ISSN 2220-3192 (online)

World Journal of *Pharmacology*

World J Pharmacol 2012 August 9; 1(4): 55-64

www.wjgnet.com

WJP World Journal of Pharmacology

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

Editorial Board

2011-2015

The *World Journal of Pharmacology* Editorial Board consists of 100 members, representing a team of worldwide experts in pharmacology. They are from 23 countries, including Australia (4), Canada (2), China (5), Denmark (1), Egypt (1), France (1), Germany (2), Greece (4), Hungary (1), India (4), Iran (1), Israel (3), Italy (15), Japan (6), Netherlands (4), New Zealand (1), Poland (1), Saudi Arabia (1), South Korea (1), Spain (5), Turkey (3), United Kingdom (8), and United States (26).

EDITOR-IN-CHIEF

Geoffrey Burnstock, *London*

GUEST EDITORIAL BOARD MEMBERS

Jia-You Fang, *Taoyuan*
Ming-Fa Hsieh, *Chung Li*

MEMBERS OF THE EDITORIAL BOARD

Australia

Jonathon C Arnold, *Sydney*
Brian Dean, *Melbourne*
Xiao-Jun Du, *Melbourne*
Cherrie A Galletly, *Adelaide*

Canada

Sylvain G Bourgoin, *Québec*
Pierre A Guertin, *Quebec*

China

George G Chen, *Hong Kong*
Li-Wu Fu, *Guangzhou*
Qin He, *Chengdu*

Denmark

Morten Grunnet, *Copenhagen*

Egypt

Nagwa M Nour El Din, *Alexandria*

France

Rene Bruno, *Marseille*

Germany

Axel Becker, *Magdeburg*
Walter E Haefeli, *Heidelberg*

Greece

Panagiotis G Anagnostis, *Thessaloniki*
Ekaterini Chatzaki, *Alexandroupolis*
Moses Elisaf, *Ioannina*
Panagiotis Ferentinos, *Athens*

Hungary

Albert Császár, *Budapest*

India

VN Balaji, *Bangalore*
Chiranjib Chakraborty, *Vellore*
Naibedya Chattopadhyay, *Lucknow*
SJS Flora, *Gwalior*

Iran

Mehrdad Hamidi, *Zanjan*

Israel

Galila Agam, *Beer-Sheva*
Eliezer Flescher, *Tel Aviv*
Moshe Gavish, *Haifa*

Italy

Francesca Borrelli, *Naples*
Franco Borsini, *Pomezia*
Silvio Caccia, *Milan*
Giuseppe Maurizio Campo, *Messina*
Raffaele Capasso, *Naples*
Dario Cattaneo, *Milan*
Davide Cervia, *Viterbo*
Emilio Clementi, *Milano*
Massimo Collino, *Torino*
Vincenzo Cuomo, *Rome*
Tullio Florio, *Genova*
Vittorio Gentile, *Napoli*
Guido Grassi, *Milan*
Mario Grassi, *Trieste*
Annalisa Guaragna, *Napoli*

Japan

Jun Fang, *Kumamoto*
Takahisa Furuta, *Hamamatsu*
Mitsuko Furuya, *Yokohama*
Osamu Handa, *Kyoto*
Hideaki Hara, *Gifu*
Zhi-Qing Hu, *Tokyo*

Netherlands

Arjan Blokland, *Maastricht*
Eliyahu Dremencov, *Groningen*
Elisa Giovannetti, *Amsterdam*
Hidde J Haisma, *Groningen*

New Zealand

Hesham Al-Sallami, *Dunedin*

Poland

Wladyslawa Anna Daniel, *Krakow*

Saudi Arabia

Mohamed Haidara, *Abha*

South Korea

Ki Churl Chang, *Jinju*

Spain

José Luis Arias-Mediano, *Granada*
Pedro Emilio Bermejo, *Madrid*
Fermín Sánchez de Medina, *Granada*
Leandro Fernández-Pérez, *Gran Canaria*
Tomas Herraiz, *Madrid*

Turkey

Sule Apikoglu-Rabus, *Istanbul*
Fatih Canan, *Bolu*
Saygin S Eker, *Bursa*

United Kingdom

Charalambos Antoniades, *Oxford*
Christopher John Bushe, *New Malden*
David J Chambers, *London*
Michael J Curtis, *London*
Rossen M Donev, *Swansea*
Marco Falasca, *London*
David J Grieve, *Belfast*

United States

James David Adams Jr, *Los Angeles*
Gustav Akk, *St. Louis*

Charles Antzelevitch, *Utica*
Hugo Ruben Arias, *Glendale*
Dominick L Auci, *Escondido*
Ross J Baldessarini, *Belmont*
Oleg A Barski, *Louisville*
Chengpeng Bi, *Kansas*
Marco Bortolato, *Los Angeles*
Josh Burk, *Williamsburg*
William K Chan, *Stockton*
James J Chen, *Jefferson*
Zhe-Sheng Chen, *New York*
Beek Yoke Chin, *Boston*
Ting-Chao Chou, *New York*
John A Dani, *Houston*
Keith M Erikson, *Greensboro*
Eric R Fedyk, *Cambridge*
Mitchell P Fink, *Los Angeles*
Bolin Geng, *Waltham*
Neeraj Gupta, *Cambridge*
James P Hardwick, *Rootstown*
David W Hein, *Louisville*
Lawrence A Hill, *Salt Lake*
Andrew G Horti, *Baltimore*
Baskaran Rajasekaran, *Pittsburgh*

WJP World Journal of Pharmacology

Contents

Bimonthly Volume 1 Number 4 August 9, 2012

Contents

World Journal of Pharmacology
Volume 1 Number 4 August 9, 2012

EDITORS FOR THIS ISSUE

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

NAME OF JOURNAL
World Journal of Pharmacology

ISSN
ISSN 2220-3192 (online)

LAUNCH DATE
February 9, 2012

FREQUENCY
Bimonthly

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

EDITOR-IN-CHIEF
Geoffrey Burnstock, PhD, DSc, FAA, FRCS(Hon), FRCP (Hon), FmedSci, FRS, Professor, Autonomic Neuroscience Centre, University College Medical School, Royal Free Campus, Rowland Hill Street, London NW3 2PF, United Kingdom

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

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

PUBLICATION DATE
August 9, 2012

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

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

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2220-3192/g_info_20100722180909.htm

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

World Journal of Pharmacology

Online Submissions: http://www.wjgnet.com/esps/
wjpharmaco@wjgnet.com
doi:10.5497/wjp.v1.i4.55

World J Pharmacol 2012 August 9; 1(4): 55-64
ISSN 2220-3192 (online)
© 2012 Baishideng. All rights reserved.

EDITORIAL

Small inhibitors of ADP-ribosylation factor activation and function in mammalian cells

Sylvain G Bourgoin, Mohammed-Amine El Azreq

Sylvain G Bourgoin, Mohammed-Amine El Azreq, Centre de Recherche en Rhumatologie et Immunologie, Centre de Recherche du CHUQ-CHUL, Quebec G1V 4G2, Canada
Sylvain G Bourgoin, Département de Microbiologie-Infectiologie et Immunologie, Faculté de Médecine, Université Laval, Quebec G1V 4G2, Canada
Author contributions: Bourgoin SG and El Azreq MA contributed equally to this paper.
Supported by A Grant from the Canadian Institutes of Health Research (MOP-14790) to Bourgoin SG
Correspondence to: Sylvain G Bourgoin, PhD, Professor, Centre de Recherche en Rhumatologie et Immunologie, Centre de Recherche du CHUL, 2705 boul. Laurier, local T1-49, Quebec G1V 4G2, Canada. sylvain.bourgoin@crchul.ulaval.ca
Telephone: +1-418-6564141-46136 Fax: +1-418-6542765
Received: October, 9, 2011 Revised: February 18, 2012
Accepted: July 12, 2012
Published online: August 9, 2012

Abstract

Small GTP-binding proteins of the ADP-ribosylation factor (Arf) family control various cell functional responses including protein transport and recycling between different cellular compartments, phagocytosis, proliferation, cytoskeletal remodelling, and migration. The activity of Arfs is tightly regulated. GTPase-activating proteins (GAPs) inactivate Arfs by stimulating GTP hydrolysis, and guanine nucleotide exchange factors (GEFs) stimulate the conversion of inactive GDP-bound Arf to the active GTP-bound conformation. There is increasing evidence that Arf small GTPases contribute to cancer growth and invasion. Increased expression of Arf6 and of Arf-GEPs, or deregulation Arf-GAP functions have been correlated with enhanced invasive capacity of tumor cells and metastasis. The spatiotemporal specificity of Arf activation is dictated by their GEFs that integrate various signals in stimulated cells. Brefeldin A (BFA), which inactivates a subset of Arf-GEFs, has been very useful for assessing the function of Golgi-localized Arfs. However, specific inhibitors to investigate the individual function of BFA-sensitive and insensitive Arf-GEFs are lacking. In recent years, specific screens have been developed, and new inhibitors with improved selectivity and potency to study cell functional responses regulated by BFA-sensitive and BFA-insensitive Arf pathways have been identified. These inhibitors have been instrumental for our understanding of the spatiotemporal activation of Arf proteins in cells and demonstrate the feasibility of developing small molecules interfering with Arf activation to prevent tumor invasion and metastasis.

© 2012 Baishideng. All rights reserved.

Key words: Golgi; Vesicular transport; Migration; Adhesion; Actin

Peer reviewer: Tzyh-Chyuan Hour, PhD, Associate Professor, Institute of Biochemistry, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan, China

Bourgoin SG, El Azreq MA. Small inhibitors of ADP-ribosylation factor activation and function in mammalian cells. *World J Pharmacol* 2012; 1(4): 55-64 Available from: URL: http://www.wjgnet.com/2220-3192/full/v1/i4/55.htm DOI: http://dx.doi.org/10.5497/wjp.v1.i4.55

ADP-RIBOSYLATION FACTORS

Introduction

ADP-ribosylation factors (Arf) are small monomeric GDP/GTP-binding proteins (20 kDa) that belong to the Ras superfamily, which is divided into five subfamilies: Ras, Rho, Rab, Ran and Arf[1]. Like other small GTPases Arfs are regulated through a cycle of GTP binding and GTP hydrolysis, which activates and inactivates them, respectively[2]. The Arf subfamily comprises six isoforms (numbered from 1 to 6) grouped into three classes based on their amino acid sequence homology. Arf class I is

composed of Arf1, 2, and 3, and class Ⅱ of Arf4 and 5, while Arf6 is the unique member of class Ⅲ[3]. Arfs are not found in prokaryotes, but are expressed in yeast (Arf2-3) and in complex eukaryotic organisms such as mammals (Arf1-6). The homology between yeast and mammalian Arfs is over 74%[4] and mammalian Arfs are able to functionally substitute for Arf depletion in yeast.

