vinall LE	MUC5AC	England	2002	0.385	0.069	2.164	-1.083	0.279
Marques T	MUC5AC	Portugal	2005	1.900	0.322	11.200	0.709	0.478
Wang RQ	MUC5AC pericancer	China	2006	0.125	0.027	0.580	-2.657	0.008
Boltin D	MUC5AC gland	Israel	2012	1.440	0.250	8.292	0.408	0.683
Boltin D	MUC5AC surface	Israel	2012	0.463	0.018	11.678	-0.467	0.640
				0.447	0.211	0.949	-2.097	0.036

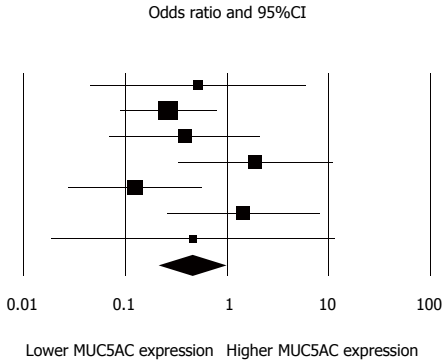


Figure 4 Nested meta-analysis of sub-studies demonstrating decrease MUC5AC expression in the gastric epithelium of *Helicobacter pylori* positive than in *Helicobacter pylori* negative patients. Including 5 papers and 7 studies.

in MUC5AC and MUC6 achieved statistical significance when patients positive and negative for *H. pylori* infection were compared.

Interestingly, there is increase in MUC1 expression in *H. pylori* positive patients (not significant). MUC1 is the main membrane-bound mucin in the gastrointestinal epithelium, and in addition to direct protection against bacteria and toxic material functions as a receptor, with cross talk ability with intracellular, cytoplasmatic proteins such as β-catenin, glycogen synthase kinase, APC and E-cadherin. Increase in MUC1 expression may facilitate the activation of the Wnt pathway and nuclear NF-κB. The effect of *H. pylori* infection on MUC1 should be further explored, since this may be a way for the bug to exert its carcinogenesis potential toward gastric adenocarcinoma or MALT lymphoma.

Other mucins studies did not present a stable direction for mucin expression when *H. pylori* positive and negative patients were compared. Nested collections of these studies for MUC2, STn, Tn, T, T1, TN, PcA, AB-PAS and MUC1 could not reach statistical significance (Figure 2A, 2C-E).

Our paper has several limitations. First, our meta-analysis is based on studies that used different immunohistochemical methods, antibodies against many kinds of mucins that manufactured by different companies. Second, we could only use studies comparing proportion of positive expression, and to exclude 9 papers that compared average scores. Third, the study performed in different populations of patients as well as different *H. pylori* species, about which we have no data. Thus, caution should be taken in interpreting the results.

In conclusion, *H. pylori* may inhibit MUC5AC expression by the human gastric epithelium, and thus facilitate colonization. In contrast, increased MUC6 expression may help inhibiting colonization using MUC6 antibiotics properties.

COMMENTS

Background

Three main mucin types are expressed in the gastric mucosa: MUC1, a membrane-bound mucin, MUC5AC and MUC6, which are secreted mucins. MUC5AC is expressed mainly at the superficial epithelium and MUC6 in the glands. Change in mucin expression was described in *Helicobacter pylori* (*H. pylori*) infection, which may contribute to the bug infectivity and harm for the integrity of gastric mucosa.

Research frontiers

Mucins are high molecular weight glycoproteins which give the mucus unstirred layer of the stomach the quality of viscosity, and protect the mucosa form bacterial invasion. Mucin and *H. pylori* have a complicated relationship. On the one hand Helicobacter pylori is adopted to live in the mucin environment, enable moving in the viscous material by liquefying the surrounding mucin using urease and higher pH, and on the other hand mucin has antibiotic effect

against the bug that control its proliferation and aggressiveness.

Innovation and breakthrough

In this meta-analysis, the authors looked at studies that investigated the relationship between *H. pylori* and mucin expression in the gastric mucosa. The possible hypothesis that the bug suppresses mucin synthesis, secretion and expression is controversial and some small studies gave confusing results. Mucin secretion could prevent aggressive behavior of *H. pylori* by inhibition of the bug proliferation and movement, but also supplies a preferred environment for the bug survival, protected from acid and pepsin.

Applications

The study results suggest that manipulation of mucin secretion by the gastric mucosa may contribute to better eradication therapy of *H. pylori*.

Terminology

H. pylori infection may cause gastritis, duodenitis, gastric ulcer, duodenal ulcer, gastric adenocarcinoma or lymphoma. The mucous layer, composed of mucins, are the barrier from bacterial invasion and have an important role in the body defense mechanisms.

Peer-review

Author presented a well-constructed meta-analysis of studies assessing the effect of Helicobacter pylori on gastric mucin secretion. This study is makes sense of the contradictory results in the literature. The discussion is excellent and elegantly provides a physiological basis for the results observed.

REFERENCES

1 **Niv Y**. H. pylori/NSAID--negative peptic ulcer--the mucin theory. *Med Hypotheses* 2010; **75**: 433-435 [PMID: 20444554 DOI: 10.1016/j.mehy.2010.04.015]

2 **Tanaka S**, Mizuno M, Maga T, Yoshinaga F, Tomoda J, Nasu J, Okada H, Yokota K, Oguma K, Shiratori Y, Tsuji T. H. pylori decreases gastric mucin synthesis via inhibition of galactosyltransferase. *Hepatogastroenterology* 2003; **50**: 1739-1742 [PMID: 14571831]

3 **Kawakubo M**, Ito Y, Okimura Y, Kobayashi M, Sakura K, Kasama S, Fukuda MN, Fukuda M, Katsuyama T, Nakayama J. Natural antibiotic function of a human gastric mucin against Helicobacter pylori infection. *Science* 2004; **305**: 1003-1006 [PMID: 15310903 DOI: 10.1126/science.1099250]

4 **Ho SB**, Takamura K, Anway R, Shekels LL, Toribara NW, Ota H. The adherent gastric mucous layer is composed of alternating layers of MUC5AC and MUC6 mucin proteins. *Dig Dis Sci* 2004; **49**: 1598-1606 [PMID: 15573912]

5 **Byrd JC**, Yan P, Sternberg L, Yunker CK, Scheiman JM, Bresalier RS. Aberrant expression of gland-type gastric mucin in the surface epithelium of Helicobacter pylori-infected patients. *Gastroenterology* 1997; **113**: 455-464 [PMID: 9247464 DOI: 10.1053/gast.1997.v113.pm9247464]

6 **Ota H**, Nakayama J, Momose M, Hayama M, Akamatsu T, Katsuyama T, Graham DY, Genta RM. Helicobacter pylori infection produces reversible glycosylation changes to gastric mucins. *Virchows Arch* 1998; **433**: 419-426 [PMID: 9849856 DOI: 10.1007/s004280050269]

7 **Barresi G**, Giuffrè G, Vitarelli E, Grosso M, Tuccari G. The immunoexpression of Tn, sialyl-Tn and T antigens in chronic active gastritis in relation to Helicobacter pylori infection. *Pathology* 2001; **33**: 298-302 [PMID: 11523928 DOI: 10.1080/00313020126324]

8 **Vinall LE**, King M, Novelli M, Green CA, Daniels G, Hilkens J, Sarner M, Swallow DM. Altered expression and allelic association of the hypervariable membrane mucin MUC1 in Helicobacter pylori gastritis. *Gastroenterology* 2002; **123**: 41-49 [PMID: 12105832 DOI: 10.1053/gast.2002.34157]

9 **Cohen M**, Drut R, Cueto Rúa E. SIALYL-Tn antigen distribution in Helicobacter pylori chronic gastritis in children: an immunohistochemical study. *Pediatr Pathol Mol Med* 2003; **22**: 117-129 [PMID: 12556292 DOI: 10.1080/pdp.22.2.117.129]

10 **Xia HH**, Yang Y, Lam SK, Wong WM, Leung SY, Yuen ST, Elia G, Wright NA, Wong BC. Aberrant epithelial expression of trefoil family factor 2 and mucin 6 in Helicobacter pylori infected gastric antrum, incisura, and body and its association with antralisation. *J Clin Pathol* 2004; **57**: 861-866 [PMID: 15280409 DOI: 10.1136/jcp.2003.015487]

11 **Kocer B**, Ulas M, Ustundag Y, Erdogan S, Karabeyoglu M, Yldrm O, Unal B, Cengiz O, Soran A. A confirmatory report for the close interaction of Helicobacter pylori with gastric epithelial MUC5AC expression. *J Clin Gastroenterol* 2004; **38**: 496-502 [PMID: 15220684 DOI: 10.1097/01.mcg.0000123168.86324.1b]

12 **Marques T**, David L, Reis C, Nogueira A. Topographic expression of MUC5AC and MUC6 in the gastric mucosa infected by Helicobacter pylori and in associated diseases. *Pathol Res Pract* 2005; **201**: 665-672 [PMID: 16325508 DOI: 10.1016/j.prp.2005.03.007]

13 **Wang RQ**, Fang DC. Effects of Helicobacter pylori infection on mucin expression in gastric carcinoma and pericancerous tissues. *J Gastroenterol Hepatol* 2006; **21**: 425-431 [PMID: 16509869 DOI: 10.1111/j.1440-1746.2005.04006.x]

14 **Boltin D**, Halpern M, Levi Z, Vilkin A, Morgenstern S, Ho SB, Niv Y. Gastric mucin expression in Helicobacter pylori-related, nonsteroidal anti-inflammatory drug-related and idiopathic ulcers. *World J Gastroenterol* 2012; **18**: 4597-4603 [PMID: 22969235 DOI: 10.3748/wjg.v18.i33.4597]

15 **Kawano S**, Tsujii M, Nagano K, Ogihara T, Tanimura H, Hayashi N, Ito T, Sato N, Kamada T, Tamura K. Different effect of Helicobacter pylori on the human gastric antral and body mucosal intracellular mucin. *Scand J Gastroenterol* 1990; **25**: 997-1003 [PMID: 1702231 DOI: 10.3109/00365529008997625]

16 **Morgenstern S**, Koren R, Moss SF, Fraser G, Okon E, Niv Y. Does Helicobacter pylori affect gastric mucin expression? Relationship between gastric antral mucin expression and H. pylori colonization. *Eur J Gastroenterol Hepatol* 2001; **13**: 19-23 [PMID: 11204804 DOI: 10.1097/00042737-200101000-00004]

17 **Morgenstern S**, Koren R, Fraser G, Okon E, Niv Y. Gastric corpus mucin expression after partial gastrectomy, in relation to colonization with Helicobacter pylori. *J Clin Gastroenterol* 2001; **32**: 218-221 [PMID: 11246347 DOI: 10.1097/00004836-200103000-00007]

18 **Fichman S**, Niv Y. Histological changes in the gastric mucosa after Helicobacter pylori eradication. *Eur J Gastroenterol Hepatol* 2004; **16**: 1183-1188 [PMID: 15489580 DOI: 10.1097/00042737-200411000-00017]

19 **Vilkin A**, Levi Z, Morgenstern S, Shmuely H, Gal E, Hadad B, Hardi B, Niv Y. Higher gastric mucin secretion and lower gastric acid output in first-degree relatives of gastric cancer patients. *J Clin Gastroenterol* 2008; **42**: 36-41 [PMID: 18097287 DOI: 10.1097/MCG.0b013e3181574d39]

20 **Kang HM**, Kim N, Park YS, Hwang JH, Kim JW, Jeong SH, Lee DH, Lee HS, Jung HC, Song IS. Effects of Helicobacter pylori Infection on gastric mucin expression. *J Clin Gastroenterol* 2008; **42**: 29-35 [PMID: 18097286 DOI: 10.1097/MCG.0b013e3180653cb7]

21 **Niv Y**, Boltin D, Halpern M, Cohen M, Levi Z, Vilkin A, Morgenstern S, Manugian V, St Lawrence E, Gagneux P, Kaur S, Sharma P, Batra SK, Ho SB. Membrane-bound mucins and mucin terminal glycans expression in idiopathic or Helicobacter pylori, NSAID associated peptic ulcers. *World J Gastroenterol* 2014; **20**: 14913-14920 [PMID: 25356051 DOI: 10.3748/wjg.v20.i40.14913]

P- Reviewer: Boltin D, Ierardi E **S- Editor**: Yu J
L- Editor: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9437

World J Gastroenterol 2015 August 21; 21(31): 9437-9441
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Adenocarcinoma of the third and fourth portions of the duodenum: The capsule endoscopy value

Feliciano Chanana Paquissi, Ana Henriqueta Filipe Bunga Pimentel Lima, Maria de Fátima do Nascimento Vieira Lopes, Francisco Viamontes Diaz

Feliciano Chanana Paquissi, Department of Medicine, Clínica Girassol, Luanda 1215, Angola

Ana Henriqueta Filipe Bunga Pimentel Lima, Service of Clinical Pathology, Clínica Girassol, Luanda 1215, Angola

Maria de Fátima do Nascimento Vieira Lopes, Service of Gastroenterology, Clínica Girassol, Luanda 1215, Angola

Francisco Viamontes Diaz, Department of General Surgery, Clínica Girassol, Luanda 1215, Angola

Author contributions: Paquissi FC and Lima AHFBP designed the report; Lopes MFNV performed the analyses of endoscopic aspects of the report; Paquissi FC collected the patient's clinical data; Lima AHFBP performed the analyses of pathological aspects of the report; Diaz FV performed the analyses of surgical aspects of the report; Paquissi FC and Lima AHFBP compiled the data and wrote the paper; all authors read and approved the final manuscript.

Correspondence to: Feliciano Chanana Paquissi, MD, Resident of Department of Medicine, Clínica Girassol, Street Comandante Gika number 225, Luanda 1215, Angola. fepaquissi@gmail.com
Telephone: +55-11-949975972

Received: May 11, 2014
Peer-review started: May 12, 2014
First decision: June 10, 2014
Revised: July 8, 2014
Accepted: August 28, 2014
Article in press: August 28, 2014

Published online: August 21, 2015

Abstract

Primary adenocarcinoma of the small intestine occurs in over 50% of cases in the duodenum. However, its location in the third and fourth duodenal portions occurs rarely and is a diagnostic challenge. The aim of this work is to report an adenocarcinoma of the third and fourth duodenal portions, emphasizing its diagnostic difficulty and the value of video capsule endoscopy. A man, 40 years old, with no medical history, with abdominal discomfort and progressive fatigue, presented four months ago with one episode of moderate melena. The physical examination was normal, except for mucosal pallor. Blood tests were consistent with microcytic, hypochromic iron deficiency anemia with 7.8 g/dL hemoglobin. The upper and lower endoscopy were normal. Additional work-up with video capsule endoscopy showed a polypoid lesion involving the third and fourth portions of the duodenum. Biopsy showed a moderately differentiated adenocarcinoma. Abdominal computed tomography showed a wall thickening from the third duodenal portion to the proximal jejunum, without distant metastasis. The patient underwent segmental resection (distal duodenum and proximal jejunum) with duodenojejunostomy. The surgical specimen histology confirmed the biopsy diagnosis, with transmural infiltration, without nodal involvement. Conclusion: Adenocarcinoma of the third and fourth portions of the duodenum is difficult to diagnose and capsule endoscopy is of great value.

Key words: Duodenum; Duodenal cancer; Adenocarcinoma; Endoscopy; Video capsule endoscopy

Core tip: Third and/or fourth duodenal portion adenocarcinoma is a rare disease, associated with a vague clinical picture and a diagnostic challenge. Capsule endoscopy has shown a higher accuracy compared to conventional endoscopic methods. This case reports the occurrence of adenocarcinoma of the third and fourth duodenal portions and the value of capsule endoscopy to minimize the diagnostic difficulty.

Paquissi FC, Lima AHFBP, Lopes MFNV, Diaz FV. Adenocarcinoma of the third and fourth portions of the duodenum: The capsule endoscopy value. *World J Gastroenterol* 2015; 21(31): 9437-9441 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9437.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9437

INTRODUCTION

The small intestine is approximately 75% of the length and 90% of the mucosal surface of the gastrointestinal tract but represents only 2% to 5% of all primary malignant gastrointestinal tumors. Tumors in the small intestine are about 13 to 18 times less common than colon cancer, despite its exposure to a variety of endogenous and exogenous harmful substances[1,2]. Histologically, there are four subtypes of malignant tumors of the small intestine: adenocarcinomas (around 40%); neuroendocrine tumors (35% to 40%); lymphomas (15%) and sarcomas (11% to 13%)[3,4]. When distributed by segments, adenocarcinomas are more common in the duodenum and proximal jejunum, neuroendocrine tumors and lymphomas are more common in the distal portions, while sarcomas have diffuse distribution[3,5].

Duodenal adenocarcinoma represents approximately 0.5% of all malignant gastrointestinal tumors and the most studied of them are those located in the first and second (most periampullary) portions[6,7]. The location of this tumor in the third and/or fourth duodenal portion is rare, presents with non-specific symptoms and is of difficult diagnosis using conventional endoscopic methods[8]. In this paper is presented an adenocarcinoma of the third and fourth portions of the duodenum, with review of the literature, emphasizing the difficulties and the value of video capsule endoscopy for diagnosis.

CASE REPORT

A man, 40 years old, with no medical history, presented with epigastric and mesogastric discomfort and progressive fatigue, with four months of evolution; and reported an episode of melena in moderate quantity. Physical examination was normal except for mucosal pallor. The laboratory findings were consistent with microcytic, hypochromic iron deficiency anemia with 3.49 × 10^6/mm^3 RBC, 7.8 g/dL hemoglobin, 27.5% hematocrit, 79 fL MCV, 22.5 pg MCH, 28.4 g/dL MCHC, 22.6% RDW, 915000/mm^3 platelets, and 13 ng/mL serum ferritin. Urine analysis, serum liver function test (LFT), hemolysis markers, and serum electrolytes were unremarkable. Upper gastrointestinal endoscopy (until the 2nd duodenal portion) and colonoscopy were normal. Further investigation, using video capsule endoscopy, in the outpatient setting, showed polypoid lesions involving the third and fourth portions of the duodenum (Figure 1). The biopsy showed a moderately differentiated adenocarcinoma. Abdominal CT showed a wall thickening involving the third and fourth portions of the duodenum and proximal jejunum, with no clear cleavage lines with adjacent structures without evidence of nodal and distant metastasis (Figure 2). The patient underwent a segmental resection of the duodenum (third and fourth portions) and proximal jejunum, with duodenojejunostomy. The pathological examination of the surgical specimen confirmed a moderately differentiated adenocarcinoma infiltrating the wall of the organ without lymph node metastasis (Figure 3). The patient underwent a follow-up by oncology.

DISCUSSION

This case represents a rare location of primary duodenal adenocarcinoma[8] in a younger patient compared to the average peak incidence of duodenal adenocarcinoma shown in literature (seventh decade of life), with a slight predominance for males[9]. The patient was younger, and there was no known condition associated with early occurrence, such as inflammatory bowel disease[10], familial adenomatous polyposis, or hereditary nonpolyposis colorectal cancer, in which cancer presents earlier (median 39 years)[11] in his personal and/or family medical history .

The clinical picture of adenocarcinoma in the third and fourth portions occurs with rather non-specific symptoms. Unlike periampullary tumors, whose main clinical picture is jaundice and other clinical aspects from the obstruction of the hepatobiliary-pancreatic system[7], in third and fourth portion tumors there are non-specific symptoms such as vague abdominal pain, weight loss, anemia symptoms, but no frank bleeding, and more rarely, bowel obstruction dominates the clinical picture[12,13]. In this case, the duration of symptoms before diagnosis was 4 mo, that is within the average literature range (from 1.4 to 8 mo)[7] One study showed worse 2-year survival rate associated with 4 mo or longer duration[14].

In routine work-up, both upper and lower endoscopy were normal. This situation is a substratum for missing tumors in the third and/or fourth portions, and is often worsened by the low index of clinical suspicion, which usually delays the diagnosis, resulting in advanced disease at diagnosis and decreasing

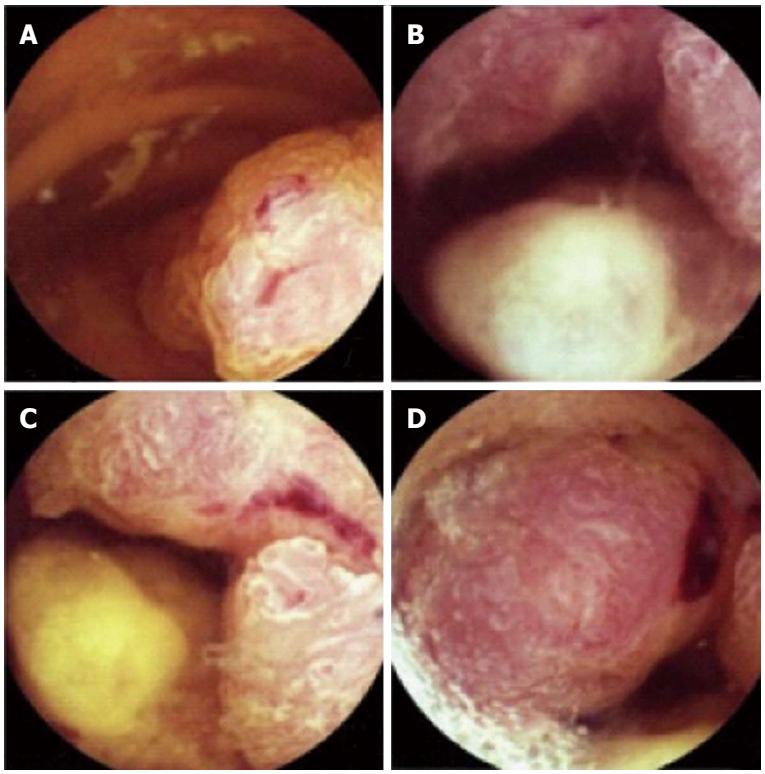


Figure 1 Capsule endoscopy findings in the 3rd and 4th portions of the duodenum. A: Polypoid and multilobular lesion; B, C: Partially obstructing the lumen; C, D: Ulcerated with low-flow bleeding.

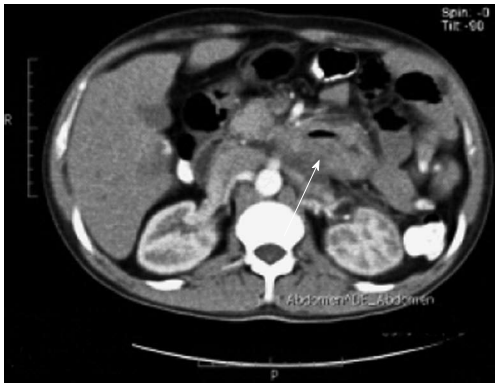


Figure 2 Abdominal computed tomography scan demonstrating a thickening of the wall involving the 3rd and 4th portions of duodenum, narrowing its lumen (arrows), without clear lines of cleavage with adjacent structures and with no evidence of nodal and distant metastasis.

the rate of potentially curative resections[7,15]. After nondiagnostic conventional endoscopic tests, in the setting of iron deficiency anemia, it is worth having a high index of suspicion for tumors beyond the second portion and to carry on the work-up using a method of greater accuracy for these tumors, the endoscopic capsule[8,16].

Capsule endoscopy is a non-invasive procedure that uses a wireless endoscopic device that allows imaging of the gastrointestinal tract. In this case, it was a valuable tool that allowed complete small bowel exploration in the ambulatory setting. The main indications for its use are the evaluation of obscure gastrointestinal bleeding, and Crohn's disease[17,18]. Its sensitivity and specificity for diagnosing a small bowel tumor is 88.9% to 95% and 75% to 95% respectively, in the setting of obscure gastrointestinal bleeding[16,19]. Tumors are found in about 3% to 9% of patients undergoing this procedure for evaluation of obscure gastrointestinal bleeding, and 50% to 60% were malignant[18]. Video capsule endoscopy has also been used for the evaluation of patients with certain disorders that increase the risk of tumors of the small intestine, such as familial adenomatous polyposis[18].

Treatment of primary duodenal adenocarcinoma depends on the location and staging. In this case, a segmental resection of the duodenum (3rd and 4th portions) and the proximal jejunum (20 cm from duodenojejunal flexure), with primary duodenojejunostomy was performed. This approach was preferred to more extensive resection, because it provides equivalent survival rates to extensive resections (since it is possible to achieve negative margins), with the benefit of lower morbidity than that associated with pancreaticoduodenectomy[20] and even better survival, as was shown in one study[21]. Currently, extensive pancreaticoduodenectomy applies more to tumors of the proximal duodenum (1st and 2nd portions)[22].

The pathological examination of the surgical specimen confirmed a moderately differentiated adenocarcinoma, which is the most common histological grade[3,23], that infiltrates the three layers of the wall, without invasion of adjacent organs or metastasis

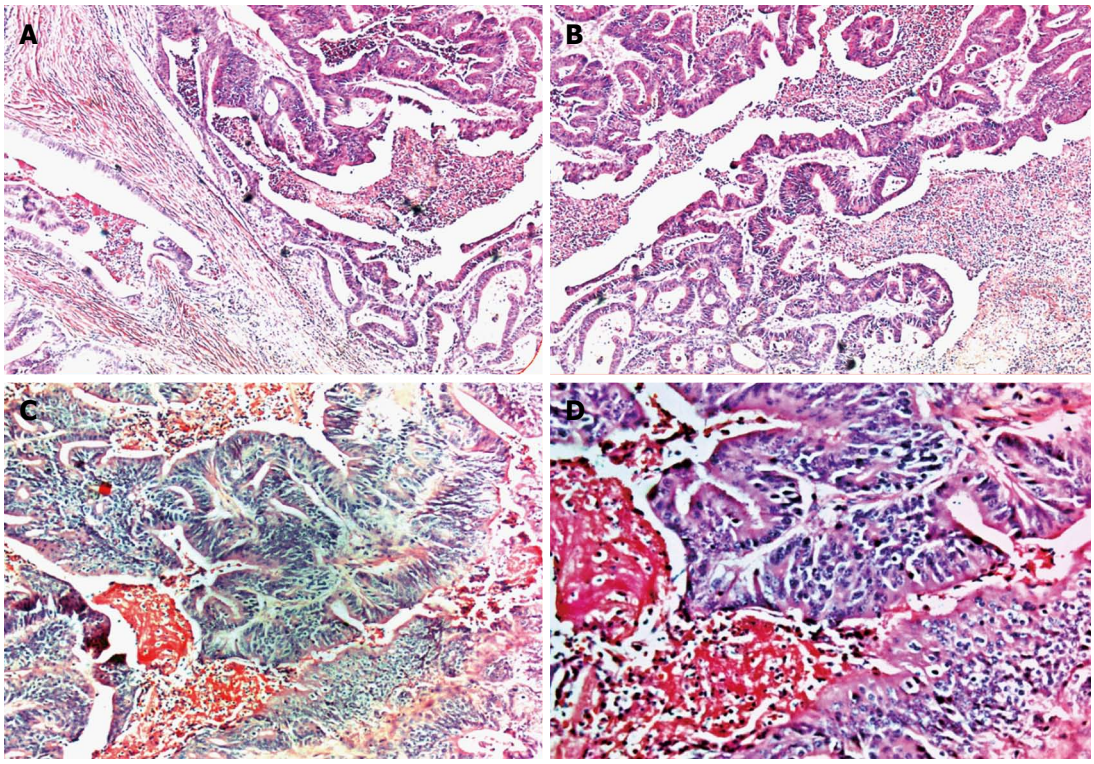


Figure 3 Histological findings of surgical specimen demonstrating moderately differentiated adenocarcinoma, infiltrating the wall thickness (A) (HE, magnification × 5), with areas of cribriform appearance due to fusion of glands and areas of necrosis (B) (HE, magnification × 10); A higher magnification, demonstrating dysplastic aspect of epithelium, loss of polarity and cell dysplasia (C) (HE, magnification × 100) and (D) (HE, magnification × 200). HE: Hematoxylin and eosin.

to the lymph nodes, and surgical margins were negative for tumor cells. Therefore, it was a stadium Ⅱ tumor (T3 N0 M0), that is the most frequent stage for adenocarcinoma in this site[12,24]. Despite negative margins and no lymph node involvement, the combination of 3 variables present in this case - tumor extension, histological grade and transmural invasion - are associated with poor prognosis[6].

With regard to the adjuvant treatment, there is no established protocol for small bowel adenocarcinomas, due to the lack of randomized trials[25]; and the few available data from retrospective studies have shown no statistically significant overall survival benefit[26]. As with treatment, there is no established follow-up protocol for patients with resected adenocarcinoma of the small intestine. In this case, the patient continued follow-up by oncologist.

Malignant tumors of the small intestine, although rare, should be part of the differential diagnosis in the investigation of obscure gastrointestinal bleeding and the high index of suspicion and appropriate use of endoscopic capsule are of great value.

COMMENTS

Case characteristics

A 40-year-old male presenting with abdominal discomfort and progressive fatigue due to severe anemia by continuous bleeding from third and fourth portions duodenal cancer.

Clinical diagnosis

Small intestine examination with video capsule endoscopy revealed a multilobular tumor, ulcerated with low-flow bleeding lesion in the third and fourth duodenal portions.

Differential diagnosis

Upper gastrointestinal endoscopy (until the 2nd duodenal portion) and colonoscopy were performed to rule out stomach and colon bleeding respectively.

Laboratory diagnosis

Blood tests demonstrated RBC 3.49 × 10^6/mm^3; hemoglobin 7.8 g/dL; MCH 79 fL and serum ferritin 13 ng/mL. Metabolic panel and liver function tests were within normal limits.

Imaging diagnosis

Abdominal computed tomography demonstrated a wall thickening involving third and fourth duodenal portions and proximal jejunum, without evidence of nodal and distant metastasis.

Pathological diagnosis

The histopathological examination of the surgical specimen demonstrated a three layer infiltrating adenocarcinoma, without lymph node invasion, with free surgical margins.

Treatment

Segmental resection of the duodenum (3rd and 4th portions) and proximal jejunum (20 cm from duodenojejunal flexure) was performed, with primary duodenojejunostomy.

Experiences and lessons

Malignant tumors of the third and fourth duodenal portions are a diagnostic challenge using conventional endoscopic tests; a high index of suspicion and appropriate use of the endoscopic capsule is of great value for early diagnosis.

Peer-review

The authors reported primary adenocarcinoma of the 3rd/4th portions of the duodenum in a 40-year-old man. Blood tests revealed microcytic, hypochromic iron deficiency anemia. Upper gastrointestinal endoscopy and colonoscopy were normal, but the polypoid lesions with low-flow bleeding were observed by video capsule endoscopy.

REFERENCES

1 **Aparicio T**, Zaanan A, Svrcek M, Laurent-Puig P, Carrere N, Manfredi S, Locher C, Afchain P. Small bowel adenocarcinoma: epidemiology, risk factors, diagnosis and treatment. *Dig Liver Dis* 2014; **46**: 97-104 [PMID: 23796552 DOI: 10.1016/j.dld.2013.04.013]

2 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]

3 **Dabaja BS**, Suki D, Pro B, Bonnen M, Ajani J. Adenocarcinoma of the small bowel: presentation, prognostic factors, and outcome of 217 patients. *Cancer* 2004; **101**: 518-526 [PMID: 15274064 DOI: 10.1002/cncr.20404]

4 **Bilimoria KY**, Bentrem DJ, Wayne JD, Ko CY, Bennett CL, Talamonti MS. Small bowel cancer in the United States: changes in epidemiology, treatment, and survival over the last 20 years. *Ann Surg* 2009; **249**: 63-71 [PMID: 19106677 DOI: 10.1097/SLA.0b013e31818e4641]

5 **Pan SY**, Morrison H. Epidemiology of cancer of the small intestine. *World J Gastrointest Oncol* 2011; **3**: 33-42 [PMID: 21461167 DOI: 10.4251/wjgo.v3.i3.33]

6 **Ryder NM**, Ko CY, Hines OJ, Gloor B, Reber HA. Primary duodenal adenocarcinoma: a 40-year experience. *Arch Surg* 2000; **135**: 1070-1074; discussion 1074-1075 [PMID: 10982512 DOI: 10.1001/archsurg.135.9.1070]

7 **Solej M**, D'Amico S, Brondino G, Ferronato M, Nano M. Primary duodenal adenocarcinoma. *Tumori* 2008; **94**: 779-786 [PMID: 19267092 DOI: 10.1700/396.4655]

8 **Eliakim R**, Fischer D, Suissa A, Yassin K, Katz D, Guttman N, Migdal M. Wireless capsule video endoscopy is a superior diagnostic tool in comparison to barium follow-through and computerized tomography in patients with suspected Crohn's disease. *Eur J Gastroenterol Hepatol* 2003; **15**: 363-367 [PMID: 12655255 DOI: 10.1097/01.meg.0000050000.68425.7e]

9 **Kim MJ**, Choi SB, Han HJ, Park PJ, Kim WB, Song TJ, Suh SO, Choi SY. Clinicopathological analysis and survival outcome of duodenal adenocarcinoma. *Kaohsiung J Med Sci* 2014; **30**: 254-259 [PMID: 24751389 DOI: 10.1016/j.kjms.2013.12.006]

10 **Xie J**, Itzkowitz SH. Cancer in inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 378-389 [PMID: 18200660 DOI: 10.3748/wjg.14.378]

11 **Björk J**, Akerbrant H, Iselius L, Bergman A, Engwall Y, Wahlström J, Martinsson T, Nordling M, Hultcrantz R. Periampullary adenomas and adenocarcinomas in familial adenomatous polyposis: cumulative risks and APC gene mutations. *Gastroenterology* 2001; **121**: 1127-1135 [PMID: 11677205 DOI: 10.1053/gast.2001.28707]

12 **Tocchi A**, Mazzoni G, Puma F, Miccini M, Cassini D, Bettelli E, Tagliacozzo S. Adenocarcinoma of the third and fourth portions of the duodenum: results of surgical treatment. *Arch Surg* 2003; **138**: 80-85 [PMID: 12511157 DOI: 10.1001/archsurg.138.1.80]

13 **Bang CS**, Yoon JH, Choi SH, Eom JH, Lee YS, Lee YH, Han SH. Primary Adenocarcinoma of Duodenum Located in Third Portion Cured by Wedge Resection. *Korean J Helicobacter Up Gastrointest Res* 2013; **13**: 263-266 [DOI: 10.7704/kjhugr.2013.13.4.263]

14 **Delcore R**, Thomas JH, Forster J, Hermreck AS. Improving resectability and survival in patients with primary duodenal carcinoma. *Am J Surg* 1993; **166**: 626-630; discussion 630-631 [PMID: 7903846 DOI: 10.1016/S0002-9610(05)80668-3]

15 **Stell D**, Mayer D, Mirza D, Buckels J. Delayed diagnosis and lower resection rate of adenocarcinoma of the distal duodenum. *Dig Surg* 2004; **21**: 434-438; discussion 438-439 [PMID: 15665538 DOI: 10.1159/000083470]

16 **Hartmann D**, Schmidt H, Bolz G, Schilling D, Kinzel F, Eickhoff A, Huschner W, Möller K, Jakobs R, Reitzig P, Weickert U, Gellert K, Schultz H, Guenther K, Hollerbuhl H, Schoenleben K, Schulz HJ, Riemann JF. A prospective two-center study comparing wireless capsule endoscopy with intraoperative enteroscopy in patients with obscure GI bleeding. *Gastrointest Endosc* 2005; **61**: 826-832 [PMID: 15933683 DOI: 10.1016/S0016-5107(05)00372-X]

17 **Ladas SD**, Triantafyllou K, Spada C, Riccioni ME, Rey JF, Niv Y, Delvaux M, de Franchis R, Costamagna G. European Society of Gastrointestinal Endoscopy (ESGE): recommendations (2009) on clinical use of video capsule endoscopy to investigate small-bowel, esophageal and colonic diseases. *Endoscopy* 2010; **42**: 220-227 [PMID: 20195992 DOI: 10.1055/s-0029-1243968]

18 **Eliakim R**. Video capsule endoscopy of the small bowel. *Curr Opin Gastroenterol* 2010; **26**: 129-133 [PMID: 20145540 DOI: 10.1097/MOG.0b013e328334df17]

19 **Pennazio M**, Santucci R, Rondonotti E, Abbiati C, Beccari G, Rossini FP, De Franchis R. Outcome of patients with obscure gastrointestinal bleeding after capsule endoscopy: report of 100 consecutive cases. *Gastroenterology* 2004; **126**: 643-653 [PMID: 14988816 DOI: 10.1053/j.gastro.2003.11.057]

20 **Bakaeen FG**, Murr MM, Sarr MG, Thompson GB, Farnell MB, Nagorney DM, Farley DR, van Heerden JA, Wiersema LM, Schleck CD, Donohue JH. What prognostic factors are important in duodenal adenocarcinoma? *Arch Surg* 2000; **135**: 635-641; discussion 641-642 [PMID: 10843358 DOI: 10.1001/archsurg.135.6.635]

21 **Kaklamanos IG**, Bathe OF, Franceschi D, Camarda C, Levi J, Livingstone AS. Extent of resection in the management of duodenal adenocarcinoma. *Am J Surg* 2000; **179**: 37-41 [PMID: 10737576 DOI: 10.1016/S0002-9610(99)00269-X]

22 **Halfdanarson TR**, McWilliams RR, Donohue JH, Quevedo JF. A single-institution experience with 491 cases of small bowel adenocarcinoma. *Am J Surg* 2010; **199**: 797-803 [PMID: 20609724 DOI: 10.1016/j.amjsurg.2009.05.037]

23 **Hung FC**, Kuo CM, Chuah SK, Kuo CH, Chen YS, Lu SN, Chang Chien CS. Clinical analysis of primary duodenal adenocarcinoma: an 11-year experience. *J Gastroenterol Hepatol* 2007; **22**: 724-728 [PMID: 17444863 DOI: 10.1111/j.1440-1746.2007.04935.x]

24 **Edge SB**, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. American Joint Committee on Cancer (AJCC) Cancer staging manual. 7th ed. New York: Springer-Verlag, 2010: 718

25 **Singhal N**, Singhal D. Adjuvant chemotherapy for small intestine adenocarcinoma. *Cochrane Database Syst Rev* 2007; (**3**): CD005202 [PMID: 17636789 DOI: 10.1002/14651858.CD005202.pub2]

26 **Overman MJ**, Kopetz S, Lin E, Abbruzzese JL, Wolff RA. Is there a role for adjuvant therapy in resected adenocarcinoma of the small intestine. *Acta Oncol* 2010; **49**: 474-479 [PMID: 20397775 DOI: 10.3109/02841860903490051]

P- Reviewer: Charoenphandhu N **S- Editor**: Ma YJ
L- Editor: O'Neill M **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9442

World J Gastroenterol 2015 August 21; 21(31): 9442-9447
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Pancreatic paraganglioma with draining vessels

Yoshitsugu Misumi, Toshio Fujisawa, Hirotsugu Hashimoto, Koichi Kagawa, Tamaki Noie, Hideyuki Chiba, Hajime Horiuchi, Yasushi Harihara, Nobuyuki Matsuhashi

Yoshitsugu Misumi, Toshio Fujisawa, Koichi Kagawa, Nobuyuki Matsuhashi, Department of Gastroenterology, NTT Medical Center Tokyo, Tokyo 141-8625, Japan

Hirotsugu Hashimoto, Hajime Horiuchi, Department of Pathology, NTT Medical Center Tokyo, Tokyo 141-8625, Japan

Tamaki Noie, Yasushi Harihara, Department of Surgery, NTT Medical Center Tokyo, Tokyo 141-8625, Japan

Hideyuki Chiba, Department of Gastroenterology, Omori Red Cross Hospital, Tokyo 141-8625, Japan

Author contributions: Misumi Y, Fujisawa T, Kagawa K, Chiba H and Matsuhashi N contributed to examinations for the disease; Hashimoto H and Horiuchi H contributed to pathological diagnosis; Noie T and Harihara Y contributed to tumour resection by surgery; Misumi Y, Fujisawa T and Matsuhashi N contributed to writing the manuscript; all authors finally approved the authorship.

Institutional review board statement: The study was reviewed and approved by the NTT Medical Center Tokyo Institutional Review Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All authors have no known conflicts of interest associated with this case report.

Correspondence to: Toshio Fujisawa, MD, PhD, Department of Gastroenterology, NTT Medical Center Tokyo, 5-9-22 Higashi-Gotanda, Shinagawa, Tokyo 141-8625, Japan. toshio.fujisawa@east.ntt.co.jp

Telephone: +81-3-34486111
Fax: +81-3-34486541

Received: April 9, 2015
Peer-review started: April 9, 2015
First decision: April 23, 2015
Revised: May 11, 2015
Accepted: June 26, 2015
Article in press: June 26, 2015
Published online: August 21, 2015

Abstract

A pancreatic paraganglioma is a rare neoplasm that is difficult to distinguish from a pancreatic neuroendocrine tumour. Here we present a case of pancreatic paraganglioma that was surgically resected following preoperative diagnosis of a pancreatic neuroendocrine tumour. Careful evaluation of the endoscopic ultrasonography findings revealed abundant draining vessels, which could have led to a correct preoperative diagnosis of pancreatic paraganglioma.

Key words: Paraganglioma; Neuroendocrine tumour; Draining vessels; Pancreatic tumour

Core tip: Pancreatic paraganglioma is a rare disease, which has been reported only in 24 cases ever. Invasive procedures toward paragangliomas carry the potential risk of catastrophic complications due to unexpected release of large quantities of catecholamines. An accurate diagnosis of paraganglioma, therefore, is important before invasive procedures. Draining vessels from the tumour are sometimes observed in a general paraganglioma. However, it is still unclear in the pancreatic paraganglioma. In the present report, usefulness of draining vessels for a diagnosis of pancreatic paraganglioma was investigated by reviewing past cases.

Misumi Y, Fujisawa T, Hashimoto H, Kagawa K, Noie T, Chiba H, Horiuchi H, Harihara Y, Matsuhashi N. Pancreatic paraganglioma with draining vessels. *World J Gastroenterol* 2015; 21(31): 9442-9447 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9442.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9442

INTRODUCTION

Paragangliomas are rare catecholamine-secreting neuroendocrine tumours that arise from neuroendocrine cells of the extra-adrenal autonomic paraganglia and affect about 1 in 2000000 of the population[1]. The head, neck, and retroperitoneum are the most commonly affected sites, but pancreatic paragangliomas are extremely rare[2]. To our knowledge, only 24 cases of pancreatic paraganglioma have been previously reported in the English literature (see Table 1)[1-20]. In these previous reports, the pancreatic paragangliomas were often preoperatively misdiagnosed as pancreatic neuroendocrine tumours (pNETs) because the radiological characteristics of pancreatic paragangliomas resemble those of pNETs. We herein report a patient with a pancreatic paraganglioma, which was surgically resected following a preoperative diagnosis of a pNET, and discuss features associated with the disorder.

CASE REPORT

An asymptomatic 47-year-old woman with a pancreatic tumour that was detected by ultrasonography was referred to our hospital. The patient's past medical history and physical examination were unremarkable. Initial laboratory studies, including levels of tumour markers such as carcinoembryonic antigen, carbohydrate antigen 19-9, and α-fetoprotein, showed no abnormalities. An examination of pancreatic endocrine hormones showed that the insulin level was slightly elevated [14.6 μU/mL (5-10 μU/mL)], but the elevation was considered non-specific because blood glucose (108 mg/dL) and C-peptide levels [3.30 (0.78-5.19) ng/dL] were within normal levels. Levels of other hormones were also within their normal ranges. Transabdominal ultrasonography showed a 1.5-cm low-echoic tumour at the pancreas head. Dynamic computed tomography (CT) also revealed a 1.5-cm well demarcated tumour at the pancreas head (Figure 1A and B). The tumour was very strongly enhanced in the arterial phase and still faintly enhanced in the portal vein phase. Maximum intensity projection of the arterial phase in a coronal view clearly revealed the tumour feeding artery from the inferior pancreaticoduodenal artery and the draining vein into the portal vein (Figure 1C). No abnormalities were found in the bile duct, pancreatic duct, or liver. Compared with contiguous pancreatic parenchyma, the tumour appeared as a low-intensity lesion in a T1-weighted magnetic resonance image (MRI) and as a high-intensity lesion in a T2-weighted MRI (Figure 1D and E, respectively). Endoscopic ultrasonography (EUS) showed a well demarcated, low, and uneven echoic tumour surrounded by draining vessels emanating from the tumour (Figure 1F and G). We did not attempt EUS-guided fine needle aspiration (FNA) for a pathological examination because of the possible risk of bleeding due to the extremely abundant vascularity of the tumour. On the basis of these imaging findings, we made a preoperative diagnosis of a nonfunctional pNET. Pancreaticoduodenectomy was performed. The resected specimen included a tumour measuring 1.5 cm × 1.2 cm (Figure 2A). The tumour was located at the caudal part of pancreas head and partially adjacent to the second portion of duodenum. The tumour was isolated from major arteries and veins. Histological examination revealed a classical Zellballen pattern, with nests of cells surrounded by thick capsules (Figures 2B and C). The tumour was surrounded by normal pancreatic parenchyma. Immunohistochemistry examination of the tumour cells showed positive staining for CD56, synaptophysin, and chromogranin A and negative staining for insulin, glucagon, gastrin, and somatostatin. The tumour cells were surrounded by S-100 protein-positive sustentacular cells (Figure 2D). These results suggested that the tumour cells were differentiated toward neuroendocrine cells, but did not express any islet hormones. Epithelial membrane antigen staining was negative in the tumour cells but positive in the pancreatic ducts remaining within the tumour (Figure 2E). This result indicated that branches of the pancreatic duct were within the tumour. Taking into consideration the tumour location and pancreatic ducts remaining within the tumour, we inferred that the tumour arose from the pancreas. On the basis of these pathological findings, a diagnosis of pancreatic paraganglioma was established. The patient was followed for more than one year after surgery, but no recurrence was confirmed.

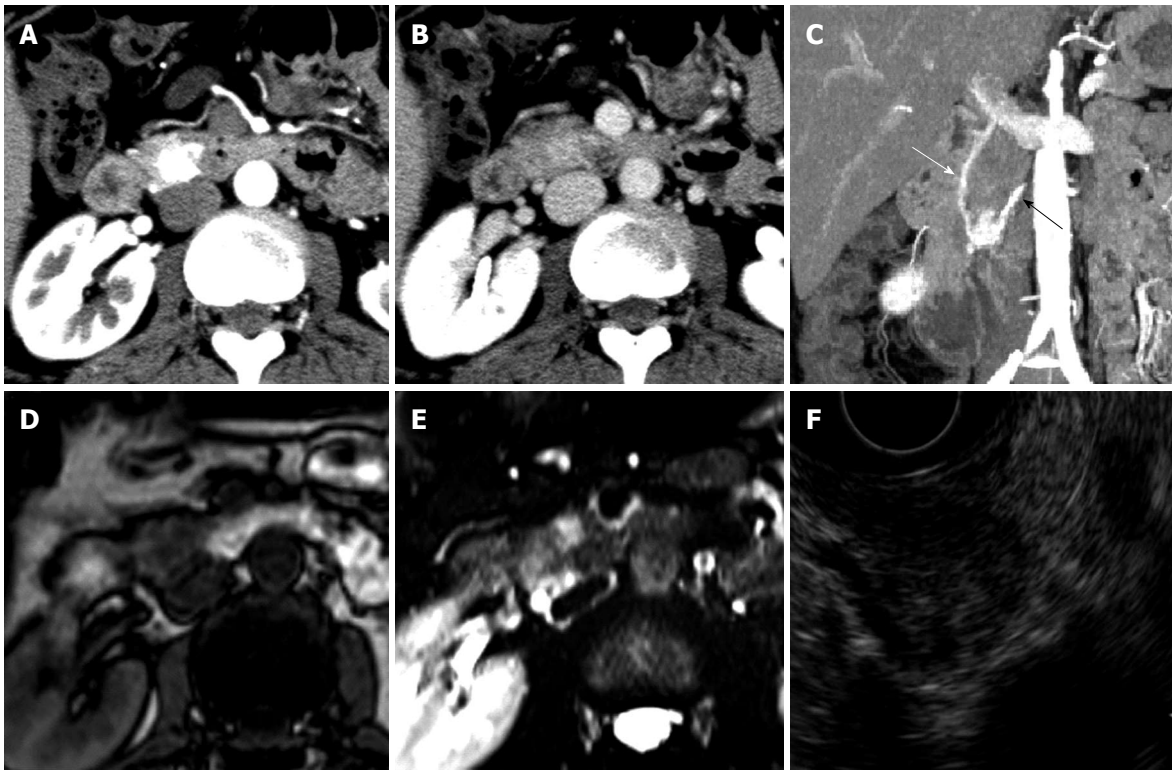
DISCUSSION

Pancreatic paragangliomas are radiologically similar to pNETs. In fact, the case reported here and 6 of the 24 other cases reported in the literature were preoperatively misdiagnosed as pNETs[1,8-12]. Fortunately, no complications occurred during the preoperative examinations or surgical procedures in the present case. However, invasive examinations and surgery of paragangliomas carry the potential risk of catastrophic complications due to unexpected release of large quantities of catecholamines. For example, a patient with pancreatic paraganglioma, who was misdiagnosed preoperatively as having pancreatic cancer, died 34 h after surgery because of unanticipated catecholamine release[17]. The use

Table 1 Summary of reports of 25 cases of pancreatic paragangliomas

Age (yr)	Sex	Tumour size (cm)	Location in pancreas	Preoperative diagnosis	Presence/absence of draining vessels	Outcome	Ref.
62	M	1.5	Body	-	NE	Autopsy	[3]
75	F	15	Tail	Pancreatic cyst	NE	-	[4]
70	F	3	Head	Pancreatic cyst	NE	-	[4]
72	F	14	Head	Cystadenoma	NE	2 yr alive	[5]
47	M	10	Body	Pancreatic cyst	NE	6 yr alive	[6]
-	-	-	Head	-	NE	2 yr alive	[7]
-	-	-	Head	-	NE	4 yr alive	[7]
45	F	8	Head	Retroperitoneal tumour	NE	5 yr alive	[8]
58	M	8	Head	Neuroendocrine tumour	NE	-	[8]
61	M	2.5	Uncus	Neuroendocrine tumour	Absent in the presented figures	5 yr alive	[9]
85	M	6	Head	Neuroendocrine tumour	NE	-	[10]
72	F	4	Uncus	Nonfunctional neuroendocrine tumour	Absent in the presented figures	-	[11]
57	F	6.5	Head	Non-functioning islet cell tumour	Stated as present	-	[12]
57	F	2	Uncus	Neuroendocrine tumour	NE	4 yr alive	[1]
50	M	3	Head	Extra-adrenal paraganglioma	Present in the presented figures	-	[13]
51	F	5	Uncus	Pancreatic cancer	NE	3 yr alive	[14]
40	F	4.5	Uncus	-	Stated as absent	-	[15]
66	M	6	Head	-	Absent in the presented figures	14 mo alive	[2]
65	F	2	Uncus	-	NE	-	[16]
30	F	6.4	Tail	-	NE	Died 34 h after surgery	[17]
19	F	9	Head	Sarcoma	Present in the presented figures	-	[18]
55	F	19	Tail	Malignant pancreatic tumour	NE	-	[19]
50	F	6	Head	Paraganglioma, fine needle aspiration	NE	4 yr alive	[20]
63	M	4	Head	Functional pancreatic paraganglioma	NE	-	[20]
47	F	1.5	Head	Nonfunctional neuroendocrine tumour	Present	1 yr alive	Present case

NE: Not evaluable.



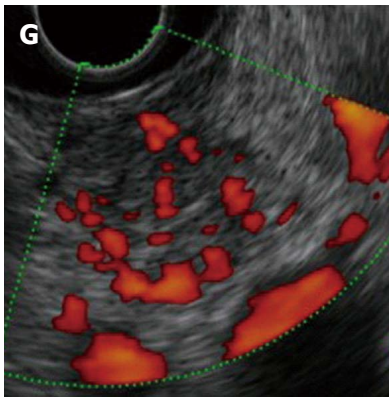


Figure 1 Images of the pancreatic tumour. A: Dynamic computed tomography (CT) in the arterial phase; B: Dynamic CT in the portal phase. 1.5-cm well demarcated tumour at the pancreas head was very strongly enhanced in the arterial phase and still faintly enhanced in the portal phase; C: Maximum intensity projection of the arterial phase in a coronal view. Black arrow indicates the feeding artery from the inferior pancreaticoduodenal artery and white arrow indicates the draining vein into the portal vein; D: T1-weighted (T1W) magnetic resonance image (MRI); E: T2-weighted (T2W) MRI. The tumour appeared as a low-intensity lesion in T1W and as a high-intensity lesion in T2W; F: Endoscopic ultrasonography (EUS) images without power flow scanning; G: EUS images with power flow scanning. The low and uneven echoic tumour was surrounded by draining vessels.

