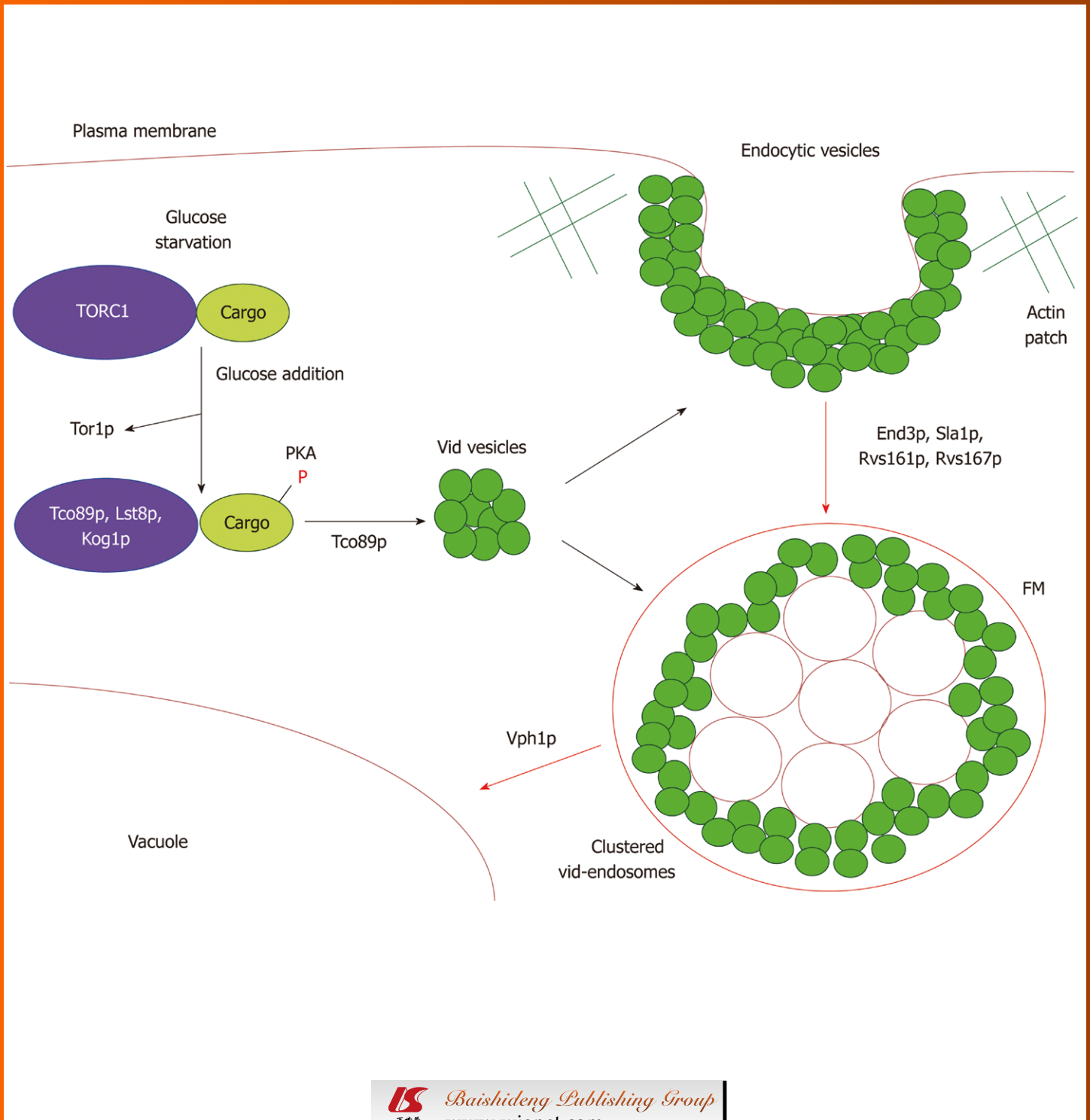
ISSN 1949-8454 (online)

World Journal of *Biological Chemistry*

World J Biol Chem 2011 November 26; 2(11): 239-251



Baishideng Publishing Group
www.wjgnet.com

WJBC

World Journal of Biological Chemistry

A peer-reviewed, online, open-access journal of biological chemistry

Editorial Board

2009-2013

The *World Journal of Biological Chemistry* Editorial Board consists of 523 members, representing a team of worldwide experts in biochemistry and molecular biology. They are from 40 countries, including Argentina (1), Australia (7), Austria (3), Belgium (6), Brazil (5), Bulgaria (1), Canada (20), Chile (1), China (36), Czech Republic (1), Denmark (1), Finland (3), France (14), Germany (17), Greece (3), India (9), Iran (2), Israel (6), Italy (26), Japan (42), Lithuania (1), Mauritius (1), Mexico (2), Netherlands (6), New Zealand (1), Norway (4), Portugal (4), Romania (1), Russia (2), Singapore (4), South Africa (1), South Korea (17), Spain (18), Sweden (4), Switzerland (3), Thailand (2), Turkey (1), Ukraine (1), United Kingdom (18), and United States (228).

Chun Liang, *Hong Kong*
Feng Liu, *Nanjing*
Shu-Wen Liu, *Guangzhou*
Pei-Yuan Qian, *Hong Kong*
Lei Ren, *Xiamen*
Hong-Bo Shao, *Yantai*
Tao Tao, *Xiamen*
Karl Tsim, *Hong Kong*
Paulus S Wang, *Taipei*
Ling-Yun Wu, *Beijing*
Zhi-Heng Xu, *Beijing*
Yong-Bin Yan, *Beijing*
Tang-Bin Yang, *Beijing*
Zeng-Ming Yang, *Xiamen*
Xue-Wu Zhang, *Guangzhou*
Yiguo Zhang, *Chongqing*
Hai-Meng Zhou, *Beijing*
Rong-Jia Zhou, *Wuhan*
Xiao-Feng Zheng, *Beijing*
Wei-Guo Zhu, *Beijing*
Chao-Chun Zou, *Hangzhou*

Czech Republic

Petr Draber, *Prague*

Denmark

Rasmus Hartmann-Petersen, *Copenhagen*

Finland

Ville-Petteri Mäkinen, *Helsinki*
Mikko Juhani Nikinmaa, *Turku*
Mika Rämet, *Tampere*

France

Yannick Allanore, *Paris*
Olivier Berteau, *Jouy En Josas*
Jean-Yves Bouet, *Toulouse*
Anthony William Coleman, *Lyon*
Cristine Alves da Costa, *Valbonne*
Yannick Goumon, *Strasbourg*
Herve Hoste, *Toulouse*
Anne Imberty, *Grenoble*
Eric J Kremer, *Montpellier*
Florian Lesage, *Sophia-Antipolis*
Jean-Louis Mergny, *Lyon*
Sylvie Rebuffat, *Paris*
Norbert Rolland, *Grenoble*
Sandrine Sagan, *Paris*

Germany

Maik Behrens, *Nuthetal*
Matthias Eckhardt, *Bonn*
Harald Genth, *Hannover*
Martin Gotte, *Muenster*
Christian Hallermann, *Muenster*
Michael Hecker, *Greifswald*
Bernhard Lüscher, *Aachen*
Werner Müller, *Mainz*
Jörg Nickelsen, *Planegg-Martinsried*
Wolfgang Obermann, *Bochum*
Matthias Ocker, *Marburg*
Satish Raina, *Borstel*
Michael Ristow, *Jena*
M Lienhard Schmitz, *Giessen*
Klaus Schulze-Osthoff, *Tübingen*
Gerhild van Echten-Deckert, *Bonn*

Greece

Evangelia Papadimitriou, *Patras*
Maria Papagianni, *Thessaloniki*
Georgia Sotiropoulou, *Rion-Patras*

India

Subrata Chattopadhyay, *Mumbai*
Virendra S Gomase, *Latur*
Siddhartha S Jana, *Kolkata*
Sunil Kumar Manna, *Hyderabad*
Vinay K Nandicoori, *New Delhi*
MN Ponnuswamy, *Chennai*
Manoj Raje, *Chandigarh*
Shio Kumar Singh, *Varanasi*
TP Singh, *New Delhi*

Iran

Mehrdad Mohri, *Mashhad*
Seyed Nasser Ostad, *Tehran*

Israel

Shoshana Bar-Nun, *Tel Aviv*
Shaul Mordechai, *Beer Sheva*
Zvi Naor, *Tel Aviv*
Edgar Pick, *Tel Aviv*
Eitan Shaulian, *Jerusalem*
Varda Shoshan-Barmatz, *Beer Sheva*

Italy

Andrea Battistoni, *Rome*
Annamaria Bevilacqua, *Milan*
Antonio Brunetti, *Catanzaro*
Santina Bruzzone, *Genova*
Gaetano Cairo, *Milano*
Giovanna De Chiara, *Rome*
Rita De Santis, *Pomeza*
Rosario Donato, *Perugia*
Vittorio Gentile, *Naples*
Fabio Grizzi, *Milan*
Maria Luisa Mangoni, *Rome*
Luca Munaron, *Torino*
Antonio Musarò, *Rome*
Sergio Papa, *Bari*
Alberto Passi, *Varese*
Rinaldo Pellicano, *Turin*
Luca Rampoldi, *Milan*
Andrea Rasola, *Padova*
Gianfranco Risuleo, *Rome*
Vito Ruggiero, *Pomezia*
Roberto Scatena, *Rome*
Massimo Stefani, *Florence*
Andrea Trabocchi, *Florence*
Carlo Ventura, *Bologna*
Elena Zocchi, *Genova*

Japan

Naohiko Anzai, *Tokyo*
Noriko Fujiwara, *Nishinomiya*
Yoshiaki Furukawa, *Yokohama*
Hiroshi Harada, *Kyoto*
Makoto Hashimoto, *Tokyo*
Tadashi Hatanaka, *Kaga-gun*
Eiichi Hinoi, *Kanazawa*
Satoshi Inoue, *Tokyo*
Takaki Ishikawa, *Osaka*
Yoshizumi Ishino, *Fukuoka*
Hiroaki Itamochi, *Yonago*
Hideaki Kaneto, *Osaka*
Koichi Kato, *Okazaki*
Eiichi N Kodama, *Sendai*
Kenji Kuwasako, *Miyazaki*
Katsumi Maenaka, *Fukuoka*
Hisao Masai, *Tokyo*
Shin-Ichiro Miura, *Fukuoka*
Eiji Miyoshi, *Suita*
Ryuichi Morishita, *Suita*
Yasu S Morita, *Osaka*
Tatsuya Sakamoto, *Setouchi*
Toshiyasu Sasaoka, *Toyama*
Hiroshi Shibuya, *Bunkyo*
Toru Shimizu, *Sendai*
Hiroshi Takahashi, *Tottori*
Takashi Takeuchi, *Yonago*
Tomohiro Tamura, *Sapporo*
Kengo Tanabe, *Tokyo*
Takuji Tanaka, *Gifu*
Ikuo Tooyama, *Otsu*
Hirokazu Tsukahara, *Fukui*
Toshimitsu Uede, *Sapporo*
Nobutaka Wakamiya, *Asahikawa*
Ji-Yang Wang, *Yokohama*
Richard W Wong, *Kanazawa*
Sho-Ichi Yamagishi, *Kurume*
Michiaki Yamashita, *Yokohama*
Kiyotsugu Yoshida, *Tokyo*

Lithuania

Arunas Ramanavicius, *Vilnius*

Mauritius

Theeshan Bahorun, *Reduit*

Mexico

Alejandra Bravo, *Morelos*
Gerardo Corzo, *Morelos*

Netherlands

Egbert J Boekema, *Groningen*
N Bovenschen, *Utrecht*
Bart Maarten Gadella, *Utrecht*
Leo Nijtmans, *Nijmegen*
MAM van Steensel, *Maastricht*
Ronald JA Wanders, *Amsterdam*

New Zealand

Alexander V Peskin, *Christchurch*

Norway

K Kristoffer Andersson, *Oslo*
Ugo Moens, *Tromsø*
J Preben Morth, *Oslo*
Herve Seligmann, *Oslo*

Portugal

Manuel Aureliano, *Faro*
Carlos Alberto da Silva Conde, *Porto*
Carlos Bandeira Duarte, *Cantanhede*
Ceu Figueiredo, *Porto*

Romania

Anca V Gafencu, *Bucharest*

Russia

Vladimir S Bondar, *Krasnoyarsk*
Ilya V Demidyuk, *Moscow*

Singapore

Sohail Ahmed, *Singapore*
Surajit Bhattacharyya, *Singapore*
Kah-Leong Lim, *Singapore*
Jianxing Song, *Singapore*

