ISSN 2220-315X (online)

World Journal of *Experimental Medicine*

World J Exp Med 2012 August 20; 2(4): 65-85

Baishideng Publishing Group
www.wjgnet.com

WJEM World Journal of Experimental Medicine

A peer-reviewed, online, open-access journal of experimental medicine

Editorial Board

2011-2015

The *World Journal of Experimental Medicine* Editorial Board consists of 104 members, representing a team of worldwide experts in experimental medicine. They are from 30 countries, including Argentina (3), Australia (4), Belgium (2), Brazil (1), Canada (2), China (11), Czech Republic (1), Denmark (2), France (4), Germany (3), Greece (3), India (4), Ireland (1), Israel (1), Italy (7), Japan (6), Lebanon (1), Malaysia (2), Mexico (1), Norway (1), Saudi Arabia (1), Singapore (1), Slovenia (1), South Korea (5), Spain (3), Sweden (3), Switzerland (1), Turkey (2), United Kingdom (2), and United States (25).

EDITOR-IN-CHIEF
De-Ling Kong, *Tianjin*
Atsushi Mizoguchi, *Boston*
Baohong Zhang, *Greenville*

GUEST EDITORIAL BOARD MEMBERS
Nan-Shan Chang, *Tainan*
Kow-Tong Chen, *Tainan*
Chih-Ping Hsu, *Hsin-Chu*
Hung-Jen Liu, *Taichung*

MEMBERS OF THE EDITORIAL BOARD

Argentina
Beatriz Basso, *Córdoba*
Cristina Ester Carnovale, *Rosario*
Angel Catalá, *La Plata*

Australia
Filip Braet, *Sydney*
Xianlan Cui, *Launceston*
Xiao-Jun Du, *Melbourne*
HS Nagaraja, *Queensland*

Belgium
Olivier Bruyere, *Liege*
Nathalie Cools, *Edegem*

Brazil
Niels Olsen Saraiva Câmara, *Sao Paulo*

Canada
Alfonso Iorio, *Hamilton*
Xiaoyan Jiang, *Vancouver*

China
Long Chen, *Nanjing*
Heng-Mi Cui, *Nanjing*
Volodymyr Dvornyk, *Hong Kong*
Jian-Xin Gao, *Shanghai*
Chun-Yan Ji, *Jinan*
Yang-Fu Jiang, *Chengdu*

Czech Republic
Jan Bernardy, *Brno*

Denmark
Shan Gao, *Aarhus*
Per Hildebrandt, *Frederiksberg*

France
Nadia Alfaidy, *Grenoble*
Abdel Aouacheria, *Lyon*
Jean-Marc Cavaillon, *Paris*
Jean-Marc Egly, *Illkirch*

Germany
Sorin Armeanu-Ebinger, *Tübingen*
Magali Cucchiarini, *Homburg*
Mohamed Hassan, *Duesseldorf*

Greece
Effie K Basdra, *Athens*
Maria Dalamaga, *Athens*
Moses Elisaf, *Ioannina*

India
Malay Chatterjee, *Kolkata*
Vijay Chauthaiwale, *Ahmedabad*
Bibhu Ranjan Das, *Mumbai*
Satya N Das, *New Delhi*

Ireland
Steven G Gray, *Dublin*

Israel
Elena Feinstein, *Ness Ziona*

Italy
Alessandro Busca, *Turin*
Giovanni Di Salvo, *Naples*
Francesco Dieli, *Palermo*
Amalia Forte, *Naples*
Umberto Galderisi, *Naples*
Gabriele Grassi, *Trieste*
Fabio Grizzi, *Rozzano*

Japan
Winn Aung, *Chiba*

Hiroshi Fukazawa, *Mito*
Hideaki Hara, *Gifu*
Toshio Hattori, *Sendai*
Atsushi Hosui, *Suita*
Peng Huang, *Okayama*

Lebanon

Hala Gali-Muhtasib, *Beirut*

Malaysia

Gam Lay Harn, *Penang*
Kamsiah Jaarin, *Kuala Lumpur*

Mexico

Javier Camacho, *Mexico*

Norway

Brynjar Foss, *Stavanger*

Saudi Arabia

Mostafa M El-Naggar, *Jazan*

Singapore

Ivy Ho, *Singapore*

Slovenia

Damjan Glavac, *Ljubljana*

South Korea

Dalwoong Choi, *Seoul*
Kang-Yell Choi, *Seoul*
Joohun Ha, *Seoul*
Eui-Bae Jeung, *Cheongju*
Chang-Duk Jun, *Gwangju*

Spain

Isabel Andia, *Bilbao*
Javier Arias-Diaz, *Madrid*
Vicente Felipo, *Valencia*

Sweden

Karl O Fagerstrom, *Kagerod*
Robert Hahn, *Tullinge*
Susanne Jacobsson, *Örebro*

Switzerland

Florian Bihl, *Bellinzona*

Turkey

Ali Kudret Adiloglu, *Ankara*

Mutay Aslan, *Antalya*

United Kingdom

Dominique Bonnet, *London*
David Gilham, *Manchester*

United States

Anshu Agrawal, *Irvine*
Mikhail Alexeyev, *Mobile*
Robert J Amato, *Houston*
Raymond T Bartus, *San Diego*
Ajay Singh Behl, *Minneapolis*
Fabian Benencia, *Athens*
Arun Bhunia, *West Lafayette*
Ramireddy Bommireddy, *Tucson*
Michael Borchers, *Cincinnati*
Alexander Bukreyev, *Galveston*
Lu Cai, *Louisville*
Arvind Chhabra, *Farmington*
Yingzi Cong, *Galveston*
Akram Da'darah, *North Grafton*
Liutao Du, *Los Angeles*
Nejat Düzgüneş, *San Francisco*
Charles E Egwuagu, *Bethesda*
Lianchun Fan, *Indianapolis*
Bingliang Fang, *Houston*
Seshu K Gudlavalleti, *Omaha*
Diane M Harper, *Leawood*
Mohamed I Husseiny, *Los Angeles*
Miroslaw Janowski, *Baltimore*

WJEM World Journal of Experimental Medicine

Contents

Bimonthly Volume 2 Number 4 August 20, 2012

World Journal of Experimental Medicine
Volume 2 Number 4 August 20, 2012

Contents

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Yuan Zhou*
Responsible Electronic Editor: *Xiao-Mei Zheng*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Jin-Lei Wang*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Experimental Medicine

ISSN
ISSN 2220-315X (online)

LAUNCH DATE
December 20, 2011

FREQUENCY
Bimonthly

EDITING
Editorial Board of *World Journal of Experimental Medicine*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: wjem@wjgnet.com
http://www.wjgnet.com

EDITOR-IN-CHIEF
De-Ling Kong, PhD, Professor, Institute of Molecular Biology, Nankai University, Tianjin 300071, China

Atsushi Mizoguchi, MD, PhD, Associate Professor in Pathology, Harvard Medical School, Molecular Pathology Unit, Massachusetts General Hospital, CNY149-6024, 13th Steert, Charlestown, MA 02114, United States

Baohong Zhang, PhD, Assistant Professor of Biology, Department of Biology, East Carolina University, Greenville, NC 27858, United States

EDITORIAL OFFICE
Jian-Xia Cheng, Director
Jin-Lei Wang, Vice Director
World Journal of Experimental Medicine
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: wjem@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpg@baishideng.com
http://www.wjgnet.com

PUBLICATION DATE
August 20, 2012

COPYRIGHT
© 2012 Baishideng. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

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/2220-315x/g_info_20100722180909.htm.

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

World Journal of Experimental Medicine

Online Submissions: http://www.wjgnet.com/esps/
wjem@wjgnet.com
doi:10.5493/wjem.v2.i4.65

World J Exp Med 2012 August 20; 2(4): 65-69
ISSN 2220-315X (online)
© 2012 Baishideng. All rights reserved.

EDITORIAL

Role of human papillomavirus in the pathogenesis of oral squamous cell carcinoma

Anastasios K Markopoulos

Anastasios K Markopoulos, Department of Oral Medicine/Pathology, Aristotle University, School of Dentistry, University Campus, 54124 Thessaloniki, Greece
Author contributions: Markopoulos AK solely contributed to this paper.
Correspondence to: Anastasios K Markopoulos, DDS, MS, PhD, Professor, Department of Oral Medicine/Pathology, Aristotle University, School of Dentistry, University Campus, 54124 Thessaloniki, Greece. anmark@dent.auth.gr
Telephone: +30-2310-999523 Fax: +30-2310-999532
Received: November 11, 2011 Revised: June 6, 2012
Accepted: July 27, 2012
Published online: August 20, 2012

Abstract

Oral cancer is one of the most common cancers and it constitutes a major health problem particularly in developing countries. Oral squamous cell carcinoma (OSCC) represents the most frequent of all oral neoplasms. Several risk factors have been well characterized to be associated with OSCC with substantial evidences. While tobacco and alcohol are the primary risk factors for OSCC development, many epidemiological studies report a strong association with human papillomavirus (HPV) in a subset of OSCC. This article presents our current knowledge on the relationship between HPV and development of OSCC. HPVs are DNA viruses that specifically target the basal cells of the epithelial mucosa. Most experimental data are consistent with the hypothesis that HPV plays a causal role in oral carcinogenesis. Genotypes, such as HPV1 infect epidermal cells, whereas HPV6, 11, 16 and 18 infect epithelial cells of the oral cavity and other mucosal surfaces. Several studies have shown that there is an increased risk of head and neck cancer in the two major HPV 16 oncogenes E6 and E7 -positive patients. The presence of antibodies to HPV E6 and E7 proteins was found to be more associated with tumors of the oro-pharynx than of the oral cavity. However, HPV alone appears to be insufficient as the cause of OSCC but requires other co-factors. Although a viral association within a subset of OSCC has been shown, the molecular and histopathological characteristics of these tumors have yet to be clearly defined.

© 2012 Baishideng. All rights reserved.

Key words: Human papillomavirus; Oral squamous cell carcinoma

Peer reviewer: Javier Camacho, PhD, Centro de Investigación y de Estudios Avanzados del IPN, Department of Pharmacology, Avenida Instituto Politécnico Nacional 2508, C.P. 07360 Mexico City, Mexico

Markopoulos AK. Role of human papillomavirus in the pathogenesis of oral squamous cell carcinoma. *World J Exp Med* 2012; 2(4): 65-69 Available from: URL: http://www.wjgnet.com/2220-315X/full/v2/i4/65.htm DOI: http://dx.doi.org/10.5493/wjem.v2.i4.65

INTRODUCTION

Worldwide, oral cancer accounts for 2%-4% of all cancer cases. In some regions, such as Pakistan, oral cancer reaches the 10% of all cancers, and around 45% in India[1,2]. Within the EU the highest oral cancer incidence rates for males are found in France and Hungary (6%) and the lowest rates in Greece and Cyprus[3,4]. In 2009 over 300 000 new cases of oral and oropharyngeal cancer were diagnosed worldwide. During the same time period, over 7000 affected individuals died of these cancers[5]. Oral cancer includes a group of neoplasms affecting any region of the oral cavity, pharyngeal regions and salivary glands. However, this term is almost synonymous with oral squamous cell carcinoma (OSCC), which represents the most frequent of all oral neoplasms. It is estimated that more of 90% of all oral neoplasms are OSCC[6]. It is well established that a strong association exists between

genetic factors, environment and oral cancer. Several factors are involved in the pathogenesis of oral cancer, such as age, gender, ethnicity, lifestyle, genetic background, status of health and exposure to one or more oncogenic factors[7]. Tobacco use and alcohol consumption are well-known risk factors. Micronutrient deficiencies and poor oral hygiene[8-10] have also been associated with increased risk. However, the observation that several patients with oral cancer have not been exposed to these risk factors suggests that additional causes may promote oral carcinogenesis. Thus, other agents, such as viruses, are being investigated.

HUMAN PAPILLOMAVIRUS AND OSCC

The human papillomavirus (HPV) family consists of more than 100 genotypes, classified in accordance with the ability to infect and transform epithelial cells. HPVs are DNA viruses that specifically target the basal cells of the epithelial mucosa[11].

Genotypes, such as HPV1 infect epidermal cells, whereas HPV6, 11, 16 and 18 infect epithelial cells of the oral cavity and other mucosal surfaces. The ability of HPV to transform epithelial cells is divided into high-risk and low-risk types. Low-risk types are associated with development of benign lesions such as warts, while infections with high-risk types may progress to malignant lesions[12].

The HPV involvement in oral carcinogenesis was supported on the basis of the following evidences: (1) the strongly established etiological role of HPV in cervical SCC[13,14]; (2) the epithelial tropism of HPV; (3) the similarity between oral and genital epithelia[15]; and (4) the detection of HPV genotypes in samples of OSCC[16-20].

The HPV involvement in oral and oropharyngeal carcinogenesis was first proposed in 1983 by Syrjänen *et al*[16]. Her results showed that 40% of the laryngeal and oral cancers contained histological and morphological similarities with HPV-infected lesions, and 50% of the samples demonstrated HPV structural proteins by immunohistochemistry[21].

Since then, several studies have focused on HPV detection in oral cancer but results have been conflicting[22-24]. It was found that the prevalence of HPV detection varies broadly, depending on the population, on the location of the cancerous lesion, type of specimen, and detection method. By contrast, HPV was more frequently detected in OSCCs of the oropharynx and tonsil than at other head and neck sites[25-27]. However, in a systematic review that was performed by Syrjänen *et al*[28] it was suggested that a potentially important causal association between HPV (specifically HPV16) and OSCC exists. In a recent study the overall HPV prevalence was 10.5% in oral cavity carcinomas and was higher in female than in male cases. Ninety five percent of HPV-positive cases were infected by a single HPV type. HPV 16 was the most prevalent type and was found in 95.5% of HPV-positive oral cavity carcinoma cases[29].

Furthermore, other studies have proved the existence of a synergistic effect between HPV and alcohol. The risk of head and neck cancer was statistically significantly increased in heavy alcohol users detected with the virus, compared to that of HPV-negative cancer drinkers. Therefore, it has been proposed that alcohol can biologically modify mucosal tissue, increasing its permeability to viral infection, or by influencing the immune response to HPV[30].

It is believed that one of the major events of HPV-induced carcinogenesis is the integration of the HPV genome into a host chromosome. HPV genome integration often occurs near fragile sites of the human genome, but there are no apparent sites for integration and no evidence for insertional mutagenesis[31].

Slightly modifying Koch's postulates, in order to establish a relationship between a causative virus and a disease, four criteria are needed: (1) viral genome to be present in tumor lesions or in tumor cells; (2) virus must be isolated from a pathologic lesion and grown in culture; (3) cultured virus should cause disease when inoculated into a healthy organism; and (4) virus must be reisolated from the inoculated host and identified as being identical to the original specific causative factor. However, the use of Koch's postulates to establish disease causation does not fully apply to these phenomena, since the etiology of cancer is multifactorial[32]. In the contrary, other authors suggest that the incidence of HPV infection in the oral cavities of healthy population is very low and therefore other risk factors are most likely responsible to promote oral carcinogenesis[33].

GENITAL HPV INFECTION AND RISK OF OSCC

Several studies examined the incidence of second cancers after an initial diagnosis of ano-genital cancers[34,35] and have showed that there is an increased risk of head and neck cancer as well as other HPV-associated ano-genital cancers in the two major HPV 16 oncogenes E6 and E7 -positive patients. This association between HPV-associated anogenital cancers and head and neck cancer was further strengthened by two larger studies[36,37].

Additionally, the presence of antibodies to HPV E6 and E7 proteins was found to be more associated with tumors of the oro-pharynx than of the oral cavity[12].

A recent study has shown that besides the classical horizontal transmission during the sexual life, a vertical transmission occurs in approximately 20% of case HPV positive people. In these individuals, HPV-DNA is detected in amniotic fluid, foetal membranes, blood and placental trophoblastic cells, all suggesting HPV infection in utero, i.e. prenatal transmission[38].

HPV TESTING

HPV testing is critical for the estimation of HPV prevalence in various oral diseases. HPV testing is usually based

on PCR method. General or consensus primers targeting L1 gene are most frequently used for HPV detection because they are able to identify several HPV genotypes at the same time. Sampling techniques together with widely divergent PCR methods in different studies explain most of the variability in HPV prevalence among OSCC and control samples[29]. *In situ* hybridization and *in situ* oncogenic protein staining techniques have also increased sensitivity and specificity and are used for HPV testing. These techniques have allowed not only the detection of HPV in cytological smears or histopathological immunesections but also the determination of the topographic site of the infection[39]. According to recent studies, HPV-positive squamous cell carcinomas have intact *p16* gene and wild type *p53* compared to HPV negative ones[40]. Other authors have noted that a distinctive mark of the presence of HPV in oral cancer could be found in *p16* nuclear or cytoplasmic overexpression[41,42]. However, one goal of the scientific research is to find new biological markers able to identify the set(s) of genes involved in oral carcinogenesis.