Arf functions

Class Ⅰ / Ⅱ Arfs: Class I and Ⅱ Arfs exhibit several functional homologies since they are generally associated with the Golgi apparatus and regulate the secretory machinery in mammal cells[5]. Arf class I (Arf1, 2 and 3) and class Ⅱ (Arf4 and 5) are functionally redundant since silencing them separately has no measurable impact on Golgi morphology in mammalian cells[6]. Active GTP-bound class Ⅰ / Ⅱ Arfs translocate reversibly to Golgi membrane compartments while inactive Arfs are cytosolic[5]. Once associated with the membranes, these Arfs recruit several coat and cargo proteins such as COPI (coatomer), clathrin adaptor protein 1 (AP-1), or Golgi-localized γ-ear-containing Arf binding proteins (GGA), that are essential for vesicular biogenesis and anterograde ER-Golgi and retrograde Golgi-ER traffic[5,7]. Sequential activation and inactivation of Arfs is required for regulation of ER-to-Golgi and Golgi-to-ER membrane traffic. Thus, Arf1-GTP in the Golgi membrane is required for vesicle budding, while Arf1 inactivation or dissociation from the membrane is necessary for vesicle detachment from the Golgi and coat disassembly[8]. Class Ⅰ / Ⅱ Arfs also regulate several signalling pathways, in particular those issued from lipid metabolism, by stimulating the production of phosphatidic acid (PA) and of phosphatidylinositol 4,5-bisphosphate (PIP2) by phospholipase D (PLD) and PI4-/PI(4)P5-kinases [PI(4)P5K], respectively[9,10].

Class Ⅲ Arfs: There are fewer structural and functional homologies between Arf6 and other Arfs compared to those between class I and Ⅱ Arfs. Contrary to other Arfs, Arf6-GTP is rarely found in the Golgi, and translocates from the cytosol to the plasma or endosomal membranes in cells[7]. Arf6 contributes to membrane trafficking essentially *via* the regulation of lipid metabolism. PLD and PI(4)P5K are amongst the major effectors of Arf6[11]. Stimulation of PLD1[12] and possibly PLD2[13] by Arf6 produces PA, and that of PI(4)P5K generates PIP2[14-16]. Arf6-dependent synthesis of PIP2 can regulate proteins involved in exocytosis and endocytosis[15,16], and the Arf6-PLD signalling axis is involved in regulated secretion[17]. Internalization of several G protein-coupled receptors (GPCRs) from the cell surface to endosomal compartments[18] and remodelling of the actin cytoskeleton[7] are also regulated by Arf6.

REGULATION OF ADP-RIBOSYLATION FACTORS ACTIVITY

The exchange of GDP for GTP on Arfs is catalyzed by guanine nucleotide exchange factors (GEFs) while the hydrolysis of bound GTP is highly accelerated by GTPase-activating proteins (GAPs)[19]. The active form of Arf is characterized by a structural conformation that deploys a N-terminally myristoylated arm that facilitates membrane binding[7].

Guanine nucleotide-exchange factors for Arfs

Arf-GEFs determine the amount and the spatiotemporal distribution of active Arfs by interacting with GDP-bound Arfs[2]. Despite low sequence homology Arf-GEFs share one common central region of 200 amino acids similar to that of yeast Sec7p protein and known as the Sec7 domain. Arf-GEFs interact with Arfs through their Sec7 domain, which is necessary and sufficient for GEF activity[20]. The Sec7 domain is regulated by other domains and contributes to substrate specificity of various Arf-GEFs[20].

The crystallographic structure of the isolated or Arf1-bound Sec7 domain has been resolved[21-23]. A hydrophobic groove composing the catalytic core of the Sec7 domain is delineated by two conserved regions interacting with the switch I and switch II domains of Arfs. The Sec7 domain contains an invariant glutamate residue necessary for GEF activity. Interaction between GDP-bound Arf and the GEF brings the invariant glutamate to a position that catalyzes the expulsion of the bound GDP from the GTP-binding site by charge repulsion. Then Arf binds GTP and the Arf-GTP complex dissociates from the GEF[23]. The Sec7 domain is targeted by Brefeldin A (BFA), a fungal metabolite secreted by *Eupenicillium brefeldianum*, and designated by early studies as an inhibitor of secretion in mammalian cells[24]. The Arf-GEFs are divided into two major classes based on their molecular weight, sequence homology, and sensitivity to BFA.

High-molecular weight GEFs: Also called large Arf-GEFs due to their molecular weight (> 100 kDa), these proteins are sensitive to BFA[25,26]. In addition to the common Sec7 domain, these Arf-GEFs share other functional domains such as Dimerization and Cyclophilin-Binding, Homology Upstream of Sec7 and Homology Downstream of Sec7[27,28]. The large Arf-GEFs found in mammals include the Golgi-specific Brefeldin A-resistance factor (GBF)[29], and the Brefeldin A-inhibited GEF (BIG) families[30]. Gea1p/Gea2p expressed by *S. cerevisiae*[31] and GNOM expressed by *Arabidopsis thaliana*[32] are GBF orthologs, while the Sec7p protein is the only BIG ortholog characterized in yeast[28,33]. In mammalian cells, large Arf-GEFs preferentially localize to the Golgi where they activate Arf class I and Ⅱ. The rapid and reversible BFA-induced dislocation of the Golgi is due to inhibition of Arf-GEF functions[33]. Over-expression of GBF[34] or of BIG[35] can overcome BFA-mediated Golgi disassembly.

Low-molecular weight GEFs: The best-characterized small Arf-GEFs are proteins of the EFA6, Brefeldin A-Resistant Arf-GEFs (BRAG), and cytohesin families. These proteins are insensitive to BFA. They are gener-

ally cytosolic and are recruited to the plasma membrane in activated cells[33]. Their central Sec7 domain is flanked by other functional domains that contribute directly or indirectly to the regulation of their activity. The Pleckstrin Homology (PH) domain in the C-terminus interacts with membrane PIP2/PIP3 and can be in part responsible for small Arf-GEF translocation to membranes[36]. However, the PH domain of the BRAG family members seems to be insensitive to membrane PIP2/PIP3[37]. The coiled-coil (CC) domain in the C-terminus of EFA6 or in the N-terminus of cytohesin family Arf-GEFs allows the formation of homodimers or of hetreodimers with other CC domain-containing proteins. The BRAG family contains an additional PDZ domain and IQ motifs[27,28].

Small Arf-GEFs exhibit some preferences for Arf family members due to substantial specificities in the Sec7 domain[38]. EFA6 catalyzes the GDP/GTP exchange exclusively on Arf6 *in vitro* and *in vivo*[39]. The other small Arf-GEFs can activate all the Arfs *in vitro* or *in vivo* depending on the cellular context[28,33].

Due to their distinct cellular localization, the roles of small Arf-GEFs are different from those of the large Arf-GEFs. EFA6D is ubiquitously expressed in mammals, EFA6A is restricted to the brain and the intestinal epithelium, and EFA6B to spleen, thymus, pancreas, and the placenta[40]. The EFA6 family members regulate actin remodelling[41,42] and polarization of the intestinal epithelium by maintaining the integrity of tight junctions[43]. The expression of BRAG2 is ubiquitous and that of other BRAG family members is more restricted to the nervous system (BRAG1/3)[44,45]. In non-neuronal cells, BRAG2 is localized in endosomes where it preferentially activates Arf6[37,46,47]. BRAG2 inhibition results in a defect in recycling to the cell surface of proteins such as integrins[46], which interferes with cell adhesion, transferrin receptors, and E-cadherin[47].

Cytohesins (1-4) are found in vertebrates[28], and their orthologs are expressed in flies[48] and in amoebae[49]. Though *in vitro* cytohesins are more potent on Arf class I [19], *in vivo* they preferentially catalyze the GDP/GTP exchange on Arf6[28,50]. The expression of cytohesin-2/3 is ubiquitous in mammalian cells, and that of cytohesin-1 is more confined to leukocytes[51]. Cytohesin-1 regulates integrin functions in T cells[50], dendritic cells[52], monocytes[53,54], and neutrophils[55-57], which affect their capacity to adhere and migrate. In non immune cells cytohesins control the recycling to the cell surface of integrins[58], and GPCRs[59,60]. Cytohesins interact with the insulin receptor complex and regulate insulin-mediated signalling in mammals[36,61-63] and flies[48].

INHIBITORS OF ADP-RIBOSYLATION FACTOR ACTIVATION BY Sec7 PROTEINS

Inhibitors of the high-molecular weight Arf-GEFs

BFA: BFA causes Golgi collapse through inhibition of anterograde/retrograde transport between the ER and the Golgi and induces tubulation of recycling endosomes, which mix with the *trans*-Golgi network[64,65]. BFA-induced effect is caused by inhibition of multiple Golgi-associated GEFs that act on Arfs[66,67], mainly Arf class I and Ⅱ. Arf1 recruits the coat protein COPI to the *cis*-Golgi enabling ER-to-Golgi vesicle transport[68]. Activation of Arf1 and possibly of Arf5 by GBF1, a *cis*-Golgi GEF, regulates the association of COPI with vesicular tubular clusters[29]. In contrast BIG1 and BIG2 localize to the *trans*-Golgi where Arf1 initiates the assembly of AP-1/clathrin coat protein complexes[69]. BFA acts to stabilize an abortive complex between the GEFs and Arf in the GDP-bound form[70]. The exchange activity of cytohesin-2 and of cytohesin-1 is not inhibited by BFA[20,71]. Swapping the Sec7 domain of cytohesin-2 with Gea proteins confers resistance to BFA[70]. The determinant of BFA sensitivity was analyzed by comparing the Sec7 domain of the yeast BFA-sensitive GEFs, Gea1p/Gea2p and Sec7p[70,72], and of bovine p200, also known as GBF1 in humans, with that of BFA-resistant GEFs[73,74]. The sec7 residues Y695 of Gea1p, D965 and M975 of Sec7p were conserved in all BFA-sensitive GEFs whereas the amino acids corresponding to F190, S198 and P208 in the Sec7 domain of cytohesin-2 were found in BFA-insensitive GEFs. Double substitutions F190Y/A191S or S198D/P208M (S199D/P209M in cytohesin-1) are sufficient for confering BFA sensitivity to the cytohesin-2 Sec7 domain[70,72]. These Sec7 residues that are critical for binding and stabilizing BFA in an abortive complex with Arf1 lie in a core region forming a direct interaction with Arf1[22,75,76]. Note that not all Golgi Arf-GEFs can make a stable abortive complex with their preferred Arf substrates. No abortive complex formation between GBF1 localized to the ERGIC and the *cis*-Golgi and Arf class Ⅱ that functions in ER-to-Golgi traffic was detected[77].

LM11: Virtual screening was initiated to discover small inhibitors that fit in a pocket at the Arf1/cytohesin-2 Sec7 domain interface identified in the crystal structure of the Arf1-GDP/cytohesin-2 complex[78]. The authors characterized a compound, LM11, that targets the Arf1-GDP/cytohesin-2 complex and acts as a non-competitive inhibitor. Structure-activity analysis indicated that LM11 in the Arf1-GDP/cytohesin-2 complex interacts with K38 in the switch 1 region of Arf1. Mutation of K38 renders Arf1 insensitive to inhibition by LM11 while $Arf1^{K38}$ activation by a BFA-sensitive Sec7 domain remains sensitive to BFA[79]. Residues R152 and N201 of the cytohesin-2 sec7 domain are conserved in several Arf-GEFs and contribute to LM11 sensitivity. LM11 does not distinguish between BFA-insensitive and sensitive Arf-GEFs. Activation of Arf1 and Arf5 by the Sec7 domain of BIG1 was LM11-sensitive *in vitro*. Similarly to BFA, LM11 interferes with Arf1 but not Arf6 activation *in vitro*[23,80]. LM11 inhibits Arf1 in cells. Treatment of MDCK cells with LM11 causes dispersion of *cis*-Golgi markers, an effect that is reminiscent of GBF1 inhibition

by BFA[68,69], and cytohesin-2-dependent migration[78].

Golgicide A: Golgicide A (GCA) was identified using a high-throughput screen for small inhibitors that protect cells from Shiga toxin toxicity[81]. GCA disperses *medial*- and *cis*-Golgi with rapid cytoplasmic redistribution of the coat protein COPI[82]. The effect of GCA differs from that of BFA in that the coat protein AP-1 and GGA3 remain associated with the *trans*-Golgi network (TGN) until the Golgi collapses. Dispersion of the coat protein COPI but not of the TGN-associated coat protein complexes suggests that GCA may target GBF1[68]. The morphological effects of GCA on *medial*- and *cis*-Golgi were reminiscent of those caused by dominant-inactive GBF1[83] and silencing of GBF1[84]. Canine GBF1 is naturally resistant to BFA due to leucine substitution for methionine 832 within the Arf-binding site of GBF1[34,70]. M832L substitution in BFA-sensitive GBF1 renders the GEF resistant to both BFA and GCA[82], suggesting that the binding site of GCA overlaps with that of BFA within the GBF1-Arf1 interfacial region. Over-expression of GBF1-M832L in GCA-sensitive cells protected them from the morphological and functional effects of the compound on the Golgi and on protein secretion, respectively. As expected, treatment with GCA caused a decrease in Arf1 activation and over-expression of the GBF1-mutant in cells reversed GCA effects on Arf1-GTP levels[82].

Replication of Hepatitis C virus (HCV) and of enteroviruses depends on GBF1[85,86]. Treatment with GCA of cells that support productive infection of viral particles reduces HCV replication and interferes with the biogenesis of virions in an Arf1-dependent manner[85]. Inactivation or silencing of Arf1 redistributes viral proteins of the replication complex from ER-like vesicular membranes to the rims of lipid droplets[87]. GCA reduces the RNA levels of protein 3A of enterovirus poliovirus and coxsackievirus B3[88-90], a protein of the replication complex interacting directly with GBF1. The effect of GCA on viral RNA replication was countered by over-expressing GBF1 or the GCA-resistant GBF1-M832L, further supporting the dogma that GCA targets GBF1[86]. This study suggests that viral proteins of the replication cycle hijack GBF1 and reduce GBF1-induced Arf activation to support the biogenesis of viral particles. GCA is more selective than BFA to dissect molecular mechanisms of the HCV and enterovirus replication cycles[85,87].

Exo1: Exo1 was identified using a phenotypic screen based on the export of the vesicular stomatitis glycoprotein fused to GFP (VSVG-GFP) from the ER to the Golgi[91]. Exo1 reproduces the effects of BFA such as the tubulation and collapse of the Golgi[64]. Though Exo1 induces a rapid release of Arf1 from the Golgi and disappearance of COPI-coated vesicles, its effects differ from those of BFA in that redistribution of the TGN-associated coat proteins is delayed and the endocytic pathways are not affected[92]. Unlike BFA and GCA[70,82], Exo1 does not interfere with the nucleotide exchange activity of various Arf-GEFs on Arf1. Whether Exo1 has a stimulatory effect on Arf-GAPs by interfering with the assembly/stability of the COPI coat with the Arf1-GTP-GAP complex required for GTP hydrolysis or through another mechanism remains to be clarified[77,92].

Exo2: Exo2 was discovered using the imaging screen described by Yarrow *et al*[91] to search for inhibitors of the secretory pathway. In BSC1 and Vero cells Exo2 has no significant effect on the integrity of the TGN or on retrograde trafficking of cholera toxin from the plasma membrane to the ER through the TGN[93]. The effect of Exo2 is cell type specific since it disperses the TGN in HeLa cells and retards trafficking of Shiga toxin to the ER[94]. Unlike BFA Exo2 does not induce tubulation of early endosomes and mixing with the TGN but disrupts the COPI-dependent anterograde transport of VSVG-GFP from the ER to the Golgi[77,92,93]. Though the data suggest that Exo2 interferes with the functions of Arfs or Arf-GEFs localized to the ER-Golgi intermediate compartment or the TGN, the targets of Exo2 remain unknown. Exo2 reduces Arf1 activation by the Sec7 domain of BIG1 but the effect is weak compared to BFA[95]. Whether Arf activation by GBF1 is sensitive to Exo2 remains to be established.

LG186: Based on the 3D model structure of Sec7 domains in complex with BFA[22,23], Exo2 was used as a prototype molecule to design an inhibitor of GBF1[95]. The drug was engineered to fill a pocket overlapping the BFA binding site at the interface between the Sec7 domain of GBF1 and Arf1-GDP[22,75]. Though LG186 slightly inhibited the exchange activity of the Sec7 domain of BIG1 compared to BFA *in vitro*, demonstration that LG186 is an inhibitor of GBF1 was not convincing[95]. Nevertheless, cells treatment with LG186 reduces Arf activation, induces dissociation of the COPI vesicle coat from the Golgi and accumulation of GBF1 on the ER-Golgi intermediate membrane compartment as described for BFA[77] and for GCA[82]. An interesting property of LG186 is the inhibition of canine GBF1 activity which is insensitive to inhibition by BFA and GCA[34,82] due to a methionine substitution in its Sec7 domain. Characterization of LG186 as a selective GBF1 inhibitor awaits further confirmation.

Inhibitors of the low-molecular weight Arf-GEFs

RNA Aptamers M69 and K61: RNA aptamers are nucleic acid species that have the capacity to bind specifically to target proteins with high affinity and to interfere with their functions *in vivo* and *in vitro*. RNA aptamers are identified using a technical procedure named SELEX (systematic evolution of ligands by exponential enrichment) which consist of repeated rounds of *in vitro* selection by screening the affinities of large RNA libraries for a specific target[96]. Famulok and colleagues used recombinant cytohesin-1[97] and cytohesin-2[98] as baits to identify bind-

ing partners from libraries containing 10^{14} and 10^{15} RNA aptamers, respectively.

The M69 RNA aptamer was reported to interact with full-length cytohesin-1 and to target the Sec7 domain but not the PH or the C-terminal domains of cytohesin-1[97]. *In vitro*, M69 inhibited GDP/GTP exchange on Arf1 catalyzed by full-length cytohesin-1 and cytohesin-2 or their Sec7 domains, but not that stimulated by the large Arf-GEF Gea2, thereby demonstrating specificity for the cytohesin family[97]. A vaccinia virus-based intramer expression system was used to allow expression of the M69 RNA aptamer in mammalian cells. Over-expression of M69 in Jurkat cells inhibited adhesion to the integrin ligand ICAM-1 and impacted cell morphology by affecting actin cytoskeleton remodelling[97]. These *in vivo* effects of M69 are reminiscent of those described for catalytically inactive cytohesin-1 on Jurkat cell adhesion[99] and spreading[100].

Another RNA aptamer, K61, discriminating cytohesin-2 from cytohesin-1, was also identified by the group of Dr Famulok[98]. K61 RNA aptamer binds more tightly to CC-Sec7 (K_d = 400 nmol/L) than to Sec7 alone (K_d = 800 nmol/L) or to Sec7-PH (K_d = 1000 nmol/L) domains of cytohesin-1, but is capable of inhibiting nucleotide exchange on Arf1 catalyzed by cytohesin-2 *in vitro*. When used to transfect HeLa cells, both the K61 RNA aptamer and cytohesin-2 siRNA, but not the cytohesin-1 siRNA, markedly decreased serum-induced ERK phosphorylation and serum-mediated transcriptional activation[98]. Of note, targeting the CC domain of cytohesin-2 with aptamer K61 has the same inhibitory effect as that of the GEF-deficient E156K mutant of cytohesin-2 on serum-mediated transcriptional activation. Though an indirect effect of aptamer K61 on Arf activation due to miss-localisation of cytohesin-2 through inhibition of protein interaction with cellular partners other than Arfs cannot be excluded, another study showed a role for cytohesin-2 and Arf6 activation in GPCR-mediated signalling through the ERK/MAP kinase pathway[101].

Sec7 inhibitor H3: Inhibitory RNA aptamers were subsequently used to screen libraries for small molecules that could displace the binding of fluorescent M69 RNA aptamer from the cytohesin-1 Sec7 domain and act as inhibitors of cytohesins[63]. One of the cell permeable inhibitors, named Sec7 inhibitor H3 (secinH3), was found to bind to and to inhibit the GEF activity of cytohesin-1, -2, and -3, and steppke, an insect ortholog for cytohesins[48,63]. The affinity of secinH3 for other Arf-GEFs such as EFA6 was very low and their nucleotide exchange activity was not inhibited by this compound[63]. Cell treatment with a high concentration of secinH3 does not disrupt the Golgi, contrary to a low dose of BFA, which precludes a role for cytohesins in maintaining Golgi morphology[63]. Feeding mice with secinH3 resulted in hepatic insulin resistance[63] and insulin-induced signalling is disrupted in flies fed with secinH3[48]. Treatment of HepG2 hepatocytes with secinH3 abrogates the association of cytohesins and Arf6 with the insulin-receptor complex[63]. More recently, secinH3 was reported to inhibit glucose-stimulated insulin secretion in cultivated rat and human β pancreatic cells through inhibition of the cytohesin-2/Arf6 signalling axis and downstream activation of Rac1 and Cdc42[102].

The valproic acid-induced neurite outgrowth in N1E-115 neuroblastoma cells, which involves cytohesin-2 and downstream activation of Arf6, was reversed by treatment with secinH3[103]. Similarly secinH3 interferes with preadipocyte 3T3-L1 cell migration through inhibition of the cytohesin-2/Arf6 signalling axis[104]. SecinH3 was also reported to inhibit the growth of human lung cancer cells through inhibition of cytohesin-2-mediated autophosphorylation of ERB receptors, but independently of cytohesin-2-induced Arf activation[105].

SecinH3 inhibits several human neutrophil functions such as degranulation and superoxide anion production *via* the regulation of the cytohesin-1-Arf6-PLD signalling axis[57]. Cytohesin-1 was first characterized as a regulator of β-2 integrin-dependent adhesion through direct interaction with LFA-1[99]. As anticipated, secinH3 inhibited activation of the β-2 integrin LFA-1 in neutrophils[55]. Adhesion of Jurkat T cells to the specific β-2 integrin substrate ICAM-1[106] and ICAM-1-dependent adhesion of neutrophils to endothelial cells[55] were inhibited by secinH3. Though secinH3 inhibits the function of LFA-1, we reported that secinH3 activates Mac-1, another neutrophil β-2 integrin, and stimulates Mac-1-dependent responses such as migration, adhesion to fibrinogen and phagocytosis[56]. SecinH3 was also reported to regulate the internalization of β-1 integrins in mammalian cells[58].

Using secinH3 as a template, virtual screening of a library of small chemicals led to the discovery of more potent inhibitors of cytohesin-1 such as secin16, secin69, secin132[106] and secinB7[107]. Secin16 and secinB7 were up to 10-fold and 26-fold more potent in inhibiting nucleotide exchange on Arf1 catalyzed by cytohesin when compared to secinH3, respectively. SecinH3, as well as lower concentrations of secin16 or secinB7, markedly reduced the proliferation of PC9 cells[107].

TARGETING ADP-RIBOSYLATION FACTORS FOR ANTICANCER AND ANTI-INFLAMATORY THERAPIES

Recent evidence indicates that the dynamics of β2 integrin activation and of other neutrophil functional responses are controlled by Arf6 GDP-GTP cycling, which is regulated by the Arf6-GEF cytohesin-1[55-57], and ARAP3[108], a dual GAP for Arf6 and RhoA. Therefore, the use of small inhibitors of cytohesin-1 causing down regulation of Arf6 may be a therapeutic option in autoimmune diseases to prevent tissue damage and inflammation associated with excessive neutrophil responses.

Arf6 is highly expressed in some breast carcino-

Table 1 Targets and effects of the ADP-ribosylation factor inhibitors

Inhibitor	Arf regulators	Arf	Phenotype/function	Cell type
Brefeldin A	GBF1	Arf1	Tubulation and collapse of Golgi apparatus	Mammalian cells
	Gea1p/2p	Arf5		
	Sec7p	Arf1p	Inhibition of protein secretion	Yeast
LM11	Cytohesin-2	Arf1	Dispersion of Golgi markers	MDCK cells
	BIG1	Arf5	Inhibition of migration	
Golgicide A	GBF1	Arf1	Protection from Shiga toxin toxicity	Mammalian cells
			Dispersion of cis- and medial-Golgi	
			Inhibition of protein secretion	
			Inhibition of viral replication:	
			(hepatitis C virus; enteroviruses; poliovirus; coxsackievirus)	
EXO1	ArfGAPs	Arf1	Tubulation, collapse of Golgi	Mammalian cells
EXO2	BIG1	Arf1	Dispersion of the trans-Golgi network	HeLa cells
			Interfere with shiga toxin trafficking to the endoplasmic reticulum	
LG186	BIG1	Arf1	Dissociation of COP I vesicles from the Golgi	HeLa cells
	GBF1			
M69 aptamer	Cytohesin-1	Arf1	Actin remodelling	Jurkat T cells
	Cytohesin-2		Inhibition of adhesion	
K61 aptamer	Cytohesin-1	Arf1	Inhibition of serum-induced ERK MAP Kinase activation	HeLa cells
	Cytohesin-2			
SecinH3	Cytohesin-1	Arf1	Insulin resistance	HepG2 cells; rat and
	Cytohesin-2	Arf6		human β pancreatic cells
	Cytohesin-3		Inhibition of superoxide anion production	Neutrophils
			Inhibition of degranulation	
			Activation of Mac-1-dependent adhesion, phagocytosis and migration	
			Inhibition of LFA-1 dependent adhesion	
			Inhibition of migration	3T3-L1 cells
			Inhibition of neuritis outgrowth	N1E-115 neublastoma cells
			Regulation of β-1 integrin internalization and of β-1 integrin-dependent adhesion	HeLa cells
	Steppke		Insulin resistance	Fly cells

Arf: ADP-ribosylation factor; GBF: Golgi-specific Brefeldin A-resistance factor; BIG: Brefeldin A-inhibited GEF.

mas[109], glioma cell lines[110], as well as in primary lung cancers[111]. Several studies have correlated the levels of Arf6 protein expression with increased cell motility[110] and the invasive phenotype[109,110,112]. The invasive capacity and the metastatic behavior of cancer cells are under the control of Arf6[113]. Silencing of Arf6 or expression of dominant negative mutants reduces cancer cell motility and invasion[109,110,112,113]. In addition to enhanced expression of Arf6, aberrant functions of regulators of Arfs (including subsets of Arf-GEFs and Arf-GAPs) have also been associated with the invasive and metastatic phenotype of cancer cells[114]. The expression of GEP100/BRAG2 (an Arf-GEF for Arf6) is increased in invasive breast carcinoma compared to non-invasive breast tumors[115]. The recruitment of GEP100 to EGF receptors in breast cancer cells activates Arf6 and stimulates cell invasion[115]. As previously reported for Arf6 in breast carcinomas[109], silencing of GEP100 reduces the invasive and metastatic phenotypes of these breast cancer cells[115]. Elevated expression of EFA6, another Arf6-GEF, recently reported in low and high-grade glioma tissues, has also been linked to cell invasiveness[116]. Although there is no available inhibitor of Arf6 activation by GEP100 or EFA6A, the proof of concept that small-inhibitors of Arf-GEFs could be used for cancer therapeutics is now emerging[114]. Depending on the cellular context the selective inhibition of Arf6-GEFs may block cell motility[78], invasion and metastasis whereas targeting Arf1-GEFs would induce inhibition of cancer cell growth[117].

CONCLUSION

The availability of inhibitors of Arfs with selectivity for individual BFA-sensitive Arf-GEFs localized to the Golgi were instrumental to understanding the spatiotemporal activation and function of Golgi-associated Arfs in anterograde (ER-to-Golgi) and retrograde (Golgi-to-ER) transport mechanisms in cells. Inhibitors that target the BFA-insensitive pathways have been developed (Table 1). However these inhibitors are specific for the cytohesin family of Arf-GEFs and cannot be used to discriminate between the Arf pathways regulated by cytohesin-1, cytohesin-2 or cytohesin-3. Though there is no inhibitor for the BRAG family of Arf-GEFs, several proofs of principle indicate that Arf-GEFs are druggable targets *in vivo* and that the design of compounds targeting the Arf/Arf-GEF complexes could yield inhibitors with potential for development into anti-cancer and anti-inflammatory drugs.

REFERENCES

1 **Takai Y**, Sasaki T, Matozaki T. Small GTP-binding proteins. *Physiol Rev* 2001; **81**: 153-208
2 **Donaldson JG**, Jackson CL. ARF family G proteins and their