South Africa

Ugo Ripamonti, *Johannesburg*

South Korea

Jae Youl Cho, *Chuncheon*
Cheol Yong Choi, *Suwon*
Dalwoong Choi, *Seoul*
Hueng-Sik Choi, *Gwangju*
Kang-Yell Choi, *Seodemun Gu*
Sin-Hyeog Im, *Gwangju*
Byeong-Churl Jang, *Daegu*
Min-Seon Kim, *Seoul*
Byoung-Mog Kwon, *Daejeon*
Seong-Wook Lee, *Yongin*
Sung Joong Lee, *Seoul*
Lee Bok Luel, *Busan*
Yuseok Moon, *Yangsan*
Jongsun Park, *Taejeon*
Dong Min Shin, *Seoul*
Young-Joon Surh, *Seoul*
Kweon Yu, *Daejon*

Spain

Jose M Andreu, *Madrid*
Joaquin Arino, *Cerdanyola del Valles*
Joaquín Arribas, *Barcelona*
Jesus Avila, *Madrid*
Antonio Casamayor, *Cerdanyola*
Antonio Celada, *Barcelona*
Francisco Ciruela, *Barcelona*
Senena Corbalan, *Murcia*
Antonio Felipe, *Barcelona*
Tino Krell, *Granada*
Pedro A Lazo, *Salamanca*
Wolfgang Link, *Madrid*
Jorge Martín-Pérez, *Madrid*
Faustino Mollinedo, *Salamanca*
Guillermo Montoya, *Madrid*
Rosario Muñoz, *Madrid*
Julia Sanz-Aparicio, *Madrid*
Manuel Vázquez-Carrera, *Barcelona*

Sweden

Bo Åkerström, *Lund*
Leonard Girnita, *Stockholm*
Johan Lennartsson, *Uppsala*
John Ulf Rannug, *Stockholm*

Switzerland

Dietmar Benke, *Zürich*
Dietbert Neumann, *Zürich*
Roger Schneiter, *Fribourg*

Thailand

Pimchai Chaiyen, *Bangkok*
Veerapol Kukongviriyapan, *Khon Kaen*

Turkey

Necla Çağlarırmak, *Manisa*

Ukraine

Eugene S Kryachko, *Kiev*

United Kingdom

Per Bullough, *Sheffield*
Wayne Grant Carter, *Nottingham*
Marco Falasca, *London*
Julian Leether Griffin, *Cambridge*
Kristiina Hilden, *Nottingham*
Adam D Hughes, *Argyll*
Lin-Hua Jiang, *Leeds*
Zhi-Liang Lu, *Edinburgh*
Peter Monk, *Sheffield*
Elizabeth Lara Ostler, *Brighton*
Ihtesham Ur Rehman, *London*
Eugenio Sanchez-Moran, *Birmingham*
Cliff Taggart, *Belfast*
David J Timson, *Belfast*
Patrick J Twomey, *Suffolk*
Elisabetta Verderio, *Nottingham*
Stephen Geoffrey Ward, *Bath*
Lu-Gang Yu, *Liverpool*

United States

Ruhul Abid, *Boston*
Nihal Ahmad, *Wisconsin*
Stephen Alexander, *Columbia*
Andrei T Alexandrescu, *Storrs*
Seth L Alper, *Boston*
Suresh V Ambudkar, *Maryland*
Douglas Andres, *Lexington*
Insoo Bae, *Washington*
Scott R Baerson, *University*
Omar Bagasra, *Orangeburg*
Yidong Bai, *San Antonio*
Andrei V Bakin, *Buffalo*
Joe B Blumer, *Charleston*
Jonathan S Bogan, *New Haven*
Joseph T Brozinick, *Indianapolis*
Michael Bruce Butterworth, *Pittsburgh*
Nickolay Brustovetsky, *Indianapolis*
Huaibin Cai, *Bethesda*
Blanca Camoretti-Mercado, *Chicago*
Daniel GS Capelluto, *Blacksburg*
Subrata Chakrabarti, *Boston*
Subbaiah C Chalivendra, *Colorado*
Yongchang Chang, *Phoenix*
Yung-Fu Chang, *Ithaca*
Xian-Ming Chen, *Omaha*
Guanjun Cheng, *Philadelphia*
Wen-Hsing Cheng, *College Park*
Xiaodong Cheng, *Galveston*
Kuo-Chen Chou, *San Diego*
John William Christman, *Chicago*
Daret St Clair, *Lexington*
Katalin Csiszar, *Honolulu*
Mu-Shui Dai, *Portland*
Siddhartha Das, *El Paso*
John S Davis, *Nebraska*
Channing Joseph Der, *Chapel Hill*
Nikolay V Dokholyan, *Chapel Hill*
Jing-Fei Dong, *Houston*
Zheng Dong, *Augusta*
Sinisa Dovat, *Madison*
Guangwei Du, *Houston*
Penelope Duerksen-Hughes, *Loma Linda*
Sherine Elsawa, *Rochester*
Ahmed Faik, *Athens*
Huizhou Fan, *Piscataway*
Yong Fan, *Pittsburgh*
Qingming Fang, *Pittsburgh*
Victor Faundez, *Atlanta*
Changjian Feng, *Albuquerque*
Jay William Fox, *Charlottesville*
Irwin Fridovich, *Durham*
Yuchang Fu, *Birmingham*
Alexandros Georgakilas, *Greenville*
Shibnath Ghatak, *Charleston*
Alasdair M Gilfillan, *Bethesda*
Jeffrey M Gimble, *Baton Rouge*
Antonio Giordano, *Philadelphia*
Channe Gowda, *Hershey*
Vsevolod V Gurevich, *Nashville*
James Hagman, *Denver*
Tsonwin Hai, *Columbus*
Yusuf A Hannun, *Charleston*
Dee Harrison-Findik, *Omaha*
Ian S Haworth, *Los Angeles*
Tong-Chuan He, *Chicago*
L Shannon Holliday, *Gainesville*
Shangwei Hou, *Philadelphia*
Chuanshu Huang, *Tuxedo*
Shile Huang, *Shreveport*
Yan Huang, *Charleston*
Johnny Huard, *Pittsburgh*
Hieronim Jakubowski, *Newark*
Xinhua Ji, *Frederick*
Yu Jiang, *Pittsburgh*
Victor X Jin, *Columbus*

WJBC World Journal of Biological Chemistry

Contents

Monthly Volume 2 Number 11 November 26, 2011

Contents

World Journal of Biological Chemistry
Volume 2 Number 11 November 26, 2011

NAME OF JOURNAL
World Journal of Biological Chemistry

LAUNCH DATE
February 26, 2010

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

EDITING
Editorial Board of *World Journal of Biological Chemistry,*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: wjbc@wjgnet.com
http://www.wjgnet.com

PUBLISHING
Baishideng Publishing Group Co., Limited,
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-3115-8812
Telephone: +852-5804-2046
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

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

PUBLICATION DATE
November 26, 2011

ISSN
ISSN 1949-8454 (online)

EDITORIAL OFFICE
Na Ma, Director
World Journal of Biological Chemistry
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: wjbc@wjgnet.com
http://www.wjgnet.com

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1949-8454/g_info_20100316155305.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/1949-8454office

World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/1949-8454office
wjbc@wjgnet.com
doi:10.4331/wjbc.v2.i11.239

World J Biol Chem 2011 November 26; 2(11): 239-245
ISSN 1949-8454 (online)

TOPIC HIGHLIGHT

Hui-Ling Chiang, PhD, *Series Editor*

Vacuole import and degradation pathway: Insights into a specialized autophagy pathway

Abbas A Alibhoy, Hui-Ling Chiang

Abbas A Alibhoy, Hui-Ling Chiang, Department of Cellular and Molecular Physiology, Penn State University College of Medicine, Hershey, PA 17033, United States
Author contributions: Alibhoy AA and Chiang HL wrote the review article together.
Supported by NIH grant R01GM 59480 and the PA Tobacco Settlement Fund to Hui-Ling Chiang
Correspondence to: Hui-Ling Chiang, PhD, Professor of Cellular and Molecular Physiology, Department of Cellular and Molecular Physiology, Penn State University College of Medicine, Hershey, PA 17033, United States. hxc32@psu.edu
Telephone: +1-717-5310860 Fax: +1-717-5317667
Received: July 29, 2011 Revised: August 30, 2011
Accepted: November 6, 2011
Published online: November 26, 2011

Abstract

Glucose deprivation induces the synthesis of pivotal gluconeogenic enzymes such as fructose-1,6-bisphosphatase, malate dehydrogenase, phosphoenolpyruvate carboxykinase and isocitrate lyase in *Saccharomyces cerevisiae*. However, following glucose replenishment, these gluconeogenic enzymes are inactivated and degraded. Studies have characterized the mechanisms by which these enzymes are inactivated in response to glucose. The site of degradation of these proteins has also been ascertained to be dependent on the duration of starvation. Glucose replenishment of short-term starved cells results in these proteins being degraded in the proteasome. In contrast, addition of glucose to cells starved for a prolonged period results in these proteins being degraded in the vacuole. In the vacuole dependent pathway, these proteins are sequestered in specialized vesicles termed vacuole import and degradation (Vid). These vesicles converge with the endocytic pathway and deliver their cargo to the vacuole for degradation. Recent studies have identified that internalization, as mediated by actin polymerization, is essential for delivery of cargo proteins to the vacuole for degradation. In addition, components of the target of rapamycin complex 1 interact with cargo proteins during glucose starvation. Furthermore, Tor1p dissociates from cargo proteins following glucose replenishment. Future studies will be needed to elaborate on the importance of internalization at the plasma membrane and the subsequent import of cargo proteins into Vid vesicles in the vacuole dependent degradation pathway.

Key words: Vacuole import and degradation; Fructose-1,6-bisphosphatase; Vacuole; Proteasome; Autophagy; Target of rapamycin complex 1; Actin polymerization; Endocytosis

Peer reviewers: Hong-Gang Wang, PhD, Professor, Lois High Berstler Professor of Pharmacology, Penn State College of Medicine, Penn State Hershey Cancer Institute, CH74, 500 University Drive, PO Box 850, Hershey, PA 17033-0850, United States; Hoyun Lee, Senior Scientist and Professor, Tumor Biology Group, Regional Cancer Program, Sudbury Regional Hospital, 41 Ramsey Lake Road, Sudbury, Ontario P3E 5J1, Canada; Michiaki Yamashita, PhD, Chief, Food Biotechnology Section, National Research Institute of Fisheries Science, 2-12-4 Fukuura, Yokohama 236-8648, Japan; Bin Shan, MD, PhD, Assistant Professor, Department of Medicine, Tulane University School of Medicine, 1430 Tulane Ave. SL-9, New Orleans, LA 70112, United States

Alibhoy AA, Chiang HL. Vacuole import and degradation pathway: Insights into a specialized autophagy pathway. *World J Biol Chem* 2011; 2(11): 239-245 Available from: URL: http://www.wjgnet.com/1949-8454/full/v2/i11/239.htm DOI: http://dx.doi.org/10.4331/wjbc.v2.i11.239

INTRODUCTION

A principal function of eukaryotic cells is to mediate trafficking of proteins and lipids between organelles for maintaining homeostasis. Vesicles facilitate such transport between organelles. The different types of transport vesicles range from coat protein complex I (COPI) vesicles,

COPII vesicles and clathrin-coated vesicles[1-3]. Of these, clathrin-coated vesicles mediate transport of proteins and lipids from the plasma membrane to early endosomes, and from the Golgi to endosomes[1]. COPI vesicles are involved in retrograde transport from the Golgi to the ER and in the intra-Golgi transport while COPII vesicles further the transport from the ER to the Golgi[2,3].

The level of proteins in a cell is dictated by protein synthesis and protein degradation. Some proteins are degraded in the proteasome while others are degraded at the site of the lysosome[4-7]. The proteasomal degradation pathway entails the use of E1, E2 and E3 enzymes for ubiquitinating target proteins[4,5]. This is followed by the subsequent targeting of these ubiquitinated proteins to the proteasome for degradation[4,5]. On the other hand, the lysosome degrades proteins and organelles using acid hydrolases[6,7]. The targeting of proteins for degradation at the lysosome occurs *via* numerous pathways[6,7]. Macroautophagy is one pathway that facilitates the degradation of such proteins. This non-selective catabolic process, which is regulated by *ATG* genes, enables cells to degrade their own components in the lysosome as a means to survive periods of nutrient deprivation and other stresses[8-11]. Besides the non-selective degradation of proteins, chaperone-mediated autophagy best exemplifies a selective autophagy pathway which utilizes luminal and cytosolic chaperones to target specific proteins (containing the KFERQ sequence) to the lysosome for degradation[12-15]. As such, autophagy is imperative for biological processes such as life span extension, cell development and growth[16,17]. Moreover, deregulated macroautophagy can result in numerous diseases[16,17].