HPV SEROLOGY

The immune response to HPV infection involves both the cell-mediated and humoral responses. However, serological evidence is circumstantial since it provides only data on prior exposure to HPV. Since not all patients with HPV-associated cancers have detectable HPV antibodies, serum antibody determination may be a limited biomarker for HPV infection and carcinogenesis. Serum antibodies to HPV capsid proteins (virus-like particles) are thought to be a marker of lifetime HPV infection[43,44]. Antibodies against HPV E6 and E7 proteins are associated with increased risk of HPV-associated cancer[45,46] but are rather linked more with tumors from the oro-pharynx than from the oral cavity[12].

The use of HPV viral load in oral biopsies in conjunction with serological markers may serve to identify a subset of HPV-associated oral cancers in which HPV is biologically active.

PROGNOSIS AND FAVORABLE OUTCOME

Several lines of evidence suggest that HPV-positive and HPV-negative HNSCC represent distinct subgroups with different biological, epidemiological and prognostic profiles[7,47]. Recent data suggest that a positive HPV status represents an important prognostic factor and is associated with a favorable outcome in head and neck cancer. Many studies confirmed that HPV-positive OSCC have a better prognosis compared with those that are HPV negative[48-51]. There is an approximate 30% absolute survival difference at 5 years (HPV-positive = 60% *vs* HPV-negative = 30%)[52]. The favorable outcome for patients harboring HPV-positive cancer cannot be easily explained. It has been proposed that HPV-positive cancer arises through a different mechanism or exhibits less genetic instability i.e., shows a lower degree of aneuploidy and a tendency to have fewer chromosomal aberrations, when compared to HPV-negative cancer[53]. In contrast, there appears to be a subgroup of HPV-positive patients whose clinical prognosis is worse than the typical HPV-positive patient. This subgroup has higher smoking rates, higher rates of *p53* mutations and higher expression of EGFR and Bcl-xL[27].

EXPERIMENTAL EVIDENCE

Experimental evidence regarding the role of HPV in oral carcinogenesis is limited both *in vitro* and in animal experimentation. The lack of suitable experimental animal models has hindered research into HPV cancers for many years. In one of the most significant studies it has been shown that oral keratinocytes could not be transformed by HPV alone but required further mutations in other oncogenes[54].

CONCLUSION

The vast amounts of epidemiological, molecular pathological and *in vitro* experimental data are consistent with the hypothesis that HPV does indeed have a causal role in oral carcinogenesis. However, HPV alone appears to be insufficient as the cause of OSCC but requires other co-factors. Although a viral association within a subset of OSCC has been shown, the molecular and histopathological characteristics of these tumors have yet to be clearly defined. Since HPV16 is involved in the development of some oral cancers, the implementation of a vaccine program for HPV 16 and 18 may prove to be beneficial in preventing not only cervical cancer, but possibly HPV16-positive oral cancers as well.

REFERENCES

1 **Landis SH**, Murray T, Bolden S, Wingo PA. Cancer statistics, 1999. *CA Cancer J Clin* 1999; **49**: 8-31, 1
2 **Siddiqui IA**, Farooq MU, Siddiqui RA, Rafi SMT. Role of toluidine blue in early detection of oral cancer. *Pak J Med Sci* 2006; **22**: 184-187
3 **Boyle P**, Ferlay J. Cancer incidence and mortality in Europe, 2004. *Ann Oncol* 2005; **16**: 481-488
4 **International Agency for Research on Cancer**. GLOBOCAN 2008: Cancer Incidence, Mortality and Prevalence Worldwide in 2008. Available from: URL: http://globocan.iarc.fr/
5 **Brinkman BM**, Wong DT. Disease mechanism and biomarkers of oral squamous cell carcinoma. *Curr Opin Oncol* 2006; **18**: 228-233
6 **Choi S**, Myers JN. Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. *J Dent Res* 2008; **87**: 14-32
7 **Llewellyn CD**, Johnson NW, Warnakulasuriya KA. Risk factors for oral cancer in newly diagnosed patients aged 45 years and younger: a case-control study in Southern England. *J Oral Pathol Med* 2004; **33**: 525-532
8 **Garrote LF**, Herrero R, Reyes RM, Vaccarella S, Anta JL, Ferbeye L, Muñoz N, Franceschi S. Risk factors for cancer of the oral cavity and oro-pharynx in Cuba. *Br J Cancer* 2001; **85**:

46-54
9 **Sánchez MJ**, Martínez C, Nieto A, Castellsagué X, Quintana MJ, Bosch FX, Muñoz N, Herrero R, Franceschi S. Oral and oropharyngeal cancer in Spain: influence of dietary patterns. *Eur J Cancer Prev* 2003; **12**: 49-56
10 **Talamini R**, Vaccarella S, Barbone F, Tavani A, La Vecchia C, Herrero R, Muñoz N, Franceschi S. Oral hygiene, dentition, sexual habits and risk of oral cancer. *Br J Cancer* 2000; **83**: 1238-1242
11 **zur Hausen H**, de Villiers EM. Human papillomaviruses. *Annu Rev Microbiol* 1994; **48**: 427-447
12 **Ragin CC**, Modugno F, Gollin SM. The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. *J Dent Res* 2007; **86**: 104-114
13 **zur Hausen H**, de Villiers EM, Gissmann L. Papillomavirus infections and human genital cancer. *Gynecol Oncol* 1981; **12**: S124-S128
14 **Bosch FX**, Lorincz A, Muñoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol* 2002; **55**: 244-265
15 **Thompson IO**, van der Bijl P, van Wyk CW, van Eyk AD. A comparative light-microscopic, electron-microscopic and chemical study of human vaginal and buccal epithelium. *Arch Oral Biol* 2001; **46**: 1091-1098
16 **Syrjänen KJ**, Pyrhönen S, Syrjänen SM, Lamberg MA. Immunohistochemical demonstration of human papilloma virus (HPV) antigens in oral squamous cell lesions. *Br J Oral Surg* 1983; **21**: 147-153
17 **Gillison ML**, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 2000; **92**: 709-720
18 **Smith EM**, Hoffman HT, Summersgill KS, Kirchner HL, Turek LP, Haugen TH. Human papillomavirus and risk of oral cancer. *Laryngoscope* 1998; **108**: 1098-1103
19 **Miller CS**, Johnstone BM. Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982-1997. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001; **91**: 622-635
20 **Schwartz SM**, Daling JR, Doody DR, Wipf GC, Carter JJ, Madeleine MM, Mao EJ, Fitzgibbons ED, Huang S, Beckmann AM, McDougall JK, Galloway DA. Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. *J Natl Cancer Inst* 1998; **90**: 1626-1636
21 **Syrjänen K**, Syrjänen S, Lamberg M, Pyrhönen S, Nuutinen J. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. *Int J Oral Surg* 1983; **12**: 418-424
22 **Cruz IB**, Snijders PJ, Steenbergen RD, Meijer CJ, Snow GB, Walboomers JM, van der Waal I. Age-dependence of human papillomavirus DNA presence in oral squamous cell carcinomas. *Eur J Cancer B Oral Oncol* 1996; **32B**: 55-62
23 **Miller CS**, White DK. Human papillomavirus expression in oral mucosa, premalignant conditions, and squamous cell carcinoma: a retrospective review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996; **82**: 57-68
24 **Syrjänen SM**, Syrjänen KJ. HPV infections of the oral mucosa. In: Syrjänen KJ, Syrjänen SM, editors. Papillomavirus infections in human pathology. New York: John Wiley and Sons, 2000: 379-412
25 **Mellin H**, Friesland S, Lewensohn R, Dalianis T, Munck-Wikland E. Human papillomavirus (HPV) DNA in tonsillar cancer: clinical correlates, risk of relapse, and survival. *Int J Cancer* 2000; **89**: 300-304
26 **D'Souza G**, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, Westra WH, Gillison ML. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007; **356**: 1944-1956
27 **Ang KK**, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010; **363**: 24-35
28 **Syrjänen S**, Lodi G, von Bültzingslöwen I, Aliko A, Arduino P, Campisi G, Challacombe S, Ficarra G, Flaitz C, Zhou HM, Maeda H, Miller C, Jontell M. Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. *Oral Dis* 2011; **17** Suppl 1: 58-72
29 **St Guily JL**, Jacquard AC, Prétet JL, Haesebaert J, Beby-Defaux A, Clavel C, Agius G, Birembaut P, Okaïs C, Léocmach Y, Soubeyrand B, Pradat P, Riethmuller D, Mougin C, Denis F. Human papillomavirus genotype distribution in oropharynx and oral cavity cancer in France--The EDiTH VI study. *J Clin Virol* 2011; **51**: 100-104
30 **Smith EM**, Ritchie JM, Summersgill KF, Hoffman HT, Wang DH, Haugen TH, Turek LP. Human papillomavirus in oral exfoliated cells and risk of head and neck cancer. *J Natl Cancer Inst* 2004; **96**: 449-455
31 **Ziegert C**, Wentzensen N, Vinokurova S, Kisseljov F, Einenkel J, Hoeckel M, von Knebel Doeberitz M. A comprehensive analysis of HPV integration loci in anogenital lesions combining transcript and genome-based amplification techniques. *Oncogene* 2003; **22**: 3977-3984
32 **Haverkos HW**. Viruses, chemicals and co-carcinogenesis. *Oncogene* 2004; **23**: 6492-6499
33 **Migaldi M**, Pecorari M, Forbicini G, Nanni N, Grottola A, Grandi T, Delle Donne G, Leocata P, Trovato D, Sgambato A. Low prevalence of human papillomavirus infection in the healthy oral mucosa of a Northern Italian population. *J Oral Pathol Med* 2012; **41**: 16-20
34 **Bjørge T**, Hennig EM, Skare GB, Søreide O, Thoresen SO. Second primary cancers in patients with carcinoma in situ of the uterine cervix. The Norwegian experience 1970-1992. *Int J Cancer* 1995; **62**: 29-33
35 **Rabkin CS**, Biggar RJ, Melbye M, Curtis RE. Second primary cancers following anal and cervical carcinoma: evidence of shared etiologic factors. *Am J Epidemiol* 1992; **136**: 54-58
36 **Frisch M**, Biggar RJ. Aetiological parallel between tonsillar and anogenital squamous-cell carcinomas. *Lancet* 1999; **354**: 1442-1443
37 **Hemminki K**, Dong C, Frisch M. Tonsillar and other upper aerodigestive tract cancers among cervical cancer patients and their husbands. *Eur J Cancer Prev* 2000; **9**: 433-437
38 **Syrjänen S**. Current concepts on human papillomavirus infections in children. *APMIS* 2010; **118**: 494-509
39 **Pannone G**, Santoro A, Papagerakis S, Lo Muzio L, De Rosa G, Bufo P. The role of human papillomavirus in the pathogenesis of head & amp; neck squamous cell carcinoma: an overview. *Infect Agent Cancer* 2011; **6**: 4
40 **Burns JE**, Baird MC, Clark LJ, Burns PA, Edington K, Chapman C, Mitchell R, Robertson G, Soutar D, Parkinson EK. Gene mutations and increased levels of p53 protein in human squamous cell carcinomas and their cell lines. *Br J Cancer* 1993; **67**: 1274-1284
41 **Gillespie MB**, Rubinchik S, Hoel B, Sutkowski N. Human papillomavirus and oropharyngeal cancer: what you need to know in 2009. *Curr Treat Options Oncol* 2009; **10**: 296-307
42 **Goon PK**, Stanley MA, Ebmeyer J, Steinsträsser L, Upile T, Jerjes W, Bernal-Sprekelsen M, Görner M, Sudhoff HH. HPV & amp; head and neck cancer: a descriptive update. *Head Neck Oncol* 2009; **1**: 36
43 **Kirnbauer R**, Hubbert NL, Wheeler CM, Becker TM, Lowy DR, Schiller JT. A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. *J Natl Cancer Inst* 1994; **86**: 494-499
44 **Carter JJ**, Koutsky LA, Wipf GC, Christensen ND, Lee SK, Kuypers J, Kiviat N, Galloway DA. The natural history of human papillomavirus type 16 capsid antibodies among a

cohort of university women. *J Infect Dis* 1996; **174**: 927-936
45 **Meschede W**, Zumbach K, Braspenning J, Scheffner M, Benitez-Bribiesca L, Luande J, Gissmann L, Pawlita M. Antibodies against early proteins of human papillomaviruses as diagnostic markers for invasive cervical cancer. *J Clin Microbiol* 1998; **36**: 475-480
46 **Stanley M**. Antibody reactivity to HPV E6 and E7 oncoproteins and early diagnosis of invasive cervical cancer. *Am J Obstet Gynecol* 2003; **188**: 3-4
47 **Schlecht NF**. Prognostic value of human papillomavirus in the survival of head and neck cancer patients: an overview of the evidence. *Oncol Rep* 2005; **14**: 1239-1247
48 **Lindquist D**, Romanitan M, Hammarstedt L, Näsman A, Dahlstrand H, Lindholm J, Onelöv L, Ramqvist T, Ye W, Munck-Wikland E, Dalianis T. Human papillomavirus is a favourable prognostic factor in tonsillar cancer and its oncogenic role is supported by the expression of E6 and E7. *Mol Oncol* 2007; **1**: 350-355
49 **Lassen P**. The role of Human papillomavirus in head and neck cancer and the impact on radiotherapy outcome. *Radiother Oncol* 2010; **95**: 371-380
50 **Weinberger PM**, Yu Z, Haffty BG, Kowalski D, Harigopal M, Brandsma J, Sasaki C, Joe J, Camp RL, Rimm DL, Psyrri A. Molecular classification identifies a subset of human papillomavirus--associated oropharyngeal cancers with favorable prognosis. *J Clin Oncol* 2006; **24**: 736-747
51 **Licitra L**, Perrone F, Bossi P, Suardi S, Mariani L, Artusi R, Oggionni M, Rossini C, Cantù G, Squadrelli M, Quattrone P, Locati LD, Bergamini C, Olmi P, Pierotti MA, Pilotti S. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. *J Clin Oncol* 2006; **24**: 5630-5636
52 **Gillison ML**. HPV and prognosis for patients with oropharynx cancer. *Eur J Cancer* 2009; **45** Suppl 1: 383-385
53 **Dahlstrand H**, Dahlgren L, Lindquist D, Munck-Wikland E, Dalianis T. Presence of human papillomavirus in tonsillar cancer is a favourable prognostic factor for clinical outcome. *Anticancer Res* 2004; **24**: 1829-1835
54 **Park NH**, Gujuluva CN, Baek JH, Cherrick HM, Shin KH, Min BM. Combined oral carcinogenicity of HPV-16 and benzo(a)pyrene: an in vitro multistep carcinogenesis model. *Oncogene* 1995; **10**: 2145-2153

S- Editor Li JY **L- Editor** A **E- Editor** Zheng XM

World Journal of Experimental Medicine

Online Submissions: http://www.wjgnet.com/esps/
wjem@wjgnet.com
doi:10.5493/wjem.v2.i4.70

World J Exp Med 2012 August 20; 2(4): 70-74
ISSN 2220-315X (online)
© 2012 Baishideng. All rights reserved.

EDITORIAL

Immunology of tuberculosis

Qing Zhang, Isamu Sugawara

Qing Zhang, Isamu Sugawara, Center of Tuberculosis Diagnosis and Treatment, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai 200433, China
Author contributions: Zhang Q and Sugawara I equally contributed to this paper.
Correspondence to: Dr. Isamu Sugawara, MD, PhD, Professor of Medicine, Center of Tuberculosis Diagnosis and Treatment, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai 200433, China. s6671112@sea.plala.or.jp
Telephone: +81-424-724853
Received: November 10, 2011 Revised: April 5, 2012
Accepted: July 27, 2012
Published online: August 20, 2012

Abstract

Various T cells and macrophages as well as cytokines are involved in the immunopathogenesis of tuberculosis (TB). A better understanding of immunology of TB can not only lead to the discovery of new immunodiagnostic tools, accelerate and facilitate the assessment of new therapeutic methods, but also find new treatment regimens. In this highlight topic we cover the latest developments in the role of T cells, macrophages, Natural killer (NK) cells, invariant NK T (iNKT) cells and γδ T cells with TB infection. Histologically, TB displays exudative inflammation, proliferative inflammation and productive inflammation depending on the time course. T cells first recognize antigen within the mycobacterially-infected lung, and then activate, differentiate, but the first T cell activation occurs in the draining lymph nodes of the lung. When protective T cells reach sufficient numbers, they can stop bacterial growth. Except for T cells, neutrophils also participate actively in defense against early-phase TB. NK cells are innate lymphocytes which are a first line of defense against mycobacterial infection. Human NK cells use the NKp46, NCRs and NKG2D receptors to lyse *Mycobacterium TB*-infected monocytes and alveolar macrophages. NK cells produce not only interferon-γ, but also interleukin (IL)-22, which is induced by IL-15 and DAP-10. iNKT cells show different phenotypes and functions. Many iNKT cells are CD4+, few iNKT cells are CD8+, while an additional fraction of iNKT cells are negative for both CD4 and CD8. γδ T cells represent an early innate defense in antimycobacterial immunity. Studies done in humans and animal models have demonstrated complex patterns of γδ T cell immune responses during chronic TB. Human alveolar macrophages and monocytes can serve as antigen presentation cells for γδ T cells. Furthermore, the predominance of Vγ9Vδ2 T cells in TB has been confirmed.

© 2012 Baishideng. All rights reserved.

Key words: Mycobacteria; Tuberculosis; Immunology; T cells; Macrophages; Dendritic cells; Invariant natural killer T cell; Neutrophils; Cytokine

Peer reviewer: Fabian Benencia, PhD, Assistant Professor of Immunology, Department of Biomedical Sciences, College of Osteopathic Medicine and BME, Russ College of Engineering and Technology, Academic Research Center, Ohio University Athens, OH 45701, United States

Zhang Q, Sugawara I. Immunology of tuberculosis. *World J Exp Med* 2012; 2(4): 70-74 Available from: URL: http://www.wjgnet.com/2220-315X/full/v2/i4/70.htm DOI: http://dx.doi.org/10.5493/wjem.v2.i4.70

INTRODUCTION

Tuberculosis (TB) is a great threat to developing countries as well as developed countries, fuelled by human immunodeficiency virus infection, drug resistance and migration of population. Various T cells are integrated in the immunopathogenesis of TB. A better understanding of this issue can not only drive the progress of new immunodiagnostic tools, accelerate and facilitate the evaluation of new therapeutic methods, but also improve new treatment tools. Here, we will update the readers on the latest developments in this field and, in particular, focus on T lymphocytes, several T cell subsets, macrophages and cytokines involved in TB immunology.

INFLAMMATORY PROCESS OF TUBERCULOSIS

Histopathologically, TB displays exudative inflammation, proliferative inflammation and productive inflammation depending on the time course. Using animal experiments and an inhalation exposure system, the pathologic condition of the infected animals was followed up for 1 year[1]. Exudative inflammation was observed for the first ten days. Thereafter, granulomas, which corresponded to foci of proliferative inflammation, were formed. Cavity formation was not recognized in animal TB, except for rabbits. Using rabbit models, Dr. Arthur Dannenberg described the pathology of TB in more detail[1]. There are five stages: onset, symbiosis, early stages of caseous necrosis, interplay of cell-mediated immunity and tissue damaging delayed-type hypersensitivity, and liquefaction and cavity formation. In stage 1, tubercle bacilli are usually destroyed or inhibited by the mature resident alveolar macrophages that ingest them. If bacilli are not destroyed, they grow and eventually destroy the alveolar macrophages. In stage 2, bacilli grow logarithmically within the immature nonactivated macrophages. These macrophages enter a tubercle from the bloodstream. This stage is termed symbiosis because bacilli multiply locally without apparent damage to the host, and macrophages accumulate and divide. In stage 3, the stage at which caseous necrosis first occurs, the number of viable bacilli becomes stationary because their growth is inhibited by the immune response to tuberculin-like antigens released from bacilli. Stage 4 is the stage that usually determines whether the disease becomes clinically apparent. Cell-mediated immunity plays a major role in this situation. The cytotoxic delayed- type hypersensitivity immune response kills these macrophages, causing enlargement of the caseous center and progression of the disease. If good cell-mediated immunity develops, a mantle of highly activated macrophages surrounds the caseous necrosis. In stage 5, bacilli evade host defenses. When liquefaction of the caseous center occurs, the bacilli multiply extracellularly, frequently attaining very large numbers. The high local concentration of tuberculin-like products derived from these bacilli causes a tissue-damaging delayed-type hypersensitivity response that erodes the bronchial wall, forming a cavity.

T CELL ACTIVATION AGAINST MYCOBACTERIUM TUBERCULOSIS

In human, a TB index case may infect a contact person through cough and expectoration, so the lung is the primary route of infection and often the main tissue exhibiting TB. Infectious droplet nuclei are deposited in the alveolar spaces of the contact person where *Mycobacterium tuberculosis (M. tuberculosis)* can be phagocytosed by alveolar macrophages, epithelial cells, dendritic cells (DC) and neutrophils[2,3]. Alveolar macrophages and DC are then believed to transport *M. tuberculosis* to local lymph nodes where T cell activation occurs and expand. DC play important and indispensable roles in the initiation and maintenance of protective immune responses following mycobacterial infection. The kind of immune responses initiated by DC determines the character of immune responses mounted by the host against the pathogen. The profile of cytokines and chemokines secreted by the result of infection of DC by mycobacteria further plays an important role in defining the course of mycobacterial infection. Activation of the phagocytic host cell is much required to limit growth of *M. tuberculosis*; as in the absence of activation, disease outcome is extremely poor. Effective phagocyte activation requires a specific cellular response, as infected hosts lacking specific components of the acquired response have a poor outcome[4]. While acquired cellular protection is expressed rapidly following systemic challenge with *M. tuberculosis*, it is less rapid in the lung. Slow expression of protection in the lung allows mycobacteria to grow and modulate the infection site. Until recently it has not been clear whether the slow response to aerosol delivery of bacteria resulted from limited availability of antigen or inhibition of antigen-presentation by *M. tuberculosis*. Several studies show that the first T cell activation occurs in the draining lymph node (DLN) of the lung 8-10 d following initial challenge. The activation of T cells correlated temporally with the arrival of bacteria and availability of antigen in the DLN, however conditions for T cell activation were unique to the DLN as the presence of antigen-producing bacteria in the lung and spleen did not result in initial activation of T cells[5,6]. While delivery of lipopolysaccharide (LPS) to the *M. tuberculosis*-infected lung failed to accelerate T cell priming[5], increasing the bacterial dose did accelerate the response modestly suggesting that both antigen burden and refractory cells serve to slow the response. Thus, protective memory cells will not become activated until they recognize antigen, i.e., more than 8 d post infection. Once T cells become activated they differentiate into effector T cells that migrate to the lung. By day 14 of infection, when activated T cells first arrive in the lung, bacteria are within alveolar macrophages, myeloid DC and neutrophils[4]. T cells can recognize antigen within the mycobacterially-infected lung but the antigen presentation is not optimal. It takes time for the protective T cells to reach sufficient numbers to stop bacterial growth. T cells can be divided into two subsets, Th1 and Th2, on the basis of the cytokines they produce. In tuberculosis, Th1 plays a major role in defense against tuberculosis. Th1 cells suppress Th2 cells. CD4 + T cells have unambiguously been identified as the most important lymphocyte subset for mediating protection. CD4 T lymphocytes differentiate in the peripheral tissues to adopt a variety of fates such as the Th-1 cells, which produce interferon (IFN)-γ to down-regulate Th2 responses and Th-2 cells, which produce interleukin (IL)-4. CD8 T lymphocytes produce predominantly IFN-γ. Though CD4 response is greater than the CD8 response, the latter can provide protection in the absence of CD4 help[7]. During active TB there is a local pulmonary immune response characterized by α/β T cells and strongly enhanced *M. tuberculosis* antigen-specific Th1 responses, with large amounts of locally secreted IFN-γ[8].

ALVEOLAR MACROPHAGES IN TUBERCULOSIS DEVELOPMENT

When tubercle bacilli reach alveoli, they are phagocytosed by resident alveolar macrophages. Though tubercle bacilli are killed by alveolar macrophages, tubercle bacilli can also kill macrophages through apoptosis. What is the fate of tubercle bacilli once they enter the phagosomes of macrophages? Alveolar macrophages of aerially infected guinea pigs were collected by bronchoalveolar lavage. At 12 d after infection, one out of about 10 000 alveolar macrophages of various sizes contained many tubercle bacilli[9]. This indicates that certain alveolar macrophages permit *M. tuberculosis* to replicate in the phagosomes, although most of tubercle bacilli are killed by activated alveolar macrophages. It will be of great interest to examine the survival mechanism of *M. tuberculosis* at the single-cell level, but we still do not know why macrophages targeted by tubercle bacilli cannot kill the bacilli.

IFN-γ knockout mice were infected with avirulent H37Ra or BCG Pasteur, multinucleated giant cells were recognized in the granulomatous lesions. The lesions also contained tubercle bacilli and consisted of multinucleated cell clusters, being immunopositive with anti-Mac-3 antibody. The multinucleated giant cells were transformed alveolar macrophages. We subsequently infected various cytokine-knockout mice with M. TB, but no Langhans' multinucleated giant cells were recognized the granulomas. Therefore, it seems that formation of multinucleated giant cells requires optimal combinations and concentrations of various cytokines, and the level of IFN-γ, at least, has to be significantly low.

CYTOKINES IN PROTECTION AGAINST TB

IFN-γ and tumor necrosis factor (TNF) have long been implicated as regulators of T cell responses in mycobacterial disease[10]. The technique of gene targeting (knockout) has swept through biomedical research. IFN-γ, TNF-α, interferon response factor (IRF)-1, nuclear factor (NF)-IL6, NF-κB p50, signal transducer and activator of transcription (STAT)1 and STAT4 knockout mice succumbed to *M. tuberculosis* infection over time. There appears to be a cytokine and transcription factor hierarchy in experimental tuberculosis. The results indicate that these molecules play major roles in defense against the disease, IFN-γ and TNF-α being the leading players in this respect[11].

Figure 1 shows the cytokine hierarchy associated with important molecules in experimental tuberculosis. The transcription factors such as NF-κB, STAT1, STAT4, IRF-1 and NF-IL6 are important molecules in defense against tuberculosis. The molecules [toll-like receptor (TLR)2, TLR6 and MyD-88] are less important in defense against tuberculosis in our hands[11].

ROLE OF NEUTROPHILS

The role of neutrophils in the development of tuberculosis remained unknown for a long time. We utilized

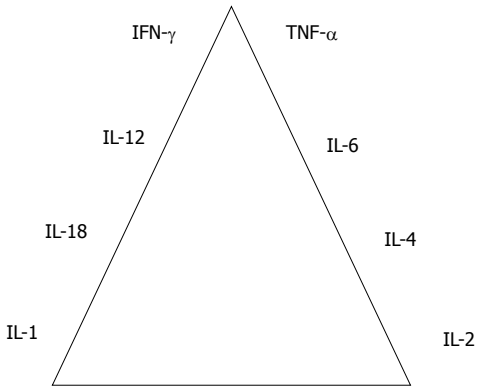

Figure 1 Cytokine hierarchy in immunology of tuberculosis. IFN: Interferon; IL: Interleukin; TNF: Tumor necrosis factor.

LPS-induced transient neutrophilia in the lungs[9]. LPS (50 μg/mL) was administered intratracheally to male Fischer rats, which were then infected with *M. tuberculosis via* an airborne route. Intratracheal injection of LPS significantly blocked the development of pulmonary granulomas and significantly reduced the number of pulmonary colony-forming units (CFU). Treatment with amphotericin B (an LPS inhibitor) or neutralizing anti-rat neutrophil antibody reversed the development of pulmonary lesions. LPS-induced transient neutrophilia prevented early mycobacterial infection. The timing of LPS administration was important. When given intratracheally at least 10 d after aerial infection, LPS did not prevent the development of tuberculosis. Neutrophils obtained by bronchoalveolar lavage killed *M. tuberculosis* bacilli. These results indicate clearly that neutrophils participate actively in defense against early-phase tuberculosis.

ROLE OF NATURAL KILLER CELLS

Natural killer (NK) cells are innate lymphocytes which are a first line of defense against infection. NK cells can kill autologous infected cells without prior sensitization, and are believed to play a pivotal role in innate immunity to microbial pathogens. In mouse model, NK cells are activated and produce IFN-γ during the early response to pulmonary tuberculosis[9] and NK cell-produced IFN-γ regulates the anti-mycobacterial resistance mediated by neutrophils[12]. However animal models do not give a clear answer to whether NK cells is important in *M. tuberculosis* infection *in vivo*. Depletion of NK cells had no effect on bacterial replication in the lung of immunocompetent mice[13], suggesting that NK cells may be redundant in the presence of intact adaptive immunity. Surprisingly, IFN-γ knockout mice, which are impaired in their ability to clear mycobacteria, cleared them as effectively as wild-type mice when NK cells were depleted, suggesting that NK cells can inhibit protective immunity[14].

Human NK cells use the NKp46, the natural cytotoxicity receptors (NCRs) and NKG2D receptors to lyse *M. tuberculosis*-infected monocytes and alveolar macrophages[15], through damage of infected cells and secretion of cytokines, such as IFN-γ[16]. Inhibitory receptors of NK

cells include killer immunoglobulin-like receptors (KIRs) and the NKG2A: CD94 dimer and NK cell activation can also be triggered by loss of inhibitory ligands from the cell surface. In addition, NK cells can also be activated by cytokines, including type I interferons, IL-12 and IL-18. NK cells are a potent and early source of cytokines, particularly IFN-γ, but they can also produce Th2-associated cytokines, such as IL-5 and IL-13, and the regulatory cytokine IL-10[17]. NK cell NKp46 expression and cytotoxicity are reduced in freshly isolated peripheral blood mononuclear cells (PBMCs) from tuberculosis patients, which may be attributable to suppression by monocytes and IL-10. Recent studies have found that NK cells produce IL-22[18], which was induced by IL-15 and DAP-10, an adaptor protein that is known to be involved in NK cell activation, in response to *M. tuberculosis*. Rohan Dhiman *et al*[19] also found that IL-22 can restrict growth of *M. tuberculosis* in macrophages by enhancing phagolysosomal fusion[19]. Nonetheless to fully understand the importance of NK cells in *M. TB* infection it may be necessary to differentiate their contributions at different stages of disease.

ROLE OF NKT CELLS

Certain T subsets, such as NKT cells and γδ T cells, have features of innate immune cells including a partially activated phenotype, a rapid response following detection of infected cells, and the modulation of other cell types. Together with NK cells, these cell subsets are functionally defined as innate lymphocytes. CD1d-restricted invariant NKT (iNKT) cells are a conserved subset of T cells that express an invariant T cell receptor (TCR) α chain (Vα24-Jα18 in humans, and Vα14-Jα18 in mice) paired with TCR β chains encoded by one or a few Vβ gene segments (Vβ11 in humans, and predominantly Vβ2, 7 and 8 in mice). These cells show different phenotypes and functions[20]. Many iNKT cells are CD4+, and they have been mainly associated with the induction of Th2 cytokines such as IL-4, IL-5, IL-13. This subset is believed to play a prominent role in suppression of autoimmune or chronic inflammatory diseases, and in promoting allergic conditions such as asthma. Few iNKT cells are CD8+, and most of those express only the CD8α subunit, which means that they likely express only CD8αα homodimers. An additional fraction of iNKT cells are negative for both CD4 and CD8 (DN T cells). They have been found to produce predominantly IFN-γ and other Th1-associated cytokines. Studies of human iNKT cells have shown that they have the ability to kill *M. tuberculosis* organisms within infected macrophages, possibly through their production of the peptide granulysin[21]. Im *et al*[22] found that the percentages of iNKT cells among total circulating T cells in TB patients were not significantly different compared to those in healthy controls. However, TB patients showed a selective reduction of the proinflammatory CD4-CD8- (DN) iNKT cells with a proportionate increase in the CD4+ iNKT cells. The mouse model of tuberculosis has been used by Sada-Ovalle *et al*[23] to find that iNKT cells have a direct bactericidal effect on *M. tuberculosis*, and protect mice against aerosol *M. tuberculosis* infection[23]. Their activation requires CD1d expression by infected macrophages as well as IL-12 and IL-18. In addition, pharmacological activation of iNKT cells with the synthetic ligand aGalCer often enhances host resistance to infection. iNKT cell use several mechanisms to modify host immunity. These include induction of DC maturation, secondary activation of effector cells (NK cells) or recruitment of inflammatory cells to the site of infection[24,25]. Thus, by being an early producer of IFN-γ and suppressing intracellular bacterial growth, iNKT cells function as an important part of the early immune response against *M. tuberculosis* that affect both the innate and the adaptive arms of the immune response.

ROLE OF γΔ T CELLS

Antigen-specific γδ T cells represent an early innate defense that may play a role in antimycobacterial immunity. Studies done in humans and animal models have demonstrated complex patterns of γδ T cell immune responses during early mycobacterial infections and chronic TB. Like αβ T lymphocytes, γδ T cells carry antigen TCR that vary in the physical properties of their ligand-binding sites. γδ T cells are frequently activated by a variety of pathogens including *M. tuberculosis*[26]. Mice lacking γδ T cells succumb more rapidly than control mice following intravenous challenge with virulent *M. tuberculosis*; however, such a difference has not been observed following infection by the aerosol route. γδ T cells constitute a whole system of functionally specialized subsets that have been implicated in the innate responses against tumors and pathogens, the regulation of immune responses, cell recruitment and activation, and tissue repair[27]. Human alveolar macrophages and monocytes can serve as antigen presentation cells (APCs) for γδ T cells. Furthermore, the predominance of Vγ9Vδ2 T cells in TB disease has been confirmed[28]. When MTB-activated CD4+ and γδ T cells from healthy tuberculin-positive donors were analyzed for cytokine production in response to *M. tuberculosis* -infected monocytes, both groups secreted large amounts of IFN-γ[29]. Previous studies have also demonstrated an increased proliferative activity of Vγ9Vδ2 T cells from patients with TB[30], but reduced production of IFN-γ, compared with that of healthy tuberculin-positive donors[31]. Additionally, Dieli *et al*[32] reported that decrease of Vγ9Vδ2 T cell effector functions involves not only IFN-γ production but also expression of granulysin[32].

CONCLUSION

γδ T cells appear to combine properties of both adaptive and innate immunities. The identification of unusual compounds that are recognized by human γδ T cells but not by αβ T cells has recently stimulated great interest in the development of γδ T cell-based therapies.

REFERENCES

1 **Dannenberg AM**. Pathogenesis of human pulmonary tu-

berculosis: Insights from the rabbit model. Washington, DC: ASM Pres, 2006
2 **Tailleux L**, Pham-Thi N, Bergeron-Lafaurie A, Herrmann JL, Charles P, Schwartz O, Scheinmann P, Lagrange PH, de Blic J, Tazi A, Gicquel B, Neyrolles O. DC-SIGN induction in alveolar macrophages defines privileged target host cells for mycobacteria in patients with tuberculosis. *PLoS Med* 2005; **2**: e381
3 **Kang PB**, Azad AK, Torrelles JB, Kaufman TM, Beharka A, Tibesar E, DesJardin LE, Schlesinger LS. The human macrophage mannose receptor directs Mycobacterium tuberculosis lipoarabinomannan-mediated phagosome biogenesis. *J Exp Med* 2005; **202**: 987-999
4 **Cooper AM**. Cell-mediated immune responses in tuberculosis. *Annu Rev Immunol* 2009; **27**: 393-422
5 **Wolf AJ**, Desvignes L, Linas B, Banaiee N, Tamura T, Takatsu K, Ernst JD. Initiation of the adaptive immune response to Mycobacterium tuberculosis depends on antigen production in the local lymph node, not the lungs. *J Exp Med* 2008; **205**: 105-115
6 **Reiley WW**, Calayag MD, Wittmer ST, Huntington JL, Pearl JE, Fountain JJ, Martino CA, Roberts AD, Cooper AM, Winslow GM, Woodland DL. ESAT-6-specific CD4 T cell responses to aerosol Mycobacterium tuberculosis infection are initiated in the mediastinal lymph nodes. *Proc Natl Acad Sci USA* 2008; **105**: 10961-10966
7 **Ngai P**, McCormick S, Small C, Zhang X, Zganiacz A, Aoki N, Xing Z. Gamma interferon responses of CD4 and CD8 T-cell subsets are quantitatively different and independent of each other during pulmonary Mycobacterium bovis BCG infection. *Infect Immun* 2007; **75**: 2244-2252
8 **Herrera MT**, Torres M, Nevels D, Perez-Redondo CN, Ellner JJ, Sada E, Schwander SK. Compartmentalized bronchoalveolar IFN-gamma and IL-12 response in human pulmonary tuberculosis. *Tuberculosis* (Edinb) 2009; **89**: 38-47
9 **Sugawara I**, Udagawa T, Yamada H. Rat neutrophils prevent the development of tuberculosis. *Infect Immun* 2004; **72**: 1804-1806
10 **Cooper AM**, Adams LB, Dalton DK, Appelberg R, Ehlers S. IFN-gamma and NO in mycobacterial disease: new jobs for old hands. *Trends Microbiol* 2002; **10**: 221-226
11 **Sugawara I**, Yamada H, Shi R. Pulmonary tuberculosis in various gene knockout mice with special emphasis on roles of cytokines and transcription factors. *Current Resp Rev* 2005; **1**: 7-13
12 **Feng CG**, Kaviratne M, Rothfuchs AG, Cheever A, Hieny S, Young HA, Wynn TA, Sher A. NK cell-derived IFN-gamma differentially regulates innate resistance and neutrophil response in T cell-deficient hosts infected with Mycobacterium tuberculosis. *J Immunol* 2006; **177**: 7086-7093
13 **Junqueira-Kipnis AP**, Kipnis A, Jamieson A, Juarrero MG, Diefenbach A, Raulet DH, Turner J, Orme IM. NK cells respond to pulmonary infection with Mycobacterium tuberculosis, but play a minimal role in protection. *J Immunol* 2003; **171**: 6039-6045
14 **Woolard MD**, Hudig D, Tabor L, Ivey JA, Simecka JW. NK cells in gamma-interferon-deficient mice suppress lung innate immunity against Mycoplasma spp. *Infect Immun* 2005; **73**: 6742-6751
15 **Doolan DL**, Hoffman SL. IL-12 and NK cells are required for antigen-specific adaptive immunity against malaria initiated by CD8+ T cells in the Plasmodium yoelii model. *J Immunol* 1999; **163**: 884-892
16 **Vankayalapati R**, Garg A, Porgador A, Griffith DE, Klucar P, Safi H, Girard WM, Cosman D, Spies T, Barnes PF. Role of NK cell-activating receptors and their ligands in the lysis of mononuclear phagocytes infected with an intracellular bacterium. *J Immunol* 2005; **175**: 4611-4617
17 **Maroof A**, Beattie L, Zubairi S, Svensson M, Stager S, Kaye PM. Posttranscriptional regulation of II10 gene expression allows natural killer cells to express immunoregulatory function. *Immunity* 2008; **29**: 295-305
18 **Wolk K**, Witte E, Wallace E, Döcke WD, Kunz S, Asadullah K, Volk HD, Sterry W, Sabat R. IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. *Eur J Immunol* 2006; **36**: 1309-1323
19 **Dhiman R**, Indramohan M, Barnes PF, Nayak RC, Paidipally P, Rao LV, Vankayalapati R. IL-22 produced by human NK cells inhibits growth of Mycobacterium tuberculosis by enhancing phagolysosomal fusion. *J Immunol* 2009; **183**: 6639-6645
20 **Swann JB**, Coquet JM, Smyth MJ, Godfrey DI. CD1-restricted T cells and tumor immunity. *Curr Top Microbiol Immunol* 2007; **314**: 293-323
21 **Gansert JL**, Kiessler V, Engele M, Wittke F, Röllinghoff M, Krensky AM, Porcelli SA, Modlin RL, Stenger S. Human NKT cells express granulysin and exhibit antimycobacterial activity. *J Immunol* 2003; **170**: 3154-3161
22 **Im JS**, Kang TJ, Lee SB, Kim CH, Lee SH, Venkataswamy MM, Serfass ER, Chen B, Illarionov PA, Besra GS, Jacobs WR, Chae GT, Porcelli SA. Alteration of the relative levels of iNKT cell subsets is associated with chronic mycobacterial infections. *Clin Immunol* 2008; **127**: 214-224
23 **Sada-Ovalle I**, Chiba A, Gonzales A, Brenner MB, Behar SM. Innate invariant NKT cells recognize Mycobacterium tuberculosis-infected macrophages, produce interferon-gamma, and kill intracellular bacteria. *PLoS Pathog* 2008; **4**: e1000239
24 **Fujii S**, Shimizu K, Hemmi H, Steinman RM. Innate Valpha14(+) natural killer T cells mature dendritic cells, leading to strong adaptive immunity. *Immunol Rev* 2007; **220**: 183-198
25 **Nakamatsu M**, Yamamoto N, Hatta M, Nakasone C, Kinjo T, Miyagi K, Uezu K, Nakamura K, Nakayama T, Taniguchi M, Iwakura Y, Kaku M, Fujita J, Kawakami K. Role of interferon-gamma in Valpha14+ natural killer T cell-mediated host defense against Streptococcus pneumoniae infection in murine lungs. *Microbes Infect* 2007; **9**: 364-374
26 **Behar SM**, Boom WH.Unconventional T Cells. In: Kaufmann SHE, Britton WJ, editors. Handbook of Tuberculosis. Weinheim: Wiley-VCH, 2008; 157–183
27 **Girardi M**. Immunosurveillance and immunoregulation by gammadelta T cells. *J Invest Dermatol* 2006; **126**: 25-31
28 **De Libero G**, Casorati G, Giachino C, Carbonara C, Migone N, Matzinger P, Lanzavecchia A. Selection by two powerful antigens may account for the presence of the major population of human peripheral gamma/delta T cells. *J Exp Med* 1991; **173**: 1311-1322
29 **Gioia C**, Agrati C, Goletti D, Vincenti D, Carrara S, Amicosante M, Casarini M, Giosue S, Puglisi G, Rossi A, Colizzi V, Pucillo LP, Poccia F. Different cytokine production and effector/memory dynamics of alpha beta+ or gamma delta+ T-cell subsets in the peripheral blood of patients with active pulmonary tuberculosis. *Int J Immunopathol Pharmacol* 2003; **16**: 247-252
30 **Dieli F**, Friscia G, Di Sano C, Ivanyi J, Singh M, Spallek R, Sireci G, Titone L, Salerno A. Sequestration of T lymphocytes to body fluids in tuberculosis: reversal of anergy following chemotherapy. *J Infect Dis* 1999; **180**: 225-228
31 **Sánchez FO**, Rodríguez JI, Agudelo G, García LF. Immune responsiveness and lymphokine production in patients with tuberculosis and healthy controls. *Infect Immun* 1994; **62**: 5673-5678
32 **Dieli F**, Sireci G, Caccamo N, Di Sano C, Titone L, Romano A, Di Carlo P, Barera A, Accardo-Palumbo A, Krensky AM, Salerno A. Selective depression of interferon-gamma and granulysin production with increase of proliferative response by Vgamma9/Vdelta2 T cells in children with tuberculosis. *J Infect Dis* 2002; **186**: 1835-1839

S- Editor Li JY **L- Editor** A **E- Editor** Zheng XM

World Journal of Experimental Medicine

Online Submissions: http://www.wjgnet.com/esps/
wjem@wjgnet.com
doi:10.5493/wjem.v2.i4.75

World J Exp Med 2012 August 20; 2(4): 75-77
ISSN 2220-315X (online)
© 2012 Baishideng. All rights reserved.

OBSERVATION

Yosuke Kakisaka, MD, *Series Editor*

Abdominal migraine reviewed from both central and peripheral aspects

Yosuke Kakisaka, Mitsugu Uematsu, Zhong I Wang, Kazuhiro Haginoya

Yosuke Kakisaka, Mitsugu Uematsu, Kazuhiro Haginoya, Department of Pediatrics, Tohoku University School of Medicine, Sendai, Miyagi 980-8574, Japan
Yosuke Kakisaka, Zhong I Wang, Neurological Institute, The Cleveland Clinic, Cleveland, OH 44195, United States
Author contributions: Kakisaka Y, Uematsu M, Wang ZI and Haginoya K all contributed to the substantial contributions to this manuscript, drafting the article or revising it critically for important intellectual content, and final approval of the version to be published.
Correspondence to: Yosuke Kakisaka, MD, Department of Pediatrics, Tohoku University School of Medicine, 1-1 Seiryo-Machi, Aobaku, Sendai, Miyagi 980-8574, Japan. kakisuke@mui.biglobe.ne.jp
Telephone: +81-22-7177287 Fax: +81-22-7177290
Received: October 9, 2011 Revised: April 8, 2012
Accepted: July 27, 2012
Published online: August 20, 2012

Abstract

Despite the 2%-5% prevalence of abdominal migraine (AM) during childhood, the precise mechanism remains unknown. In this review, we present recent studies on AM and speculate its mechanism from both peripheral and central nervous system aspects. Although the main symptoms of AM exist at the peripheral level, previous studies have reported possible dysfunction of central nervous system, including photophobia, phonophobia and abnormal visual evoked responses. Recently, a case has been reported with AM combined with "Alice in Wonderland" syndrome with visual and/or bodily distortions, which serves as another piece of evidence of central dysfunction. Another case reported an AM patient having peculiar stereotypical ecchymosis in the legs and buttocks associated with pain attack, which implied possible involvement of peripheral nervous system. Although further investigations and accumulation of AM cases are still needed, we believe that the schema hypothesized here is helpful to plan further experimental approach to clarify the mechanism of this peculiar disease.

© 2012 Baishideng. All rights reserved.

Key words: Migraine equivalent; Abdominal migraine; Central hypersensitivity; Visceral hypersensitivity; Sumatriptan

Peer reviewer: Feng Tao, MD, PhD, Assistant Professor, Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, 720 Rutland Ave. / Ross 355, Baltimore, MD 21025, United States

Kakisaka Y, Uematsu M, Wang ZI, Haginoya K. Abdominal migraine reviewed from both central and peripheral aspects. *World J Exp Med* 2012; 2(4): 75-77 Available from: URL: http://www.wjgnet.com/2220-315X/full/v2/i4/75.htm DOI: http://dx.doi.org/10.5493/wjem.v2.i4.75

INTRODUCTION

It was in 1921 when abdominal migraine (AM) was first described[1]. Thanks to efforts by previous investigators[2], AM is now regarded as the abdominal equivalent to migraine and has been classified not only in the International Classification of Headache Disorders[3], but also in the Rome III criteria for functional gastrointestinal disorders[4]. To date, the mechanism of AM still remains unknown. In this review, we will present recent studies on AM and speculate its mechanism from both peripheral and central nervous system aspects, to add new knowledge to our understanding of this disease.

CENTRAL SYMPTOMS ACCOMPANYING ABDOMINAL PAIN IN AM

Although the main symptom of AM exists at the pe-

ripheral level, as the word "abdominal" indicates in the disease name, previous studies have reported possible dysfunction of central nervous system, including photophobia, phonophobia and abnormal visual evoked response[5,6]. The percentage of visual disturbance and phonophobia in AM were reported to be about 20% and 10%, respectively[5,7]. As expected, these percentages were markedly lower than that of migraine, for which 77% were reported to have photophobia, and 69% had phonophobia[5]. Mortimer and Good showed similar response pattern in visual evoked potential in cases with migraine and AM, e.g., higher amplitude of fast wave activity than healthy controls and the presence of paroxysmal sharp wave activity[6]. The existence of central nervous system symptoms in both AM and migraine indicates that AM is not only a peripheral disorder, but may also carry certain CNS process similar to migraine.

Patients with AM were reported to have central nervous system hypersensitivity. Recently, Hamed reported a case with AM coexisting with Alice in Wonderland syndrome (AWS)[8]. The symptoms are characterized with perceptual abnormalities, distortion of body image, and alternation in subjective time sense, as Alice has in the story written by Lewis Carroll[9]. AWS can also be observed in patients with migraine[10]. The 20-year-old patient, as presented in the case from Hamed, had abdominal colic pain, which fits to diagnostic criteria of AM. This patient had a strong family history of migraine. Hamed stated that, despite normal brain magnetic resonance imaging and electroencephalography, medical examinations including transcranial magnetic stimulation and evoked potentials revealed enhanced cortical excitability in multiple brain lesions[8].

PERIPHERAL SYMPTOM ACCOMPANYING TO ABDOMINAL PAIN IN AM

AM belongs to the category of functional gastrointestinal disorders, which are thought to be related to background visceral hypersensitivity[4]. Recently, we reported a peculiar phenomenon seen in a patient diagnosed with AM who had ecchymosis in the legs and buttocks associated with abdominal pain[11]. No currently known hypothesis could clearly explain the peculiar phenomenon. It is known that a subset of patients with migraine have similar ecchymosis in areas covered with trigeminal nerve, where the main site for migraine headache would be[12,13]. Wesselmann and Lai showed that, in their rat model, insult to a pelvic organ resulted in extravasation in the skin region innervated by the affected nerves[14]. In the process of investigating the mechanism of referred pain from visceral organ, they developed a rat model for pain caused by uterine inflammation. Experimental visceral inflammation in their rats pretreated with evans blue dye resulted in dye extravasation in the skin over the abdomen, groin, lower back, thighs, perineal area and proximal tail. They speculated that the neuronal pathway accounting for the phenomenon may include dichotomizing afferent fibers, afferent-afferent interactions *via* a spinal cord pathway, or a sympathetic reflex[14]. When viewed in terms of human anatomy, the ecchymosis of legs and buttock can be accounted with neuronal input of visceral pain to sacral segment of spinal cord[15]. Based on these reports, we hypothesize the following mechanism to explain the peculiar skin phenomenon in our case[16]. First, under predisposing visceral hypersensitivity associated with AM, the visceral nerves responsible for abdominal nociception, especially those innerved by the sacral levels of spinal cord, are activated. This change then results in the occurrence of ecchymosis in the legs and buttock.

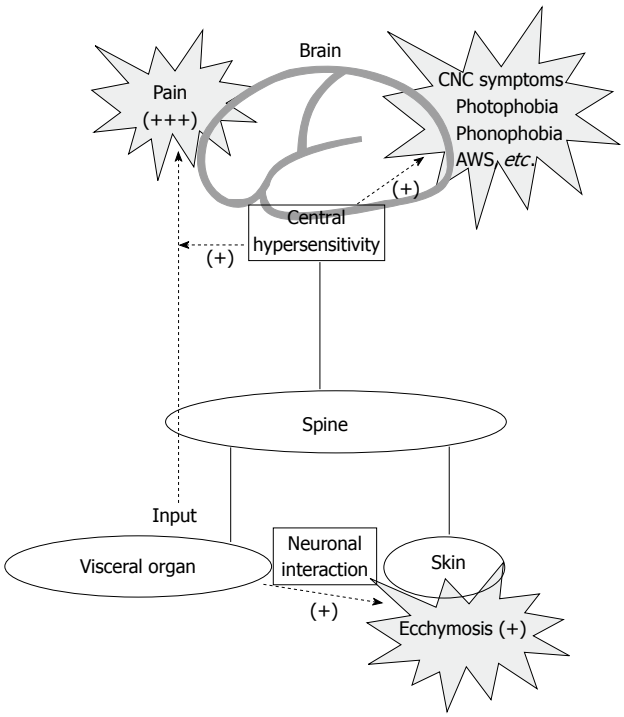

Figure 1 The central and peripheral network for symptoms relating to abdominal migraine is shown. CNS: Central nervous system; AWS: Alice wonderland syndrome.

POSSIBLE MECHANISM OF TRIPTAN TO TREAT AM-RELATED PAIN

Triptan (serotonergic agonists) has been to treat acute symptom of AM[7,11,16]. This agent has been generally thought to act on several regions, e.g., 5-HT1B receptors on the meningeal vasculature, and 5-HT1D receptors on trigeminal nerve terminals projecting to the dural vasculature and to the brain stem trigeminal nuclei[17]. Jeong *et al*[18] reported that, using their Sprague-Dawley rat model, sumatriptan brought pain relief by inhibiting GABAergic and glutamatergic synaptic transmission within the midbrain periaqueductal grey matter (PAG), a center of pain control, *via* a 5-hydroxytryptamine (5-HT) 1B and 5-HT1D receptor mediated decrease in neurotransmitter release[18]. Their result supported the central sensitization mechanism of

AM pain relief. It is important to note that, central effect of sumatriptan may not be the only way to pain relief from a report of Vera-Portocarrero's group[19]. They evaluated in two models the effects of systemic/rostral ventromedial medulla (RVM) sumatriptan administration on visceral pain, and the role of RVM, a center of pain modulation, in the process of pain relief by sumatriptan. They developed a rat model for experimental pancreatitis by intravenous injection of dibutyltin dichloride, and another rat model for irritable bowel syndrome by intracolonic instillation of sodium butyrate. They observed the effects of systemic/RVM administration of 5-HT1B/D antagonists on systemic/RVM sumatriptan action. Systemic sumatriptan elicited a dose- and time-related blockade to pain in both models that was blocked by systemic administration of either 5-HT1B or 5-HT1D antagonists, but not by RVM administration of these agents. Sumatriptan administered into the RVM similarly produced dose and time-related blockade of referred hypersensitivity in both models. The pain was blocked by RVM administration of the 5-HT1B antagonists but not the 5-HT1D antagonists. Based on the results, the authors speculated that sumatriptan suppresses either inflammatory or noninflammatory visceral pain, most likely through peripheral 5-HT1B/D receptors. They also mentioned that actions at 5-HT1B receptors within the RVM offer an additional potential site of action for the modulation of visceral pain by triptans.

CONCLUSION

We hypothesize a comprehensive central and peripheral interaction schema to explain the symptoms of AM. As shown in Figure 1, visceral organ produced pain and increased cortical excitability, which in turn induce central symptoms such as photophobia, phonophobia, and AWS; with intricate interactions from the central and peripheral nervous system, effects such as ecchymosis are shown on the peripheral level. Although further investigations and accumulation of AM cases are still needed, we believe that, the schema hypothesized here is helpful to plan further experimental approach to clarify the mechanism of this peculiar disease.

ACKNOWLEDGMENTS

The corresponding author dedicates this paper to his wife Haruka and his lovely kids Atsushi and Satomi, and also to his family and colleagues who have always given him intellectual and moral support.

REFERENCES

1 **Buchanan JA**. The abdominal crisis of migraine. *J Nerv Ment Dis* 1921; **54**: 406-412
2 **Cuvellier JC**, Lépine A. Childhood periodic syndromes. *Pediatr Neurol* 2010; **42**: 1-11
3 **Headache Classification Subcommittee of the International Headache Society**. The International Classification of Headache Disorders: 2nd edition. *Cephalalgia* 2004; **24** Suppl 1: 9-160
4 **Rasquin A**, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, Walker LS. Childhood functional gastrointestinal disorders: child/adolescent. *Gastroenterology* 2006; **130**: 1527-1537
5 **Abu-Arafeh I**, Russell G. Prevalence and clinical features of abdominal migraine compared with those of migraine headache. *Arch Dis Child* 1995; **72**: 413-417
6 **Mortimer MJ**, Good PA. The VER as a diagnostic marker for childhood abdominal migraine. *Headache* 1990; **30**: 642-645
7 **Russell G**, Abu-Arafeh I, Symon DN. Abdominal migraine: evidence for existence and treatment options. *Paediatr Drugs* 2002; **4**: 1-8
8 **Hamed SA**. A migraine variant with abdominal colic and Alice in Wonderland syndrome: a case report and review. *BMC Neurol* 2010; **10**: 2
9 **Lippman CW**. Certain hallucinations peculiar to migraine. *J Nerv Ment Dis* 1952; **116**: 346-351
10 **Golden GS**. The Alice in Wonderland syndrome in juvenile migraine. *Pediatrics* 1979; **63**: 517-519
11 **Kakisaka Y**, Wakusawa K, Haginoya K, Uematsu M, Tsuchiya S. Abdominal migraine associated with ecchymosis of the legs and buttocks: does the symptom imply an unknown mechanism of migraine? *Tohoku J Exp Med* 2010; **221**: 49-51
12 **DeBroff BM**, Spierings EL. Migraine associated with periorbital ecchymosis. *Headache* 1990; **30**: 260-263
13 **Nozzolillo D**, Negro C, Nozzoli C, de Rini A, Marco V, Passarella B. Migraine associated with facial ecchymoses ipsilateral to the symptomatic side. *J Headache pain* 2004; **5**: 256-259
14 **Wesselmann U**, Lai J. Mechanisms of referred visceral pain: uterine inflammation in the adult virgin rat results in neurogenic plasma extravasation in the skin. *Pain* 1997; **73**: 309-317
15 **Crossman AR**. Neuroanatomy. In: Standring S, editor. Gray's anatomy: the anatomical basis of clinical practice. 40th ed. New York: Churchill Livingstone, 2008: 225-236
16 **Kakisaka Y**, Wakusawa K, Haginoya K, Saito A, Uematsu M, Yokoyama H, Sato T, Tsuchiya S. Efficacy of sumatriptan in two pediatric cases with abdominal pain-related functional gastrointestinal disorders: does the mechanism overlap that of migraine? *J Child Neurol* 2010; **25**: 234-237
17 **Bigal ME**, Krymchantowski AV, Ho T. Migraine in the triptan era: progresses achieved, lessons learned and future developments. *Arq Neuropsiquiatr* 2009; **67**: 559-569
18 **Jeong HJ**, Chenu D, Johnson EE, Connor M, Vaughan CW. Sumatriptan inhibits synaptic transmission in the rat midbrain periaqueductal grey. *Mol Pain* 2008; **4**: 54
19 **Vera-Portocarrero LP**, Ossipov MH, King T, Porreca F. Reversal of inflammatory and noninflammatory visceral pain by central or peripheral actions of sumatriptan. *Gastroenterology* 2008; **135**: 1369-1378

S- Editor Li JY **L- Editor** A **E- Editor** Zheng XM

World Journal of Experimental Medicine

Online Submissions: http://www.wjgnet.com/esps/
wjem@wjgnet.com
doi:10.5493/wjem.v2.i4.78

World J Exp Med 2012 August 20; 2(4): 78-85
ISSN 2220-315X (online)
© 2012 Baishideng. All rights reserved.

REVIEW

Potential roles of longan flower and seed extracts for anti-cancer

Chih-Cheng Lin, Yuan-Chiang Chung, Chih-Ping Hsu

Chih-Cheng Lin, Department of Biotechnology, Yuanpei University, Hsinchu City 30015, Taiwan, China
Yuan-Chiang Chung, Department of Surgery, Cheng-Ching General Hospital, Taichung City 40764, Taiwan, China
Chih-Ping Hsu, Department of Medical Laboratory Science and Biotechnology, Yuanpei University, Hsinchu City 30015, Taiwan, China
Author contributions: Lin CC and Chung YC contributed equally to this article; Lin CC and Hsu CP substantially contributed to conception and design, acquisition of data, analysis and interpretation of data; Lin CC and Chung YC drafted the article and revised it critically for important intellectual content; Chung YC and Hsu CP contributed to final approval of the version to be published.
Supported by A research grand from Cheng-Ching General Hospital, CH10000142
Correspondence to: Chih-Ping Hsu, PhD, Department of Medical Laboratory Science and Biotechnology, Yuanpei University, No. 306 Yuanpei Street, Hsinchu City 30015, Taiwan, China. hsucp@mail.ypu.edu.tw
Telephone: +886-3-6108166 Fax: +886-3-6102312
Received: February 21, 2012 Revised: July 21, 2012
Accepted: July 27, 2012
Published online: August 20, 2012

Abstract

Polyphenol-rich plants are known to possess benefits to human health. Recent studies have revealed that many Traditional Chinese Medicines (TCMs) are rich sources of polyphenols and exhibit antioxidant and anti-inflammatory activities, and these TCMs have been shown experimentally to overcome some chronic diseases, including cancer. Longan flowers and seeds, two TCMs traditionally used for relieving pain and urinary diseases, have been revealed in our recent reports and other studies to possess rich amounts of polyphenolic species and exhibit strong anti-oxidant activity, and these could be applied for the treatment of diabetes and cancer. Herein, we review the recent findings regarding the benefits of these two TCMs in the treatment of human cancer and the possible cellular and molecular mechanisms of both substances.

© 2012 Baishideng. All rights reserved.

Key words: Longan flower; Longan seed; Cancer; Oncoprotein; Tumor suppressor; Traditional Chinese Medicine

Peer reviewer: Ramireddy Bommireddy, PhD, Assistant Professor, Bio5 Institute, MRB Rm 323, 1656 E Mabel St, Tucson, AZ 85724-5217, United States

Lin CC, Chung YC, Hsu CP. Potential roles of longan flower and seed extracts for anti-cancer. *World J Exp Med* 2012; 2(4): 78-85 Available from: URL: http://www.wjgnet.com/2220-315X/full/v2/i4/78.htm DOI: http://dx.doi.org/10.5493/wjem.v2.i4.78

INTRODUCTION

Cancer has become the most common disease threatening public health, which has led to interventions for the prevention and treatment of this disease. Based on the fact that cancer exhibits a slow, stepwise development, and requires several years to become a life-threatening disease, it is regarded largely as a preventable disease[1-3]. Although improvements in medical techniques have been made in recent years, some types of cancer are still difficult to cure, even following advanced treatment. The challenge mainly arises owing to the recurrence, chemoresistance and distal metastasis of progressing cancer, which have become the important focuses of novel detection methods and treatment strategies[4]. Epidemiological studies show a correlation between increasing consumption of phenolic compounds and a reduced risk of cancer[5-8]. Plants are the primary source of polyphenols, and some have been regarded as forming part of a healthy diet for many years, such as tea, soybean, pome-

granate, and pine nuts[9]. Traditional Chinese Medicine (TCM) has been developed in China for more than two thousand years. TCMs comprise various forms of herbal medicine and complemental therapy such as acupuncture, massage (Tui na), exercise (qigong), and dietary therapy. The pharmacopoeia of TCM, named the Compendium of Materia Medica, records hundreds of medicinal substances such as plants, minerals and animal products, and their health beneficial action in the body. Different parts of plants, such as the leaves, roots, stems, flowers, and seeds, are used. Several clinically-used chemotherapeutic drugs are derived from TCMs, such as camptothecin, isolated from the "happy tree" (*Camptotheca accuminata*); etoposide, semi-synthesized from a compound of *Podophyllum emodi var. chinense*; vincristin and vinblastin, isolated from the Madagascar periwinkle (*Catharanthus roseus*); and paclitaxel, purified from *Taxus chinensis*[10,11]. Recent studies have further revealed that some TCMs or their components exhibit anti-tumor activities towards several types of cancer, such as liver[12], lung[13], gastric[14], nasopharyngeal[15] and colorectal cancer[16]. Non-steroidal anti-inflammatory drugs have been documented in animal and human studies to reduce the risk of colorectal cancer and adenomatous polyps and have been implicated as a cancer prevention and treatment strategy[1,17-19]. Longan flowers and seeds have been analyzed and were found to possess rich amounts of polyphenols, including proanthocyanidin A2, (-)-epicatechin, gallic acid and ellagic acid, and exhibit strong antioxidant and inflammatory activities[20-24]. Recently, several studies by our research group have further revealed that longan flower and seed extracts exhibit an anti-cancer activity towards colorectal, liver, lung, cervical and breast cancer. Herein, we review the recent findings regarding the benefits of these two TCMs in the treatment of human cancer and the possible cellular and molecular mechanisms of both substances.

LONGAN FLOWERS AND SEEDS IN TCM

Longan (*Dimocarpus Longan* Lour.) is a subtropical fruit grown throughout Asia, with southern China including Taiwan being the main center of commercial production. The longan fruit is a famous summer fruit and is used as a TCM as a stomachic, febrifuge, and vermifuge, and also as an antidote to poison[24]. In agriculture, off-season induction of flowering in longan trees is a desirable economic goal and is accomplished through the application of gibberellin biosynthesis inhibitors[25]. To increase the size and quality of the fruit, an important operation is to prune or remove flower spikes in the cluster. Longan flowers are sold in herb markets and due to their fresh and fruity aroma are mainly used to prepare an infusion that is drunk for pleasure in Taiwan. As described in a TCM pharmacopoeia named Herbal Quanzhou, drinking the water extract of longan flowers could overcome micturition, urgency and voiding dysfunction. The powder of dried longan seeds can be used for treating bleeding, dampness, hernia, lymphomegaly of the neck and armpit, odour, scabies and eczema, as described in another TCM pharmacopoeia named Chinese Herbal Medicine. The National Herbal Compendium of China also records that longan seed powder is generally applied for stomach pain and as a styptic. The multiple medical functions of longan flowers and seeds, and especially the reduction of swelling as recorded in the TCM pharmacopoeia, imply that these two TCMs can be applied in cases of microbial infection, inflammation, and metabolic diseases. Evidence to this end has been revealed by current scientific methods during the past decade.

Anti-oxidant activity and effect on inflammation and metabolic disorders of longan flower extracts

Although multiple medicinal applications of longan flowers are recorded in TCM pharmacopoeia, the scientific evidence related to their effect on human health has been accumulating over recent years. We have demonstrated that the hot water reflux or ethanol extract of the longan flower, contained abundant proantocyanidins and rarely anthocyanins, suppresses nitric oxide and prostaglandin E2 production in lipopolysaccharide -stimulated macrophage cell line RAW264.7 and may be the potential source of natural dietary anti-oxidants and anti-inflammatory agent[20]. The longan flower extract (LFE), analyzed by Professor Hwang and colleagues, exhibits a strong anti-oxidant activity, which is mainly due to (-)epicatechin and proanthocyanidin A2[26]. Proanthocyanidin-rich substances such as grape seed extracts have been implicated as preventive agents against cardiovascular disease and cancer, which are strongly associated with chronic inflammation diseases[9,27,28]. In another study by Professor Hwang, consumption of LFE reduced the blood pressure and oxidative markers such as plasma thiobarbituric acid and liver antioxidant enzyme activity in fructose-fed rats. Insulin action signaling, such as insulin receptor substrate-1 and glucose transporter 4, is enhanced in these rats, indicating the effect of LFE on overcoming insulin resistance[29]. Recent study has also revealed that feeding LFE to high-caloric-diet rats results in reduction of body weight, size of epididymal fat, serum triglycerides and atherogenic index. The main mechanism is downregulation of pancreas lipase, sterol regulatory element binding protein-1c and fatty acid synthase, and upregulation of low-density lipoprotein receptor and peroxisome proliferator-activated-receptor α expression, as well as promotion of fecal triglyceride excretion[30]. These studies indicate that LFE can not only be applied for the treatment of urinary disorders, but also has the potential for use as a preventive or treatment agent for metabolic diseases.

Anti-colorectal cancer effects of LFE

Polyphenol-rich extracts have been demonstrated to exert anti-cancer effects[31-33]. Owing to the rich amount of phenolics in LFE, our research team explored its possible role in human malignancy diseases. We selected colorectal cancer as the first subject, because it has been

the most common cancer type in Taiwan since 2007, when the dietary behavior of Taiwanese became more westernized[34]. Recent studies have revealed that the water reflux extract of longan flowers is enriched with two major compounds, (-)-epicatechin and proanthocyanidin A2[26], which are also found in grape seed extract as active anti-colorectal cancer agents[35,36]. Based on these reports, we proposed that LFE could play a possible role in colorectal cancer (CRC) prevention and treatment. In our recent study[37], we treated two CRC cell lines, SW480 and Colo 320DM, which are derived from Duke's B and Duke's C patients respectively, with LFE, and found an inhibitory effect on the proliferation of these two cell lines in a dose- and time-dependent manner. LFE treatment also affected the anchorage-independent growth of these two cell lines in soft agar, an *in vitro* assay to test the cancer cells clonogenic growth, and anoikis resistance, and was closely correlated with *in vivo* tumorigenesis[38,39]. The uncontrolled cell division, clonogenic growth and anoikis resistance are the main malignant properties leading cancer cells to unlimited growth and tumorigenesis *in vivo*[38-40]. The results strongly indicate that LFE is capable of influencing the malignant potential of CRC cells, which implies a role of LFE in the prevention and treatment of colorectal cancer. The mechanisms of LFE in anti-CRC growth are mainly due to cell-cycle arrest in the S phase and induction of mitochondria-mediated apoptosis. Previously, Kaur *et al*[36] reported that proanthocyanidin-rich grape seed extracts inhibited the proliferation of colorectal cancer cells due to their ability to cease G1-phase arrest of the cell cycle. However, LFE elevated cyclin E levels and downregulated cyclin A levels and arrested CRC cells in the DNA synthesis phase of the cell cycle. The elevation of cyclin E levels in both LFE-treated CRC cells was correlated with S-phase cell accumulation. Cyclin A is synthesized during the S phase and its functional disruption can inhibit chromosomal DNA replication[41-43]. Together with the accumulation of cyclin E, we conclude that the effect of LFE on the cell division cycle is mainly due to hampered DNA synthesis. LFE also exhibits selected apoptosis induction in Colo 320DM rather than SW480. Induction of apoptosis is another possible mechanism by which the anti-proliferative effect of LFE on colorectal cancer cells may be exerted. In the present study, we show that LFE treatment could induce apoptosis in one of the tested CRC cells (Colo 320DM). The apoptotic cells represented DNA fragmentation, mitochondrial membrane potential loss and the activation of caspase 3. The Bcl-2 family has been demonstrated as the main mechanism of naturally-occurring phytochemicals-induced apoptosis in cancer cells[44-47]. Bcl-2 inhibits Bax action and protects cytochrome C preservation in mitochondria, which leads to the maintenance of mitochondria membrane potential and keeping cell survive. The decrease in Bcl-2 level in LFE-treated Colo 320DM cells was correlated with apoptosis, indicating that the mechanism of apoptosis induction by LFE in this cell line is mainly due to suppression of the anti-apoptotic protein Bcl-2. LFE failed to induce apoptosis in SW480 cells, which may be associated with some DNA damage repair mechanisms and tumor suppressor gene mutations such as *p53* in SW480. This is an intriguing issue worthy of further investigation.

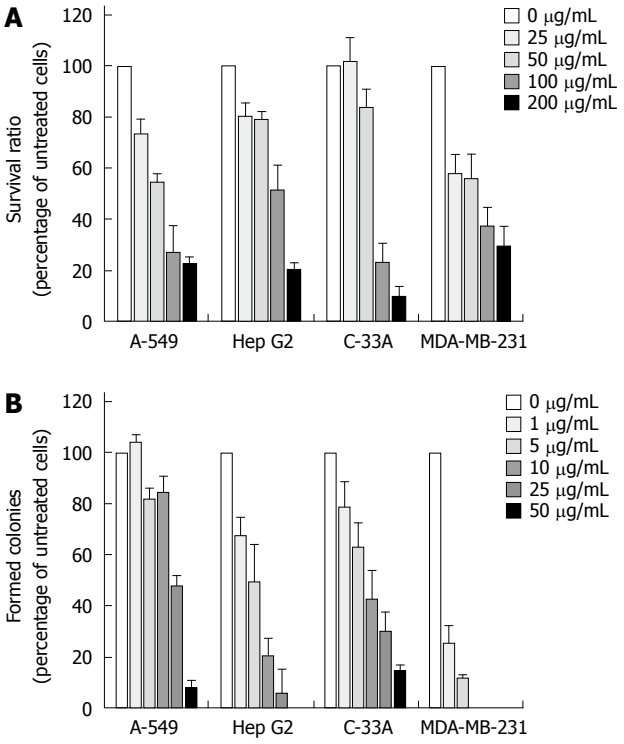

Figure 1 The inhibitory effect of longan seed extract on the growth of cancer cells. The longan seed extract (LSE)-inhibited growth assessed herein consists of cancer cell proliferation by (A) trypan blue assay and (B) colony forming activity. For the cell proliferation assay, 100 000 cancer cells as indicated were seeded in 60-mm sterile plastic dishes and treated with different concentrations of LSE. After incubation at 37 ℃ for 48 h, cells were detached by treatment with trypsin, and the suspended cells were stained with trypan blue. The viable cells were then counted under a phase contrast microscope. Colony formation activity was assessed by seeding 200 cells into 60-mm dishes then treating these cells with different concentrations of LSE. After incubation for 14 d, the formed colonies that contained more than 50 cells were regarded as one colony, and the numbers of colonies in the dishes were counted. The data represent the average of three independent experiments and are expressed as the mean ± SD.

DISCUSSION AND RESULTS

Potential role of longan seed extract in anti-CRC and other types of carcinomas

Longan seed extract (LSE) has been shown to consist of gallic acid, corilagin and ellagic acid as the main active components, resulting in anti-oxidant and tyrosinase activities[21]. As gallic acid, ellagic acid and corilagin have been investigated in terms of their effects against different types of human cancer, we proposed that LSE, rich in these three components, could exhibit anti-cancer activity. Our recent study showed that LSE is capable of inhibiting cell proliferation and clonogenic growth in SW480, HT-29 and Colo 320DM, indicating a similar role of LSE to that of LFE in anti-CRC cells[48]. Recently, we further tested LSE with regards to the growth inhibition

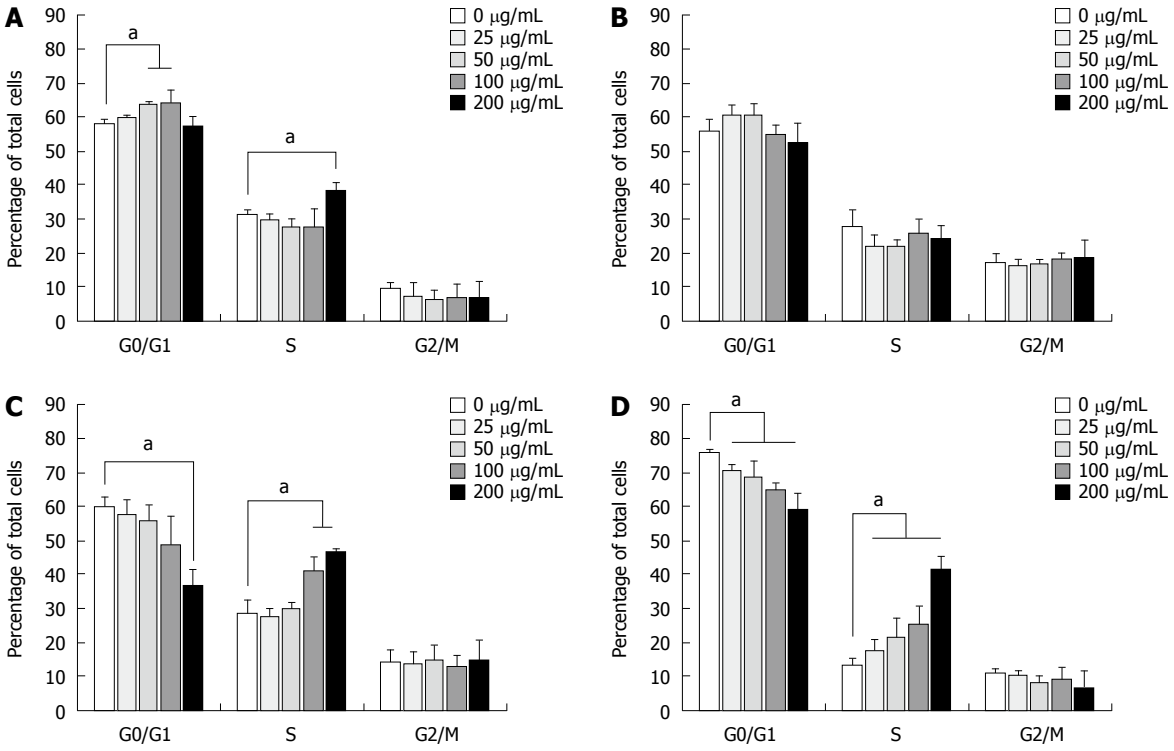

Figure 2 Cell cycle analysis of longan seed extract -treated cancer cells. About 1 × 10^6 cells in 10 mL medium in 100 mm plate were treated with increasing concentrations of longan seed extract as indicated, then incubated at 37 ℃ for 48 h. The (A) A-549, (B) Hep G2, (C) C-33A and (D) MDA-MB-231 cells harvested by trypsinization were fixed in 70% alcohol at -20 ℃ for 2 h and then reconstituted in phosphate-buffered saline. The cells were stained with propidium iodide solution in the dark at room temperature for 30 min. The stained cells were then analyzed using the FL-2A parameter of a flow cytometer to obtain the DNA content of the cells, and the distribution in each cell-cycle phase was determined using Modfit software. Data are expressed as a percentage of the total cells. Data represent the average of three independent experiments, and are expressed as the mean ± SD. $^aP < 0.05$.

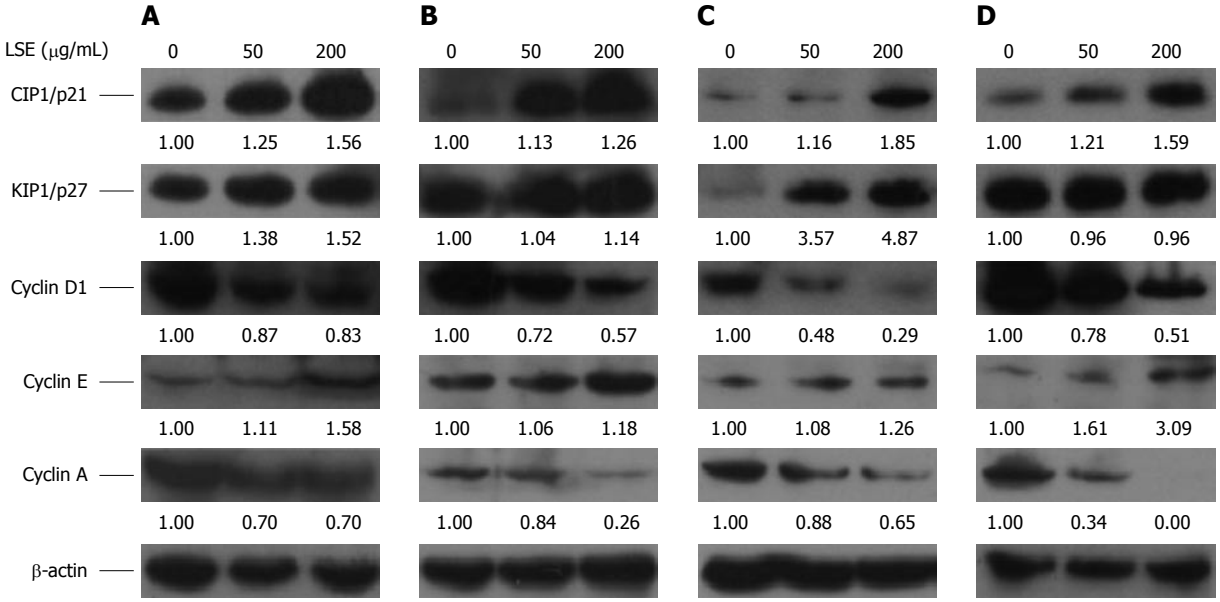

Figure 3 Immunoblots of cell cycle-controlling proteins in longan seed extract-treated cancer cells. About 50 and 200 μg/mL longan seed extract (LSE)-treated (A) A-549, (B) Hep G2, (C) C-33A and (D) MDA-MB-231 cells were incubated at 37 ℃ for 48 h. The harvested cells were lysed in Triton X 100-containing hypotonic buffer as per previous reports (Hsu *et al*[37], 2010; Chung *et al*[48], 2010) at 4 ℃ for 30 min. Cell lysates were centrifuged and the protein concentrations in the supernatants were determined using a bicinchoninic acid protein detection kit. Cell protein lysates were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes and immunoblotted to show cyclin D1, cyclin E, cyclin A, CIP1/p21 and KIP1/p27, with the β-actin level used as the loading control. The images are representative results from three independent experiments. The density of each protein band was measured using ImageJ software and protein expression was normalized to β-actin, and the relative amount of each protein band was referenced to the untreated control.

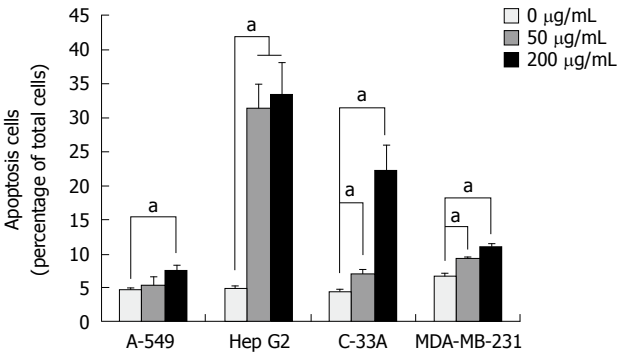

Figure 4 Detection of longan seed extract-induced apoptotic cells. Phosphatidylserine is usually distributed in the inner fleet of the plasma membrane. When cells are undergoing apoptosis, this phospholipid translocates to the outer fleet, and can be recognized by a bacteria glycoprotein named annexin V. We used annexin V conjugated with fluorescein isothiocyanate (FITC) as an apoptosis indicator and analyzed the apoptotic cells by flow cytometry. Briefly, 50 and 200 μg/mL longan seed extract-treated cells were incubated at 37 ℃ for 48 h. The treated cells were then suspended and stained with annexin V conjugated with FITC. Ten thousand cells were analyzed by flow cytometry using FL-1 as the parameter. Data are taken from the averages of three independent experiments and expressed as the mean ± SD. $^{a}P < 0.05$.

of lung adenocarcinoma cell line A549, hepatocellular carcinoma cell line Hep-G2, cervix carcinoma cell line C33A, and breast carcinoma cell line MDA-MB-231. The proliferation and colony-forming activity of these cancer cell lines were suppressed gradually by increasing LSE concentrations, and MDA-MB-231 was more sensitive to LSE than the other types of cancer cells (Figure 1). As shown in Figure 1A, the viability of MDA-MB-231 cells decreased to lower than 60% of the untreated cells following 25 μg/mL LSE treatment. A similar effect occurred in 50 μg/mL LSE-treated A549 cells and 100 μg/mL LSE-treated C33A and HepG2 cells. The colony-forming activity of these cancer cells was also suppressed. As shown in Figure 1B, the colonies of MDA-MB-231 cells were lower than 40% of the control colonies at 1 μg/mL LSE and 0% at more than 10 μg/mL LSE. Similar effects were seen in HepG2 cells at 10 and 50 μg/mL LSE, respectively. The colonies of C33A and A549 cells were lower than 40% compared to control cells at 25 μg/mL LSE, and 10%-20% compared to control cells appeared at 50 μg/mL LSE. Together, these results indicate that LSE is capable of influencing the proliferation and clonogenesis of colorectal, lung, liver, cervix and breast cancer cells.

LSE influences the expression of cell cycle-associated oncoproteins and suppressors

Our previous study demonstrated that LSE induced the cell cycle arrest of CRC cells in the DNA synthesis phase and suppressed cyclin D1 and cyclin A expression. Recently, we examined the effect of LSE on the cell-cycle progression of lung (A549), liver (HepG2), cervix (C33A) and breast cancer cells (MDA-MB-231). The S phase obviously increased in LSE-treated C33A and MDA-MB-231 cells, while the G1 phase increased in A549 cells (Figure 2). We further analyzed cell cycle-modulating proteins including cyclin D1, cyclin E, cyclin A, CIP/p21 and KIP/p27 in LSE-treated cells, and found that LSE systemically suppressed cyclin D1 and cyclin A expression and concomitantly enhanced CIP/p21 and KIP/p27 (Figure 3). The growth inhibition effect of naturally-occurring products on human cancer cells may arise from downregulation of oncoproteins or enhancement of tumor suppressor proteins[49-51]. Overexpression of cyclin D1 has been shown to play a pivotal role in promoting cancer cell proliferation, focus formation, tumorigenesis, drug resistance and metastasis, and has long been regarded as one of the important oncoproteins[52-56]. Loss of expression or function of CIP1/p21 and KIP1/p27 has been implicated in the genesis or progression of many human malignancies[57,58]. The suppression of cyclin D1 and enhancement of CIP1/p21 and KIP1/p27 expression by LSE treatment imply that the inhibitory effects of LSE on cancer cell growth may arise from the regulation of these proteins. The related cellular events are cell-cycle arrest in LSE-treated cancer cells, as these proteins are cell cycle-controlling proteins. Cyclin D1 and cyclin E are generated after mitogen stimulation in quiescent cells. These two proteins associate with CDK4/CDK6 and CDK2 and then enter the nucleus to phosphorylate/inactivate retinoblastoma protein, which leads cells to enter into the S phase[59]. Cyclin/CDKs activity is negatively regulated by inhibitors of cyclin-dependent kinases (INKs). CIP1/p21 and KIP1/p27 belong to the Kip/Cip family of INKs, which associate with CDK4,6/cyclin D1 and CDK2/cyclin E, A complex and inactivate their kinase activity by interfering with ATP binding and the cyclin/CDK structure[60]. Fifty μg/mL LSE-treated A549 cells exhibit decreased cyclin D1 and E and elevated CIP1/p21 and KIP1/p27, indicating the molecular mechanism of the LSE-induced G1-phase arrest. However, other cancer cells treated with the same concentration of LSE show no change or increased cyclin E and exhibit S-phase arrest (MDA-MB-231) or little change in the cell cycle (C33A, HepG2). S-phase arrest of LSE-treated cells occurs in MDA-MB-231 cells treated with more than 25 μg/mL LSE and C33A and A549 cells treated with more than 200 μg/mL LSE. Cyclin A, which is synthesized in the G1/S transition and associates with CDK2 to promote the S/G2 phase, is remarkably decreased in all LSE-treated MDA-MB-231 cells and in A549 and C33A cells treated with 200 μg/mL LSE. Our previous study indicated that elevated cyclin E and decreased cyclin A may be the key mechanism leading cancer cells to arrest in the S phase[37]. The results further confirm the distribution of cyclin E and A in S-phase arrest of cancer cells. The results taken together indicate that LSE interferes with tumor-promoting activities and cell-cycle progression by suppression of oncoproteins such as cyclin D1 and A and enhancement of suppressors such as CIP1/p21 and KIP1/p27.

LSE induced mitochondria-mediated apoptosis

Apoptosis is the essential mechanism by which unwanted

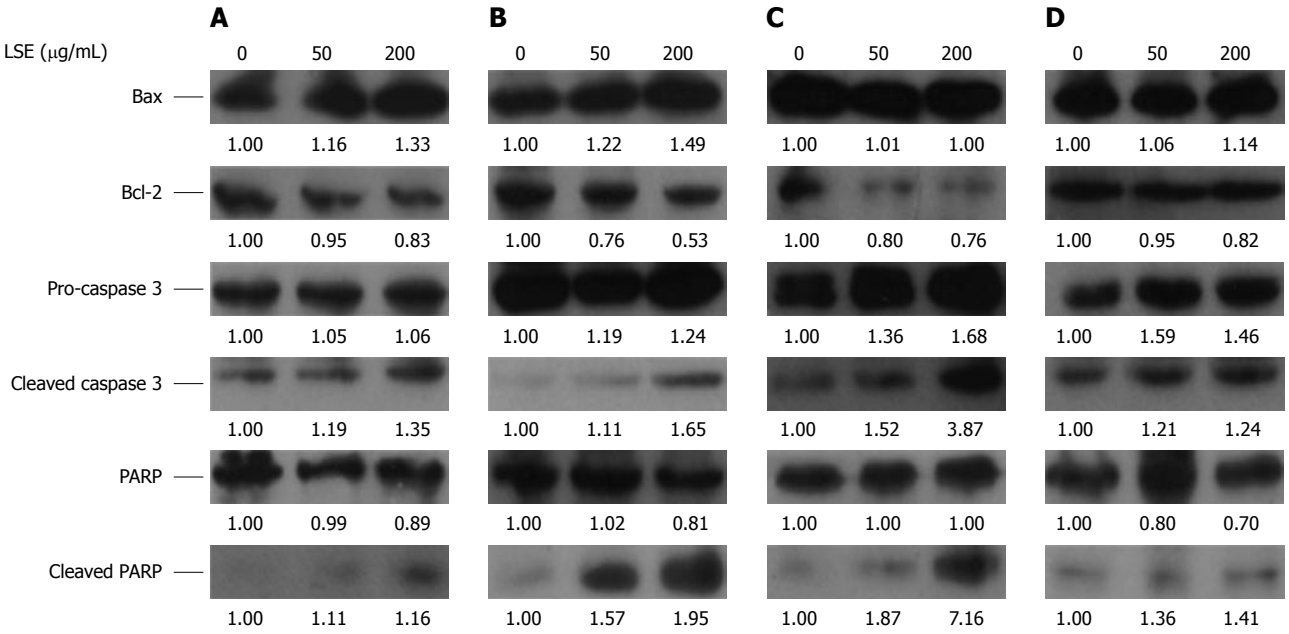

Figure 5 Immunoblots of apoptosis-associated proteins in longan seed extract-treated cancer cells. The same cell lysates from longan seed extract (LSE)-treated (A) A-549, (B) Hep G2, (C) C-33A and (D) MDA-MB-231 cells as described in Figure 3 were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes and immunoblotted to show the levels of Bax, Bcl-2, pro-Caspase 3, cleaved-caspase 3, poly (ADP-ribose) polymerase-1 (PARP-1) and cleaved-PARP. The images are representative of three independent experiments.

or damaged cells are eliminated during development or maintenance of tissue homeostasis in multiple cellular organisms[61,62]. Defective regulation of apoptosis has been closely associated with many human chronic diseases such as neural degeneration, autoimmune disease, AIDS and cancer[63]. Inducing cancer cells into apoptosis is the main mechanism by which anti-neoplasm drugs or natural products suppress cancer cell growth[62]. In our previous study, induction of apoptosis was found to be another possible mechanism of the antiproliferative activity of LSE on CRC cells[48]. However, one CRC cell line appeared to be resistant to LSE-induced apoptosis. Result of present study further showed that LSE selectively induced significant apoptosis in HepG2 and C33A cells but only a slight elevation of apoptosis in A549 and MDA-MB-231 cells (Figure 4). Caspase 3 was activated by the proteolytic cleavage of upstream apoptosome and the consequent cleavage of substrates such as PARP. Many reports suggest that the activation of caspase 3 is a common event during polyphenolics-induced apoptosis of cancer cells[35-37,64]. Our previous study showed that LSE enhances caspase 3 activity in CRC cells and induces apoptosis. Our recent results further showed that LSE induced pro-caspase 3 expression and cleavage (Figure 5). LSE-induced caspase 3 activation and apoptosis may operate through the Bcl-2 family of proteins. The Bcl-2 family members are important mediators of mitochondria-induced apoptosis in cancer cells[61,65,66]. These proteins form multimers, which act as pores in cell membranes, controlling the flow of molecules[67]. Bcl-2 has been associated with apoptosis inhibition, whereas expression of Bax has been associated with apoptosis induction[68,69]. Bcl-2 inhibits apoptosis by inhibiting the release of cytochrome c (Apaf 2) and apoptosis inducing factor (AIF) from the mitochondria to the cytoplasm, and by limiting the activation of caspase 3 by inhibiting its activator protein, Apaf 1[70]. Recent studies indicate that the ratio of Bax:Bcl-2 proteins is the determining factor in transmitting the apoptosis signal[46,67,71,72]. In our study, the Bcl-2 levels were decreased in LSE-treated cancer cells, especially in sensitive cells[48]; however, the change in Bax levels in these cells are not affected, indicating that LSE-induced apoptosis is mainly due to the regulation of the level of Bcl-2.

CONCLUSION

The longan is one of the most important fruits in China, economically speaking. The flowers and seeds of the longan were regarded as waste for a long time, and failed to be utilized. However, according to TCM pharmacopoeia, longan flowers and seeds possess multiple pharmaceutical applications. Recent advanced biotechnology and pharmacology techniques allow us to gain a deeper insight into the functions of these two TCMs using scientific methods. LFE could suppress oxidation and inflammation, decrease blood pressure, triglycerides and body weight, and overcome metabolic diseases such as diabetes mellitus. We provide data to demonstrate that LFE is also capable of inducing cell-cycle arrest and apoptosis, at least in colorectal cancer cells, implying that LFE could also be applied as an anti-neoplasm agent. LSE systemically inhibits colorectal, lung, liver, cervix and breast cancer cells, indicating the utilization of LSE in cancer prevention and treatment. LSE could suppress oncoproteins such as cyclin D1, A and Bcl-2 and elevate suppressors

such as caspase 3, Bax, CIP1/p21 and KIP1/p27. This may provide a possible role of LSE as a multiple-target therapeutic agent to control abnormal growth and malignancy in cancer. The *in vivo* efficacy of both LFE and LSE in mice tumorigenesis is the next important issue for further investigation, and indeed this research is ongoing in our research team. In conclusion, recent advanced studies have validated the novel pharmaceutical functions of LFE and LSE, especially their anti-cancer functions, and have provided scientific evidence to further the application of these two TCMs in the pharmaceutical industry.

REFERENCES

1 **Algra AM**, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. *Lancet Oncol* 2012; **13**: 518-527
2 **Tanaka T**, Shnimizu M, Moriwaki H. Cancer chemoprevention by carotenoids. *Molecules* 2012; **17**: 3202-3242
3 **Narayanan BA**. Chemopreventive agents alters global gene expression pattern: predicting their mode of action and targets. *Curr Cancer Drug Targets* 2006; **6**: 711-727
4 **Ribatti D**. Cancer stem cells and tumor angiogenesis. *Cancer Lett* 2012; **321**: 13-17
5 **Brown EM**, Gill CI, McDougall GJ, Stewart D. Mechanisms underlying the anti-proliferative effects of berry components in in vitro models of colon cancer. *Curr Pharm Biotechnol* 2012; **13**: 200-209
6 **Ros E**. Health benefits of nut consumption. *Nutrients* 2010; **2**: 652-682
7 **Halliwell B**. Antioxidants and human disease: a general introduction. *Nutr Rev* 1997; **55**: S44-S9; discussion S44-S9
8 **Surh YJ**. Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer* 2003; **3**: 768-780
9 **Kaur M**, Agarwal C, Agarwal R. Anticancer and cancer chemopreventive potential of grape seed extract and other grape-based products. *J Nutr* 2009; **139**: 1806S-1812S
10 **Efferth T**, Fu YJ, Zu YG, Schwarz G, Konkimalla VS, Wink M. Molecular target-guided tumor therapy with natural products derived from traditional Chinese medicine. *Curr Med Chem* 2007; **14**: 2024-2032
11 **Efferth T**, Li PC, Konkimalla VS, Kaina B. From traditional Chinese medicine to rational cancer therapy. *Trends Mol Med* 2007; **13**: 353-361
12 **Wu P**, Dugoua JJ, Eyawo O, Mills EJ. Traditional Chinese Medicines in the treatment of hepatocellular cancers: a systematic review and meta-analysis. *J Exp Clin Cancer Res* 2009; **28**: 112
13 **Tian G**, Guo L, Gao W. Use of compound Chinese medicine in the treatment of lung cancer. *Curr Drug Discov Technol* 2010; **7**: 32-36
14 **Wu M**, Yao B. Advances in TCM treatment of gastric cancer and studies on the apoptosis. *J Tradit Chin Med* 2002; **22**: 303-307
15 **Cho WC**, Chen HY. Clinical efficacy of traditional Chinese medicine as a concomitant therapy for nasopharyngeal carcinoma: a systematic review and meta-analysis. *Cancer Invest* 2009; **27**: 334-344
16 **Tan KY**, Liu CB, Chen AH, Ding YJ, Jin HY, Seow-Choen F. The role of traditional Chinese medicine in colorectal cancer treatment. *Tech Coloproctol* 2008; **12**: 1-6; discussion 6
17 **Cathcart MC**, Lysaght J, Pidgeon GP. Eicosanoid signalling pathways in the development and progression of colorectal cancer: novel approaches for prevention/intervention. *Cancer Metastasis Rev* 2011; **30**: 363-385
18 **Schrör K**. Pharmacology and cellular/molecular mechanisms of action of aspirin and non-aspirin NSAIDs in colorectal cancer. *Best Pract Res Clin Gastroenterol* 2011; **25**: 473-484
19 **Johnson CC**, Hayes RB, Schoen RE, Gunter MJ, Huang WY. Non-steroidal anti-inflammatory drug use and colorectal polyps in the Prostate, Lung, Colorectal, And Ovarian Cancer Screening Trial. *Am J Gastroenterol* 2010; **105**: 2646-2655
20 **Ho SC**, Hwang LS, Shen YJ, Lin CC. Suppressive effect of a proanthocyanidin-rich extract from longan (Dimocarpus longan Lour.) flowers on nitric oxide production in LPS-stimulated macrophage cells. *J Agric Food Chem* 2007; **55**: 10664-10670
21 **Rangkadilok N**, Sitthimonchai S, Worasuttayangkurn L, Mahidol C, Ruchirawat M, Satayavivad J. Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. *Food Chem Toxicol* 2007; **45**: 328-336
22 **Rangkadilok N**, Worasuttayangkurn L, Bennett RN, Satayavivad J. Identification and quantification of polyphenolic compounds in Longan (Euphoria longana Lam.) fruit. *J Agric Food Chem* 2005; **53**: 1387-1392
23 **Soong YY**, Barlow PJ. Isolation and structure elucidation of phenolic compounds from longan (Dimocarpus longan Lour.) seed by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr A* 2005; **1085**: 270-277
24 **Yang B**, Jiang YM, Shi J, Chen F, Ashraf M. Extraction and pharmacological properties of bioactive compounds from longan (Dimocarpus longan Lour.) fruit - A review. *Food Research International* 2011; **44**: 1837-1842
25 **Manochai P**, Sruamsiri P, Wiriya-alongkorn W, Naphrom D, Hegele M, Bangerth F. Year around off season flower induction in longan (Dimocarpus longan, Lour.) trees by KClO3 applications: potentials and problems. *Sci Hortic* 2005; **104**: 379-390
26 **Hsieh MC**, Shen YJ, Kuo YH, Hwang LS. Antioxidative activity and active components of longan (Dimocarpus longan Lour.) flower extracts. *J Agric Food Chem* 2008; **56**: 7010-7016
27 **Pan MH**, Lai CS, Wu JC, Ho CT. Molecular mechanisms for chemoprevention of colorectal cancer by natural dietary compounds. *Mol Nutr Food Res* 2011; **55**: 32-45
28 **Kidd PM**. Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea, and grape seed extracts. *Altern Med Rev* 2009; **14**: 226-246
29 **Tsai HY**, Wu LY, Hwang LS. Effect of a proanthocyanidin-rich extract from longan flower on markers of metabolic syndrome in fructose-fed rats. *J Agric Food Chem* 2008; **56**: 11018-11024
30 **Yang DJ**, Chang YY, Hsu CL, Liu CW, Lin YL, Lin YH, Liu KC, Chen YC. Antiobesity and hypolipidemic effects of polyphenol-rich longan (Dimocarpus longans Lour.) flower water extract in hypercaloric-dietary rats. *J Agric Food Chem* 2010; **58**: 2020-2027
31 **Yang CS**, Wang H. Mechanistic issues concerning cancer prevention by tea catechins. *Mol Nutr Food Res* 2011; **55**: 819-831
32 **Wilken R**, Veena MS, Wang MB, Srivatsan ES. Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. *Mol Cancer* 2011; **10**: 12
33 **Korkina LG**, De Luca C, Kostyuk VA, Pastore S. Plant polyphenols and tumors: from mechanisms to therapies, prevention, and protection against toxicity of anti-cancer treatments. *Curr Med Chem* 2009; **16**: 3943-3965
34 Department of Health. 2011. Available from: URL: http://www.doh.gov.tw/CHT2006/DM/DM2_2.aspx?now_fod_list_no=11965&class_no=440&level_no=5. Accessed on December 20, 2011
35 **Hsu CP**, Lin YH, Chou CC, Zhou SP, Hsu YC, Liu CL, Ku

FM, Chung YC. Mechanisms of grape seed procyanidin-induced apoptosis in colorectal carcinoma cells. *Anticancer Res* 2009; **29**: 283-289
36 **Kaur M**, Singh RP, Gu M, Agarwal R, Agarwal C. Grape seed extract inhibits in vitro and in vivo growth of human colorectal carcinoma cells. *Clin Cancer Res* 2006; **12**: 6194-6202
37 **Hsu CP**, Lin YH, Zhou SP, Chung YC, Lin CC, Wang SC. Longan flower extract inhibits the growth of colorectal carcinoma. *Nutr Cancer* 2010; **62**: 229-236
38 **Coates JM**, Galante JM, Bold RJ. Cancer therapy beyond apoptosis: autophagy and anoikis as mechanisms of cell death. *J Surg Res* 2010; **164**: 301-308
39 **Chiarugi P**, Giannoni E. Anoikis: a necessary death program for anchorage-dependent cells. *Biochem Pharmacol* 2008; **76**: 1352-1364
40 **Westhoff MA**, Fulda S. Adhesion-mediated apoptosis resistance in cancer. *Drug Resist Updat* 2009; **12**: 127-136
41 **Müller GA**, Engeland K. The central role of CDE/CHR promoter elements in the regulation of cell cycle-dependent gene transcription. *FEBS J* 2010; **277**: 877-893
42 **Piechaczyk M**, Farràs R. Regulation and function of JunB in cell proliferation. *Biochem Soc Trans* 2008; **36**: 864-867
43 **Pines J**, Hunter T. p34cdc2: the S and M kinase? *New Biol* 1990; **2**: 389-401
44 **Geng F**, Tang L, Li Y, Yang L, Choi KS, Kazim AL, Zhang Y. Allyl isothiocyanate arrests cancer cells in mitosis, and mitotic arrest in turn leads to apoptosis via Bcl-2 protein phosphorylation. *J Biol Chem* 2011; **286**: 32259-32267
45 **Brito PM**, Simões NF, Almeida LM, Dinis TC. Resveratrol disrupts peroxynitrite-triggered mitochondrial apoptotic pathway: a role for Bcl-2. *Apoptosis* 2008; **13**: 1043-1053
46 **Mantena SK**, Baliga MS, Katiyar SK. Grape seed proanthocyanidins induce apoptosis and inhibit metastasis of highly metastatic breast carcinoma cells. *Carcinogenesis* 2006; **27**: 1682-1691
47 **Meeran SM**, Katiyar SK. Grape seed proanthocyanidins promote apoptosis in human epidermoid carcinoma A431 cells through alterations in Cdki-Cdk-cyclin cascade, and caspase-3 activation via loss of mitochondrial membrane potential. *Exp Dermatol* 2007; **16**: 405-415
48 **Chung YC**, Lin CC, Chou CC, Hsu CP. The effect of Longan seed polyphenols on colorectal carcinoma cells. *Eur J Clin Invest* 2010; **40**: 713-721
49 **Weng JR**, Bai LY, Chiu CF, Wang YC, Tsai MH. The dietary phytochemical 3,3'-diindolylmethane induces G2/M arrest and apoptosis in oral squamous cell carcinoma by modulating Akt-NF-κB, MAPK, and p53 signaling. *Chem Biol Interact* 2012; **195**: 224-230
50 **Rajasekaran D**, Elavarasan J, Sivalingam M, Ganapathy E, Kumar A, Kalpana K, Sakthisekaran D. Resveratrol interferes with N-nitrosodiethylamine-induced hepatocellular carcinoma at early and advanced stages in male Wistar rats. *Mol Med Report* 2011; **4**: 1211-1217
51 **Melchini A**, Costa C, Traka M, Miceli N, Mithen R, De Pasquale R, Trovato A. Erucin, a new promising cancer chemopreventive agent from rocket salads, shows anti-proliferative activity on human lung carcinoma A549 cells. *Food Chem Toxicol* 2009; **47**: 1430-1436
52 **Musgrove EA**, Caldon CE, Barraclough J, Stone A, Sutherland RL. Cyclin D as a therapeutic target in cancer. *Nat Rev Cancer* 2011; **11**: 558-572
53 **Wang C**, Lisanti MP, Liao DJ. Reviewing once more the c-myc and Ras collaboration: converging at the cyclin D1-CDK4 complex and challenging basic concepts of cancer biology. *Cell Cycle* 2011; **10**: 57-67
54 **Alao JP**. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. *Mol Cancer* 2007; **6**: 24
55 **Fu M**, Wang C, Li Z, Sakamaki T, Pestell RG. Minireview: Cyclin D1: normal and abnormal functions. *Endocrinology* 2004; **145**: 5439-5447
56 **Tashiro E**, Tsuchiya A, Imoto M. Functions of cyclin D1 as an oncogene and regulation of cyclin D1 expression. *Cancer Sci* 2007; **98**: 629-635
57 **Starostina NG**, Kipreos ET. Multiple degradation pathways regulate versatile CIP/KIP CDK inhibitors. *Trends Cell Biol* 2012; **22**: 33-41
58 **Abukhdeir AM**, Park BH. P21 and p27: roles in carcinogenesis and drug resistance. *Expert Rev Mol Med* 2008; **10**: e19
59 **Malumbres M**, Barbacid M. To cycle or not to cycle: a critical decision in cancer. *Nat Rev Cancer* 2001; **1**: 222-231
60 **Pavletich NP**. Mechanisms of cyclin-dependent kinase regulation: structures of Cdks, their cyclin activators, and Cip and INK4 inhibitors. *J Mol Biol* 1999; **287**: 821-828
61 **Park JW**, Choi YJ, Jang MA, Lee YS, Jun DY, Suh SI, Baek WK, Suh MH, Jin IN, Kwon TK. Chemopreventive agent resveratrol, a natural product derived from grapes, reversibly inhibits progression through S and G2 phases of the cell cycle in U937 cells. *Cancer Lett* 2001; **163**: 43-49
62 **Scatena R**. Mitochondria and cancer: a growing role in apoptosis, cancer cell metabolism and dedifferentiation. *Adv Exp Med Biol* 2012; **942**: 287-308
63 **Thompson CB**. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995; **267**: 1456-1462
64 **Engelbrecht AM**, Mattheyse M, Ellis B, Loos B, Thomas M, Smith R, Peters S, Smith C, Myburgh K. Proanthocyanidin from grape seeds inactivates the PI3-kinase/PKB pathway and induces apoptosis in a colon cancer cell line. *Cancer Lett* 2007; **258**: 144-153
65 **Green DR**, Reed JC. Mitochondria and apoptosis. *Science* 1998; **281**: 1309-1312
66 **Reed JC**. Double identity for proteins of the Bcl-2 family. *Nature* 1997; **387**: 773-776
67 **Reed JC**. Balancing cell life and death: bax, apoptosis, and breast cancer. *J Clin Invest* 1996; **97**: 2403-2404
68 **Oltvai ZN**, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 1993; **74**: 609-619
69 **Zhan Q**, Fan S, Bae I, Guillouf C, Liebermann DA, O'Connor PM, Fornace AJ. Induction of bax by genotoxic stress in human cells correlates with normal p53 status and apoptosis. *Oncogene* 1994; **9**: 3743-3751
70 **Rossé T**, Olivier R, Monney L, Rager M, Conus S, Fellay I, Jansen B, Borner C. Bcl-2 prolongs cell survival after Bax-induced release of cytochrome c. *Nature* 1998; **391**: 496-499
71 **Chresta CM**, Masters JR, Hickman JA. Hypersensitivity of human testicular tumors to etoposide-induced apoptosis is associated with functional p53 and a high Bax: Bcl-2 ratio. *Cancer Res* 1996; **56**: 1834-1841
72 **Reed JC**, Miyashita T, Takayama S, Wang HG, Sato T, Krajewski S, Aimé-Sempé C, Bodrug S, Kitada S, Hanada M. BCL-2 family proteins: regulators of cell death involved in the pathogenesis of cancer and resistance to therapy. *J Cell Biochem* 1996; **60**: 23-32

S- Editor Li JY **L- Editor** A **E- Editor** Zheng XM

World Journal of Experimental Medicine

Online Submissions: http://www.wjgnet.com/esps/
wjem@wjgnet.com
www.wjgnet.com

World J Exp Med 2012 August 20; 2(4): I
ISSN 2220-315X (online)
© 2012 Baishideng. All rights reserved.

Acknowledgments to reviewers of *World Journal of Experimental Medicine*

We acknowledge our sincere thanks to our reviewers. Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of our World Series Journals. Both the editors of the journals and authors of the manuscripts submitted to the journals are grateful to the following reviewers for reviewing the articles (either published or rejected) over the past period of time.

Jan Bernardy, MVD, PhD, Assistant Professor, Swine Clinic, Veterinary Faculty, Palackého str No. 1, 61300 Brno, Czech Republic

Nadia Alfaidy, PhD, iRTSV-Biology of Cancer and infection, INSERM U1036, 17 rue des Martyrs, 38054 Grenoble, France

Sorin Armeanu-Ebinger, Professor, Department of Pediatric Surgery, Children's Hospital, University of Tuebingen, Hoppe-Seyler-Str.3, 72076 Tübingen, Germany

Effie K Basdra, DMD, Associate Professor, Department of Biological Chemistry, University of Athens Medical School, 75 M. Asias street – Goudi, GR-11527 Athens, Greece

Arun Bhunia, BVSc, PhD, Professor of Molecular Food Microbiology, Department of Food Science, Department of Comparative Pathobiology, Purdue University, 745 Agriculture Mall Dr., West Lafayette, IN 47907, United States

Steven G Gray, PhD, Translational Cancer Research Group, Department of Clinical Medicine, Trinity Centre for Health Sciences, Rm 2.103, Institute of Molecular Medicine, St James's Hospital, Dublin 8, Ireland

Winn Aung, MBBS, PhD, Diagnostic Imaging Program, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan

Gam Lay Harn, PhD, Associate Professor, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia

Dalwoong Choi, Associate Professor, Department of Environmental Health, College of Health Sciences, Korea University, JungLeung-3-Dong, SungBook-Gu, Seoul 136-703, South Korea

Karl O Fagerstrom, PhD, Associate Professor, Fagerstrom Consulting, Jordkull 3670, Kagerod 26878, Sweden

Ali Kudret Adiloglu, MD, Associate Professor, S.B. Ankara Egitim ve Arastlrma Hastanesi, Mikrobiyoloji Laboratuvarl Klinik lefi, Ulucanlar Cd., 19. Sok. No: 16/6 Israil Evleri 06500, Emek, Ankara, Turkey

Anshu Agrawal, PhD, Associate Adjunct Professor, C-240A, Med/Sci-I, Division of Basic and Clinical Immunology, Department of Medicine, University of California, Irvine, CA 92697, United States

World Journal of Experimental Medicine

Online Submissions: http://www.wjgnet.com/esps/
wjem@wjgnet.com
www.wjgnet.com

World J Exp Med 2012 August 20; 2(4): I
ISSN 2220-315X (online)
© 2012 Baishideng. All rights reserved.

Events Calendar 2012

January 15-20, 2012
Fungal Pathogens: From Basic Biology to Drug
Santa Fe, NM, United States

January 20-20, 2012
Exploiting Bacteriophages for Bioscience, Biotechnology and Medicine
London, United Kingdom

January 22-27, 2012
Biology of Spirochetes
Ventura, CA, United States

February 7-12, 2012
Gene Silencing by Small RNAs
Vancouver, British Columbia, Canada

March 4 -10, 2012
Malaria Experimental Genetics
Hinxton, Cambridge, United Kingdom

March 12-13, 2012
2nd Annual International Conference on BioInformatics and Computational Biology Special Track: Stem Cell Research
Singapore

March 12-13, 2012
BICB 2012: 2nd Annual International Conference on BioInformatics and Computational Biology (updated)
Global Science and Technology Forum
Thailand

March 18-21, 2012
Annual Conference of the Association for General and Applied Microbiology
Tubingen, Germany

March 31-April 3, 2012
22nd European Congress of Clinical Microbiology and Infectious Diseases ECCMID
London, United Kingdom

April 2-4, 2012
Electron transfer at the microbe-mineral interface
Norwich, United Kingdom

April 18, 2012
6th Broadening Microbiology Horizons in Biomedical Science Meeting
Stratford-Upon-Avon, United Kingdom

May 6-12, 2012
4th ASM Conference on Prokaryotic Cell Biology and Development
Montreal, Canada

May 7-19, 2012
Bioinformatics and comparative genomes analyses
Napoli, Italy

May 8 - 10, 2012
Exploring Human Host-Microbiome Interactions in Health and Disease
Cambridge, United Kingdom

May 30-31, 2012
European Lab Automation
Hamburg, Germany

June 3-8, 2012
Anaerobes in Health and Disease; How to Isolate, Identify and Look for Resistance in a Cost-Effective Way
Szeged, Hungary

June 16-21, 2012
Gene transcription in yeast
Girona, Spain

June 21-22, 2012
Swiss Joint Annual Meeting
St. Gallen, Switzerland

July 22-27, 2012
15th International Conference on Experimental Mechanics
University of Porto
Portugal

July 29-August 2, 2012
XV IS-MPMI Kyoto 2012.
International Congress on Molecular Plant-Microbe Interactions
Kyoto, Japan

August 18-22, 2012
The 30th World Congress of Biomedical Laboratory Science
Berlin, Germany

August 25-September 1, 2012
Update on Indications, Interactions and Complications in the Use of Pharmaceuticals
Honolulu, Hawaii, United States

September 7-9, 2012
International Congress of Maritime Medicine
Odessa, Ukraine

October 14-24, 2012
Medical Ethics and Legal Medicine
Miami, Florida, United States

December 1-6, 2012
Qatar Health 2012
Doha, Qatar

World Journal of Experimental Medicine

Online Submissions: http://www.wjgnet.com/esps/
wjem@wjgnet.com
www.wjgnet.com

World J Exp Med 2012 August 20; 2(4): I-V
ISSN 2220-315X (online)
© 2012 Baishideng. All rights reserved.

GENERAL INFORMATION

World Journal of Experimental Medicine (*World J Exp Med*, *WJEM*, online ISSN 2220-315X, DOI: 10.5493) is a bimonthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 104 experts in experimental medicine from 30 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 open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

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 *WJEM* 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 *WJEM* is an OA journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJEM* 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.

Aims and scope

WJEM aims to rapidly report rapidly new theories, methods and techniques for prevention, diagnosis, treatment, rehabilitation and nursing in the field of experimental medicine. *WJEM* covers topics concerning clinical laboratory medicine (applied and basic research in hematology, body fluid examination, cytomorphology, genetic diagnosis of hematological disorders, thrombosis and hemostasis, and blood typing and transfusion), biochemical examination (applied and basic research in laboratory automation and information system, biochemical methodology, and biochemical diagnostics), clinical microbiology (microbiological laboratory quality control and management; microbiological specimen collection and its influencing factors; conventional, automatic or molecular detection of clinical microorganisms; monitoring of bacterial and fungal drug resistance, drug resistance mechanisms, and rational application of antibiotics; monitoring and control of nosocomial infections), immunodiagnostics (laboratory diagnosis of infectious diseases, tumor markers and their application, laboratory diagnosis of autoimmune diseases, and immunotechnology), clinical laboratory management (laboratory quality control and management, traceability and calibration, information management system and laboratory automation, and laboratory biosafety management), and experimental medicine-related traditional medicine, and integrated Chinese and Western medicine. The journal also publishes original articles and reviews that report the results of experimental medicine-related applied and basic research in fields such as immunology, physiopathology, cell biology, pharmacology, medical genetics, and pharmacology of Chinese herbs.

Columns

The columns in the issues of *WJEM* will include: (1) Editorial: To introduce and comment on the substantial advance and its importance in the fast-developing areas; (2) Frontier: To review the most representative achievements and comment on the current research status in the important fields, and propose directions for the 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 Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (6) Review: To systemically review the most representative progress and unsolved problems in the major scientific disciplines, comment on the current research status, and make suggestions on the future work; (7) Original Articles: To originally report the innovative and valuable findings in experimental medicine; (8) Brief Articles: To briefly report the novel and innovative findings in experimental medicine; (9) Case Report: To report a rare or typical case; (10) Letters to the Editor: To discuss and make reply to the contributions published in *WJEM*, or to introduce and comment on a controversial issue of general interest; (11) Book Reviews: To introduce and comment on quality monographs of experimental medicine; and (12) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on the research in experimental medicine.

Name of journal
World Journal of Experimental Medicine

ISSN
ISSN 2220-315X (online)

Editor-in-Chief
De-Ling Kong, PhD, Professor, Institute of Molecular Biology, Nankai University, Tianjin 300071, China

Atsushi Mizoguchi, MD, PhD, Associate Professor in Pathology, Harvard Medical School, Molecular Pathology Unit, Massachusetts General Hospital, CNY149-6024, 13th Steert, Charlestown, MA 02114, United States

Baohong Zhang, PhD, Assistant Professor of Biology, Department of Biology, East Carolina University, Greenville, NC 27858, United States

Editorial Office
World Journal of Experimental Medicine
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjem@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-85381891
Fax: +86-10-85381893

Indexed and Abstracted in
Digital Object Identifier.

Published by
Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

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

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc.* The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJEM* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

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 Baishideng Publishing Group Co., Limited, 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 ICMJE 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 Submis-

sion System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2220-315x/g_info_20100722180909.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjem@wjgnet.com, or by telephone: +86-10-85381891. 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 ICMJE, 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: +86-10-85381892 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 *WJR*, 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 and brief articles, 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/2220-315x/g_info_20100725072755.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/index.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/2220-315x/g_info_20100725073806.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.*

Examples for paper writing
Editorial: http://www.wjgnet.com/2220-315x/g_info_20100725071851.htm

Frontier: http://www.wjgnet.com/2220-315x/g_info_20100725071932.htm

Topic highlight: http://www.wjgnet.com/2220-315x/g_info_20100725072121.htm

Observation: http://www.wjgnet.com/2220-315x/g_info_20100725072232.htm

Guidelines for basic research: http://www.wjgnet.com/2220-315x/g_info_20100725072344.htm

Guidelines for clinical practice: http://www.wjgnet.com/2220-315x/g_info_20100725072543.htm

Review: http://www.wjgnet.com/2220-315x/g_info_20100725072656.htm

Original articles: http://www.wjgnet.com/2220-315x/g_info_20100725072755.htm

Brief articles: http://www.wjgnet.com/2220-315x/g_info_20100725072920.htm

Case report: http://www.wjgnet.com/2220-315x/g_info_20100725073015.htm

Letters to the editor: http://www.wjgnet.com/2220-315x/g_info_20100725073136.htm

Book reviews: http://www.wjgnet.com/2220-315x/g_info_20100725073214.htm

Guidelines: http://www.wjgnet.com/2220-315x/g_info_20100725073300.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJEM*. The revised version including manuscript and high-resolution image figures (if any) should be re-submitted online (http://www.wjgnet.com/2220-315xoffice/). The author should send the copyright transfer letter, responses to the reviewers, English language Grade B certificate (for non-native speakers of English) and final manuscript checklist to wjem@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/2220-315X/g_info_20100725073726.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/2220-315X/g_info_20100725073445.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
WJEM 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
WJEM is an international, peer-reviewed, OA, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 600 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.