STEM CELLS AND DEVELOPMENT
Volume 25, Number 3, 2016

DOI: 10.1089/scd.2015.0278

Spheroid Mesenchymal Stem Cells and Mesenchymal Stem Cell-Derived Microvesicles: Two Potential Therapeutic Strategies

Lili Xie,[1] Mao Mao,[2] Liang Zhou,[1] and Bing Jiang[1]

Mesenchymal stem cells (MSCs) have drawn worldwide attention of scientists and clinicians due to their ability to differentiate into other cell lineages, secrete paracrine factors, modulate inflammation and immunity, and also due to the effectiveness of MSCs in treating degenerative diseases. Recent studies have shown that, when cultured in spheroids, MSCs have greater differentiation ability, and increased anti-inflammatory and immunomodulatory capacities compared with traditional two-dimensional (2D) cultures. Furthermore, spheroid MSCs can be used on scale-up productions in clinically relevant manufacturing platforms. Microvesicles (MVs) are small membranous vesicles that can transfer proteins, genetic materials, and lipids to cells. MVs derived from MSCs (MSC-MVs) are not only emerging as potent transfer agents for molecular information, but also are effective in a series of tissue repair and anti-tumor experiments. Therefore, both spheroid MSCs and MSC-MVs have great potential in experimental and clinical applications. In this review, the characteristics, therapeutic applications and potential clinical translational opportunities of spheroid MSCs and MSC-MVs were discussed.

Introduction

Mesenchymal stem cells (MSCs) are multipotent cells that give rise to various cell types of the mesodermal germ layer, including adipogenic, osteogenic, and chondrogenic lineages [1,2]. While MSCs were originally isolated by Friedenstein et al. from the bone marrow [3], nowadays they can be easily purified from a variety of adult or embryonic tissues including placenta, adipose tissue, and umbilical cord [4]. MSCs can contribute to the recovery of liver [5,6], heart [7–9], brain [10,11], and vasculature [12] from injuries, though the exact mechanisms are still not completely understood. Many cell transplantation studies have shown that only a few exogenous stem cells differentiated after grafting into tissues, suggesting mechanisms other than cell differentiation are involved [13]. Indeed, mounting evidences support a mechanism of paracrine action between MSCs and their host tissues that underlines MSC therapy [14]. As human MSCs can be easily obtained and produced in large quantities in vitro, and as there has been almost no adverse effect reported in experimental and clinical studies of allogeneic MSC transplantations, MSCs have been widely investigated for their therapeutic uses in regenerative medicine.

MSCs are typically cultured in 2D monolayer conditions (2D-MSCs) [15]. Despite its success in cell-based therapies in experimental settings, low efficacy and safety issues remain to be concerned [16], preventing their application in clinical uses. Ways to enhance the efficacy of MSCs and to reduce the risk of carcinogenicity have been the main focus of study for the past few decades. Many cell types can form three-dimensional (3D) architecture including hepatocytes [17], cancer cells [18] and, more recently, stem cells [19,20]. Among many studies, Lee et al. was one of the first groups who found that intravenously injected single cell suspension of MSCs can form microemboli-like aggregates in the lung, which was found to have upregulated expression of multiple genes including an anti-inflammatory molecule, TSG-6, and thereby led to increased cardiac function in a mouse model of myocardial infarction [21]. Since then, spheroid MSCs have gained much attention as an effective therapeutic agent due to their greater anti-inflammatory effects, differentiation capacities, and enhanced cell survival than conventional 2D-MSCs [22]. Spheroids MSCs have a 3D microstructure consisting of a core, which contains aggregated cells tightly adhering to each other, and a monolayer of cells surrounding the core [23]. Thus far, a variety of culturing conditions and spheroid-forming devices have been developed to facilitate production of spheroid MSCs [24–26].

Microvesicles (MVs), earlier referred to as microparticles, exosomes and ectosomes, are membranous small vesicles that can transfer protein, messenger RNA (mRNA) and micro RNA (miRNA) into cells, invoking changes of the

[1]Department of Ophthalmology, The Second Xiangya Hospital, Central South University, Changsha, China.
[2]Departments of Ophthalmology and Anatomy, Institute for Human Genetics, UCSF School of Medicine, San Francisco, California.

gene expression, multiplication, and differentiation of the recipient cells [27]. Compared to MSCs, MVs not merely play a pivotal and indispensible role in regeneration and tissue repair, but also are more durable and preservable [28,29], inducing stronger protective effect that fall off over time at a lower rate. In addition, because of their small size, systemic administration of MVs instead of MSCs may reduce the safety concerns such as vascular occlusions about MSCs [30,31].

Despite potential therapeutic benefits shown by numerous experimental studies, the results obtained from clinical trials of MSC treatment were not as beneficial as those described in experimental conditions. Many problems such as donor variability, immune-mediated rejection, loss of functional properties, even malignant transformation, remain to be solved [32]. Besides these problems, standardization of culture protocols, treatment timing and method, and selections of donor cells need further investigation.

In this review, we summarized the characteristics of spheroid MSCs and MSC-MVs and their therapeutic applications in diseases such as cancer, ischemic diseases, and cerebrovascular diseases (CVDs). The potential clinical translational opportunities and central challenges associated with future clinical applications were also discussed.

Characteristics of Spheroid MSCs

Biological properties of spheroid MSCs

Increased anti-inflammatory properties. Although the mechanism of MSCs in regulating inflammation have not been exactly defined, their anti-inflammatory properties are of great importance for MSCs to play a role in repairing damaged organs. Inflammation is part of the complex biological process that involves action of immune cells, blood vessels, and molecular mediators. Spheroid MSCs can affect the activity of immune cells (especially macrophages [33]), increase the expression of anti-inflammatory genes and proteins [34], and control the development of blood vessels. Through formation of sphere-like structures, spheroid MSCs can be self-activated partially through cellular stress responses to secrete anti-inflammatory molecules such as prostaglandin E2 (PGE2) [35]. Secretion of PGE2 from activated MSCs can influence the activities of macrophages, the primary player during the onset and development of inflammation [33], and transform them from an original proinflammatory M1 to an anti-inflammatory M2 phenotype [35]. In addition to PGE2, upregulation of a series of other inflammatory modulating cytokines including TNFα-stimulated protein 6 (TSG6), leukemia inhibitory factor (LIF), stanniocalcin 1 (STC-1), and interleukin 1 receptor antagonist (IL1RN) [17,36] have also been shown. Signaling pathways that mediate these expression changes were thought to include stress response pathways such as caspase-dependent IL1 signaling [36] and the activation of NFκB [35].

Enhanced differentiation capacity. The conventional 2D culture method of MSCs lacks a tight control of cell fate, which results in low differentiation efficiency and impairs the clinical applicable values of 2D-MSCs. In contrast, many studies have showed that spheroid MSCs have better differentiation capacity. For example, Wang et al. showed that spheroid MSCs have greatly increased differentiation efficiency toward adipocytes and osteoblasts [37] when cultured on a micro-patterned substrate, which controls MSC spheroid dimension and maintains their cellular function and behavior. Similarly, spheroid MSCs also showed enhanced chondrogenic differentiation potential [38] and better cardiomyogenic differentiation ability compared with 2D cultured MSCs [39]. The enhanced differentiation capacity of spheroid MSCs involves the following molecular mechanisms: (i) the Rho/Rho-associated kinase pathway is activated, which triggers spheroid formation and gap junction mediated intercellular communication [38]. (ii) The culture substrates used in the 3D culture, such as chitosan membranes, can trigger calcium influx and enhance intracellular calcium signaling, which increases the gene expression of N-cadherin and noncanonical WNT proteins, and enhances cell migration, aggregation, and cell differentiation [39]. (iii) Hypoxia-related signaling cascades and enhanced cell–cell interactions can contribute to the increased differentiation capacity of MSCs. Through activating p38, hypoxic environment in spheroids upregulates the expression of TGF-β3, leading to enhanced differentiation capacity of spheroid MSCs [40].

Enhanced cell survival. In a randomized, controlled study of patient with chronic ischemic heart disease, Assmus et al. showed that intracoronary infusion of 2D-adult progenitor cells had only limited effects on restoration of functions of left ventricles, due to poor survival of those transplanted cells [41]. Similar findings were also shown by a study using MSCs for regenerating and repopulating the damaged myocardium [42]. Poor survival of transplanted MSCs is mainly due to withdrawal of survival growth factors and hypoxia in the ischemic tissue environment [42]. In contrast, the mild hypoxic condition established in the core of MSC spheroids resembles that of ischemic injured tissues that does not have enough oxygen and nutrients. Therefore, MSCs cultured in spheroids might survive the harsh hypoxic conditions of ischemic tissues to which they are transplanted. Indeed, as shown by Bhang et al., adipose-derived spheroid MSCs have enhanced cell survival in ischemic tissues compared with 2D-MSCs [43]. Likewise, gingival-derived spheroid MSCs show better therapeutic efficacy for the treatment of oral mucositis due to a similar mechanism [44]. Furthermore, through improving cell survival and increasing secretion of paracrine factors, spheroid MSCs can significantly enhance the angiogenic efficacy, thus improving the therapeutic outcome [45].

3D spheroid/tissue formation technologies

In early studies of 3D cultures, MSC spheroids form in a noncontact manner in which no noncellular supports or scaffolds are added. The hanging drop 3D culture technique, for example, which puts cell suspension in a drop and allows cell aggregation at the bottom of the drop due to gravity, is one of the wildly used method for 3D cultures of MSC spheroids [46]. With the development of culture technology, 3D spheroids can now be produced by growing cells on scaffolds such as chitosan membranes [47], chitosan-hyaluronan membranes [48], and porous polyurethane scaffolds [49]. Scaffold cultures of MSC spheroids can be used for mass productions, but the spheroid size cannot be well controlled. As spheroid size can affect the therapeutic ability of spheroid MSCs by modifying their angiogenic capacity [49], numerous mass production approaches and

3D spheroid formation ''micro'' devices have been developed to produce uniform shapes of MSC spheroids. For example, Derda et al. reported the generation of paper-supported 3D cell culture [50] method, which allows spheroid formation in a well-controlled way. In industry, ''micro'' devices are currently being used on scale-up productions to reduce manufacturing costs, thus showing better application value [51].

Therapeutic Application of Spheroid MSCs

Microenvironmental niches have a significant impact on the fate of MSCs to self-renew or to differentiate [52], and on cell survival [53]. Due to lack of suitable microenvironment, 2D-MSCs cannot secrete multiple bioactive substances continuously [54]. However, the 3D arrangement of cells in MSC spheroids can promote communication between cells and their environment, thus providing a more suitable microenvironment. The use of 3D spheroid MSCs has been shown to be a simple and more effective strategy in experimental studies and clinical trials than conventional 2D cultures.

Osteoarthropathy

Osteoarthropathy such as osteoarthritis, rheumatoid arthritis, degenerative arthritis, encompasses a series of diseases leading to arthralgia, arthrocele, and dysarthrosis. Recent studies have demonstrated that the lesions of cartilage and/or synovium instead of bone is the cause of osteoarthropathy. MSCs have been considered as an attractive cell source for the therapy of cartilage lesions because of their chondrogenic differentiation potential in vitro. However, transplanted 2D-MSCs lack stringent control of chondrogenic differentiation, which results in endochondral ossification instead of acquisition of a stable chondrogenic phenotype [55]. Recent studies using spheroid MSCs have demonstrated advantages over 2D-MSCs in treatments of osteoarthropathy. Arufe et al. showed that spheroid MSCs derived from synovial membranes have a more stringent differentiation into normal chondrocyte-like cells [56]. Likewise, MSCs from umbilical cord stroma also showed direct chondrogenic differentiation through spheroid formation [57]. Moreover, micromass coculture of human articular chondrocytes and MSCs can form spheroids that lead to stable cartilage tissue formation in vitro [58]. Despite the success of spheroid MSCs in osteoarthropathy treatment in experimental conditions, problems such as low treatment efficiency still need to be solved to achieve clinical success. For example, due to avascularity, nutrient consumption and waste generation in synovial joint, how to enhance survival and function of the transplanted MSCs in a nutrient-poor environment, remains to be resolved. As demonstrated by Farrell et al., although the survival of MSCs in the central region of spheroids is lower than those at the construct periphery, a subset population of cells from the central region remains viable (20%–40%) [59]. The MSC subpopulation may not merely have the potential of cartilage differentiation, but can survive and flourish in the hypoxic and nutrient-poor environment suggesting population heterogeneity exists [60]. Likewise, through comparing different characters of three subpopulations of MSCs, Arufe et al. showed that spheroids formed from CD271-enriched and CD73-enriched MSCs from normal human synovial membranes were better than CD106(+) MSCs in intrinsic cartilage repair [61]. This result implies that selecting MSC subpopulations that are more resistant to metabolic challenges or better mimic the behavior of native cartilage cells might be a feasible way to optimize the efficacy on the treatment of osteoarthropathy.

Ischemic diseases

Besides osteoarthropathy, MSCs have also been considered useful in prevention and therapy of ischemic diseases, especially of cerebrum and heart ischemia, which are two leading causes of disabling and death worldwide. Since last decade, stem cell therapy is emerging as an innovative approach to restore functions of different organs that have ischemia-reperfusion injuries (IRIs). It is worthwhile mentioning that, however, after injection of 2D-MSCs, the hypoxic microenvironment, local inflammation, and blood vessel damage are major causes for low efficiency of stem cell therapy [62]. Recent experimental studies have shown that transplantation of spheroid MSCs represents an innovative treatment solution for cardiac ischemia [63] and hind limb ischemia [45]. In three aspects, spheroid MSCs have advantages over 2D-MSCs for the treatment of ischemic diseases (Fig. 1). (i) The volume and diameter of cells released from spheroids are about 1/4 and 1/2 of 2D-MSCs, respectively [22]. Since spheroid MSCs are uniformly smaller in size than 2D-MSCs, they can pass through blood vessels more easily and thus markedly reducing the chance of vascular obstructions and stroke [64,65]. (ii) The enhanced and long-term survival of spheroid MSCs transplanted into the ischemic region raises the efficacy of stem cell therapy [43]. (iii) Transplantation of spheroid MSCs can enhance vascularization and increase the functional microvessel density of ischemic tissue comparing to 2D-MSCs [49,45]. However, the ability of spheroid MSCs in stimulating angiogenesis and thus treating ischemic injuries can be affected by culture parameters such as culture size and external oxygenation. For example, MSC spheroids with a size of 10,000 cells cultured under 2% O_2 exhibited better production of vascular endothelial growth factor (VEGF) than spheroids containing 60,000 cells that were cultured under 20% O_2 [66]. Therefore, careful considerations should be given to both culture size and local oxygen tension to achieve optimal and consistent therapeutic results.

Characteristics of MSC-MVs

MVs are small membranous vesicles present in many kinds of body fluids and in interstitial spaces between cells [67]. MVs encompass a heterogonous population of vesicles including exosomes that are 50–100 nm in size, and ectosomes (also known as shredding vesicles) whose sizes are between 100 and 1,000 nm [29,68]. Exosomes and ectosomes are very similar but vary in biogenesis. Exosomes have an endosome origin and are released when multivescular endosome fuses with the plasma membrane. On the contrary, ectosomes form by directly budding from the plasma membrane [69]. Because both exosomes and ectosomes coexist in vitro and in vivo and cannot be separated by current methodologies, they are collectively called MVs [70]. MVs can serve as a vehicle to

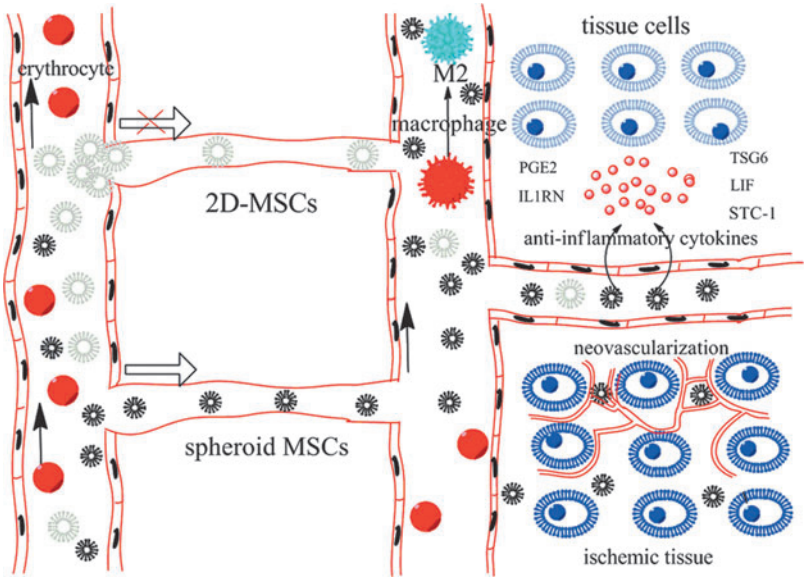

FIG. 1. Advantages of spheroid MSCs in trafficking and therapeutic efficacy to ischemia. (1) 2D-MSCs cause vascular obstructions most likely at the precapillary site, especially when they gather together. Since the volume of cells released from spheroids is about 1/4 of the 2D-MSCs' (nearly half in diameter), spheroid MSCs can easily pass through blood vessels and are less likely to get clogged up than 2D-MSCs (2) Compared with an equal number of 2D-MSCs, spheroid MSCs have upregulated expression of anti-inflammatory cytokines. The upregulated expression of anti-inflammatory cytokines can transform macrophages from an original proinflammatory M1 to anti-inflammatory M2 phenotype (3) Transplantation of spheroid MSCs can enhance vascularization and increase the functional microvessel density of ischemic tissue compared with 2D-MSCs. 2D, two-dimensional; MSCs, mesenchymal stem cells; TSG6, TNFα stimulated protein 6; IL1RN, interleukin 1 receptor antagonist; PGE2, prostaglandin E2; STC-1, stanniocalcin 1; LIF, leukemia inhibitory factor.

transfer a variety of bioactive cargoes such as proteins, lipids, mRNA, and miRNA, and play a vital role in cell–cell communication and tissue regeneration [71–75] (Fig. 2). Notably, the cargos of MVs are dependent on the cell type of origin, subjected to changes during preparation and can be influenced by local microenvironment [76,77]. Recent technical advances in transcriptomics, proteomics, lipidomics, and bioinformatics have revealed the composition of these cargoes, which provides the basis for understanding the therapeutic roles of MVs in diseases [78,79].

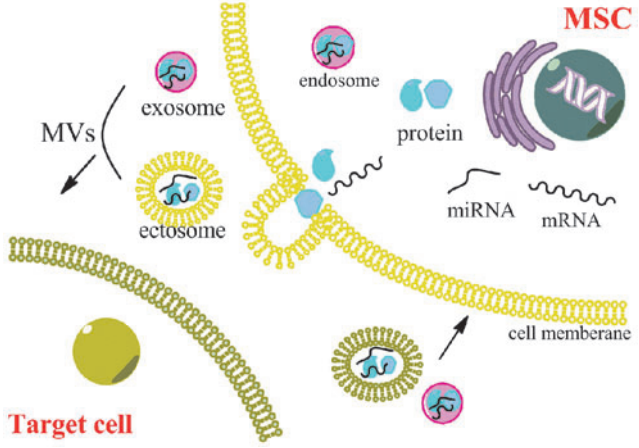

FIG. 2. MVs as mediators of cell–cell communication. MVs encompass a heterogonous population of vesicles including exosomes and ectosomes. Exosomes have an endosome origin and are released when multivescular endosome fuses with the plasma membrane. On the contrary, ectosomes form by directly budding from the plasma membrane. With the change of signal factors coming from microenvironment, MVs derived from MSCs transferred protein, mRNA, miRNA, and lipids to target cells, thus altering their physical activities. Exchange of MVs is bi-directional, which means target cells can also transfer genetic and protein information to MSCs via MVs. MV, microvesicle; mRNA, messenger RNA; miRNA, micro RNA.

Cargoes of MSC-MVs

Proteins. The importance of MV proteins were first indicated by proteomic studies of MSC-conditioned media, which showed that the therapeutic effect of MSCs is at least partially due to the membrane-bound and intracellular proteins [80]. Using liquid chromatography-tandem mass spectrometry, Kim et al. identified 730 proteins in MVs derived from human bone marrow MSCs with a high confidence level [78]. The MSC-MV proteome reflects characteristics of both MVs and MSCs. Among these 730 proteins, 420 proteins were found in MVs from other cell sources and included those associated with MV biogenesis, trafficking, and docking processes. In addition, 122 MV proteins were also shared by two MSC proteomes and included surface receptors and signaling molecules controlling self-renewal and differentiation of MSCs. Functional analysis of the MV proteome indicates that MV proteins are involved in a variety of processes such as cell proliferation, adhesion, signaling, and morphogenesis. In addition to individual proteins, protein complexes have also been noted. Lai et al. reported the detection of a functional 20S proteasomes with 7α subunits and 7β subunits. The 20S proteasome were thought to ameliorate tissue damage by degrading misfolded proteins and synergizing functions of other constituents in exosome [81].

Messenger RNA. MVs can horizontally transfer mRNA to recipient cells. In a study performed by Bruno et al., 239 transcripts were identified in MVs from human bone marrow MSCs by microarray analyses [82]. The mRNA in these MVs is a subset of cellular mRNA associated with mesenchymal phenotypes that controls the transcription, cell proliferation, and immunoregulation [82]. In addition, Zhu et al. demonstrated that human MSC-MVs express mRNA of some pivotal MSC paracrine factors, particularly angiopoietin-1 [83]. More

importantly, the delivered mRNA can be translated into proteins in target cells and change their phenotypes [84]. Deregibus et al. showed that MVs derived from human endothelial progenitor cells trigger angiogenesis after being internalized into endothelial cells [85]. Cargos transferred by these MVs include mRNA associated with PI3K/AKT signaling pathway that is known to have angiogenic and antiapoptotic functions. When MVs were incubated with RNase, the curative effects declined, suggesting the major biological effect of MVs is through mRNA. Because of their prominent roles in gene regulation, mRNA transferred by MVs is of prime importance for a variety of biological effectors carried out by MVs.

Micro RNA. In addition to mRNA, MVs may transfer noncoding RNAs such as small regulatory miRNA into target cells [86]. miRNA are small RNA molecules containing 22 nucleotides and functioning in post-transcriptional regulation of gene expression. Recent studies have indicated that the exchange of miRNA carried by MVs between neighboring cells plays an integral part of cell–cell communication of MSCs and damaged cells. The exchange of miRNA can be bidirectional. miRNA from damaged cells can reprogram MSCs to exhibit features of damaged cells. On the other hand, miRNA from MSCs can allow resident cells in damaged tissues to reenter cell cycle and/or reprogram damaged cells to survive cell death. Chen et al. demonstrated that MVs can carry miRNAs, which are present predominantly in a precursor form instead of a mature form [87,88]. By sequencing analysis, Koh et al. showed that miRNA composition of MVs from human embryonic stem cell-derived MSCs represented a small subset of those in the intracellular compartment of MSCs. Among the miRNAs, high levels of let-7 family of miRNA are present in both intracellular and extracellular (in MVs) of MSCs, suggesting their involvement in intercellular communications [89]. The let-7 family of miRNA in MSC-MCs can regulate self-renewal and differentiation of MSCs by regulating their downstream target, HSF4A. Likewise, other families of miRNA have been identified in MSC-MVs and shown to prevent apoptosis [90], promote neural plasticity [91] and neural growth [92], suppress angiogenesis [93], and inhibit tumor growth [94] by regulating their targets.

Lipids. Although the lipidomes of MSC-MVs have not been fully characterized, studies on MVs from other cell types and body fluids are available [95]. Lipid composition of MVs from different cellular source varies but is mainly composed of a lipid bilayer similar to the plasma membrane from which the vesicle derives. Compared to the plasma membrane, MV lipids are enriched with phosphatidylserine, disaturated phosphatidylethanolamine, disaturated phosphatidylcholine, sphingomyelin, ganglioside GM3, and cholesterol. The lipid-bilayer of MVs is thought to provide stability and structural rigidity to MVs, and it has been shown to serve as suitable carriers for membrane proteins, thereby allowing noncanonical cell–cell communication [79].

Therapeutic Application of MSC-Derived MVs

Previously MVs were considered as a way for cells to discard unwanted cellular materials. Nowadays it is known that MVs are involved in delivery of bioactive information and cell–cell communication throughout the body [76,96]. Because of their ability to transfer membrane and cytoplasmic constituents from source cells, MVs can achieve the endocrine or paracrine effects of MSCs with no need for trans-differentiation into tissues [94,97]. In addition, MVs not only express specific antigens of cells from which they originate and thus maintaining some of their properties [98], but also have less risk to be rejected by recipient tissues after injecting [30,31]. Therefore, it is hopeful for MVs to be used as a replacement of cell-based therapy for the treatment of certain diseases.

Cancer

In cancer, MVs can serve as biomarkers and effectors on cell–cell interactions [99,100]. Therefore MVs might play a role in anticancer therapy [101,102], although their potential carcinogenicity cannot be ignored [103].

Through the transmission of their cargoes to cancer cells, MSC-MVs can have potent anticancer effect. For instance, miRNA-23b transferred by MSC-MVs can downregulate the expression of a target gene, MARCKS, thus promoting breast cancer cell dormancy [104]. Moreover, through the transmission of miRNA-16, a miRNA known to target VEGF, MSC-MVs can significantly suppress the expression of VEGF, which is related to inadequate blood vessel formation in breast cancer both in vitro and in vivo [93]. Moreover, MSC-MVs can be an efficient drug delivery vector of anticancer drugs, which can exert the antitumor function in cooperation with the MSC-MVs [105]. However, on the other hand, as MVs are modulators of their microenvironment, MSC-MVs derived from tumor tissues may own some oncogenic properties resembling their cells of origin. The level of tumor marker protein, cytokines and miRNA in tumor tissue-derived MSC-MVs is higher than in normal tissue derived-MSC-MVs, while the level of tumor suppressor miRNA in tumor tissue-derived MSC-MVs is lower [106]. Therefore, the carcinogenic risk of tumor tissue-derived MSC-MVs should not be ignored [106], and if possible, needs to be reduced in years to come. On the other hand, searching for overlapping miRNAs that are carried by both cancer cell-derived MSC-MVs and cancer tissues may lead to discovery of novel biomarkers for cancer progression [107].

Cerebrovascular diseases

CVDs such as stroke, transient ischemic attack, and intracerebral hemorrhage are one of the three principal diseases causing death worldwide. Ever since 2001, stem cells have been used to treat stroke [108]. The therapeutic effect of MSCs is through promoting neuronal plasticity, angiogenesis, and immunemodulation [109] instead of through replacing damaged cells. This is not only because MSC-MVs can transfer genetic information [110], but also because they have several potential advantages in treatment of CVD.

In the central nervous system, miRNA plays an important role in neuronal development and maturation, and also is involved in fine regulation of adult neuronal plasticity [89]. In addition, miRNA can also play a role in pathological conditionals like stroke. Xin et al. observed that miR-133b can be transferred by MSC-MVs to neurons and astrocytes in culture and in an animal model [89,90]. The transferred miRNA-133b decreased CTGF expression and thus attenuated the glial scar, inhibited RhoA expression, and enhanced neurite regrowth. Nevertheless, effective delivery of miRNA into the brain is a bottleneck of miRNA treatment for CVDs, and a

variety of strategies have been developed to deliver miRNA, most of which used synthetic materials. Because of their small sizes and biogenic nature, MSC-MVs can easily pass through the blood–brain barrier and be internalized to the target cells. Thus, MSC-MVs can be an effective strategy for therapeutic miRNA delivery to the brain under pathological circumstances. In fact, systemic administration of MSC-MVs alone was found to be effective in promoting neurological outcome and neurovascular remodeling [111]. Moreover, because genetically manipulated MSC-MVs can be produced in large amounts, MSC-MVs are promising in being commercialized as a powerful therapeutic agent. A third way to improve the effect of MSC-MV therapy is to improve its target specificity by altering the expression of cell type-specific adhesive molecules at the membrane surface of MSC-MVs [112]. Overall, MSC-MVs can be a promising strategy to effectively treat complicated diseases such as the CVDs without apparent advert side effects.

Kidney diseases

Kidney diseases usually have a common course of progression from renal inadequacy, kidney failure, and then to uremia. If not controlled effectively, kidney diseases may have a poor prognosis. In fact, as many as 20% of patients diagnosed with acute tubular necrosis were shown to progress to chronic kidney disease (CKD) stage IV in 18–24 months [113]. In various kinds of cell therapies for kidney diseases, MSC therapy has attracted much attention because it can effectively attenuate renal inflammation, endoplasmic reticulum stress, oxidative stress, and apoptosis [114,115].

As showed by Tögel et al., the protective effect of MSCs for kidney after IRI was mainly due to paracrine mechanisms [116]. Consistently, Bi et al. confirmed that conditioned media of MSCs could directly protect renal tubule cells from death caused by cisplatin. Also, it is needless to inject the MSCs into body to spur the desired protective response [117]. Moreover, Gatti et al. demonstrated that it is MSC-MVs that mimic the favorable function of MSCs treatment for acute kidney injury (AKI) and CKD consequent to IRI [118]. Taken together, these observations support a pivotal and indispensible role of MSC-MVs in stem cell therapy for kidney diseases. As previously mentioned, through horizontal transfer of mRNAs and miRNA associated with gene transcription, cell proliferation, and immunomodulation, MSC-MVs can inhibit apoptosis and stimulate proliferation of tubular epithelial cells [81,118,119]. Remarkably, for the first time, Tomasoni et al. showed that MSC-MVs could contribute to the expression of growth factor receptors on the tubular cells for the treatment of AKI, thus inducing stronger protective effect [120]. Additionally, MSC-MVs can be a safe choice of therapy. Compared with transplanting MSCs directly, administrating MSC-MVs can avoid the potential risk of mal-differentiation [121] and tumorgenicity [32] of transplanted cells. In addition, injection of MSC-MVs can improve confinement caused by lack of specific homing and ability to target cells [122].

Clinical Translation Opportunities

Although numerous laboratory studies have highlighted the curative effects of spheroid MSCs and MSC-MVs, few clinical trials have been made so far because of lack of large datasets and further studies on details in effectiveness. Thus, to accelerate the development of clinical translational research, consideration should be given to establishment of standards and improvement of efficacy.

For spheroid MSC, one way to increase the treatment efficacy is to use coculture models. For example, 3D scaffolds coseeded with human endothelial progenitor and MSCs can promote angiogenesis within 7 days [123]. Moreover, selection of MSC subpopulations that are more resistant to metabolic challenge or better mimic the behavior of naive target cells might maintain the consistency of stem cell therapy [57,59]. Finally, genetic modulation of MSC spheroids has been applied in some experiments to increase the secretion of beneficial molecules such as growth factors and to promote functional recovery [27].

As mediators of cell–cell communication, MSC-MVs have shown great potential for anticancer therapy and tissue regeneration [124]. In addition, engineered MVs can serve as a novel drug delivery system. For instance, exosome encapsulated anti-inflammatory drugs have been delivered noninvasively to treat brain inflammatory-related diseases [125]. Moreover, it is worthwhile to explore the unique applications of MSC-MVs with different origins because of their cell type-specific characteristics. For example, GC-MSC-MVs can be new biomarkers for the treatment of gastric cancer [107]. For further accelerating clinical translation, highly quantitative studies on MSC-MVs should be performed in future. Recently, Chevillet et al. performed a quantitative and stoichiometric analysis of miRNA and exosomes isolated from multiple source of body fluids and found that standard preparation of exosomes contains only a small fraction of miRNA in the plasma, suggesting a novel mechanism for MV mediated cell–cell communication [126]. Similar quantitative approaches can be taken to evaluate the relationship among mRNA, miRNA, protein, and MSC-MVs, which will provide more information for the functional boundaries for MSC-MV-mediated delivery in future.

Conclusion Remarks

Although there are numerous positive effects of traditional 2D MSC treatment for degenerative diseases [94], ischemic diseases [127], and sterile tissue injuries [128], major problems with cell therapies are the limited cell availability, engraftment, and carcinogenicity. Spheroid MSCs and MSC-MVs, on the contrary, display superior therapeutic effect and fewer side effects. Spheroid MSCs show improved biological properties that increase its potential clinical translational opportunities, partially because they mimic much better the in vivo environment of a real tissue. As mediators in delivery of bioactive information and cell–cell communication, MVs could achieve endocrine or paracrine effects of MSCs with no need for trans-differentiation. Furthermore, engineered MVs could serve as a novel gene/drug delivery system [126,129], which suggests that MSC-MVs can be custom-engineered to be more appropriate for stem cell therapy. Following a better insight of the two kinds of potential therapeutic strategies, comparison and contrast should be made on curative effects, culture conditions, treatment timing, and method in more details in the near future to optimize stem cell therapy. Moreover, it is worthwhile to explore ways to

integrate the superiorities of both of the two potential therapeutic strategies.

Acknowledgments

This work was supported by the Natural Science Foundation of China (NSFC 81271004, NSFC 81371012 to Bing Jiang and NSFC 81200697 to Liang Zhou).

Author Disclosure Statement

No competing financial interests exist.

References

1. Russell KC, DG Phinney, MR Lacey, BL Barrilleaux, KE Meyertholen and KC O'Connor. (2010). In vitro high-capacity assay to quantify the clonal heterogeneity in trilingage potential of mesenchymal stem cells reveals a complex hierarchy of lineage commitment. Stem Cells 28:788–798.
2. Menssen A, T Häupl, M Sittinger, B Delorme, P Charbord and J Ringe. (2011). Differential gene expression profiling of human bone marrow-derived mesenchymal stem cells during adipogenic development. BMC Genomics 12:461.
3. Friedenstein AJ, JF Gorskaja and NN Kulagina. (1976). Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 4:267–274.
4. Huang L, C Niu and B Willard. (2015). Proteomic analysis of porcine mesenchymal stem cells derived from bone marrow and umbilical cord: implication of the proteins involved in the higher migration capability of bone marrow mesenchymal stem cells. Stem Cell Res Ther 6:77.
5. Li H, B Zhang, Y Lu, M Jorgensen, B Petersen and S Song. (2011). Adipose tissue-derived mesenchymal stem cell-based liver gene delivery. J Hepatol 54:930–938.
6. Ezquer M, F Ezquer, M Ricca, C Allers and P Conget. (2011). Intravenous administration of multipotent stromal cells prevents the onset of non-alcoholic steatohepatitis in obese mice with metabolic syndrome. J Hepatol 55:1112–1120.
7. Van Linthout S, K Savvatis, K Miteva, J Peng, J Ringe, K Warstat, C Schmidt-Lucke, M Sittinger, HP Schultheiss and C Tschöpe. (2011). Mesenchymal stem cells improve murine acute coxsackievirus B3-induced myocarditis. Eur Heart J 32:2168–2178.
8. Li Y, Y Hiroi, S Ngoy, R Okamoto, K Noma, CY Wang, HW Wang, Q Zhou, F Radtke, R Liao and JK Liao. (2011). Notch1 in bone marrow-derived cells mediates cardiac repair after myocardial infarction. Circulation 123:866–876.
9. Samper E, A Diez-Juan, JA Montero and P Sepúlveda. (2013). Cardiac cell therapy: boosting mesenchymal stem cells effects. Stem Cell Rev 9:266–280.
10. van elthoven CT, A Kavelaars, F van Bel and CJ Heijnen. (2010). Repeated mesenchymal stem cell treatment after neonatal hypoxia-ischemia has distinct effects on formation and maturation of new neurons and oligodendrocytes leading to restoration of damage, corticospinal motor tract activity, and sensorimotor function. J Neurosci 30:9603–9611.
11. Miranda CO, CA Teixeira, MA Liz, VF Sousa, F Franquinho, G Forte, P Di Nardo, P Pinto-Do-Ó and MM Sousa. (2011). Systemic delivery of bone marrow-derived mesenchymal stromal cells diminishes neuropathology in a mouse model for Krabbe's disease. Stem Cells 29:1738–1751.
12. Ishii M, R Shibata and Y Numaguchi. (2011). Enhanced angiogenesis by transplantation of mesenchymal stem cell sheet created by a novel magnetic tissue engineering method. Arterioscler Thromb Vasc Biol 31:2210–2215.
13. Wang D, F Zhang, W Shen, M Chen, B Yang, Y Zhang and K Cao. (2011). Mesenchymal stem cell injection ameliorates the inducibility of ventricular arrhythmias after myocardial infarction in rats. Int J Cardiol 152:314–320.
14. Guiducci S, M Manetti, E Romano, B Mazzanti, C Ceccarelli, S Dal Pozzo, AF Milia, S Bellando-Randone, G Fiori, et al. (2011). Bone marrow-derived mesenchymal stem cells from early diffuse systemic sclerosis exhibit a paracrine machinery and stimulate angiogenesis in vitro. Ann Rheum Dis 70:2011–2021.
15. Tsai KS, SH Yang, YP Lei, CC Tsai, HW Chen, CY Hsu, LL Chen, HW Wang, SA Miller, et al. (2011). Mesenchymal stem cells promote formation of colorectal tumors in mice. Gastroenterology 141:1046–1056.
16. Funes JM, S Henderson, R Kaufman, JM Flanagan, M Robson, B Pedley, S Moncada and C Boshoff. (2014). Oncogenic transformation of mesenchymal stem cells decreases Nrf2 expression favoring in vivo tumor growth and poorer survival. Mol Cancer 13:20.
17. Ananthanarayanan A, B Nugraha, M Triyatni, S Hart, S Sankuratri and H Yu. (2014). Scalable spheroid model of human hepatocytes for hepatitis C infection and replication. Mol Pharm 11:2106–2114.
18. Huang YJ and SH Hsu. (2014). Acquisition of epithelial-mesenchymal transition and cancer stem-like phenotypes within chitosan-hyaluronan membrane-derived 3D tumor spheroids. Biomaterials 35:10070–10079.
19. Huang AH, MJ Farrell, M Kim and RL Mauck. (2010). Long-term dynamic loading improves the mechanical properties of chondrogenic mesenchymal stem cell-laden hydrogel. Eur Cell Mater 19:72–85.
20. Coutu DL, J Cuerquis, R El Ayoubi, KA Forner, R Roy, M François, M Griffith, D Lillicrap, AM Yousefi, MD Blostein and J Galipeau. (2011). Hierarchical scaffold design for mesenchymal stem cell-based gene therapy of hemophilia B. Biomaterials 32:295–305.
21. Lee RH, AA Pulin, MJ Seo, DJ Kota, J Ylostalo, BL Larson, L Semprun-Prieto, P Delafontaine and DJ Prockop. (2009). Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 5:54–63.
22. Bartosh TJ, JH Ylöstalo, A Mohammadipoor, N Bazhanov, K Coble, K Claypool, RH Lee, H Choi and DJ Prockop. (2010). Aggregation of human mensenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties. Proc Natl Acad Sci U S A 107:13724–13729.
23. Hoyer M, C Meier, A Breier, J Hahner, G Heinrich, N Drechsel, M Meyer, C Rentsch, LA Garbe, et al. (2015). In vitro characterization of self-assembled anterior cruciate ligament cell spheroids for ligament tissue engineering. Histochem Cell Biol 143:289–300.
24. Huang GS, CS Tseng, B Linju Yen, LG Dai, PS Hsieh and SH Hsu. (2013). Solid freeform-fabricated scaffolds designed to carry multicellular mesenchymal stem cell

spheroids for cartilage regeneration. Eur Cell Mater 26:179–194.
25. Futrega K, JS Palmer, M Kinney, WB Lott, MD Ungrin, PW Zandstra and MR Doran. (2015). The microwell-mesh: a novel device and protocol for the high throughput manufacturing of cartilage microtissues. Biomaterials 62:1–12.
26. Wise JK, AI Alford, SA Goldstein and JP Stegemann. (2014). Comparison of uncultured marrow mononuclear cells and culture-expanded mesenchymal stem cells in 3D collagen-chitosan microbeads for orthopedic tissue engineering. Tissue Eng Part A 20:210–224.
27. Tseng TC and SH Hsu. (2014). Substrate-mediated nanoparticle/gene delivery to MSC spheroids and their applications in peripheral nerve regeneration. Biomaterials 35:2630–2641.
28. Pant S, H Hilton and ME Burczynski. (2012). The multifaceted exosome: biogenesis role in normal and aberrant cellular function, and frontiers for pharmacological and biomaker opportunities. Biochem Pharmacol 83:1484–1494.
29. Cocucci E and J Meldolesi. (2015). Ectosomes and exosomes: shedding the confusion between extracellular vesicles. Trends Cell Biol 25:364–372.
30. Akao Y, A Iio, T Itoh, S Noguchi, Y Itoh, Y Ohtsuki and T Naoe. (2011). Microvesicle-mediated RNA molecule delivery system using monocytes/macrophages. Mol Ther 19:395–399.
31. Cocucci E, G Racchetti and J Meldolesi. (2009). Shedding microvesicles: artefacts no more. Trends Cell Biol 19:43–51.
32. Jeong JO, JW Han, JM Kim, HJ Cho, C Park, N Lee, DW Kim and YS Yoon. (2011). Malignant tumor formation after transplantation of short-term cultured bone marrow mesenchymal stem cell in experimental myocardial infarction and diabetic neuropathy. Cir Res 108 1340–1347.
33. Soehnlein O and L Lindbom. (2010). Phagocyte partnership during the onset and resolution of inflammation. Nat Rev Immunol 10:427–439.
34. Yeh HY, BH Liu, M Sieber and SH Hsu. (2014). Substrate-dependent gene regulation of self-assembled human MSC spheroids on chitosan membranes. BMC Genomics 15:10.
35. Ylöstalo JH, TJ Bartosh, K Coble and DJ Prockop. (2012). Human mesenchymal stem/stromal cells cultured as spheroids are self-activated to produce prostaglandin E2 that directs stimulated macrophages into an anti-inflammatory phenotype. Stem Cells 30:2283–2296.
36. Bartosh TJ, JH Ylöstalo, N Bazhanov, J Kuhlman and DJ Prockop. (2013). Dynamic compaction of human mesenchymal stem/precursor cells into spheres self-activates caspase-dependent IL1 signaling to enhance secretion of modulator of inflammation and immunity (PEG2, TSG6, and STC1). Stem Cells 31:2443–2456.
37. Wang W, K Itaka, S Ohba, N Nishiyama, UI Chung, Y Yamasaki and K Kataoka. (2009). 3D spheroids culture system on micropatterned substrates for improved differentiation efficiency of multipotent mesenchymal stem cells. Biomaterials 30:2705–2715.
38. Hsu SH, GS Huang, SY Lin, F Feng, TT Ho and YC Liao. (2012). Enhanced chondrogenic differentiation potential of human gingival fibroblasts by spheroid formation on chitosan membranes. Tissue Eng Part A 18:67–79.
39. Yeh HY, BH Liu and SH Hsu. (2012). The calcium-dependent regulation of spheroid formation and cardiomyogenic differentiation for MSCs on chitosan membranes. Biomaterials 33:8943–8954.
40. Yoon HH, SH Bhang, JY Shin, J Shin and BS Kim. (2012). Enhanced cartilage formation via three-dimensional cell engineering of human adipose-derived stem cells. Tissue Eng Part A 18:1949–1956.
41. Assmus B, J Honold, V Schächinger, MB Britten, U Fischer-Rasokat, R Lehmann, C Teupe, K Pistorius, H Martin, et al. (2006). Transcoronary transplantation of progenitor cells after myocardial infarction. N Engl J Med 335:1222–1232.
42. Zhu W, J Chen, X Cong and X Chen. (2006). Hypoxia and serum deprivation-induced apoptosis in mesenchymal stem cell. Stem Cells 24:416–425.
43. Bhang SH, SW Cho, WG La, TJ Lee, HS Yang, AY Sun, SH Baek, JW Rhie and BS Kim. (2011). Angiogenesis in ischemic tissue produced by spheroid grafting of human adipose-derived stromal cells. Biomaterials 32:2734–2747.
44. Zhang Q, AL Nguyen, S Shi, C Hill, P Wilder-Smith, TB Krasieva and AD Le. (2012). Three-dimensional spheroid culture of human gingiva-derived mesenchymal stem cells enhances mitigation of chemotherapy-induced oral mucositis. Stem Cells Dev 21:937–947.
45. Bhang SH, S Lee, JY Shin, TJ Lee and BS Kim. (2012). Transplantation of cord blood mesenchymal stem cells as spheroids enhances vascularization. Tissue Eng Part A 18:2138–2147.
46. Murphy KC, SY Fang and JK Leach. (2014). Human mesenchymal stem cell spheroids in fibrin hydrogels exhibit improved cell survival and potential for bone healing. Cell Tissue Res 357:91–99.
47. Hsu SH, GS Huang and F Feng. (2012). Isolation of the multipotent MSC subpopulation from human gingival fibroblasts by culturing on chitosan membranes. Biomaterials 33:2642–2655.
48. Huang GS, LG Dai, BL Yen and SH Hsu. (2011). Spheroid formation of mesenchymal stem cells on chitosan and chitosan-hyaluronan membranes. Biomaterials 32:6929–6945.
49. Laschke MW, TE Schank, C Scheuer, S Kleer, S Schuler, W Metzger, D Eglin, M Alini and MD Menger. (2013). Three-dimensional spheroids of adipose-derived mesenchymal stem cells are potent initiators of blood vessel formation in porous polyurethane scaffolds. Acta Biomater 9:6876–6884.
50. Derda R, A Laromaine, A Mammoto, SK Tang, T Mammoto, DE Ingber and GM Whitesides. (2009). Papaer-supported 3D cell culture for tissue-based bioassays. Proc Natl Acad Sci U S A 106:18457–18462.
51. Ota H and N Miki. (2013). Microtechnology-based three-dimensional spheroid formation. Front Biosci (Elite Ed) 5:37–48.
52. Burdick JA and G Vunjak-Novakovic. (2009). Engineered microenvironments for controlled stem cell differentiation. Tissue Eng Part A 15:205–219.
53. Uzer G, WR Thompson, B Sen, Z Xie, SS Yen, S Miller, G Bas, M Styner, CT Rubin, et al. (2015). Cell mechanosensitivity to extremely low-magnitude signals is enabled by a LINCed nucleus. Stem Cells 33:2063–2076.
54. Watt FM and BL Hogan. (2000). Out of Eden: stem cells and their niches. Science 287:1427–1430.
55. Pelttari K, A Winter, E Steck, K Goetzke, T Hennig, BG Ochs, T Aigner and W Richter. (2006). Premature induction of hypertrophy during in vitro chondrogenesis of

human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice. Arthritis Rheum 54:3254–3266.
56. Arufe MC, A De la Fuente, I Fuentes-Boquete, FJ De Toro and FJ Blanco. (2009). Differentiation of synovial CD-105(+) human mesenchymal stem cells into chondrocyte-like cells through spheroid formation. J Cell Biochem 108:145–155.
57. Arufe MC, A De la Fuente, J Mateos, I Fuentes, FJ De Toro and FJ Blanco. (2011). Analysis of the chondrogenic potential and secretome of mesenchymal stem cells derived from human umbilical cord stroma. Stem Cells Dev 20:1199–1212.
58. Giovannini S, J Diaz-Romero, T Aigner, P Heini, P Mainil-Varlet and D Nesic. (2010). Micromass co-culture of human articular chondrocytes and human bone marrow mesenchymal stem cells to investigate stable neocartilage tissue formation in vitro. Eur Cells Mater 20:245–259.
59. Farrell MJ, MB Fisher, AH Huang, JI Shin, KM Farrell and RL Mauck. (2014). Functional properties of bone marrow-derived MSC-based engineered cartilage are unstable with very long-term in vitro culture. J Biomech 47:2173–2182.
60. Farrel MJ, JI Shin, LJ Smith and RL Mauck. (2015). Functional consequences of glucose and oxygen deprivation on engineered mesenchymal stem cell-based cartilage constructs. Osteoarthritis Cartilage 23:134–142.
61. Arufe MC, A De la Fuente, I Fuentes, FJ de Toro and FJ Blanco. (2010). Chondrogenic potential of subpopulations of cells expressing mesenchymal stem cell markers derived from human synovial membranes. J Cell Biomech 111:834–845.
62. Hill E, T Boontheekul and DJ Mooney. (2006). Regulating activation of transplanted cells controls tissue regeneration. Proc Natl Acad Sci U S A 103:2494–2499.
63. Ceccaldi C, R Bushkalova, C Alfarano, O Lairez, D Calise, P Bourin, C Frugier, C Rouzaud-Laborde, D Cussac, et al. (2014). Evaluation of polyelectrolyte complex-based scaffolds for mesenchymal stem cell therapy in cardiac ischemia treatment. Acta Biomater 10:901–911.
64. Ge J, L Guo, S Wang, Y Zhang, T Cai, RC Zhao and Y Wu. (2014). The size of mesenchymal stem cells is a significant cause of vascular obstructions and stroke. Stem Cell Rev 10:295–303.
65. Guo L, J Ge, Y Zhou, S Wang, RC Zhao and Y Wu. (2014). Three-dimensional spheroid-cultured mesenchymal stem cells devoid of embolism attenuate brain stroke injury after intra-arterial injection. Stem Cells Dev 23: 978–989.
66. Skiles ML, B Hanna, L Rucker, A Tipton, A Brougham-Cook and JO Blanchette. (2015). ASC spheroid geometry and culture oxygenation differentially impact induction of pre-angiogenic behaviors in endothelial cells. Cell Transplant 24:2323–2335.
67. Schifferli JA. (2011). Microvesicles are messengers. Semin Immunopathol 33:393–394.
68. György B, ME Hung, XO Breakefield and JN Leonard. (2015). Therapeutic application of extracellular vesicles: clinical promise and open questions. Annu Rev Pharmacol Toxicol 55:439–464.
69. Théry C, M Ostrowski and E Segura. (2009). Membrane vesicles as conveyors of immune responses. Nav Rev Immunol 9:581–593.
70. Biancone L, S Bruno, MC Dereqibu, C Tetta and G Camussi. (2012). Therapeutic potential of mesenchymal stem cell-derived microvesicles. Nephrol Dial Transplant 27:3037–3042.
71. Sabin K and N Kikyo. (2014). Microvesicles as mediators of tissue regeneration. Transl Res 163:286–295.
72. Maumus M, C Jorgensen and D Noël. (2013). Mesenchymal stem cells in regenerative medicine applied to rheumatic diseases: role of secretome and exosomes. Biochimie 95:2229–2234.
73. Quesenberry PJ, LR Goldberg, JM Aliotta, MS Dooner, MG Pereira, S Wen and G Camussi. (2014). Cellular phenotype and extracellular vesicles: basic and clinical considerations. Stem Cells Dev 23:1429–1436.
74. Huang YC, O Parolini and L Deng. (2013). The potential role of microvesicles in mesenchymal stem cell-based therapy. Stem Cells Dev 22:841–844.
75. Katsuda T, R Tsuchiya, N Kosaka, Y Yoshioka, K Takagaki, K Oki, F Takeshita, Y Sakai, M Kuroda and T Ochiya. (2013). Human adipose-tissue-derived mesenchymal stem cells secrete functional neprilysin-bound exosomes. Sci Rep 3:1197.
76. Ratajczak J, M Wysoczynski, F Hayek, A Janowska-Wieczorek and MZ Ratajczak. (2006). Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. Leukemia 20:1487–1495.
77. Camussi G, MC Deregibus, S Bruno, V Cantaluppi and L Biancone. (2010). Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int 78:838–848.
78. Kim HS, DY Choi, SJ Yun, SM Choi, JW Kang, JW Jung, D Hwang, KP Kim and DW Kim. (2012). Proteomic analysis of microvesicles derived from human mesenchymal stem cells. J Proteome Res 11:839–849.
79. Choi DS, DK Kim, YK Kim and YS Gho. (2013). Proteomics, transcriptomics and lipidomics of exosomes and ectosomes. