ISSN 1948-0210 (online)

World Journal of *Stem Cells*

World J Stem Cells 2011 April 26; 3(4): 25-42

Baishideng Publishing Group
www.wjgnet.com

WJSC World Journal of Stem Cells

A peer-reviewed, online, open-access journal of stem cells

Editorial Board

2009-2013

The *World Journal of Stem Cells* Editorial Board consists of 284 members, representing a team of worldwide experts in stem cells research. They are from 28 countries, including Australia (5), Austria (1), Belgium (3), Brazil (2), Canada (7), China (19), Czech Republic (2), Denmark (4), Finland (3), France (5), Germany (14), Hungary (3), India (3), Iran (1), Israel (4), Italy (13), Japan (18), Netherlands (4), Norway (2), Singapore (10), South Korea (15), Spain (6), Sweden (2), Switzerland (3), Turkey (2), United Arab Emirates (1), United Kingdom (15), and United States (117).

WJSC

World Journal of Stem Cells

Contents

Monthly Volume 3 Number 4 April 26, 2011

World Journal of Stem Cells
Volume 3 Number 4 April 26, 2011

Contents

NAME OF JOURNAL
World Journal of Stem Cells

LAUNCH DATE
December 31, 2009

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

EDITING
Editorial Board of *World Journal of Stem Cells*,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1891
Fax: +86-10-8538-1893
E-mail: wjsc@wjgnet.com
http://www.wjgnet.com

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

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

PUBLICATION DATE
April 26, 2011

ISSN
ISSN 1948-0210 (online)

EDITORIAL OFFICE
Jin-Lei Wang, Director
World Journal of Stem Cells
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1891
Fax: +86-10-8538-1893
E-mail: wjsc@wjgnet.com
http://www.wjgnet.com

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1948-0210/g_info_20100313165700.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/1948-0210office

World Journal of Stem Cells

Online Submissions: http://www.wjgnet.com/1948-0210office
wjsc@wjgnet.com
doi:10.4252/wjsc.v3.i4.25

World J Stem Cells 2011 April 26; 3(4): 25-33
ISSN 1948-0210 (online)

EDITORIAL

Adipose-derived stromal cells: Their identity and uses in clinical trials, an update

Louis Casteilla, Valérie Planat-Benard, Patrick Laharrague, Béatrice Cousin

Louis Casteilla, Valérie Planat-Benard, Patrick Laharrague, Béatrice Cousin, Université de Toulouse, UPS, UMR 5241 Métabolisme, Plasticité et Mitochondrie, BP 84225, F-31 432 Toulouse Cedex 4, France
Louis Casteilla, Valérie Planat-Benard, Patrick Laharrague, Béatrice Cousin, CNRS, UMR 5241 Métabolisme, Plasticité et Mitochondrie, BP 84 225, F-31 432 Toulouse, France
Patrick Laharrague, Laboratoire d'Hématologie, CHU Toulouse, France
Author contributions: All authors wrote the paper.
Supported by FP7 program (CADSCADE), Etablissement Français du Sang Pyrénées-Mediterranée, Fondation pour la Recherche Médicale (Programme "Vieillissement Cardiovadsculaire Normal et Pathologique", project DCV20070409252) and CTP "transpyreneen stem cells group"
Correspondence to: Louis Casteilla, PhD, CNRS, University Paul Sabatier, UMR 5241 BP 84225, F-31 432 Toulouse Cedex 4, France. louis.casteilla@inserm.fr
Telephone: +33-5-62170904 Fax: +33-5-62170905
Received: March 31, 2010 Revised: December 14, 2010
Accepted: December 21, 2010
Published online: April 26, 2011

Abstract

In adults, adipose tissue is abundant and can be easily sampled using liposuction. Largely involved in obesity and associated metabolic disorders, it is now described as a reservoir of immature stromal cells. These cells, called adipose-derived stromal cells (ADSCs) must be distinguished from the crude stromal vascular fraction (SVF) obtained after digestion of adipose tissue. ADSCs share many features with mesenchymal stem cells derived from bone marrow, including paracrine activity, but they also display some specific features, including a greater angiogenic potential. Their angiogenic properties as well as their paracrine activity suggest a putative tumor-promoting role for ADSCs although contradictory data have been published on this issue. Both SVF cells and ADSCs are currently being investigated in clinical trials in several fields (chronic inflammation, ischemic diseases, *etc.*). Apart from a phase Ⅲ trial on the treatment of fistula, most of these are in phase Ⅰ and use autologous cells. In the near future, the end results of these trials should provide a great deal of data on the safety of ADSC use.

Key words: Mesenchymal stem cells; Stroma cells; Cell therapy; White adipose tissue

Peer reviewers: Mikhail G Kolonin, PhD, Centre for Stem Cell Research, Institute of Molecular Medicine, University of Texas Health Science Center at Houston, 1825 Pressler st., Rm. 630-F, Houston, TX 77030, United States; Soo-Hong Lee, PhD, Assistant Professor, College of Life Science, CHA Stem Cell Institute, CHA University, 606-16 Yeoksam 1-dong, Gangnam-gu, Seoul 135-081, South Korea

Casteilla L, Planat-Benard V, Laharrague P, Cousin B. Adipose-derived stromal cells: Their identity and uses in clinical trials, an update. *World J Stem Cells* 2011; 3(4): 25-33 Available from: URL: http://www.wjgnet.com/1948-0210/full/v3/i4/25.htm DOI: http://dx.doi.org/10.4252/wjsc.v3.i4.25

INTRODUCTION

The interest in adipose-derived multipotent stroma cells has increased greatly, largely because it is easy to obtain large amounts of these cells *via* the liposuction process, using only local anesthesia. Since many aspects of adipose-derived stromal cells (ADSCs) are fully described in recent reviews[1], we will focus on specific points that are poorly discussed elsewhere and will also comment on the clinical uses of these cells based on our own expertise in the field of ischemia.

WHAT ARE ADSCS AND WHAT THEY DO?

Mesenchymal stem cells (MSC) were first described as

Table 1 Differentiated phenotype given rise from adipose-derived stromal cell and interactive effects of these cells with immune and cancer cells

Phenotype given rise in *in vitro* system	Ref.
Classic mesenchymal phenotype (adipocyte, osteoblast, chondrocyte)	[1]
Hematopoietic supporting cells	[25]
Other phenotypes	Vascular cells (Smooth-muscle cells, Endothelial)[16]
	Neurones[1]
	Cardiomyocyte and skeletal cells in the required presence of 5 azacytidine[16]
Modulation of inflammation and immune suppressive functions	Rheumatoid arthritis[31]
	GVH[30]
	Autoimmune encephalomyelitis[32]
Anti-cancer effect	Tumor progression inhibition[34]
Pro-cancer effects	Tumor progression growth[35,37]

immature cells in adult bone marrow, able to give rise to mesenchymal lineages such as osteoblasts, chondrocytes and adipocytes[2]. These cells are selected by adhesion onto Petri dish before expansion. Under low culture density, distinct colonies are formed, each one deriving from a single precursor cell, the CFU-F. It has been estimated that MSCs represent a very low proportion (0.01%-0.0001%) of the nucleated cells in adult human bone marrow[3]. In 2005, the International Society for Cellular Therapy established the minimal criteria for MSC definition. Three criteria were proposed: adherence to plastic, specific surface antigen (CD73+, CD90+, CD105+, CD45-, CD34-, CD14 or CD11b-, CD79- or CD19-, HLA-DR) and *in vitro* capability to give rise to adipocytes, osteoblasts and chondrocytes[4]. A clarification published later proposed the term "multipotent mesenchymal stromal cells," since only a cell subset seems able to display self-renewal properties[5,6]. It is also necessary to add to this definition the supporting effect of these cells, particularly on hematopoietic stem cells[7].

For a long time, as first described in the 1960s, a protocol similar to that used to purify MSCs from bone marrow was used to purify adherent immature cells called preadipocytes[1]. To obtain these cells, fat pads should be minced and incubated with collagenase in order to dissociate the extracellular matrix. Afterwards, floating mature adipocytes are separated from the pelleted stromal vascular fraction (SVF). Since the SVF comprises a heterogeneous cell population, the final isolation step consists in plating these cells in order to select the adherent population by successive washings. With the appropriate cocktail of differentiating agents, these cells can give rise adipocytes, demonstrating *a posteriori*, the existence of adipose progenitors in the stromal fraction of adipose tissues, irrespective of the age of the patient. These preadipocytes were later demonstrated to be multipotent and were named ADSCs[8]. However, since their self-renewal has not been definitively established and, in agreement with the statement of International Society for Cellular Therapy[5], we prefer to use the term "stromal" instead of "stem". In contrast to MSCs, when freshly prepared and during the first rounds of proliferation, these cells express the CD34 antigen[9]. The frequency of these cells is much higher in adipose tissue (100 to 500 fold higher) than that of MSCs in bone marrow. Although numerous authors use the same term, MSC, both for cells derived from bone marrow and for those derived from adipose tissue, several differences have been described at genomic, proteomic and functional levels[10-12], suggesting that MSCs and ADSCs are different and that MSCs are probably more committed towards osteoblastic and chondrogenic lineages than ADSCs[1].

A confusing point is that the term ADSCs or adipose-derived stem/stroma cells is also used for crude SVF. This fraction is highly heterogeneous and contains many cell subsets including native ADSC, mature endothelial and hematopoietic cells, the latter representing a large portion of this fraction (up to 20%)[9]. In this context, these cells cannot be named stem or mesenchymal cells. In our opinion, the term ADSCs should be restricted only to the cells described below, although a more precise classification depending on cell potential may exist.

The functional properties of mesenchymal stromal cells can be summarized as: multipotency, functional cell support that could be termed stromagenesis, modulation of immuno-inflammatory functions. These last two properties may be connected. Most of these effects are believed to be mediated *via* paracrine activity[13,14]. In this context, it is noteworthy that fat is considered as a true endocrine tissue and that adipose lineage cells display a strong secretory activity[15]. Many reviews focus on these features in bone marrow and adipose-tissue-derived cells[1,7,15,16]. With the exception of the classic mesenchymal phenotype (adipocyte, osteoblast, chondrocyte), no study has clearly demonstrated a complete and functional differentiation of mesenchymal-like cells. Most often, the phenotype is only established by the detection of some markers of differentiated phenotypes. A recent study suggested that MSCs could give rise to "intermediate biphenotypic cells" which co-express cell-specific markers whilst maintaining the stromal phenotype but without truly becoming functional[17]. Such findings strongly support the use of the term "differentiated phenotype-like cells" to define these cells after differentiation, as they only mimic true differentiated cells without displaying all the features of them.

In Table 1 we have summarized the differentiated phenotypes arising from ADSCs and the interactive ef-

fects of these cells with immune and cancer cells. We would like to focus our comments on some specific or neglected aspects. For ADSCs, it is noteworthy that among the various potentials of these cells, many reports concern the cardiovascular field, including both *in vivo* and functional evaluations[9,18-20]. In this context, we successively demonstrated strong angiogenic features in ischemic hindlimb, myocardial infarction and wound-healing situations, associated or not with irradiation[9,21,22]. It appears that ADSCs are more efficient in this field than their bone marrow counterparts, and a direct comparison in the same set of experiments indicated a better angiogenic effect of ADSCs than MSCs[23]. Another functional characteristic initially attributed to MSCs is their ability to support hematopoiesis[6,24]. ADSCs also appear to posess this property, although they appear less efficient in supporting immature hematopoietic cells[25]. Another key feature of bone marrow MSCs is their ability to modulate immune and inflammatory functions[26]. This property has been clearly demonstrated and is currently being investigated in a clinical trial, although the exact underlying mechanisms and the molecules involved are still being discussed and could be species-dependent[27,28]. Based on the complexity of the effects, it is reasonable to suggest that they are due to a combination of numerous molecules, and that these could display some redundancy. Such redundant factors could explain the discrepancies between the different studies. Immunosuppressive capacity and modulation of inflammation are shared with ADSCs, which seem very efficient both *in vitro* and in different *in vivo* situations[29-32]. This immunosuppressive effect associated with the angiogenic properties of ADSC raises questions about the interactions between these cells and cancer cells. This is crucial as a positive relationship between obesity and cancer is well-known[33]. We recently demonstrated that ADSCs strongly inhibit pancreatic cancer cell line proliferation, both *in vitro* and *in vivo,* and induce tumor cell death by altering cell cycle progression[34]. These data appear to be contradictory to the description of angiogenic properties of these cells in ischemic situations and to four recent reports demonstrating that ADSCs could promote tumor growth[35-38]. Among these reports, the work published by Donnerberg' s group is particularly interesting as it is the only report on the effect of ADSCs on primary cancer cells rather than cell lines, and also because it describes an investigation of the interactions between ADSCs and dormant or active cancer cells that were purified using different stem cell markers[35]. The authors concluded that ADSCs could trigger the growth of tumors from active cancer cells but not from dormant cells. Furthermore, when all reports are compared it appears that a positive effect of ADSCs on tumor cells is observed when these cells are co-injected with cancer cells or transplanted at the beginning of the tumor process. In contrast, a negative effect can be observed when ADSCs are implanted in pre-existing tumors[34]. Thus, we can suggest that, partner dependent, reactive cross-talk can take place between ADSCs and other cell types in order to maintain proper development of the tissue and a correct balance between proliferation and differentiation.