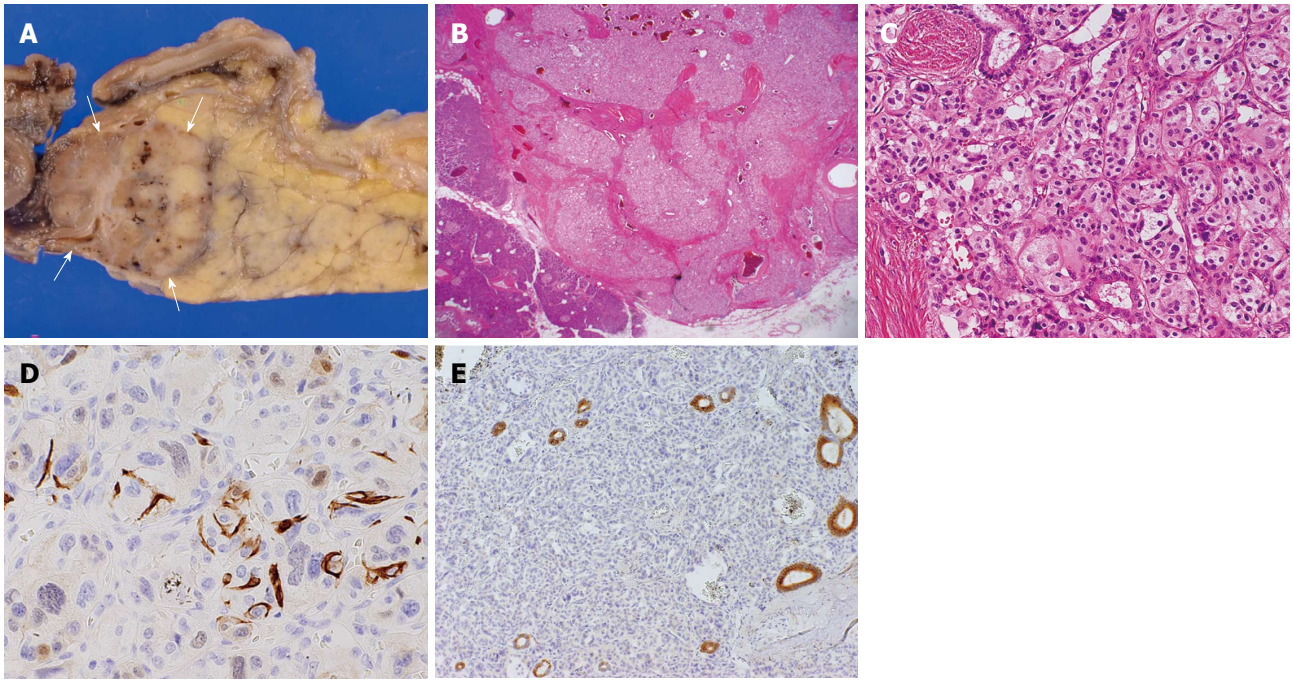


Figure 2 Histological examinations of the pancreatic tumour. A: Macroscopic findings of the resected specimen. Arrows indicate the tumour in the pancreas head; B, C: Hematoxylin and eosin staining showing tumour cells in a Zellballen pattern at magnifications of × 10 (B) and × 200 (C); D: Immunohistochemistry of S-100 protein displaying S-100 protein-positive sustentacular cells surrounding the tumour cells (magnification × 200); E: Immunohistochemistry of epithelial membrane antigen displaying pancreatic duct branches remaining within the tumour (magnification × 100).

of EUS-guided FNA without preparations against catecholamine release could also be dangerous. These dangers highlight the importance of an accurate diagnosis of pancreatic paraganglioma.

Preoperative imaging may provide guidance for an accurate diagnosis. Areas of signal flow void (referred to as a "salt and pepper" pattern[21]) are often observed on MRIs of highly vascularized paragangliomas. Additionally, enlarged feeding arteries and early contrast filling of the draining veins are observed on dynamic CT images. This feature of draining veins has been reported to be useful in distinguishing pancreatic paraganglioma from pNET[12]. In the present case, the draining vein was confirmed by dynamic CT, but "salt and pepper" pattern was absent on the MRI. In the 24 other cases (Table 1), one has been reported to have draining veins[12], and one has been reported to be devoid of such veins[15]. The presence or absence of draining veins was not mentioned in the text of the reports of the remaining 22 cases, but examination of images presented in the reports suggest that two cases[13,18] had and three cases[2,9,11]

did not have draining veins (presented material did not allow evaluation of draining veins in the remaining 17 cases). Formation of the tumour draining vasculature is supposed to depend on a variety of growth factors secreted from the tumour itself[22]. Most pancreatic paragangliomas seem to have a characteristic to induce abundant draining veins, although details of the mechanism of the vessel induction remain to be elucidated. In summary, draining veins could be observed in 50% (four of eight) of evaluable cases. We conclude that evaluation of imaging findings, especially identifying draining veins around the tumour, may be useful in making a correct preoperative diagnosis of pancreatic paraganglioma.

COMMENTS

Case characteristics

A 47-year-old woman with a pancreatic tumour detected by ultrasonography was referred to our hospital.

Clinical diagnosis

The patient showed no symptom.

Differential diagnosis

Solid tumors (adenocarcinoma, neuroendocrine tumours, Solid-pseudopapillary tumor, acinar cell carcinoma, and paraganglioma), cystic tumors (serous cystic neoplasm).

Laboratory diagnosis

Laboratory studies, including levels of tumour markers such as carcinoembryonic antigen, carbohydrate antigen 19-9, and α-fetoprotein, showed no abnormalities, but insulin level was slightly elevated [14.6 μU/mL].

Imaging diagnosis

Computed tomography showed a 1.5-cm well demarcated tumour at the pancreas head, which was very strongly enhanced in the arterial phase and still faintly enhanced in the portal vein phase.

Pathological diagnosis

Histological examination showed a classical Zellballen pattern, and tumour cells of positive staining for CD56, synaptophysin, and chromogranin A, that were surrounded by S-100 protein-positive sustentacular cells.

Treatment

The patient received pancreaticoduodenectomy.

Related reports

Pancreatic paraganglioma has been reported only in 24 cases ever.

Term explanation

Paraganglioma is rare catecholamine-secreting neuroendocrine tumour that arises from neuroendocrine cells of the extra-adrenal autonomic paraganglia

Experiences and lessons

Identifying draining veins around the tumour is useful in making a correct preoperative diagnosis of pancreatic paraganglioma.

Peer-review

The authors have summarized 25 cases of pancreatic paraganglioma including the present case and reported usefulness of tumour draining vessels for distinguishing pancreatic paraganglioma from pancreatic neuroendocrine tumour.

REFERENCES

1 **Tsukada A**, Ishizaki Y, Nobukawa B, Kawasaki S. Paraganglioma of the pancreas: a case report and review of the literature. *Pancreas* 2008; **36**: 214-216 [PMID: 18376320 DOI: 10.1097/01.MPA.0000311841.35183.45]

2 **Lightfoot N**, Santos P, Nikfarjam M. Paraganglioma mimicking a pancreatic neoplasm. *JOP* 2011; **12**: 259-261 [PMID: 21546704]

3 **Goodof I**, Lischer C. Tumor of the carotid body and the pancreas. *Arch Pathol* 1943; **35**: 6

4 **Bartley O**, Ekdahl PH, Hultén L. Paraganglioma simulating pancreatic cyst. *Acta Chir Scand* 1966; **132**: 289-297 [PMID: 5929097]

5 **Cope C**, Greenberg SH, Vidal JJ, Cohen EA. Nonfunctioning nonchromaffin paraganglioma of the pancreas. *Arch Surg* 1974; **109**: 440-442 [PMID: 4368962]

6 **Zamir O**, Amir G, Lernau O, Ne'eman Z, Nissan S. Nonfunctional paraganglioma of the pancreas. *Am J Gastroenterol* 1984; **79**: 761-763 [PMID: 6486113]

7 **Howard JM**, Jordan JL, Reber HA. Surgical disease of the pancreas. Philadelphia: Lea and Febiger, 1987

8 **Malthouse SR**, Robinson L, Rankin SC. Ultrasonic and computed tomographic appearances of paraganglioma simulating pancreatic mass. *Clin Radiol* 1992; **45**: 271-272 [PMID: 1395386]

9 **Fujino Y**, Nagata Y, Ogino K, Watahiki H, Ogawa H, Saitoh Y. Nonfunctional paraganglioma of the pancreas: report of a case. *Surg Today* 1998; **28**: 209-212 [PMID: 9525014]

10 **Parithivel VS**, Niazi M, Malhotra AK, Swaminathan K, Kaul A, Shah AK. Paraganglioma of the pancreas: literature review and case report. *Dig Dis Sci* 2000; **45**: 438-441 [PMID: 10711464]

11 **Ohkawara T**, Naruse H, Takeda H, Asaka M. Primary paraganglioma of the head of pancreas: contribution of combinatorial image analyses to the diagnosis of disease. *Intern Med* 2005; **44**: 1195-1196 [PMID: 16357461]

12 **Kim SY**, Byun JH, Choi G, Yu E, Choi EK, Park SH, Lee MG. A case of primary paraganglioma that arose in the pancreas: the Color Doppler ultrasonography and dynamic CT features. *Korean J Radiol* 2008; **9** Suppl: S18-S21 [PMID: 18607119 DOI: 10.3348/kjr.2008.9.s.s18]

13 **Sangster G**, Do D, Previgliano C, Li B, LaFrance D, Heldmann M. Primary retroperitoneal paraganglioma simulating a pancreatic mass: a case report and review of the literature. *HPB Surg* 2010; **2010**: 645728 [PMID: 21188160 DOI: 10.1155/2010/645728]

14 **Zengel B**, Alacacioglu A, Yagci A, Postaci H, Erdinc I, Ozguzer A, Denecli A. Primary paraganglioma of the pancreas: Review of literature and a case report. *Firat Tip Dergisi* 2010; **15**: 3

15 **He J**, Zhao F, Li H, Zhou K, Zhu B. Pancreatic paraganglioma: A case report of CT manifestations and literature review. *Quant Imaging Med Surg* 2011; **1**: 41-43 [PMID: 23256053 DOI: 10.3978/j.issn.2223-4292.2011.08.02]

16 **Higa B**, Kapur U. Malignant paraganglioma of the pancreas. *Pathology* 2012; **44**: 53-55 [PMID: 22157694 DOI: 10.1097/PAT.0b013e32834e42b6]

17 **Wang ZL**, Fu L, Zhang Y, Babu SR, Tian B. An asymptomatic pheochromocytoma originating from the tail of the pancreas. *Pancreas* 2012; **41**: 165-167 [PMID: 22173833 DOI: 10.1097/MPA.0b013e31822362d0]

18 **Al-Jiffry BO**, Alnemary Y, Khayat SH, Haiba M, Hatem M. Malignant extra-adrenal pancreatic paraganglioma: case report and literature review. *BMC Cancer* 2013; **13**: 486 [PMID: 24138700 DOI: 10.1186/1471-2407-13-486]

19 **Borgohain M**, Gogoi G, Das D, Biswas M. Pancreatic paraganglioma: An extremely rare entity and crucial role of immunohistochemistry for diagnosis. *Indian J Endocrinol Metab* 2013; **17**: 917-919 [PMID: 24083178 DOI: 10.4103/2230-8210.117217]

20 **Zhang L**, Liao Q, Hu Y, Zhao Y. Paraganglioma of the pancreas: a potentially functional and malignant tumor. *World J Surg Oncol*

2014; **12**: 218 [PMID: 25030833 DOI: 10.1186/1477-7819-12-218]
21 **Wieneke JA**, Smith A. Paraganglioma: carotid body tumor. *Head Neck Pathol* 2009; **3**: 303-306 [PMID: 20016787 DOI: 10.1007/s12105-009-0130-5]
22 **Ruddell A**, Croft A, Kelly-Spratt K, Furuya M, Kemp CJ. Tumors induce coordinate growth of artery, vein, and lymphatic vessel triads. *BMC Cancer* 2014; **14**: 354 [PMID: 24886322 DOI: 10.1186/1471-2407-14-354]

P- Reviewer: Koike Y, Sinha R, Takahashi T, Verbeke CS
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Liu XM

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9448

World J Gastroenterol 2015 August 21; 21(31): 9448-9452
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Immunoglobulin G4-related autoimmune pancreatitis and sialadenitis: A case report

Ru-Ying Fan, Jian-Qiu Sheng

Ru-Ying Fan, Jian-Qiu Sheng, Department of Gastroenterology, Beijing Military General Hospital, Beijing 100700, China

Author contributions: Fan RY designed, wrote and revised the paper; Sheng JQ collected case data.

