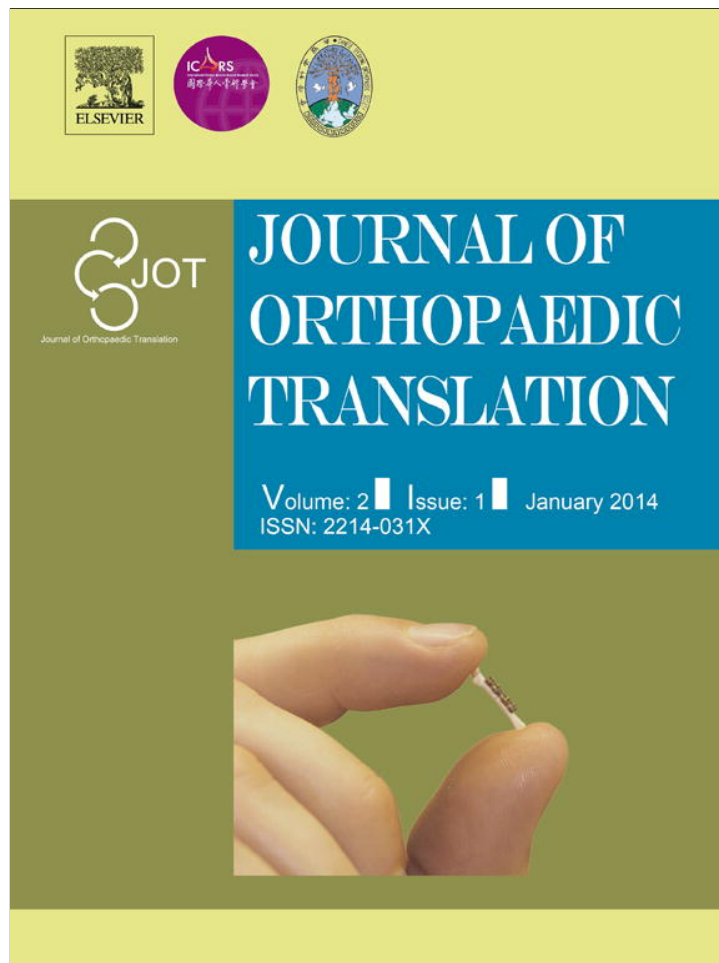


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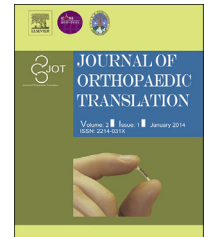
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REVIEW ARTICLE

Circulating mesenchymal stem cells and their clinical implications

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Received 15 June 2013; received in revised form 14 October 2013; accepted 18 November 2013

Available online 15 December 2013

KEYWORDS

Circulating MSCs;
Allogenic MSCs;
Cell therapy

Summary Circulating mesenchymal stem cells (MSCs) is a new cell source for tissue regeneration and tissue engineering. The characteristics of circulating MSCs are similar to those of bone marrow-derived MSCs (BM-MSCs), but they exist at a very low level in healthy individuals. It has been demonstrated that MSCs are able to migrate to the sites of injury and that they have some distinct genetic profiles compared to BM-MSCs. The current review summarizes the basic knowledge of circulating MSCs and their potential clinical applications, such as mobilizing the BM-MSCs into circulation for therapy. The application of MSCs to cure a broad spectrum of diseases is promising, such as spinal cord injury, cardiovascular repair, bone and cartilage repair. The current review also discusses the issues of using of allogeneic MSCs for clinical therapy.

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Introduction

Mesenchymal stem cells (MSCs) are non-haematopoietic cells which can be easily isolated from bone marrow and

other tissues, such as adipose, umbilical cord, and peripheral blood. The MSCs have a multipotent capacity to differentiate into a variety of other cell types, including osteoblasts, adipocytes, chondrocytes, myoblasts, and neurons [1,2]. In response to stimuli, MSCs have the ability of homing to the target tissue. Also, MSCs have been shown to be immunosuppressive and anti-inflammatory; they do not express major histocompatibility complex-II (MHC-II), CD80, CD86, and CD40, and minimally express MHC-I on the

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cell surface [1,3]. These characteristics have made MSCs a promising cell source for tissue engineering.

Circulating MSCs, also called peripheral blood-derived MSCs (PB-MSCs), were initially discovered as fibroblast-like cells and were later confirmed by many investigators as peripheral blood-borne colony-forming units [4,5]. Usually, they exist at a very low level in healthy individuals, but under some pathological conditions, the number of circulating MSCs is greatly increased [6,7]. In 2007, we reviewed some studies/knowledge about circulating MSCs and their relationship with bone marrow-derived MSCs (BM-MSCs) [8]. In this current review, we give a brief summary of the current studies about circulating MSCs and analyse the clinical application of circulating MSCs in tissue regeneration.

Biological characteristics of circulating MSCs

As there are no consistent defining characteristics of MSCs among researchers, the International Society for Cellular Therapy has proposed three criteria that have been generally accepted to categorise progenitor cells as MSCs: (1) adherence to plastic; (2) specific surface antigen expression; and (3) multipotent differentiation potential. Circulating MSCs also fulfil these requirements.

The frequency of BM-MSCs in humans under normal conditions is very low, ranging from 1 in 10^4 to 1 in 10^5 bone marrow mononuclear cells (MNCs) [9]. Compared with BM-MSCs, the frequency of circulating MSCs in humans is even lower, in the order of 1 in 10^8 peripheral blood MNCs [10]. Fernandez and co-workers [11] successfully identified cells with features of MSCs in growth-factor-mobilized peripheral blood cells from breast cancer patients. The MSCs identified by Fernandez and co-workers [11] expressed collagen I, collagen III, fibronectin, CD106, CD54, SH2, and SH3, but did not express antigens CD34, CD45, and CD14. However, they did not find any stromal cells in normal peripheral blood cells which were not mobilized by growth factors. Three years later, Zvaifler and co-workers [12] successfully identified mesenchymal precursor cells in the blood of normal individuals, and these cells were referred to as blood-derived mesenchymal precursor cells. These blood-

derived mesenchymal precursor cells also did not express CD34, CD45, and CD14, but were positive for vimentin, collagen I, bone morphogenetic protein receptors IA (BMPRI A) and BMPRI B. In the stromal cells obtained from mobilized peripheral blood cells, two cell populations were observed; fibroblast-like cells and some small round cells. Zvaifler and co-workers [12] also observed that both fibroblast-like cells and some large round cells exist in the predominant cells, and they found that culture conditions are an important factor which can modify the morphology of the progenitor cells.

Despite the difficulty in detection, to date, circulating MSCs have been detected and isolated from various species, such as guinea pig, mouse, rabbit, rat, and humans [8]. We have successfully isolated and cultured PB-MSCs from adult Sprague Dawley rats under normal conditions. The BM-MSCs and PB-MSCs showed similar characteristics of cell proliferation and multi-differentiation potentials. We compared the expression of a set of surface markers, and found that CD73 may be an important indicator to distinguish PB-MSCs from BM-MSCs (as summarized in Fig. 1). PB-MSCs are also plastic-adherent and have multi-differentiation potential. They can be differentiated into adipocytes, chondrocytes, and osteocytes under certain conditions, as proved by previous publications and our unpublished study.

MSCs homing

The capacity of MSCs to home and migrate to the target tissue is an important determinant for the clinical use of MSCs. For example, for bone formation to occur, MSCs must migrate to the bone surface, where they differentiate into osteoblasts and deposit bone matrix. There is a prevailing view that circulating MSCs engraft more rapidly than BM-MSCs [13]. Although the mechanisms for MSCs homing/migration and the mediators and chemotactic signalling involved are not fully identified, a likely paradigm for MSCs migration has already come into being. It is proposed that the mechanism used for MSCs homing is similar to the well-documented trafficking and homing of haematopoietic stem cells to the bone marrow, and leukocytes into the inflamed tissues [14,15]. Of course, there are some

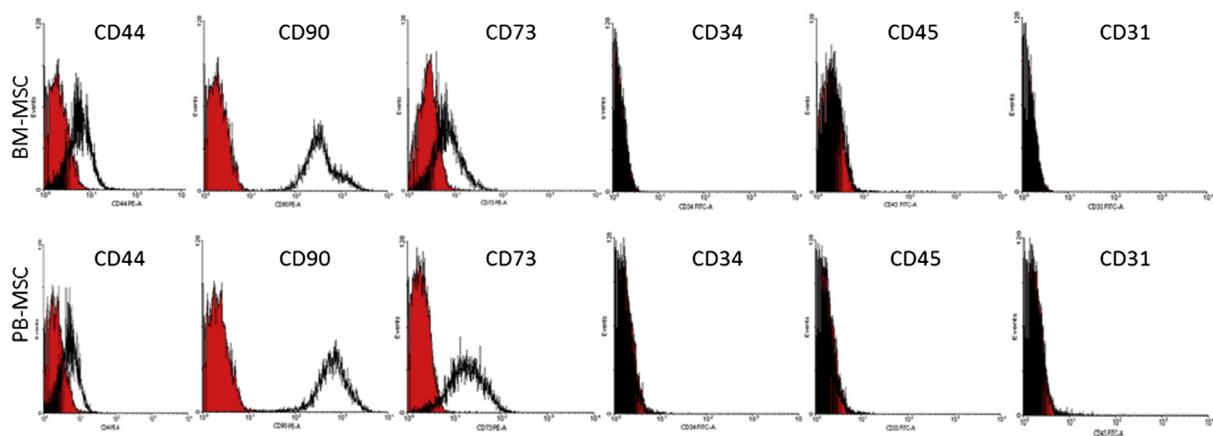


Figure 1 Surface markers of rat peripheral blood-derived mesenchymal stem cells (PB-MSCs) and bone marrow-derived MSCs (BM-MSCs), as analysed by flow cytometry. Isotype controls are represented in red, and the analysis with antibodies for cellular surface markers are represented in black. The PB-MSCs and BM-MSCs shared similar phenotypic CD markers.

differences among them. For example, the MSCs do not express ligands to endothelial selectins, such as P-selectin glycoprotein ligand-1 (PSGL-1) or sialyl Lewis X carbohydrates, so MSCs are not able to bind to chimeric constructs of the endothelial selectins CD62E (E-selectin) and CD62P (P-selectin).

Recently, the chemokine stromal cell-derived factor-1 (SDF1)/CXC chemokine receptor-4 (CXCR4) axis has been recognized as controlling the migration of MSCs [16,17]. CXCR4, a 352 amino acid rhodopsin-like G-protein-coupled receptor, binds with SDF1 and induces intracellular signaling transduction through several divergent pathways which are related to chemotaxis, cell survival, proliferation, and gene transcription. Our previous study also showed that CXCR4 was involved in MSCs homing and engraftment to tumours, the migration potential of MSCs toward tumour cells was enhanced with the upregulation of CXCR4 when MSCs were exposed to tumour conditioned medium, and the SDF1 inhibitor, AMD3100, could partly abolish the MSCs migration toward tumour cells [18]. A big issue concerning the SDF1/CXCR4 axis is that CXCR4 is usually absent on the surface of MSCs culture-expanded *in vitro* [16,19,20]. To overcome this problem, stimulating the expression of CXCR4 is one of the strategies for enhancing the migration capacity of MSCs [21–23].

MSCs for tissue regeneration

The capacity of MSCs is outstanding, such as multi-differentiation, immunosuppression, and immune privilege. In addition, they do not have the ethical issues like embryonic stem cells. These unique privileges make MSCs a promising cell source for cytotераpy. There is accumulating evidence that MSCs can be used to cure a broad spectrum of diseases, such as spinal cord injury, cardiovascular repair, bone and cartilage repair.

For the use of MSCs in cardiovascular repair, it has been demonstrated that systemically injected MSCs migrate to the infarcted myocardium at various time points, and some of the MSCs undergo differentiation to a cardiac phenotype, whereas other cells participate in angiogenesis in the infarcted area [24]. MSCs can survive very well in a xenogenic environment while retaining their abilities. In another study involving the autologous delivery of bone marrow cells into the infarct border zone in patients who had a myocardial infarction, all patients were alive and well at 3–9 months after surgery, and 80% (5/6) of the patients showed greatly improved infarct tissue perfusion; 70% (4/6) of the patients showed enhanced global left-ventricular function [25].

Also, there are many examples of the application of MSCs in the area of bone and cartilage repair. Quarto et al. [26] reported that local delivery of bone marrow stem cells combined with macroporous hydroxyapatite scaffolds results in substantial improvement in repairing large defects in long bones. Similarly, Solchaga et al. [27] reported the use of a combination of scaffold and bone marrow to treat osteochondral defects in rabbits. The results indicate that bone marrow loading appears to accelerate the first stage of the repair process after 3 weeks. In a caprine model of osteoarthritis, it has been demonstrated that local delivery

of MSCs to the injured joints can stimulate marked regeneration of the medial meniscus, the implanted MSCs can be detected in the newly formed tissues, and the normally seen progressive destruction in this model is retarded [28]. The effect of systemically injected MSCs on fracture healing is evaluated in a mouse fracture healing model; the results indicate that MSCs transplantation significantly increases cartilage and bone content and improves the fracture healing by improving the callus biomechanical properties [29]. To treat femoral head necrosis in rabbit models, intravenous transplantation of MSCs has been proven to migrate directionally and survive in the necrotic femoral heads without immunological rejection [30].

Most of the studies were conducted using bone marrow or other tissue-derived MSCs. However, the application of PB-MSCs is rarely reported. The main reason is that isolation and culture of PB-MSCs is not as easy as other tissue derived-MSCs. In 2006, our group demonstrated that PB-MSCs could enhance bone regeneration in the rabbit ulna critical-sized bone defect model [31].

Although the application of MSCs to cure a broad spectrum of diseases is promising, the efficacy still needs to be improved, especially for the differentiation of MSCs into specified functional cells *in vivo*. Many genes, chemicals, and cytokines have been used to enhance the efficacy of transplanted MSCs recently. For example, administration of interleukin 6 has been found to enhance the ability of MSCs to repair liver after CCL₄-induced fibrosis in mice [32]. Systemically administrated MSCs transduced with BMP2 and α 4 integrin have been demonstrated to increase bone density in a mouse model of osteopenia [33]. To manipulate specific gene expression in MSCs that may enhance their differentiation, proliferation, migration, and survival would be the future research directions.

Mobilization of MSCs

As mentioned, the characteristics of BM-MSCs and PB-MSCs are similar. Although BM-MSCs can be isolated and cultured very easily from bone marrow to fulfil the requirement of clinical use, the process of isolating MSCs from patients is

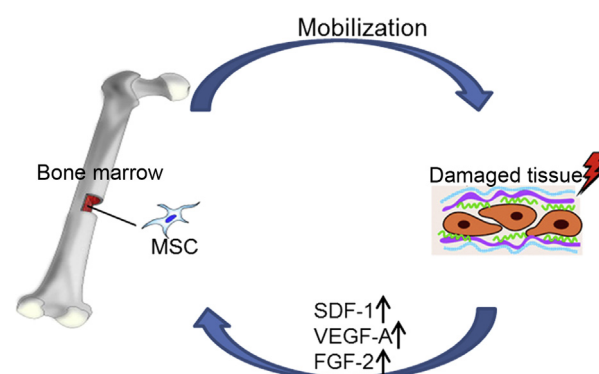


Figure 2 Schematic illustration of the migration of mesenchymal stem cells (MSCs) to the site of injury. MSCs reside in the bone marrow until stimulated by alarm signals, such as stromal cell-derived factor-1 (SDF1), vascular endothelial growth factor A (VEGF-A), and fibroblast growth factor 2 (FGF-2) in the plasma.

complicated and painful. Compared with BM-MSCs, the number of PB-MSCs is rarer in the blood and the isolation method is not well defined. For clinical application, the PB-MSCs seem to be more promising as long as we can find the solutions to effectively isolate and culture them *in vitro*. The general method used to isolate PB-MSCs is Ficoll-Paque, which may affect the proliferation and survival of PB-MSCs, and the components of the medium also need to be evaluated, such as supplements of some growth factors. Circulating MSCs are present in the peripheral blood in minimal concentrations under normal conditions. However, their numbers significantly increase in the blood after injury, such as bone fracture [34] and large acute burns [35]. Also, the increase in circulating MSCs is seen in patients with some chronic diseases, such as osteoporosis [36], breast cancer [11], and bone sarcomas [6]. It is believed that these increased MSCs may be released from the bone marrow. Our previous work has demonstrated that some osteoblasts involved in fracture healing are

systemically mobilized and recruited to the fracture site from remote bone marrow, rather than passively leaked into the circulation and to the bone injury site [37]. How do MSCs in the bone marrow know where and when injuries happen? It is well known that various cytokines and a complex interaction of chemical alarm signals are released from the injured tissues, and these alarm signals are sent to MSCs through some pathways that have not yet been clearly elucidated. For example, significant increases of SDF1, Vascular endothelial growth factor A (VEGF-A), and Fibroblast growth factor 2 (FGF-2) in the plasma have been detected in patients with ST elevation acute myocardial infarction [38]. When MSCs in the bone marrow received these alarm signals, some of these can be mobilized into the bloodstream depending on how serious the injuries are, as illustrated in Fig. 2.

Administration of some cytokines can mimic the alarm signals and mobilize MSCs into blood. This can increase the number of MSCs in the blood, and makes the isolation of

Table 1 Summary of publications using allogeneic mesenchymal stem cells (MSCs) in cell therapy applications.

| Diseases/animal model | Recipient species | Outcome | Refs |
|---|-------------------|---|------|
| Sjögren syndrome | Mouse, human | MSCs suppressed autoimmunity and restored salivary gland secretory function in both mouse models and Sjögren syndrome patients. | [47] |
| Ischaemic cardiomyopathy | Human | Allogeneic MSCs are as safe and effective as autologous MSCs in patients with left ventricular dysfunction due to ischaemic cardiomyopathy. | [46] |
| Drug-resistant polymyositis and dermatomyositis | Human | MSCs transplantation is safe and effective in patients with drug-resistant polymyositis or dermatomyositis. | [48] |
| Femoral segmental defect | Rat | Bone morphogenetic protein 2 (BMP-2) engineered allogeneic MSCs can repair critical bone defects to the same degree as rats treated with BMP-2 engineered autologous MSCs. | [49] |
| Spinal cord injury | Human | Neuropathic pain subsided from intermittent 10/10 to once/week 3/10 VAS. Recovery of muscle, bowel and sexual function was noted, along with a decrease in American spinal injury association (ASIA) score to "D". | [50] |
| Physéal bone bridge | Rabbit | No significant difference was found between rabbits with transplanted autogenous MSCs and rabbits with transplanted allogeneic MSCs either in the femur length, or in its valgus deformity. | [51] |
| Osteogenesis imperfect | Human | MSCs can engraft after transplantation, differentiate to osteoblasts as well as skin fibroblasts, and produce clinical benefits. | [52] |
| Acute myocardial infarction | Sheep | MSCs reduce infarct size and prevent subsequent adverse cardiac remodelling. | [53] |
| Multiple sclerosis | Human | The disease course was stabilized after the transplantation. | [54] |
| Systemic lupus erythematosus | Human | MSCs transplantation resulted in the induction of clinical remission and improvement in organ dysfunction in drug-resistant systemic lupus erythematosus patients. No transplantation-related adverse event was observed. | [55] |
| Cartilage defects | Pig | Quantification analyses for arthroscopy, histology, and magnetic resonance imaging revealed a better outcome in the MSCs-treated knees. | [56] |
| Acute myocarditis | Rat | The allogeneic administration of Fetal membrane-derived MSCs (FM-MSC) attenuated myocardial dysfunction and inflammation, and the host cell-mediated immune response was attenuated. | [57] |
| Recessive dystrophic epidermolysis bullosa | Human | MSCs administration resulted in an improvement in the skin of two patients, and the observed clinical benefit lasted for 4 months. | [58] |
| Ulcerative colitis | Human | Reduce the clinical and morphological indices of inflammatory activity in 34 (72.7%) patients. | [59] |
| Acute kidney injury | Rat | Reduce late renal fibrosis and loss of renal function in surviving animals. | [60] |

circulating MSCs possible. Recently, researchers have found that granulocyte-colony stimulating factor (G-CSF), a cytokine that contributes to haematopoietic progenitor cells and endothelial progenitor cells mobilization [39–41], can also be used to mobilize MSCs [42]. By using fibrin microbeads that can bind matrix-dependent cells, Kassir et al. [42] have isolated PB-MSCs from adult healthy human donors treated with G-CSF. Although they only get PB-MSCs from eight out of 11 samples, and the yield is lower than bone marrow, they made a big progress compared with the conventional method. In 2011, Iwasaki et al. [43] reported that the hepatocyte growth factor could also stimulate the migration of circulating mesoangioblasts (circulating mesenchymal cells that co-express endothelial makers).

Other issues of circulating MSCs: autologous versus allogeneic transplantation

Studies on circulating MSCs are rare in the literature. To our best understanding, circulating MSCs are a special subset of MSCs that are found in the circulation. However, their origin may be from bone marrow and other pools of MSCs in the body, and their release is tightly controlled by systemic and local factors (such as inflammatory cytokines, hormones, and growth factors). Stem cell homing refers to the inherent ability of stem cells to navigate toward a specific location (such as injury, inflammation, or infection) through unfamiliar areas. Stem cell mobilization means stem cells assemble and move toward specific stimuli or signals (directional movement), whereas cell migration is the inherent ability of cell movement and the migration may be directional or random. There are debates on the use of autologous and allogeneic MSCs through local and systemic administration for cell therapy applications. When administered systemically, MSCs may share certain characters with circulating MSCs. Because circulating MSCs are difficult to obtain from the blood, currently, our knowledge about them comes largely from studies of BM-MSCs.

Autologous MSCs transplantation means that the patient receives their own MSCs, with no risk of graft-versus-host disease. The disadvantage of autologous MSCs transplantation is that a longer time is needed (several days or weeks) for MSCs cell preparation, and the treatment window may be missed. In addition, sometimes the quality of autologous MSCs cannot be guaranteed in patients with comorbidities or advanced age [44,45].

Allogeneic MSCs transplantation is a procedure in which the patient receives MSCs from other donors. Allogeneic MSCs can be used as an “off-the-shelf” therapeutic agent, thus avoiding the painful bone marrow aspiration and culture delays prior to treatment. The potential risk from using allogeneic MSCs is rejection. However, so far, there have been no reports of serious adverse side effects following allogeneic systemic and local administration of MSCs, including immunological complications in animals and human studies reported. There is increasing evidence to prove that MSCs are immunosuppressive cells and that allogeneic MSCs may be used with similar therapeutic efficacy to autologous MSCs. The study carried out by Hare et al. [46] demonstrated that allogeneic MSCs are as safe and as effective as autologous MSCs in patients with

ischaemic cardiomyopathy. They also found that allogeneic MSCs did not stimulate significant donor-specific alloimmune reactions. So, allogeneic MSCs transplantation seems to be more promising, as it ensures that patients can get treatment at the best time with well-prepared allogeneic MSCs. Publications showing administration of allogeneic MSCs for curing various diseases are summarized in Table 1.

To date, no standard procedure for therapeutic MSC administration has been agreed on, which remains one of the critical issues for the clinical application of MSCs. For instance, the following questions still have open answers. How many MSCs should be used for patients with different diseases? When is the best time for MSC application? What kind of MSCs is most suitable? Which routine of administration and how often should MSCs be given (once/week or twice/week)? All these issues are valid ones and should be addressed properly through carefully designed animal and clinical trials before large-scale clinical applications of MSCs can be trialled and accepted in patients with certain disease conditions.

Conclusion

In conclusion, MSCs play an important role in the regenerative processes of many tissues. Circulating MSCs is a new cell source for tissue regeneration and tissue engineering. The implication of circulating MSCs in diseases is more promising, although many questions remain to be clarified, such as MSC mobilization from the bone marrow, homing mechanisms to specific tissues, and functional roles *in vivo*. In clinical practice, the use of allogeneic MSCs is more practical and may become the main cell source for clinical cell therapy. There are still many questions needing answers, and these answers have to come from carefully designed animal and clinical trials.

Conflicts of interest

The authors indicate no potential conflicts of interest.

Acknowledgements

This work was supported by a grant from the National Natural Science Foundation of China (NSFC Nos. 81172177, 81371946) and the Hong Kong Government Research Grant Council, General Research Fund (CUHK471110 and CUHK470813) to Li Gang. This work was also supported, in part, by the National Basic Science and Development Programme (973 Programme, 2012CB518105) and SMART program seed funding, Lui Che Woo Institute of Innovation Medicine, The Chinese University of Hong Kong.

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