The possible difference between native and cultured cells remains an open question. Indeed, as it now appears that extensive proliferation can achieve reprogramming, it is not possible to exclude the possibility that the features attributed to mesenchymal cells, defined after a large number of doublings in classic culture conditions, could be more related to the culture process than to the intrinsic properties of native mesenchymal cells.

WHERE DO ADSCs COME FROM?

To better understand the physiological importance and role of a cell, it is vital to know and understand their development. Nearly all animal species have developed strategies to handle energy stores, in the form of white adipose tissue (WAT), according to their particular needs. Most mammals have both intra-abdominal and subcutaneous fat pads[39]. In humans, WAT development mainly occurs during the last trimester of intra-uterine life. At birth, fat represents around 16% of body weight. In adult humans, this tissue is dispersed throughout the body with major intra-abdominal (around the omentum, intestines, and perirenal areas) and subcutaneous depots (buttocks, thighs, and abdomen). Additionally, WAT is found in many other areas: the retro-orbital space, face, extremities, bone marrow. It is noteworthy that major differences in the metabolic properties and patterns of gene expression within different fat depots have been described and could be related to different pathogenic states[39]. Although adipose tissue development is often associated with an increase in the risk of metabolic diseases and morbidity, Tran *et al*[40] neatly demonstrated that subcutaneous fat is intrinsically different from visceral fat and protects against metabolic disorders. Endocrine and paracrine activity would explain a large part of these differences. It is clear that all deposits are not equivalent and this must be kept in mind when analyzing precursors present in tissue and particularly in ADSCs. Indeed, at least in mice, the potential of ADSC differ according to the location of adipose tissue from which they are purified[41].

Like muscle and bone, adipose tissue is generally regarded as having a mesodermal origin, even though no studies have been performed tracing the precise lineage. However, as with the bones and muscles of the skull, it was recently reported that adipose cell lineages originate from the neural crest during development[42]. So, both neuroectoderm and mesoderm could give rise to local adipose tissue. This dual origin (ectoderm and mesoderm) could be also true during development, as was proposed for MSCs[43]. Indeed, during development neuroepithelial cells supply an Initial and transient wave of MSC differentiation[43].

Very few data are available on the early development of adipose tissue in humans. In 1965, Wassermann was the first to study the development of WAT in compari-

son with other organs in humans. Through a careful histological study he demonstrated that adipose depots develop from primitive organs[44]. Within these primitive organs, clusters of adipocytes emerge from a bulk of mesenchymal cells related to the development of the vascular network, giving rise to fatty lobules. The most differentiated cells are far-distant from capillaries. Vascularization therefore plays a major role in the development of adipose tissues. Angiogenesis and adipogenesis appear coordinated in time and space. In evaluating the particular relationship between adipose lineage cells and endothelial cells, we have established that many adipose precursors express the surface marker CD34, a protein also present at the surface of immature cells and endothelial cells[9]. Since we have also provided evidence of a true angiogenic potential *in vitro* and *in vivo*, we hypothesized that these adipose precursors could commit to the endothelial lineage under appropriate conditions. Bouloumié's group drew similar conclusions from *in vivo* studies[19]. The precise location of native ADSCs inside adipose tissue has not been determined. Indeed, one set of experiments suggests that ADSCs display pericyte properties, as proposed for all MSCs[45,46]. This conclusion is not consistent with one of our recent reports[47] in which immunohistological analysis revealed that native ASCs exhibited specific morphological features with long protrusions. Moreover, native ASCs were found scattered in AT stroma and did not express *in vivo* pericytic markers such as NG2, CD140b or alpha-smooth muscle actin, which appeared during the culture process. More recently, employing a non-invasive assay to follow fat mass reconstitution *in vivo*, Rodeheffer *et al*[48] identified a subpopulation of early adipocyte progenitor cells (Lin^-, $CD29^+$, $CD34^+$, $Sca\text{-}1^+$, $CD24^+$) resident in adult WAT. Using genetically marked mice, Tang *et al*[49] found that most adipocytes descend from a pool of proliferating progenitors that are already committed, either prenatally or early in postnatal life. These progenitors reside at least in part in the mural cell compartment of the adipose vasculature, but not in the vasculature of other tissues. These data could be related to the hypothesis that naïve MSCs originate from a subset of human perivascular cells that express both pericyte and MSCs markers *in situ* (CD146, NG2, PDGF-Rβ)[45,46]. Therefore, adipose-derived stroma cells could differentiate from various types of vascular cell types, probably located within the WAT itself. However, it appears that the developmental origin of white preadipocytes differs according to the location[50] and this opens up the question of possible differences in the potential of preadipocytes/ADSC.

TOWARDS CLINICAL TRIALS

Table 2 showed the different steps for a clinical trial. There is great interest in adipose tissue as a source of therapeutic cells as the cells are obtained from adults, thereby avoiding ethical concerns, and use tissue which is abundant and easy to obtain, even when compared with bone marrow where sampling requires general anesthesia. Another advantage is that, as the frequency of ADSC is much higher in adipose tissue than those of MSC in bone marrow, a large number of cells can be obtained without a large number of passages. In this way, the risk of culture-induced chromosomal abnormality senescence is greatly decreased[51].

From the point of view of safety and adverse-side effects,two other key issues are the possibility of undesirable differentiation and the possibility of interaction between ADSCs and resident cancer cells. Concerning the first point, undesirable calcifications have been observed after the transplantation of BN-MNC cells in the heart after infarction[52]. More recently, cysts and microcalcifications were detected in 4 out of 70 patients after breast reconstruction using lipoaspirate associated with crude SVF[53]. To our knowledge, no other cases of undesirable differentiation have been described, suggesting either that such events are rare or have not been fully and systematically evaluated. However, this issue has been discussed and analyzed in recent pre-clinical papers[54]. No definitive conclusion can be reached on the possible interaction between ADSCs and cancer cells as contradictory reports have been published,as discussed previously. We can merely stress that a risk cannot be excluded in the context of pathologies associated with cancer.

In Tables 3 and 4, we have indexed all the clinical trials that we found using adipose, derived and stem as keywords on the "clinicaltrial" and "pubmed" websites. As discussed previously, there is some confusion about the use of the term adipose-derived stroma/stem cells either for ADSCs or crude SVF. All trials using crude SVF and ADSC are presented in Tables 3 and 4 respectively. Based on the ease of obtaining crude SVF, it is not surprising to find the first published clinical application describing the use of this cell fraction in a case report concerning a massive defect of the calvaria after injury[55]. In this report, crude SVF was mixed with fibrin glue. Three months after the reconstruction, CT-scans showed new bone formation and near complete calvarial continuity around the site of the damage. Unfortunately, no another related papers have been published since this seminal report. It is surprising not to find more reported trials particularly in the context of breast reconstruction. This could be due to the peculiar status for SVF cells which can be extemporaneously obtained in the operating room and thus escape the classic legislation on cell therapy. This might be considered regrettable since, as stated above, one trial described undesirable events (cysts and microcalcification)[53] and the possible risk of a cancer promoting effect of any cells derived from adipose tissue is questionable. Moreover, it is noteworthy that two clinical trials dedicated to the treatment of cirrhosis with autologous SVF were suspended although no reason was disclosed. Other trials are investigating the effect of SVF in the cardiovascular field, including acute myocardial infarction. This is an ideal situation for testing SVF effects as, in a clinical setting, it is not possible to use expanded cells which

Table 2 The different steps for a clinical trialand cancer cells

Steps of clinical trial	Elements of discussion
Design	Autologous
	Immunocompatibility
	Lag of time between fat sampling and delivery
	Amount of cells
	Allogenic
	Histocompability issue
	If bank, ready to use treatment
	Inclusion criteria
	Too broad: leads to wrong conclusions associated with great variability and independent parameters,
	Too restricted: enrolment difficulties associated with non relevant and inadequate parameters
	Exclusion criteria
	Too broad: enrolment difficulties associated with non relevant and inadequate parameters
	Too restricted: risks of adverse side effects associated with interactions between transplanted cells and undesirable context
	Number of patients: statistically defined
	Objective and well-established criteria of safety and efficacy
	Uni or multicenter analysis
	Standardization of procedures between centers
	Efficiency of enrolment
Sampling	Liposuction:
	Local anesthesia
	Fat depot
	Technique (no ultra-sound)
	Anti-coagulant
Culture	Opened or closed system
	Bovine or human-derived products
	Number of passages
	Quality and Safety control
	Release criteria
Injection	iv
	Poorly invasive but large distribution and mostly trapped in lung
	im or intra tissue
	More invasive
	More restricted localization
	Pressure challenge for adipose-derived stromal cells
Monitoring of the tolerance and the safety	Criteria: pain, wound healing, inflammation, immunology, tumor
	Kinetics for analysis
	Short and long term safety
Monitoring of the results	Objective criteria, standardisation of procedures
Analysis of the results	Adequate statisitic
	Stick to primary and secondary aims

require a delay for cultivation unless the therapeutic cells are allogenic and already frozen. This would require the existence of a cell bank, which is not yet the case for these cells. For these applications, a positive outcome may be unlikely as similar protocols have already been tested with bone marrow mono-nuclear cells (BM-MNC and not MSC) with negative results in most cases[56]. By the same reasoning, there may be some optimism for the use of SVF in the treatment of critical ischemia hindlimb as the injection of BM-MNC was relatively successful even after 2 years[57,58].

Only the clinical trials described in Table 3 correspond to the use of ADSCs after purification and expansion. ADSC were systematically evaluated for their ability to rebuild volume in depressed scars following the subcutaneous injection of ADSC which differentiated towards adipocytes. This trial of 36 patients was completed in 2007 but has not, to our knowledge, yet been documented in any peer-reviewed international journal. Only one report relates to a field in which ADSCs have been evaluated for their reconstructive properties based on their classic mesenchymal differentiation potentials, specifically in the field of bone or cartilage reconstruction when the osteogenic or chondrogenic potentials of ADSC are well-established and widely investigated[59]. In this case report with 36 mo of follow-up, the defect was successfully reconstructed with a microvascular flap using beta-tricalcium phosphate, autologous ADSCs and bone morphogenetic protein-2 to trigger their osteogenesis. Although this result is encouraging, a case report cannot give prove a general effect and no conclusions can be definitively drawn until phase Ⅰ and Ⅱ trials have been conducted. Most other ADSC trials concern fistula complications that result from tissue degeneration following an uncontrolled inflammatory process. In a noteworthy case report, Garcia-Olmo *et al*[60] found that expanded ADSCs are more efficient than the freshly-prepared in treating Crohn's disease. The trials on fistula indicate that ADSCs are very efficient in controlling inflammation and improving the healing process[22,61,62]. Garcia-Olmo *et al*[61,62]

Table 3 Clincial trials using stromal vascular fraction

Clinical trials with SVF	Design	Results	Ref
Traumatic calvaria defect	Autologous SVF + fibrin glue: case report	Success	{Lendeckel, 2004 #483}
Breast reconstruction after lumpectomy	Autologous fat + autologous SVF. No arm control	Cysts and Microcalcifications (4/70 patients)	{Yoshimura, 2008 #481}
	Autologous fat + autologous SVF, phase Ⅳ		{Yoshimura, 2008 #481} NCT00616135*
Lipodystrophy Ⅰ	Autologous SVF + fat Phase Ⅰ	recruiting	NCT00715546*
Non revascularizable myocardium	Autologous SVF Injection into the left ventricle	ongoing	NCT00426868*
Treatment of Pts With ST-Elevation Myocardial Infarction	Autologous SVF, Phase Ⅰ Injection into the left ventricle	Ongoing	NCT00442806*
	Autologous SVF, 2 doses against placebo, Phase Ⅱ, Ⅲ, Intracoronary injection	Not yet open	NCT01216995*
Diabetes Ⅰ	"Activated" autologous SVF, phase Ⅰ / Ⅱ iv administration	recruiting	NCT00703599*
Diabetes Ⅱ	"Activated" autologous SVF, phase Ⅰ / Ⅱ	recruiting	NCT00703612*
Liver cirrhosis	Autologous SVF Intrahepatic arterial administration	Suspended suspended	NCT00913289* NCT01062750

The clinical trials that are indexed in this table were retrieved using adipose, derived and stem in clinicaltrial and PubMed websites. As discussed in the text, there is some confusion about the use of the term adipose-derived stroma/stem cells. This term should be restricted to cultured mesenchymal stem cells. In fact, it can recover the use of the crude and strongly heterogeneous stroma fraction just after its recovery after fat digestion. The trials listed in this table correspond to the use of such fraction (*identifier on Clinicaltrials website: *http://clinicaltrials.gov/ct2/results?term=adipose+derived+cells). SVF: Stromal vascular fraction.