DIVERSE PATHWAYS TRANSPORT PROTEINS TO THE YEAST VACUOLE

The yeast vacuole is homologous to the mammalian lysosome and is required for cellular processes such as osmoregulation, protein maturation and protein degradation[18,19]. The function of the yeast vacuole is dependent on the targeting of vacuole resident proteins into this organelle. For instance, the acid hydrolase carboxypeptidase Y is transported from the Golgi to the vacuole for maturation by the Vps pathway[20,21]. This process involves around 40 *VPS* genes[20,21]. In addition, plasma membrane proteins and other extracellular components can be internalized and targeted to the vacuole by endocytosis[18,19]. In contrast, proteins can also be transported from the cytoplasm into vacuole. Enzymes such as aminopeptidase I and α-mannosidase are synthesized in their inactive forms in the cytoplasm and are transported to the vacuole *via* the Cvt pathway[8,9,16]. This pathway shares many common components with the macroautophagy pathway and the pexophagy pathway[8,9,16].

Changes in nutrient conditions can influence the transport of proteins and organelles to the vacuole. For example, when *Saccharomyces cerevisiae* are starved of nitrogen, the macroautophagy pathway delivers proteins sequestered in autophagosomes to the vacuole for degradation[8,9,16]. Tor1p, a protein kinase and rapamycin target, is a component of the target of rapamycin complex 1 (TORC1) that regulates cell growth in response to stimuli such as nutrients and cellular stresses[8,9]. It achieves this by regulating gene transcription, protein translation, ribosomal biogenesis and macroautophagy[8,9]. Moreover, the macroautophagy pathway is inhibited by Tor1p and is induced by rapamycin even in the absence of nitrogen starvation[8,9,16]. Another example, the pexophagy pathway targets peroxisomes to the vacuole for degradation when yeast is shifted from growth in media containing oleic acid to that containing glucose[22].

INDUCTION, INACTIVATION AND DEGRADATION OF GLUCONEOGENIC ENZYMES

The pivotal regulatory enzyme in the gluoconeogenesis pathway, fructose-1,6-bisphosphatase (FBPase), is induced in *Saccharomyces cerevisiae* by glucose starvation. When cells are replenished with media containing fresh glucose, FBPase is inactivated and degraded[23-25]. This type of inactivation is termed catabolite inactivation[26,27]. Additionally, it has been determined that other gluconeogenic enzymes such as malate dehydrogenase (MDH2), phosphoenolpyruvate carboxykinase (Pck1p) and isocitrate lyase (Icl1p) are also inactivated by glucose[28-30]. Of these, catabolite inactivation of FBPase has been studied exhaustively for the vacuole dependent pathway[29,31,32]. In the course of catabolite inactivation, it has been ascertained that FBPase is phosphorylated and inactivated by cAMP-dependent protein kinase or protein kinase A (PKA)[29,33,34].

The site of degradation of FBPase is dependent on the duration of starvation. The Wolf lab has demonstrated that following glucose replenishment, FBPase is modified by ubiquitination and is degraded in the proteasome[35-38]. However, our lab has shown that FBPase is degraded in the vacuole in cells that have been starved of glucose for 3 d[23,27,31]. We examined the degradation of FBPase using a Δ*pep4*Δ*prb1*Δ*prc1* vacuole mutant containing deletions of proteinases A, B and C[29]. In this strain, it was observed that when glucose was added to cells that have been starved for 1 d, FBPase was degraded normally. In contrast, when glucose was added to cells that were starved for 3 d, FBPase degradation was impaired. This indicates that while vacuole proteinases are not essential for FBPase degradation in 1 d starved cells, they are essential for cells starved for 3 d. More significantly, our lab has recently demonstrated that other gluconeogenesis enzymes such as MDH2, Pck1p and Icl1p also share the same degradation characteristics as FBPase[29,30].

THE VACUOLE IMPORT AND DEGRADATION PATHWAY

The vacuole import and degradation (Vid) pathway is a

selective autophagy pathway that mediates degradation of FBPase, MDH2, Pck1p and Icl1p in the vacuole after glucose starvation for 3 d[23,27,29-31]. The genes involved in this pathway are cumulatively called *VID* genes[32,39,40]. Upon characterizing the wild-type and Δ*pep4* strains by fractionation of lysates in a size column, it was ascertained that four peaks were found to contain FBPase[32]. The first peak contained the plasma membrane protein Pma1p and the fourth peak was enriched for Vid vesicles[32]. By kinetic analysis, it was determined that Vid vesicles are transitional carriers in the Vid pathway[41]. Thus, gluconeogenic enzymes destined for degradation by the Vid pathway associate with these vesicles prior to their delivery to the vacuole. Through *in vitro* assays, it was discerned that FBPase was imported into purified Vid vesicles[42]. In addition, the plasma membrane protein Vid22p, the cytosolic heat shock proteins Ssa1p and Ssa2p, and the peptidylprolyl isomerase Cyclophilin A are required for the sequestration of FBPase into Vid vesicles[42,43].

Cells lacking the ubiquitin conjugating enzyme 1 gene were observed to block the formation of Vid vesicles, thereby suggestive of a role in Vid vesicle biogenesis[44]. Vid24p has been characterized as a peripheral protein on Vid vesicles and is extensively used to study the trafficking of Vid vesicles in response to glucose[45]. Data from our recent studies suggest that the Vid pathway converges with the endocytic pathway[40]. COPI coatomer proteins have also been identified as peripheral proteins on Vid vesicles and they recruit Vid24p to Vid vesicles[40]. Furthermore, the coatomer subunit Sec28p traffics to endosomes and is distributed on retrograde vesicles forming on the vacuole membrane in response to glucose refeeding[40]. Following the merging of the Vid vesicles with endosomes, the Vid-endosome clusters transport their cargo to the vacuole, a step which requires Vph1p[40]. This was determined in the Δ*vph1* strain where FBPase is distributed in the lumen of FM-containing endosomes[40]. FM is an endocytic dye that stains the endosomes and reaches the vacuole[40].

ENDOCYTOSIS AND ACTIN POLYMERIZATION ARE REQUIRED FOR DELIVERY OF CARGO PROTEINS TO THE VACUOLE FOR DEGRADATION

Having previously demonstrated that the Vid pathway merges with the endocytic pathway, it would be significant to elaborate on this finding. One possible explanation is that the Vid vesicles may merge with the endocytic vesicles that are forming on the plasma membrane. Hence, it follows that FBPase may also be targeted to the plasma membrane. In order to investigate this, the distribution of FBPase was visualized at the ultra-structural level[46]. This revealed that in wild type and in Δ*pep4* strains, FBPase is distributed near the plasma membrane and in irregularly shaped intracellular structures at 15 min following glucose replenishment. Based on the FBPase distribution, it can be inferred that the Vid pathway utilizes the early steps of the endocytic pathway to deliver FBPase to the vacuole for degradation. Upon purifying the FBPase containing intracellular structures, it was determined that these were enriched for the endosomal marker Pep12p and the Vid vesicle marker Vid24p. This suggests that following glucose replenishment, Vid vesicles may cluster or aggregate with endosomes to form large FBPase-containing structures.

The distribution of FBPase near the plasma membrane indicates that the early steps of endocytosis are required for the Vid pathway. Moreover, it has been previously reported that the early steps of endocytosis are mediated by actin polymerization in yeast[47-52]. Actin polymerization comprises recruitment and interplay of different actin related proteins at the site of internalization. Proteins such as End3p, Sla1p and Pan1p are recruited at the early steps and promote initiation of actin polymerization assembly. Subsequently, Myo3p and Myo5p are mobilized during the later stages of actin polymerization. Finally, Rvs161p and Rvs167p facilitate scission of endocytic vesicles[47-52]. Initially, the distribution of FBPase was studied in a yeast strain where the *END3* gene had been deleted[46]. It was ascertained that FBPase distribution in the plasma membrane, endosome and Vid vesicle fractions were reduced in the Δ*end3* mutant in comparison to that observed in the positive control Δ*vph1* strain. These results highlight a requirement for the early steps of endocytosis in mediating association of FBPase with Vid vesicles. Owing to a requirement for actin polymerization in mediating pinching off of endocytic vesicles from the plasma membrane, FBPase degradation was studied in mutants that impaired actin polymerization at different steps[46]. As such, FBPase degradation was inhibited in mutant strains such as Δ*end3* and Δ*sla1*. In summation, association of FBPase with Vid vesicles required the actin polymerization genes.

It was determined that the gluconeogenesis enzymes FBPase and MDH2 displayed a low distribution to actin patches (sites of actin polymerization) in the wild type yeast strain during glucose starvation. However, there was an increased distribution of FBPase and MDH2 to actin patches by 30 min following glucose replenishment. By 60 min of glucose addition, less co-localization of FBPase and MDH2 with actin patches was observed. As such, the cargo proteins were targeted to actin patches on the plasma membrane[46]. As means for determining whether Vid vesicles are targeted to the actin patches, Vid vesicle markers Vid24p and Sec28p distribution was examined in wild type cells[46]. Interestingly, Sec28p and Vid24p were observed to be distributed to the actin patches during glucose starvation and for up to 30 min following glucose replenishment. Sec28p and Vid24p demonstrated less co-localization to the actin patches by 60 min of glucose addition. Hence, it can be inferred that Vid vesicles are targeted to actin patches during glucose starvation and for up to 30 min following glucose replen-

ishment. Moreover, Sec28p and Vid24p association with actin patches was prolonged in cells lacking the *RVS167* gene (involved in scission of endocytic vesicles from the plasma membrane)[46]. This demonstrates that actin polymerization is required for Vid-endocytic vesicles to pinch off from the plasma membrane.

Based on these studies, we contend that Sec28p and Vid24p are present at actin patches during glucose starvation. These are also sites of endocytic vesicle formation from the plasma membrane. During glucose replenishment, FBPase and MDH2 are imported into free Vid vesicles and into Vid vesicles at the site of the actin patches. Endocytic vesicles are then released into the cytoplasm to become small endosomes with Vid vesicles clustering or aggregating to form irregularly shaped structures. In this manner, the Vid-endosome clusters serve to deliver their cargo to the vacuole for degradation.

THE TOR COMPLEX 1 INTERACTS WITH MULTIPLE CARGO PROTEINS TARGETED FOR DEGRADATION IN THE VID PATHWAY

In order to garner a more cumulative understanding of the Vid pathway, it is imperative to know how cells recognize cargo proteins that are destined for degradation. To address these goals, we sought to identify cellular proteins that interacted with FBPase. Under previously characterized growth conditions, putative FBPase interacting proteins were purified by affinity chromatography. The bound material was subjected to MALDI analysis. This facilitated in identifying Tco89p among other cellular protein candidates[30]. Tco89p is a component of the TORC1 which is also comprised of Tor1p, Kog1p and Lst8p[53,54].

The role of Tco89p in the Vid pathway was characterized by examining the degradation of FBPase, MDH2, Icl1p and Pck1p in the Δ*tco89* mutant[30]. It was determined that while FBPase, MDH2, Iclp and Pck1p were degraded in wild-type cells, the degradation of these proteins was impaired in Δ*tco89* cells. This indicates that Tco89p is required for the vacuolar dependent degradation of multiple proteins selected for the Vid pathway. It was also ascertained that components of TORC1 interacted with FBPase, MDH2, Icl1p and Pck1p during glucose starvation[30]. Through kinetic studies, it was established that Tor1p was dissociated from cargo proteins after the addition of glucose. Interestingly, Tco89p remained associated with FBPase after the addition of glucose. As such, Tor1p and Tco89p may dictate different functions in the Vid pathway. This also alludes to an inhibitory function of Tor1p association in mediating cargo protein degradation. This was verified by observing that cells overexpressing the *TOR1* gene exhibited a delay in FBPase degradation. As it has been demonstrated that Tor1p is inhibitory to FBPase degradation, it was hypothesized that treatment of wild-type cells with rapamycin would promote degradation of cargo protein. However, it was determined that the addition of rapamycin impaired FBPase degradation in wild-type cells following glucose replenishment. Furthermore, *TOR1* overexpressing cells inhibited the sequestering of cargo proteins into Vid vesicles. Similar results were also observed in cells lacking the *TCO89* gene. Surprisingly, *TOR1* deletion has little effect on FBPase degradation.

It was next determined whether Vid vesicle biogenesis was affected in cells overexpressing *TOR1* or in cells lacking this gene[30]. Vid24p, a peripheral protein on Vid vesicles, was used to study the biogenesis of Vid vesicles. From differential centrifugation, it was ascertained that a fraction of Vid24p was detected in the Vid vesicle-enriched fraction in wild-type cells. Similarly, in Δ*tor1* cells, a fraction of Vid24p was also detected in the Vid vesicle-enriched fraction. However, low levels of Vid24p were present in the Vid vesicle-enriched fraction in cells overexpressing *TOR1*. In support, low levels of Vid24p were also found in the Vid vesicle-enriched fraction in Δ*tco89* cells. These results suggest that *TOR1* and *TCO89* are involved in the biogenesis of Vid vesicles.