Institutional review board statement: The study was reviewed and approved by the Beijing Military General Hospital Institutional Review Board.

Informed consent statement: Study participant provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors declare no conflict of interest.

Correspondence to: Ru-Ying Fan, MD, Department of Gastroenterology, Beijing Military General Hospital, No. 5 Nanmencang, Dongcheng District, Beijing 100700, China. fanry2@163.com
Telephone: +86-10-66721168
Fax: +86-10-66721629

Received: January 14, 2015
Peer-review started: January 16, 2015
First decision: April 13, 2015
Revised: April 27, 2015
Accepted: June 26, 2015
Article in press: June 26, 2015
Published online: August 21, 2015

Abstract

Immunoglobulin G4 (IgG4)-related disease is a rare systemic disease. A 67-year-old male presented at our institution with mild upper abdominal pain and jaundice for 20 d. Laboratory results revealed high levels of IgG4 (15.4 g/L, range: 0.08-1.4 g/L). Computed tomography (CT) showed significant enlargement of the entire pancreas and a capsule-like low-density rim surrounding the whole pancreas. Positron emission tomography/CT revealed increased uneven metabolism of the entire pancreas. Both magnetic resonance cholangiopancreatography and endoscopic retrograde cholangiopancreatography showed stenosis of the distal common bile duct and proximal main pancreatic duct, and dilation of the proximal common bile duct and extra- and intra-hepatic bile ducts. He was diagnosed with IgG4-related autoimmune pancreatitis. The patient was treated with prednisone for 14 mo. The patient responded well to prednisone but upon cessation of the corticosteroid developed enlargement of the submandibular gland. The patient's serum IgG4 was elevated at 23.9 g/L. It is important to maintain treatment, so the patient was again treated with prednisone and had a good response. Follow-up of IgG4-related disease is thus necessary.

Key words: Immunoglobulin G4; Immunoglobulin G4-related disease; Autoimmune pancreatitis; Sialadenitis

Core tip: Immunoglobulin G4 (IgG4)-related disease is a rare systemic disease which affects many organs. Here we report a case of a patient with IgG4-related disease involving the pancreas and metachronous sialadenitis.

Fan RY, Sheng JQ. Immunoglobulin G4-related autoimmune pancreatitis and sialadenitis: A case report. *World J Gastroenterol* 2015; 21(31): 9448-9452 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9448.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9448

INTRODUCTION

Immunoglobulin G4 (IgG4)-related disease (RD) is a rare and often misdiagnosed systemic disease with multiple clinical manifestations and multiorgan involvement[1], though metachronous manifestations are rare. We present a unique case of metachronous IgG4-related autoimmune pancreatitis (AIP) and sialadenitis.

CASE REPORT

A 67-year-old male presented at our institution in March 2012 with mild upper abdominal pain and jaundice for 20 d. The physical examination was unremarkable except for moderate yellowing of the patient's skin and sclera. Laboratory results revealed high levels of alanine aminotransferase (357 U/L, range: 0-40 U/L), aspartate aminotransferase (362 U/L, range: 0-40 U/L), γ-glutamyltransferase (595.0 U/L, range: 8-50 U/L), alkaline phosphatase (750 U/L, range: 20-110 U/L), total bilirubin (52.70 μmol/L, range: 4.3-22.5 μmol/L), and direct serum bilirubin (29.50 μmol/L, range: 0-8.84 μmol/L). Serum amylase and glucose were within normal ranges, as were carbohydrate antigen, carcinoembryonic antigen, and alpha-fetoprotein. The erythrocyte sedimentation rate was elevated at 32 mm/h (range: 0-15 mm/h). Total IgG was elevated (21.5 g/L, range: 6.0-16.0 g/L), IgG4 was about 11 times the normal limit (15.4 g/L, range: 0.08-1.4 g/L), and γ-globulin was 33.03% (range: 10.7%-20.0%). Antinuclear antibody, anti-mitochondrial antibody, and smooth muscle antibody were all negative.

Radiographs of the chest were unremarkable. Computed tomography (CT) showed significant enlargement of the entire pancreas and a capsule-like low-density rim surrounding the whole pancreas. The proximal portion of the main pancreatic duct was stenotic and no significant dilation of the distal duct was observed. CT also revealed stenosis of the distal common bile duct, dilation of the proximal common bile duct, and dilation of the extra- and intra-hepatic bile ducts. Close examination of the CT scan showed heterogeneous enhancement of the pancreas where the density of the pancreatic body and tail was lower than that of the pancreatic head (Figure 1).

Endoscopic retrograde cholangiopancreatography (ERCP) found stenosis of the distal common bile duct (about 5 cm in length), dilation of the proximal common bile duct (approximately 2 cm maximum diameter), and dilation of the extra- and intra-hepatic bile ducts. The proximal main pancreatic duct was stenotic (about 5 cm in length) and associated with slight dilation of the distal main pancreatic duct.

Positron emission tomography (PET)/CT revealed increased uneven metabolism of the entire pancreas, and especially of the pancreatic head (Figure 2).

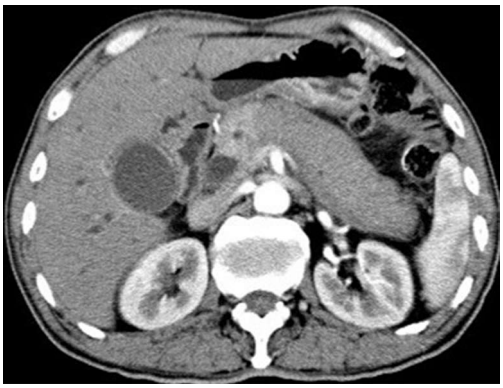


Figure 1 Computed tomography of the enlarged sausage-like pancreas and heterogeneous enhancement of the pancreas in March 2012 prior to steroid treatment.

Thickening of the walls of the gall bladder and dilation of the intrahepatic bile duct was also observed. Other organs were normal.

The patient was diagnosed with AIP. Stents were placed in the bile and pancreatic ducts to manage the patient's jaundice. The patient was then treated with prednisone at 40 mg/d for 3 wk, tapered by 2.5-5 mg/d every 1-2 wk with a maintenance dose of 7.5 mg/d over a period of 6 mo. Total steroid administration lasted for 14 mo.

Follow-up

During steroid administration, the patient was seen in our hospital in June 2012, November 2012, and August 2014. At the initial follow-up, the patient was asymptomatic and his liver enzyme and IgG4 levels were within the normal ranges. Abdominal CTs in June and November 2012 showed a normal pancreatic form (Figure 3A, B). Follow-up magnetic resonance cholangiopancreatography (MRCP) and ERCP showed reduced dilation of the common bile duct (approximately 1.5 cm maximum diameter) and the extra- and intra-hepatic bile ducts. Slight stenosis of the distal common bile duct and mild dilation of the distal main pancreatic duct were also observed. At the second follow-up period in November 2012, the stents in the bile and pancreatic ducts were removed by ERCP.

In 2013, the patient was diagnosed with Mikulicz syndrome and was treated with oral prednisone for 50 d in a local hospital. The patient responded well to the prednisone but upon cessation of the corticosteroid developed enlargement of the submandibular gland. In August 2014, the patient was admitted to our hospital after 8 mo of bilateral submandibular gland enlargement. The patient reported that he did not feel any abdominal discomfort. Physical examination revealed swollen but non-tender bilateral submandibular glands and no jaundice was noted.

Blood values were unremarkable except for serum IgG4 which was elevated at 23.9 g/L. Anti-Sjögren

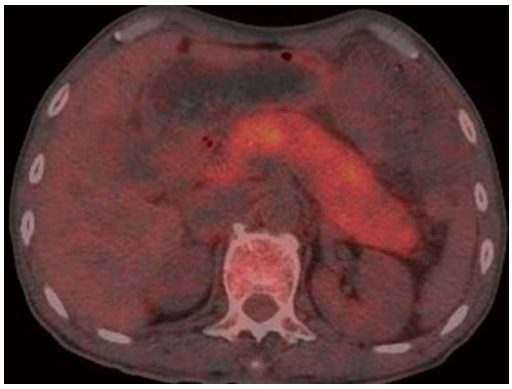


Figure 2 Positron emission tomography/computed tomography showing increased uneven metabolism of the entire pancreas in March 2012.

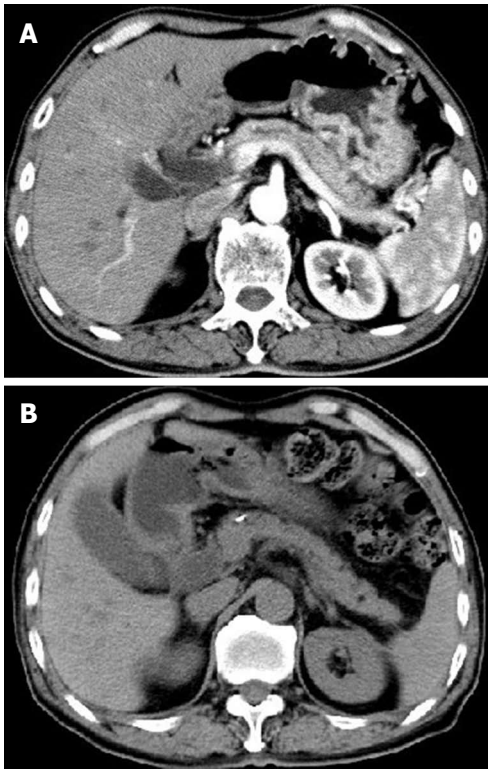


Figure 3 Computed tomography in June and November 2012 revealing the normalized pancreas after steroid treatment.

syndrome-related antigen A and Sjögren syndrome type B antigen were both negative. Repeat X-rays of the chest were unremarkable. Abdominal CT and MRCP showed similar findings to those in June and November 2012 (Figure 4, Figure 5). Salivary gland ultrasonography revealed symmetrical bilateral swelling of the submandibular and parotid glands. Magnetic resonance imaging of the salivary glands showed symmetrical bilateral enlargement of the submandibular (4.0 × 2.6 × 5.2 cm) (Figure 6) and parotid (4.7 × 4.5 × 5.8 cm) glands.

The patient was again treated with 40 mg/d prednisone for 4 wk, following which, ultrasonography of the submandibular and parotid glands was normal.

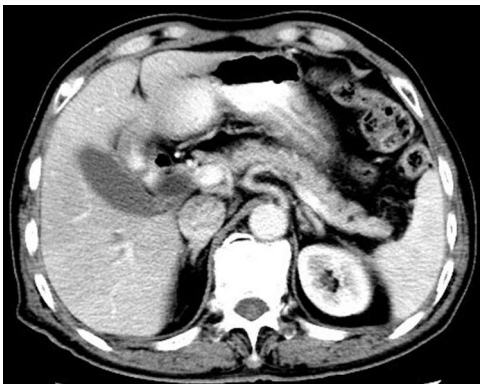


Figure 4 Computed tomography in August 2014 also showing the normal pancreas.

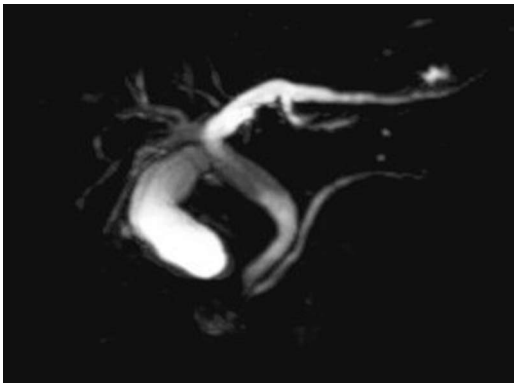


Figure 5 Magnetic resonance cholangiopancreatography showing stenosis of the distal common bile duct and proximal main pancreatic duct, and dilation of the proximal common bile duct and extra- and intra-hepatic bile ducts.

DISCUSSION

Diagnosis of autoimmune pancreatitis

The concept of AIP was first proposed by Yoshida *et al*[2] in 1995. The incidence of AIP was reported as 0.82 per 100000 cases, or roughly 4%-13% of chronic pancreatitis cases. The male to female ratio has been reported as 2.85:1.0 with 95% of patients over 45 years of age[3]. AIP is currently classified into 2 subgroups, type 1 and type 2. Type 1 AIP, the most common, frequently involves extra-pancreatic organs and is accompanied by IgG4-positive plasmacytes and/or elevated serum IgG4 levels. IgG4-RD was proposed to describe this condition[4,5], and AIP type 1 is a pancreatic manifestation of IgG4-RD.

As this case presented with elevated serum IgG4, was responsive to corticosteroid treatment, and had pancreatic imaging consistent with the diagnosis of AIP, it met the Asia and International Consensus Diagnostic Criteria for AIP[6,7]. It is important to discriminate AIP from pancreatic and bile duct carcinoma. Misdiagnosis of carcinoma can possibly lead to unnecessary surgery. In past years, 2.5% of AIP cases in North America underwent pancreaticoduodenectomy due to misdiagnosis of pancreatic cancer[8]. In this case, on CT

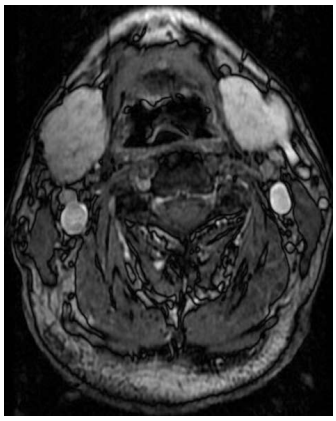


Figure 6 Magnetic resonance imaging of the salivary glands showing symmetrical bilateral enlargement of the submandibular gland (4.0 × 2.6 × 5.2 cm).

the pancreas appeared consistent in shape (sausage-like) with a diagnosis of AIP. ERCP showed stenosis of the distal common bile and proximal main pancreatic duct without significant dilation of the main distal pancreatic duct. Systemic PET/CT examination showed inflammatory characteristics in the entire pancreas. The tumor markers CA19-9, carcinoembryonic antigen, and alpha-fetoprotein were normal. Furthermore, the patient was very responsive to corticosteroids and at follow-up there was no sign of a tumor, so cancer of the pancreas and bile duct was ruled out.

Treatment of AIP

As per the treatment guidelines, this case was treated with oral corticosteroids and stents placed in the proximal main pancreatic duct and the distal common bile duct[9]. Follow-up imaging showed restoration of normal pancreatic morphology, but stenosis of the distal common bile duct and proximal main pancreatic duct remained, indicating that the pancreas recovered more quickly than the bile and pancreatic ducts. This founding was similar to another study reported in the literature[10]. Because fibrotic lesions have a poor response to steroids[11], IgG4-related fibrosis of the pancreatic and bile ducts was not completely ruled out. In accordance with published guidelines[9], steroids should be maintained for at least 3 years for proper management of AIP. This case did not meet this as steroids were only administered for 14 mo, so it was possible that the steroid maintenance therapy was not sufficient for this patient.

IgG4-related sialadenitis

After withdrawal of the steroid for 7 mo, the patient developed swelling of the bilateral submandibular glands. Subsequent steroid treatment for 50 d was effective, but upon cessation, the patient developed bilateral parotid edema. Examination showed normal pancreatic morphology but bilateral symmetrical swelling of the submandibular and parotid glands. Serum IgG4 was again elevated. Together with a history of AIP, this condition met the diagnostic criteria of IgG4-RD[12]. IgG4-RD often simultaneously involves multiple organs, but sialadenitis here was asynchronous with AIP. The involvement of autoimmune mechanisms in IgG4-RD is an underlying possibility[13], but it is not clear if different organs have different pathogenesis.

Prognosis

IgG4-RD responds well to treatment with corticosteroids, but there is a high likelihood of recurrence after ceasing steroid administration, and approximately 92% of AIP recurs within 3 years[14]. Continued administration of corticosteroids may be effective in preventing recurrence. Currently, it is not clear if IgG4-RD is related to neoplastic processes, but it is worth noting that there are reports in the literature of cancer with AIP and/or IgG4-related sialadenitis[15,16]. This patient will continue to be monitored for future recurrence.

COMMENTS

Case characteristics

A 67-year-old male with IgG4-related disease involving the pancreas and metachronous sialadenitis.

Clinical diagnosis

Autoimmune pancreatitis and sialadenitis.

Differential diagnosis

Pancreatic cancer, primary sclerosing cholangitis, lymphoma.

Laboratory diagnosis

Total bilirubin 52.70 μmol/L; direct bilirubin 29.50 μmol/L; IgG4 15.4 g/L.

Imaging diagnosis

Computed tomography showed significant enlargement of the entire pancreas and a capsule-like low-density rim surrounding the whole pancreas. Magnetic resonance imaging of the salivary glands indicated symmetrical bilateral enlargement of the submandibular and parotid glands.

Treatment

The patient was treated with prednisone.

Related reports

Immunoglobulin G4-related disease (IgG4-RD) is a systemic disease which affects multiple organs and follow up is important.

Term explanation

IgG4-RD is a systemic disease with multiple clinical manifestations and multiorgan involvement.

Experiences and lessons

For the patient with autoimmune pancreatitis, attention should be paid to multiorgan involvement during follow-up.

Peer-review

This article reported a case of multiple manifestations of IgG4-RD, including the pancreas and parotids and it is helpful to obtain more attention for this underdiagnosed disease.

REFERENCES

1 **Stone JH**, Zen Y, Deshpande V. IgG4-related disease. *N Engl J Med* 2012; **366**: 539-551 [PMID: 22316447 DOI: 10.1056/NEJMra1104650]

2 **Yoshida K**, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. *Dig Dis Sci* 1995; **40**: 1561-1568 [PMID: 7628283 DOI: 10.1007/BF02285209]

3 **Naitoh I**, Nakazawa T, Ohara H, Ando T, Hayashi K, Tanaka H, Okumura F, Miyabe K, Yoshida M, Sano H, Takada H, Joh T. Clinical significance of extrapancreatic lesions in autoimmune pancreatitis. *Pancreas* 2010; **39**: e1-e5 [PMID: 19924018 DOI: 10.1097/MPA.0b]

4 **Umehara H**, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T, Matsui S, Sumida T, Mimori T, Tanaka Y, Tsubota K, Yoshino T, Kawa S, Suzuki R, Takegami T, Tomosugi N, Kurose N, Ishigaki Y, Azumi A, Kojima M, Nakamura S, Inoue D. A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details. *Mod Rheumatol* 2012; **22**: 1-14 [PMID: 21881964 DOI: 10.1007/s10165-011-0508-6]

5 **Umehara H**, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T, Matsui S, Yoshino T, Nakamura S, Kawa S, Hamano H, Kamisawa T, Shimosegawa T, Shimatsu A, Nakamura S, Ito T, Notohara K, Sumida T, Tanaka Y, Mimori T, Chiba T, Mishima M, Hibi T, Tsubouchi H, Inui K, Ohara H. Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. *Mod Rheumatol* 2012; **22**: 21-30 [PMID: 22218969 DOI: 10.1007/s10165-011-0571-z]

6 **Otsuki M**, Chung JB, Okazaki K, Kim MH, Kamisawa T, Kawa S, Park SW, Shimosegawa T, Lee K, Ito T, Nishimori I, Notohara K, Naruse S, Ko SB, Kihara Y. Asian diagnostic criteria for autoimmune pancreatitis: consensus of the Japan-Korea Symposium on Autoimmune Pancreatitis. *J Gastroenterol* 2008; **43**: 403-408 [PMID: 18600383 DOI: 10.1007/s00535-008-2205-6]

7 **Shimosegawa T**, Chari ST, Frulloni L, Kamisawa T, Kawa S, Mino-Kenudson M, Kim MH, Klöppel G, Lerch MM, Löhr M, Notohara K, Okazaki K, Schneider A, Zhang L. International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the International Association of Pancreatology. *Pancreas* 2011; **40**: 352-358 [PMID: 21412117 DOI: 10.1097/MPA.0b013e3182142fd2]

8 **Hardacre JM**, Iacobuzio-Donahue CA, Sohn TA, Abraham SC, Yeo CJ, Lillemoe KD, Choti MA, Campbell KA, Schulick RD, Hruban RH, Cameron JL, Leach SD. Results of pancreaticoduodenectomy for lymphoplasmacytic sclerosing pancreatitis. *Ann Surg* 2003; **237**: 853-858; discussion 858-859 [PMID: 12796582 DOI: 10.1097/01.SLA.0000071516.54864.C1]

9 **Kamisawa T**, Okazaki K, Kawa S, Shimosegawa T, Tanaka M. Japanese consensus guidelines for management of autoimmune pancreatitis: III. Treatment and prognosis of AIP. *J Gastroenterol* 2010; **45**: 471-477 [PMID: 20213336 DOI: 10.1007/s00535-010-0221-9]

10 **Du SY**, Fang L, Zhang MG, Fan YH. Clinical and Imaging analysis of bile duct damage caused by autoimmune pancreatitis. *Clin J Gastroenterol Hepatol* 2014; **23**: 686-688

11 **Nakazawa T**, Naitoh I, Ando T, Hayashi K, Okumura F, Miyabe K, Yoshida M, Ohara H, Joh T. A case of advanced-stage sclerosing cholangitis with autoimmune pancreatitis not responsive to steroid therapy. *JOP* 2010; **11**: 58-60 [PMID: 20065555]

12 **Okazaki K**, Uchida K, Ikeura T, Takaoka M. Current concept and diagnosis of IgG4-related disease in the hepato-bilio-pancreatic system. *J Gastroenterol* 2013; **48**: 303-314 [PMID: 23417598 DOI: 10.1007/s00535-012-0744-3]

13 **Yamamoto M**, Naishiro Y, Suzuki C, Kokai Y, Suzuki R, Honda S, Abe T, Takahashi H, Shinomura Y. Proteomics analysis in 28 patients with systemic IgG4-related plasmacytic syndrome. *Rheumatol Int* 2010; **30**: 565-568 [PMID: 19609529 DOI: 10.1007/s00296-009-1030]

14 **Kamisawa T**, Shimosegawa T, Okazaki K, Nishino T, Watanabe H, Kanno A, Okumura F, Nishikawa T, Kobayashi K, Ichiya T, Takatori H, Yamakita K, Kubota K, Hamano H, Okamura K, Hirano K, Ito T, Ko SB, Omata M. Standard steroid treatment for autoimmune pancreatitis. *Gut* 2009; **58**: 1504-1507 [PMID: 19398440 DOI: 10.1136/gut.2008.172908]

15 **Inoue H**, Miyatani H, Sawada Y, Yoshida Y. A case of pancreas cancer with autoimmune pancreatitis. *Pancreas* 2006; **33**: 208-209 [PMID: 16868495 DOI: 10.1097/01.mpa.0000232329]

16 **Gill J**, Angelo N, Yeong ML, McIvor N. Salivary duct carcinoma arising in IgG4-related autoimmune disease of the parotid gland. *Hum Pathol* 2009; **40**: 881-886 [PMID: 19200575 DOI: 10.1016/j.humpath.2008.10.020]

P- Reviewer: Buijs J, Berger Z, Haruta I **S- Editor**: Yu J
L- Editor: Cant MR **E- Editor**: Zhang DN

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i31.9453

World J Gastroenterol 2015 August 21; 21(31): 9453-9456
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Growth hormone used to control intractable bleeding caused by radiation-induced gastritis

Liang Zhang, Wen-Jie Xia, Zheng-Sen Zhang, Xin-Liang Lu

Liang Zhang, Wen-Jie Xia, Zheng-Sen Zhang, Xin-Liang Lu, Department of Gastroenterology, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, Zhejiang Province, China

Author contributions: Lu XL was responsible for the patient's care and follow-up; Zhang ZS reviewed the literature; Xia WJ and Zhang L wrote the paper.

Conflict-of-interest statement: The authors declare no conflict of interest.

Correspondence to: Xin-Liang Lu, PhD, Department of Gastroenterology, Second Affiliated Hospital, School of Medicine, Zhejiang University, No. 88 Jiefang Road, Hangzhou 310009, Zhejiang Province, China. xinliang.lu@yahoo.com
Telephone: +86-571-87022776
Fax: +86-571-87022776

Received: February 3, 2015
Peer-review started: February 6, 2015
First decision: April 13, 2015
Revised: May 11, 2015
Accepted: June 10, 2015
Article in press: June 10, 2015
Published online: August 21, 2015

Abstract

Intractable bleeding caused by radiation-induced gastritis is rare. We describe a 69-year-old man with intractable hemorrhagic gastritis induced by postoperative radiotherapy for the treatment of esophageal carcinoma. Although anti-secretory therapy with or without octreotide was initiated for hemostasis over three months, melena still occurred off and on, and the patient required blood transfusions to maintain stable hemoglobin. Finally growth hormone was used in the treatment of hemorrhage for two weeks, and hemostasis was successfully achieved. This is the first report that growth hormone has been used to control intractable bleeding caused by radiation-induced gastritis.

Key words: Growth hormone; Upper gastrointestinal bleeding; Radiation-induced gastritis

Core tip: Intractable bleeding caused by radiation-induced gastritis is rare. We describe a 69-year-old man with intractable hemorrhagic gastritis induced by postoperative radiotherapy for the treatment of esophageal carcinoma. Anti-secretory therapy with or without octreotide seemed in vain. Finally growth hormone was used in the treatment of hemorrhage for two weeks, and hemostasis was successfully achieved. This is the first report that growth hormone has been used to control intractable bleeding caused by radiation-induced gastritis.