Table 4 Clincial trials using adipose-derived stromal cell

Clinical trials with ADSC	Design	Results	Ref
Maxillary reconstruction	Autologous ADSC case report	Success	{Mesimaki, 2009 #480}
Cryptoglandular origin fistula with or without Crohn's disease	Autologous ADSC phase Ⅰ / Ⅱ intra-tissue	ADSCs more effective (P = 0.001). Recurrence rate with ADSC = 17.6%	{Garcia-Olmo, 2008 #451; Garcia-Olmo, 2009 #449}
	Autologous ADSC Phase Ⅱ, 2 arms (fibrin glue, fibrin glue + ADSC)	Ongoing, not recruiting	NCT00115466*
Crohn's disease fistula	Autologous ADSC, phase Ⅰ and Ⅱ	Phase Ⅰ, complete Phase Ⅱ recruiting	NCT00992485* NCT01011244*
	Autologous ADSC, phase Ⅰ	Phase Ⅰ / Ⅱ recruiting	NCT01157650*
	Allogenic ADSC: phase Ⅰ / Ⅱ 20×10^6 then 40×10^6	recruiting	NCT00999115*
Complex Perianal Fistulas not associated to Crohn's disease	Autologous ADSC, phase Ⅲ three arms (fibrine, ADSC, fibrin glue + ADSC; 20×10^6 then 40×10^6 when no effect)	Completed (214 enrolled patients)	NCT00475410*
	Long term safety	Recruiting	NCT01020825*
Depressed Scar	Autologous ADSC predifferentiated towards adipocyte, phase Ⅱ, Ⅲ	Complete	NCT00992147*
Chronic critical limb Ischemia	Autologous ADSC, phase Ⅰ im 100×10^6	Recruiting	NCT01211028*
Chronic critical limb Ischemia in diabetic patients	Autologous ADSC, phase Ⅰ / Ⅱ iv administration	Recruiting	NCT01079403*
Fecal incontinence	Autologous ADSC, phase Ⅰ	Recruiting	NCT01011686*
GVHD	Autologous ADSC iv 10^6/kg	4/5 alive (after a median follow-up of 40 mo)	{Fang, 2007 #469}
	Autologous ADSC Three arms no administration, iv 10^6/kg or 3×10^6/kg	Recruiting	NCT01222039
Secondary Progressive Multiple Sclerosis	Autologous ADSC phase Ⅰ / Ⅱ 3 arms (iv 10^6 and 4×10^6/kg against no intervention)	Recruiting	NCT01056471*

The clinical trials that are indexed in this table were retrieved using adipose, derived and stem in clinicaltrial and Pubmed websites. As discussed in the text, there is some confusion about the use of the term adipose-derived stroma/stem cells that can be used for crude SVF. This term should be restricted to cultured adipose derived mesenchymal stem cells and the table lists the trials using such cells (*identifier on Clinicaltrials website: *http://clinicaltrials.gov/ct2/results?term=adipose+derived+cells). ADSC: Adipose-derived stromal cell; GVHD: Graft-versus-host disease.

published the conclusions of randomized phase I and Ⅱ trials in which they compared injection of ADSC into rectal mucosa with fibrin glue to fibrin glue alone on 25 patients with complex perianal fistulas associated or not with Crohn's disease. Following a first dose of 20 million ADSCs, a second dose of 40 million was administered 8 wk later in cases where there was no initial healing. Patients were considered healed when a total epithelialization of the external opening was evident after 8 wk. Seventy one percent of patients treated with ADSC and fibrin glue displayed fistula healing compared to 16% observed in patients treated with fibrin glue alone ($P < 0.001$). This positive effect on healing is all the more remarkable as it is otherwise poorly documented, even in rodent models[22]. Two further clinical trials have focused on Graft *versus* Host disease,.an application which is not surprising given the efficiency of MSCs at the clinical level[63] and the similarity in immunomodulation properties between MSCs and ADSCs[29,30]. Multiple sclerosis can be also included in the field of the modulation of inflammation/immune response. In a rodent model, it was demonstrated that intravenously injected ADSCs can home to the lymph nodes and brain and that they act by suppressing the autoimmune response in early phases of disease as well as by inducing local neuroregeneration by endogenous progenitors in animals with established disease[32]. The strong immunosuppressive effects of ADSC reported by various independent groups naturally led to investigation the effect of ADSC on MS. Based on the positive and well-documented positive effects of intravenous administration of MSCs, positive effects of ADSCs[63] are also expected in this field. Only two trials have investigated the effect of ADSCs on chronic critical limb ischemia, one after intra-muscular injections, the second with intravenous injections in diabetic patients. A futher planned trial also intends to use allogenic ADSCs in the context of fistula. This trial is important as it could open up the field of ADSC-associated regenerative medicine.

CONCLUSION

The various clinical trials demonstrate that fat-derived therapy is not a dream, but is becoming a reality. Surprisingly, results so far suggest that the efficiency of ADSCs in regenerative medicine could be related more to their capacity to modulate immunity and/or inflammation than to their differentiation potentials. The physiological relevance of this phenomenon needs to be better documented, as this could lead to improved efficiency and perhaps new therapeutic possibilities for these cells. However, it is reasonable to speculate that one type of cells will not be able to cover all therapeutic applications and that it will be necessary to fully delineate the respective applications for the various types of cells. In this context, it is reasonable to suggest that, each time ADSCs display effects more or less similar to other cell types, the inherent advantages of adipose tissue.will favour its use over cells from other sources.

REFERENCES

1 **Gimble JM**, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ Res* 2007; **100**: 1249-1260
2 **Friedenstein AJ**, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luriá EA, Ruadkow IA. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. *Exp Hematol* 1974; **2**: 83-92
3 **Pittenger MF**, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; **284**: 143-147
4 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317
5 **Horwitz EM**, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 2005; **7**: 393-395
6 **Sacchetti B**, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* 2007; **131**: 324-336
7 **Uccelli A**, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008; **8**: 726-736
8 **Zuk PA**, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002; **13**: 4279-4295
9 **Planat-Benard V**, Silvestre JS, Cousin B, André M, Nibbelink M, Tamarat R, Clergue M, Manneville C, Saillan-Barreau C, Duriez M, Tedgui A, Levy B, Pénicaud L, Casteilla L. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. *Circulation* 2004; **109**: 656-663
10 **Noël D**, Caton D, Roche S, Bony C, Lehmann S, Casteilla L, Jorgensen C, Cousin B. Cell specific differences between human adipose-derived and mesenchymal-stromal cells despite similar differentiation potentials. *Exp Cell Res* 2008; **314**: 1575-1584
11 **Wagner W**, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, Blake J, Schwager C, Eckstein V, Ansorge W, Ho AD. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. *Exp Hematol* 2005; **33**: 1402-1416
12 **Kern S**, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* 2006; **24**: 1294-1301
13 **Caplan AI**, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem* 2006; **98**: 1076-1084
14 **Phinney DG**, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair--current views. *Stem Cells* 2007; **25**: 2896-2902
15 **Wang P**, Mariman E, Renes J, Keijer J. The secretory function of adipocytes in the physiology of white adipose tissue. *J Cell Physiol* 2008; **216**: 3-13
16 **Casteilla L**, Planat-Bénard V, Cousin B, Silvestre JS, Laharrague P, Carrière G, Carrière A, Pénicaud L. Plasticity of adipose tissue: a promising therapeutic avenue in the treatment of cardiovascular and blood diseases? *Arch Mal Coeur Vaiss* 2005; **98**: 922-926
17 **Rose RA**, Jiang H, Wang X, Helke S, Tsoporis JN, Gong N, Keating SC, Parker TG, Backx PH, Keating A. Bone marrow-