From localization studies, it was determined that Tor1p and Tco89p were both distributed on endosomes emerging from the plasma membrane[30]. Moreover, these proteins were also detected on vesicles forming from the vacuole membrane. Such vesicles have been termed retrograde vesicles and these findings furnish support for previous observations where retrograde vesicles containing Sec28p could form on the vacuole membrane. Based on these results, it is proposed that TORC1 cycles between the plasma membrane and the vacuole. This facilitates in maintaining the size of the vacuole *via* the anterograde and retrograde transport pathways.

CURENT MODEL FOR THE VID PATHWAY

Based on the aforementioned studies, we propose the following model for the Vid pathway (Figure 1). Growth of yeast cells under glucose starvation induces synthesis of gluconeogenic enzymes such as FBPase, MDH2, Icl1p and Pck1p. During glucose starvation, Vid24p and Sec28p are distributed as peripheral proteins on free Vid vesicles and on Vid vesicles clustering around endocytic vesicles at the sites of actin polymerization. Tor1p, Tco89p and proteins involved in the early steps of actin polymerization, such as End3p and Sla1p, are believed to play a role in Vid vesicle biogenesis. Additionally, members of TORC1 interact with FBPase and other cargo proteins during glucose starvation. Following glucose replenishment, Tor1p dissociates from cargo proteins, thereby enabling cargo proteins to be phosphorylated by PKA. It should be noted that both the TORC1 and PKA pathways regulate cell growth with respect to nutrient availability[55]. However, there are conflicting reports concerning the order of involvement of these two pathways. As such, dissociation of Tor1p from cargo proteins may precede phosphorylation of cargo proteins by PKA

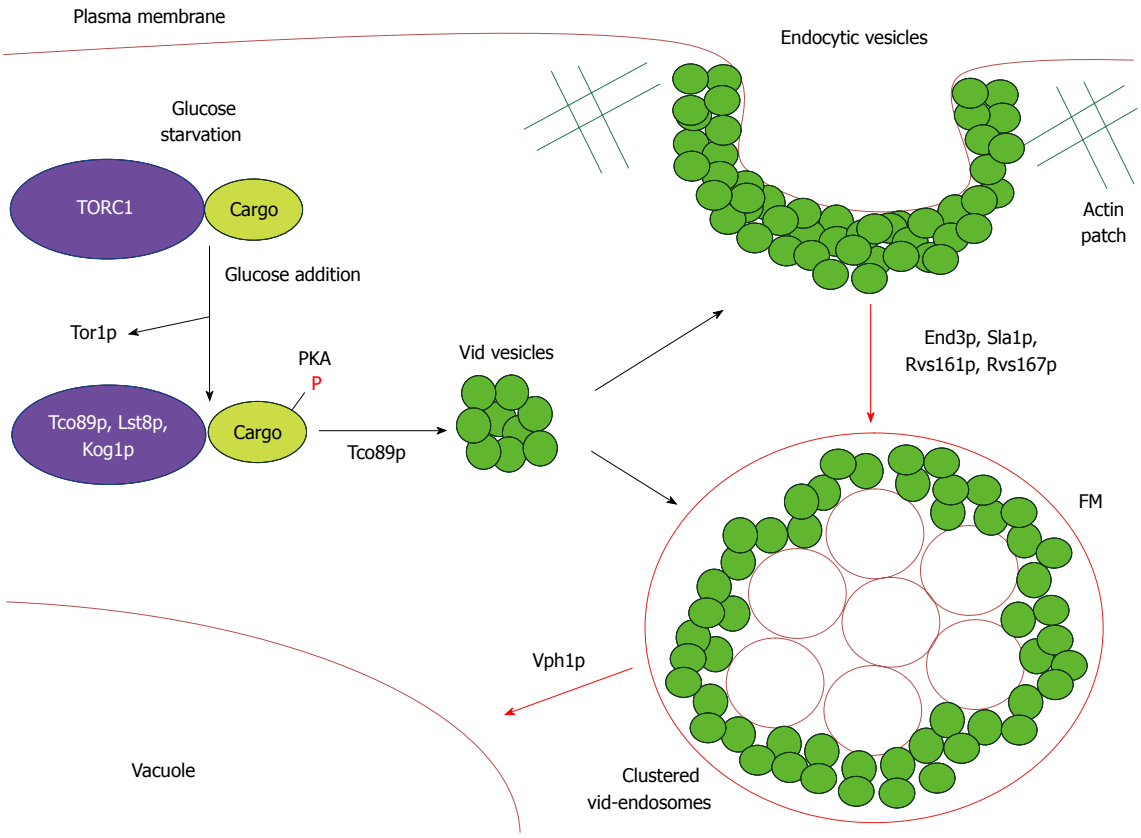


Figure 1 A model for the vacuole import and degradation pathway. Gluconeogenic enzymes (cargo) targeted for degradation by the Vid pathway associate with the TORC1 complex during glucose starvation. Tor1p dissociates from this complex following glucose replenishment. Thereafter, the cargo proteins are phosphorylated by PKA. Tco89p mediates sequestration of the cargo proteins into free Vid vesicles and into Vid vesicles clustered around endocytic vesicles forming on the plasma membrane. Proteins involved in the later steps of actin polymerization, namely Rvs161p and Rvs167p, mediate scission of the endocytic vesicles and these are released into the cytoplasm as small endosomes. The Vid vesicles aggregate around these endosomes to form irregularly shaped structures. Vph1p is required for the transport of the Vid-endosome clusters to the vacuole.

and *vice versa*. Moreover, the TORC1 and PKA pathways could also be acting in concert *via* parallel pathways[55]. Further studies will be required to clarify this relationship. Following inactivation of cargo proteins, Tco89p is required to sequester cargo proteins into the Vid vesicles aggregating around endocytic vesicles forming from the plasma membrane. Thereafter, proteins involved in the later steps of actin polymerization, namely Rvs161p and Rvs167p, mediate scission of endocytic vesicles and these are released into the cytoplasm as small endosomes. The Vid vesicles accrue around these endosomes to form irregularly shaped structures. The transport of the Vid-endosome clusters to the vacuole for degradation of cargo proteins requires the *VPH1* gene. We further postulate that the Vid pathway uses a specialized endocytic pathway. As such, we surmise that our model delineates multiple events ranging from cargo protein interaction with TORC1 to merging of the Vid pathway with the endocytic pathway at the actin patches as vital steps in the vacuole dependent degradation of gluconeogenic enzymes.

FUTURE DIRECTIONS

Despite having elucidated novel components of the Vid pathway such as TORC1 and actin polymerization, innumerable questions remain to be answered. For instance, what is the origin of Vid vesicles? Are they derived from retrograde vesicles that emerge from the vacuole membrane? Or do Vid vesicles originate from the site of plasma membrane internalization? We have demonstrated that endocytic mutants, such as Δ*rvs161* and Δ*rvs167*, that impair plasma membrane internalization, result in a prolonged distribution of peripheral Vid vesicle proteins such as Sec28p and Vid24p at the site of actin patches. Moreover, these endocytic mutants impede FBPase degradation following glucose replenishment. From these observations, one could argue that Vid vesicles are distributed at the site of plasma membrane internalization. Perhaps Vid vesicles are a specialized type of endosome? Interestingly, in support of this hypothesis, it should be noted that the Vid-endosome clusters observed following glucose re-feeding are reminiscent of multivesicular bodies. Furthermore, the significance of the requirement of internalization for FBPase degradation warrants further investigation. Are cargo proteins secreted out into the periplasmic space and internalized at the site of actin polymerization? Perhaps this may facilitate in sequestration of cargo proteins into Vid vesicles.

Another intriguing aspect of the regulation of gluco-

neogenic enzymes is what mechanisms determine degradation of proteins *via* the ubiquitin-proteasome pathway *vs* the vacuole dependent Vid pathway. It has been demonstrated that the site of degradation is governed by disparate modifications of cargo proteins following glucose replenishment. Cargo proteins are ubiquitinated before degradation in the proteasome[35-38]. On the other hand, cargo proteins are phosphorylated *via* PKA prior to their targeting to the vacuole[56]. However, the signaling cascade that mediates the transition from the ubiquitin-proteasome pathway to the Vid pathway requires elucidation.

FBPase has been described among components of excretory secretory products from *Clonorchis sinensis* adult worms and indicate that FBPase may serve as a marker in diagnosing clonorchiasis-associated hepatic fibrosis[57]. In addition, FBPase has also been identified as a bio-marker for assessing damage to the proximal renal tubules[58]. Furthermore, it has been determined that gluconeogenesis is upregulated in patients suffering from Type Ⅱ diabetes[59]. Managlinat dialanetil (an FBPase inhibitor) has been demonstrated to show promise in the treatment of Type Ⅱ diabetes[59]. As such, an understanding of how to activate or inactivate the Vid pathway could prove invaluable in identifying targets for developing therapies against diseases caused by aberrant gluconeogenesis in humans. Thus, addressing the abovementioned questions will form the core of our future investigations in our quest to better characterize this unique autophagy pathway.

REFERENCES

1 **McMahon HT**, Mills IG. COP and clathrin-coated vesicle budding: different pathways, common approaches. *Curr Opin Cell Biol* 2004; **16**: 379-391
2 **Duden R**. ER-to-Golgi transport: COP I and COP II function (Review). *Mol Membr Biol* 2003; **20**: 197-207
3 **Gu F**, Aniento F, Parton RG, Gruenberg J. Functional dissection of COP-I subunits in the biogenesis of multivesicular endosomes. *J Cell Biol* 1997; **139**: 1183-1195
4 **Varshavsky A**. Regulated protein degradation. *Trends Biochem Sci* 2005; **30**: 283-286
5 **Goldberg AL**. Functions of the proteasome: from protein degradation and immune surveillance to cancer therapy. *Biochem Soc Trans* 2007; **35**: 12-17
6 **Mortimore GE**, Hutson NJ, Surmacz CA. Quantitative correlation between proteolysis and macro- and microautophagy in mouse hepatocytes during starvation and refeeding. *Proc Natl Acad Sci USA* 1983; **80**: 2179-2183
7 **Seglen PO**, Gordon PB, Holen I. Non-selective autophagy. *Semin Cell Biol* 1990; **1**: 441-448
8 **Mizushima N**, Klionsky DJ. Protein turnover via autophagy: implications for metabolism. *Annu Rev Nutr* 2007; **27**: 19-40
9 **Wang CW**, Klionsky DJ. The molecular mechanism of autophagy. *Mol Med* 2003; **9**: 65-76
10 **Klionsky DJ**. The molecular machinery of autophagy: unanswered questions. *J Cell Sci* 2005; **118**: 7-18
11 **Dunn WA**. Studies on the mechanisms of autophagy: formation of the autophagic vacuole. *J Cell Biol* 1990; **110**: 1923-1933
12 **Kaushik S**, Cuervo AM. Chaperone-mediated autophagy. *Methods Mol Biol* 2008; **445**: 227-244
13 **Massey A**, Kiffin R, Cuervo AM. Pathophysiology of chaperone-mediated autophagy. *Int J Biochem Cell Biol* 2004; **36**: 2420-2434
14 **Cuervo AM**, Dice JF. Age-related decline in chaperone-mediated autophagy. *J Biol Chem* 2000; **275**: 31505-31513
15 **Chiang HL**, Dice JF. Peptide sequences that target proteins for enhanced degradation during serum withdrawal. *J Biol Chem* 1988; **263**: 6797-6805
16 **Mizushima N**, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature* 2008; **451**: 1069-1075
17 **Cuervo AM**. Autophagy: in sickness and in health. *Trends Cell Biol* 2004; **14**: 70-77
18 **Bryant NJ**, Stevens TH. Vacuole biogenesis in Saccharomyces cerevisiae: protein transport pathways to the yeast vacuole. *Microbiol Mol Biol Rev* 1998; **62**: 230-247
19 **Jones EW**. Three proteolytic systems in the yeast saccharomyces cerevisiae. *J Biol Chem* 1991; **266**: 7963-7966
20 **Robinson JS**, Klionsky DJ, Banta LM, Emr SD. Protein sorting in Saccharomyces cerevisiae: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases. *Mol Cell Biol* 1988; **8**: 4936-4948
21 **Rothman JH**, Stevens TH. Protein sorting in yeast: mutants defective in vacuole biogenesis mislocalize vacuolar proteins into the late secretory pathway. *Cell* 1986; **47**: 1041-1051
22 **Yuan W**, Stromhaug PE, Dunn WA. Glucose-induced autophagy of peroxisomes in Pichia pastoris requires a unique E1-like protein. *Mol Biol Cell* 1999; **10**: 1353-1366
23 **Chiang HL**, Schekman R. Regulated import and degradation of a cytosolic protein in the yeast vacuole. *Nature* 1991; **350**: 313-318
24 **Gancedo C**. Inactivation of fructose-1,6-diphosphatase by glucose in yeast. *J Bacteriol* 1971; **107**: 401-405
25 **Gamo FJ**, Navas MA, Blazquez MA, Gancedo C, Gancedo JM. Catabolite inactivation of heterologous fructose-1,6-bisphosphatases and fructose-1,6-bisphosphatase-beta-galactosidase fusion proteins in Saccharomyces cerevisiae. *Eur J Biochem* 1994; **222**: 879-884
26 **Schork SM**, Thumm M, Wolf DH. Catabolite inactivation of fructose-1,6-bisphosphatase of Saccharomyces cerevisiae. Degradation occurs via the ubiquitin pathway. *J Biol Chem* 1995; **270**: 26446-26450
27 **Schork SM**, Bee G, Thumm M, Wolf DH. Site of catabolite inactivation. *Nature* 1994; **369**: 283-284
28 **Minard KI**, McAlister-Henn L. Glucose-induced degradation of the MDH2 isozyme of malate dehydrogenase in yeast. *J Biol Chem* 1992; **267**: 17458-17464
29 **Hung GC**, Brown CR, Wolfe AB, Liu J, Chiang HL. Degradation of the gluconeogenic enzymes fructose-1,6-bisphosphatase and malate dehydrogenase is mediated by distinct proteolytic pathways and signaling events. *J Biol Chem* 2004; **279**: 49138-49150
30 **Brown CR**, Hung GC, Dunton D, Chiang HL. The TOR complex 1 is distributed in endosomes and in retrograde vesicles that form from the vacuole membrane and plays an important role in the vacuole import and degradation pathway. *J Biol Chem* 2010; **285**: 23359-23370
31 **Chiang HL**, Schekman R, Hamamoto S. Selective uptake of cytosolic, peroxisomal, and plasma membrane proteins into the yeast lysosome for degradation. *J Biol Chem* 1996; **271**: 9934-9941
32 **Hoffman M**, Chiang HL. Isolation of degradation-deficient mutants defective in the targeting of fructose-1,6-bisphosphatase into the vacuole for degradation in Saccharomyces cerevisiae. *Genetics* 1996; **143**: 1555-1566
33 **Mazón MJ**, Gancedo JM, Gancedo C. Inactivation of yeast fructose-1,6-bisphosphatase. In vivo phosphorylation of the enzyme. *J Biol Chem* 1982; **257**: 1128-1130
34 **Jiang Y**, Davis C, Broach JR. Efficient transition to growth on fermentable carbon sources in Saccharomyces cerevisiae requires signaling through the Ras pathway. *EMBO J* 1998;