Zhang L, Xia WJ, Zhang ZS, Lu XL. Growth hormone used to control intractable bleeding caused by radiation-induced gastritis. *World J Gastroenterol* 2015; 21(31): 9453-9456 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i31/9453.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i31.9453

INTRODUCTION

Although upper gastrointestinal bleeding caused by

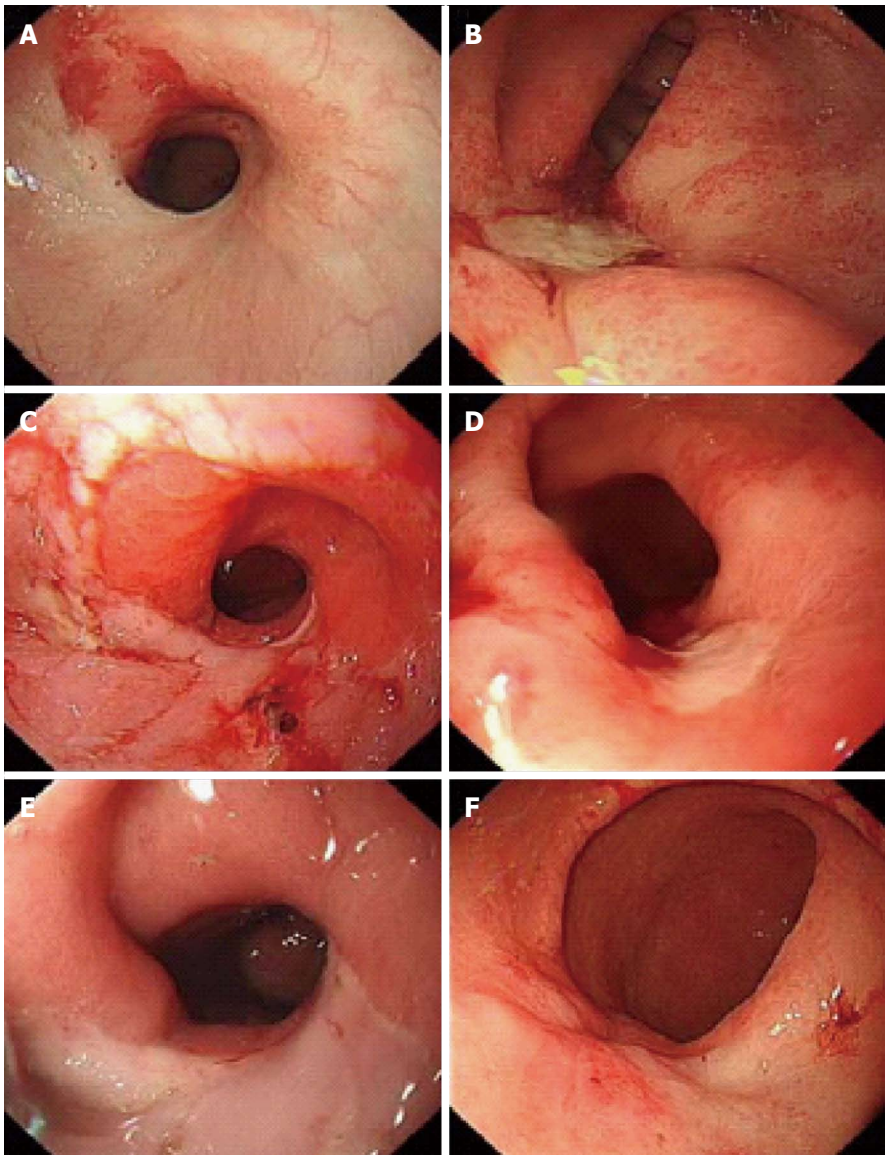


Figure 1 Endoscopic view. A: Diffuse edematous hyperemia and active bleeding from the esophagus-stomach anastomosis after radiation therapy; B: An ulcer with bleeding in the antrum after radiation therapy; C: Active oozing of blood from the esophagus-stomach anastomosis after three months of anti-secretory therapy; D: An ulcer with active bleeding after three months of anti-secretory therapy; E: No active oozing of blood from the esophagus-stomach anastomosis two months after growth hormone therapy; F: An ulcer scar without active bleeding two months after growth hormone therapy.

radiation-induced gastritis is infrequent, it is a very serious complication and is difficult to deal with. Nowadays, several methods were reported for treating upper gastrointestinal bleeding caused by radiation-induced gastritis, but there is still no standard treatment[1,2]. We encountered a case of intractable bleeding caused by radiation-induced gastritis which was induced by postoperative radiotherapy for the treatment of esophageal carcinoma. Successful hemostasis was achieved with treatment of growth hormone. This is the first report that growth hormone has been used to control intractable bleeding caused by radiation-induced gastritis.

CASE REPORT

A 69-year-old man was diagnosed with esophageal cancer and had a radical surgery for esophageal carcinoma in June 2013. The tumor was pathologically diagnosed as high differentiation squamous-cell carcinoma. After surgery, from July 2013 to December 2013, he was treated with the chemotherapy regimen of nedaplatin 130 mg plus tegafur, gimeracil and oteracil potassium capsules 60 mg b.i.d. for 6 times. In February 2014, he was treated with radiotherapy (CTV D95 5880cGy/28F, PTV D95 5040cGy/28F). Three months after radiotherapy, he began to appear paroxysmal burning epigastric pain with melena and severe anemia, and laboratory examination indicated that hemoglobin dropped to 43 g/L. Gastroscopy was performed and indicated a diffuse edematous hyperemia and active bleeding from the gastric mucosa close to the esophagus-stomach anastomosis (Figure 1A) and the antrum, and an ulcer with bleeding

in the greater curvature (Figure 1B). As hemoglobin level went up and down, blood transfusion was administered once or twice a week. Furthermore, the dosage of esomeprazole was enhanced from 40 mg Q12H to 40 mg Q8H, and combined with the use of octreotide for hemostasis over three months. Melena still occurred off and on, and the patient required blood transfusions to maintain stable hemoglobin. Thus gastroscopy was performed for evaluating the patient' s condition. It indicated active oozing of blood from the gastric mucosa close to the esophagus-stomach anastomosis (Figure 1C), and the gastric ulcer that was smaller than before with active bleeding (Figure 1D). A total of 16 units of packed red blood cells were transfused to maintain the level of hemoglobin at about 70 g/L.

Finally growth hormone (15 U QOD ih, 5 U mixed in 50 mL saline for 5 times, po) was used in the treatment of hemorrhage for two weeks, and hemostasis was successfully achieved. Without blood transfusions, the hemoglobin level improved to 90 g/L one month after the growth hormone therapy. The patient did not experience melena any more. The fecal occult blood test was negative, and the follow-up gastroscopy indicated no active oozing of blood from hemorrhagic lesions close to the esophagus-stomach anastomosis (Figure 1E), and an ulcer scar in the antrum without active bleeding (Figure 1F). The patient still receives proton pump inhibitor up to now, no gastrointestinal bleeding recurs, and his hemoglobin levels improve to 124 g/L without blood transfusions or iron supplements.

DISCUSSION

Radiation-induced gastritis may be more frequent than we realized, for many patients do not have obvious symptoms. The tolerance dose of radiation in the stomach and intestine is 45 Gy and 55 Gy, respectively[3]. In our case, the patient was treated with 58.8 Gy in CTV and 50.4 Gy in PTV. It induced intractable hemorrhagic gastritis. The primary typical injury of radiation-induced gastritis is acute inflammation of the gastric mucosa. When injury progresses, it might lead to mucosal ischemia, ulceration, and telangiectasis[4].

The standard treatment method for radiation-induced hemorrhagic gastritis has not been established. Argon plasma coagulation has been reported for successful hemostasis of radiation-induced hemorrhagic gastritis, colitis and proctitis[3,5-7]. However, our patient had extensive lesions, thus we could not use this strategy. Meanwhile, prednisolone[1,2], hyperbaric oxygen therapy[8], aminocaproic acid[4], and endoscopic band ligation[9] have also been reported for treating radiation-induced hemorrhagic gastritis. In our case, although anti-secretory therapy with or without octreotide was initiated for hemostasis over three months, melena still occurred off and on, and the patient required blood transfusions to maintain stable hemoglobin.

Growth hormone was reported to be able to increase protein synthesis, attenuate protein catabolism and stimulate cell proliferation and differentiation to facilitate wound healing, and in animal models it was also reported useful for gastric ulcer healing[10-12]. Thus in this case, we thought that it might be efficient for hemostasis. Maybe part of growth hormone taken orally could not have very good effect because of the function of digestion. Thus in this situation, digestive enzyme could not function well, and growth hormone could have an effect on the surface of the ulcer to facilitate the ulcer healing. Finally growth hormone (15 U QOD ih, 5 U mixed in 50 mL saline for 5 times, po) was used in the treatment of hemorrhage for two weeks, and hemostasis was successfully achieved. Without blood transfusions, the hemoglobin level improved to 90 g/L one month after growth hormone therapy.

Up to now, the patient still receives proton pump inhibitor, no gastrointestinal bleeding recurs, and his hemoglobin levels improve to 124 g/L without blood transfusions or iron supplements. Thus we recommend that growth hormone therapy can be tried as a first choice for intractable bleeding caused by radiation-induced gastritis.

COMMENTS

Case characteristics

A 69-year-old man presented paroxysmal burning epigastric pain with melena and severe anemia.

Clinical diagnosis

The abdomen is soft with minimal tenderness but no rebound tenderness.

Differential diagnosis

Gastric ulcer and recurrent esophageal cancer.

Laboratory diagnosis

Hemoglobin dropped to 43 g/L.

Imaging diagnosis

Gastroscopy was performed and indicated a diffuse edematous hyperemia and active bleeding from the gastric mucosa close to the esophagus-stomach anastomosis and the antrum, and an ulcer with bleeding in the greater curvature.

Treatment

Anti-secretory therapy with or without octreotide was initiated for hemostasis over three months, melena still occurred off and on, and the patient required blood transfusions to maintain stable hemoglobin. Finally growth hormone was used in the treatment of hemorrhage for two weeks, and hemostasis was successfully achieved.

Related reports

Intractable bleeding caused by radiation-induced gastritis is rare. Previous case reports indicated that argon plasma coagulation (APC), prednisolone, hyperbaric oxygen therapy, aminocaproic acid, and endoscopic band ligation might be useful for intractable bleeding.

Term explanation

APC is a medical endoscopic procedure which is primarily used to control bleeding from gastrointestinal tract lesions.

Experiences and lessons

Growth hormone therapy can be tried as a first choice for intractable bleeding caused by radiation-induced gastritis.

Peer-review

This interesting article is the first report that growth hormone has been used to control intractable bleeding caused by radiation-induced gastritis. The authors did good work effort. While because of the rarity, it still needs multicentric studies and long-term outcome and prognosis evaluation.

REFERENCES

1 **Yun HG**, Kim HY, Kim do Y, Lim YJ. Successful treatment of intractable bleeding caused by radiation-induced hemorrhagic gastritis using oral prednisolone: a case report. *Cancer Res Treat* 2015; **47**: 334-338 [PMID: 25327495]

2 **Zhang L**, Xie XY, Wang Y, Wang YH, Chen Y, Ren ZG. Treatment of radiation-induced hemorrhagic gastritis with prednisolone: a case report. *World J Gastroenterol* 2012; **18**: 7402-7404 [PMID: 23326152 DOI: 10.3748/wjg.v18.i48.7402]

3 **Shukuwa K**, Kume K, Yamasaki M, Yoshikawa I, Otsuki M. Argon plasma coagulation therapy for a hemorrhagic radiation-induced gastritis in patient with pancreatic cancer. *Intern Med* 2007; **46**: 975-977 [PMID: 17603236]

4 **Grover N**, Johnson A. Aminocaproic acid used to control upper gastrointestinal bleeding in radiation gastritis. *Dig Dis Sci* 1997; **42**: 982-984 [PMID: 9149051]

5 **Wada S**, Tamada K, Tomiyama T, Yamamoto H, Nakazawa K, Sugano K. Endoscopic hemostasis for radiation-induced gastritis using argon plasma coagulation. *J Gastroenterol Hepatol* 2003; **18**: 1215-1218 [PMID: 12974916]

6 **Venkatesh KS**, Ramanujam P. Endoscopic therapy for radiation proctitis-induced hemorrhage in patients with prostatic carcinoma using argon plasma coagulator application. *Surg Endosc* 2002; **16**: 707-710 [PMID: 11972221 DOI: 10.1007/s00464-001-8164-0]

7 **Tjandra JJ**, Sengupta S. Argon plasma coagulation is an effective treatment for refractory hemorrhagic radiation proctitis. *Dis Colon Rectum* 2001; **44**: 1759-1765; discussion 1771 [PMID: 11742157]

8 **Banerjee N**, Javed A, Deepak D, Pawar M, Chattopadhyay TK. Hyperbaric oxygen therapy: an adjuvant treatment modality for chemo-radiation induced hemorrhagic gastritis. *Trop Gastroenterol* 2011; **32**: 248-250 [PMID: 22332352]

9 **Staiano T**, Grassia R, Iiritano E, Bianchi G, Dizioli P, Buffoli F. Treatment of radiation-induced hemorrhagic gastritis with endoscopic band ligation. *Gastrointest Endosc* 2010; **72**: 452-453 [PMID: 20226458]

10 **Beckert S**, Class N, Farrahi F, Coerper S. Growth hormone enhances gastric ulcer healing in rats. *Med Sci Monit* 2004; **10**: BR255-BR258 [PMID: 15277985]

11 **Póvoa G**, Diniz LM. Growth hormone system: skin interactions. *An Bras Dermatol* 2011; **86**: 1159-1165 [PMID: 22281905]

12 **Breederveld RS**, Tuinebreijer WE. Recombinant human growth hormone for treating burns and donor sites. *Cochrane Database Syst Rev* 2014; **9**: CD008990 [PMID: 25222766 DOI: 10.1002/14651858.CD008990.pub3]

P- Reviewer: Al-Azzawi Y, Makhoul E **S- Editor**: Yu J
L- Editor: Wang TQ **E- Editor**: Liu XM

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327