derived mesenchymal stromal cells express cardiac-specific markers, retain the stromal phenotype, and do not become functional cardiomyocytes in vitro. *Stem Cells* 2008; **26**: 2884-2892
18 **Rehman J**, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, Pell CL, Johnstone BH, Considine RV, March KL. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 2004; **109**: 1292-1298
19 **Miranville A**, Heeschen C, Sengenès C, Curat CA, Busse R, Bouloumié A. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. *Circulation* 2004; **110**: 349-355
20 **Casteilla L**, Planat-Bénard V, Cousin B, Laharrague P, Bourin P. Vascular and endothelial regeneration. *Curr Stem Cell Res Ther* 2010; **5**: 141-144
21 **Mazo M**, Planat-Bénard V, Abizanda G, Pelacho B, Léobon B, Gavira JJ, Peñuelas I, Cemborain A, Pénicaud L, Laharrague P, Joffre C, Boisson M, Ecay M, Collantes M, Barba J, Casteilla L, Prósper F. Transplantation of adipose derived stromal cells is associated with functional improvement in a rat model of chronic myocardial infarction. *Eur J Heart Fail* 2008; **10**: 454-462
22 **Ebrahimian TG**, Pouzoulet F, Squiban C, Buard V, André M, Cousin B, Gourmelon P, Benderitter M, Casteilla L, Tamarat R. Cell therapy based on adipose tissue-derived stromal cells promotes physiological and pathological wound healing. *Arterioscler Thromb Vasc Biol* 2009; **29**: 503-510
23 **Kim Y**, Kim H, Cho H, Bae Y, Suh K, Jung J. Direct comparison of human mesenchymal stem cells derived from adipose tissues and bone marrow in mediating neovascularization in response to vascular ischemia. *Cell Physiol Biochem* 2007; **20**: 867-876
24 **Koç ON**, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, Lazarus HM. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. *J Clin Oncol* 2000; **18**: 307-316
25 **Corre J**, Barreau C, Cousin B, Chavoin JP, Caton D, Fournial G, Penicaud L, Casteilla L, Laharrague P. Human subcutaneous adipose cells support complete differentiation but not self-renewal of hematopoietic progenitors. *J Cell Physiol* 2006; **208**: 282-288
26 **Jones BJ**, McTaggart SJ. Immunosuppression by mesenchymal stromal cells: from culture to clinic. *Exp Hematol* 2008; **36**: 733-741
27 **Siegel G**, Schäfer R, Dazzi F. The immunosuppressive properties of mesenchymal stem cells. *Transplantation* 2009; **87**: S45-S49
28 **Ren G**, Su J, Zhang L, Zhao X, Ling W, L'huillie A, Zhang J, Lu Y, Roberts AI, Ji W, Zhang H, Rabson AB, Shi Y. Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. *Stem Cells* 2009; **27**: 1954-1962
29 **Puissant B**, Barreau C, Bourin P, Clavel C, Corre J, Bousquet C, Taureau C, Cousin B, Abbal M, Laharrague P, Penicaud L, Casteilla L, Blancher A. Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells. *Br J Haematol* 2005; **129**: 118-129
30 **Yañez R**, Lamana ML, García-Castro J, Colmenero I, Ramírez M, Bueren JA. Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. *Stem Cells* 2006; **24**: 2582-2591
31 **González MA**, Gonzalez-Rey E, Rico L, Büscher D, Delgado M. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. *Arthritis Rheum* 2009; **60**: 1006-1019
32 **Constantin G**, Marconi S, Rossi B, Angiari S, Calderan L, Anghileri E, Gini B, Bach SD, Martinello M, Bifari F, Galiè M, Turano E, Budui S, Sbarbati A, Krampera M, Bonetti B. Adipose-derived mesenchymal stem cells ameliorate chronic experimental autoimmune encephalomyelitis. *Stem Cells* 2009; **27**: 2624-2635
33 **Roberts DL**, Dive C, Renehan AG. Biological mechanisms linking obesity and cancer risk: new perspectives. *Annu Rev Med* 2010; **61**: 301-316
34 **Cousin B**, Ravet E, Poglio S, De Toni F, Bertuzzi M, Lulka H, Touil I, André M, Grolleau JL, Péron JM, Chavoin JP, Bourin P, Pénicaud L, Casteilla L, Buscail L, Cordelier P. Adult stromal cells derived from human adipose tissue provoke pancreatic cancer cell death both in vitro and in vivo. *PLoS One* 2009; **4**: e6278
35 **Zimmerlin L**, Donnenberg AD, Rubin JP, Basse P, Landreneau RJ, Donnenberg VS. Regenerative therapy and cancer: in vitro and in vivo studies of the interaction between adipose-derived stem cells and breast cancer cells from clinical isolates. *Tissue Eng Part A* 2011; **17**: 93-106
36 **Lin G**, Yang R, Banie L, Wang G, Ning H, Li LC, Lue TF, Lin CS. Effects of transplantation of adipose tissue-derived stem cells on prostate tumor. *Prostate* 2010; **70**: 1066-1073
37 **Prantl L**, Muehlberg F, Navone NM, Song YH, Vykoukal J, Logothetis CJ, Alt EU. Adipose tissue-derived stem cells promote prostate tumor growth. *Prostate* 2010; **70**: 1709-1715
38 **Zhang Y**, Daquinag A, Traktuev DO, Amaya-Manzanares F, Simmons PJ, March KL, Pasqualini R, Arap W, Kolonin MG. White adipose tissue cells are recruited by experimental tumors and promote cancer progression in mouse models. *Cancer Res* 2009; **69**: 5259-5266
39 **Gesta S**, Tseng YH, Kahn CR. Developmental origin of fat: tracking obesity to its source. *Cell* 2007; **131**: 242-256
40 **Tran TT**, Yamamoto Y, Gesta S, Kahn CR. Beneficial effects of subcutaneous fat transplantation on metabolism. *Cell Metab* 2008; **7**: 410-420
41 **Prunet-Marcassus B**, Cousin B, Caton D, André M, Pénicaud L, Casteilla L. From heterogeneity to plasticity in adipose tissues: site-specific differences. *Exp Cell Res* 2006; **312**: 727-736
42 **Billon N**, Iannarelli P, Monteiro MC, Glavieux-Pardanaud C, Richardson WD, Kessaris N, Dani C, Dupin E. The generation of adipocytes by the neural crest. *Development* 2007; **134**: 2283-2292
43 **Takashima Y**, Era T, Nakao K, Kondo S, Kasuga M, Smith AG, Nishikawa S. Neuroepithelial cells supply an initial transient wave of MSC differentiation. *Cell* 2007; **129**: 1377-1388
44 **Wassermann P**. The development of adipose tissue. In: Fenn WO, Rahn H, editors. Handbook of Physiology. Washington, DC: American Physiological Society, 1965: 105
45 **Traktuev DO**, Merfeld-Clauss S, Li J, Kolonin M, Arap W, Pasqualini R, Johnstone BH, March KL. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a perienDothelial location, and stabilize endothelial networks. *Circ Res* 2008; **102**: 77-85
46 **Crisan M**, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badylak S, Buhring HJ, Giacobino JP, Lazzari L, Huard J, Péault B. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 2008; **3**: 301-313
47 **Maumus M**, Peyrafitte JA, D'Angelo R, Fournier-Wirth C, Bouloumié A, Casteilla L, Sengenès C, Bourin P. Native human adipose stromal cells: localization, morphology and phenotype. *Int J Obes* (Lond) 2011; Epub ahead of print
48 **Rodeheffer MS**, Birsoy K, Friedman JM. Identification of white adipocyte progenitor cells in vivo. *Cell* 2008; **135**: 240-249
49 **Tang W**, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, Tallquist MD, Graff JM. White fat progenitor cells reside in the adipose vasculature. *Science* 2008; **322**: 583-586
50 **Gesta S**, Blüher M, Yamamoto Y, Norris AW, Berndt J,

Kralisch S, Boucher J, Lewis C, Kahn CR. Evidence for a role of developmental genes in the origin of obesity and body fat distribution. *Proc Natl Acad Sci USA* 2006; **103**: 6676-6681

51 **Tarte K**, Gaillard J, Lataillade JJ, Fouillard L, Becker M, Mossafa H, Tchirkov A, Rouard H, Henry C, Splingard M, Dulong J, Monnier D, Gourmelon P, Gorin NC, Sensebé L. Clinical-grade production of human mesenchymal stromal cells: occurrence of aneuploidy without transformation. *Blood* 2010; **115**: 1549-1553

52 **Yoon YS**, Park JS, Tkebuchava T, Luedeman C, Losordo DW. Unexpected severe calcification after transplantation of bone marrow cells in acute myocardial infarction. *Circulation* 2004; **109**: 3154-3157

53 **Yoshimura K**, Sato K, Aoi N, Kurita M, Hirohi T, Harii K. Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. *Aesthetic Plast Surg* 2008; **32**: 48-55; discussion 56-57

54 **Bel A**, Planat-Bernard V, Saito A, Bonnevie L, Bellamy V, Sabbah L, Bellabas L, Brinon B, Vanneaux V, Pradeau P, Peyrard S, Larghero J, Pouly J, Binder P, Garcia S, Shimizu T, Sawa Y, Okano T, Bruneval P, Desnos M, Hagège AA, Casteilla L, Pucéat M, Menasché P. Composite cell sheets: a further step toward safe and effective myocardial regeneration by cardiac progenitors derived from embryonic stem cells. *Circulation* 2010; **122**: S118-S123

55 **Lendeckel S**, Jödicke A, Christophis P, Heidinger K, Wolff J, Fraser JK, Hedrick MH, Berthold L, Howaldt HP. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. *J Craniomaxillofac Surg* 2004; **32**: 370-373

56 **Menasche P**. Cell-based therapy for heart disease: a clinically oriented perspective. *Mol Ther* 2009; **17**: 758-766

57 **Tateishi-Yuyama E**, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, Shimada K, Iwasaka T, Imaizumi T. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet* 2002; **360**: 427-435

58 **Matoba S**, Tatsumi T, Murohara T, Imaizumi T, Katsuda Y, Ito M, Saito Y, Uemura S, Suzuki H, Fukumoto S, Yamamoto Y, Onodera R, Teramukai S, Fukushima M, Matsubara H. Long-term clinical outcome after intramuscular implantation of bone marrow mononuclear cells (Therapeutic Angiogenesis by Cell Transplantation [TACT] trial) in patients with chronic limb ischemia. *Am Heart J* 2008; **156**: 1010-1018

59 **Mesimäki K**, Lindroos B, Törnwall J, Mauno J, Lindqvist C, Kontio R, Miettinen S, Suuronen R. Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. *Int J Oral Maxillofac Surg* 2009; **38**: 201-209

60 **Garcia-Olmo D**, Herreros D, Pascual M, Pascual I, De-La-Quintana P, Trebol J, Garcia-Arranz M. Treatment of enterocutaneous fistula in Crohn's Disease with adipose-derived stem cells: a comparison of protocols with and without cell expansion. *Int J Colorectal Dis* 2009; **24**: 27-30

61 **Garcia-Olmo D**, Garcia-Arranz M, Herreros D. Expanded adipose-derived stem cells for the treatment of complex perianal fistula including Crohn's disease. *Expert Opin Biol Ther* 2008; **8**: 1417-1423

62 **Garcia-Olmo D**, Herreros D, Pascual I, Pascual JA, Del-Valle E, Zorrilla J, De-La-Quintana P, Garcia-Arranz M, Pascual M. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. *Dis Colon Rectum* 2009; **52**: 79-86

63 **Le Blanc K**, Ringdén O. Immunomodulation by mesenchymal stem cells and clinical experience. *J Intern Med* 2007; **262**: 509-525

S- Editor Wang JL **L- Editor** Hughes D **E- Editor** Zheng XM

World Journal of Stem Cells

Online Submissions: http://www.wjgnet.com/1948-0210office
wjsc@wjgnet.com
doi:10.4252/wjsc.v3.i4.34

World J Stem Cells 2011 April 26; 3(4): 34-42
ISSN 1948-0210 (online)

ORIGINAL ARTICLE

Non-random tissue distribution of human naïve umbilical cord matrix stem cells

Dharmendra Kumar Maurya, Chiyo Doi, Marla Pyle, Raja Shekar Rachakatla, Duane Davis, Masaaki Tamura, Deryl Troyer

Dharmendra Kumar Maurya, Chiyo Doi, Marla Pyle, Raja Shekar Rachakatla, Masaaki Tamura, Deryl Troyer, Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, United States
Duane Davis, Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506, United States
Author contributions: Maurya DK, Doi C, Rachakatla RS and Tamura M were responsible for the study design, experimental work, data evaluation and analysis, and drafting the manuscript; Pyle M, Davis D and Troyer D were consulted extensively in the experimental design and interpretation of results, as well as in the preparation of the manuscript; Tamura M was the research supervisor and participated in the study design, assessment of the results and drafting the manuscript.
Supported by (in part) the Kansas State University (KSU) Terry C. Johnson Center for Basic Cancer Research, the KSU College of Veterinary Medicine Dean's Fund, KSU Targeted Excellence, Kansas State Legislative Appropriation and NIH1R21CA135599
Correspondence to: Masaaki Tamura, Associate Professor, Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, United States. mtamura@vet.ksu.edu
Telephone: +1-785-5324825 Fax: +1-785-5324557
Received: September 13, 2010 Revised: January 10, 2011
Accepted: January 17, 2011
Published online: April 26, 2011

Abstract

AIM: To determine the tissue and temporal distribution of human umbilical cord matrix stem (hUCMS) cells in severe combined immunodeficiency (SCID) mice.

METHODS: For studying the localization of hUCMS cells, tritiated thymidine-labeled hUCMS cells were injected in SCID mice and tissue distribution was quantitatively determined using a liquid scintillation counter at days 1, 3, 7 and 14. Furthermore, an immunofluorescence detection technique was employed in which anti-human mitochondrial antibody was used to identify hUCMS cells in mouse tissues. In order to visualize the distribution of transplanted hUCMS cells in H&E stained tissue sections, India Black ink 4415 was used to label the hUCMS cells.

RESULTS: When tritiated thymidine-labeled hUCMS cells were injected systemically (iv) in female SCID mice, the lung was the major site of accumulation at 24 h after transplantation. With time, the cells migrated to other tissues, and on day three, the spleen, stomach, and small and large intestines were the major accumulation sites. On day seven, a relatively large amount of radioactivity was detected in the adrenal gland, uterus, spleen, lung, and digestive tract. In addition, labeled cells had crossed the blood brain barrier by day 1.

CONCLUSION: These results indicate that peripherally injected hUCMS cells distribute quantitatively in a tissue-specific manner throughout the body.

Key words: Human umbilical cord matrix stem cells; Immunohistochemistry; India black ink loaded cells; Radio-labeled transplanted cells; Tissue distribution

Peer reviewers: Alp Can, MD, PhD, Professor, Laboratory for Stem Cell Science, Department of Histology and Embryology, Ankara University, School of Medicine, Sihhiye, Ankara 06100, Turkey; Herman S Cheung, Professor, Department of Biomedical Engineering, University of Miami, 219 A McArthur Annex, 1251 Memorial Drive, Coral Gables, FL 33146, United States

Maurya DK, Doi C, Pyle M, Rachakatla RS, Davis D, Tamura M, Troyer D. Non-random tissue distribution of human naïve umbilical cord matrix stem cells. *World J Stem Cells* 2011; 3(4): 34-42

Available from: URL: http://www.wjgnet.com/1948-0210/full/v3/i4/34.htm DOI: http://dx.doi.org/10.4252/wjsc.v3.i4.34

INTRODUCTION

Although embryonic stem cells (ESCs) have significant potential in treatment of many medical disorders, there are moral/ethical issues surrounding their derivation which hamper their clinical use. In addition, the limited number of cells at initial harvest necessitates extensive *in vitro* expansion. There are fewer moral/ethical concerns in relation to postnatal stem cells, and it was found recently that umbilical cord matrix contains an inexhaustible, non-controversial source of stem cells[1-11]. These multipotent stem cells, called umbilical cord matrix stem (UCMS) or Wharton's jelly stem cells, are isolated from the mesenchyme-like cushioning material called 'Wharton's jelly' found between the vessels of the umbilical cord[7]. UCMS cells have several properties that encourage their development as therapeutic agents. Namely, they (1) are isolated in large numbers; (2) are negative for CD34 and CD45; (3) grow vigorously and can be frozen/ thawed; (4) can be clonally expanded; and (5) can easily be engineered to express exogenous proteins[9-11].

Over the past decade, stem cells have been the focus of investigations for the treatment of diseases ranging from chronic heart failure to diabetes and multiple sclerosis. The process of stem-cell-based repair of acute injury involves homing and engraftment of the stem cell of interest to the site of injury, followed by either differentiation of the stem cell to indigenous end-organ cells or liberation of paracrine factors that lead to preservation and/or optimization of organ function[12].

Tumors are composed of both tumor cells and non-malignant benign cells. The "benign" tumor compartment includes blood vessels, stromal fibroblasts, and infiltrating inflammatory cells[13]. Stromal fibroblasts offer structural support for malignant cells and influence the behavior and aggressiveness of cancers[13]. The formation of tumor stroma resembles wound healing and scar formation[14]. Malignant cells induce *de novo* formation of connective tissue in order to provide enough stroma to support cancer growth[15,16]. The homing of stem cells to tumors and other areas of inflammation is well established[17-20]. There are also a number of reports showing that genetically engineered stem cells efficiently deliver therapeutic proteins to cancers and other sites of inflammation[7,19,21-24]. Wharton's jelly umbilical cord stem cells have the ability to traffic selectively to tumors[20]. Our previous studies indicate that human umbilical cord matrix stem (hUCMS) cells were attracted toward SDF-1 and VEGF *in vitro*[19]. We also found that hUCMS cells alone or hUCMS cells engineered to secrete the cytokine interferon (IFN)-β (hUCMS-IFN-β) are capable of reducing the growth of MDA 231 human breast carcinoma cells[19,25] and H358 lung bronchioloalveolar carcinoma cells (our un-published results). These observations open new possibilities for the treatment of various cancers and other types of diseases using stem cells. The potential of stem cell-based therapies for treating a myriad of human and animal diseases led us to study the trafficking of UCMS cells in un-engineered severe combined immunodeficiency (SCID) mice over time and to evaluate whether some tissues in the animal preferentially recruit UCMS cells. To address these aims, we analyzed the tissue distribution of hUCMS cells using sensitive radioisotope tracking coupled with histochemistry and immunohistochemistry.

MATERIALS AND METHODS

Culture of hUCMS cells

Human UCMS cells were harvested from term deliveries at the time of birth, with the mother's consent. The methods used to isolate and culture hUCMS cells were previously described[11]. Human UCMS cells were maintained in low serum defined medium (DM), a mixture of 56% low glucose DMEM (Invitrogen, Carlsbad, CA), 37% MCBD 201 (Sigma, St. Louis, MO) and 2% fetal bovine serum (FBS, Atlanta Biologicals Inc, Norcross, GA) containing 1 × insulin transferring selenium-X (Invitrogen), 1 × ALBUMax1 (Invitrogen), 1 × Pen/Strep (Invitrogen), 10 nmol/L dexamethasone (Sigma), 100 μmol/L ascorbic acid 2-phosphate (Sigma), 10 ng/mL epidermal growth factor (R&D Systems, Minneapolis, MN), and 10 ng/mL platelet derived growth factor-BB (R&D Systems). Cells were incubated at 37℃ in an incubator with 5% CO_2 at saturating humidity. When cells had reached 70%-80% confluency, they were detached with 0.25% trypsin-EDTA (Invitrogen), the trypsin was inactivated with fresh media, and the cells were centrifuged at 250 × *g* for 5 min and re-plated in a ratio of 1:3. Human UCMS cells from passages 6-8 were utilized for the present study.

Animals

To evaluate the homing of hUCMS cells, female SCID mice, 6-8 wk of age, with CB17 background were purchased from Charles River (Wilmington, MA). Mice were held for 10 d after arrival to allow them to acclimatize. All animal experiments were done under strict adherence with the Institutional Animal Care and Use Committee protocol as set by Kansas State University.

Retention of tritiated thymidine in hUCMS cells

hUCMS cells were radiolabeled by incubating 1 × 10^6 cells with 1 μCi tritiated thymidine for 24 h. For measuring the retention of radioactivity, the cells were washed with 1 × PBS and fed with fresh medium without tritiated thymidine. Retention of radioactivity was measured on days 1, 3, 7 and 10 post-labeling. Cells were harvested at given time points and radioactivity incorporated into the cells was

measured by a Packard liquid scintillation counter Tri-Carb 2100TR (Perkin Elmer Life Science Boston, MA).

Tracking of tritiated thymidine-labeled hUCMS cells

For studying the localization of hUCMS cells, 1.5×10^6 cells were radiolabeled with 9 μCi tritiated thymidine (^{3}H-hUCMS cells). The next day the cells were washed twice with 1 × PBS and lifted from the culture dish by trypsinization. Cells were counted and washed with PBS. Finally, cells (2.5×10^6/mL) were suspended in PBS. Each mouse was inoculated with 0.5×10^6 cells in 200 μL PBS through tail vein injection. Randomized individual animals were kept in a clean room in cages with HEPA filters. Feces and urine were collected daily during the experimental period. Mice were sacrificed on days 1, 3, 7 and 14 after ^{3}H-hUCMS cell injection and various tissues were collected. All tissues were homogenized in 9-20 volumes of PBS (a larger volume of PBS was used for small tissues, such as adrenal glands) and 0.5 mL homogenate was used for liquid scintillation counting in duplicate.

Immunofluorescence detection

Anti-human mitochondrial antibody was used to identify hUCMS cells in mouse tissues. For immunofluorescence staining, tissue sections (6 μm) were washed with phosphate buffered saline-0.2% Triton X-100 (PBS-TX) and fixed with 70% ethanol and acetone (1:1) mixture. This was followed by washing with three changes of PBS-TX. Tissue sections were blocked with 5% normal goat serum in PBS-TX for 30 min, and then incubated with anti-human mitochondrial antibody produced in mouse (1:500, Chemicon, Temecula, CA), in PBS-TX overnight. The tissues were washed three times with PBS-TX and incubated with Alexa Fluor 488 conjugated secondary antibody raised in goat (1:500, Molecular Probes, Carlsbad, CA) for 3 h. The tissues were incubated for 30 min in Hoechst 33342 (10 μg/mL, Sigma) as a nuclear counterstain followed by a triple rinse with PBS-TX. The antigen localization was observed using epifluorescence microscopy (Nikon Eclipse). Images were captured using a Roper Cool Snap ES camera and Metamorph 7 software.

India black ink labeling of stem cells

In order to visualize transplanted hUCMS cells in H&E stained tissue sections, we developed a method to label the cells with waterproof drawing ink, India Black ink 4415 (Sanford, Oak Brook, IL). The ink was diluted 1:10 in ultrapure water and sterilized by autoclaving. To label cells for injection, the diluted ink was added to DM at a dilution of 1:20, for a final ink concentration of 0.5% and cells were incubated for 24 h. After lifting labeled cells by trypsinization, excess India Black ink was washed away with 1 × PBS. The labeled cells suspended in PBS (0.5×10^6 cells/200 μL PBS) were intravenously injected through the tail vein. Mice were sacrificed by CO_2 asphyxiation on days 1, 3, 7 and 14 after injection. The mice were perfused with 20 mL saline containing 0.05% heparin and then with 20 mL 10% formalin in saline. Tissues were dissected, fixed in 10% formalin, and paraffin-embedded. To determine the distribution of the hUCMS cells, tissue sections were stained with hematoxylin and eosin.

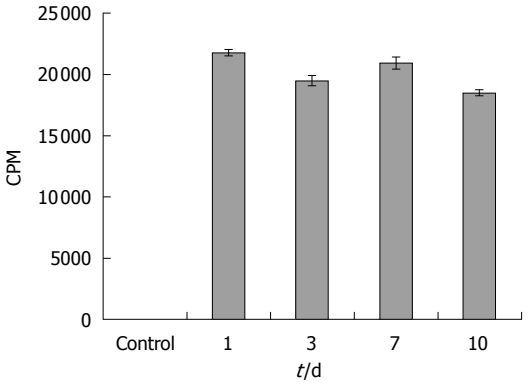

Figure 1 Retention of tritiated thymidine in human umbilical cord matrix stem cells *in vitro*. Human umbilical cord matrix stem cells were radiolabeled by incubating 1 × 10^6 cells with 1 μCi tritiated thymidine for 24 h followed by washing with 1 × PBS and kept in the growth medium without tritiated thymidine. Retention of radioactivity was measured on days 1, 3, 7 and 10 post-labeling.

Statistical analysis

Data is represented as mean ± SE values from 5 animals.

RESULTS

First, retention of the tritiated thymidine in hUCMS cells was evaluated using hUCMS cells in culture. Over 90% of the radioactivity was retained in the cells for at least 10 d (Figure 1). It is therefore suggested that tritiated thymidine labeled hUCMS cells can be utilized to determine their tissue localization.

Although a preliminary mouse study using 0.5×10^6 hUCMS cells labeled with 1.0 μCi tritiated thymidine distinctively identified major tissue distribution sites, the specific radioactivity per gram of tissue was not high. Therefore, 0.5×10^6 cells radiolabeled with 3.0 μCi tritiated thymidine were utilized for the tissue distribution study. The specific activity of the injected cells at the time of injection was determined to be 529 900 ± 33 470 c/min per 1×10^6 cells (0.53 c/min per cell). Use of higher radioactivity per cell also provides a longer period of observation and greater sensitivity.

Observation of tissue radioactivity, human mitochondrial antigen, and India Black ink labeled cells made it clear that after administration of hUCMS cell *via* the tail vein, lung and spleen are the first target organs for engraftment of hUCMS cells. Tissue distributions of radioactivity throughout the body at various time points are presented in Figure 2. Considerable specific radioactivity was detected in the lung and spleen after 1 d, whereas little activity was detected in other tissues (Figure 2, white bars). However, hUCMS cells re-distributed to other tissues with time. On day 3, relatively large amounts of specific

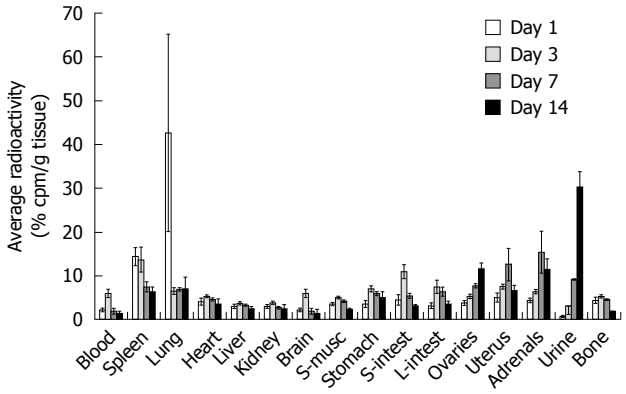

Figure 2 Average tissue distribution of radiolabeled human umbilical cord matrix stem cells 1 d (white bars), 3 d (light grey bars), 7 d (dark grey bars) and 14 d (black bars) after the cell injection. Mice were injected through a lateral tail vein with 5 × 10^5 human umbilical cord matrix stem cells labeled with tritiated thymidine and sacrificed 1, 3, 7, and 14 d after the injection. All tissues were individually dissected and homogenized with 9-20 volumes of 1 × PBS. Radioactivity was measured in duplicate determinations as described in the Methods. All presented data are normalized by subtracting non-specific radioactivity from respective control tissues and presented as mean ± SE. Data are expressed as% average activity per gram tissue.

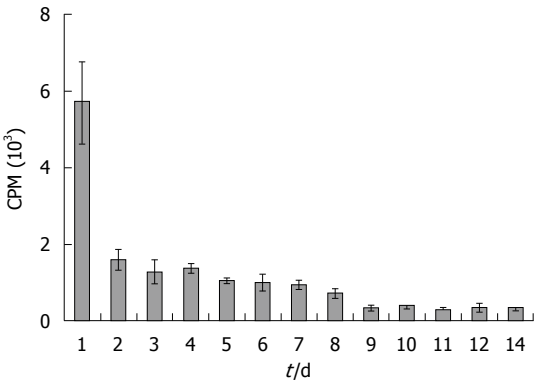

Figure 3 Amount of urinary excretion of radioactivity. After injection of radiolabeled human umbilical cord matrix stem cells urine was collected daily and excretion of radioactivity in urine was determined. The excretion levels are normalized by urine volume and expressed as total radioactivity/day/mouse.

radioactivity were found in the spleen and digestive tract (Figure 2, light grey bars). On day 7, the highest specific radioactivity was detected in the adrenal glands (Figure 2, dark grey bars). It is noteworthy that on day 7, the second highest specific radioactivity was in the uterus. On day 14, specific radioactivity in various tissues was reduced in comparison to earlier days. The ovaries, adrenal glands and uterus showed the highest retention of radioactivity (Figure 2, black bars). The radioactivity retention in the lung and spleen were also relatively high.

Since the characteristics of gene expression in hUCMS cells are similar to those in bone marrow mesenchymal stem cells[10], and since recruitment of mesenchymal stem cells to bone marrow is possible, migration of UCMS cells to bone marrow was determined by measuring the radioactivity in whole femur homogenate. The maximum amount of radioactivity in femur homogenate was detected on day 1, and with time the amount of radioactivity decreased slowly (total radioactivity observed per gram femur bone at days 1, 3, 7 and 14 was 7.6%, 5.23%, 5.18% and 3.44%, respectively). This may suggest that bone marrow is an important distribution site for circulating mesenchymal stem cells, and that UCMS cells mimic behavior of bone marrow mesenchymal stem cells.

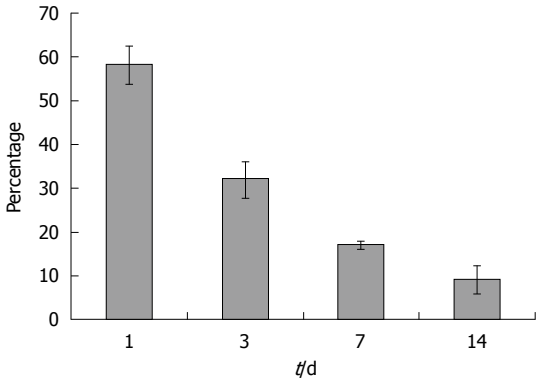

Figure 4 Percentage of radioactivity recovered on different days. Amount of radioactivity detected in different tissues and urine on days 1, 3, 7 and 14 after radiolabeled human umbilical cord matrix stem cells injection was compared with total radioactivity injected. Data are presented in term of percentage radioactivity.

No radioactivity was detected in the feces (data not shown). Elimination of radioactivity by urinary excretion was significant. A large quantity of the radioactivity, approximately 2.75% of total recovered radioactivity, was excreted *via* this route within 24 h of injection (Figure 3). However, urinary excretion of the radioactivity gradually decreased with time (Figure 3). The total amount of radioactivity recovered from all tissues analyzed and from urine was calculated to be 58, 33, 17 and 10% of total injected radioactivity on days 1, 3, 7 and 14, respectively (Figure 4).

To further substantiate the radioactivity-based tissue distribution of hUCMS cells, immunofluorescence staining was applied to all tissues. Although hUCMS cells were detected in lung, intestine, and brain at days 1 and 3 (Figure 5), they were undetectable by this method in most other tissues even 1 d after injection. However, we were able to detect hUCMS cells at day 7 in the intestine (Figure 5).

As another method to validate the radiolabeled cell distribution in various tissues, and to better observe morphology in paraffin embedded sections, we developed a technique in which cells were loaded with India Black ink before injection. In this method, hUCMS cells loaded with India Black ink were observed as black particle-loaded cells in H & E stained slides. By this method we were able to detect the cells in brain, lung, and stomach on day 1 (Figure 6). The India Black ink-loaded hUCMS cells were also detected on days 3, 7 and 14 in the lung, but not in the uterus, spleen, kidney, *etc.* (Figure 6).

DISCUSSION

Increasing evidence suggests that adult and postnatal

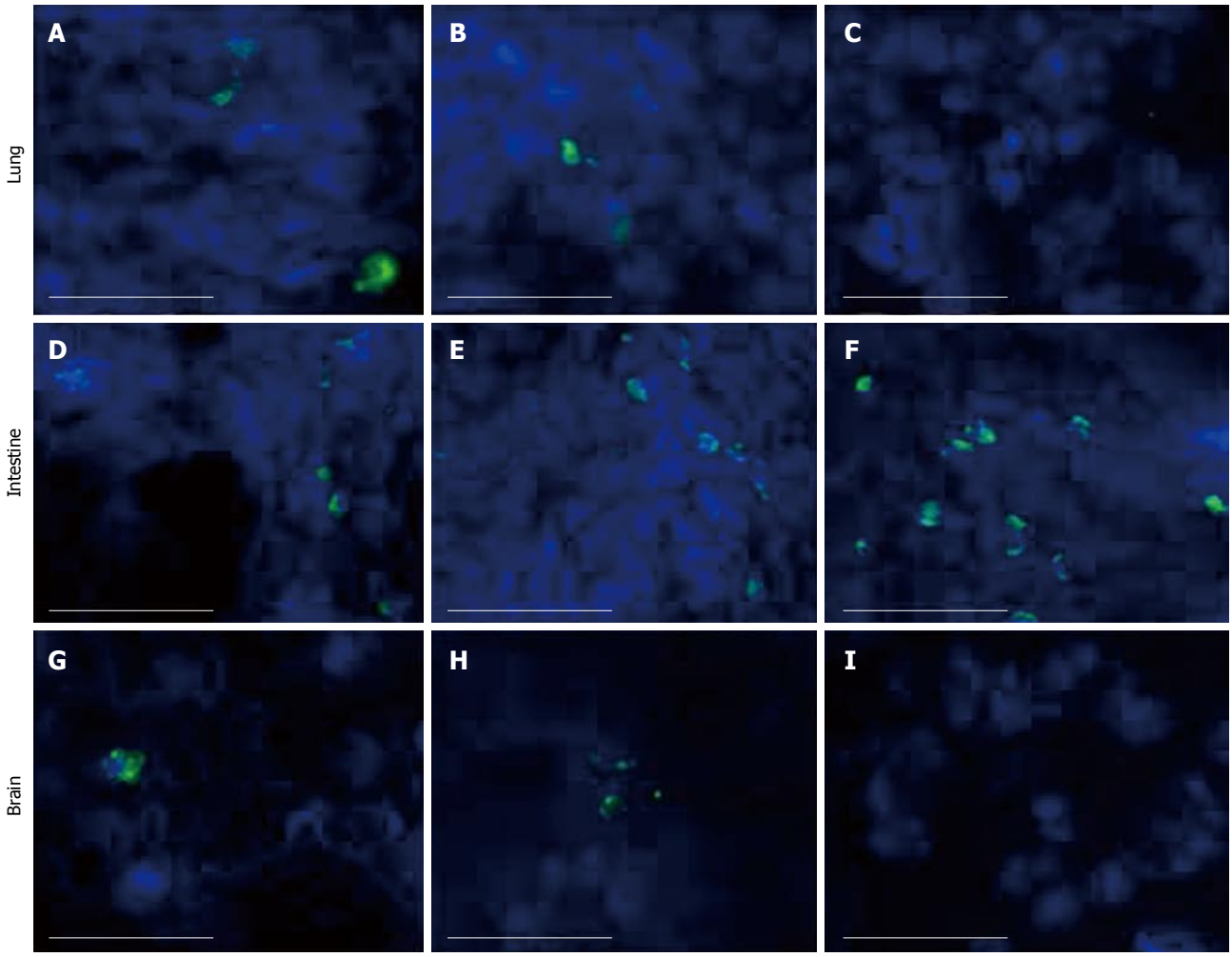

Figure 5 Detection of human umbilical cord matrix stem cells by immunofluorescence staining on days 1, 3 and 7. Human umbilical cord matrix stem cells were detected in lung (A-C), intestine (D-F) and brain (G-I) using anti-human mitochondrial antibody. DAPI was used as counter stain for nuclear staining. All images were captured using 40 × objective. Scale bar = 50 μm.

stem cells are valuable resources for various medical therapies. Among many tissue-originated multipotent stem cells, UCMS cells are very usable due to their abundance, low immunogenicity[26], and simplicity of harvest and *in vitro* expansion[7,11]. However, the tissue distribution characteristics of transplanted UCMS cells in the normal body have not been studied previously. Many of the aforementioned therapies require systemic transplantation of stem cells. Therefore, their tissue distribution in physiological and pathological conditions is important. Here we report the distribution of hUCMS cells in SCID mice after systemic transplantation using the sensitive tracking method of radiolabeled cell detection combined with histochemical and immunohistochemical detection of hUCMS cells.

The lung and spleen were found to be the major distribution tissues at 24 h after transplantion (Figure 2, white bars). The spleen continued to be the highest distribution site even 3 d after transplantation (Figure 2, light grey bars). Since the spleen is the site for clearance of most foreign and damaged cells present in circulation, the splenic distribution may be associated with clearing of damaged or dead hUCMS cells. Splenic clearance may also be related to the initial clearance of radioactivity in the urine after transplantation (Figure 3).

On day three, in addition to the spleen, the gastrointestinal tract, including the stomach and small and large intestines, were the primary distribution organs (Figure 2, white grey bars). However, this distribution pattern was changed on day 7, when the highest distribution was found to be in the adrenal glands and uterus (Figure 2, dark grey bars). The lung, spleen and intestine show lesser amounts by day 7. This pattern was not changed at 14 d after transplantation (Figure 2, black bars) although the total radioactivity recovered was significantly decreased.

On the other hand, although a significant amount of radioactivity was distributed in skeletal muscle, the India Black ink labeled UCMS cells were not detected in skeletal muscle. This is perhaps due to the fact that the UCMS cells distrubuted through such a large amount of skeletal muscle. Therefore, although the total amount of calculated radioactivity in muscle is high, the specific activity

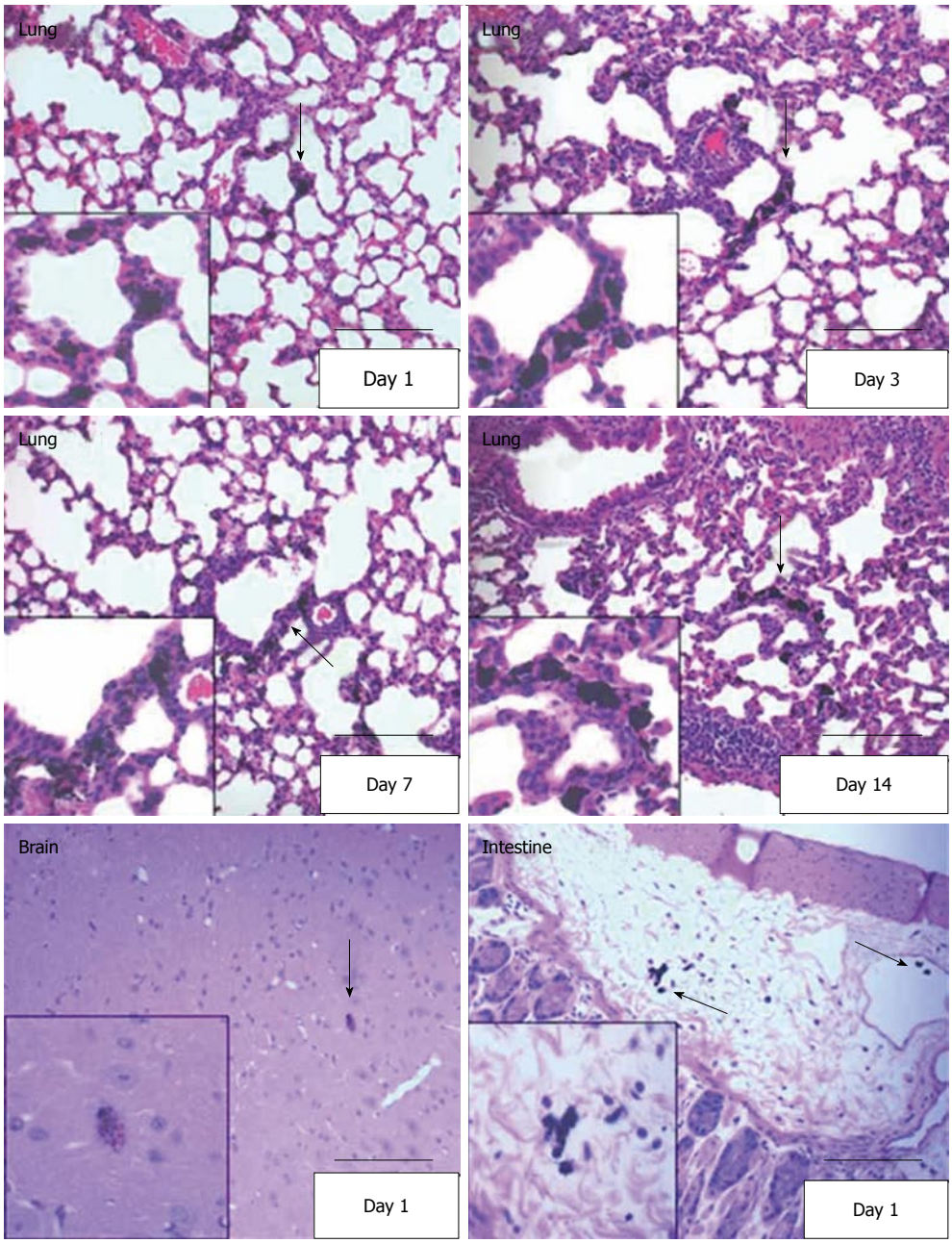

Figure 6 Visualization of human umbilical cord matrix stem cells in hematoxylin and eosin stained tissue sections. Human umbilical cord matrix stem (hUCMS) cells were loaded with India Black ink and injected through the tail vein. hUCMS cells were detected in lung, intestine and brain on the days indicated in the panels at 200 × magnification. Red arrows in the 200 × pictures indicate India Black ink-labeled hUCMS cells which were magnified at 400 × magnification and presented as inset in each panel. Scale bar = 50 μm.

(radioactivity per gram tissue) is low, and individual cells may have escaped detection. To study the distribution of hUCMS cells in bone marrow, radioactivity in whole femur homogenate was determined. A noticeable amount of radioactivity was detected in the femur homogenate. This may suggest that hUCMS cells localize in the bone marrow niche and potentially contribute to bone marrow function.

The primary distribution sites were visually confirmed by detection of hUCMS cells using immunohistochemical methods and India Black ink labeling of the hUCMS cells. Careful observation of the morphology of the transplanted cells in H&E tissue sections, immunohistochemical and India Black ink labeling and the time course study suggest that the majority of transplanted cells detected in various tissues are intact and were not engulfed by macrophages or other phagocytic cells. Transplanted UCMS cells remained as characteristically large cells and many of them were detected adjacent to lung vasculature even 14 d after transplantation (Figure 6). Therefore, a significant portion of the transplanted UCMS cells appears to be intact for a lengthy period. However, a por-

tion of the transplanted cells appears to be destroyed and removed from circulation, and free tritiated thymidine is excreted into the urine. Therefore, it is difficult to rule out the possibility that a small portion of radiolabeled hUCMS cells may have been engulfed by macrophages and redistributed with macrophage migration. However, all three detection methods were in good agreement and identified the lung, gastrointestinal tract, and brain as primary tissue distribution sites at days 1 and 3 after cell transplantation. This agreement of UCMSC distribution between the three methods supports the suggestion that India Black ink labeled cells behaved as unlabeled cells do *in vivo,* although it is difficult to completely exclude the possibility that India Black ink labeled cells may have been sequestered differently. The high level of radioactivity distribution to the adrenal glands may be misleading, since we were unable to detect the intact hUCMS cells in adrenal glands by immunohistochemical and histochemical methods. Since the adrenal glands and ovaries are small (approximately 30 mg/gland), a small artifact in the radioactive signal would be exaggerated mathematically. In addition, it is possible that metabolized or free tritiated thymidine may have been redistributed to these tissues. We expected to find that the lung was the primary distribution site in the early stages of transplantation because our previous studies indicated that systemically transplanted IFN-β-expressing hUCMS cells significantly reduce the growth of lung metastasized MDA 231 human breast carcinoma xenografts[19,25]. This efficient lung distribution indicates that UCMS cells may have substantial therapeutic potential in various lung diseases including cancer, asthma and pneumonia, when UCMS cells are used to deliver therapeutic molecules and/or express therapeutic genes.

The distribution of hUCMS cells in the uterus and ovaries is of interest. The present study clearly indicates that the major distribution site in the reproductive organs is the uterus rather than the ovaries. Since both organs are sites of extensive remodeling during the estrous cycle[27], the stem cells may be responding to chemoattractants similar to those associated with wound healing or tumors. The observation of relatively high distribution in the intestinal tract also correlates with localization to regions of rapid tissue turnover, since the epithelium is replaced every 4-7 d. Radiolabeled hUCMS cell distribution to uterus, ovaries, and digestive tract suggests that transplanted UCMS cells may play a role in tissue remodeling associated with aggressive cell division. Although the hUCMS cells have been shown to traffic to tumor tissues in a manner similar to bone marrow mesenchymal stem cells and neural stem cells[25], the present results suggest that a portion of systemically transplanted UCMS cells are likely to distribute to other tissues that contain actively dividing cells.

One of the most interesting findings is that there was radioactivity in the brain 1, 3, and 7 d after transplantation. This finding was confirmed by immunohistochemical detection and India Black ink labeling of hUCMS cells. Since the cells were not detected in the blood-brain barrier vasculature, they evidently crossed the blood brain barrier. Although this barrier is comprised primarily of tight junctions between endothelial cells and poses a formidable obstacle to the delivery of many therapeutic agents to the central nervous system[28], UCMS cells could cross this barrier and potentially be used as delivery vehicles for therapeutics to the brain. In support of this speculation, systemically administered neuronal stem cells engineered to express a pro-drug processing enzyme were found to migrate into the brain and to exhibit therapeutic properties[22]. Furthermore, bone marrow MSC or fetal stem cells physiologically cross blood-brain barrier and play an important role in blood vessel remodeling[29] and in healing of brain injury[30].

Since detection of the radiolabeled cells is very sensitive as compared to histochemical and immunohistochemical detection of the cells, it was possible to monitor the distribution of the hUCMS cells in most tissues *via* radiolabeling. Immunofluorescence and India Black ink labeling made it possible to clarify the exact location of the hUCMS cells within many organs, but were less sensitive than radiolabeling. A possible explanation for this sensitivity difference is that in the radioactive method we have homogenized whole tissues whereas the immunofluorescence and India ink labeling methods were carried out on sections only 5-10 μM thick, so it is possible that cells may have been present in higher numbers in other parts of the organ.

In conclusion, intravenously administered hUCMS cells exhibited time-specific tissue distribution in SCID mice. The lung was a major distribution site at 24 h after transplantation. With time, major distribution sites changed to the gastrointestinal tract at 3 and 7 d after transplantation. In addition to the gastrointestinal tract, both ovaries and uterus were found to be distribution sites. The tissue distributions were confirmed by immunohistochemical and histological observations of hUCMS cells in tissue sections. Morphological analysis revealed that systemically administered hUCMS cells can be efficently distributed in brain. These findings indicate that UCMS cells are potentially useful tools for the treatment of various diseases in the lung, gastrointestinal tract, and brain.

COMMENTS

Background

The authors recently found that human umbilical cord matrix stem (hUCMS) cells alone, or hUCMS cells transduced with interferon (IFN)-β, significantly reduce the growth of human breast carcinoma cells and human lung bronchioloalveolar carcinoma cells *in vitro* and *in vivo*. These findings open a new area for the therapeutic application of stem cells for multiple types of cancer.

Research frontiers

Stem cells can be derived from a variety of sources such as embryos [embryonic stem cells (ESCs)], bone marrow stem cells, fetal tissues, cord blood, *etc*. ESCs have moral/ethical issues surrounding their derivation. Although postnatal stem

cells offer fewer concerns, many postnatal stem cells derived from the sources listed above also have drawbacks which hamper their clinical use. these include invasive tissue collection and prolonged *in vitro* expansion times due to the very limited number of cells which can be initially harvested. Recently, it was found that umbilical cord matrix contains an inexhaustible, non-controversial source of stem cells. Therefore, studying quantitative tissue distribution of hUCMS cells in animals provides fundamentally important knowledge for the use of this stem cell type in the future.

Innovations and breakthroughs

Previous studies indicate that hUCMS cells were attracted toward SDF-1 and VEGF *in vitro* (Rachakatla *et al*, 2008). The authors also found that hUCMS cells alone or hUCMS cells engineered to secrete the IFN-β (hUCMS-IFN-β) are capable of reducing the growth of human breast carcinoma cells and lung bronchioloalveolar carcinoma cells. These observations open a new era in the treatment of various cancers and other types of diseases using stem cells. Intravenously administered hUCMS cells exhibited time-specific tissue distribution in severe combined immunodeficiency (SCID) mice. The lung was a major distribution site at 1 d after transplantation. Major distribution sites changed to the gastrointestinal tract at 3 and 7 d after transplantation. Morphological analysis revealed that systemically administered hUCMS cells can also be efficently distributed in the brain. These results suggest that hUCMS cells could be used as a therapeutic and/or therapeutic delivery tools for abnormalities in these tissues.

Applications

Since hUCMS cells are shown to be poorly immunogenic (Weiss *et al*, 2006; Cho *et al*, 2008), it is conceivable that hUCMS cells could be used as allogeneic therapeutics or therapeutic gene and/or protein delivery tools for inflammatory diseases and various types of cancer. Conveniently, hUCMS cells appear to be tropic to inflamatory tissues and tumors (Rachakatla *et al*, 2008; Ayuzawa *et al*, 2009; Matsuzuka *et al*, 2010).

Peer review

In the presented manuscript authors showed the tissue distribution pattern of iv injected hUCMS cells into SCID mice. Their results indicated that the first site of engraftment is lung, a finding which may be anticipated since the route of administration was intravenous.

REFERENCES

1 **Campard D**, Lysy PA, Najimi M, Sokal EM. Native umbilical cord matrix stem cells express hepatic markers and differentiate into hepatocyte-like cells. *Gastroenterology* 2008; **134**: 833-848

2 **Can A**, Karahuseyinoglu S. Concise review: human umbilical cord stroma with regard to the source of fetus-derived stem cells. *Stem Cells* 2007; **25**: 2886-2895

3 **Friedman R**, Betancur M, Boissel L, Tuncer H, Cetrulo C, Klingemann H. Umbilical cord mesenchymal stem cells: adjuvants for human cell transplantation. *Biol Blood Marrow Transplant* 2007; **13**: 1477-1486

4 **Hoynowski SM**, Fry MM, Gardner BM, Leming MT, Tucker JR, Black L, Sand T, Mitchell KE. Characterization and differentiation of equine umbilical cord-derived matrix cells. *Biochem Biophys Res Commun* 2007; **362**: 347-353

5 **La Rocca G**, Anzalone R, Corrao S, Magno F, Loria T, Lo Iacono M, Di Stefano A, Giannuzzi P, Marasà L, Cappello F, Zummo G, Farina F. Isolation and characterization of Oct-4+/HLA-G+ mesenchymal stem cells from human umbilical cord matrix: differentiation potential and detection of new markers. *Histochem Cell Biol* 2009; **131**: 267-282

6 **Lu LL**, Liu YJ, Yang SG, Zhao QJ, Wang X, Gong W, Han ZB, Xu ZS, Lu YX, Liu D, Chen ZZ, Han ZC. Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. *Haematologica* 2006; **91**: 1017-1026

7 **Mitchell KE**, Weiss ML, Mitchell BM, Martin P, Davis D, Morales L, Helwig B, Beerenstrauch M, Abou-Easa K, Hildreth T, Troyer D, Medicetty S. Matrix cells from Wharton's jelly form neurons and glia. *Stem Cells* 2003; **21**: 50-60

8 **Seshareddy K**, Troyer D, Weiss ML. Method to isolate mesenchymal-like cells from Wharton's Jelly of umbilical cord. *Methods Cell Biol* 2008; **86**: 101-119

9 **Troyer DL**, Weiss ML. Wharton's jelly-derived cells are a primitive stromal cell population. *Stem Cells* 2008; **26**: 591-599