17: 6942-6951
35 **Schork SM**, Bee G, Thumm M, Wolf DH. Catabolite inactivation of fructose-1,6-bisphosphatase in yeast is mediated by the proteasome. *FEBS Lett* 1994; **349**: 270-274
36 **Hämmerle M**, Bauer J, Rose M, Szallies A, Thumm M, Düsterhus S, Mecke D, Entian KD, Wolf DH. Proteins of newly isolated mutants and the amino-terminal proline are essential for ubiquitin-proteasome-catalyzed catabolite degradation of fructose-1,6-bisphosphatase of Saccharomyces cerevisiae. *J Biol Chem* 1998; **273**: 25000-25005
37 **Horak J**, Regelmann J, Wolf DH. Two distinct proteolytic systems responsible for glucose-induced degradation of fructose-1,6-bisphosphatase and the Gal2p transporter in the yeast Saccharomyces cerevisiae share the same protein components of the glucose signaling pathway. *J Biol Chem* 2002; **277**: 8248-8254
38 **Regelmann J**, Schüle T, Josupeit FS, Horak J, Rose M, Entian KD, Thumm M, Wolf DH. Catabolite degradation of fructose-1,6-bisphosphatase in the yeast Saccharomyces cerevisiae: a genome-wide screen identifies eight novel GID genes and indicates the existence of two degradation pathways. *Mol Biol Cell* 2003; **14**: 1652-1663
39 **Brown CR**, McCann JA, Hung GG, Elco CP, Chiang HL. Vid22p, a novel plasma membrane protein, is required for the fructose-1,6-bisphosphatase degradation pathway. *J Cell Sci* 2002; **115**: 655-666
40 **Brown CR**, Wolfe AB, Cui D, Chiang HL. The vacuolar import and degradation pathway merges with the endocytic pathway to deliver fructose-1,6-bisphosphatase to the vacuole for degradation. *J Biol Chem* 2008; **283**: 26116-26127
41 **Huang PH**, Chiang HL. Identification of novel vesicles in the cytosol to vacuole protein degradation pathway. *J Cell Biol* 1997; **136**: 803-810
42 **Brown CR**, McCann JA, Chiang HL. The heat shock protein Ssa2p is required for import of fructose-1, 6-bisphosphatase into Vid vesicles. *J Cell Biol* 2000; **150**: 65-76
43 **Brown CR**, Cui DY, Hung GG, Chiang HL. Cyclophilin A mediates Vid22p function in the import of fructose-1,6-bisphosphatase into Vid vesicles. *J Biol Chem* 2001; **276**: 48017-48026
44 **Shieh HL**, Chen Y, Brown CR, Chiang HL. Biochemical analysis of fructose-1,6-bisphosphatase import into vacuole import and degradation vesicles reveals a role for UBC1 in vesicle biogenesis. *J Biol Chem* 2001; **276**: 10398-10406
45 **Chiang MC**, Chiang HL. Vid24p, a novel protein localized to the fructose-1, 6-bisphosphatase-containing vesicles, regulates targeting of fructose-1,6-bisphosphatase from the vesicles to the vacuole for degradation. *J Cell Biol* 1998; **140**: 1347-1356
46 **Brown CR**, Dunton D, Chiang HL. The vacuole import and degradation pathway utilizes early steps of endocytosis and actin polymerization to deliver cargo proteins to the vacuole for degradation. *J Biol Chem* 2010; **285**: 1516-1528
47 **Engqvist-Goldstein AE**, Drubin DG. Actin assembly and endocytosis: from yeast to mammals. *Annu Rev Cell Dev Biol* 2003; **19**: 287-332
48 **Kaksonen M**, Sun Y, Drubin DG. A pathway for association of receptors, adaptors, and actin during endocytic internalization. *Cell* 2003; **115**: 475-487
49 **Toret CP**, Drubin DG. The budding yeast endocytic pathway. *J Cell Sci* 2006; **119**: 4585-4587
50 **Dewar H**, Warren DT, Gardiner FC, Gourlay CG, Satish N, Richardson MR, Andrews PD, Ayscough KR. Novel proteins linking the actin cytoskeleton to the endocytic machinery in Saccharomyces cerevisiae. *Mol Biol Cell* 2002; **13**: 3646-3661
51 **Warren DT**, Andrews PD, Gourlay CW, Ayscough KR. Sla1p couples the yeast endocytic machinery to proteins regulating actin dynamics. *J Cell Sci* 2002; **115**: 1703-1715
52 **Howard JP**, Hutton JL, Olson JM, Payne GS. Sla1p serves as the targeting signal recognition factor for NPFX(1,2)D-mediated endocytosis. *J Cell Biol* 2002; **157**: 315-326
53 **Reinke A**, Anderson S, McCaffery JM, Yates J, Aronova S, Chu S, Fairclough S, Iverson C, Wedaman KP, Powers T. TOR complex 1 includes a novel component, Tco89p (YPL180w), and cooperates with Ssd1p to maintain cellular integrity in Saccharomyces cerevisiae. *J Biol Chem* 2004; **279**: 14752-14762
54 **Wedaman KP**, Reinke A, Anderson S, Yates J, McCaffery JM, Powers T. Tor kinases are in distinct membrane-associated protein complexes in Saccharomyces cerevisiae. *Mol Biol Cell* 2003; **14**: 1204-1220
55 **Ramachandran V**, Herman PK. Antagonistic interactions between the cAMP-dependent protein kinase and Tor signaling pathways modulate cell growth in Saccharomyces cerevisiae. *Genetics* 2011; **187**: 441-454
56 **Cui DY**, Brown CR, Chiang HL. The type 1 phosphatase Reg1p-Glc7p is required for the glucose-induced degradation of fructose-1,6-bisphosphatase in the vacuole. *J Biol Chem* 2004; **279**: 9713-9724
57 **Zheng M**, Hu K, Liu W, Hu X, Hu F, Huang L, Wang P, Hu Y, Huang Y, Li W, Liang C, Yin X, He Q, Yu X. Proteomic analysis of excretory secretory products from Clonorchis sinensis adult worms: molecular characterization and serological reactivity of a excretory-secretory antigen-fructose-1,6-bisphosphatase. *Parasitol Res* 2011; **109**: 737-744
58 **Kepka A**, Dariusz Szajda S, Stypułkowska A, Waszkiewicz N, Jankowska A, Chojnowska S, Zwierz K. Urinary fructose-1,6-bisphosphatase activity as a marker of the damage to the renal proximal tubules in children with idiopathic nephrotic syndrome. *Clin Chem Lab Med* 2008; **46**: 831-835
59 **Wang Y**, Tomlinson B. Managlinat dialanetil, a fructose-1,6-bisphosphatase inhibitor for the treatment of type 2 diabetes. *Curr Opin Investig Drugs* 2007; **8**: 849-858

S- Editor Cheng JX **L- Editor** O'Neill M **E- Editor** Zheng XM

World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/1949-8454office
wjbc@wjgnet.com
doi:10.4331/wjbc.v2.i11.246

World J Biol Chem 2011 November 26; 2(11): 246-251
ISSN 1949-8454 (online)

BRIEF ARTICLE

Inhibitory role of TACE/ADAM17 cytotail in protein ectodomain shedding

Xiaojin Li, Liliana Pérez, Huizhou Fan

Xiaojin Li, Liliana Pérez, Huizhou Fan, Department of Physiology and Biophysics, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854, United States
Author contributions: Fan H conceived the study; all authors were involved in experimental design; Li X and Pérez L performed the experiments; Fan H, Li X and Pérez L analyzed the data; Fan H wrote the manuscript.
Supported by Grants from the National Institutes of Health, No. AG029859; the National Center of the American Heart Association, No. 0330335N; the New Jersey Commission on Cancer Research (NJCCR 703010) to Fan H
Correspondence to: Huizhou Fan, MD, PhD, Department of Physiology and Biophysics, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 683 Hoes Lane West, Piscataway, NJ 08854, United States. fanhu@umdnj.edu
Telephone: +1-732-2354607 Fax: +1-732-2355038
Received: August 22, 2011 Revised: October 12, 2011
Accepted: October 19, 2011
Published online: November 26, 2011

Abstract

AIM: To determine if the cytotail of the principal sheddase tumor necrosis factor-α converting enzyme (TACE; ADAM17) controls protein ectodomain shedding.

METHODS: Site-directed mutagenesis was performed to derive TACE variants. The resulting TACE expression plasmids with amino acid substitutions in the extracellular, cysteine-rich disintegrin domain (CRD) and/or deleted cytotail, along with an expression vector for the enhanced green fluorescence protein were transfected into shedding-defective M1 mutants stably expressing transmembrane L-selectin or transforming growth factor (TGF)-α. The expression levels of the TACE substrates at the cell surface were determined by flow cytometry.

RESULTS: Consistent with published data, a single point mutation (C600Y) in the CRD led to shedding deficiency. However, removal of the cytotail from the C600Y TACE variant partially restored ectodomain cleavage of TGF-α and L-selectin. Cytotail-deleted mutants with any other substituting amino acid residues in place of Cys600 displayed similar function compared with tailless C600Y TACE.

CONCLUSION: The cytotail plays an inhibitory role, which becomes evident when it is removed from an enzyme with another mutation that affects the enzyme function.

Key words: ADAM17; Ectodomain shedding; L-selectin; Tumor necrosis factor-α converting enzyme

Peer reviewer: Shiyou Chen, PhD, Assistant Professor, University of Georgia, 501 DW Brooks Drive, Athens, GA 30602, United States

Li X, Pérez L, Fan H. Inhibitory role of TACE/ADAM17 cytotail in protein ectodomain shedding. *World J Biol Chem* 2011; 2(11): 246-251 Available from: URL: http://www.wjgnet.com/1949-8454/full/v2/i11/246.htm DOI: http://dx.doi.org/10.4331/wjbc.v2.i11.246

INTRODUCTION

Protein ectodomain shedding serves as an important means for regulating the function of cell surface proteins required for a variety of physiological processes[1]. For example, generation of freely diffusible epidermal growth factor receptor (EGFR) ligands including transforming growth factor (TGF)-α from their transmembrane precursors is essential for the development of multiple organs[2-4]. Ectodomain shedding is also crucial for pathogenesis. Thus, overproduction of soluble EGFR ligands causes cellular transformation[5]; generation of the cir-

culating cytokine tumor necrosis factor (TNF)-α from its transmembrane precursor is responsible for cachexia, septic shock and other inflammatory conditions[6-8].