regulators: roles in membrane transport, development and disease. *Nat Rev Mol Cell Biol* 2011; **12**: 362-375
3 **Kahn RA**, Cherfils J, Elias M, Lovering RC, Munro S, Schurmann A. Nomenclature for the human Arf family of GTP-binding proteins: ARF, ARL, and SAR proteins. *J Cell Biol* 2006; **172**: 645-650
4 **Kahn RA**, Kern FG, Clark J, Gelmann EP, Rulka C. Human ADP-ribosylation factors. A functionally conserved family of GTP-binding proteins. *J Biol Chem* 1991; **266**: 2606-2614
5 **Donaldson JG**, Honda A, Weigert R. Multiple activities for Arf1 at the Golgi complex. *Biochim Biophys Acta* 2005; **1744**: 364-373
6 **Volpicelli-Daley LA**, Li Y, Zhang CJ, Kahn RA. Isoform-selective effects of the depletion of ADP-ribosylation factors 1-5 on membrane traffic. *Mol Biol Cell* 2005; **16**: 4495-4508
7 **D'Souza-Schorey C**, Chavrier P. ARF proteins: roles in membrane traffic and beyond. *Nat Rev Mol Cell Biol* 2006; **7**: 347-358
8 **Nie Z**, Randazzo PA. Arf GAPs and membrane traffic. *J Cell Sci* 2006; **119**: 1203-1211
9 **Exton JH**. Regulation of phospholipase D. *FEBS Lett* 2002; **531**: 58-61
10 **Brown HA**, Sternweis PC. Stimulation of phospholipase D by ADP-ribosylation factor. *Methods Enzymol* 1995; **257**: 313-324
11 **Donaldson JG**. Multiple roles for Arf6: sorting, structuring, and signaling at the plasma membrane. *J Biol Chem* 2003; **278**: 41573-41576
12 **Perez-Mansilla B**, Ha VL, Justin N, Wilkins AJ, Carpenter CL, Thomas GM. The differential regulation of phosphatidylinositol 4-phosphate 5-kinases and phospholipase D1 by ADP-ribosylation factors 1 and 6. *Biochim Biophys Acta* 2006; **1761**: 1429-1442
13 **Hiroyama M**, Exton JH. Localization and regulation of phospholipase D2 by ARF6. *J Cell Biochem* 2005; **95**: 149-164
14 **Béglé A**, Tryoen-Tóth P, de Barry J, Bader MF, Vitale N. ARF6 regulates the synthesis of fusogenic lipids for calcium-regulated exocytosis in neuroendocrine cells. *J Biol Chem* 2009; **284**: 4836-4845
15 **Aikawa Y**, Martin TF. ARF6 regulates a plasma membrane pool of phosphatidylinositol(4,5)bisphosphate required for regulated exocytosis. *J Cell Biol* 2003; **162**: 647-659
16 **Aikawa Y**, Martin TF. ADP-ribosylation factor 6 regulation of phosphatidylinositol-4,5-bisphosphate synthesis, endocytosis, and exocytosis. *Methods Enzymol* 2005; **404**: 422-431
17 **Vitale N**, Chasserot-Golaz S, Bader MF. Regulated secretion in chromaffin cells: an essential role for ARF6-regulated phospholipase D in the late stages of exocytosis. *Ann N Y Acad Sci* 2002; **971**: 193-200
18 **Hunzicker-Dunn M**, Gurevich VV, Casanova JE, Mukherjee S. ARF6: a newly appreciated player in G protein-coupled receptor desensitization. *FEBS Lett* 2002; **521**: 3-8
19 **Jackson CL**, Casanova JE. Turning on ARF: the Sec7 family of guanine-nucleotide-exchange factors. *Trends Cell Biol* 2000; **10**: 60-67
20 **Chardin P**, Paris S, Antonny B, Robineau S, Béraud-Dufour S, Jackson CL, Chabre M. A human exchange factor for ARF contains Sec7- and pleckstrin-homology domains. *Nature* 1996; **384**: 481-484
21 **Goldberg J**. Structural basis for activation of ARF GTPase: mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. *Cell* 1998; **95**: 237-248
22 **Mossessova E**, Gulbis JM, Goldberg J. Structure of the guanine nucleotide exchange factor Sec7 domain of human arno and analysis of the interaction with ARF GTPase. *Cell* 1998; **92**: 415-423
23 **Renault L**, Guibert B, Cherfils J. Structural snapshots of the mechanism and inhibition of a guanine nucleotide exchange factor. *Nature* 2003; **426**: 525-530
24 **Misumi Y**, Misumi Y, Miki K, Takatsuki A, Tamura G, Ikehara Y. Novel blockade by brefeldin A of intracellular transport of secretory proteins in cultured rat hepatocytes. *J Biol Chem* 1986; **261**: 11398-11403
25 **Cherfils J**, Melançon P. On the action of Brefeldin A on Sec7-stimulated membrane-recruitment and GDP/GTP exchange of Arf proteins. *Biochem Soc Trans* 2005; **33**: 635-638
26 **Zeghouf M**, Guibert B, Zeeh JC, Cherfils J. Arf, Sec7 and Brefeldin A: a model towards the therapeutic inhibition of guanine nucleotide-exchange factors. *Biochem Soc Trans* 2005; **33**: 1265-1268
27 **Mouratou B**, Biou V, Joubert A, Cohen J, Shields DJ, Geldner N, Jürgens G, Melançon P, Cherfils J. The domain architecture of large guanine nucleotide exchange factors for the small GTP-binding protein Arf. *BMC Genomics* 2005; **6**: 20
28 **Casanova JE**. Regulation of Arf activation: the Sec7 family of guanine nucleotide exchange factors. *Traffic* 2007; **8**: 1476-1485
29 **Claude A**, Zhao BP, Kuziemsky CE, Dahan S, Berger SJ, Yan JP, Armold AD, Sullivan EM, Melançon P. GBF1: A novel Golgi-associated BFA-resistant guanine nucleotide exchange factor that displays specificity for ADP-ribosylation factor 5. *J Cell Biol* 1999; **146**: 71-84
30 **Togawa A**, Morinaga N, Ogasawara M, Moss J, Vaughan M. Purification and cloning of a brefeldin A-inhibited guanine nucleotide-exchange protein for ADP-ribosylation factors. *J Biol Chem* 1999; **274**: 12308-12315
31 **Peyroche A**, Paris S, Jackson CL. Nucleotide exchange on ARF mediated by yeast Gea1 protein. *Nature* 1996; **384**: 479-481
32 **Geldner N**, Anders N, Wolters H, Keicher J, Kornberger W, Muller P, Delbarre A, Ueda T, Nakano A, Jürgens G. The Arabidopsis GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. *Cell* 2003; **112**: 219-230
33 **Gillingham AK**, Munro S. The small G proteins of the Arf family and their regulators. *Annu Rev Cell Dev Biol* 2007; **23**: 579-611
34 **Niu TK**, Pfeifer AC, Lippincott-Schwartz J, Jackson CL. Dynamics of GBF1, a Brefeldin A-sensitive Arf1 exchange factor at the Golgi. *Mol Biol Cell* 2005; **16**: 1213-1222
35 **Shinotsuka C**, Waguri S, Wakasugi M, Uchiyama Y, Nakayama K. Dominant-negative mutant of BIG2, an ARF-guanine nucleotide exchange factor, specifically affects membrane trafficking from the trans-Golgi network through inhibiting membrane association of AP-1 and GGA coat proteins. *Biochem Biophys Res Commun* 2002; **294**: 254-260
36 **Klarlund JK**, Tsiaras W, Holik JJ, Chawla A, Czech MP. Distinct polyphosphoinositide binding selectivities for pleckstrin homology domains of GRP1-like proteins based on diglycine versus triglycine motifs. *J Biol Chem* 2000; **275**: 32816-32821
37 **Someya A**, Sata M, Takeda K, Pacheco-Rodriguez G, Ferrans VJ, Moss J, Vaughan M. ARF-GEP(100), a guanine nucleotide-exchange protein for ADP-ribosylation factor 6. *Proc Natl Acad Sci USA* 2001; **98**: 2413-2418
38 **Pasqualato S**, Ménétrey J, Franco M, Cherfils J. The structural GDP/GTP cycle of human Arf6. *EMBO Rep* 2001; **2**: 234-238
39 **Pasqualato S**, Renault L, Cherfils J. Arf, Arl, Arp and Sar proteins: a family of GTP-binding proteins with a structural device for 'front-back' communication. *EMBO Rep* 2002; **3**: 1035-1041
40 **Sakagami H**, Suzuki H, Kamata A, Owada Y, Fukunaga K, Mayanagi H, Kondo H. Distinct spatiotemporal expression of EFA6D, a guanine nucleotide exchange factor for ARF6, among the EFA6 family in mouse brain. *Brain Res* 2006; **1093**: 1-11
41 **Franco M**, Peters PJ, Boretto J, van Donselaar E, Neri A, D' Souza-Schorey C, Chavrier P. EFA6, a sec7 domain-containing exchange factor for ARF6, coordinates membrane recy-

cling and actin cytoskeleton organization. *EMBO J* 1999; **18**: 1480-1491
42 **Derrien V**, Couillault C, Franco M, Martineau S, Montcourrier P, Houlgatte R, Chavrier P. A conserved C-terminal domain of EFA6-family ARF6-guanine nucleotide exchange factors induces lengthening of microvilli-like membrane protrusions. *J Cell Sci* 2002; **115**: 2867-2879
43 **Luton F**, Klein S, Chauvin JP, Le Bivic A, Bourgoin S, Franco M, Chardin P. EFA6, exchange factor for ARF6, regulates the actin cytoskeleton and associated tight junction in response to E-cadherin engagement. *Mol Biol Cell* 2004; **15**: 1134-1145
44 **Inaba Y**, Tian QB, Okano A, Zhang JP, Sakagami H, Miyazawa S, Li W, Komiyama A, Inokuchi K, Kondo H, Suzuki T. Brain-specific potential guanine nucleotide exchange factor for Arf, synArfGEF (Po), is localized to postsynaptic density. *J Neurochem* 2004; **89**: 1347-1357
45 **Murphy JA**, Jensen ON, Walikonis RS. BRAG1, a Sec7 domain-containing protein, is a component of the postsynaptic density of excitatory synapses. *Brain Res* 2006; **1120**: 35-45
46 **Dunphy JL**, Moravec R, Ly K, Lasell TK, Melancon P, Casanova JE. The Arf6 GEF GEP100/BRAG2 regulates cell adhesion by controlling endocytosis of beta1 integrins. *Curr Biol* 2006; **16**: 315-320
47 **Hiroi T**, Someya A, Thompson W, Moss J, Vaughan M. GEP100/BRAG2: activator of ADP-ribosylation factor 6 for regulation of cell adhesion and actin cytoskeleton via E-cadherin and alpha-catenin. *Proc Natl Acad Sci USA* 2006; **103**: 10672-10677
48 **Fuss B**, Becker T, Zinke I, Hoch M. The cytohesin Steppke is essential for insulin signalling in Drosophila. *Nature* 2006; **444**: 945-948
49 **Shina MC**, Müller R, Blau-Wasser R, Glöckner G, Schleicher M, Eichinger L, Noegel AA, Kolanus W. A cytohesin homolog in Dictyostelium amoebae. *PLoS One* 2010; **5**: e9378
50 **Kolanus W**. Guanine nucleotide exchange factors of the cytohesin family and their roles in signal transduction. *Immunol Rev* 2007; **218**: 102-113
51 **Ogasawara M**, Kim SC, Adamik R, Togawa A, Ferrans VJ, Takeda K, Kirby M, Moss J, Vaughan M. Similarities in function and gene structure of cytohesin-4 and cytohesin-1, guanine nucleotide-exchange proteins for ADP-ribosylation factors. *J Biol Chem* 2000; **275**: 3221-3230
52 **Quast T**, Tappertzhofen B, Schild C, Grell J, Czeloth N, Förster R, Alon R, Fraemohs L, Dreck K, Weber C, Lämmermann T, Sixt M, Kolanus W. Cytohesin-1 controls the activation of RhoA and modulates integrin-dependent adhesion and migration of dendritic cells. *Blood* 2009; **113**: 5801-5810
53 **Hajishengallis G**, Wang M, Liang S. Induction of distinct TLR2-mediated proinflammatory and proadhesive signaling pathways in response to Porphyromonas gingivalis fimbriae. *J Immunol* 2009; **182**: 6690-6696
54 **Sendide K**, Reiner NE, Lee JS, Bourgoin S, Talal A, Hmama Z. Cross-talk between CD14 and complement receptor 3 promotes phagocytosis of mycobacteria: regulation by phosphatidylinositol 3-kinase and cytohesin-1. *J Immunol* 2005; **174**: 4210-4219
55 **El azreq MA**, Bourgoin SG. Cytohesin-1 regulates human blood neutrophil adhesion to endothelial cells through β2 integrin activation. *Mol Immunol* 2011; **48**: 1408-1416
56 **El Azreq MA**, Garceau V, Bourgoin SG. Cytohesin-1 regulates fMLF-mediated activation and functions of the β2 integrin Mac-1 in human neutrophils. *J Leukoc Biol* 2011; **89**: 823-836
57 **El Azreq MA**, Garceau V, Harbour D, Pivot-Pajot C, Bourgoin SG. Cytohesin-1 regulates the Arf6-phospholipase D signaling axis in human neutrophils: impact on superoxide anion production and secretion. *J Immunol* 2010; **184**: 637-649
58 **Oh SJ**, Santy LC. Differential effects of cytohesins 2 and 3 on beta1 integrin recycling. *J Biol Chem* 2010; **285**: 14610-14616
59 **Laroche G**, Giguère PM, Dupré E, Dupuis G, Parent JL. The N-terminal coiled-coil domain of the cytohesin/ARNO family of guanine nucleotide exchange factors interacts with Galphaq. *Mol Cell Biochem* 2007; **306**: 141-152
60 **Giguère P**, Rochdi MD, Laroche G, Dupré E, Whorton MR, Sunahara RK, Claing A, Dupuis G, Parent JL. ARF6 activation by Galpha q signaling: Galpha q forms molecular complexes with ARNO and ARF6. *Cell Signal* 2006; **18**: 1988-1994
61 **Li HS**, Shome K, Rojas R, Rizzo MA, Vasudevan C, Fluharty E, Santy LC, Casanova JE, Romero G. The guanine nucleotide exchange factor ARNO mediates the activation of ARF and phospholipase D by insulin. *BMC Cell Biol* 2003; **4**: 13
62 **Oatey PB**, Venkateswarlu K, Williams AG, Fletcher LM, Foulstone EJ, Cullen PJ, Tavaré JM. Confocal imaging of the subcellular distribution of phosphatidylinositol 3,4,5-trisphosphate in insulin- and PDGF-stimulated 3T3-L1 adipocytes. *Biochem J* 1999; **344 Pt 2**: 511-518
63 **Hafner M**, Schmitz A, Grüne I, Srivatsan SG, Paul B, Kolanus W, Quast T, Kremmer E, Bauer I, Famulok M. Inhibition of cytohesins by SecinH3 leads to hepatic insulin resistance. *Nature* 2006; **444**: 941-944
64 **Klausner RD**, Donaldson JG, Lippincott-Schwartz J. Brefeldin A: insights into the control of membrane traffic and organelle structure. *J Cell Biol* 1992; **116**: 1071-1080
65 **Lippincott-Schwartz J**, Yuan LC, Bonifacino JS, Klausner RD. Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER. *Cell* 1989; **56**: 801-813
66 **Donaldson JG**, Finazzi D, Klausner RD. Brefeldin A inhibits Golgi membrane-catalysed exchange of guanine nucleotide onto ARF protein. *Nature* 1992; **360**: 350-352
67 **Helms JB**, Rothman JE. Inhibition by brefeldin A of a Golgi membrane enzyme that catalyses exchange of guanine nucleotide bound to ARF. *Nature* 1992; **360**: 352-354
68 **Zhao X**, Claude A, Chun J, Shields DJ, Presley JF, Melançon P. GBF1, a cis-Golgi and VTCs-localized ARF-GEF, is implicated in ER-to-Golgi protein traffic. *J Cell Sci* 2006; **119**: 3743-3753
69 **Zhao X**, Lasell TK, Melançon P. Localization of large ADP-ribosylation factor-guanine nucleotide exchange factors to different Golgi compartments: evidence for distinct functions in protein traffic. *Mol Biol Cell* 2002; **13**: 119-133
70 **Peyroche A**, Antonny B, Robineau S, Acker J, Cherfils J, Jackson CL. Brefeldin A acts to stabilize an abortive ARF-GDP-Sec7 domain protein complex: involvement of specific residues of the Sec7 domain. *Mol Cell* 1999; **3**: 275-285
71 **Meacci E**, Tsai SC, Adamik R, Moss J, Vaughan M. Cytohesin-1, a cytosolic guanine nucleotide-exchange protein for ADP-ribosylation factor. *Proc Natl Acad Sci USA* 1997; **94**: 1745-1748
72 **Sata M**, Moss J, Vaughan M. Structural basis for the inhibitory effect of brefeldin A on guanine nucleotide-exchange proteins for ADP-ribosylation factors. *Proc Natl Acad Sci USA* 1999; **96**: 2752-2757
73 **Mansour SJ**, Herbrick JA, Scherer SW, Melançon P. Human GBF1 is a ubiquitously expressed gene of the sec7 domain family mapping to 10q24. *Genomics* 1998; **54**: 323-327
74 **Morinaga N**, Moss J, Vaughan M. Cloning and expression of a cDNA encoding a bovine brain brefeldin A-sensitive guanine nucleotide-exchange protein for ADP-ribosylation factor. *Proc Natl Acad Sci USA* 1997; **94**: 12926-12931
75 **Cherfils J**, Ménétrey J, Mathieu M, Le Bras G, Robineau S, Béraud-Dufour S, Antonny B, Chardin P. Structure of the Sec7 domain of the Arf exchange factor ARNO. *Nature* 1998; **392**: 101-105
76 **Robineau S**, Chabre M, Antonny B. Binding site of brefeldin A at the interface between the small G protein ADP-ribosylation factor 1 (ARF1) and the nucleotide-exchange factor Sec7 domain. *Proc Natl Acad Sci USA* 2000; **97**: 9913-9918
77 **Chun J**, Shapovalova Z, Dejgaard SY, Presley JF, Melançon P. Characterization of class I and II ADP-ribosylation factors

(Arfs) in live cells: GDP-bound class II Arfs associate with the ER-Golgi intermediate compartment independently of GBF1. *Mol Biol Cell* 2008; **19**: 3488-3500
78 **Viaud J**, Zeghouf M, Barelli H, Zeeh JC, Padilla A, Guibert B, Chardin P, Royer CA, Cherfils J, Chavanieu A. Structure-based discovery of an inhibitor of Arf activation by Sec7 domains through targeting of protein-protein complexes. *Proc Natl Acad Sci USA* 2007; **104**: 10370-10375
79 **Flisiak S**, Zeeh JC, Guibert B, Cherfils J, Zeghouf M. An Arf1 GTPase mutant with different responses to GEF inhibitors. *Biochem Biophys Res Commun* 2008; **377**: 156-160
80 **Zeeh JC**, Zeghouf M, Grauffel C, Guibert B, Martin E, Dejaegere A, Cherfils J. Dual specificity of the interfacial inhibitor brefeldin a for arf proteins and sec7 domains. *J Biol Chem* 2006; **281**: 11805-11814
81 **Saenz JB**, Doggett TA, Haslam DB. Identification and characterization of small molecules that inhibit intracellular toxin transport. *Infect Immun* 2007; **75**: 4552-4561
82 **Sáenz JB**, Sun WJ, Chang JW, Li J, Bursulaya B, Gray NS, Haslam DB. Golgicide A reveals essential roles for GBF1 in Golgi assembly and function. *Nat Chem Biol* 2009; **5**: 157-165
83 **Szul T**, Garcia-Mata R, Brandon E, Shestopal S, Alvarez C, Sztul E. Dissection of membrane dynamics of the ARF-guanine nucleotide exchange factor GBF1. *Traffic* 2005; **6**: 374-385
84 **Manolea F**, Claude A, Chun J, Rosas J, Melançon P. Distinct functions for Arf guanine nucleotide exchange factors at the Golgi complex: GBF1 and BIGs are required for assembly and maintenance of the Golgi stack and trans-Golgi network, respectively. *Mol Biol Cell* 2008; **19**: 523-535
85 **Matto M**, Sklan EH, David N, Melamed-Book N, Casanova JE, Glenn JS, Aroeti B. Role for ADP ribosylation factor 1 in the regulation of hepatitis C virus replication. *J Virol* 2011; **85**: 946-956
86 **van der Linden L**, van der Schaar HM, Lanke KH, Neyts J, van Kuppeveld FJ. Differential effects of the putative GBF1 inhibitors Golgicide A and AG1478 on enterovirus replication. *J Virol* 2010; **84**: 7535-7542
87 **Goueslain L**, Alsaleh K, Horellou P, Roingeard P, Descamps V, Duverlie G, Ciczora Y, Wychowski C, Dubuisson J, Rouillé Y. Identification of GBF1 as a cellular factor required for hepatitis C virus RNA replication. *J Virol* 2010; **84**: 773-787
88 **Wessels E**, Duijsings D, Lanke KH, Melchers WJ, Jackson CL, van Kuppeveld FJ. Molecular determinants of the interaction between coxsackievirus protein 3A and guanine nucleotide exchange factor GBF1. *J Virol* 2007; **81**: 5238-5245
89 **Wessels E**, Duijsings D, Lanke KH, van Dooren SH, Jackson CL, Melchers WJ, van Kuppeveld FJ. Effects of picornavirus 3A Proteins on Protein Transport and GBF1-dependent COP-I recruitment. *J Virol* 2006; **80**: 11852-11860
90 **Wessels E**, Duijsings D, Niu TK, Neumann S, Oorschot VM, de Lange F, Lanke KH, Klumperman J, Henke A, Jackson CL, Melchers WJ, van Kuppeveld FJ. A viral protein that blocks Arf1-mediated COP-I assembly by inhibiting the guanine nucleotide exchange factor GBF1. *Dev Cell* 2006; **11**: 191-201
91 **Yarrow JC**, Feng Y, Perlman ZE, Kirchhausen T, Mitchison TJ. Phenotypic screening of small molecule libraries by high throughput cell imaging. *Comb Chem High Throughput Screen* 2003; **6**: 279-286
92 **Feng Y**, Yu S, Lasell TK, Jadhav AP, Macia E, Chardin P, Melancon P, Roth M, Mitchison T, Kirchhausen T. Exo1: a new chemical inhibitor of the exocytic pathway. *Proc Natl Acad Sci USA* 2003; **100**: 6469-6474
93 **Feng Y**, Jadhav AP, Rodighiero C, Fujinaga Y, Kirchhausen T, Lencer WI. Retrograde transport of cholera toxin from the plasma membrane to the endoplasmic reticulum requires the trans-Golgi network but not the Golgi apparatus in Exo2-treated cells. *EMBO Rep* 2004; **5**: 596-601
94 **Spooner RA**, Watson P, Smith DC, Boal F, Amessou M, Johannes L, Clarkson GJ, Lord JM, Stephens DJ, Roberts LM. The secretion inhibitor Exo2 perturbs trafficking of Shiga toxin between endosomes and the trans-Golgi network. *Biochem J* 2008; **414**: 471-484
95 **Boal F**, Guetzoyan L, Sessions RB, Zeghouf M, Spooner RA, Lord JM, Cherfils J, Clarkson GJ, Roberts LM, Stephens DJ. LG186: An inhibitor of GBF1 function that causes Golgi disassembly in human and canine cells. *Traffic* 2010; **11**: 1537-1551
96 **Weigand JE**, Suess B. Aptamers and riboswitches: perspectives in biotechnology. *Appl Microbiol Biotechnol* 2009; **85**: 229-236
97 **Mayer G**, Blind M, Nagel W, Böhm T, Knorr T, Jackson CL, Kolanus W, Famulok M. Controlling small guanine-nucleotide-exchange factor function through cytoplasmic RNA intramers. *Proc Natl Acad Sci USA* 2001; **98**: 4961-4965
98 **Theis MG**, Knorre A, Kellersch B, Moelleken J, Wieland F, Kolanus W, Famulok M. Discriminatory aptamer reveals serum response element transcription regulated by cytohesin-2. *Proc Natl Acad Sci USA* 2004; **101**: 11221-11226
99 **Kolanus W**, Nagel W, Schiller B, Zeitlmann L, Godar S, Stockinger H, Seed B. Alpha L beta 2 integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1, a cytoplasmic regulatory molecule. *Cell* 1996; **86**: 233-242
100 **Geiger C**, Nagel W, Boehm T, van Kooyk Y, Figdor CG, Kremmer E, Hogg N, Zeitlmann L, Dierks H, Weber KS, Kolanus W. Cytohesin-1 regulates beta-2 integrin-mediated adhesion through both ARF-GEF function and interaction with LFA-1. *EMBO J* 2000; **19**: 2525-2536
101 **Gsandtner I**, Charalambous C, Stefan E, Ogris E, Freissmuth M, Zezula J. Heterotrimeric G protein-independent signaling of a G protein-coupled receptor. Direct binding of ARNO/cytohesin-2 to the carboxyl terminus of the A2A adenosine receptor is necessary for sustained activation of the ERK/MAP kinase pathway. *J Biol Chem* 2005; **280**: 31898-31905
102 **Jayaram B**, Syed I, Kyathanahalli CN, Rhodes CJ, Kowluru A. Arf nucleotide binding site opener [ARNO] promotes sequential activation of Arf6, Cdc42 and Rac1 and insulin secretion in INS 832/13 β-cells and rat islets. *Biochem Pharmacol* 2011; **81**: 1016-1027
103 **Yamauchi J**, Miyamoto Y, Torii T, Mizutani R, Nakamura K, Sanbe A, Koide H, Kusakawa S, Tanoue A. Valproic acid-inducible Arl4D and cytohesin-2/ARNO, acting through the downstream Arf6, regulate neurite outgrowth in N1E-115 cells. *Exp Cell Res* 2009; **315**: 2043-2052
104 **Torii T**, Miyamoto Y, Sanbe A, Nishimura K, Yamauchi J, Tanoue A. Cytohesin-2/ARNO, through its interaction with focal adhesion adaptor protein paxillin, regulates preadipocyte migration via the downstream activation of Arf6. *J Biol Chem* 2010; **285**: 24270-24281
105 **Bill A**, Schmitz A, Albertoni B, Song JN, Heukamp LC, Walrafen D, Thorwirth F, Verveer PJ, Zimmer S, Meffert L, Schreiber A, Chatterjee S, Thomas RK, Ullrich RT, Lang T, Famulok M. Cytohesins are cytoplasmic ErbB receptor activators. *Cell* 2010; **143**: 201-211
106 **Stumpfe D**, Bill A, Novak N, Loch G, Blockus H, Geppert H, Becker T, Schmitz A, Hoch M, Kolanus W, Famulok M, Bajorath J. Targeting multifunctional proteins by virtual screening: structurally diverse cytohesin inhibitors with differentiated biological functions. *ACS Chem Biol* 2010; **5**: 839-849
107 **Bill A**, Blockus H, Stumpfe D, Bajorath J, Schmitz A, Famulok M. A homogeneous fluorescence resonance energy transfer system for monitoring the activation of a protein switch in real time. *J Am Chem Soc* 2011; **133**: 8372-8379
108 **Gambardella L**, Anderson KE, Nussbaum C, Segonds-Pichon A, Margarido T, Norton L, Ludwig T, Sperandio M, Hawkins PT, Stephens L, Vermeren S. The GTPase-activating protein ARAP3 regulates chemotaxis and adhesion-dependent processes in neutrophils. *Blood* 2011; **118**: 1087-1098
109 **Hashimoto S**, Onodera Y, Hashimoto A, Tanaka M, Hama-

guchi M, Yamada A, Sabe H. Requirement for Arf6 in breast cancer invasive activities. *Proc Natl Acad Sci USA* 2004; **101**: 6647-6652

110 **Hu B**, Shi B, Jarzynka MJ, Yiin JJ, D'Souza-Schorey C, Cheng SY. ADP-ribosylation factor 6 regulates glioma cell invasion through the IQ-domain GTPase-activating protein 1-Rac1-mediated pathway. *Cancer Res* 2009; **69**: 794-801

111 **Knizhnik AV**, Kovaleva OB, Laktionov KK, Mochal'nikova VV, Komel'kov AV, Chevkina EM, Zborovskaia IB. [Arf6, RalA and BIRC5 protein expression in non small cell lung cancer]. *Mol Biol* (Mosk) 2011; **45**: 307-315

112 **Tague SE**, Muralidharan V, D'Souza-Schorey C. ADP-ribosylation factor 6 regulates tumor cell invasion through the activation of the MEK/ERK signaling pathway. *Proc Natl Acad Sci USA* 2004; **101**: 9671-9676

113 **Muralidharan-Chari V**, Hoover H, Clancy J, Schweitzer J, Suckow MA, Schroeder V, Castellino FJ, Schorey JS, D'Souza-Schorey C. ADP-ribosylation factor 6 regulates tumorigenic and invasive properties in vivo. *Cancer Res* 2009; **69**: 2201-2209

114 **Vigil D**, Cherfils J, Rossman KL, Der CJ. Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy? *Nat Rev Cancer* 2010; **10**: 842-857

115 **Morishige M**, Hashimoto S, Ogawa E, Toda Y, Kotani H, Hirose M, Wei S, Hashimoto A, Yamada A, Yano H, Mazaki Y, Kodama H, Nio Y, Manabe T, Wada H, Kobayashi H, Sabe H. GEP100 links epidermal growth factor receptor signalling to Arf6 activation to induce breast cancer invasion. *Nat Cell Biol* 2008; **10**: 85-92

116 **Li M**, Ng SS, Wang J, Lai L, Leung SY, Franco M, Peng Y, He ML, Kung HF, Lin MC. EFA6A enhances glioma cell invasion through ADP ribosylation factor 6/extracellular signal-regulated kinase signaling. *Cancer Res* 2006; **66**: 1583-1590

117 **Ohashi Y**, Iijima H, Yamaotsu N, Yamazaki K, Sato S, Okamura M, Sugimoto K, Dan S, Hirono S, Yamori T. AMF-26, a novel inhibitor of the Golgi system, targeting ADP-ribosylation factor 1 (Arf1) with potential for cancer therapy. *J Biol Chem* 2012; **287**: 3885-3897

S- Editor Yan JL **L- Editor** A **E- Editor** Zheng XM

World Journal of Pharmacology

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

World J Pharmacol 2012 August 9; 1(4): I
ISSN 2220-3192 (online)
© 2012 Baishideng. All rights reserved.

Acknowledgments to reviewers of *World Journal of Pharmacology*

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

Tzyh-Chyuan Hour, PhD, Associate Professor, Institute of Biochemistry, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan, China

Bolin Geng, PhD, Principal Scientist, Medicinal Chemistry, Infection iMed, AstraZeneca R and D Boston, 35 Gatehouse Drive, Waltham, MA02451, United States

Josh Burk, Associate Professor, Department of Psychology, College of William and Mary, 540 Landrum Drive, Williamsburg, VA 23187, United States

Moses Elisaf, Professor of Internal Medicine, University of Ioannina, Medical School, Department of Internal Medicine, 451 10 Ioannina, Greece

Tullio Florio, MD, PhD, Professor of Pharmacology, Dipartimento di Oncologia, Biologia e Genetica, Universita' di Genova, Viale Benedetto XV, 2, 16132 Genova, Italy

Swaran JS Flora, PhD, Associate Director, Scientist 'G' (Associate Director), Head, Division of Pharmacology and Toxicology, Defence Research and Development Establishment, Jhansi Road, Gwalior 474 002, India

WJP World Journal of Pharmacology

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

World J Pharmacol 2012 August 9; 1(4): I
ISSN 2220-3192 (online)
© 2012 Baishideng. All rights reserved.