10 **Weiss ML**, Medicetty S, Bledsoe AR, Rachakatla RS, Choi M, Merchav S, Luo Y, Rao MS, Velagaleti G, Troyer D. Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells* 2006; **24**: 781-792

11 **Weiss ML**, Troyer DL. Stem cells in the umbilical cord. *Stem Cell Rev* 2006; **2**: 155-162

12 **Penn MS**, Khalil MK. Exploitation of stem cell homing for gene delivery. *Expert Opin Biol Ther* 2008; **8**: 17-30

13 **Hanahan D**, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57-70

14 **Dvorak HF**. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 1986; **315**: 1650-1659

15 **Hasebe T**, Sasaki S, Sugitoh M, Ono M, Saitoh N, Ochiai A. Highly proliferative intratumoral fibroblasts and a high proliferative microvessel index are significant predictors of tumor metastasis in T3 ulcerative-type colorectal cancer. *Hum Pathol* 2001; **32**: 401-409

16 **Kuniyasu H**, Abbruzzese JL, Cleary KR, Fidler IJ. Induction of ductal and stromal hyperplasia by basic fibroblast growth factor produced by human pancreatic carcinoma. *Int J Oncol* 2001; **19**: 681-685

17 **Aboody KS**, Brown A, Rainov NG, Bower KA, Liu S, Yang W, Small JE, Herrlinger U, Ourednik V, Black PM, Breakefield XO, Snyder EY. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc Natl Acad Sci USA* 2000; **97**: 12846-12851

18 **Nakamizo A**, Marini F, Amano T, Khan A, Studeny M, Gumin J, Chen J, Hentschel S, Vecil G, Dembinski J, Andreeff M, Lang FF. Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. *Cancer Res* 2005; **65**: 3307-3318

19 **Rachakatla RS**, Marini F, Weiss ML, Tamura M, Troyer D. Development of human umbilical cord matrix stem cell-based gene therapy for experimental lung tumors. *Cancer Gene Ther* 2007; **14**: 828-835

20 **Studeny M**, Marini FC, Champlin RE, Zompetta C, Fidler IJ, Andreeff M. Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. *Cancer Res* 2002; **62**: 3603-3608

21 **Aboody KS**, Najbauer J, Danks MK. Stem and progenitor cell-mediated tumor selective gene therapy. *Gene Ther* 2008; **15**: 739-752

22 **Aboody KS**, Najbauer J, Schmidt NO, Yang W, Wu JK, Zhuge Y, Przylecki W, Carroll R, Black PM, Perides G. Targeting of melanoma brain metastases using engineered neural stem/progenitor cells. *Neuro Oncol* 2006; **8**: 119-126

23 **Kumar S**, Chanda D, Ponnazhagan S. Therapeutic potential of genetically modified mesenchymal stem cells. *Gene Ther* 2008; **15**: 711-715

24 **Studeny M**, Marini FC, Dembinski JL, Zompetta C, Cabreira-Hansen M, Bekele BN, Champlin RE, Andreeff M. Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents. *J Natl Cancer Inst* 2004; **96**: 1593-1603

25 **Rachakatla RS**, Pyle MM, Ayuzawa R, Edwards SM, Marini FC, Weiss ML, Tamura M, Troyer D. Combination treatment of human umbilical cord matrix stem cell-based interferon-beta gene therapy and 5-fluorouracil significantly reduces growth of metastatic human breast cancer in SCID mouse lungs. *Cancer Invest* 2008; **26**: 662-670

26 **Weiss ML**, Anderson C, Medicetty S, Seshareddy KB, Weiss

RJ, VanderWerff I, Troyer D, McIntosh KR. Immune properties of human umbilical cord Wharton's jelly-derived cells. *Stem Cells* 2008; **26**: 2865-2874

27 **Zoubina EV**, Fan Q, Smith PG. Variations in uterine innervation during the estrous cycle in rat. *J Comp Neurol* 1998; **397**: 561-571

28 **Karnovsky MJ**. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. *J Cell Biol* 1967; **35**: 213-236

29 **Galimi F**, Summers RG, van Praag H, Verma IM, Gage FH. A role for bone marrow-derived cells in the vasculature of noninjured CNS. *Blood* 2005; **105**: 2400-2402

30 **Tan XW**, Liao H, Sun L, Okabe M, Xiao ZC, Dawe GS. Fetal microchimerism in the maternal mouse brain: a novel population of fetal progenitor or stem cells able to cross the blood-brain barrier? *Stem Cells* 2005; **23**: 1443-1452

S- Editor Wang JL **L- Editor** Hughes D **E- Editor** Zheng XM

World Journal of Stem Cells

Online Submissions: http://www.wjgnet.com/1948-0210office
wjsc@wjgnet.com
www.wjgnet.com

World J Stem Cells 2011 April 26; 3(4): I
ISSN 1948-0210 (online)

Acknowledgments to reviewers of *World Journal of Stem Cells*

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

Borhane Annabi, PhD, Professor, Department of Chemistry, Biomed Research Centre, Université du Québec à Montréal Montreal, Quebec, H2X 2J6, Canada

Tomoki Aoyama, Associate Professor, Department of Human Health Sciences, Graduate school of Medicine, KyotoUniversity, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

Jeremy M Crook, PhD, Department of Stem Cell Medicine, O'Brien Institute, 42 Fitzroy Street, Melbourne 3065, Australia

Hoeon Kim, PhD, Biotherapeutic Division, Aprogen Inc., E18, KAIST, 335 Gwahangno, Yuseong-gu, Daejeon 305-701, South Korea

Steven Shoei-Lung Li, Professor, Institute of Medicine, Kaohsiung Medical University, Kasohsiung 807, Taiwan, China

Najimi Mustapha, PhD, Laboratory of Pediatric Hepatology and Cell Therapy, Avenue Hippocrate 10/1301, 1200 Brussels, Belgium

Stefano Pluchino, MD, PhD, CNS Repair Unit-DIBIT2, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Via Olgettina, 58, 20132 Milan, Italy

Naiara Zoccal Saraiva, DVM, MSc, FCAV-UNESP-Jaboticabal, Via de Acesso Prof. Paulo Donato Castellane, s/n, CEP 14884-900, Jaboticabal, SP, Brazil

Frank JT Staal, PhD, Professor, Department of Immunohematology and Blood Transfusion, LeidenUniversityMedicalCenter, Albinusdreef 2, building 1, L1-36, PO Box 9600, 2300 RC LEIDEN, Netherlands

Takashi Tada, Professor, Stem Cell Engineering, Institute for Frontier Medical Sciences, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

Andre Van Wijnen, PhD, Department of Cell Biology, Rm S3-322, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, United States

Shu Wang, Associate Professor, Department of Biological Sciences, National University of Singapore, Singapore; Group Leader, Institute of Bioengineering and Nanotechnology, Singapore Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, Singapore 138669, Singapore

Ernst Wolvetang, Associate Professor, Stem Cell Engineering Group, Australian Institute for Bioengineering and Nanotechnology, Level 4, Building 75, St Lucia campus, University of Queensland, 4072, Brisbane, Australia

Young-Sup Yoon, MD, PhD, Director of Stem Cell Biology, Associate Professor (Tenure), Emory University School of Medicine, United States

World Journal of Stem Cells

Online Submissions: http://www.wjgnet.com/1948-0210office
wjsc@wjgnet.com
www.wjgnet.com

World J Stem Cells 2011 April 26; 3(4): I
ISSN 1948-0210 (online)

Events Calendar 2011

March 26, 2011
Stem Cell Agency Governance Subcommittee Meeting, Crowne Plaza SFO, 1177 Airport Blvd, Burlingame, CA, United States

January 29-February 2, 2011
LabAutomation2011, Palm Springs, CA, United States

February 4, 2011
7th annual Swiss Stem Cell Network meeting, Swiss Federal Institute of Technology in Lausanne, Switzerland

March 1, 2011
The 6th Annual Stem Cell Summit, 11 Fulton Street, New York City, NY, United States

March 22, 2011
StemCONN 2011, Farmington, CT, United States

March 27-31, 2011
SBS 17th Annual Conference and Exhibition, Orlando, FL, United States

April 6-8, 2011
EMBO Conference-Advances in Stem Cell Research: Development, Regeneration and Disease, Institut Pasteur, Paris, France

April 7-10, 2011
2011 CSHL Meeting on Stem Cell Engineering & Cell Therapy, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States

April 25-26, 2011
International Conference on Stem Cell Research, Hotel Equatorial Penang, Malaysia

April 27, 2011
6th Annual Wisconsin Stem Cell Symposium, BioPharmaceutical Technology Center, Madison, WI, United States

May 9-11, 2011
The World Stem Cells and Regenerative Medicine Congress 2011, Victoria Park Plaza, London, United Kingdom

May 23-24, 2011
The 4th Annual Israeli Stem Cell Meeting, Beit Sourasky, Chaim Sheba Medical Center, Israel

May 26-27, 2011
7th annual Stem Cell Research & Therapeutics Conference, Boston, MA, United States

September 20-24, 2011
2011 CSHL Meeting on Stem Cell Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States

October 2011
3rd Annual World Stem Cells & Regenerative Medicine Congress Asia 2011, Seoul, South Korea

WJSC World Journal of Stem Cells

Online Submissions: http://www.wjgnet.com/1948-0210office
wjsc@wjgnet.com
www.wjgnet.com

World J Stem Cells 2011 April 26; 3(4): I-V
ISSN 1948-0210 (online)

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Stem Cells (*World J Stem Cells*, *WJSC*, online ISSN 1948-0210, DOI: 10.4252), is a monthly, open-access (OA), peer-reviewed journal supported by an editorial board of 284 experts in stem cell from 28 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 *WJSC* 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 *WJSC* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJSC* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

The major task of *WJSC* is to report rapidly original articles and comprehensive reviews on basic laboratory investigations of stem cells and their application in clinical care and treatment of patients. *WJSC* is designed to cover all aspects of stem cells, including: Embryonic, neural, hematopoietic, mesenchymal, tissue-specific, and cancer stem cells; the stem cell niche; stem cell genomics and proteomics; and stem cell techniques and their application in clinical trials. Papers published in *WJSC* will cover the biology, culture, differentiation and application of stem cells from all stages of their development, from germ cell to embryo and adult.

Columns

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

Name of journal

World Journal of Stem Cells

ISSN

ISSN 1948-0210 (online)

Indexed and Abstracted in

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

Published by

Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the

authors except where indicated otherwise.

Biostatistical editing

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

Conflict-of-interest statement

In the interests of transparency and to help reviewers assess any potential bias, *WJSC* 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 comform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

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

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

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

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/1948-0210office. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1948-0210/g_info_20100313165700.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjsc@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 International Committee of Medical Journal Editors, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-

Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

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

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

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

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-85381891 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 *WJSC*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

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

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

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

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1948-0210/g_info_list.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/1948-0210/g_info_20100313172144.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/1948-0210/g_info_20100313165833.htm

Frontier: http://www.wjgnet.com/1948-0210/g_info_20100313170509.htm

Topic highlight: http://www.wjgnet.com/1948-0210/g_info_20100313170618.htm

Observation: http://www.wjgnet.com/1948-0210/g_info_20100313170727.htm

Guidelines for basic research: http://www.wjgnet.com/1948-0210/g_info_20100313170855.htm

Guidelines for clinical practice: http://www.wjgnet.com/1948-0210/g_info_20100313171012.htm

Review: http://www.wjgnet.com/1948-0210/g_info_20100313171124.htm

Original articles: http://www.wjgnet.com/1948-0210/g_info_20100313171239.htm

Brief articles: http://www.wjgnet.com/1948-0210/g_info_20100313171358.htm

Case report: http://www.wjgnet.com/1948-0210/g_info_20100313171504.htm

Letters to the editor: http://www.wjgnet.com/1948-0210/g_info_20100313171613.htm

Book reviews: http://www.wjgnet.com/1948-0210/g_info_20100313171713.htm

Guidelines: http://www.wjgnet.com/1948-0210/g_info_20100313171803.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

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

Editorial Office

World Journal of Stem Cells

Editorial Department: Room 903, Building D,
Ocean International Center, No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjsc@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-8538-1891
Fax: +86-10-8538-1893

Language evaluation

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

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/1948-0210/g_info_20100313172045.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1948-0210/g_info_20100313172000.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

WJSC 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

WJSC is an international, peer-reviewed, Open-Access, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. The related standards are as follows. Publication fee: 1300 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.