TNF-α converting enzyme (TACE) is a principal sheddase that cleaves not only transmembrane TNF-α, but also a large number of other membrane proteins[9]. As a member of the large family of a disintegrin and metalloprotease domains (ADAM), TACE or ADAM17 is a membrane-anchored metalloprotease (Figure 1)[10,11]. The protease is biosynthesized as a zymogen, in which the catalytic domain is led by an amino-terminal prodomain, and followed in succession by a cysteine-rich disintegrin domain (CRD), a transmembrane segment and a carboxyl terminal cytotail. The TACE zymogen is enzymatically inactive because the prodomain interacts with the active site, causing inaccessibility to protein substrates.

Ectodomain shedding by TACE is a tightly regulated process. Accordingly, a variety of stimuli including growth factors, inflammatory mediators, ionophores, carcinogens and tumor promoters can induce shedding[12-14]. However, the cellular and molecular mechanism that controls TACE-mediated shedding remains unclear. Although prodomain removal is a prerequisite for TACE to gain catalytic activity[15-17], it does not seem sufficient for shedding activation, because an increase in prodomain removal is not observed following stimulation[11]. Therefore, activation of shedding appears to be through modulation of mature TACE.

It has been established that signaling pathways involving two mitogen-activated protein kinases (MAPKs), Erk and p38, mediate the activation of shedding in response to various stimuli[12,13,18]. The serine- and threonine-rich TACE cytotail is suspected to play a role in the regulation of TACE function. In particular, both MAPKs have been shown to phosphorylate directly the TACE cytotail at Thr-735[18-20]. Erk activity-dependent phosphorylation at Ser-819 has also been demonstrated. Furthermore, Ser-791 is phosphorylated in resting cells, and undergoes dephosphorylation in response to growth factor stimulation[21]. However, mutation of these phosphorylation sites individually or in combination, and even removal of the entire cytotail have no detectable effects on shedding[21-24]. Thus, the function of the TACE cytotail remains illusive.

There is also evidence suggesting a role for the CRD in TACE-mediated shedding[9,24-26]. We have previously demonstrated that a substitution (C600Y) within the CRD results in enzyme inactivity[9]. Interestingly, in an attempt to examine the function of other cysteines in the CRD, we found that deletion of the cytotail partially restores the shedding activity in the C600Y TACE variant. This finding suggests an inhibitory role for the cytotail in ectodomain shedding and resolves a long mystery with regarding the function of the TACE cytotail, which becomes apparent only when there is another defect in the enzyme.

MATERIALS AND METHODS

Reagents

Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), bovine serum albumin (BSA), penicillin, streptomycin, 1,10-phenanthroline, EDTA, paraformaldehyde and inorganic salts were purchased from Sigma-Aldrich (St. Louis, MO). Mouse monoclonal antibodies recognizing the ectodomain of L-selectin and TGF-α have been described previously[12,21,27]. Phycoerythrin (PE)-conjugated goat anti-mouse IgG (whole molecule) was purchased from Jackson ImunoResearch Laboratories (West Grove, PA, United States). Cell Lifters were purchased from Corning Inc. (Corning, NY, United States).

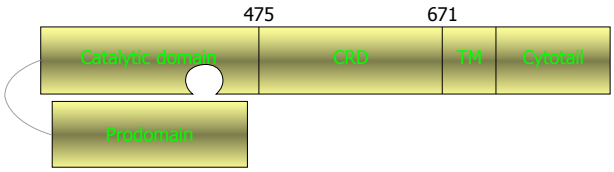


Figure 1 Schematic drawing of tumor necrosis factor-α converting enzyme domain structure. Lengths of domains are not in proportion. The amino acids marking the beginning and the end of the cysteine-rich/disintegrin domain (CRD) are numbered. TM: Transmembrane domain.

Expression vectors

pRK5-based plasmids for expressing wild-type murine TACE, C600Y TACE, cytotail-truncated TACE (ΔC) and the ΔC derivatives containing C600Y and C600A mutations (i.e. ΔC/C600Y and ΔC/C600A, respectively) have been described previously[9]. Additional ΔC constructs carrying an alanine in place of each of the remaining 25 cysteines in the CRD (i.e. ΔC/C478A, ΔC/C489A, ΔC/C501A, ΔC/C502A, ΔC/C506A, ΔC/C514A, ΔC/C521A, ΔC/C522A, ΔC/C525A, ΔC/C534A, ΔC/C542A, ΔC/C548A, ΔC/C555A, ΔC/C567A, ΔC/C573A, ΔC/C578A, ΔC/C582A, ΔC/C591A, ΔC/C593A, ΔC/C603A, ΔC/C604A, ΔC/C611A, ΔC/C630A, ΔC/C635A and ΔC/C641A) were constructed for this work by polymerase chain reactions using the high fidelity Pfu DNA polymerase (Stratagene, La Jolla, CA, United States). Sequence authenticity of the inserts in the final expression vectors was confirmed by automated DNA sequencing performed by the UMDNJ-Robert Wood Johnson Medical School DNA Core Facility.

Cell lines and culture conditions

The shedding-defective M1-L-selectin and M1-TGF-α cells, which overexpress transmembrane L-selectin and TGF-α, respectively, have been described previously[22]. The cell lines were maintained as adherent cultures using DMEM supplemented with 8% FBS and the antibiotics penicillin and streptomycin.

Assays of L-selectin and TGF-α ectodomain shedding

The enzyme activity of wild-type or mutant TACE was determined with cell-based shedding assays using two-color flow cytometry as described previously[28]. M1-L-selectin and M1-TGF-α cells were transiently cotransfected with a TACE expression vector (or the control RK5 vector) and a mammalian expression plasmid for

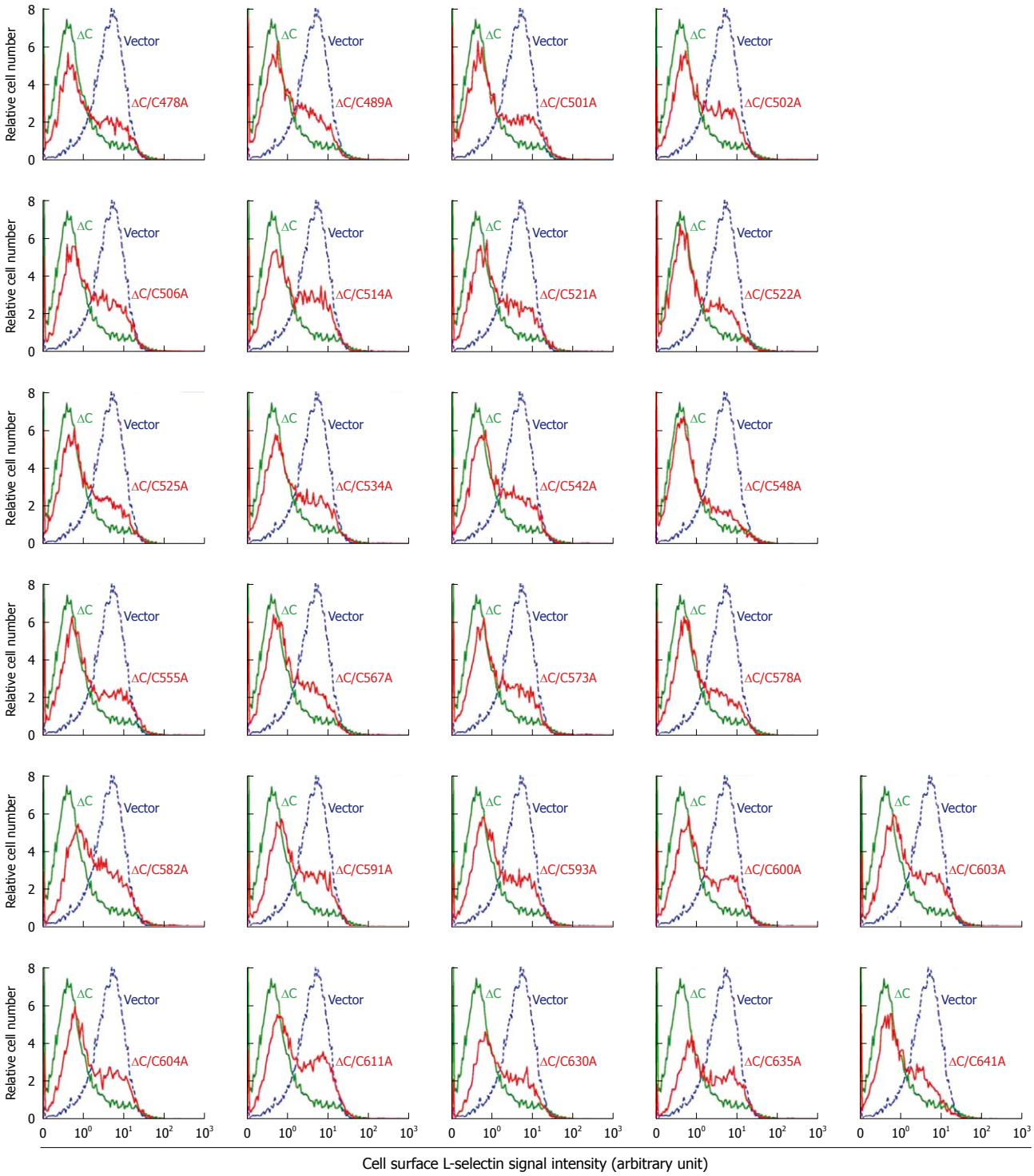


Figure 2 L-selectin ectodomain shedding activity in cytotail-deleted tumor necrosis factor-α converting enzyme constructs with cysteine to alanine substitutions. The shedding-defective M1-L-selectin cells, which stably express transmembrane L-selectin, were transiently cotransfected with the transfection marker green fluorescence protein (GFP), and indicated tumor necrosis factor-α converting enzyme (TACE) vectors or the control pRK5 plasmid at a ratio of 1:3. Transmembrane L-selectin on the surface of live unfixed cells was immunostained with anti-L-selectin and a PE-conjugated secondary antibody, and was detected by flow cytometry. GFP-positive cells were gated as cells expressing the cotransfected TACE construct. Green: ΔC TACE; Red: ΔC TACE with a cysteine to alanine substitution at indicated position; dotted blue lines, pRK5.

enhanced green fluorescence protein (GFP) at a ratio of 3:1. Twenty hours after transfection, cell culture plates were placed on ice and the medium was replaced with cold PBS, supplemented with 10 mmol/L 1, 10-phenanthroline (a metalloproteases inhibitor), 5 mmol/L EDTA, 2% BSA and 0.1% NaN_3 (PEB). Cells were scraped off the plates with a Cell Lifter, collected and centrifuged at 1000 r/min at 4 ℃ for 5 min. Following the removal of the supernatant, cells were resuspended in 50 μL PEB containing 500 ng DREG56 (anti-L-

