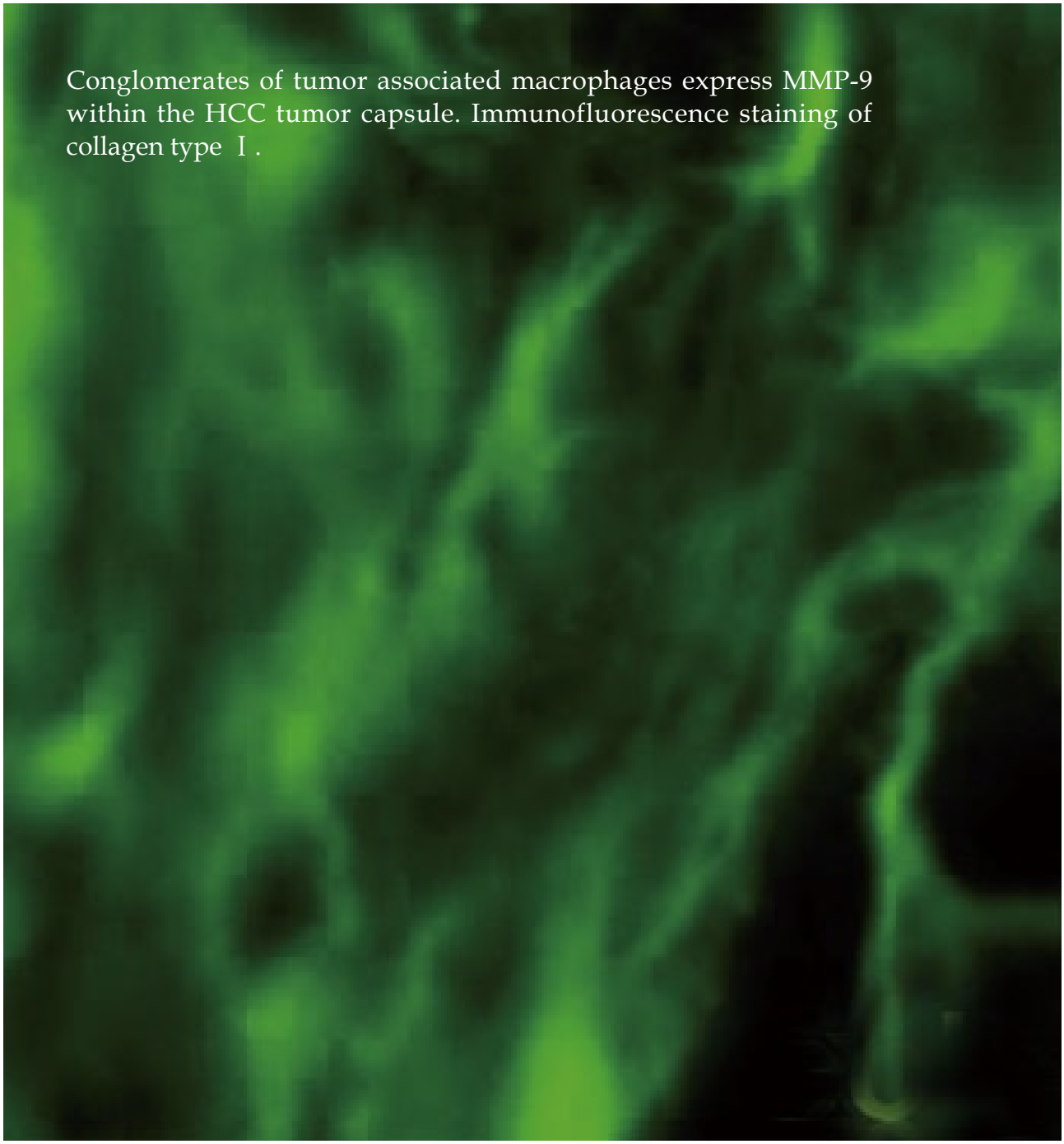
ISSN 1948-5182 (online)

World Journal of Hepatology

World J Hepatol 2010 May 27; 2(5): 171-202

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



Conglomerates of tumor associated macrophages express MMP-9 within the HCC tumor capsule. Immunofluorescence staining of collagen type Ⅰ.

WJH World Journal of Hepatology

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

Editorial Board

2009-2013

The *World Journal of Hepatology* Editorial Board consists of 572 members, representing a team of worldwide experts in hepatology. They are from 45 countries, including Argentina (4), Australia (7), Austria (2), Bangladesh (1), Belgium (3), Botswana (2), Brazil (8), Brunei Darussalam (1), Bulgaria (1), Canada (10), Chile (1), China (89), Egypt (3), Finland (1), France (15), Gambia (1), Germany (28), Greece (8), Hungary (3), India (20), Ireland (1), Israel (7), Italy (65), Japan (45), Malaysia (1), Mexico (4), Netherlands (4), Pakistan (2), Poland (1), Portugal (1), Philippines (1), Romania (1), Saudi Arabia (1), Singapore (4), South Korea (17), Spain (22), Sri Lanka (1), Sudan (1), Switzerland (2), Thailand (6), Tunisia (2), Turkey (13), United Kingdom (17), United States (144), and Venezuela (1).

Egypt

Finland

France

Gambia

Germany

Greece

Hungary

India

Ireland

Israel

Italy

Luca Aasaloni, *Bologna*
Giovanni Addolorato, *Rome*
Luigi E Adinolfi, *Naples*
Pietro Andreone, *Bologna*
M Appetecchia, *Rome*
Antonio Ascione, *Napoli*
Ferruccio Bonino, *Milano*
Bruno D Bruno, *Benevento*
Savino Bruno, *Milano*
Melchiorre Cervello, *Palermo*
Claudio Chiesa, *Rome*
Stefano Colagrande, *Firenze*
Massimo G Colombo, *Milan*
Samuele De Minicis, *Montegranaro*
Alessandro Vitale, *alessandro*
Fabio Farinati, *Padova*
Paolo Feltracco, *Padova*
Domenico Ferri, *Bari*
Amalia Gastaldelli, *Pisa*
Domenico Girelli, *Verona*
Fernando Goglia, *Benevento*
Alessandro Grasso, *Savona*
Ignazio Grattagliano, *Bari*
Pietro Invernizzi, *Milan*
Francesco Izzo, *Naples*
Amedeo Lonardo, *Modena*
Malaguarnera Lucia, *Trecastagni*
Massimo Di Maio, *Rossano*
Melania Manco, *Rome*
Andrea Mancuso, *Palermo*
F Marotta, *Milano*
Fabio Marra, *Florence*
Roberto Mazzanti, *Florence*
Giulia Morsica, *Milan*
Antonio Moschetta, *Bari*
Massimo Negrini, *Ferrara*
Andrea Nicolini, *Pisa*
Giuseppe R Nigri, *Rome*
Valerio Nobili, *Rome*
Valentina Pallottini, *Rome*
Adriano M Pellicelli, *Rome*
Marcello Persico, *Naples*
Massimo Pinzani, *Firenze*
Giovanni Polimeni, *Messina*
Camillo Porta, *Pavia*
Piero Portincasa, *Bari*
Emilio Quaia, *Trieste*
Giuseppe Remuzzi, *Bergamo*
Domenico Ribatti, *Bari*
Massimo Roncalli, *Rozzano*
Carlo Sabbà, *Bari*
Orazio Schillaci, *Rome*
Gaetano Serviddio, *Foggia*
Aurelio Sonzogni, *Bergamo*
Paolo Sorrentino, *Salerno*
Enea Spada, *Roma*
Giovanni Tarantino, *Naples*
Luciano Tarantino, *Naples*
Claudio Tiribelli, *Trieste*
Pierluigi Toniutto, *Udine*
Pietro Vajro, *Naples*
Luca Vigano, *Torino*

Japan

Yuichiro Eguchi, *Saga*
Munechika Enjoji, *Fukuoka*
Jiro Fujimoto, *Osaka*
Atsushi Hosui, *Osaka*
Kazuo Ikeda, *Nagoya*
Toru Ishikawa, *Niigata*
Yoshiaki Iwasaki, *Okayama*
Satoru Kakizaki, *Gunma*
Naoya Kato, *Tokyo*
Takumi Kawaguchi, *Kurume*
Kiminori Kimura, *Tokyo*
Tsuneo Kitamura, *Chiba*
Keiichi Kubota, *Tochigi*
Sabina Mahmood, *Okayama*
Hitoshi Maruyama, *Chiba*
Sachiko Matsuhashi, *Saga*
Toshihiro Mitaka, *Sapporo*
Eiji Miyoshi, *Yamada-oka Suita*
Zenichi Morise, *Toyoake Aichi*
Ryuichi Morisihita, *Osaka*
Yoshiki Murakami, *Kyoto*
Satoru Murata, *Tokyo*
Atsushi Nakajima, *Kanagawa*
Yasuni Nakanuma, *Kanazawa*
Waka Ohishi, *Hiroshima*
Morikazu Onji, *Matsuyama*
Toshiji Saibara, *Nankoku*
Hiroaki Shiba, *Tokyo*
Ikuo Shoji, *Hyogo*
Ryo Sudo, *Yokohama*
Yoshio Sumida, *Nara*
Shinji Tanaka, *Tokyo*
Takuji Tanaka, *Gifu*
Akihiko Tsuchida, *Tokyo*
Takato Ueno, *Kurume*
Shinichi Ueno, *Kagoshima*
Kiyohito Yagi, *Osaka*
Yo-ichi Yamashita, *Hiroshima*
Teruyoshi Yanagita, *Saga*
Shuang-Qin Yi, *Kanazawa*
Hiroshi Yoshida, *Tokyo*
Hitoshi Yoshiji, *Nara*

Malaysia

Kamsiah Jaarin, *Kuala Lumpur*

Mexico

Norberto C Chavez-Tapia, *Tlalpan*
Javier Lizardi Cervera, *Tlalpan CP*
Saúl Villa-Treviño, *México DF*
Florencia V Vorackova, *México DF*

Netherlands

Robert Jacobus de Knegt, *Rotterdam*
TU Hoogenraad, *Heidelberglaan*
Maarten E Tushuizen, *MB Amsterdam*
Robert C Verdonk, *RB Groningen*

Pakistan

Syed Hamid Ali, *Karachi*
Huma IQ TI, *Islamabad*

Poland

Maria ES Lotowska, *Bialystok*

Portugal

Felix Dias Carvalho, *Porto*

Philippines

Janus P Ong, *Manila*

Romania

Eugen Georgescu, *Craiova*

Saudi Arabia

Ahmed Helmy, *Riyadh*

Singapore

Wei Ning Chen, *Singapore*
Si-Shen Feng, *Singapore*
Lang Zhuo, *Singapore*
Chun-Tao Wai, *Singapore*

South Korea

Sang Hoon Ahn, *Seoul*
Sun Pyo Hong, *Yongin*
Byung Ihn Choi, *Seoul*
Seok Joo Han, *Seoul*
Kyung Lib Jang, *Busan*
Bum-Joon Kim, *Seoul*
Dong Goo Kim, *Seoul*
Kyung Sik Kim, *Seoul*
Meehyein Kim, *Yongin*
Young Chul Kim, *Seoul*
Mi-Kyung Lee, *Jeonnam*
Young-Ik Lee, *Taejon*
Kwan-Kyu Park, *Daegu*
Hyunchul Rhim, *Seoul*
In Kyoung Lim, *Gyunggi-do*
Dae-Yeul Yu, *Daejeon*
Jong Won Yun, *Kyungbuk*

Spain

Jose AG Agundez, *Badajoz*
Maria Angeles, *Madrid*
Agustin Castiella, *Mendaro*
Ruben Ciria, *Cordoba*
Joan Clari, *Barcelona*
Maria Buti Ferret, *Barcelona*

Puri Fortes, *Pamplona*
Joan Genescà, *Barcelona*
María J Gómez-Lechón, *Valencia*
Arias Jaime, *Madrid*
Ángeles Pajares María, *Madrid*
Jordi Muntane, *Cordoba*
Jose JG Marin, *Salamanca*
Julia P Onsurbe, *Barcelona*
Albert Parés, *Barcelona*
Sonia Ramos, *Madrid*
Cristina Ripoll, *Madrid*
Isabel F Romero, *Barcelona*
Marta R Romero, *Salamanca*
Juan Macias Sanchez, *Sevilla*
Juan Sastre, *Valencia*
Manuel Vázquez-Carrera, *Barcelona*

Sri Lanka

EGD Shaman Rajindrajith, *Ragama*

Sudan

Hatim MY Mudawi, *Khartoum*

Switzerland

Beat Mullhaupt, *Zurich*
Maurer A Christoph, *Liestal*

Thailand

Nattiya Hirankarn, *Bangkok*
Somchai Pinlaor, *Khon Kaen*
Yong Poovorawan, *Bangkok*
Abhasnee Sobhonslidsuk, *Bangkok*
Chanitra Thuwajit, *Bangkok*
Sopit Wongkham, *Khon Kaen*

Tunisia

Olfa Bahri, *Tunis-Belvedere*
Chadli Dziri, *Tunis*

Turkey

Inci Alican, *Istanbul*
Ahmet Atessahin, *Elazig*
Yasemin Hatice Balaban, *Ankara*
Hayrullah Derici, MD, *Izmir*
Cigdem Ulukaya Durakbasa, *Istanbul*
Muhsin MM Harputluoglu, *Malatya*
Abdurrahman Kadayifci, *Gaziantep*
Adnan Kadayifci, *Antalya*
Ali Sazci, *Kocaeli*
Ilker Tasci, *Ankara*
Mehmet Yalniz, *Elazig*
Serkan Yener, *Izmir*
Yusuf Yilmaz, *Istanbul*

United Kingdom

Alastair David Burt, *Newcastle*
David O Cosgrove, *London*
Anil Dhawan, *London*
Indra Neil Guha, *Nottingham*
Phillip M Harrison, *London*
Hübscher SG Hübscher, *Birmingham*
Long R Jiao, *London*
AT Koulaouzidis, *Edinburgh*
Patricia Lalor, *Birmingham*
David A Lomas, *Cambridge*
Rajeshwar P Mookerjee, *London*
Gareth J Morris-Stiff, *Wales*
Kathryn L Nash, *Southampton*
Derek Anthony O'Reilly,
Christian P Selinge, *Bolton*
Konstantinos Tziomalos, *London*
Feng Wu, *Oxford*

United States

Gary A Abrams, *Montgomery*
Hassan H A-Kader, *Tucson*
Hans-Olov Adami, *Massachusetts*
Joseph Ahn, *Maywood*
Shannon Marie Bailey, *Alabama*
Numan Cem Balci, *St Louis MO*
Edmund J Bini, *New York*
Victor E Buckwold, *Frederick*
Roniel Cabrera, *Gainesville*
Guoqing Cao, *Indiana*
Disaya Chavalitdhamrong, *New York*
Chien-Shing Chen, *Loma Linda*
Fei Chen, *Morgantown*
Su Chen, *San Antonio*
Youhai H Chen, *Philadelphia*
Anne M Covey, *New York*
Mark J Czaja, *New York*
Srikanta Dash, *New Orleans*
Anthony JB Demetris, *Pittsburgh*
Sridevi Devaraj, *California*
Lisa Ross Dixon, *Gainesville*
Terrence M Donohue, *Omaha*
Q Ping Dou, *Detroit*
Murray N Ehrinpreis, *Detroit*
Marwan Ghazi Fakih, *Buffalo*
Shengyun Fang, *Maryland*
Claus J Fimmel, *Illinois*
Robert Anthony Fisher, *Virginia*
Samuel W French, *Torrance*
Phillip A Furman, *Princeton*
M Eric Gershwin, *California*
Jalal K Ghali, *Michigan*
Grace Liejun Guo, *Kansas City*
Dieter Haemmerich, *Charleston*
Young S Hahn, *Charlottesville*
Stephen A Harrison, *Texas*
Dee Harrison-Findik, *Nebraska*
Sidhartha Hazari, *Louisiana*
Thomas S Helling, *Jackson*
Alan W Hemming, *Florida*
Iryna S Hepburn, *Evans*
Ai-Xuan L Holterman, *Chicago*
Ke-Qin Hu, *California*
Guangcun Huang, *Ohio*
Wendong Huang, *California*
Rachel M Hudacko, *New Brunswick*
Michael John Jacobs, *Michigan*
Hartmut W Jaeschke, *Kansas City*
Ravi Jhaveri, *North Carolina*
Lynt B Johnson, *Washington*
Neil Louis Julie, *Bethesda*
Sanjay Kakar, *San Francisco*
Sanjeeva P Kalva, *Boston*
Jing X Kang, *Massachusetts*
Hetal Karsan, *Georgia*
Emmet B Keeffe, *California*
Nancy Ellen Kemeny, *New York*
Andrew Scott Kennedy, *Cary*
Kusum K Kharbanda, *Omaha*
David H Kirn, *California*
Hyam Lerner Leffert, *La Jolla*
Stacee Marie Lerret, *Milwaukee*
Fengzhi Li, *New York*
Wei Li, *Houston*
Shuang Liu, *Indiana*
Su Hao Lo, *Davis*
Daniel G Maluf, *Richmond*
Jose E Manautou, *Storrs*
Richard S Mangus, *Indiana*
Mary Ko Manibusan, *Virginia*
Paul Martin, *Miami,*
Jochen Mattner, *Ohio*
James A McCubrey, *North Carolina*
Valentina Medici, *Sacramento*
George Michalopoulos, *Pittsburgh*
Smruti R Mohanty, *Illinois*
John T Moore, *GlaxoSmithKline*
Ravi Murthy, *Texas*
Laura E Nagy, *Cleveland*
Sagar U Nigwekar, *Rochester*
Eileen M O'Reilly, *New York*
Kevin FS O'Carroll, *Hershey*
Melissa Kay Osborn, *Atlanta*
Helieh Saatara Oz, *Kentucky*
Igor P Pogribny, *Arkansas*
Nicholas C Popescu, *Bethesda Maryland*
Daniel S Pratt, *Boston*
Ratna B Ray, *Louis*
Nancy Reau, *Chicago*
Janardan K Reddy, *Chicago*
Martin J Ronis, *Little Rock*
Phillip Ruiz, *Florida*
Tanios B Saab, *Columbus*
Adnan Said, *Madison*
Neeraj Saxena, *Georgia*
Raymund R Saxena, *Minnesota*
Ann Scheimann, *Baltimore*
Timothy M Schmitt, *Charlottesville*
Bernd Schnabl, *La Jolla*
Kunwar Shailubhai, *Pennsylvania*
Muhammad Y Sheikh, *California*
Perry Shen, *Winston-Salem*
Viji Shridhar, *Rochester*
Shivendra D Shukla, *Missouri*
Ashwani K Singal, *Galveston*
Keshav K Singh, *New York*
Omar Skalli, *Shreveport*
Byoung-Joon Song, *Maryland*
Branko Stefanovic, *Tallahassee*
Stephen Strom, *Pennsylvania*
Xiao Su, *San Francisco*
Wing-Kin Syn, *North Carolina*
Gyongyi Szabo, *Massachusetts*
Shinako Takada, *Houston*
Yueming Tang, *Chicago*
John M Taylor, *Philadelphia*
Swee H The, *Springfield*

WJH World Journal of Hepatology

Contents

Monthly Volume 2 Number 5 May 27, 2010

Contents

World Journal of Hepatology
Volume 2 Number 5 May 27, 2010

ABOUT COVER

Roderfeld M, Rath T, Lammert F, Dierkes C, Graf J, Roeb E. Innovative immunohistochemistry identifies MMP-9 expressing macrophages at the invasive front of murine HCC

World J Hepatol 2010; 2(5): 175-179

http://www.wjgnet.com/1948-5182/full/v2/i5/175.htm

AIM AND SCOPE

World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a monthly, open-access, peer-reviewed journal supported by an editorial board of 572 experts in hepatology from 45 countries.

The major task of *WJH* is to report rapidly the most recent results in basic and clinical research on hepatology, including: liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology.

NAME OF JOURNAL
World Journal of Hepatology

LAUNCH DATE
October 31, 2009

SPONSOR
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

EDITING
Editorial Board of *World Journal of Hepatology*,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0038
Fax: +86-10-8538-1893
E-mail: wjh@wjgnet.com
http://www.wjgnet.com

PUBLISHING
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

SUBSCRIPTION
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

ONLINE SUBSCRIPTION
One-Year Price 216.00 USD

PUBLICATION DATE
May 27, 2010

CSSN
ISSN 1948-5182 (online)

EDITORIAL OFFICE
Hai-Ning Zhang, Director
World Journal of Hepatology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0038
Fax: +86-10-8538-1893
E-mail: wjh@wjgnet.com
http://www.wjgnet.com

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm. If you do not have web access please wcontact the editorial office.

ONLINE SUBMISSION
http://www.wjgnet.com/1948-5182office

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i5.171

World J Hepatol 2010 May 27; 2(5): 171-174
ISSN 1948-5182 (online)

EDITORIAL

Hepatocellular carcinoma in thalassemia: A critical review

Andrea Mancuso

Andrea Mancuso, Emergency Area, Ospedali Riuniti di Sciacca (Ag), Palermo 90138, Italy
Author contributions: Mancuso A contributed solely to this paper.
Correspondence to: Andrea Mancuso, MD, Emergency Area, Ospedali Riuniti di Sciacca (Ag), Via Houel 13, Palermo 90138, Italy. mancandrea@libero.it
Telephone: +39-091-6090252 Fax: +39-091-6090252,
Received: April 12, 2010 Revised: May 12, 2010
Accepted: May 19, 2010
Published online: May 27, 2010

Abstract

Due to blood transfusions, thalassemics are often infected with either hepatitis C virus (HCV) or hepatitis B virus and often have hemochromatosis. Hepatocellular carcinoma (HCC) has emerged in thalassemics only recently as a result of the improvement in thalassemia outcomes. In fact, a prospective study estimated an HCC incidence in β-thalassemia of about 2%. Although data are scanty, HCC screening in thalassemics with risk factors for HCC should be carried out. HCV treatments have some efficacy in HCV infected thalassemics despite partial contraindication to ribavirin and iron overload. However, there are no data on how HCV treatment translates into HCC prevention. Preliminary data suggest that HCC treatment in thalassemics should generally have the same outcomes as in non-thalassemics. Although coexistence of severe comorbidities makes liver transplantation challenging, this therapeutic possibility should not be precluded for well selected HCC β-thalassemia patients. In fact, 2 transfusion dependent adult HCC β-thalassemia patients have recently undergone successful liver transplantation with a good outcome. In conclusion, HCC seems to be a developing issue in thalassemia and HCC screening should be carried out. HCC treatment, including liver transplantation, can be performed in selected patients. A multidisciplinary effort is needed for management.

Key words: Thalassemia; Hepatocellular carcinoma; Hemochromatosis; Screening; Complication; Liver transplantation

Peer reviewers: Fei Chen, PhD, Professor, Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, United States; Ali Sazci, MSc, PhD, Professor, Kocaeli University, Faculty of Medicine, Department of Medical Biology and Genetics, Umuttepe, Kocaeli 41380, Turkey

Mancuso A. Hepatocellular carcinoma in thalassemia: A critical review. *World J Hepatol* 2010; 2(5): 171-174 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i5/171.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i5.171

INTRODUCTION

Thalassemias are rare inherited hemoglobin disorders resulting in chronic hemolytic anemia, endemic particularly in the Mediterranean Area and South-East Asia[1]. Depending on severity, two clinical forms are distinguished: thalassemia major (TM), characterized by severe anemia starting during the first year of life, requiring lifelong transfusion therapy for survival, and thalassemia intermedia (TI), characterized by later onset and generally milder anemia, permitting survival without regular transfusion therapy[2].

Due to blood transfusions, many patients with β-thalassemia are infected with either hepatitis C virus (HCV) or hepatitis B virus (HBV), particularly those who were born before the 1990s[3]. Moreover, most of the patients have hemochromatosis, which is the main cause of morbidity and mortality. In the past, HCC was not a known complication of thalassemia probably because many patients did not survive long enough to develop the condition and also because there was insufficient attention to the issue. In fact, many patients died at a young age, mainly due to heart failure. Recently, the outcome of thalassemia has improved and chronic organ damage, in

particular end-stage liver disease, has become a frequent complication. Better outcome is related to the improved treatment of iron overload with iron chelating drugs in the management of thalassemia complications, particularly heart disease, and in the prevention of infectious diseases secondary to blood transfusion[4].

Hepatocellular carcinoma (HCC) is a complication of cirrhosis and it affects prognosis. Occasionally, HCC can develop on a cirrhosis-free liver if other risk factors are present. Nowadays, a range of well-established treatments exist that can improve survival of HCC patients[5-13]. In this paper, the knowledge of HCC in thalassemia will be updated. This review deals with patients with both TM and TI and excludes other thalassemia syndromes, such as Sickle cell/beta thalassemia that are clinically similar to Sickle Cell Disease and quite different from TM and TI.

EPIDEMIOLOGY OF THALASSEMIA-ASSOCIATED HCC

Awareness of HCC as a clinical complication of thalassemia has developed in the last few years. In the past, many patients did not survive long enough to develop HCC. In fact, many patients died at a young age, mainly due to heart failure[4,14]. Recently, the outcome of thalassemia has improved and chronic organ damage, in particular end-stage liver disease and HCC, has appeared as a frequent complication[4]. In fact, a recent multicenter retrospective study on patients with different thalassemia syndromes, identified 22 cases of HCC in Italian patients, 19 of whom were affected by either TM or TI[15]. Furthermore, a prospective study was conducted on HCC incidence in 108 thalassemia patients (38 with TM and 70 with TI; median age 36.8 yr) screened with liver ultrasound imaging. Seventy-two (31 TM, 41 TI) with one or more risk factors for HCC (iron overload in 72, HCV infection in 46, HBV infection in two, liver cirrhosis in 10) and 33 (4 TM, 29 TI) without risk factors were included in the study; three were excluded (1 with a previous diagnosis of HCC on Child-Pugh class C cirrhosis, and two patients who were under 18 years old). All patients with iron overload were treated with at least one iron chelating drug. Once a liver focal lesion was found at ultrasound, HCC diagnosis was confirmed following current guidelines[13]. Overall, two of the 72 with at least one HCC risk factor were found to have a newly developed HCC, with an estimated HCC incidence in thalassemia of about 2%[16].

Moreover, other new cases have been recently discovered, suggesting that HCC is becoming one of the leading clinical problem in thalassemia (unpublished data). Finally, if the future trend confirms an increase in the number of HCC in thalassemia, it would be reasonable to recommend HCC screening of all thalassemia patients with some risk factor since this could allow early treatment, leading to improved outcomes[17,18].

HCC PREVENTION IN THALASSEMIA PATIENTS

Currently, there are no effective tools for preventing HCC in thalassemia. Since hepatic disease is multifactorial in thalassemia, the theoretical possibility of preventing HCC is based on the possibility of preventing or curing HCC risk factors, namely hemochromatosis and chronic viral hepatitis, mainly due to HCV.

Hemochromatosis is a known risk factor for HCC[19,20]. Treatment of iron overload with iron chelating drugs is certainly the leading reason why thalassemia outcomes have recently improved dramatically. Since subcutaneous or intravenous deferoxamine was first shown to be an effective chelating agent[4], new oral drugs have been developed which have proven as effective as deferoxamine with better compliance[21]. Moreover, as recent evidence suggests, the use of multiple chelating drugs taken either concomitantly or sequentially seems likely to be the future route for iron overload treatment in thalassemia[22]. However, while thalassemia outcomes have been improving in recent years mainly because of iron chelation, HCC has emerged as a new complication, suggesting perhaps that current chelating regimens alone are not effective enough to prevent HCC.

Treatment of HCV-related chronic hepatitis in thalassemia using different kinds of interferon with or without ribavirin has had variable results in a number of small trials. Overall, most of the studies reported some efficacy. However, in addition to the rather small number of patients treated, there remain concerns about the safety of ribavirin. In fact, ribavirin increases not only the number of responders but also increases the need for blood transfusion[23-33].

Based on the information currently available, there is no evidence suggesting that HCV treatment can translate into HCC prevention in thalassemia. However, it is plausible that prevention of HCV infection due to blood transfusion will, in future, result in a reduction of HCC in thalassemia.

Potential treatments of thalassemia-associated HCC

Since survival of patients with thalassemia has dramatically improved in recent years[4], thalassemia should not be considered a contraindication per se to HCC treatments that are effective in non-thalassemia patients[5-13].

To date, there is little published evidence concerning the treatment of HCC in thalassemia. However, both percutaneous radio frequency thermoablation and ethanol injection[16], surgical resection[16,34] and chemoembolization (unpublished data) have been shown effective and safe for the treatment of HCC in selected patients with thalassemia. Recent determinations of the therapeutic efficacy of sorafenib showed some significant improvement in prognosis for earlier stage HCC[7,8]. It is, as yet, unknown whether sorafenib will be beneficial for the HCC associated with thalassemia.

A special mention should be made of the possibility of

liver transplantation in thalassemia patients. In fact, choice of the best treatment for HCC in thalassemia remains controversial. In particular, the possibility of treating HCC or end-stage liver disease with liver transplantation has long been rejected because of organ shortage and because thalassemia is considered a contraindication. Only one combined heart and liver transplantation has been reported and the outcome is currently not known[35]. The recent demonstration of improvements in thalassemia outcomes should prompt a reconsideration of this issue[4]. It is no longer reasonable that thalassemia should be considered a contraindication to liver transplantation, provided there is not any significant co-morbidity, namely heart disease and severe pulmonary hypertension[14]. In fact, encouraging results in this regard have been achieved in recent studies from the author's team, which showed successful liver transplantation in 2 transfusion dependent thalassemia patients at 2 different Liver Transpantation Units. Post-transplantation outcome has been satisfactory in both cases after 6 mo and 2 years follow-up evaluations, respectively. Of course, a multidisciplinary effort is needed for management.

CONCLUSION

Thalassemia is a rare disease in which the appearance of HCC as a complication is mainly the result of recently improved outcomes in developed countries. Preliminary data suggest an incidence of HCC in thalassemia of about 2%. However, since thalassemia is endemic in many under-developed countries where patients are probably not screened for HCC, it is possible that present knowledge of this issue represents only the tip of an iceberg.

Periodic liver Ultrasound HCC screening should probably be considered for thalassemia patients with risk factors for HCC.

Prevention of HCV infection through blood transfusion is nowadays the only known evidence-based means to prevent HCC in thalassemia.

HCC treatment in thalassemia patients should be the same as for non thalassemic HCC patients. Although coexistence of severe comorbidities makes the role of liver transplantation challenging, this therapeutic possibility should not be precluded for well selected HCC thalassemia patients. Of course, a multidisciplinary effort is needed for management of transplantation patients.

Many of the considerations reported in this review are extrapolated from scanty data that surely lack comprehensive evidence and they are mainly the personal opinion of the author. Multicenter international studies should be performed to strengthen these data.

REFERENCES

1 **Higgs DR**, Thein SL, Woods WG. The molecular pathology of the thalassemias. In: Weatherall DJ, Clegg B, editors. The thalassemia syndromes, 4th edn Oxford: Blackwell Science, 2001: 133-191

2 **Cappellini MD**, Caruso V, Cianciulli P, Filosa A, Galanello R, Lai ME. Guidelines for beta-thalassemia intermedia. Emothal, Sett-DiC 2005; **3**: 37-46

3 **Aach RD, Stevens CE**, Hollinger FB, Mosley JW, Peterson DA, Taylor PE, Johnson RG, Barbosa LH, Nemo GJ. Hepatitis C virus infection in post-transfusion hepatitis. An analysis with first- and second-generation assays. *N Engl J Med* 1991; **325**: 1325-1329

4 **Borgna-Pignatti C**, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, Romeo MA, Forni GL, Gamberini MR, Ghilardi R, Piga A, Cnaan A. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica* 2004; **89**: 1187-1193

5 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236

6 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917

7 **Rampone B**, Schiavone B, Martino A, Viviano C, Confuorto G. Current management strategy of hepatocellular carcinoma. *World J Gastroenterol* 2009; **15**: 3210-3216

8 **Poon D**, Anderson BO, Chen LT, Tanaka K, Lau WY, Van Cutsem E, Singh H, Chow WC, Ooi LL, Chow P, Khin MW, Koo WH. Management of hepatocellular carcinoma in Asia: consensus statement from the Asian Oncology Summit 2009. *Lancet Oncol* 2009; **10**: 1111-1118

9 **Verslype C**, Van Cutsem E, Dicato M, Arber N, Berlin JD, Cunningham D, De Gramont A, Diaz-Rubio E, Ducreux M, Gruenberger T, Haller D, Haustermans K, Hoff P, Kerr D, Labianca R, Moore M, Nordlinger B, Ohtsu A, Rougier P, Scheithauer W, Schmoll HJ, Sobrero A, Tabernero J, van de Velde C. The management of hepatocellular carcinoma. Current expert opinion and recommendations derived from the 10th World Congress on Gastrointestinal Cancer, Barcelona, 2008. *Ann Oncol* 2009; **20** Suppl 7: vii1-vii6

10 **El-Serag HB**, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1752-1763

11 **Tanwar S**, Khan SA, Grover VP, Gwilt C, Smith B, Brown A. Liver transplantation for hepatocellular carcinoma. *World J Gastroenterol* 2009; **15**: 5511-5516

12 **Llovet JM**, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. *J Hepatol* 2008; **48** Suppl 1: S20-S37

13 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430

14 **Cogliandro T**, Derchi G, Mancuso L, Mayer MC, Pannone B, Pepe A, Pili M, Bina P, Cianciulli P, De Sanctis V, Maggio A. Guideline recommendations for heart complications in thalassemia major. *J Cardiovasc Med (Hagerstown)* 2008; **9**: 515-525

15 **Borgna-Pignatti C**, Vergine G, Lombardo T, Cappellini MD, Cianciulli P, Maggio A, Renda D, Lai ME, Mandas A, Forni G, Piga A, Bisconte MG. Hepatocellular carcinoma in the thalassaemia syndromes. *Br J Haematol* 2004; **124**: 114-117

16 **Mancuso A**, Sciarrino E, Renda MC, Maggio A. A prospective study of hepatocellular carcinoma incidence in thalassemia. *Hemoglobin* 2006; **30**: 119-124

17 **Bolondi L**. Screening for hepatocellular carcinoma in cirrhosis. *J Hepatol* 2003; **39**: 1076-1084

18 **Sangiovanni A**, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R, Morabito A, De Franchis R, Colombo M. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. *Gastroenterology* 2004; **126**: 1005-1014

19 **Kowdley KV**. Iron, hemochromatosis, and hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S79-S86

20 **Elmberg M**, Hultcrantz R, Ekbom A, Brandt L, Olsson S, Olsson R, Lindgren S, Lööf L, Stål P, Wallerstedt S, Almer S, Sandberg-Gertzén H, Askling J. Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. *Gastroenterology* 2003; **125**: 1733-1741

21 **Roberts DJ**, Brunskill SJ, Doree C, Williams S, Howard J, Hyde CJ. Oral deferiprone for iron chelation in people with thalassaemia. *Cochrane Database Syst Rev* 2007; CD004839

22 **Maggio A**, Vitrano A, Capra M, Cuccia L, Gagliardotto F, Filosa A, Romeo MA, Magnano C, Caruso V, Argento C, Gerardi C, Campisi S, Violi P, Malizia R, Cianciulli P, Rizzo M, D'Ascola DG, Quota A, Prossomariti L, Fidone C, Rigano P, Pepe A, D'Amico G, Morabito A, Gluud C. Long-term sequential deferiprone-deferoxamine versus deferiprone alone for thalassaemia major patients: a randomized clinical trial. *Br J Haematol* 2009; **145**: 245-254

23 **Saffar MJ**, Saffar H, Khalilian AR, Naqshvar F. Combination therapy with interferon and ribavirin for chronic hepatitis C infection in beta-thalassaemia major. *East Mediterr Health J* 2009; **15**: 785-791

24 **Inati A**, Taher A, Ghorra S, Koussa S, Taha M, Aoun E, Sharara AI. Efficacy and tolerability of peginterferon alpha-2a with or without ribavirin in thalassaemia major patients with chronic hepatitis C virus infection. *Br J Haematol* 2005; **130**: 644-646

25 **Butensky E**, Pakbaz Z, Foote D, Walters MC, Vichinsky EP, Harmatz P. Treatment of hepatitis C virus infection in thalassemia. *Ann N Y Acad Sci* 2005; **1054**: 290-299

26 **Li CK**, Chan PK, Ling SC, Ha SY. Interferon and ribavirin as frontline treatment for chronic hepatitis C infection in thalassaemia major. *Br J Haematol* 2002; **117**: 755-758

27 **Di Marco V**, Lo Iacono O, Capra M, Grutta S, Ciaccio C, Gerardi C, Maggio A, Renda D, Almasio P, Pisa R. alpha-Interferon treatment of chronic hepatitis C in young patients with homozygous beta-thalassemia. *Haematologica* 1992; **77**: 502-506

28 **Wonke B**, Donohue SM, Hoffbrand AV, Scheuer PJ, Brown D, Dusheiko G. Recombinant alpha 2B interferon (IFN) in the treatment of chronic hepatitis C disease in thalassaemia major (TM). *Bone Marrow Transplant* 1993; **12** Suppl 1: 24-25

29 **Di Marco V**, Lo Iacono O, Capra M, Grutta S, Ciaccio C, Gerardi C, Maggio A, Renda D, Almasio P, Pisa R. Alpha interferon treatment of chronic hepatitis C in beta-thalassaemia. *Gut* 1993; **34**: S142-S143

30 **Donohue SM**, Wonke B, Hoffbrand AV, Reittie J, Ganeshaguru K, Scheuer PJ, Brown D, Dusheiko G. Alpha interferon in the treatment of chronic hepatitis C infection in thalassaemia major. *Br J Haematol* 1993; **83**: 491-497

31 **Di Marco V**, Lo Iacono O, Almasio P, Ciaccio C, Capra M, Rizzo M, Malizia R, Maggio A, Fabiano C, Barbaria F, Craxì A. Long-term efficacy of alpha-interferon in beta-thalassemics with chronic hepatitis C. *Blood* 1997; **90**: 2207-2212

32 **Telfer PT**, Garson JA, Whitby K, Grant PR, Yardumian A, Hoffbrand AV, Wonke B. Combination therapy with interferon alpha and ribavirin for chronic hepatitis C virus infection in thalassaemic patients. *Br J Haematol* 1997; **98**: 850-855

33 **Spiliopoulou I**, Repanti M, Katinakis S, Karana-Ginopoulou A, Papanastasiou DA. Response to interferon alfa-2b therapy in mutitransfused children with beta-thalassemia and chronic hepatitis C. *Eur J Clin Microbiol Infect Dis* 1999; **18**: 709-715

34 **Mancuso A**, Rigano P, Renda D, Di Salvo V, Pignatti CB, Guddo F, Buccellato A, Nicoli N, Maggio A. Hepatocellular carcinoma on cirrhosis-free liver in a HCV-infected thalassemic. *Am J Hematol* 2005; **78**: 158-159

35 **Olivieri NF**, Liu PP, Sher GD, Daly PA, Greig PD, McCusker PJ, Collins AF, Francombe WH, Templeton DM, Butany J. Brief report: combined liver and heart transplantation for end-stage iron-induced organ failure in an adult with homozygous beta-thalassemia. *N Engl J Med* 1994; **330**: 1125-1127

S- Editor Zhang HN **L- Editor** Hughes D **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i5.175

World J Hepatol 2010 May 27; 2(5): 175-179
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Innovative immunohistochemistry identifies MMP-9 expressing macrophages at the invasive front of murine HCC

Martin Roderfeld, Timo Rath, Frank Lammert, Christian Dierkes, Jürgen Graf, Elke Roeb

Martin Roderfeld, Timo Rath, Elke Roeb, Justus-Liebig-University Giessen, Department of Medicine II, Gastroenterology, Giessen 35385, Germany
Frank Lammert, Department of Internal Medicine II, Saarland University Hospital, Saarland University, Homburg 66421, Germany.
Christian Dierkes, Justus-Liebig-University Giessen, Department of Pathology, Giessen 35385, Germany.
Jürgen Graf, Philipps-University Marburg, Department of Anaesthesiology and Intensive Care, 35033 Marburg and Passenger Medical Care, Deutsche Lufthansa AG, Frankfurt 60546, Germany
Author contributions: Roderfeld M designed the study, analyzed and interpreted data, and drafted the manuscript; Rath T and Dierkes C were responsible for acquisition of data, material, technical and intellectual support, analysis, and interpretation; Lammert F and Graf J provided critical revision of the manuscript and important intellectual content; Roeb E was responsible for study design, supervision and drafting of the manuscript.
Supported by the Grants from the Deutsche Forschungsgemeinschaft (RO 957/8-1 and SFB/TRR 57) and by BMBF ZooMAP-TPC4; a Research Grant of the University Medical Center Giessen and Marburg (UKGM 10/2010 GI)
Correspondence to: Elke Roeb, Professor, Justus-Liebig-University, Department of Medicine Ⅱ, Gastroenterology, Paul-Meimberg-Str. 5, Giessen 35385, Germany. elke.roeb@innere.med.uni-giessen.de
Telephone: +49-641-9942338 Fax: +49-641-9942339
Received: January 8, 2010 Revised: April 9, 2010
Accepted: April 16, 2010
Published online: May 27, 2010

Abstract

AIM: To investigate the proteolytic contribution of tumor-associated macrophages (TAM) in tumor invasion, we analyzed whether TAM at the invasive front of small HCC in *Abcb4*$^{-/-}$-mice show an enhanced expression of MMP-9.

METHODS: Liver cryosections of the hepatocellular carcinoma (HCC) invasive front from 12 mo old *Abcb4*$^{-/-}$-mice were stained for collagen type Ⅰ and MMP-9 using Alexa488 and Alexa568 labeled secondary antibodies. Afterwards, the Alexa568 dye was bleached and the macrophage marker F4/80 was visualized using Alexa568 labeled secondary antibodies. Finally, photographs of the invasive tumor front were digitally overlaid and analyzed.

RESULTS: After complete bleaching of the primary dye, specific fluorescence staining of a third antigen, here F4/80, was successfully performed on the same histological section. With this method, we were able to identify conglomerates of matrix metalloproteinase (MMP-9) expressing macrophages within the tumor capsule of HCC.

CONCLUSION: MMP-9 expressing macrophages are involved in matrix remodelling at the invasive tumor front of HCC. The described staining protocol provides a simple yet powerful extension of conventional immuno-histochemistry, facilitating visualization of at least three different antigens plus nuclei in one single histological section.

Key words: Fluorescence staining; Hepatocellular carcinoma; Matrix metalloproteinase; Tumor associated macrophages

Peer reviewer: Juan Carlos Perazzo, MD, PhD, Professor, Department of Biological Sciences, Pathophysiology School of Pharmacy and Biochemistry, UBA, Junin 950, 5, Buenos Aires 1113, Argentina

Roderfeld M, Rath T, Lammert F, Dierkes C, Graf J, Roeb E. Innovative immunohistochemistry identifies MMP-9 expressing macrophages at the invasive front of murine HCC. *World J Hepatol* 2010; 2(5): 175-179 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i5/175.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i5.175

INTRODUCTION

Abcb4 knockout mice (formerly called *Mdr2*-knockout mice) lack the liver-specific P-glycoprotein responsible for phosphatidylcholine transport across the canalicular membrane. The absence of phospholipids from biliary fluid in *Abcb4*$^{-/-}$-mice results in bile regurgitation and portal inflammation followed by development of dysplasia and hepatocellular carcinoma (HCC)[1,2]. Therefore, *Abcb4*$^{-/-}$-mice represent an ideal animal model mimicking cholangitis-associated carcinogenesis[1,2].

The initial step in carcinogenesis (i.e. invasive and metastatic cell behavior) is the proteolytic destruction of the extracellular matrix (ECM), including the basement membrane. Numerous studies have demonstrated the pivotal role of matrix metalloproteinases (MMPs) for tumor associated ECM degradation[3-5]. In particular, the gelatinases MMP-2 and MMP-9 have gained considerable attention: in various studies on tumor invasion and metastasis. MMP-2 and MMP-9 were shown to be able to cleave collagen type Ⅳ, the main component of the basement membrane[6-8].

Previous studies demonstrated abundant expression of MMP-9 in tumor cells of HCC[6,7]. Furthermore, MMP-9 expression was associated with growth and invasiveness of HCC[6,9,10]. Apart from the malignant cells themselves, growing attention is being paid to the tumor microenvironment as a mediator of invasive and metastatic behaviour[11,12]. Large quantities of tumor-associated macrophages (TAM) are associated with poor prognosis in various types of cancer, thereby suggesting relevance of these cells for tumor progression[13]. A number of studies were able to prove the expression of a broad range of tumor promoting factors including MMP-9 by which TAM may drive tumor angiogenesis[12,14,15].

Against this background, our aim was to analyze the expression patterns and cellular sources of MMP-9 within the tumor microenvironment of HCCs related to TAM in a mouse model of hepatocarcinogenesis. We developed a modification of the well-known and traditionally conducted immunohistochemistry. This allows the parallel visualization of at least three antigens within one histological section. The described approach may represent a powerful advancement in the analytical capabilities of immunohistochemistry.

MATERIALS AND METHODS

Animals and tissue preparation

FVB/N-*Abcb4*^{tm1bor} gene-targeted mice were crossed back towards the fibrosis-susceptible BALB/cJ strain for ten generations as characterized recently[16,17]. Mice were killed at the age of twelve months and tissue samples of HCCs were fixed in 1% neutral buffered formalin for 16 h and afterwards embedded in Tissue-Tek (Sakura, Zoeterwoude, Netherlands) for cryopreservation at -80℃. The present study was performed with permission of the State of Hessen, regional council Giessen, according to section 8 of the German Law for the Protection of Animals and conforms to the *Guide for the Care and Use of Laboratory Animals* (Az: V54-19c20/15cGI20/10).

Immunofluorescence

3 μm frozen sections were blocked for 30 min with 5% bovine serum albumin, 2% goat serum (Biomeda, Foster City, USA) and 0.1% cold fish skin gelatine (Sigma-Aldrich) in PBS with 0.1% Triton (Roth, Karlsruhe, Germany) and 0.05% Tween 20 (Serva, Heidelberg, Germany). Sections were immunostained with goat anti-mouse MMP-9 antibodies, Alexa 568-conjugated donkey anti-goat IgG and afterwards with rabbit anti-mouse collagen type Ⅰ antibodies and Alexa 488-conjugated goat anti-rabbit IgG. DAPI (4',6-diamidino-2-phenylindole dihydrochloride, Sigma) was used for nucleus staining. The stained probes were covered with Dako Cytomation mounting medium (Glostrup, Denmark) and glass cover slips for microscopic analysis. Fluorescence images of the tumor invasive front were obtained under a fluorescence microscope (Leica DMRB, Wetzlar, Germany; camera: Nikon Coolpix 5400, Düsseldorf, Germany). Afterwards, the Alexa568 dye was completely bleached by illumination with green light under the microscope for 2 h. Immunostaining of macrophage antigens was performed with rat anti-F4/80 primary and goat anti-rat Alexa568 secondary antibodies. The carefully adjusted selection of antibodies derived from different host-species was crucial to avoid unintended cross-stainings. Finally, photomicrographs of the invasive tumor front were taken and analyzed digitally using Photoshop software version 9.0.2. (Adobe, München, Germany).

Primary antibodies were diluted 1:50 and secondary antibodies 1:1000 prior to use. Specificity of immunohistological stainings was verified by the use of isotype control-IgGs instead of primary antibodies. MMP-9 IgGs were purchased from R&D-Systems (#AF909, Wiesbaden, Germany), collagen type Ⅰ IgGs from Biodesign (#T40777R, Freiburg, Germany), and F4/80 IgGs from Dianova (#T-2006, Hamburg, Germany). Alexa 468- and Alexa 488-conjugated secondary antibodies were purchased from Molecular Probes (Eugene, USA).

RESULTS

Within one year all BALB/c-*Abcb4*$^{-/-}$-mice (n = 17) had developed an HCC. On average 7 ± 4 tumors were detected per mouse, with a mean tumor size of 4.5 ± 2.5 mm. Figure 1 illustrates the macroscopic and histological aspect of HCC in *Abcb4*$^{-/-}$-mice.

Following the modifications of conventional immunohistochemistry as described above, we successfully identified three antigens (MMP-9, collagen Ⅰ and F4/80) plus nuclei within the same histological section (Figure 2).

Immunofluorescence co-staining of collagen type Ⅰ and MMP-9 visualized conglomerates of MMP-9 expressing cells within the tumor capsule of HCC. After photobleaching of the red fluorescent dye, staining with

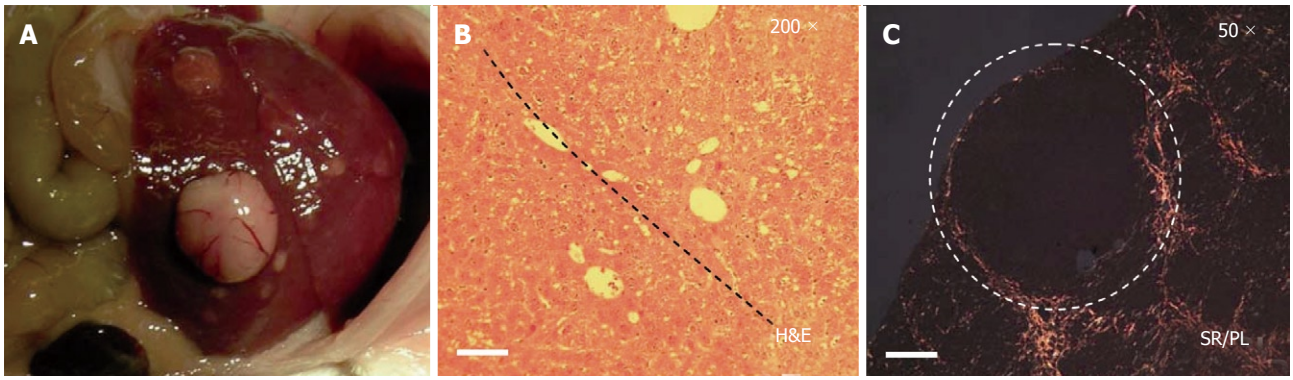


Figure 1 Macroscopic and histological aspect of HCC in BALB/c-*Abcb4*$^{-/-}$-mice. A: Representative macroscopic view of BALB/c-*Abcb4*$^{-/-}$-mouse liver at one year of age. Typically, one prominent tumor and a number of smaller tumors are visible; B: Hematoxylin and Eosin (H&E) stained liver section of the invasive tumor front (tumor on the upper right, fibrotic parenchyma on the lower left side, dashed line: HCC tumor capsule, bar represents 100 μm); C: Fifty-fold enlarged Sirius red (SR) stained liver section photographed under polarized light (PL, bar represents 400 μm). Fibrillar collagens appear in red. The tumor capsule is rich in collagen, whereas tumor stroma appears collagen-free.

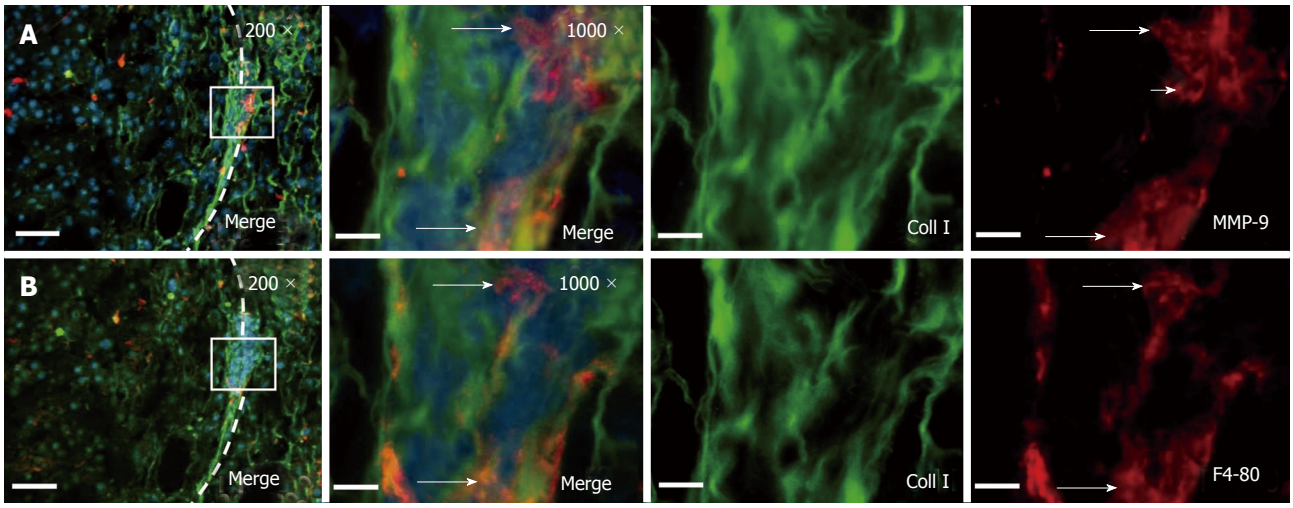


Figure 2 Conglomerates of tumor associated macrophages express MMP-9 within the HCC tumor capsule. A: Immunofluorescence co-staining of collagen type Ⅰ (green) and MMP-9 (red) displayed clusters of MMP-9 expressing cells in the tumor capsule. B: After photobleaching of the red fluorescence dye, macrophage marker F4/80 was visualized. The long arrows indicate MMP-9 positive macrophages. The shot arrow indicates an MMP-9 expressing cell which is negative for F4/80. DAPI was used for nucleus staining. The left panels provide a section overview with 200-fold magnification and bars represent 100 μm (left side of the panel: Tumor; dashed line: Tumor capsule; box: This is enlarged to 1000 × magnification in the corresponding panel, bars represent 20 μm). Original green and red channel micrographs (1000 ×) were shown. Images were derived by conventional wide field fluorescence microscopy. Representative digital overlays are shown.

macrophage marker F4/80 identified macrophages to be the main source of MMP-9 expressing cells at the invasive tumor front. Representative results of the modified immunohistochemistry are shown in Figure 2.

DISCUSSION

Previous studies reported an enhanced expression of MMP-9 in HCC by the tumor cells themselves[6,7]. Furthermore, MMP-9 expression was associated with growth and invasiveness of HCC[6,9,10]. However, growing evidence exists that, apart from malignant cells, the tumor environment may play a pivotal role with regard to invasive and metastatic cell behaviour[11,12]. Accordingly, a number of *in vitro* and *in vivo* studies demonstrated that cancer cells were able to induce MMP expression in noncancerous cells thereby eventually promoting tumor progression[8,18-20]. Representing a part of the tumor microenvironment, TAM have been shown to be essential for both tumor growth and metastasis[21-23]. A recent study by Tsagozis and coworkers in prostate cancer revealed that the expression of MMP-9 by TAM is necessary to maintain their tumor promoting phenotype[24]. In murine studies on cervical cancer, Giraudo *et al*[14] showed that enhanced expression of MMP-9 contributed to cervical carcinogenesis by promoting tumor angiogenesis.

Herein, we analyzed the expression patterns and cellular sources of MMP-9 at the invasive front of HCC with respect to tumor associated macrophages by means of immunohistochemistry. Traditionally, double immunofluorescence staining with green and red fluorescent dyes allows minimal overlapping fluorescence emission spectra. The application of the blue fluorescence channel for antigen visualization in wide field

fluorescence microscopy is not feasible yet, because of low fluorescence quantum yields of the correspondingly labeled secondary antibodies. Therefore, conventional immunohistochemistry was limited to the visualization of only two antigens plus nuclei within the same section.

To achieve within one histological section the combined illustration of the cellular sources of MMP-9 at the invasive front of HCC together with type I collagen representing the main component of fibrotic ECM the visualization of more than two antigens was essential .

We therefore developed a modification of conventional immunohistochemistry, facilitating the parallel visualization of three antigens using two fluorescent dyes. Essential for this approach is the complete wipeout of one dye (Alexa568) by green light. In contrast, green fluorescent Alexa488 dyes withstand the photobleaching without any loss of fluorescence intensity. Following the bleaching procedure, Alexa568 was re-used to tag the third antigen, the macrophage marker F4/80. Owing to the photo stability of Alexa488 towards green light, the defined region of interest was easily reproducible after the second staining.

Applying this approach, we have clearly visualized conglomerates of MMP-9 expressing macrophages within the capsule of HCCs. In accordance with data on TAM and MMP-9 expression for tumor invasion and angiogenesis in the literature, our results suggest an etiologic role for TAM and MMP-9 in the development of HCC[12,14,15,21-24].

However, whether MMP-9 expression in TAM within the HCC capsule leads to increased tumor invasiveness or eventually promotes angiogenesis remains unclear.

In summary, the staining procedure described herein has been shown to be a simple, feasible yet powerful advancement of the widely used technique of immunohistochemistry. This may be of the utmost interest for those lacking access to spectral imaging via confocal laser microscopy[25].

ACKNOWLEDGMENTS

The authors are grateful to Annette Tschuschner and Michaela Weiss for their technical support.

COMMENTS

Background

The initial step in carcinogenesis (i.e. invasive and metastatic cell behavior) is the proteolytic destruction of the extracellular matrix (ECM), including the basement membrane. Numerous reports were able to demonstrate the pivotal role of matrix-metalloproteinases (MMPs) for tumor associated ECM degradation. In particular, the gelatinases MMP-2 and MMP-9 have gained considerable attention: in various studies on tumor invasion and metastasis MMP-2 and MMP-9 were able to cleave collagen type IV, the main component of the basement membrane. Previous studies demonstrated abundant expression of MMP-9 in tumor cells of hepatocellular carcinoma (HCC). Furthermore, MMP-9 expression was associated with growth and invasiveness of HCC. Apart from the malignant cells themselves, growing attention is paid to the tumor microenvironment as a mediator of invasive and metastatic behaviour. Large quantities of tumor-associated macrophages (TAM) are related to poor prognosis in various entities of cancer, thereby suggesting relevance of these cells for tumor progression. A number of studies was able to prove the expression of a broad range of tumor promoting factors including MMP-9 by which TAM may drive tumor angiogenesis.

Research frontiers

It is unclear, whether MMP expression in TAM within the HCC may lead to increased tumor invasiveness or eventually promotes angiogenesis.

Innovations and breakthroughs

Representing a part of the tumor microenvironment, TAM have been shown to be essential for both, tumor growth and metastasis. A recent study by Tsagozis and coworkers in prostate cancer revealed that the expression of MMP-9 by TAM is necessary to maintain their tumor promoting phenotype. In murine studies on cervical cancer, Giraudo et al. showed that enhanced expression of MMP-9 contributed to cervical carcinogenesis by promoting tumor angiogenesis.

Applications

The staining procedure described herein proves to be a simple, feasible yet powerful advancement of the widely used technique of immunohistochemistry. This may be of utmost interest for those lacking access to spectral imaging via confocal laser microscopy. Applying this approach, we have clearly visualized conglomerates of MMP-9 expressing macrophages within the capsule of HCCs. In accordance with data on TAM and MMP-9 expression for tumor invasion and angiogenesis in the literature, our results suggest an etiologic role of TAM and MMP-9 for development of HCC.

Peer review

The rationale of this work is adequate. The methods employed clearly let the authors to reach the aims, and the results are appropriatle to sustain a discussion closer to the results showing well knowledge of the subject. This work is simple, direct, and interesting, and brings awareness to the subject.

REFERENCES

1 **Katzenellenbogen M**, Pappo O, Barash H, Klopstock N, Mizrahi L, Olam D, Jacob-Hirsch J, Amariglio N, Rechavi G, Mitchell LA, Kohen R, Domany E, Galun E, Goldenberg D. Multiple adaptive mechanisms to chronic liver disease revealed at early stages of liver carcinogenesis in the Mdr2-knockout mice. *Cancer Res* 2006; **66**: 4001-4010

2 **Smit JJ**, Schinkel AH, Oude Elferink RP, Groen AK, Wagenaar E, van Deemter L, Mol CA, Ottenhoff R, van der Lugt NM, van Roon MA. Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell* 1993; **75**: 451-462

3 **Classon M**, Settleman J. Emerging concepts in tumor progression and therapy. *Semin Cancer Biol* 2000; **10**: 393-397

4 **Egeblad M**, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2002; **2**: 161-174

5 **Zucker S**, Vacirca J. Role of matrix metalloproteinases (MMPs) in colorectal cancer. *Cancer Metastasis Rev* 2004; **23**: 101-117

6 **Arii S**, Mise M, Harada T, Furutani M, Ishigami S, Niwano M, Mizumoto M, Fukumoto M, Imamura M. Overexpression of matrix metalloproteinase 9 gene in hepatocellular carcinoma with invasive potential. *Hepatology* 1996; **24**: 316-322

7 **Määttä M**, Soini Y, Liakka A, Autio-Harmainen H. Differential expression of matrix metalloproteinase (MMP)-2, MMP-9, and membrane type 1-MMP in hepatocellular and pancreatic adenocarcinoma: implications for tumor progression and clinical prognosis. *Clin Cancer Res* 2000; **6**: 2726-2734

8 **Roeb E**, Dietrich CG, Winograd R, Arndt M, Breuer B, Fass J, Schumpelick V, Matern S. Activity and cellular origin of gelatinases in patients with colon and rectal carcinoma differential activity of matrix metalloproteinase-9. *Cancer* 2001; **92**: 2680-2691

9 **Cheng JC**, Chou CH, Kuo ML, Hsieh CY. Radiation-enhanced hepatocellular carcinoma cell invasion with MMP-9 expression through PI3K/Akt/NF-kappaB signal transduction pathway. *Oncogene* 2006; **25**: 7009-7018

10 **Sakamoto Y**, Mafune K, Mori M, Shiraishi T, Imamura H, Mori M, Takayama T, Makuuchi M. Overexpression

of MMP-9 correlates with growth of small hepatocellular carcinoma. *Int J Oncol* 2000; **17**: 237-243
11 **Marx J**. Cancer biology. All in the stroma: cancer's Cosa Nostra. *Science* 2008; **320**: 38-41
12 **Pahler JC**, Tazzyman S, Erez N, Chen YY, Murdoch C, Nozawa H, Lewis CE, Hanahan D. Plasticity in tumor-promoting inflammation: impairment of macrophage recruitment evokes a compensatory neutrophil response. *Neoplasia* 2008; **10**: 329-340
13 **Bingle L**, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J Pathol* 2002; **196**: 254-265
14 **Giraudo E**, Inoue M, Hanahan D. An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. *J Clin Invest* 2004; **114**: 623-633
15 **Lewis CE**, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 2006; **66**: 605-612
16 **Henkel C**, Roderfeld M, Weiskirchen R, Berres ML, Hillebrandt S, Lammert F, Meyer HE, Stühler K, Graf J, Roeb E. Changes of the hepatic proteome in murine models for toxically induced fibrogenesis and sclerosing cholangitis. *Proteomics* 2006; **6**: 6538-6548
17 **Hillebrandt S**, Goos C, Matern S, Lammert F. Genome-wide analysis of hepatic fibrosis in inbred mice identifies the susceptibility locus Hfib1 on chromosome 15. *Gastroenterology* 2002; **123**: 2041-2051
18 **Masson V**, de la Ballina LR, Munaut C, Wielockx B, Jost M, Maillard C, Blacher S, Bajou K, Itoh T, Itohara S, Werb Z, Libert C, Foidart JM, Noël A. Contribution of host MMP-2 and MMP-9 to promote tumor vascularization and invasion of malignant keratinocytes. *FASEB J* 2005; **19**: 234-236
19 **Mc Donnell S**, Chaudhry V, Mansilla-Soto J, Zeng ZS, Shu WP, Guillem JG. Metastatic and non-metastatic colorectal cancer (CRC) cells induce host metalloproteinase production in vivo. *Clin Exp Metastasis* 1999; **17**: 341-349
20 **Mook OR**, Van Overbeek C, Ackema EG, Van Maldegem F, Frederiks WM. In situ localization of gelatinolytic activity in the extracellular matrix of metastases of colon cancer in rat liver using quenched fluorogenic DQ-gelatin. *J Histochem* Cytochem 2003; **51**: 821-829
21 **Foda HD**, Zucker S. Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis. *Drug Discov Today* 2001; **6**: 478-482
22 **Luo Y**, Zhou H, Krueger J, Kaplan C, Lee SH, Dolman C, Markowitz D, Wu W, Liu C, Reisfeld RA, Xiang R. Targeting tumor-associated macrophages as a novel strategy against breast cancer. *J Clin Invest* 2006; **116**: 2132-2141
23 **Zeisberger SM**, Odermatt B, Marty C, Zehnder-Fjällman AH, Ballmer-Hofer K, Schwendener RA. Clodronate-liposome-mediated depletion of tumour-associated macrophages: a new and highly effective antiangiogenic therapy approach. *Br J Cancer* 2006; **95**: 272-281
24 **Tsagozis P**, Eriksson F, Pisa P. Zoledronic acid modulates antitumoral responses of prostate cancer-tumor associated macrophages. *Cancer Immunol Immunother* 2008; **57**: 1451-1459
25 **Roderfeld M**, Matern S, Roeb E. [Confocal laser scanning microscopy: a deep look into the cell]. *Dtsch Med Wochenschr* 2003; **128**: 2539-2542

S- Editor Zhang HN **L- Editor** Hughes D **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i5.180

World J Hepatol 2010 May 27; 2(5): 180-184
ISSN 1948-5182 (online)

BRIEF ARTICLE

Safety of pegylated interferon and ribavirin therapy for chronic hepatitis C in patients with sickle cell anemia

Hussain Issa

Hussain Issa, Division of Gastroenterology, Department of Internal Medicine, King Fahad Specialist Hospital, Dammam, Sayhat 7312-32437, Saudi Arabia
Author contributions: Issa H contributed solely to this paper.
Correspondence to: Hussain Issa, MD, Division of Gastroenterology, Department of Internal Medicine, King Fahad Specialist Hospital, Dammam, PO Box 4012, Sayhat 7312-32437, Saudi Arabia. hussain31911@yahoo.com
Telephone: +966-3-8372138
Received: January 16, 2010 Revised: May 6, 2010
Accepted: May 13, 2010
Published online: May 27, 2010

Abstract

AIM: To evaluate the safety and efficacy of combined pegylated interferon and ribavirin for the treatment of chronic hepatitis C (HCV) in patients with sickle cell anemia (SCA).

METHODS: Fifty-two patients with SCA and HCV were treated over a period of 7 years from June 2002 to July 2009. Their medical records were reviewed for: age at treatment, sex, body mass index, Hb level at the start of therapy and on follow-up, hemoglobin electrophoresis, liver function tests, G6PD level, LDH, bilirubin, HCV-RNA viral load, HCV genotype, liver biopsy, duration of treatment, and side effects. All were treated with pegylated interferon and a standard dose of ribavirin. The treatment was continued for 24 wk for those with genotype 2 and 3 and for 48 wk for those with genotype 1 and 4.

RESULTS: Fifty-two patients (30 females and 22 males) were treated. Their mean age was 29.5 years (range 15-54 years). HCV genotype was determined in 48 and 15 had liver biopsy. Their mean pre-treatment HCV-RNA viral load was 986330 IU/mL (range 12762-3329282 IU/mL). The liver biopsy showed grade Ⅰ in 6 and grade Ⅱ in 9 and stage Ⅰ in 13 and stage Ⅱ in 2. Only 8 were receiving hydroxyurea at the time of treatment. All tolerated the treatment well and none experienced a decrease in their Hb which required blood transfusion pre, during or after therapy. There were no hematological side effects attributable to ribavirin at the usual recommended dose. Thirty-seven (71.2%) achieved SVR at 6 mo after the end of treatment. The remaining 15 were non-responders. Two of them showed an ETR but had a relapse. The remaining 13 had a relatively significant HCV-RNA viral load with a mean HCV-RNA viral load of 1829741.2 IU/mL (900000-3329282 IU/mL) and eight of them had HCV genotype 1, four had HCV genotype 4, and one had HCV genotype 5.

CONCLUSION: Patients with SCA and HCV can be treated with pegylated interferon and ribavirin at the usual recommended dose. This is even so in those who are not receiving hydroxyurea. The treatment is safe and effective and the response rate is comparable to those without SCA.

Key words: Sickle cell anemia; Chronic hepatitis C; Treatment

Peer reviewers: Melissa Kay Osborn, MD, Emory University Hospital Midtown, 550 Peachtree Street, 7th Floor Medical Office Tower, Atlanta, GA 30308, United States; Ana Carolina Ferreira Netto Cardoso, MD, Hopital Beaujon, 100, bd du Gal-Leclerc, Clichy 92110, France

Issa H. Safety of pegylated interferon and ribavirin therapy for chronic hepatitis C in patients with sickle cell anemia. *World J Hepatol* 2010; 2(5): 180-184 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i5/180.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i5.180

INTRODUCTION

Sickle cell anemia (SCA) is one of the common hemo-

globinopathies in the world. In the Eastern Province of Saudi Arabia, SCA is common with a reported sickle cell trait frequency up to 25% and a sickle cell anemia frequency of about 2%[1,2]. It is well known that SCA can affect any part of the body and one of the common organs to be affected is the hepatobiliary system. This can manifest in several different ways including cholelithiasis, choledocholithiasis, hepatic crisis, hepatic sequestration and cholestatic jaundice as well as transfusion related hepatitis B and C[3-8]. The exact frequency of hepatitis B and C in patients with SCA in Saudi Arabia is not known but the average annual incidence of seropositivity per 100 000 population was 104.6 for HBV and 78.4 for HCV[9]. An 18.2% prevalence of antibodies to HCV was reported among sickle cell patients in the Central region of Saudi Arabia[10]. The life expectancy of patients with SCA has improved considerably, attributable to better understanding of the disease, improved medical and surgical management and the use of hydroxyurea[11-13]. However, this will put SCA patients with chronic hepatitis B and C at risk of developing liver cirrhosis, hepatocellular carcinoma and liver failure if their hepatitis B and C are not treated. In spite of the markedly improved results in the treatment of chronic hepatitis C using the combined therapy of pegylated interferon and ribavirin, patients with SCA and chronic hepatitis C have not been considered suitable for such treatment because of the believed risk that ribavirin will induce hemolysis and severe anemia which can aggravate their already existing anemia[14-16]. Recently however there have been three publications reporting success of such treatment in patients with SCA and HCV C although the number of patients reported was small[17-20]. This report describes our experience in the management of 52 SCA patients with HCV using pegylated interferon and ribavirin at the usual recommended dose.

MATERIALS AND METHODS

In June 2002, we started treating SCA patients with HCV using pegylated interferon and ribavirin at Qatif Central Hospital, Saudi Arabia. This was approved by the ethical and research committee and an informed written consent was obtained from each patient explaining the type and duration of treatment as well as the possible side effects. This was a retrospective study and the following information was noted: age at treatment, sex, body mass index, Hb level at the start of treatment and on follow-up, hemoglobin electrophoresis, liver function tests, G6PD level, LDH, bilirubin, HCV-RNA viral load, HCV genotype, liver biopsy, duration of treatment, and side effects. HCV serology screening was done using a third generation ELISA test and confirmatory RIBA test. HCV-RNA was detected and assayed using an automated extraction system (Cobas Ampliprep). HCV Quantification and Detection was performed using Abbott Real Time M2000 RT instrument and the COBAS TaqMan HCV Test (RT-PCR technology) on the COBAS AmliPrep system (Roche) These assays detect and quantitate genotypes (1-6). An Internal Control is included in the assays to monitor any possible amplification inhibitors.

Prior to treatment, all patients had complete blood count, liver function tests, thyroid function tests, alpha fetoprotein, autoimmune profile, HBV and HIV screening and an abdominal ultrasound as part of their work-up. None of our patients had co-infection with HIV or HBV. The Hb level, LDH and bilirubin levels were monitored and checked initially, and during follow-up at 2 wk, 4 wk, 8 wk, 12 wk, 16 wk, 24 wk, 32 wk, 40 wk and 48 wk. The METAVIR fibrosis grading scale was used to grade and stage liver fibrosis[21]. Treatment was initiated within two to three weeks post liver biopsy. Only 48 out of 52 patients were HCV genotyped as this facility was not available in our hospital at the beginning of the study. All our patients were treated with pegylated interferon alpha-2a (Pegasys 180 micrograms pre-filled syringes, Roche) 180 microgram subcutaneously, once per week or pegylated interferon alpha-2b (Peginterone, Schering-plough) 1 ug/kg subcutaneously, once per week and ribavirin. Ribavirin Rebetol (200 mg capsules, Schering-Plough) or Copegus 200 mg tablets, Roche) was used in a dose of 400 mg twice daily for those with HCV genotype 2 and 3 as well as those in whom HCV genotype was not known and 600 mg twice daily for those with HCV genotype 1, 4 and 5. The treatment was continued for 24 wk for those with genotype 2, 3 and those in whom HCV genotype was not known and for 48 wk for those with genotype 1, 4 and 5. Early virological response (EVR), end of treatment response (ETR) and sustained viral response (SVR) were documented when HCV RNA was undetectable or < 2 log, in comparison with baseline viral load at 12 wk from treatment (EVR), undetectable HCV-RNA at the end of treatment (ETR) and at 6 mo following completion of treatment respectively (SVR).

RESULTS

Fifty-two patients with SCA and HCV (Table 1) were treated, comprising 30 females and 22 males. Their mean age at the time of treatment was 29.5 years (range 15-54 years). Their mean HbS was 74.4% (66%-89%) and their mean HbF was 22.5% (9.6%-33.6%). Twelve (23.1%) had G6PD deficiency. HCV genotype was done in only 48. This indicated genotype 2 in 20; genotype 1 in 13, genotype 4 in 8, genotype 3 in 6 and one had genotype 5. Their mean pretreatment quantitative HCV- RNA level was 986330 IU/mL (range 12762-3329282 IU/mL). Fifteen underwent liver biopsy. This showed grade Ⅰ in 6 and grade Ⅱ in 9 and stage Ⅰ in 13 and stage Ⅱ in 2. Only 8 were receiving hydroxyurea at the time of treatment. All patients completed their therapy and in none of them was the treatment reduced or discontinued because of major side effects, hemolysis or bone marrow suppression. None of our patients suffered Hb drop which required blood transfusion pre, during or after

Table 1 Clinical data of SCA patients with chronic hepatitis

Total No. of patients	52
Sex	30 F: 22 M
Mean age (range)	29.5 (15-54) years
Mean HbS (range)	74.4% (66%-89%)
Mean HbF (range)	22.5% (9.6%-33.6%)
G6PD deficiency	12 (23.1%)
Mean HCV-RNA viral load (range)	986330 IU/mL (range 12762-3329282 IU/mL)
HCV genotype	
genotype 1	13 (8 non-responders)
genotype 2	20 (2 non-responders)
genotype 3	6
genotype 4	8 (4 non-responders)
genotype 5	1 (1 non-responder)
not available	4
Mean Hb level	
At start of treatment	10.2 g/dL (7.5-11.5 g/dL)
At 3 mo of treatment	10.4 g/dL (8.9-11.2 g/dL)
At 6 mo of treatment	10.35 g/dL (8.5-11.9 g/dL)
At the end of treatment	10.3 g/dL (8.2-11.7 g/dL)
SVR at 6 mo after the end of treatment	37 (71.2%)

SCA: Sickle cell anemia; SVR: Sustained viral response; HCV: Chronic hepatitis C.

therapy. Their mean hemoglobin level at the start of treatment was 10.2 g/dL (7.5-11.5 g/dL). There was no significant change in hemoglobin, LDH and bilirubin levels during or at the end of treatment. Their mean Hb level at 3 mo was 10.4 g/dL (8.9-11.2 g/dL), at 6 mo was 10.35 g/dL (8.5-11.9 g/dL) and at the end of treatment was 10.3 g/dL (8.2-11.7 g/dL). The lowest Hb level at the beginning of treatment was 7.5 g/dL. This patient did not receive pre-treatment blood transfusion and his Hb improved during treatment and at the end of treatment. His mean Hb during treatment was 9.6 g/dL (7.5-11.2 g/dL). The main adverse effects encountered in some of our patients were flu-like symptoms, headache, and loss of appetite and generalized body ache. These adverse effects were transient and tolerated by all patients. There were no more episodes of acute vaso-occlusive crisis, acute chest syndrome or other complications of sickle cell anemia while undergoing therapy. Thirty-seven (71.2%) of our patients achieved SVR at 6 mo after the end of treatment. The remaining 15 were non-responders. Two of them with an HCV genotype 2 showed an end of treatment response but had a relapse. The remaining 13 non responders had relatively significant HCV viral load with a mean HCV-RNA viral load 1829741.2 IU/mL (900000-3329282 IU/mL). Eight of them had HCV genotype 1, four had HCV genotype 4, and one had HCV genotype 5.

DISCUSSION

Patients with SCA frequently need blood transfusions to treat the various complications including acute and chronic anemia, splenic and hepatic sequestration crisis, acute chest syndrome, and priapism and central nervous system crisis. The need for blood transfusion in these patients starts early especially in those with hemolytic crisis, splenic sequestration crisis and acute chest syndrome. This puts them at potential risk for alloimmunization, iron overload and chronic viral hepatitis B and C[7,8]. Hepatitis C is one of the most common blood-born infections worldwide. This is more so in those who receive frequent blood transfusions, especially patients with thalassemia and SCA[10]. It is also well known that patients with HCV if left untreated are susceptible to liver damage, liver cirrhosis, hepatocellular carcinoma and liver failure. The combination of iron overload and HCV can lead to more rapidly progressive liver disease. The life expectancy of patients with SCA is known to have been shorter than the general population and they usually die of SCA-related complications. This however is not the case nowadays and as a result of better understanding of SCA and its complications, the use of hydroxyurea as well as improved care, SCA patients are living longer[11-13]. In the past there was no well proven treatment for HCV, but recent progress has made the treatment and cure of HCV possible[14-16]. The standard treatment for HCV is a combined therapy using pegylated interferon and ribavirin. Treatment response depends on several factors including HCV genotype[14-16]. A sustained virological response in up to 60% of HCV patients with genotype 1 and 4 and up to 90% in those with genotype 2 and 3 has been reported[15]. For many years, patients with SCA and HCV were considered to be unsuitable for such treatment. One reason for this is that ribavirin may induce hemolysis and severe anemia which can further aggravate patients' already existing anemia. Swaim *et al*[17] were the first to report successful treatment of two patients with SCA and HCV using interferon alpha-2b and ribavirin. Ancel *et al*[18] treated ten patients with HCV, five with thalassemia and five with SCA. Eight received pegylated interferon plus ribavirin while the other two were treated with pegylated interferon as a monotherapy. Nine out of 10 (90%) achieved a virological response and 6 (60%) went on to achieve sustained virological response after treatment was completed. Ayyub *et al*[20] reported eight patients with SCA and HCV who were treated with pegylated interferon alpha-2a and ribavirin for one year. All eight patients had a complete early virological response and five of them maintained a sustained virological response when assessed 6 mo after the end of treatment. Our series is the largest to be reported so far. We treated 52 SCA patients with HCV and 37 (71.2%) of them showed SVR 6 months after the end of treatment. This is comparable to the results reported by others and to those for non SCA patients treated for HCV[15,16,18-20]. It is also important to note that the dose of ribavirin was not reduced in any of our patients and the non-responders had a relatively high viral load which negatively predicts the response to treatment[22]. Eight of them also had HCV genotype 1; four had HCV genotype 4, 2 had HCV genotype 2 and one had HCV genotype 5.

In all the few reports of patients with thalassemia and HCV who were treated with interferon and ribavirin, there was an increase in their transfusion requirements[18,23-26]. Hamidah *et al*[24] reported a significant increase in the transfusion requirements of one patient with thalassemia and HCV who was treated with pegylated interferon alpha-2b and ribavirin. Telfer *et al*[25] treated 11 thalassemic patients with interferon and ribavirin and reported sustained virological response in 5 of them. There was however an increase in their transfusion requirements. Ancel *et al*[18] reported a 22% increase in the transfusion requirements of five patients with thalassemia and HCV treated with pegylated interferon and ribavirin. Li *et al*[26] reported a 30% increase in the transfusion requirement of a group of patients with thalassemia treated for HCV with interferon alpha-2b and ribavirin. In general, this was due to ribavirin-induced hemolysis. This however was not the case for patients with SCA where it was shown that either there was no change in their Hb level or in some of them there was actually an increase in their Hb level[17-20]. This was also shown in our series where none of our patients required blood transfusion during or at the end of treatment and in none of them there was a need to decrease the dose of ribavirin or discontinue treatment. The reason for this difference between patients with thalassemia and SCA in this regard is not known. One contributing factor is hydroxyurea which is currently used to ameliorate the severity of SCA by increasing patients HbF levels[11,12]. It was postulated that an increase in the HbF level in these patients may decrease the chance of ribavirin-induced hemolytic anemia. Because of this it was recommended to start and maintain these patients on hydroxyurea prior to therapy with ribavirin. SCA patients from the Eastern Province of Saudi Arabia are known to have high levels of HbF which is a contributing factor for the mildness of SCA in our patients[27]. Our patients had a high mean HbF level of 22.5% (9.6%-33.6%). Only 8 of them were on hydroxyurea and their mean HbF level was 19.8% (11.5%-28.2%). These were started on hydroxyurea treatment prior to the decision to treat them for HCV and none of the remaining patients were intentionally started on hydroxyurea. There was no difference between those who were on hydroxyurea and those who did not receive hydroxyurea in terms of response to treatment or complications. We feel that the use of hydroxyurea prior to or during therapy for HCV in patients with SCA, although beneficial is not a necessity or a prerequisite for the treatment of HCV. This is especially so in our setting where our patients are already having high levels of HbF It is also well known that ribavirin causes a dose-dependent hemolytic anemia. Another measure to decrease or avoid ribavirin-induced hemolysis was to start these patients at a lower dose of ribavirin as suggested by Ancel *et al*[18]. Their patients received pegylated interferon at full dose while ribavirin was started at the lower dose of 200 mg twice daily and increased gradually until the full recommended dose was reached, usually within 4-8 wk from the beginning of therapy. This was also the case for all the patients reported by Ayyub *et al*[20]. Their patients were treated with pegylated interferon alpha-2a, 180 micrograms subcutaneously once per week and an initial dose of ribavirin 200 mg twice daily, increased gradually to 400 mg twice daily over a period of 4-8 wk. All our patients with HCV genotype 2 and 3 and those in whom the genotype was not available were started on 400 mg ribavirin twice daily and those with HCV genotype 1, 4 and 5 received 600 mg of ribavirin twice daily. None of our patients developed hemolysis, anemia or bone marrow suppression. We feel that patients with SCA and HCV can tolerate from the start the full dose of 400 mg of ribavirin twice daily for those with HCV genotypes 2 and 3 and up to 600 mg twice daily for those with genotypes 1 and 4 without subjecting them to additional major side effects.

In conclusion, patients with SCA and HCV can be treated with pegylated interferon and the usual recommended dose of ribavirin. This is even so for those who are not receiving hydroxyurea. The treatment is safe and effective and the response rate is comparable to those without SCA. There were no hematological side effects attributable to ribavirin even at the usual recommended dose. These patients however need to be followed-up closely while on therapy by a hematologist and a gastroenterologist. Further studies are important in this regard to establish definite guidelines for the treatment of SCA patients with HCV.

COMMENTS

Background

Sickle cell anemia (SCA) is one of the common hemoglobinopathies in the world. It is well known that SCA can affect any part of the body and that patients are liable to transfusion related hepatitis B and C. The life expectancy of patients with SCA has improved considerably and this is attributed to better understanding of the disease, improved medical and surgical management and the use of hydroxyurea. Improved life expectancy will put SCA patients with chronic hepatitis B and C at risk of developing liver cirrhosis, hepatocellular carcinoma and liver failure if their hepatitis B and C are not treated. In spite of the markedly improved results in the treatment of chronic hepatitis C using the combined therapy of pegylated interferon and ribavirin, patients with SCA and chronic hepatitis C were not previously considered suitable for such treatment because of the believed risk that ribavirin would induce hemolysis and severe anemia which could aggravate their already existing anemia.

Research frontiers

There have been three publications reporting success of such treatment in patients with SCA and HCV C although the number of patients reported was small and dose of ribavirin was not the standard.

Innovations and breakthroughs

The standard treatment for HCV is a combined therapy using pegylated interferon and ribavirin. Treatment response depends on several factors including HCV genotype. Patients with SCA and HCV were, for many years, considered to be unsuitable for such treatment. One reason for this is that ribavirin may induce hemolysis and severe anemia which can further aggravate already existing anemia. It is also well known that ribavirin causes a dose-dependent hemolytic anemia. In the previous studies patients were started at a lower dose of ribavirin as a measure to decrease or avoid ribavirin-induced hemolytic anemia. These patients received pegylated interferon at full dose while ribavirin was started at a lower dose of 200 mg twice daily and increased gradually until the full recommended dose was reached, usually within 4-8 wk from the beginning of therapy. This may have an impact on the RVR or SVR. All our patients with HCV genotype 2 and 3 and those in whom the genotype was not available were started on 400 mg ribavirin twice daily while those with HCV genotype 1, 4 and 5 received 600 mg of ribavirin twice daily. None of our

patients developed hemolysis, anemia or bone marrow suppression. Our series is the largest to be reported so far. We treated 52 SCA patients with HCV and 37 (71.2%) of them showed SVR. It was also postulated that an increase in the HbF level in these patients may decrease the chance of ribavirin induced hemolytic anemia. Because of this it was recommended to start and maintain these patients on hydroxyurea prior to therapy with ribavirin. Our patients had a high mean HbF level of 22.5% (9.6%-33.6%). We feel that the use of hydroxyurea prior to or during therapy for HCV in patients with SCA, although beneficial, is not a necessity or a prerequisite for the treatment of HCV. This is especially so in our setting where our patients already have high levels of HbF.

Applications

Patients with SCA and HCV can be treated safely with pegylated interferon in the usual recommended dose of ribavirin without subjecting them to additional major side effects. We feel that patients with SCA and HCV can tolerate from the start the full dose of 400 mg of ribavirin twice daily for those with HCV genotypes 2 and 3 and up to 600 mg twice daily for those with genotypes 1 and 4. This is even so for those who are not receiving hydroxyurea. The treatment is safe and effective and the response rate is comparable to those without SCA.

Peer review

The author presents a nice-sized case series of sickle-cell anemia patients treated for hepatitis C successfully. The paper adds to the literature and provides evidence that this population can be safely given ribavirin.

REFERENCES

1 **Perrine RP**, Pembrey ME, John P, Perrine S, Shoup F. Natural history of sickle cell anemia in Saudi Arabs. A study of 270 subjects. *Ann Intern Med* 1978; **88**: 1-6
2 **Al-Awamy BH**. Sickle cell anemia, its clinical manifestations and their management. *Saudi Med J* 1987; 9: 553-562
3 **Krauss JS**, Freant LJ, Lee JR. Gastrointestinal pathology in sickle cell disease. *Ann Clin Lab Sci* 1998; **28**: 19-23
4 **Shao SH**, Orringer EP. Sickle cell intrahepatic cholestasis: approach to a difficult problem. *Am J Gastroenterol* 1995; **90**: 2048-2050
5 **Bauer TW**, Moore GW, Hutchins GM. The liver in sickle cell disease. A clinicopathologic study of 70 patients. *Am J Med* 1980; **69**: 833-837
6 **Schubert TT**. Hepatobiliary system in sickle cell disease. *Gastroenterology* 1986; **90**: 2013-2021
7 **Hassan M**, Hasan S, Giday S, Alamgir L, Banks A, Frederick W, Smoot D, Castro O. Hepatitis C virus in sickle cell disease. *J Natl Med Assoc* 2003; **95**: 939-942
8 **DeVault KR**, Friedman LS, Westerberg S, Martin P, Hosein B, Ballas SK. Hepatitis C in sickle cell anemia. *J Clin Gastroenterol* 1994; **18**: 206-209
9 **Memish ZA**, Knawy BA, El-Saed A. Incidence trends of viral hepatitis A, B, and C seropositivity over eight years of surveillance in Saudi Arabia. *Int J Infect Dis* 2010; **14**: e115-e120
10 **Al-Faleh FZ**, Sami Ramia, S. Hepatitis C virus (HCV) infection in Saudi Arabia: A review. *Ann Saudi Med* 1997; **17**: 77-82
11 **Steinberg MH**. Hydroxyurea treatment for sickle cell disease. *Scientific World Journal* 2002; **2**: 1706-1728
12 **Steinberg MH**, Barton F, Castro O, Pegelow CH, Ballas SK, Kutlar A, Orringer E, Bellevue R, Olivieri N, Eckman J, Varma M, Ramirez G, Adler B, Smith W, Carlos T, Ataga K, DeCastro L, Bigelow C, Saunthararajah Y, Telfer M, Vichinsky E, Claster S, Shurin S, Bridges K, Waclawiw M, Bonds D, Terrin M. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. *JAMA* 2003; **289**: 1645-1651
13 **Vichinsky EP**. Comprehensive care in sickle cell disease: its impact on morbidity and mortality. *Semin Hematol* 1991; **28**: 220-226
14 **Lauer GM**, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41-52
15 **Manns MP**, McHutchinson JG, Gordon S. Peginterferon alfa-2b plus ribavirin compared to interferon alfa-2b plus ribavirin for the treatment of chronic hepatitis C: 24 week treatment analysis of a multicenter, multinational phase III randomized controlled trial. *Hepatology* 2000; **32**: A297-A297
16 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982
17 **Swaim MW**, Agarwal S, Rosse WF. Successful treatment of hepatitis C in sickle-cell disease. *Ann Intern Med* 2000; **133**: 750-751
18 **Ancel DB**, Chaslin-Ferbus D, Amiot, XJ. Treatment of chronic hepatitis C in thalassemic and sickle cell disease patients with interferon alfa2b (IFN) and ribavirin (RBV). Los Angeles, CA. *DDW* 2006: A198
19 **Ancel D**, Amiot X, Chaslin-Ferbus D, Hagege I, Garioud A, Girot R, Pol S, Grange JD. Treatment of chronic hepatitis C in sickle cell disease and thalassaemic patients with interferon and ribavirin. *Eur J Gastroenterol Hepatol* 2009; **21**: 726-729
20 **Ayyub MA**, El-Moursy SA, Khazindar AM, Abbas FA. Successful treatment of chronic hepatitis C virus infection with peginterferon alpha-2a and ribavirin in patients with sickle cell disease. *Saudi Med J* 2009; **30**: 712-716
21 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832
22 **Vichinsky EP**. Current issues with blood transfusions in sickle cell disease. *Semin Hematol* 2001; **38**: 14-22
23 **Nunez M**, Marino A, Miralles C, Berdun MA, Sola J, Hernandez-Burruezo JJ. Baseline serum hepatitis C virus (HCV) RNA level and response at week 4 are the best predictor of relapse after treatment with pegylated interferon plus ribavirin in HIV/HCV-coinfected patients. *JAIDS* 2007; **45**: 439-444
24 **Hamidah A**, Thambidorai CR, Jamal R. Peginterferon alfa-2b and ribavirin in thalassaemia/chronic hepatitis C virus-co-infected non-responder to standard interferon-based. *Med J Malaysia* 2005; **60**: 517-519
25 **Telfer PT**, Garson JA, Whitby K, Grant PR, Yardumian A, Hoffbrand AV, Wonke B. Combination therapy with interferon alpha and ribavirin for chronic hepatitis C virus infection in thalassaemic patients. *Br J Haematol* 1997; **98**: 850-855
26 **Li CK**, Chan PK, Ling SC, Ha SY. Interferon and ribavirin as frontline treatment for chronic hepatitis C infection in thalassemia major. *Br J Hematol* 2002; **117**: 755-758
27 **Al-Awamy BH**, Niazi GA, el-Mouzan MI, Altorki MT, Naeem MA. Relationship of haemoglobin F and alpha thalassaemia to severity of sickle-cell anaemia in the Eastern Province of Saudi Arabia. *Ann Trop Paediatr* 1986; **6**: 261-265

S- Editor Zhang HN **L- Editor** Hughes D **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i5.185

World J Hepatol 2010 May 27; 2(5): 185-191
ISSN 1948-5182 (online)

BRIEF ARTICLE

Construction of non-covalent single-chain Fv dimers for hepatocellular carcinoma and their biological functions

Cai-Qun Bie, Dong-Hua Yang, Xu-Jing Liang, Shao-Hui Tang

Cai-Qun Bie, Department of Gastroenterology, Shenzhen Shajing Affiliated Hospital of Guangzhou Medical University, Shenzhen 518104, Guangdong Province, China
Dong-Hua Yang, Xu-Jing Liang, Shao-Hui Tang, Department of Gastroenterology, the First Affiliated Hospital of Jinan University, Guangzhou 510630, Guangdong Province, China
Author contributions: All authors made substantial contributions to conception and design, or acquisition of experimental data, or analysis and interpretation of data; and to drafting the article or revising it critically for important intellectual content.
Supported by Guangdong Province Science and Technology Tackling Key Project, No.2006B19901014, and the New Drug Project of Guangzhou Committee of Science and Technology, No.2004Z3-E4121
Correspondence to: Dong-Hua Yang, Professor, Department of Gastroenterology, the First Affiliated Hospital of Jinan University, West No.613, Huangpu Road, Guangzhou 510630, Guangdong Province, China. thdyang@yahoo.cn
Telephone: +86-20-27722241-3819
Received: December 25, 2009 Revised: April 7, 2010
Accepted: April 14, 2010
Published online: May 27, 2010

Abstract

AIM: To create new diabodies with improved binding activity to antigen of the variable light - variable heavy (VH-VL) oriented single-chain Fv dimers genes (scFv).

METHODS: The linker between VH and VL genes was shortened to 3-5 amino acid residues and cloned into the vector pCANTAB5E. The recombinant plasmids were transformed into TG1 cells and sequenced. The positive transformed cells were infected by M13K07 helper phage to form human recombinant phage antibodies. Expressed products were identified by SDS-PAGE, Western blotting, size exclusion gel chromatography (SEC), ELISA and immunohistochemistry.

RESULTS: Three scFv (scFv-3, scFv-4, scFv-5) were constructed successfully with binding ability to hepatocellular carcinoma 3.5-6 fold greater than their parental scFv. The single-chain Fv dimer (scFv-5, termed BDM3) with the best binding ability was successfully expressed in Yeast pichlia, as shown by SDS-PAGE and Western blotting. SEC results suggested the molecular weight of the expressed products was about 61 kDa. Expressed products showed significantly stronger binding to hepatocellular carcinoma cells than scFv, still having 50% binding activity even after 16 h incubation as 37℃. The purified dimers were bound specifically to the tumor antigen of HCC.

CONCLUSION: we have generated scFv dimers by shortening a series of linkers to 3-5 amino acid residues in VH-linker-VL orientation, resulting in highly stable and affinity-improved dimeric molecules. These will become an attractive targeting moiety in immunotherapeutic and diagnostic applications for HCC.

Key words: Diabody; Antibody-targeted; Specificity; Affinity; Stability

Peer reviewer: Lang Zhuo, PhD, Team Leader and Principal Research Scientist, Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos #04-16, 138669, Singapore

Bie CQ, Yang DH, Liang XJ, Tang SH. Construction of non-covalent single-chain Fv dimers for hepatocellular carcinoma and their biological functions. *World J Hepatol* 2010; 2(5): 182-191 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i5/185.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i5.185

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer-related death. The incidence of HCC is rising around the world. The diagnosis and treatment

of HCC have undergone major changes over the past decade. Unfortunately, there is no completely safe and effective treatment available for those who have developed HCC. Antibodies are a powerful tool for use in diagnosis and therapy of the tumor, because they bind with high specificity and high affinity to a variety of molecules, notably proteins and peptides. Whole antibodies provide high target binding specificity but their use in rapid tumor targeting and in vivo imaging is limited by slow tissue penetration, long circulating half-lives and often undesirable functions[1]. Small monovalent antibody fragments (scFv and Fab) exhibit good tissue penetration but their lack of avidity results in faster off-rates and rapid clearance. Diabodies are noncovalent single-chain Fv (scFv) dimers formed by producing scFv with short (3-5 AA) linkers between their variable light (VL) and variable heavy (VH) chains. This prevents the VH and VL chains in a single molecule from associating with each other in a scFv orientation. Instead, the VL from one molecule associates with the VH from a second molecule, to form a dimer that binds antigen divalently[2]. Several studies have demonstrated that genetically engineered antitumor diabody molecules can be used as effective vehicles for radioimmunotherapy, or can effectively reduce the growth rate of human tumor xenografts[3-8]. Diabodies thus represent an improved strategy for selective tumor targeting as compared with scFv, Fab, or monoclonal antibody molecules.

In previous studies we have described the construction, screening and humanization of the scFv (termed HDM) against human hepatocellular carcinoma using phage display technology[9-11]. HDM has binding specificity to hepatocellular carcinoma and is potentially effective in tumor imaging and therapeutics. To improve its antigen-binding avidity, we constructed the scFv dimers by shorting the linkers to 3-5 amino acid residues in same orientation as their parental scFv and assayed their biological functions, anticipating good behavior in antibody-targeted immunotherapeutic and diagnostic applications.

MATERIALS AND METHODS

Construction of scFv dimers

The pCANAB5E-HDM encoding the anti-HCC scFv HDM was previously constructed in our laboratory. This scFv, which has specificity for human hepatocellular carcinoma, is in the VH-linker-VL format, where the linker consists of the sequence (Gly4Ser)$_3$. The construct is cloned into the pCANAB 5E phagemid vector immediately downstream of the pⅢ leader sequence, which directs expression to the periplasm. The gene encoding the scFv is fused, *via* an amber codon, to the pIII gene of a filamentous phage. In *supE* strains of *E. coli* (TG1), this allows expression of the scFv on the surface of phage, as a fusion with the minor coat protein pⅢ, while in *supE*- strains (HB2151) translation is terminated at the amber codon, producing soluble scFv. A series of bivalent dimers (termed BDMs) was constructed by shortening the 15 amino acid linker between the VH and VL domain to 3-5 (Gly2Ser, Gly3Ser, Gly4Ser) residues. To obtain scFv dimers, BDM, the VH domain of scFv HDM, was PCR amplified with flanking restriction sites Sft I and the partial linker sequence. The VL domain of scFv HBM was PCR amplified with flanking restriction sites Not I and the partial linker sequence. Gene splicing by overlap extension was then performed using the VH sense and VL antisense primers. All primer sequences are showed in Table 1.

Table 1 Primer sequence

	scFv-5 residues
VH	Sense: 5′-TATGGCCCAGCCGGCCATGG-3′
	Antisense: 5′-AGAACCACCACCACCTGAGGAGACGGTGACC GT-3′
VL	Sense: 5′-TCAGGTGGTGGTGGTTCTGACATTGAGCTCACCCA GTCTCCA-3′
	Antisense: 5′-TATGCGGCCGCCCGTTTCA-3′
	scFv-4 residues
VH	Sense: 5′-TATGGCCCAGCCGGCCATGG-3′
	Antisense: 5′-AGAACCACCACCTGAGGAGACGGTGACCGT-3′
VL	Sense: 5′-TCCTCAGGTGGTGGTTCTGACATTGAGCTCACCCA GTCTCCA-3′
	Antisense: 5′-TATGCGGCCGCCCGTTTCA-3′
	scFv-3 residues
VH	Sense: 5′-TATGGCCCAGCCGGCCATGG-3′
	Antisense: 5′-AGAACCACCTGAGGAGACGGTGACCGT-3′
VL	Sense: 5′-GTCTCCTCAGGTGGTTCTGACATTGAGCTCACCCA GTCTCCA-3′
	Antisense: 5′-TATGCGGCCGCCCGTTTCA-3′
	Sequencing primers
	Sense: 5′-CAA CGT GAA AAA ATTATT CGC-3′
	Antisense: 5′-GTA AAT GAA TTT TCT GTA TGA GG-3′

The PCR reactions were performed using standard conditions with 100 ng of template DNA, 200 mmol/L dNTP (TaKaRa, Japan) and 2.5 U PrimerStar polymerase (TaKaRa, Japan) with 35 cycles at 94℃ for 30 sec, 64℃ for 30 sec and 72℃ for 1 min, using an Eppendorf Thermal Cycler (Eppendorf, Germany). The amplified products were then separated by agarose gel electrophoresis and gel purified using a Gel Extraction Kit (Omega, USA). Equimolar amounts of the two products were then mixed and used in a secondary SOE PCR. For the first five cycles no primers were added, and then the two 'external' primers (VH sense and VL antisense) were added for a further 30 cycles. The resulting full length product was then gel purified as described above, cut with the restriction enzymes SfiI and NotI (TaKaRa, Japan.) and ligated onto the pCANAB5E vector molecules at their C-terminal ends with a hexahistidine epitope tag for detection and purification purposes by overnight reaction at 16℃ using 0.1 unit T4 ligase (TaKaRa, Japan). The products were transformed intoTG1 cells. 7-8 single colonies were picked from the each agar plate and grown overnight at 37℃ in shaking culture in 2 × YT media containing 2% glucose and 100 μg/mL ampicillin. The sequence of positive colonies was determined with the ABI Perkin Elmer 373A auto-mated DNA sequencer (Applied

Biosystems, Forster City, CA), using the sequencing primers. Each sequence was determined at least 2 times.

Expression, purification and biophysical analyses of soluble dimers

The positive colonies were grown at 37℃ in shaking culture in 2 × YT media containing 2% glucose and 100 μg/mL ampicillin to an OD 600 of ~0.5 and 1 × 10^{10}/mL M13K07 helper phage (Pharmacia, Amersham.) was added. The bacteria were incubated for 1 h at 37ºC before being centrifuged at 1500 g for 20 min and resuspended in 2 × YT containing 100 μg/mL ampicillin and 50 μg/mL kanamycin. Following overnight culture at 30℃ the cultures were clarified by centrifugation and the phage precipitated using 2.5 mol/L NaCl/20% PEG 8000.

Cultures of *E. coli* HB2151(*supE*-) were infected with phage containing the relevant constructs. They were then diluted 1/100 and grown at 37℃ in 2 × YT medium containing 2% glucose and 100 μg/mL ampicillin to an OD 600 of ~0.5. The bacteria were pelleted and resuspended in 2 × YT medium containing 100 μg/mL ampicillin and 1 mmol/L isopropyl-β-D-thiogalactopyranoside (IPTG). The cells were then grown for 12 h at 30℃[9,10], centrifuged at 1500 g for 20 min, extracted and harvested for soluble diabodies from the periplasm. The soluble diabodies was purified with a HiTrap Anti-E Tag antibody column (Amersham Pharmacia), utilizing the hexahistidine epitope tag on their C-terminal ends.

The relative molecular mass of each affinity purified scFv dimer was compared by size exclusion gel chromatography on a Superdex 200 HR10/30 column (Amersham Pharmacia) run in PBS at a flow rate of 0.5 mL/min, calibrated with Biorad Gel Filtration Standard proteins[12,13]. The purity of size-fractionated antibodies was monitored by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions after staining with Simply Blue Safe Stain (Invitrogen, Carlsbad, CA). The specificity of eluted fractions was determined by Western blot analysis using an anti His-Tag-peroxidase conjugated mAb (Amersham Pharmacia) followed by chemiluminescent detection (ECL Plus, Amersham Pharmacia).

ELISA analysis of binding activity of soluble scFv dimers to human hepatocellular carcinoma cell lines

The binding of soluble scFv dimers molecules was determined by ELISA. The mixing suspension of Bel-7402, SMMC-7721 and HepG-2 cells (Cell Bank of Wuhan University, China) were harvested and coated onto microtitre plates. Unoccupied sites on the plates were blocked using PBS-milk and the samples, diluted in PBS-milk from 1:1 to 1:256, were added and incubated for 2 h at room temperature. The plates were washed 5 times with PBS-milk and 5 times with PBS. The soluble scFv dimers were detected using the anti His-Tag monoclonal antibody, labeled with horseradish peroxidase (HRP) conjugate (Pharmacia). The assays were developed using o-phenylenediamine (Dako) and absorbance was read at 490 nm wavelength in Model 680 Microplate Reader (Biorad, USA) with the parental scFv fragment HBM as control.

Immunohistochemistry

To determine the antigen-binding specificity of scFv dimers, immunohistochemistry was performed. Human hepatocellular carcinoma tissue and non-hepatocellular carcinoma tissue (donated by Professor Zhong, China) sections were heated at 56℃ for 2 h, washed successively with dimethylbenzene twice for 20 min, 95% alcohol twice for 2 min, 80% alcohol once for 1 min, distilled water once for 1 min, PBS twice for 1 min. After washing, tissue sections were incubated with 3% H_2O_2 for 5 min in room temperature and the above washing steps were repeated once. Subsequently, sera (1:10) from BALB/c mice were added on the surfaces of tissue sections in a humidified atmosphere at room temperature for 10 min and the surplus sera were discarded. Purified scFv dimers were then added to the tissue sections. The tissue sections were kept in a humidified atmosphere at 4℃ overnight and then washed with PBS for 5 min. The HRP-Anti-E Tag Conjugate was added and reacted under the above conditions overnight followed by washing with PBS for 3 min. Finally, 0.05% H_2O_2/DAB substrate was added to the tissue sections for 30 min. The specimens were photographed using the Leica Photo System (Qwin).

Biophysical stability analysis

At a concentration of 10 μg/mL, soluble scFv dimers were incubated at 37℃ for up to 7 d. Samples were taken at different time points (at intervals of 12 h) and frozen at -20℃ until the end of the experiment. Samples were subsequently analyzed for binding activity to human hepatocellular carcinoma cells.

RESULTS

Construction of scFv dimers

Three BDMs with short linkers, scFv-3, scFv-4 and scFv-5, were constructed in the pCANAB5E vector as described in Materials and Methods. The pictures of PCR reaction products and digested vector on agarose gel electrophoresis are shown in Figure 1. The DNA sequence of each scFv dimmer construct was confirmed in both orientations to ensure that the correct sequence had been inserted between the VH and VL domains(gene sequences of BDMs not shown).

Expression, purification and biophysical analyses of soluble scFv dimers

The constructs were transferred to the HB2151 strain of *E.coli*. The bacteria were induced for 12 h at 30℃ with 1 mmol/L IPTG. The soluble scFv fragments was extracted and purified as described above. Affinity purified scFv

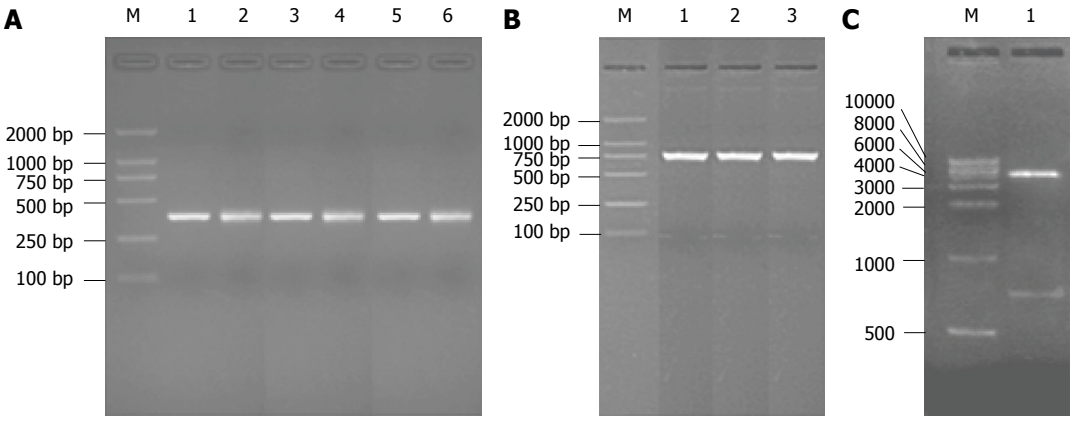


Figure 1 Agarose gel electrophoresis of PCR products. A: PCR products of VH and VL of scFv-3, scFv-4 and scFv-5. M: DNA marker DL2000; Lane 1: PCR products of the VH gene of scFv-3; Lane 2: PCR products of the VL gene of scFv-3. Lane 3: PCR products of the VH gene of scFv-4; Lane 4: PCR products of the VL gene of scFv-4. Lane 5: PCR products of the VH gene of scFv-5; Lane 6: PCR products of the VL gene of scFv-5; B: PCR products of scFv-3, scFv-4 and scFv-5. M: DNA marker DL2000; Lane 1: PCR products of the scFv-3 gene; Lane 2: PCR products of the scFv-4 gene; Lane 3: PCR products of the scFv-5 gene; C: Digested pCANAB5E vector: M: DNA marker; Lane 1: Digested pCANAB5E vector.

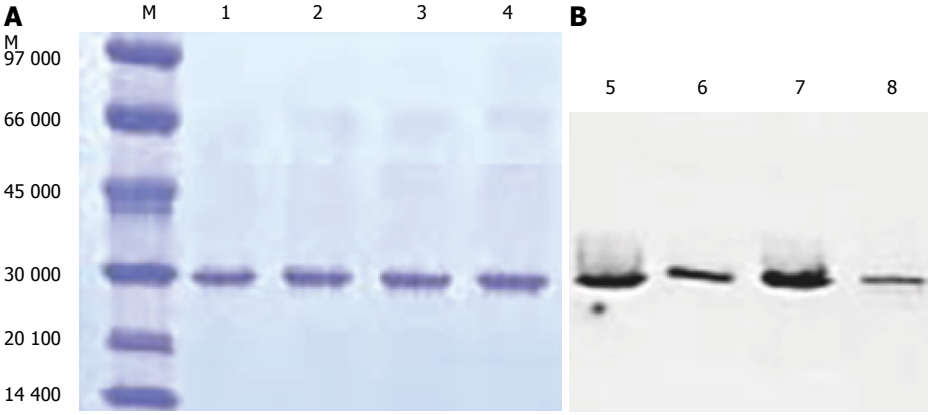


Figure 2 SDS-PAGE and Western blot. A: SDS gel electrophoresis of affinity-purified scFv fragments under reducing conditions. M: Low molecular weight markers (kDa); Lane 1: scFv-3; Lane 2: scFv-4; Lane 3: scFv-5; Lane 4: parental scFV HBM; B: Western blot. Lane 5: scFv-3; Lane 6: scFv-4; Lane 7: scFv-5; Lane 8: parental scFV HBM.

fragments and parental scFv HBM migrated as single bands with the expected molecular mass (30 kDa) on a reducing SDS-PAGE gel (Figure 2A). Western blot analysis (Figure 2B) was used to detect the C-terminal his-tag with an scFv-specific probe. The result indicated that the scFv dimers had been expressed successfully, the bands of dimers and monovalent scFv were all at 30 kDa because of the reducing conditions.

To examine the dimerization behavior of the scFv fragments, samples of scFv-3, scFv-4, scFv-5 were individually subjected to analysis by size exclusion gel chromatography on a calibrated Superdex 200 HR 10/30 column (Figure 3A). The major peaks for each of the scFv fragments showed the following distribution: ScFv-3 eluted in two peaks (25 and 28 min) corresponding to trimers (92 kDa) and dimers (61 kDa), respectively (Figure 3); ScFv-4 showed a predominant peak at an elution time of 28 min corresponding to a dimer (61 kDa) and an additional small peak indicating trimers (Figure 3); ScFv-5 was similar to ScFv-4 (not shown).

ELISA analysis of binding of soluble dimers to human hepatocellular carcinoma cell lines

Affinity constants of the scFv dimers for binding to human hepatocellular carcinoma cell lines were determined using a Model 680 Microplate Reader (Biorad) at 490 nm wavelength. All three scFv dimers showed improved binding activity compared to parental HDM (Figure 3B). Fitting the data from the equilibrium-binding curves into the non-linear regression model according to the Levenberg-Marquard method revealed a 3.5, 5.0 and 6.0-fold higher apparent affinity compared to the patental HDM for scFv-3, scFv-4 and scFv-5 respectively. The result also showed binding affinity of three dimers in a rank of scFv-5, scFv-4, scFv-3, with the binding activity decreasing according to diluted concentration.

Immunohistochemistry

The results of Immunohistochemistry were showed in Figure 4. As expected, scFv dimers react with human hepatocellular carcinoma tissue but not with non-hepatocellular carcinoma tissue. This suggests that scFv dimers have ideal specificity for binding to human hepatocellular carcinoma tissue. Therefore, the potential application of scFv dimers in clinical antibody-targeted diagnosis and therapy for HCC appears highly promising.

Stability analysis of scFv dimers

The thermostability of the dimeric molecules was analyzed. scFv-3 lost 50% of its initial binding activity

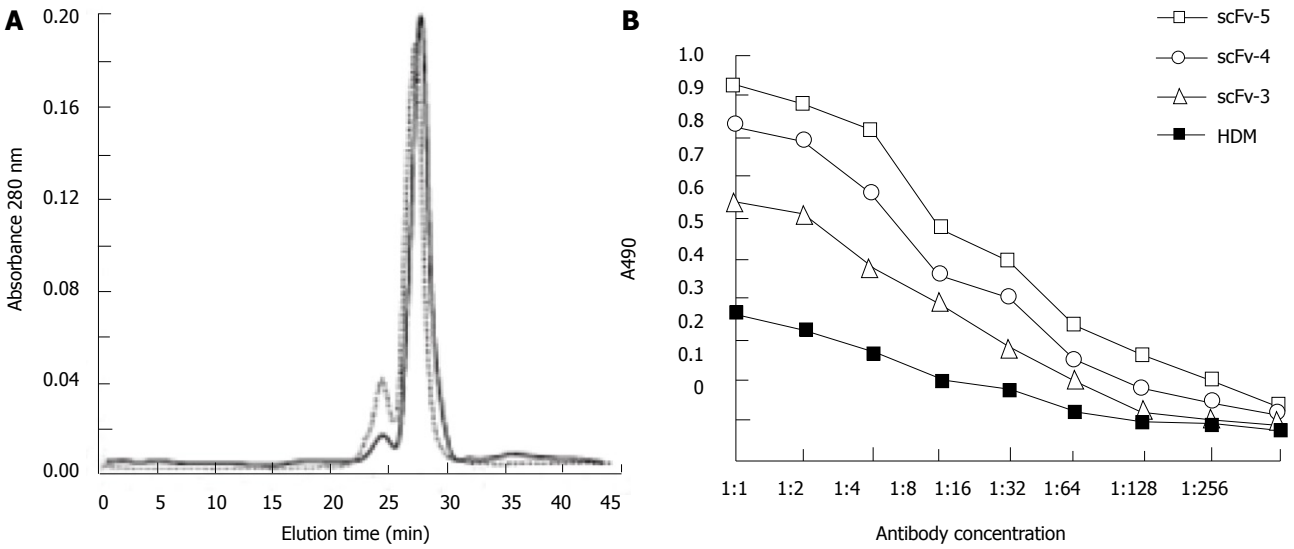


Figure 3 Analysis of soluble scFv dimers. A: Size exclusion chromatography of affinity purified scFv-3, scFv-4, scFv-5. Superimposed are elution profiles from a Superdex 200 gel filtration column of scFv-4 (solid line) and scFv-3 (dashed line). Elution peaks and molecular weight of calibration reference proteins are Gel Filtration Standard proteins (Biorad); B: Equilibrium binding curves of scFv dimers. Binding activity to hepatocellular carcinoma cell lines: ScFv-5 > scFv-4 > scFv-3 > HDM, with the tendency of decreasing binding activity going by diluted concentration. Determined By Model 680 Microplate Reader (Biorad).

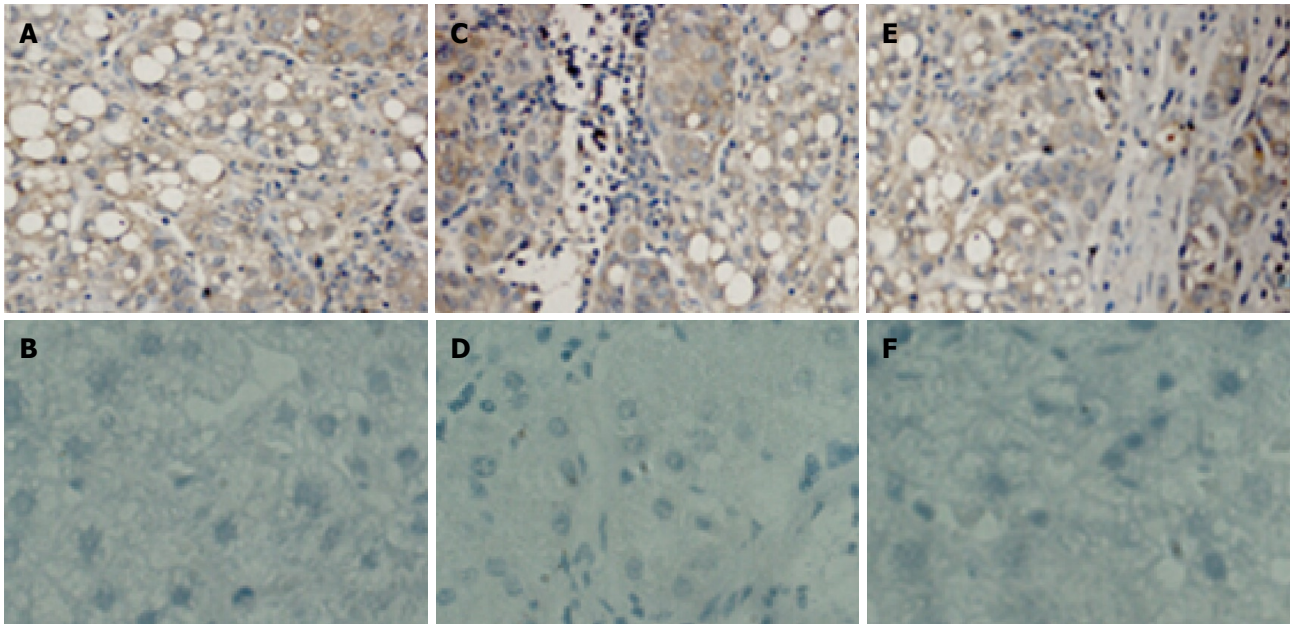


Figure 4 Immunohistochemistry of human hepatocellular carcinoma tissue. A: Reaction of scFv-3 dimer and human hepatocellular carcinoma tissue; B: Reaction of scFv-3 dimer and non- hepatocellular carcinoma tissue; C: Reaction of scFv-4 dimer and human hepatocellular carcinoma tissue; D: Reaction of scFv-4 dimer and non- hepatocellular carcinoma tissue; E: Reaction of scFv-5 dimer and human hepatocellular carcinoma tissue; F: Reaction of scFv-5 dimer and non-hepatocellular carcinoma tissue. Bound scFv dimers were detected with an antibody directed to the hexahistidine tag (Dianova) and peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dako). Staining was performed with diaminobenzidine (DAB)/H_2O_2; nuclei were counterstained with hematoxylin. As expected, scFv dimers react with human hepatocellular carcinoma tissue but not with non- hepatocellular carcinoma tissue. The image was captured at 20 × magnification.

after only 18 hrs incubation at 37℃ and completely lost binding activity after 70 hrs incubation. In contrast. scFv-4 was similar to scFv-5, with a 16 hr half life and totally lost binding activity after 60 hrs incubation.

DISCUSSION

Engineered antibodies possess considerable potential for immunotherapeutic and diagnostic applications and some engineered antibodies have already been approved by FDA in the USA for clinical uses. Antitumor antibodies must bind to tumor antigens with high affinity to achieve durable tumor retention. This has spurred efforts to generate high affinity antibodies for use in cancer therapy. In our previous work, we constructed and biopanned a recombinant phage scFv library to obtain a scFv which has specificity for human hepatocellular carcinoma. This was in the VH-linker-VL format, with the linker

consisting of the sequence (Gly4Ser)3. We then mutated and humanized the scFv to get scFv dimers (BDMs), which possess reduced immunogenicity[9-11]. The purpose of this study was to generate and characterize bivalent scFv antibody derivatives from HDMs. The most straightforward method to generate a bivalent scFv is to shorten the variable domain connecting linker peptide, thereby allowing for the non-covalent association of multiple polypeptide chains to a dimeric molecule[2,12-17], and to form a 'double-headed' fragment with two antigen binding sites that point away from each other[18]. This compactness contributes not only to low immunogenicity and high tumor penetration but also to rapid clearance from the circulation[15,19,20]. In addition to the linker length, the orientation of the variable domains was shown to impact the multimerization behavior and affinity of the polypeptide chains[13,14,21,22]. The distance between the carboxyl terminus end of VL and the amino terminus of VH is greater than that for the opposite orientation. It has, therefore, been suggested that VL-VH orientated scFvs are more constrained than VH-VL oriented fragments when connected by the same linker and therefore tend to exhibit a higher tendency to form higher molecular weight oligomers[13,14,23].

In this regard, we made several scFv dimers variants differing in linker length with a VH-linker-VL orientation. The sequence analysis showed that the sequences of VH-Gly2Ser-VL, VH- Gly3Ser-VL, VH-Gly4Ser-VL were exactly correct. Based upon size exclusion chromatographic profiles on Superdex 200, we conclude that the scFv-3, scFv-4 and scFv-5 predominantly formed stable dimers. This result is consistent with reports of other scFvs generated in the same domain orientation and with the same 3-5 amino acid linker[12,21].

The most important advantage of multivalent scFvs over monovalent scFv and Fab fragments is the gain in functional binding affinity (avidity) to target antigens. High avidity requires that scFv multimers are capable of binding simultaneously to target antigens[24]. The functional affinity of the BDMs was 3.5-6 fold that determined for the parent HDM, reflecting the gain in avidity due to dimerization and the capability of the molecule for simultaneous binding to two epitopes. The findings in immunohistochemistry and thermostability analysis have indicated that scFv dimers have improved specificity and stability and will become the paradigm for high-affinity antibody-based therapeutic and diagnostic reagents for HCC.

In conclusion, we have generated scFv dimers by shortening a series linkers to 3-5 amino acid residues in VH-linker-VL orientation,resulting in a set of highly stable and affinity-improved dimeric molecules. This work is crucial in making the antibody an attractive targeting moiety for HCC.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the second most common cause of cancer-related death in our country. Traditional therapies, such as resection, chemotherapy and radiotherapy do not have satisfactory efficacy. Single chain variable fragments (scFv) can be a good vector in targeted therapy for HCC as a result of their low molecular weight, strong tumor tissue penetration, weak immunogenicity and short half-lives in blood. They also possess the same binding ability and specificity as the whole antibody.

Research frontiers

This team previously obtained a scFv against hepatocellular carcinoma (scFv 4-16, GenBank: DQ640759) through the phage display antibody library technology. After reconstruction of affinity maturation and humanization of scFv, we attained a scFv with high affinity to HCC (scFvDM) and a humanized scFv (scFvHDM).

Innovations and breakthroughs

scFv is a monovalent antibody with the shortcoming of low affinity, poor stability, too rapid clearance from the circulation and low-level expression in bacterial expression systems. This study set out to improve its binding activity to antigen by shortening the linker of the VH-VL oriented scFv to 3-5 residues. We examined the expression and biological functions of the resulting diabodies

Applications

These diabodies may become an attractive targeting moiety in immunotherapeutic and diagnostic applications for HCC.

Peer review

The authors described an incremental improvement on the genetic engineering of HDM for targeting HCC, which remains to be one of most difficult solid tumor to manage. I recommend accepting this manuscript.

REFERENCES

1 **Carter P**. Improving the efficacy of antibody-based cancer therapies. *Nat Rev Cancer* 2001; **1**: 118-129

2 **Holliger P**, Prospero T, Winter G. "Diabodies": small bivalent and bispecific antibody fragments. *Proc Natl Acad Sci USA* 1993; **90**: 6444-6448

3 **Adams GP**, Shaller CC, Dadachova E, Simmons HH, Horak EM, Tesfaye A, Klein-Szanto AJ, Marks JD, Brechbiel MW, Weiner LM. A single treatment of yttrium-90-labeled CHX-A-C6.5 diabody inhibits the growth of established human tumor xenografts in immunodeficient mice. *Cancer Res* 2004; **64**: 6200-6206

4 **Takemura S**, Asano R, Tsumoto K, Ebara S, Sakurai N, Katayose Y, Kodama H, Yoshida H, Suzuki M, Imai K, Matsuno S, Kudo T, Kumagai I. Construction of a diabody (small recombinant bispecific antibody) using a refolding system. *Protein Eng* 2000; **13**: 583-588

5 **Takemura S**, Kudo T, Asano R, Suzuki M, Tsumoto K, Sakurai N, Katayose Y, Kodama H, Yoshida H, Ebara S, Saeki H, Imai K, Matsuno S, Kumagai I. A mutated superantigen SEA D227A fusion diabody specific to MUC1 and CD3 in targeted cancer immunotherapy for bile duct carcinoma. *Cancer Immunol Immunother* 2002; **51**: 33-44

6 **Asano R**, Kudo T, Nishimura Y, Makabe K, Hayashi H, Suzuki M, Tsumoto K, Kumagai I. Efficient construction of a diabody using a refolding system: anti-carcinoembryonic antigen recombinant antibody fragment. *J Biochem* 2002; **132**: 903-909

7 **Casey JL**, Napier MP, King DJ, Pedley RB, Chaplin LC, Weir N, Skelton L, Green AJ, Hope-Stone LD, Yarranton GT, Begent RH. Tumour targeting of humanised cross-linked divalent-Fab antibody fragments: a clinical phase I/II study. *Br J Cancer* 2002; **86**: 1401-1410

8 **Asano R**, Sone Y, Makabe K, Tsumoto K, Hayashi H, Katayose Y, Unno M, Kudo T, Kumagai I. Humanization of the bispecific epidermal growth factor receptor x CD3 diabody and its efficacy as a potential clinical reagent. *Clin Cancer Res* 2006; **12**: 4036-4042

9 **Zhou M**, Yang DH, Tang SH, Lu XH, Wang W, Li K. Construction, screening and characterization of the single chain variable fragment antibody against human hepa-

tocellular carcinoma. *Zhong Guo Bing Li Sheng Li Zha Zhi* 2005; **21**: 1246-1248
10 **Lu XH**, Yang DH, Zhou M, Tang SH. [Affinity maturation of a single-chain antibody against hepatocellular carcinoma]. *Zhonghua Gan Zang Bing Za Zhi* 2006; **14**: 19219-19225
11 **Ye G**, Yang DH, Lu XH, Zhou M, Tang SH, Wang W. Humanization of a single-chain antibody against hepatocellular carcinoma. *Zhong Guo Bing Li Sheng Li Zha Zhi* 2008; **11**: 1144-1150
12 **Atwell JL**, Breheney KA, Lawrence LJ, McCoy AJ, Kortt AA, Hudson PJ. scFv multimers of the anti-neuraminidase antibody NC10: length of the linker between VH and VL domains dictates precisely the transition between diabodies and triabodies. *Protein Eng* 1999; **12**: 597-604
13 **Dolezal O**, Pearce LA, Lawrence LJ, McCoy AJ, Hudson PJ, Kortt AA. ScFv multimers of the anti-neuraminidase antibody NC10: shortening of the linker in single-chain Fv fragment assembled in V(L) to V(H) orientation drives the formation of dimers, trimers, tetramers and higher molecular mass multimers. *Protein Eng* 2000; **13**: 565-574
14 **Kortt AA**, Lah M, Oddie GW, Gruen CL, Burns JE, Pearce LA, Atwell JL, McCoy AJ, Howlett GJ, Metzger DW, Webster RG, Hudson PJ. Single-chain Fv fragments of anti-neuraminidase antibody NC10 containing five- and ten-residue linkers form dimers and with zero-residue linker a trimer. *Protein Eng* 1997; **10**: 423-433
15 **Adams GP**, Schier R, McCall AM, Crawford RS, Wolf EJ, Weiner LM, Marks JD. Prolonged in vivo tumour retention of a human diabody targeting the extracellular domain of human HER2/neu. *Br J Cancer* 1998; **77**: 1405-1412
16 **Le Gall F**, Reusch U, Moldenhauer G, Little M, Kipriyanov SM. Immunosuppressive properties of anti-CD3 single-chain Fv and diabody. *J Immunol Methods* 2004; **285**: 111-127
17 **Pei XY**, Holliger P, Murzin AG, Williams RL. The 2.0-A resolution crystal structure of a trimeric antibody fragment with noncognate VH-VL domain pairs shows a rearrangement of VH CDR3. *Proc Natl Acad Sci USA* 1997; **94**: 9637-9642
18 **Perisic O**, Webb PA, Holliger P, Winter G, Williams RL. Crystal structure of a diabody, a bivalent antibody fragment. *Structure* 1994; **2**: 1217-1226
19 **Wu AM**, Chen W, Raubitschek A, Williams LE, Neumaier M, Fischer R, Hu SZ, Odom-Maryon T, Wong JY, Shively JE. Tumor localization of anti-CEA single-chain Fvs: improved targeting by non-covalent dimers. *Immunotechnology* 1996; **2**: 21-36
20 **Adams GP**, Tai MS, McCartney JE, Marks JD, Stafford WF 3rd, Houston LL, Huston JS, Weiner LM. Avidity-mediated enhancement of in vivo tumor targeting by single-chain Fv dimers. *Clin Cancer Res* 2006; **12**: 1599-1605
21 **Arndt MA**, Krauss J, Rybak SM. Antigen binding and stability properties of non-covalently linked anti-CD22 single-chain Fv dimers. *FEBS Lett* 2004; **578**: 257-261
22 **Carmichael JA**, Power BE, Garrett TP, Yazaki PJ, Shively JE, Raubischek AA, Wu AM, Hudson PJ. The crystal structure of an anti-CEA scFv diabody assembled from T84.66 scFvs in V(L)-to-V(H) orientation: implications for diabody flexibility. *J Mol Biol* 2003; **326**: 341-351
23 **Plückthun A**, Pack P. New protein engineering approaches to multivalent and bispecific antibody fragments. *Immunotechnology* 1997; **3**: 83-105
24 **Todorovska A**, Roovers RC, Dolezal O, Kortt AA, Hoogenboom HR, Hudson PJ. Design and application of diabodies, triabodies and tetrabodies for cancer targeting. *J Immunol Methods* 2001; **248**: 47-66

S- Editor Zhang HN **L- Editor** Hughes D **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i5.192

World J Hepatol 2010 May 27; 2(5): 192-197
ISSN 1948-5182 (online)

CASE REPORT

A successful treatment by hepatic arterial infusion therapy for advanced, unresectable biliary tract cancer

Masako Nishimura

Masako Nishimura, Department of Gastroenterology, Otsu Red Cross Shiga Hospital, 298 Wani- naka, Otsu, Shiga 520-0580, Japan
Author contributions: Nishimura M contributed solely to this paper.
Correspondence to: Masako Nishimura, MD, Department of Gastroenterology, Otsu Red Cross Shiga Hospital, 298 Wani-naka, Otsu, Shiga 520-0580, Japan. nishimu@hkg.odn.ne.jp
Telephone: +81-77-5948777 Fax: +81-77-5948778
Received: November 30, 2009 Revised: March 23, 2010
Accepted: March 30, 2010
Published online: May 27, 2010

Peer reviewers: Hiroshi Yoshida, MD, PhD, Department of Surgery, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo, 113-8603, Japan; Ruben Ciria, PhD, Uniersity Hospital Reina Sofia, Departmento De Cirugia Hepatobiliar Y Trasplante HEPÁTICO (Department of Hepatobiliary Surgery and Liver Transplantation), Avennida Menendez PidalI s/n, Cordoba 14004, Spain

Nishimura M. A successful treatment by hepatic arterial infusion therapy for advanced, unresectable biliary tract cancer. *World J Hepatol* 2010; 2(5): 192-197 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i5/192.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i5.192

Abstract

Biliary tract cancers (BTC) are relatively rare tumors, and the prognosis is extremely poor. There has been no standard chemotherapy for advanced BTC. However, recently, gemcitabine (GEM) have been used against BTC as the most active agent, and promising response rates and overall survival times with tolerable drug toxicities have been observed. In this article, two cases of advanced intrahepatic cholangiocarcinoma and unresectable metastatic gallbladder (GB) cancer are reported. They were treated with hepatic arterial infusion (HAI) chemotherapy using a combination of GEM and cisplatin, along with the systemic administration of GEM. As a consequence, multiple liver tumors, the GB cancer and metastatic lymph nodes regressed without severe drug toxicities, and favorable results (the overall survival times were 16 and 14 mo, respectively) were achieved. In conclusion, HAI therapy using GEM combined with cisplatin may be a useful and well-tolerated option for advanced BTC, especially in cases where multiple liver metastases are detected.

Key words: Hepatic arterial infusion; Gemcitabine; Cisplatin; Intrahepatic cholangiocarcinoma; Gallbladder cancer

INTRODUCTION

Biliary tract cancers (BTC) are relatively rare tumors, and their prognosis is extremely poor. The median survival period of gallbladder (GB) cancer is 6 mo, and cholangiocarcinoma (CC) also has an unfavorable prognosis, with a median survival time of 3-9 mo. In particular, intrahepatic cholangiocarcinoma (ICC) generally presents at a more advanced stage than extrahepatic CC. Therefore, its prognosis is even worse. In the past, the efficacy of conventional systemic chemotherapy in advanced, unresectable BTC was negligible. However, after the use of gemcitabine (GEM), a novel nucleoside analog, encouraging results for tumor control rates and survival time have been recently reported for BTC[1-4]. Furthermore, a chemotherapeutic regimen using GEM combined with cisplatin has been demonstrated to be superior to GEM alone and/or a combination of fluoropyrimidines and cisplatin[5-9]. Chemotherapy using hepatic arterial infusion (HAI) is a promising option for advanced and multiple hepatocellular carcinoma (HCC) and unresectable metastatic liver tumors of various origins, and this therapeutic modality has resulted in superior tumor control rates compared to systemic chemotherapy[10-13]. However, there have been few reports

on HAI therapy using GEM together with cisplatin for advanced BTC.

In this article, two cases of advanced ICC and unresectable GB cancer are reported, which showed favorable outcomes when treated with HAI therapy using a combination of GEM and cisplatin.

CASE REPORT

Case 1

A 71-year-old man was admitted to our hospital with multiple hepatic tumors. A palpable liver was found in the right upper abdominal quadrant during physical examination, whereas no other relevant pathological findings were evident. The laboratory findings showed liver dysfunction. Among the serum tumor markers, α-fetoprotein (AFP) and carbohydrate antigen (CA) 19-9 were elevated (124.3 ng/mL, normal < 10; and 62 U/mL, normal < 37, respectively), but the level of carcinoembryonic antigen (CEA) was normal. Contrast-enhanced (CE) computed tomography (CT) of the abdomen revealed a huge liver tumor measuring 11 cm in diameter in the medial segment, and multiple small-sized tumors mainly located in the anterior and medial segment of the liver, which presented with ring enhancement in the arterial phase and central necrosis (Figure 1A and B). Multiple swollen lymph nodes (LNs) were noted in liver hilus and para- aorta, accompanied by ascites. No other tumors presented as primary foci in any other tissue outside the liver. Biopsied specimens from one of the hepatic tumors revealed a moderately-differentiated adenocarcinoma with widespread necrosis, which was not accompanied by hepatic cell components (Figure 2A). Additional histological examinations disclosed positive immunostaining for cytokeratin 7, and immuno-positive AFP in the adenocarcinoma cells (Figure 2B and C), suggesting that the hepatic tumors were AFP-producing cholangiocellular carcinomas. Under the diagnosis that the current case was an ICC of UICC TMN stage Ⅲ C with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0, intensive chemotherapy was implemented, using both HAI along and intravenous (i.v.) administration. Following informed consent was given concerning the side effects of the therapy including interventional procedures and chemotherapeutic agents, 600 mg/m^2 of GEM on d 1 and 8, and 10 mg/body of cisplatin on d 1 to 3 and 8 to 10 were infused via a subcutaneously implanted port which was connected to a catheter placed in the proper hepatic artery. Concurrently, 400 mg/m^2 of GEM was administered intravenously on d 1 and 8, every 3 wk. The patient received 9 cycles of chemotherapy for 26 wk. Following completion of 2 cycles of chemotherapy, the huge tumor in the medial segment showed a reduction in diameter, and the ascitic fluid disappeared completely. However, an abnormal uptake on FDG-PET CT was observed in the hepatic tumors in both lobes, as well as the LNs in the liver hilus and para-aorta (Figure 3). After completion of 7 cycles of chemotherapy, almost all of the hepatic tumors had shrunk in size, and the swollen LNs were almost absent upon a CE-CT of the abdomen (Figure 4A). Meanwhile, a PET-CT revealed abnormal uptake only in the tumors in the medial segment, and no uptake in tumors in the right lobe and LNs (Figure 4B), suggesting that the tumor response was a partial response (PR), according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. The elevated serum levels of CA19-9 and AFP were also decreased to almost the normal range after 8 cycles of chemotherapy (Figure 5). Following 9 cycles of chemotherapy, hematological toxicities such as severe thrombocytopenia (NCI-CTC, grade 3) and leukopenia (grade 2) were observed. Therefore, a modified chemotherapeutic regimen (80% of the initial dose of GEM on d 1, and 10 mg cisplatin on d 1 and 2) was carried out biweekly thereafter. Although a greater reduction of the abnormal uptake from the tumor in the medial segment was revealed on the PET-CT after the end of 11 cycles of chemotherapy, new abnormal uptake was found in the LN in the liver hilus. Therefore, the present case was diagnosed as a pro gressive disease (PD). A new chemotherapy regimen using GEM (480 mg/m^2 for HAI and 320 mg/m^2 for i.v. administration on d 2) and oral S-1 (80 mg/body for 7 to 14 d, consecutively) every 3 wk was started.

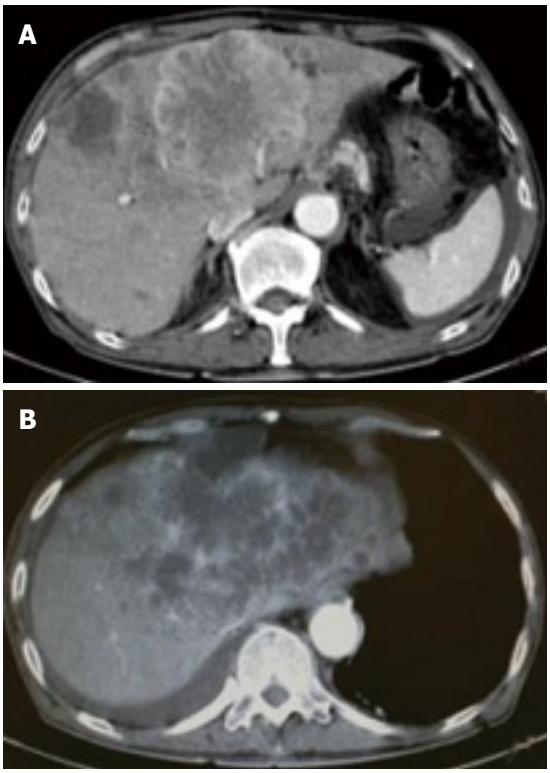


Figure 1 Contrast-enhanced (CE) computed tomography of the abdomen revealed a huge tumor, measuring 11 cm in diameter, which presented with ring enhancement in the arterial phase and central necrosis. A: In the medial segment; B: Multiple small-sized tumors mainly located in the anterior and medial segments of the liver.

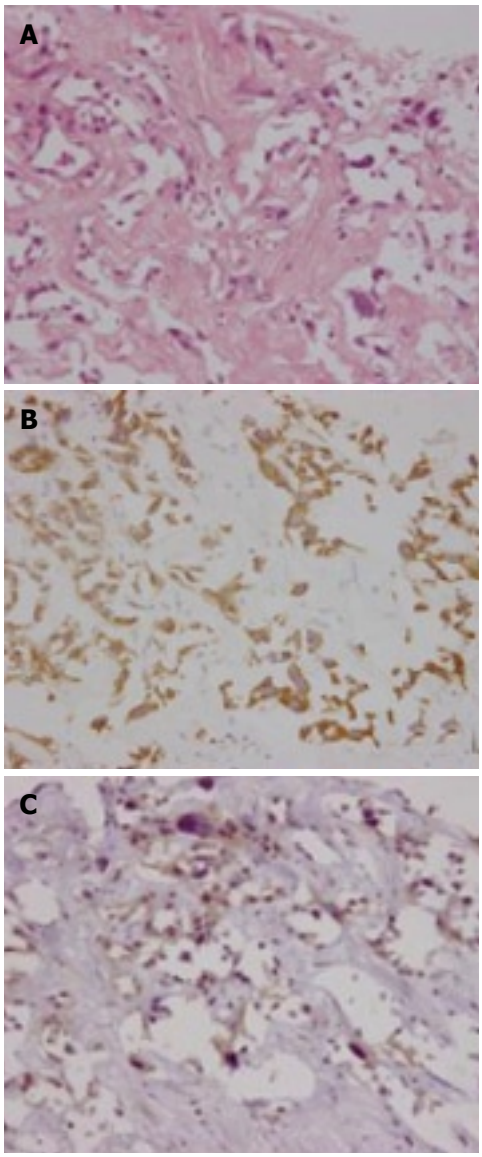


Figure 2 Biopsied specimens from one of the hepatic tumors. A: A moderately-differentiated adenocarcinoma with wide-spread necrosis, which were not accompanied by hepatic cell components (HE × 200); B: Positive immunostaining for cytokeratin 7 (× 200) in the adenocarcinoma cells; C: Immuno-positive AFP (× 200) in the adenocarcinoma cells.

Following the end of 4 cycles of the new regimen, the liver tumors and metastatic LNs became larger. An ECF regimen using epirubicin 40 mg/m^2 and cisplatin 48 mg/m^2 for HAI on d 1, and 5-FU 160 mg/m^2 for i.v. administration for 4 consecutive days, was then carried out every 3 wk. However, systemic metastases (peritoneum, bone and lung) and massive ascites were soon observed. At 16 mo after the diagnosis, the patient died of multiple organ failure.

Case 2

An 83-year-old woman presented to our hospital with abdominal discomfort and appetite loss. Physical examination revealed a swollen gallbladder but no other pathological findings. The laboratory data demonstrated liver dysfunction and an elevation in the serum levels

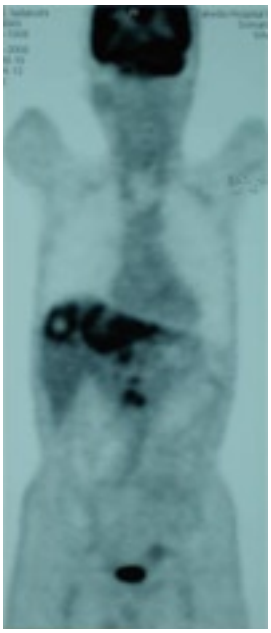


Figure 3 FDG-PET CT after the end of 2 cycles of chemotherapy revealed abnormal uptake in the hepatic tumors in both lobes and the LNs in the liver hilus and para-aorta.

of CA 19-9 and CEA (82.0 U/mL, normal < 37; 61.3 ng/mL, normal < 5, respectively). Ultrasonography and CE-CT of the abdomen revealed gall stones and a GB tumor, which invaded the adjacent liver directly. Multiple liver tumors and swollen LNs in the liver hilus were also observed. The patient's PS was 1 and, based on the diagnosis that the current case was an advanced GB carcinoma (UICC TNM stage Ⅳ), chemotherapy using a 4 wk cycle of i.v. administration of 1000 mg/m^2 of GEM on d 1, 8, 15 was started. After completion of 4 cycles of chemotherapy, the tumor increased in size in the GB, liver and LNs in the liver hilus (Figure 6A and B), and ascitic fluid appeared on CE-CT (RECIST, PD). The serum levels of CA 19-9 and CEA elevated gradually (Figure 7). Furthermore, metastasis in the lumbar vertebra was detected. Immediately thereafter, radiation therapy to the lumbar vertebrae was started. After informed consent was given, a heparin-coated catheter was placed in the common hepatic artery to supply all of the tumor vessels, including the GB and liver. Thereafter, the catheter was connected to the injection port. The gastroduodenal artery was occluded by steel coils to prevent gastroduodenal injury from anticancer drugs. Next, a new chemotherapeutic regimen comprising HAI (600 mg/m^2 of GEM on d 1 and 8, and 10 mg/body of cisplatin on d 1 to 3 and 8 to 10) and the i.v. administration of GEM (400 mg/m^2, d 1 and 8), was carried out every 3 wk for 3 cycles. After hematological toxicity (grade 2) occurred at the end of 3 cycles of chemotherapy, a modified regimen in which cisplatin administration was shortened to 2 d was instigated for 2 cycles. At the end of 3 cycles of chemotherapy with HAI, the tumors in the GB and liver decreased in size (Figure 8A and B), and the swollen LNs in the liver hilus regressed on the abdominal CT (RECIST, PR). However, following the 2 cycles of modified HAI chemotherapy, a perforation in the duodenum which was invaded directly

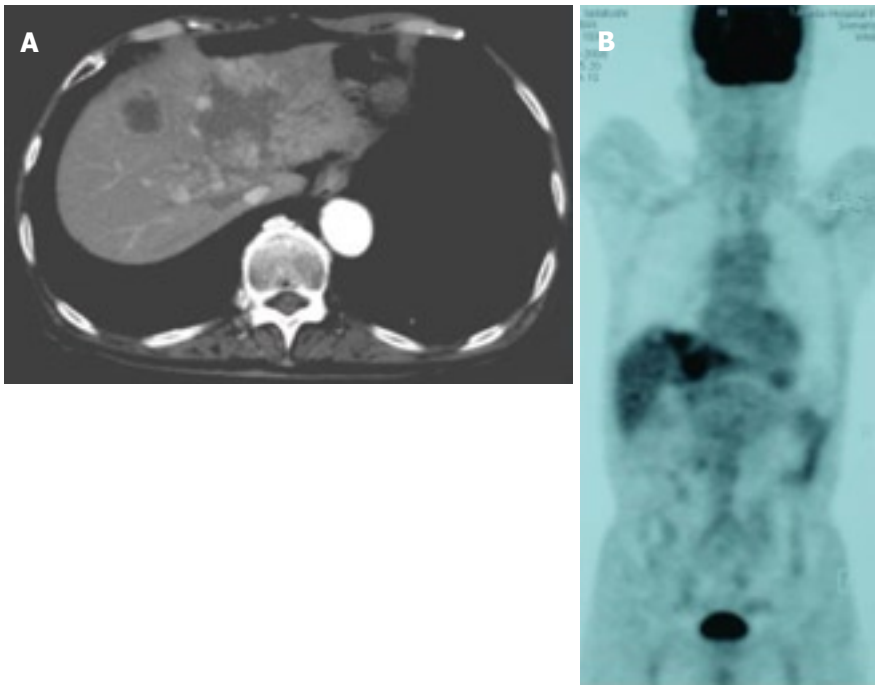


Figure 4 Images after the end of 7 cycles of chemotherapy. A: CE-CT showed a reduction in the size of a huge tumor seen in the medial segment; B: PET-CT revealed abnormal uptake only in the tumors in the medial segment, and no uptake was observed in tumors from the right lobe and the LNs in the liver hilus and para-aorta.

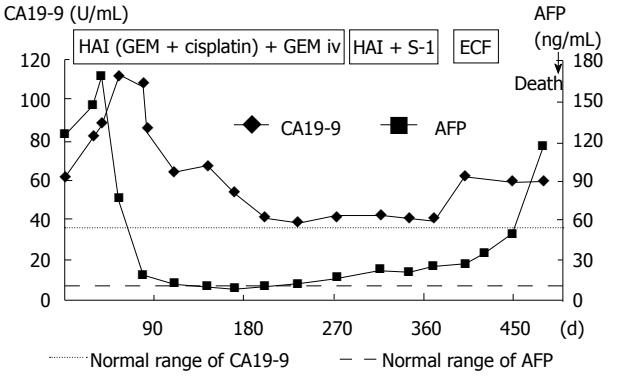


Figure 5 Clinical course and changes in the serum levels of CA19-9 and AFP.

by the GB cancer occurred. The chemotherapy was then discontinued, and 14 mo after the initial diagnosis, the patient was died of multiple organ failure.

DISCUSSION

In the past, there was no standard chemotherapy for advanced BTC i.e. GB cancer and CC. However, GEM has recently been used against BTC as the most active agent, and promising response rates and overall survival times with tolerable drug toxicities were demonstrated[1-4]. GEM has a synergistic and cytotoxic effect *in vivo* and *in vitro*, in combination with cisplatin[14,15] and capecitabine[16]. More recently, the superiority of combination chemotherapy using GEM plus cisplatin, as well as the combination of GEM with capecitabine or oxaliplatin, has been demonstrated among the GEM-only, GEM-based and 5-FU-based chemotherapeutic regimens for BTC[4,5,8,9,17-19]. The intra-arterial (i.a.) administration of GEM was used for the treatment of advanced pancreatic

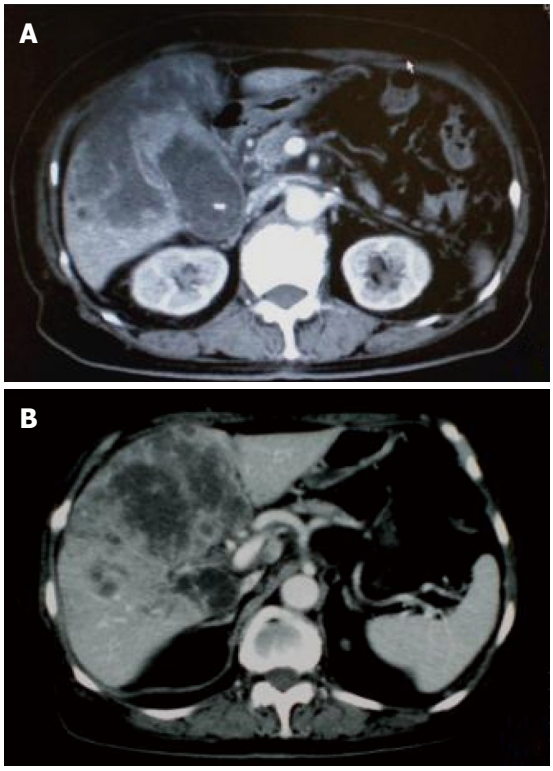


Figure 6 CE-CT after the end of 4 cycles of i.v. administration chemotherapy showed that the tumor increased in size. A: The GB tumor; B: Liver tumor.

carcinoma by Shamseddine et. al. in 2005[20]. They reported that GEM in i.a. administration could be safely escalated to 1400 mg/m^2 within a tolerable toxicity. On the other hand, in low dose FP (5-FU and cisplatin) therapy for advanced and multiple HCCs, cisplatin is administered intra-arterially at a dose of 10 mg/body for 5 consecutive days[13]. Based on the previous reports mentioned above and our judgment that the control of

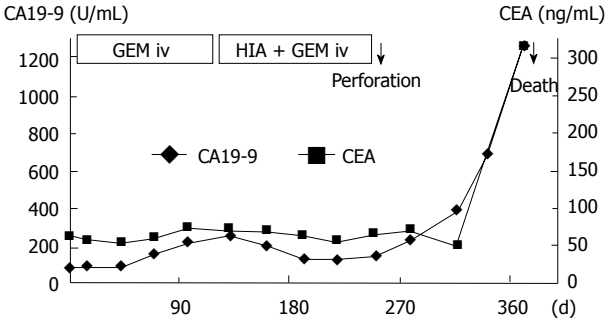


Figure 7 Clinical course and changes in the serum levels of CA19-9 and CEA.

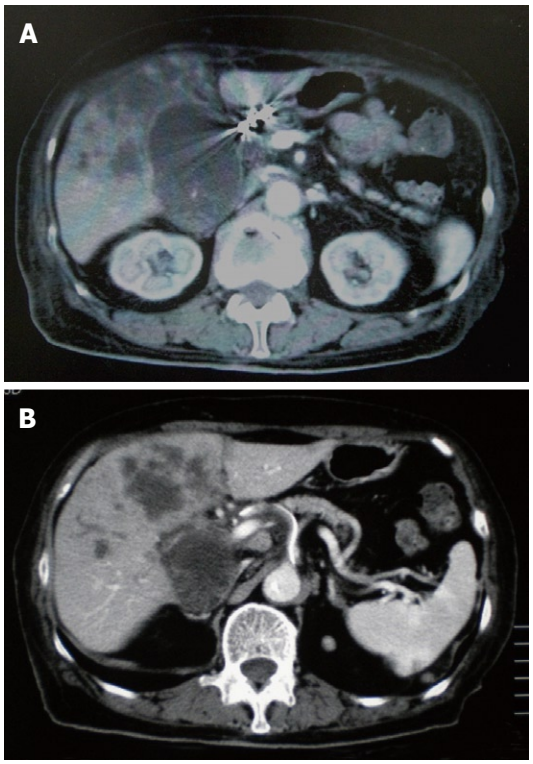


Figure 8 CE-CT after the end of 3 cycles of HAI chemotherapy showed a decrease in the size of tumor. A: The GB tumor; B: Liver tumor.

multiple liver tumors might decide the patient's survival time, HAI therapy using GEM combined with cisplatin was performed. Following consideration of treatment efficacy against distant metastases (LNs and bone), the systemic administration of GEM was used together with HAI in both cases. In case 1, after the end of 7 cycles of chemotherapy, the liver tumors had regressed, and the swollen LNs and ascitic fluid disappeared completely. In case 2, because of the PD after 4 cycles of the i.v. administration of GEM, HAI therapy was started again, and consequently favorable tumor responses were achieved. The survival times of the current two cases were also similar.

A chemotherapeutic regimen using the i.a. administration of GEM together with hepatic embolization has been performed against advanced BTC, and respectable results were achieved[21,22]; the survival time for the group treated by GEM with microspheres was 20.2 mo, whereas that of the TACE group using a combination of GEM plus cisplatin was 13.8 mo. Arterial administration can deliver anti-cancer agents at high concentrations into liver tumors, and has a longer lasting cytotoxic effect. A previous report demonstrated that the peak plasma concentration of GEM after i.a. administration was reduced to ~1/7th of that observed using the systemic i.v. route, and that no grade 3 or 4 toxicity was documented after i.a. administration of up to 1400 mg/m^2 of GEM[20]. Vogl *et al*[21] noted that the use of GEM doses (~1800 mg/m^2) higher than the recommended 1000 mg/m^2 was well tolerated if microspheres were used. Moreover, Gusani *et al*[22] showed that the median survival in unresectable CC treated with GEM-based TACE was not significantly different in patients with liver disease only, as compared to those with extra-hepatic disease. In 2008, HAI therapy using a combination of GEM and mitomycin against advanced CC revealed a poor tumor control rate, as compared to that against metastatic breast cancer and colorectal carcinoma[12]. Therefore, HAI therapy using GEM combined with cisplatin may be a useful and well-tolerated option for advanced BTC, especially in which multiple liver metastases are detected.

In recent years, targeted therapy has been carried out for advanced BTC as a second-line chemotherapy[23] and in phase Ⅱ trials[24]. Chemotherapeutic regimens using cetuximab or bevacizumab combined with GEM and oxaliplatin have also been used against advanced BTC and, at present, passable tumor- responses have been achieved[23,24]. In conclusion, HAI therapy with GEM plus cisplatin might be an effective and well-tolerated option in advanced BTC, and in the future we recommend that clinical trials of HAI therapy using GEM-based platinum or capecitabine, with or without targeted agents, should be performed for advanced BTC.

REFERENCES

1 **Knox JJ**, Hedley D, Oza A, Siu LL, Pond GR, Moore MJ. Gemcitabine concurrent with continuous infusional 5-fluorouracil in advanced biliary cancers: a review of the Princess Margaret Hospital experience. *Ann Oncol* 2004; **15**: 770-774

2 **Alberts SR**, Al-Khatib H, Mahoney MR, Burgart L, Cera PJ, Flynn PJ, Finch TR, Levitt R, Windschitl HE, Knost JA, Tschetter LK. Gemcitabine, 5-fluorouracil, and leucovorin in advanced biliary tract and gallbladder carcinoma: a North Central Cancer Treatment Group phase II trial. *Cancer* 2005; **103**: 111-118

3 **Park JS**, Oh SY, Kim SH, Kwon HC, Kim JS, J-Kim H, Kim YH. Single-agent gemcitabine in the treatment of advanced biliary tract cancers: a phase II study. *Jpn J Clin Oncol* 2005; **35**: 68- 73

4 **Yonemoto N**, Furuse J, Okusaka T, Yamao K, Funakoshi A, Ohkawa S, Boku N, Tanaka K, Nagase M, Saisho H, Sato T. A multi-center retrospective analysis of survival benefits of chemotherapy for unresectable biliary tract cancer. *Jpn J Clin Oncol* 2007; **37**: 843-851

5 **Eckel F**, Schmid RM. Chemotherapy in advanced biliary

tract carcinoma: a pooled analysis of clinical trials. *Br J Cancer* 2007; **96**: 896-902
6 **Valle JW**, Wasan H, Johnson P, Bridgewater J, Maraveyas A, Jones E, Tunney V, Swindell R, on behalf of the ABC-01 study group. Gemcitabine, alone or in combination with cisplatin, in patients with advanced or metastatic cholangiocarcinoma (CC) and other biliary tract tumors: A multicenter, randomized, phase II (the UK ABC-01) study. *Am Soc Clin Oncol* 2006: A98
7 **Lee MA**, Woo IS, Kang JH, Hong YS, Lee KS. Gemcitabine and cisplatin combination chemotherapy in intrahepatic cholangiocarcinoma as second-line treatment: report of four cases. *Jpn J Clin Oncol* 2004; **34**: 547-550
8 **Thongprasert S**, Napapan S, Charoentum C, Moonprakan S. Phase II study of gemcitabine and cisplatin as first-line chemotherapy in inoperable biliary tract carcinoma. *Ann Oncol* 2005; **16**: 279-281
9 **Meyerhardt JA**, Zhu AX, Stuart K, Ryan DP, Blaszkowsky L, Lehman N, Earle CC, Kulke MH, Bhargava P, Fuchs CS. Phase-II study of gemcitabine and cisplatin in patients with metastatic biliary and gallbladder cancer. *Dig Dis Sci* 2008; **53**: 564-570
10 **Tanaka N**, Yamakado K, Nakatsuka A, Fujii A, Matsumura K, Takeda K. Arterial chemoinfusion therapy through an implanted port system for patients with unresectable intrahepatic cholangiocarcinoma – initial experience. *Eur J Radiol* 2002; **41**: 42-48
11 **Cantore M**, Mambrini A, Fiorentini G, Rabbi C, Zamagni D, Caudana R, Pennucci C, Sanguinetti F, Lombardi M, Nicoli N. Phase II study of hepatic intraarterial epirubicin and cisplatin, with systemic 5-fluorouracil in patients with unresectable biliary tract tumors. *Cancer* 2005; **103**: 1402-1407
12 **Vogl TJ**, Zangos S, Eichler K, Selby JB, Bauer RW. Palliative hepatic intraarterial chemotherapy (HIC) using a novel combination of gemcitabine and mitomycin C: results in hepatic metastases. *Eur Radiol* 2008; **18**: 468-476
13 **Okuda K**, Tanaka M, Shibata J, Ando E, Ogata T, Kinoshita H, Eriguchi N, Aoyagi S, Tanikawa K. Hepatic arterial infusion chemotherapy with continuous low dose administration of cisplatin and 5-fluorouracil for multiple recurrence of hepatocellular carcinoma after surgical treatment. *Oncol Rep* 1999; **6**: 587-591
14 **Peters GJ**, Bergman AM, Ruiz van Haperen VW, Veerman G, Kuiper CM, Braakhuis BJ. Interaction between cisplatin and gemcitabine in vitro and in vivo. *Semin Oncol* 1995; **22**: 72-79
15 **Moufarij MA**, Phillips DR, Cullinane C. Gemcitabine potentiates cisplatin cytotoxicity and inhibits repair of cisplatin-DNA damage in ovarian cancer cell lines. *Mol Pharmacol* 2003; **63**: 862-869
16 **Sawada N**, Fujimoto-Ouchi K, Ishikawa T, Tanaka Y, Ishitsuka H. Antitumor activity of combination therapy with capecitabine plus vinorelbine, and capecitabine plus gemcitabine in human tumor xenograft models. *Proc Am Assoc Cancer Res* 2002; A5388
17 **Iyer RV**, Gibbs J, Kuvshinoff B, Fakih M, Kepner J, Soehnlein N, Lawrence D, Javle MM. A phase II study of gemcitabine and capecitabine in advanced cholangiocarcinoma and carcinoma of the gallbladder: a single-institution prospective study. *Ann Surg Oncol* 2007; **14**: 3202-3209
18 **Kim MJ**, Oh DY, Lee SH, Kim DW, Im SA, Kim TY, Heo DS, Bang YJ. Gemcitabine-based versus fluoropyrimidine-based chemotherapy with or without platinum in unresectable biliary tract cancer: a retrospective study. *BMC Cancer* 2008; **8**: 374
19 **Gebbia N**, Verderame F, Di Leo R, Santangelo D, Cicero G, Valerio M, Arcara C, Badalamenti G, Fulfaro F, Carreca I. A phase II study of oxaliplatin (O) and gemcitabine (G) first line chemotherapy in patients with advanced biliary tract cancers. *J Clin Oncol* 2005; **23** suppl 16: A4132
20 **Shamseddine AI**, Khalifeh MJ, Mourad FH, Chehal AA, Al-Kutoubi A, Abbas J, Habbal MZ, Malaeb LA, Bikhazi AB. Comparative pharmacokinetics and metabolic pathway of gemcitabine during intravenous and intra-arterial delivery in unresectable pancreatic cancer patients. *Clin Pharmacokinet* 2005; **44**: 957-967
21 **Vogl TJ**, Schwarz W, Eichler K, Hochmuth K, Hammerstingl R, Jacob U, Scheller A, Zangos S, Heller M. Hepatic intraarterial chemotherapy with gemcitabine in patients with unresectable cholangiocarcinomas and liver metastases of pancreatic cancer: a clinical study on maximum tolerable dose and treatment efficacy. *J Cancer Res Clin Oncol* 2006; **132**: 745-755
22 **Gusani NJ**, Balaa FK, Steel JL, Geller DA, Marsh JW, Zajko AB, Carr BI, Gamblin TC. Treatment of unresectable cholangiocarcinoma with gemcitabine-based transcatheter arterial chemoembolization (TACE): a single-institution experience. *J Gastrointest Surg* 2008; **12**: 129-37
23 **Paule B**, Herelle MO, Rage E, Ducreux M, Adam R, Guettier C, Bralet MP. Cetuximab plus gemcitabine-oxaliplatin (GEMOX) in patients with refractory advanced intrahepatic cholangiocarcinomas. *Oncology* 2007; **72**: 105-110
24 **Clark JW**, Meyerhardt JA, Sahani DV, Namasivayam S, Abrams TA, Stuart K, Bhargava P, Blaszkowsky LS, Jain SR, Zhu AX. Phase II study of gemcitabine, oxaliplatin in combination with bevacizumab (GEMOX-B) in patients with unresectable or metastatic biliary tract and gallbladder cancers. *J Clin Oncol* 2007; **25** suppl 18S: A4625

S- Editor Zhang HN **L- Editor** Hughes D **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i5.198

World J Hepatol 2010 May 27; 2(5): 198-200
ISSN 1948-5182 (online)

CASE REPORT

Intraoperative intracardiac thrombosis in a liver transplant patient

Lena Sibulesky, Prith Peiris, C Burcin Taner, David J Kramer, Juan M Canabal, Justin H Nguyen

Lena Sibulesky, C Burcin Taner, Justin H Nguyen, Department of Transplantation, Division of Transplant Surgery, Mayo Clinic, Jacksonville, FL 32224, United States
Prith Peiris, Department of Anesthesiology, Mayo Clinic, Jacksonville, FL 32224, United States
David J Kramer, Juan M Canabal, Departments of Transplantation and Critical Care, Mayo Clinic, Jacksonville, FL 32224, United States
Author contributions: Sibulesky L, Peiris P and Nguyen JH collected and analyzed data and wrote the paper; Kramer DJ and Canabal JM analyzed data; and Taner CB participated in writing the paper.
Correspondence to: Justin H Nguyen, MD, Division of Transplant Surgery, Department of Transplantation, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, United States. nguyen.justin@mayo.edu
Telephone: +1-904-9563262 Fax: +1-904-9563359
Received: December 25, 2010 Revised: April 15, 2010
Accepted: April 22, 2010
Published online: May 27, 2010

Abstract

A 66-year-old female with cryptogenic cirrhosis complicated by ascites, hepatic encephalopathy, variceal bleeding and malnutrition with MELD of 34 underwent orthotopic deceased donor liver transplantation performed with piggyback technique. Extensive eversion thromboendovenectomy was performed for a portal vein thrombus which resulted in an excellent portal vein flow. The liver graft was recirculated without any hemodynamic instability. Subsequently, the patient became hypotensive progressing to asystole. She was resuscitated and a transesophageal probe was inserted which revealed a mobile right atrial thrombus and an underfilled poorly contractile right ventricle. The patient was noted to be coagulopathic at the time. She became progressively more stable with a TEE showing complete resolution of the intracardiac thrombus.