Events Calendar 2012

January 8-13, 2012
Keystone Symposia on Molecular and Cellular Biology
Chemokines and Leukocyte Trafficking in Homeostasis and Inflammation
Breckenridge, CO, United States

January 22-27, 2012
International Society for Stem Cell Research
Keystone Symposia on Cardiovascular Development and Regulation
Taos, NM, United States

January 26-27, 2012
2nd Annual Pediatric Pharmacology Conference
Philadelphia, PA, United States

January 30-31, 2012
Allergy Drug Discovery and Development Conference
San Diego, CA, United States

February 3-5, 2012
Heart Failure Council of Thailand/ Heart Association of Thailand
6th Asian Pacific Congress of Heart Failure
Chiang Mai, Thailand

February 8-11, 2012
6th International Conference SUMO, Ubiquitin, UBL Proteins: Implications for Human Diseases
Houston, TX, United States

February 12-15, 2012
4th International Conference on Drug Discovery & Therapy
Dubai, United Arab Emirates

February 26-29, 2012
11th International Dead Sea Symposium on Cardiac Arrhythmias and Device Therapy
Jerusalem, Israel

February 27-28, 2012
2nd Ubiquitin Research and Drug Discovery
Las Vegas, NV, United States

February 27-28, 2012
4th Ocular Diseases & Drug Discovery
Las Vegas, NV, United States

February 27-28, 2012
Targets and Strategies in Drug Discovery Summit
Las Vegas, NV, United States

March 8-9, 2012
British Pharmacological Society
BPS Focused Meeting - Challenges in Neurotherapeutics: From Animal Models to Clinical Needs
Dublin, Ireland

March 14-17, 2012
American Society for Clinical Pharmacology and Therapeutics
2012 Annual Meeting
National Harbor, MD, United States

March 15-16, 2012
Biomarker Summit 2012
San Diego, CA, United States

March 18-23, 2012
Keystone Symposia on Molecular and Cellular Biology
Ubiquitin Signaling
Whistler, British Columbia, Canada

March 19-21, 2012
British Pharmacological Society
The Biomedical Basis of Elite Performances
London, United Kingdom

March 19-21, 2012
The Biomedical Basis of Elite Performance
the British Pharmacological Society & The Physiological Society
London, United Kingdom

March 31 - April 4, 2012
American Association for Cancer Research
103rd Annual Meeting
Chicago, IL, United States

April 11, 2012
British Pharmacological Society
Statistics Workshop
London, United Kingdom

April 21-25, 2012
Experimental Biology 2012
San Diego, CA, United States

April 23-24, 2012
British Pharmacological Society
4th BPS Focused Meeting on Cell Signaling
Leicester, United Kingdom

May 2-4, 2012
8th Annual Pediatric Clinical Trials Conference
Philadelphia, PA, United States

May 13-18, 2012
Keystone Symposia on Molecular and Cellular Biology
Drug Resistance and Persistence in Tuberculosis
Kampala, Uganda

May 16-19, 2012
International Stress and Behavior Society
17th International "Stress and Behavior" Conference
St. Petersburg, Russia

June 7-9, 2012
British Pharmacological Society
Focused Meeting on Neuropeptides
London, United Kingdom

June 9-12, 2012
The Neutrophil in Immunity
Quebec City, PQ, Canada

June 10-15, 2012
FASEB Summer Research Conferences
Retinoids
Snowmass Village, CO, United States

June 10-15, 2012
FASEB Summer Research Conferences
Trace Elements in Biology & Medicine
Steamboat Springs, CO, United States

June 13-16, 2012
International Society for Stem Cell Research
10th Annual Meeting
Yokohama, Japan

June 22-24, 2012
International Stress and Behavior Society
18th International "Stress and Behavior" North America Conference
New Orleans, LA, United States

June 23-27, 2012
International Society for Advancement of Cytometry
CYTO 2012
Leipzig, Germany

June 24-27, 2012
Eurotox 2012
Stockholm, Sweden

June 26-29, 2012
4th International Congress on Cell Membranes and Oxidative Stress
Isparta, Turkey

July 14-18, 2012
Controlled Release Society
39th Annual Meeting and Exposition
Quebec City, Canada

July 15-20, 2012
FASEB Summer Research Conferences
Protein Phosphatases
Snowmass Village, CO, United States

July 17-20, 2012
6th European Congress of Pharmacology
Granada, Spain

July 22-27, 2012
FASEB Summer Research Conferences
Tyrosine Kinase Signaling in Cancer, Disease, and Development
Snowmass Village, CO, United States

July 27-30, 2012
International Academy of Cardiology
17th World Congress on Heart Disease
Toronto, ON, Canada

July 29 - August 3, 2012
FASEB Summer Research Conferences
Integration of Genomic and Non-Genomic Steroid Receptor Actions
Snowmass Village, CO, United States

August 2-5, 2012
American Psychological Association
2012 Annual Convention
Orlando, FL, United States

August 5-9, 2012
26th Symposium of The Protein Society
San Diego, CA, United States

September 9-13, 2012
10th International Catecholamine Symposium
Pacific Grove, CA, United States

September 23-26, 2012
American College of Clinical Pharmacology
41st Annual Meeting
Chicago, IL, United States

October 13-17, 2012
Society for Neuroscience
Annual Meeting
New Orleans, LA, United States

October 14-18, 2012
ISSX 18th North American Regional Meeting
Dallas, TX, United States

October 14-18, 2012
American Association of Pharmaceutical Scientists
Annual Meeting
Chicago, IL, United States

December 18-20, 2012
British Pharmacological Society Winter Meeting
London, United Kingdom

World Journal of Pharmacology

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

World J Pharmacol 2012 August 9; 1(4): I-V
ISSN 2220-3192 (online)
© 2012 Baishideng. All rights reserved.

GENERAL INFORMATION

World Journal of Pharmacology (*World J Pharmacol*, *WJP*, online ISSN 2220-3192, DOI: 10.5497) is a bimonthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 100 experts in pharmacology from 23 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 *WJP* 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 *WJP* is an OA journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJP* 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

WJP aims to report rapidly new theories, methods and techniques for prevention, diagnosis, treatment, rehabilitation and nursing in the field of pharmacology. *WJP* covers topics concerning neuropsychiatric pharmacology, cerebrovascular pharmacology, geriatric pharmacology, anti-inflammatory and immunological pharmacology, antitumor pharmacology, anti-infective pharmacology, metabolic pharmacology, gastrointestinal and hepatic pharmacology, respiratory pharmacology, blood pharmacology, urinary and reproductive pharmacology, pharmacokinetics and pharmacodynamics, clinical pharmacology, drug toxicology, and pharmacology-related traditional medicine, and integrated Chinese and Western medicine. The journal also publishes original articles and reviews that report the results of pharmacology-related applied and basic research in fields such as immunology, physiopathology, cell biology, medical genetics, and pharmacology of Chinese herbs.

Columns

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

Name of journal

World Journal of Pharmacology

ISSN

ISSN 2220-3192 (online)

Editor-in-Chief

Geoffrey Burnstock, PhD, DSc, FAA, FRCS(Hon), FRCP (Hon), FmedSci, FRS, Professor, Autonomic Neuroscience Centre, University College Medical School, Royal Free Campus, Rowland Hill Street, London NW3 2PF, United Kingdom

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

Indexed and Abstracted in
Digital Object Identifier.

Published by
Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

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

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

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJP* 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 have 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 participant. 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/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2220-3192/g_info_20100722180909.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjpharmaco@wjgnet.com, or by telephone: +86-10-85381891. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be

typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

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

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

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

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

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

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

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-85381891 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 *WJP*, 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 less than 256 words) and structured abstracts (no less than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no less 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 less than 140 words); RESULTS (no less than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

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

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/2220-3192/g_info_20100725072755.htm.

Illustrations

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

Tables

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

Notes in tables and illustrations

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

Acknowledgments

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

REFERENCES

Coding system

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

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

PMID and DOI

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

Style for journal references

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

Style for book references

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

Format

Journals

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

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

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

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

In press

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

Organization as author

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

Both personal authors and an organization as author

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

No author given

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

Volume with supplement

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

Issue with no volume

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

No volume or issue

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

Books

Personal author(s)

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

Chapter in a book (list all authors)

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

Author(s) and editor(s)

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

Conference proceedings

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

Conference paper

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

Electronic journal (list all authors)

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

Patent (list all authors)

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

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

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

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h,

blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2220-3192/g_info_20100725073806.htm.

Abbreviations

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

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

Editorial: http://www.wjgnet.com/2220-3192/g_info_20100725071851.htm

Frontier: http://www.wjgnet.com/2220-3192/g_info_20100725071932.htm

Topic highlight: http://www.wjgnet.com/2220-3192/g_info_20100725072121.htm

Observation: http://www.wjgnet.com/2220-3192/g_info_20100725072232.htm

Guidelines for basic research: http://www.wjgnet.com/2220-3192/g_info_20100725072344.htm

Guidelines for clinical practice: http://www.wjgnet.com/2220-3192/g_info_20100725072543.htm

Review: http://www.wjgnet.com/2220-3192/g_info_20100725072656.htm

Original articles: http://www.wjgnet.com/2220-3192/g_info_20100725072755.htm

Brief articles: http://www.wjgnet.com/2220-3192/g_info_20100725072920.htm

Case report: http://www.wjgnet.com/2220-3192/g_info_20100725073015.htm

Letters to the editor: http://www.wjgnet.com/2220-3192/g_info_20100725073136.htm

Book reviews: http://www.wjgnet.com/2220-3192/g_info_20100725073214.htm

Guidelines: http://www.wjgnet.com/2220-3192/g_info_20100725073300.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJP*. The revised version including manuscript and high-resolution image figures (if any) should be re-submitted online (http://www.wjgnet.com/2220-3192office/). The author should send the copyright transfer letter, responses to the reviewers, English language Grade B certificate (for non-native speakers of English) and final manuscript checklist to wjpharmaco@wjgnet.com.

Language evaluation

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

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2220-3192/g_info_20100725073726.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2220-3192/g_info_20100725073445.htm.

Proof of financial support

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

Links to documents related to the manuscript

WJP 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

WJP is an international, peer-reviewed, OA, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 600 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.