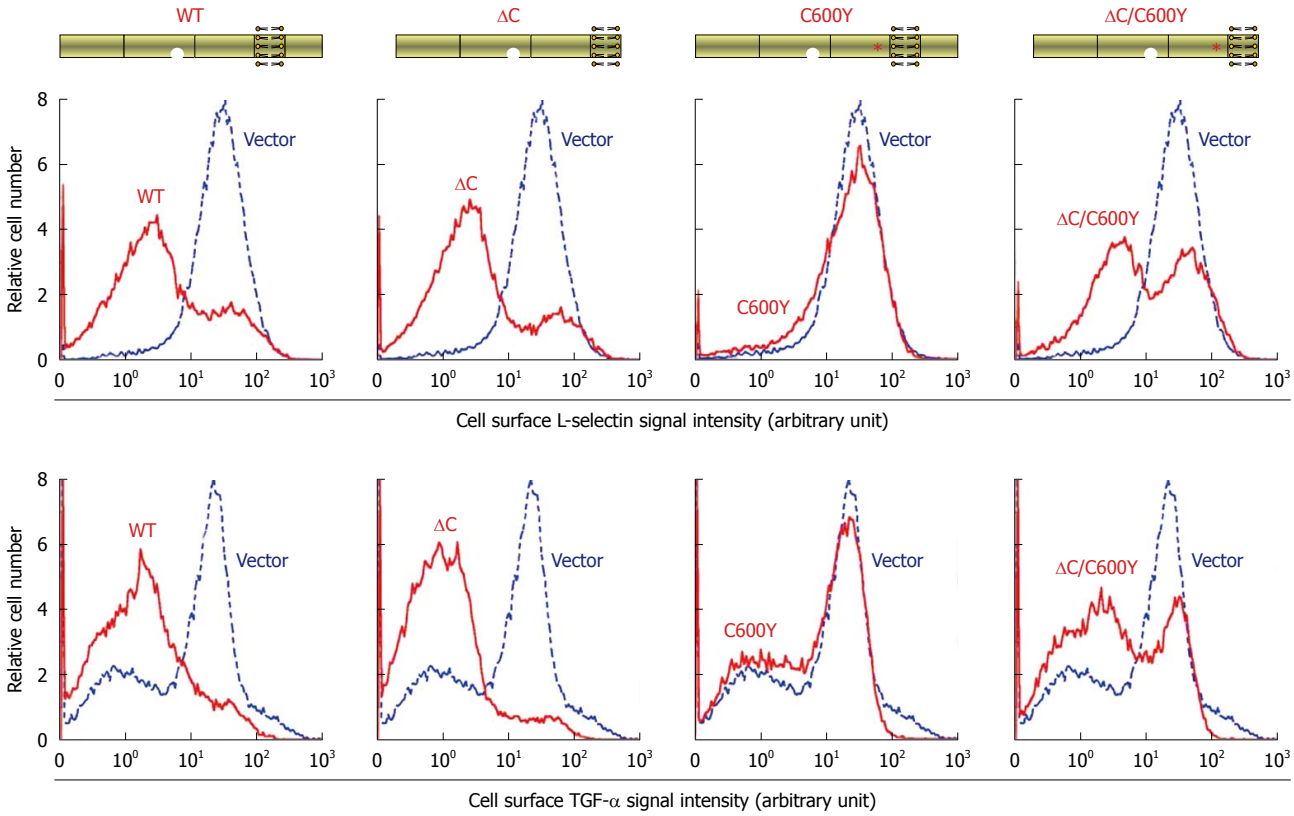


Figure 3 Deletion of the cytotail from C600Y tumor necrosis factor-α converting enzyme led to partial restoration of L-selectin and transforming growth factor α shedding. Expression plasmids for wild-type or mutated tumor necrosis factor-α converting enzyme (TACE) are schematically shown on the top of the Figure. The activity of L-selectin shedding in the TACE constructs was determined as in Figure 2. Transforming growth factor (TGF)-α shedding was determined in a similar manner except the M1-L-selectin cells were replaced with shedding-defective M1-TGF-α cells, which overexpress transmembrane TGF-α, and the anti-L-selectin was substituted with anti-TGF-α.

selectin) or 200 ng anti-TGF-α, incubated on ice for 30 min with gentle agitation, washed twice with PEB and reacted with 50 μL PE-conjugated goat anti-mouse IgG diluted in PEB (1:200) for 30 min. After another two washes with PEB, cells were fixed in 500 μL of 1% paraformaldehyde (prepared in PEB) and were immediately analyzed by flow cytometry using a Coulter Epics XL.MCL flow cytometer (Beckman Coulter, Miami, FL, United States). GFP and PE signals were simultaneously detected through the FL1 and FL2 channels, respectively. For each analysis, unstained stable cells (M1-L-selectin or M1-TGF-α) cotransfected with GFP and the control pRK5 vector without an insert, and immunostained stable cells transfected with pRK5 only (i.e. no GFP), were used to set up the instrument to obtain optimal GFP-PE signal compensation. Mock-stained parental M1 cells were used to verify the final compensatory conditions under which about 98% of the cells were recognized as GFP- and PE-doubly negative cells.

RESULTS

Previously, we have identified a TACE variant carrying a C600Y substitution within the CRD from a shedding-defective cell line. Cell-based shedding assays confirmed that the variant sheddase lacks the capacity to cleave substrates[9]. On the basis of the tertiary structure of the CRD of ADAM10, which share a high level of sequence homology to that of TACE[9], Cys-600 forms a disulfide bridge with Cys-593; also, there may be additional 12 disulfide bridges[29]. To determine if other cysteines and/or disulfide bridges are also important for TACE function, we replaced each of the remaining cysteine in the CRD with alanine. For the convenience of vector construction, the C→A mutants were constructed on the ΔC TACE backbone because previous studies have shown that deletion of the cytotail does not affect TACE function[21,23,24]. The enzyme activities of the resulting ΔC/C→A variants were determined in the M1-L-selectin cell line, which overexpresses transmembrane L-selectin but is defective in ectodomain shedding, due to the loss of one TACE allele and a mutation in the other allele resulting in an M435I substitution at the active site[9,28].

Contrary to our prediction, all the ΔC/C→A variants were largely active in L-selectin shedding (Figure 2). Particularly surprising was the L-selectin shedding activity exhibited by the ΔC/C600A variant, because we have previously shown that ΔC/C600A and ΔC/C600Y have no detectable enzyme activity, similar to the full-length C600Y TACE[9]. In that study, the inactivity of C600Y TACE was demonstrated in all three TACE-defective cell lines, with multiple substrates, and using different detec-

tive methods; however, the ΔC/C600A and ΔC/C600Y constructs were tested only with one TACE substrate using one-color flow cytometry, which cannot discriminate TACE-expressing cells from untransfected cells[9]. Therefore, we believe that the data showing enzymatic inactivity in the ΔC/C600A and ΔC/C600Y constructs were erroneous. In contrast, the shedding-proficiency in the ΔC/C600A mutant, as demonstrated using two-color flow cytometry in which eGFP served as a surrogate marker for TACE expression (Figure 2), ought to be considered reliable.

We next compared the enzyme activities of the full-length C600Y TACE, the ΔC/C600Y construct, and their respective parental forms (i.e. wild-type TACE and ΔC TACE). Consistent with published data, ΔC TACE demonstrated levels of enzyme activity that were similar to those in wild-type TACE, in the cleavage of transmembrane L-selectin and TGF-α, whereas full-length C600Y TACE completely failed to cleave these substrates (Figure 3). Evidently, the ΔC/C600Y construct partially regained L-selectin and TGF-α shedding activities, as compared to the C600Y variant (Figure 3). These results indicate that the cytotail plays an inhibitory role in ectodomain shedding, which became detectable when there was another defect in the enzyme.

DISCUSSION

TACE is responsible for ectodomain shedding of numerous membrane proteins and it is required for a variety of physiological processes, but how its activity is regulated remains unsatisfactorily defined. It was thought, immediately after the sequencing of the TACE cDNA, that the cytotail of the sheddase plays a critical role in the enzyme activity[11]. However, this hypothesis has been questioned because removal of the entire cytotail has demonstrated no detectable effect on TACE-dependent shedding[21-23,28]. The lack of an apparent effect of the cytotail deletion (in wild-type TACE) was again reproduced in this work. Nevertheless, removal of the cytotail from the shedding-defective C600Y TACE variant clearly led to a substantial restoration of enzyme activity. Thus, the cytotail plays an inhibitory role, which becomes noticeable only in the presence of another defect in the enzyme.

How the cytotail exerts an inhibitory activity in shedding has yet to be defined. It is known that the cytotail is phosphorylated in response to activators of ectodomain shedding[11,18-21]. We speculate that the phosphorylation alleviates the inhibitory activity of the cytotail, which is also achievable by the deletion of the cytotail.

Cys-600 has been predicted to participate in the formation of a disulfide bridge[29]. Interestingly, recent studies have indicated that agents that affect the redox potential regulate TACE activity[24,25]. Furthermore, protein disulfide isomerase has been shown to inhibit TACE activity through modifying the conformation of the CRD[25]. The fact that the inhibitory role of the cytotail was discovered in the context of Cys-600 mutations suggests that the cytotail of TACE regulates disulfide bridging and thus the conformation of the CRD.

In conclusion, our study, aimed at furthering the understanding of how the CRD regulates the function of TACE, has led to the recognition of a new, inhibitory role for the cytotail in ectodomain shedding. However, the original question about which, if any, cysteines other than Cys-600 in the CRD are critical for the enzyme activity of TACE has yet to be answered using full-length constructs.

ACKNOWLEDGMENTS

This work was presented to the Gordon Research Conference on Regulated Proteolysis of Cell Surface Proteins held in Davidson College, NC, United States in July, 2011.

COMMENTS

Background

Tumor necrosis factor-α converting enzyme (TACE) is responsible for the cleavage of numerous membrane proteins at the cell surface. The enzyme activity of TACE is tightly regulated by intracellular signaling pathways. The carboxyl-terminal cytotail has long been thought to function as a signaling transducer in the regulation of TACE function. However, deletion of the cytotail so far has not shown a detectable effect on protein ectodomain shedding.

Research frontiers

In addition to normal development, TACE is required for the pathogenesis of diseases including inflammation and autoimmunity. Identification of new domain function in TACE has implications for molecular cell physiology and pathophysiology, and also may help development of new therapeutic agents.

Innovations and breakthroughs

The present study identifies an inhibitory role for the TACE cytotail. This new function is detectable when the cytotail is removed from a TACE construct with a mutation in the cysteine-rich disintegrin domain, which adversely affects the enzyme function. Previous studies have failed to recognize this important role because the cytotail-truncation mutants used in those studies did not have an additional mutation.

Peer review

Despite a limited number of data presented by the authors, the newly identified function of the cytotail is both interesting and of potential significance. The introduction was somewhat unnecessarily detailed, and a few places could be revised for enhanced clarification.

REFERENCES

1 **Becherer JD**, Blobel CP. Biochemical properties and functions of membrane-anchored metalloprotease-disintegrin proteins (ADAMs). *Curr Top Dev Biol* 2003; **54**: 101-123
2 **Blobel CP**. ADAMs: key components in EGFR signalling and development. *Nat Rev Mol Cell Biol* 2005; **6**: 32-43
3 **Jackson LF**, Qiu TH, Sunnarborg SW, Chang A, Zhang C, Patterson C, Lee DC. Defective valvulogenesis in HB-EGF and TACE-null mice is associated with aberrant BMP signaling. *EMBO J* 2003; **22**: 2704-2716
4 **Zhao J**, Chen H, Peschon JJ, Shi W, Zhang Y, Frank SJ, Warburton D. Pulmonary hypoplasia in mice lacking tumor necrosis factor-alpha converting enzyme indicates an indispensable role for cell surface protein shedding during embryonic lung branching morphogenesis. *Dev Biol* 2001; **232**: 204-218
5 **Sandgren EP**, Luetteke NC, Palmiter RD, Brinster RL, Lee DC. Overexpression of TGF alpha in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. *Cell* 1990; **61**: 1121-1135

6 **Tracey KJ**, Morgello S, Koplin B, Fahey TJ, Fox J, Aledo A, Manogue KR, Cerami A. Metabolic effects of cachectin/tumor necrosis factor are modified by site of production. Cachectin/tumor necrosis factor-secreting tumor in skeletal muscle induces chronic cachexia, while implantation in brain induces predominantly acute anorexia. *J Clin Invest* 1990; **86**: 2014-2024

7 **Horiuchi K**, Kimura T, Miyamoto T, Takaishi H, Okada Y, Toyama Y, Blobel CP. Cutting edge: TNF-alpha-converting enzyme (TACE/ADAM17) inactivation in mouse myeloid cells prevents lethality from endotoxin shock. *J Immunol* 2007; **179**: 2686-2689

8 **Conway JG**, Andrews RC, Beaudet B, Bickett DM, Boncek V, Brodie TA, Clark RL, Crumrine RC, Leenitzer MA, McDougald DL, Han B, Hedeen K, Lin P, Milla M, Moss M, Pink H, Rabinowitz MH, Tippin T, Scates PW, Selph J, Stimpson SA, Warner J, Becherer JD. Inhibition of tumor necrosis factor-alpha (TNF-alpha) production and arthritis in the rat by GW3333, a dual inhibitor of TNF-alpha-converting enzyme and matrix metalloproteinases. *J Pharmacol Exp Ther* 2001; **298**: 900-908

9 **Li X**, Fan H. Loss of ectodomain shedding due to mutations in the metalloprotease and cysteine-rich/disintegrin domains of the tumor necrosis factor-alpha converting enzyme (TACE). *J Biol Chem* 2004; **279**: 27365-27375

10 **Moss ML**, Jin SL, Milla ME, Bickett DM, Burkhart W, Carter HL, Chen WJ, Clay WC, Didsbury JR, Hassler D, Hoffman CR, Kost TA, Lambert MH, Leesnitzer MA, McCauley P, McGeehan G, Mitchell J, Moyer M, Pahel G, Rocque W, Overton LK, Schoenen F, Seaton T, Su JL, Becherer JD. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. *Nature* 1997; **385**: 733-736

11 **Black RA**, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature* 1997; **385**: 729-733

12 **Fan H**, Derynck R. Ectodomain shedding of TGF-alpha and other transmembrane proteins is induced by receptor tyrosine kinase activation and MAP kinase signaling cascades. *EMBO J* 1999; **18**: 6962-6972

13 **Gechtman Z**, Alonso JL, Raab G, Ingber DE, Klagsbrun M. The shedding of membrane-anchored heparin-binding epidermal-like growth factor is regulated by the Raf/mitogen-activated protein kinase cascade and by cell adhesion and spreading. *J Biol Chem* 1999; **274**: 28828-28835