Key words: Liver transplantation; Cardiac thrombus; Reperfusion injury; Coagulopathy; Transesophageal echocardiogram

Peer reviewers: Valentina Medici, MD, PhD, Department of Internal Medicine, University of California Davis, 4150 V Street, Suite 3500, Sacramento, CA 95817, United States; Joseph Ahn, MD, Assistant Professor of Medicine, Medical Director, Liver Transplantation, Loyola University Medical Center, 2160 S. First Ave., Building 54, Room 007, Maywood, IL 60153, United States

Sibulesky L, Peiris P, Taner CB, Kramer DJ, Canabal JM, Nguyen JH. Intraoperative intracardiac thrombosis in a liver transplant patient. *World J Hepatol* 2010; 2(5): 198-200 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i5/198.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i5.198

INTRODUCTION

Intracardiac thrombosis (ICT) during orthtopic liver transplantation is a catastrophic complication that carries a very high mortality. The exact cause of this condition is not known but it appears that it has multiple etiologies including administration of antifibrinolytic drugs intraoperatively and venovenous bypass.

In this case report we describe a patient who developed ICT during orthotopic liver transplantation. No risk factors could be identified.

CASE REPORT

A 66-year-old female with cryptogenic cirrhosis was admitted to our Intensive Care Unit (ICU) with acutely worsening liver function. She had had multiple operations in the past including open cholecystectomy, total abdominal hysterectomy with salpingo-oophorectomy and appendectomy. She was noted to have a nodular liver while undergoing laparotomy for lysis of adhesions in 2007. Her liver disease was complicated by ascites, hepatic

Table 1 Patient coagulation parameters at the beginning of the case, at the time of the intracardiac thrombus diagnosis and at the end of the case

	Platelets /μL	INR	Fibrinogen (mg/dL)
Beginning of case	44 000	1.8	210
Detection of clot	13 000	6.4	< 25
End of case	55 000	1.7	196

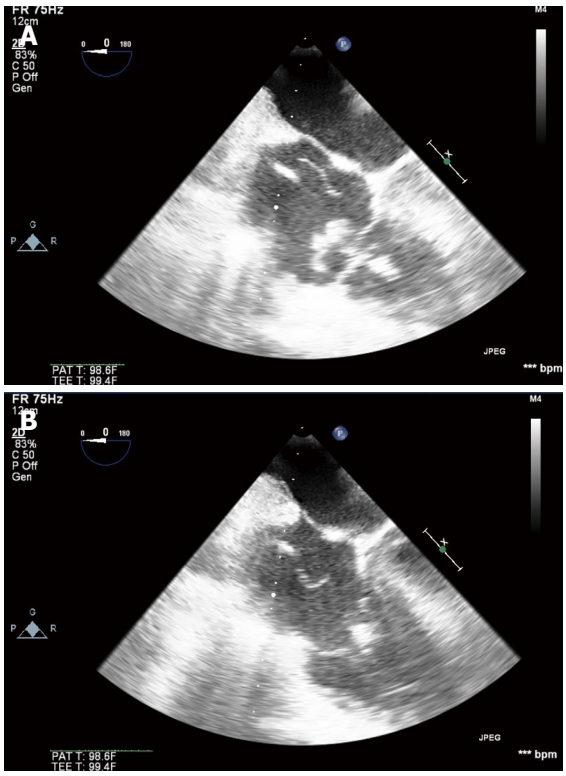


Figure 1 Transesophageal echocardiography images. A: Large thrombus in right atrium in region of tricuspid valve and a second mass in right ventricle; B: empty right atrium with small mass on tricuspid valve; right ventricle is clear.

encephalopathy, variceal bleeding and malnutrition. She was on continuous venovenous hemofiltration for renal dysfunction. Her MELD was 34. A contrast-enhanced CT revealed a cirrhotic liver with thrombus in the portal vein extending into the superior mesenteric and splenic veins. The patient did not have any known hypercoagulable disorder and the thrombus in the portal vein was attributed to her liver disease. She was treated in the ICU for 15 d when an organ became available. The orthotopic deceased donor liver transplantation was performed with piggyback technique. Extensive eversion thromboendovenectomy resulted in an excellent portal vein flow. The liver graft was recirculated after the hepatic and portal vein anastomoses were completed. The patient tolerated recirculation well without any hemodynamic instability. While the hepatic artery was dissected and mobilized for the arterial anastomosis, the patient became acutely hypotensive progressing to asystole. She was resuscitated with external cardiac massage and inotropic support. A transesophageal echocardiography (TEE) probe was emergently inserted and showed mobile right atrial thrombus and an underfilled, poorly contractile right ventricle (Figure 1A). At the time of resuscitation, INR was 6.4, fibrinogen < 25 mg/dL and platelets 13 000/μL (Table 1). No antifibrinolytic therapy was administered. Continued monitoring by TEE showed the clot adherent to the tricuspid valve (Figure 1B) and later was not seen on four chamber view indicating either spontaneous thrombolysis or propagation into the pulmonary vasculature. The right ventricular function and filling improved and the patient became hemodynamically more stable, allowing successful completion of the liver transplantation. The patient was transferred to the ICU for postoperative care on dopamine drip (10 mcg/kg per minute). The patient was weaned off pressors on POD zero and was successfully extubated on POD two. An echocardiogram performed on POD two demonstrated normal right and left ventricular size and function with ejection fraction of 57%. There was no evidence of intracardiac thrombus. The postoperative ultrasound of the liver graft revealed widely patent hepatic veins, hepatic artery and portal vein with normal flows. Ultrasound of the extremities was negative for deep vein thrombosis. The patient currently has normal liver function and continues to make good progress.

DISCUSSION

Intracardiac thrombosis (ICT) during liver transplantation is a devastating complication with poor outcomes. Warnaar *et al*[1] reported a mortality rate of 68% with a majority of patients dying in the operating room. This complication is extremely rare (1%-1.5%) and unfortunately the exact etiology is not known. It has been associated with intraoperative administration of antifibrinolytics including ε-aminocaproic acid, aprotinin and clotting factors used to decrease intraoperative bleeding and transfusion requirement[2]. Other potential risk factors described are the presence of venovenous bypass, pulmonary artery catheter, continuous venovenous hemofiltration, migration of preexisting thrombi, sepsis and disseminated intravascular coagulation. Empiric administration of antifibrinolytics and clotting factors should be strongly discouraged since the risk of ICT formation and pulmonary embolus at times outweighs the risk of intraoperative bleeding.

Systemic hypotension, increase in central venous pressure, pulmonary artery pressure, decrease in cardiac output and cardiac arrest are frequently encountered signs[2]. The definitive diagnosis is made on transesophageal echocardiography.

There is no consensus for treatment since the condition is so rare. Administration of tissue plasminogen activator into the right atrium[2,3] and heparin boluses[4] have been described although they are associated with potentially massive hemorrhage. Suction embolectomy is another treatment option.

The source of the ICT in our patient is uncertain.

It is unclear whether this clot was preformed or newly developed. After recirculation, our patient became profoundly coagulopathic. We believe that the fibrinolysis that occurred after reperfusion of the liver graft led to the lysis of the thrombus and subsequently to a successful outcome.

REFERENCES

1 **Warnaar N**, Molenaar IQ, Colquhoun SD, Slooff MJ, Sherwani S, de Wolf AM, Porte RJ. Intraoperative pulmonary embolism and intracardiac thrombosis complicating liver transplantation: a systematic review. *J Thromb Haemost* 2008; **6**: 297-302

2 **Jackson D**, Botea A, Gubenko Y, Delphin E, Bennett H. Successful intraoperative use of recombinant tissue plasminogen activator during liver transplantation complicated by massive intracardiac/pulmonary thrombosis. *Anesth Analg* 2006; **102**: 724-728

3 **Planinsic RM**, Hilmi IA, Sakai T. Prevention of intracardiac/pulmonary thrombosis during liver transplantation. *Anesth Analg* 2006; **103**: 1329

4 **Planinsic RM**, Nicolau-Raducu R, Eghtesad B, Marcos A. Diagnosis and treatment of intracardiac thrombosis during orthotopic liver transplantation. *Anesth Analg* 2004; **99**: 353-356, table of contents

S- Editor Zhang HN **L- Editor** Roemmele A **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i5.201

World J Hepatol 2010 May 27; 2(5): 201-202
ISSN 1948-5182 (online)

Huge extrahepatic portal vein aneurysm as a late complication of liver transplantation

Fabrizio di Francesco, Salvatore Gruttadauria, Settimo Caruso, Bruno Gridelli

Fabrizio di Francesco, Salvatore Gruttadauria, Settimo Caruso, Bruno Gridelli, Mediterranean Institute for Transplant and Advanced Specialized Therapies, University of Pittsburgh Medical Center, Palermo 90127, Italy
Salvatore Gruttadauria, Settimo Caruso, Bruno Gridelli, Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA 8084 3600, United States
Author contributions: Gruttadauria S and Gridelli B contributed equally to this work and designed the paper; di Francesco F and Gruttadauria S contributed equally to write the paper; Caruso S analyzed and collected data.
Correspondence to: Salvatore Gruttadauria, MD, Associate Professor of Surgery, Department of Surgery, University of Pittsburgh, Coordinator Abdominal Adult Transplant; Mediterranean Institute for Transplant and Advanced Specialized Therapies, University of Pittsburgh Medical Center in Italy (ISMETT), Via E Tricomi N 1, Palermo 90127, Italy. sgruttadauria@ismett.edu
Telephone: +39-0912192111 Fax: +39-0912192400
Received: December 16, 2009 Revised: March 30, 2010
Accepted: April 6, 2010
Published online: May 27, 2010

Abstract

A 60-year-old male underwent orthotopic liver transplantation because of hepatitis C virus related cirrhosis. After 12 d, the patient underwent re-transplantation due to primary graft non function. One year later the patient developed a thrombosis of the main portal vein needing a surgical revision. After 11 years the patient was operated on because of a clinical picture of intestinal occlusion. As an incidental finding, a large aneurysm of the main portal vein was diagnosed. The incidence of intra- and extrahepatic Portal vein aneurysms (PVAs) is not clear. To the best of our knowledge, only one case of intrahepatic PVA in a liver transplant has been reported in the literature. In addition, we have found no documented cases of extrahepatic PVAs in liver transplanted patients.

Key words: Portal vein aneurysm; Late complication; Liver transplantation; Portal hypertension; Doppler ultrasound

Peer reviewers: Lars Müller, MD, Consultant, Department of General and Thoracic Surgery, University Hospital of Schleswig-Holstein, Campus Kiel, Arnold-Heller-Str. 3, Kiel 24105, Germany; Boon Hun Yong, Associate Professor, Department of Anaesthesiology, University of Hong Kong, Hong Kong, China

di Francesco F, Gruttadauria S, Caruso S, Gridelli B. Huge extrahepatic portal vein aneurysm as a late complication of liver transplantation. *World J Hepatol* 2010; 2(5): 201-202 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i5/201.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i5.201

TO THE EDITOR

A 60-year-old male underwent orthotopic liver transplantation in Cambridge, England in 1993, because of hepatitis C virus related cirrhosis. After 12 d, the patient underwent re-transplantation due to primary graft non function. In 1994, a thrombosis of the main portal vein (MPV) was detected. As a result, the patient underwent surgical reconstruction (there were no medical records or details of the previous surgery in the patient's possession). From 1994 to 2003, the patient showed no liver function alterations. However, liver ultrasound (US) performed during follow-up showed a slow dimensional increase of the MPV (16 mm in December 1994, 18 mm in 1996, 25 mm in 2002, and 30 mm in 2003).

The patient did not undergo any other radiological examinations at any time. In June 2003, the patient was admitted to another hospital for acute intestinal occlusion, with abdominal pain and vomiting. The patient underwent explorative laparotomy with adhesiolisis. At that time no aneurysm of the portal vein was diagnosed or detected.

In July 2003, the patient was admitted to our center with a clinical picture of intestinal occlusion. 64

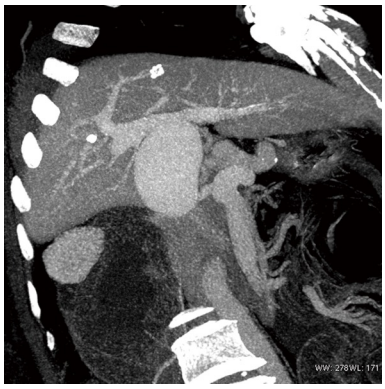


Figure 1 Multi intensity projection shows a large aneurysm (maximum diameter 6.3 cm) of the MPV that arose 1 cm after the spleno-mesenteric confluence.

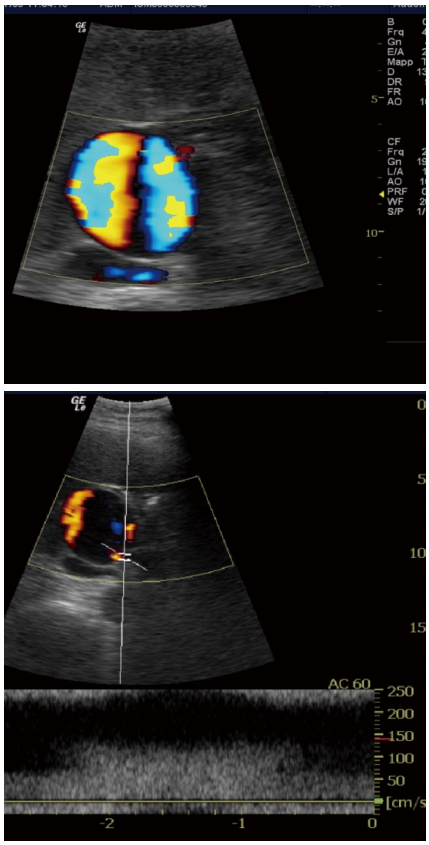


Figure 2 Color-Doppler US showsa turbulent flow inside the aneurysm (red and blue color). An increase velocity of flow isdetected at the origin of the aneurysm.

Multi Detector CT scan (Lightspeed VCT, GE) performed with and without contrast media showed an overdistension of the small bowel and right colon in the presence of a severe neoplastic lesion occluding the transverse colon. A colonscopy confirmed the lesion, which was also biopsy proven. As an incidental finding, a large aneurysm of the MPV, 6.5 cm in diameter and arising 1 cm after the spleno-mesenteric confluence, was found (Figure 1). The patient underwent surgery for colon resection; the presence of tenacious adherences made it impossible to check the portal vein situation. A mild amount of ascites and splenomegaly was detected (longitudinal diameter of 16.3 cm) with no other radiological signs of portal hypertension. At that time liver function tests were normal. Doppler US showed increased velocity near the origin of the aneurysm, and turbulent flow inside. Monophasic flow with normal velocity was detected in the intrahepatic portal branches (Figure 2).

Extrahepatic portal vein aneurysms, defined as MPV greater than 1.9 cm, are rare in non-transplanted patients and in cirrhotic patients, and only 67 cases have been documented, in the English literature. Portal vein aneurysms (PVAs) can be congenital or acquired, and even if the etiology remains unclear, there are some related conditions, such as portal hypertension, pancreatitis or trauma.

PVAs are usually asymptomatic[1-3] if small, while large PVAs can cause biliary tract obstruction, chronic portal hypertension, portal vein thrombosis, acute portal hypertension, abdominal discomfort and, though rarely, adjacent structure compression and rupture.

Our patient showed no symptoms or complications related to the aneurysm that justified surgical treatment. The patient was followed with Doppler US every three months.

The incidence of intra- and extrahepatic PVAs is not clear. To the best of our knowledge, only one case of intrahepatic PVA in a liver transplant has been reported in the literature[4]. In addition, we have found no documented cases of extrahepatic PVAs in liver transplanted patients. We believe that in our case, one or more of the three surgical interventions on the portal vein, created a weak point in the portal vein which was responsible, over a period of years, for the development of the aneurysm. In addition, at the time of the colon resection surgery, the intestinal occlusion could have increased the intra-abdominal pressure and consequently the portal pressure, leading to the enlargement of the portal aneurysm.

REFERENCES

1 **Gallego C**, Velasco M, Marcuello P, Tejedor D, De Campo L, Friera A. Congenital and acquired anomalies of the portal venous system. *Radiographics* 2002; **22**: 141-159

2 **Koc Z**, Oguzkurt L, Ulusan S. Portal venous system aneurysms: imaging, clinical findings, and a possible new etiologic factor. *AJR* 2007; **189**: 1023-1030

3 **Miyazaki K**, Takatsuki M, Eguchi S, Hidaka M, Tokai H, Hamasaki K, Tajima Y, Kanematsu T. Living donor liver transplantation for hepatitis C virus cirrhosis with a huge portal vein aneurysm. *Liver Transpl* 2008; **14**: 1221-1222

4 **Ferraz-Neto BH**, Sakabe D, Buttros DA, Resende MB, Afonso RC. Portal vein aneurysm as late complication of liver transplantation: a case report. *Transplant Proc* 2004; **36**: 970-971

S- Editor Zhang HN **L- Editor** Hughes D **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
www.wjgnet.com

World J Hepatol 2010 May 27; 2(5): I
ISSN 1948-5182 (online)

Acknowledgments to reviewers of *World Journal of Hepatology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Hepatology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those published in this issue and those rejected for this issue) during the last editing time period.

Joseph Ahn, MD, Assistant Professor of Medicine**,** Medical Director, Liver Transplantation, Loyola University Medical Center, 2160 S. First Ave., Building 54, Room 007, Maywood, IL 60153, United States

Ana Carolina Ferreira Netto Cardoso, MD, Hopital Beaujon, 100, bd du Gal-Leclerc, Clichy 92110, France

Fei Chen, PhD, Professor, Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, United States

Ruben Ciria, PhD, Uniersity Hospital Reina Sofia, Departmento De Cirugia Hepatobiliar Y Trasplante HEPÁTICO (Department of Hepatobiliary Surgery and Liver Transplantation), Avennida Menendez PidalI s/n, Cordoba 14004, Spain

Stephen Lam Chan, MD, Department of Clinical Oncology, The Chinese University of Hong Kong, Prince of Wales Hospital, 30-32 Ngan Street, Shatin, New Territories, Hong Kong, China

Wan Yee Joseph Lau, MD, Professor, Clinical Sciences Building, Prince of Wales Hospital, Shatin, New Territories, Hong Kong, China

Lars Müller, MD, Consultant, Department of General and Thoracic Surgery, University Hospital of Schleswig-Holstein, Campus Kiel, Arnold-Heller-Str. 3, Kiel 24105, Germany

Valentina Medici, MD, PhD, Department of Internal Medicine, University of California Davis, 4150 V Street, Suite 3500, Sacramento, CA 95817, United States

Zenichi Morise, MD, PhD, Department of Surgery, Fujita Health University School of Medicine, 1-98 Dengakugakubo Kutsukakecho, Toyoake, AICHI 470-1192, Japan

Melissa Kay Osborn, MD, Emory University Hospital Midtown, 550 Peachtree Street, 7th Floor Medical Office Tower, Atlanta, GA 30308, United States

Juan Carlos Perazzo, MD, PhD, Professor, Department of Biological Sciences, Pathophysiology School of Pharmacy and Biochemistry, UBA, Junin 950, 5, Buenos Aires 1113, Argentina

Ali Sazci, MSc, PhD, Professor, Kocaeli University, Faculty of Medicine, Department of Medical Biology and Genetics, Umuttepe, Kocaeli 41380, Turkey

Xun-Di Xu, MD, PhD, Department of Gastroenterological Surgery, Xiangya 2nd Hospital, Central South University, Renmin Zhong Road 139, Changsha 410011, Hunan Province, China

Boon Hun Yong, Associate Professor, Department of Anaesthesiology, University of Hong Kong, Hong Kong, China

Hiroshi Yoshida, MD, PhD, Department of Surgery, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo, 113-8603, Japan

Lang Zhuo, PhD, Team Leader and Principal Research Scientist, Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos #04-16, 138669, Singapore

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
www.wjgnet.com

World J Hepatol 2010 May 27; 2(5): I
ISSN 1948-5182 (online)

Meetings

Events Calendar 2010

January 25-26
Tamilnadu, India
International Conference on Medical Negligence and Litigation in Medical Practice

January 25-29
Waikoloa, HI, United States
Selected Topics in Internal Medicine

January 26-27
Dubai, United Arab Emirates
2nd Middle East Gastroenterology Conference

March 04-06
Bethesda, MD, United States
8th International Symposium on Targeted Anticancer Therapies

March 05-07
Peshawar, Pakistan
26th Pakistan Society of Gastroenterology & Endoscopy Meeting

March 12-14
Bhubaneswar, India
18th Annual Meeting of Indian National Association for Study of the Liver

March 25-28
Beijing, China
The 20th Conference of the Asian Pacific Association for the Study of the Liver

March 27-28
San Diego, California, United States
25th Annual New Treatments in Chronic Liver Disease

April 07-09
Dubai, United Arab Emirates
The 6th Emirates Gastroenterology and Hepatology Conference, EGHC 2010

April 14-18
Vienna, Austria
The International Liver Congress™ 2010

May 01-05
New Orleans, LA, United States
Digestive Disease Week Annual Meeting

May 06-08
Munich, Germany
The Power of Programming: International Conference on Developmental Origins of Health and Disease

June 04-06
Chicago, IL, United States
American Society of Clinical Oncologists Annual Meeting

June 16-19
Hong Kong, China
ILTS: International Liver Transplantation Society ILTS Annual International Congress

September 10-12
Montreal, Canada
International Liver Association's Fourth Annual Conference

September 12-15
Boston, MA, United States
ICAAC: Interscience Conference on Antimicrobial Agents and Chemotherapy Annual Meeting

September 16-18
Prague, Czech Republic
Prague Hepatology Meeting 2010

September 23-26
Prague, Czech Republic
The 1st World Congress on Controversies in Gastroenterology & Liver Diseases

October 15-20
San Antonio, TX, United States
ACG 2010: American College of Gastroenterology Annual Scienitfic Meeting

October 23-27
Barcelona, Spain
18th United European Gastroenterology Week

October 29-November 02
Boston, Massachusetts, United States
The Liver Meeting® 2010--AASLD's 61st Annual Meeting

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
www.wjgnet.com

World J Hepatol 2010 May 27; 2(5): I-V
ISSN 1948-5182 (online)

Instructions to authors

GENERAL INFORMATION

World Journal of Hepatology (*World J Hepatol, WJH*, online ISSN 1948-5182, DOI: 10.4254), is a monthly, openaccess, peer-reviewed journal supported by an editorial board of 572 experts in hepatology from 45 countries..

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results.

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJH* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJH* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJH* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

The major task of *WJH* is to report rapidly the most recent results in basic and clinical research on hepatology, including: liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology.

The columns in the issues of *WJH* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Article: To report innovative and original findings in hepatology; (9) Brief Article: To briefly report the novel and innovative findings in hepatology; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJH*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of hepatology; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on basic research and clinical practice in hepatology.

CSSN
ISSN 1948-5182 (online)

Published by
Beijing Baishideng BioMed Scientific Co., Ltd.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and

Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Beijing Baishideng BioMed Scientific Co., Ltd, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the International Committee of Medical Journal Editors to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www. clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/1948-5182 office. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjh@wjgnet.com, or by telephone: +86-10-59080038. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by International Committee of Medical Journal Editors, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g., Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g., Telephone: +86-10-59080039 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJH*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original

contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles, rapid communication and case reports, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1948-5182/g_info_20100107113351.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A:...; B:...; C:...; D:...; E:...; F:...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World*

J Gastroenterol 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1948-5182/g_info_20100107115140.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.

Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*

Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*

Biology: *H. pylori*, *E coli*, *etc.*

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJH*. The revised version including manuscript and high-resolution image figures (if any) should be copied on a floppy or compact disk. The author should send the revised manuscript, along with printed high-resolution color or black and white

photos, copyright transfer letter, and responses to the reviewers by courier (such as EMS/DHL).

Editorial Office

World Journal of Hepatology

Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080038
Fax: +86-10-85381893
E-mail: wjh@wjgnet.com
http://www.wjgnet.com

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/1948-5182/g_info_20100107114726.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1948-5182/g_info_20100107114601.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJH will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

Authors of accepted articles must pay a publication fee.
EDITORIAL, TOPIC HIGHLIGHTS, BOOK REVIEWS and LETTERS TO THE EDITOR are published free of charge.