14 **Pandiella A**, Massagué J. Multiple signals activate cleavage of the membrane transforming growth factor-alpha precursor. *J Biol Chem* 1991; **266**: 5769-5773

15 **Gonzales PE**, Solomon A, Miller AB, Leesnitzer MA, Sagi I, Milla ME. Inhibition of the tumor necrosis factor-alpha-converting enzyme by its pro domain. *J Biol Chem* 2004; **279**: 31638-31645

16 **Milla ME**, Leesnitzer MA, Moss ML, Clay WC, Carter HL, Miller AB, Su JL, Lambert MH, Willard DH, Sheeley DM, Kost TA, Burkhart W, Moyer M, Blackburn RK, Pahel GL, Mitchell JL, Hoffman CR, Becherer JD. Specific sequence elements are required for the expression of functional tumor necrosis factor-alpha-converting enzyme (TACE). *J Biol Chem* 1999; **274**: 30563-30570

17 **Schlöndorff J**, Becherer JD, Blobel CP. Intracellular maturation and localization of the tumour necrosis factor alpha convertase (TACE). *Biochem J* 2000; **347** Pt 1: 131-138

18 **Xu P**, Derynck R. Direct activation of TACE-mediated ectodomain shedding by p38 MAP kinase regulates EGF receptor-dependent cell proliferation. *Mol Cell* 2010; **37**: 551-566

19 **Díaz-Rodríguez E**, Montero JC, Esparís-Ogando A, Yuste L, Pandiella A. Extracellular signal-regulated kinase phosphorylates tumor necrosis factor alpha-converting enzyme at threonine 735: a potential role in regulated shedding. *Mol Biol Cell* 2002; **13**: 2031-2044

20 **Soond SM**, Everson B, Riches DW, Murphy G. ERK-mediated phosphorylation of Thr735 in TNFalpha-converting enzyme and its potential role in TACE protein trafficking. *J Cell Sci* 2005; **118**: 2371-2380

21 **Fan H**, Turck CW, Derynck R. Characterization of growth factor-induced serine phosphorylation of tumor necrosis factor-alpha converting enzyme and of an alternatively translated polypeptide. *J Biol Chem* 2003; **278**: 18617-18627

22 **Li X**, Pérez L, Pan Z, Fan H. The transmembrane domain of TACE regulates protein ectodomain shedding. *Cell Res* 2007; **17**: 985-998

23 **Reddy P**, Slack JL, Davis R, Cerretti DP, Kozlosky CJ, Blanton RA, Shows D, Peschon JJ, Black RA. Functional analysis of the domain structure of tumor necrosis factor-alpha converting enzyme. *J Biol Chem* 2000; **275**: 14608-14614

24 **Wang Y**, Herrera AH, Li Y, Belani KK, Walcheck B. Regulation of mature ADAM17 by redox agents for L-selectin shedding. *J Immunol* 2009; **182**: 2449-2457

25 **Willems SH**, Tape CJ, Stanley PL, Taylor NA, Mills IG, Neal DE, McCafferty J, Murphy G. Thiol isomerases negatively regulate the cellular shedding activity of ADAM17. *Biochem J* 2010; **428**: 439-450

26 **Amour A**, Slocombe PM, Webster A, Butler M, Knight CG, Smith BJ, Stephens PE, Shelley C, Hutton M, Knäuper V, Docherty AJ, Murphy G. TNF-alpha converting enzyme (TACE) is inhibited by TIMP-3. *FEBS Lett* 1998; **435**: 39-44

27 **Kahn J**, Walcheck B, Migaki GI, Jutila MA, Kishimoto TK. Calmodulin regulates L-selectin adhesion molecule expression and function through a protease-dependent mechanism. *Cell* 1998; **92**: 809-818

28 **Pérez L**, Kerrigan JE, Li X, Fan H. Substitution of methionine 435 with leucine, isoleucine, and serine in tumor necrosis factor alpha converting enzyme inactivates ectodomain shedding activity. *Biochem Cell Biol* 2007; **85**: 141-149

29 **Janes PW**, Saha N, Barton WA, Kolev MV, Wimmer-Kleikamp SH, Nievergall E, Blobel CP, Himanen JP, Lackmann M, Nikolov DB. Adam meets Eph: an ADAM substrate recognition module acts as a molecular switch for ephrin cleavage in trans. *Cell* 2005; **123**: 291-304

S- Editor Cheng JX **L- Editor** Kerr C **E- Editor** Zheng XM

World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/1949-8454office
wjbc@wjgnet.com
www.wjgnet.com

World J Biol Chem 2011 November 26; 2(11): I
ISSN 1949-8454 (online)

Acknowledgments to reviewers of *World Journal of Biological Chemistry*

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

Shiyou Chen, PhD, Assistant Professor, University of Georgia, 501 DW Brooks Drive, Athens, GA 30602, United States

Hoyun Lee, Senior Scientist and Professor, Tumor Biology Group, Regional Cancer Program, Sudbury Regional Hospital, 41 Ramsey Lake Road, Sudbury, Ontario P3E 5J1, Canada

Bin Shan, MD, PhD, Assistant Professor, Department of Medicine, Tulane University School of Medicine, 1430 Tulane Ave. SL-9, New Orleans, LA 70112, United States

Hong-Gang Wang, PhD, Professor, Lois High Berstler Professor of Pharmacology, Penn State College of Medicine, Penn State Hershey Cancer Institute, CH74, 500 University Drive, PO Box 850, Hershey, PA 17033-0850, United States

Michiaki Yamashita, PhD, Chief, Food Biotechnology Section, National Research Institute of Fisheries Science, 2-12-4 Fukuura, Yokohama 236-8648, Japan

WJBC

World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/1949-8454office
wjbc@wjgnet.com
www.wjgnet.com

World J Biol Chem 2011 November 26; 2(11): I
ISSN 1949-8454 (online)

Events Calendar 2011

January 19-20,
BioBusiness
London, United Kingdom

January 27-28
Predictive Human Toxicity and ADME/Tox Studies 2011
Brussels, Belgium

January 29-February 2
LabAutomation 2011
Palm Springs, United States

February 1-2
2011 Pharma Market Research Conference
Parsippany, United States

February 6-8
5th Drug Discovery for Neurodegeneration
San Diego, United States

February 7-10
3rd International Conference and Exhibition on Drug Discovery and Therapy
Dubai, United Arab Emirates

February 13-16
Natural Products Conference 2011
Sharm el Sheikh, Egypt

February 14-17
Therapeutic Approaches to Neurodegeneration - Age Modifiers, Proteostasis, and Stem Cells
Nassau, Bahamas

February 16-19
Electrochemistry Conference 2011
Sharm el Sheikh, Egypt

February 21-23
World Antibody Drug Conjugate Summit Frankfurt, Germany

February 22-24
2011 International Conference on Bioinformatics and Computational Biology III ROUND
Haikou, China

February 22-25
Medicinal Chemistry Conference 2011
Sharm el Sheikh, Egypt

February 23-25
International Conference on Bioscience, Biotechnology, and Biochemistry
Penang, Malaysia

February 26-28
2011 International Conference on Bioscience, Biochemistry and Bioinformatics
Sentaosa, Singapore

March 4
Discussion Workshop: Perfecting the ELISPOT - a time for answers
London, United Kingdom

March 4-11
Inorganic Reaction Mechanisms
Gordon Research Conferences
Galveston, United States

March 7-8
Fragments 2011 - Third RSC-BMCS Fragment-based Drug Discovery meeting
Stevenage, United Kingdom

March 9-13
10th International Conference on Alzheimers and Parkinsons Diseases
Barcelona, Spain

March 13-18
Pittcon 2011
Atlanta, United States

March 17-20
EMBO|EMBL Symposia: Seeing is Believing - Imaging the Processes of Life
Heidelberg, Germany

March 20-22
The molecular biology of inflammatory bowel diseases
Durham, United Kingdom

March 21-23
World Congress on Biotechnology
Hyderabad, India

March 23-25
BIT's 4th Annual Protein and Peptide Conference
Beijing, China

March 25-27
2011 3rd International Conference on Bioinformatics and Biomedical Technology 3rd round call for paper
Sanya, China

March 27-April 2
EMBO Practical Course - Methods in Chemical Biology
Heidelberg, Germany

April 6-8
Faraday Discussion 150: Frontiers in Spectroscopy
Basel, United States

April 6-8
Membrane Proteins: Structure and Function
Oxford, United Kingdom

April 11-12
7th SCI-RSC symposium on Proteinase Inhibitor Design
Basel, United States

April 11-14
First EuCheMS Inorganic Chemistry Conference (EICC-1)
Manchester, United Kingdom

April 18-19
Analysis of free radicals, radical modifications and redox signalling
Birmingham, United Kingdom

April 20-21
BioFine Europe Exhibition 2011
Cambridge, United Kingdom

May 1-6
46th EUCHEM Conference on Stereochemistry
Brunnen, United States

June 1-5
EMBO Conference Series - Chromatin and Epigenetics
Heidelberg, Germany

June 15-17
Spectroscopy - Detective in Science
Rostock, Germany

June 15-18
3rd International Symposium on Metallomics
Münster, Germany

July 11-13
Ubiquitin Conference
Philadelphia, United States

July 17-22
Charge Transfer in Biosystems - ESF-LFUI Conference
Obergurgl, United States

July 18-20
2nd International Congress on Analytical Proteomics
Ourense, United States

August 3-4
From beads on a string to the pearls of regulation: the structure and dynamics of chromatin
Cambridge, United Kingdom

August 7-12
15th International Conference on Biological Inorganic Chemistry (ICBIC 15)
Vancouver, United States

August 28-September 2
Microscopy Conference 2011
Kiel, Germany

WJBC World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/1949-8454office
wjbc@wjgnet.com
www.wjgnet.com

World J Biol Chem 2011 November 26; 2(11): I-V
ISSN 1949-8454 (online)

GENERAL INFORMATION

World Journal of Biological Chemistry (*World J Biol Chem*, *WJBC*, online ISSN 1949-8454, DOI: 10.4331), is a monthly, open-access (OA), peer-reviewed journal supported by an editorial board of 523 experts in biochemistry and molecular biology from 40 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 *WJBC* 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 *WJBC* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJBC* 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

The major task of *WJBC* is to rapidly report the most recent developments in the research by the close collaboration of biologists and chemists in area of biochemistry and molecular biology, including: general biochemistry, pathobiochemistry, molecular and cellular biology, molecular medicine, experimental methodologies and the diagnosis, therapy, and monitoring of human disease.

Columns

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

Name of journal

World Journal of Biological Chemistry

ISSN

ISSN 1949-8454 (online)

Indexed and Abstracted in

PubMed Central, PubMed, Digital Object Identifer, and Directory of Open Access Journals.

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 from 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, *WJBC* 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 Submission System at: http://www.wjgnet.com/1949-8454office. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1949-8454/g_info_20100316155305.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjbc@wjgnet.com, or by telephone: +86-10-85381892. 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 *WJBC*, 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/1949-8454/g_info_20100316160646.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/1949-8454/g_info_20100309232449.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/1949-8454/g_info_20100316155524.htm

Frontier: http://www.wjgnet.com/1949-8454/g_info_20100312091506.htm

Topic highlight: http://www.wjgnet.com/1949-8454/g_info_20100316155725.htm

Observation: http://www.wjgnet.com/1949-8454/g_info_20100316155928.htm

Guidelines for basic research: http://www.wjgnet.com/1949-8454/g_info_20100312092119.htm

Guidelines for clinical practice: http://www.wjgnet.com/1949-8454/g_info_20100312092247.htm

Review: http://www.wjgnet.com/1949-8454/g_info_20100316160234.htm

Original articles: http://www.wjgnet.com/1949-8454/g_info_20100316160646.htm

Brief articles: http://www.wjgnet.com/1949-8454/g_info_20100312092528.htm

Case report: http://www.wjgnet.com/1949-8454/g_info_20100316161452.htm

Letters to the editor: http://www.wjgnet.com/1949-8454/g_info_20100309232142.htm

Book reviews: http://www.wjgnet.com/1949-8454/g_info_20100312092929.htm

Guidelines: http://www.wjgnet.com/1949-8454/g_info_20100312093057.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJBC*. The revised version including manuscript and high-resolution image figures (if any) should be copied on a floppy or compact disk. The author should send the revised manuscript, along with printed high-resolution color or black and white photos, copyright transfer letter, and responses to the reviewers by courier (such as EMS/DHL).

Editorial Office

World Journal of Biological Chemistry
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjbc@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893

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/1949-8454/g_info_20100309233100.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/1949-8454/g_info_20100309232833.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
WJBC 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
WJBC is an international, peer-reviewed, Open-Access, 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. The related standards are as follows. Publication fee: 1300 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.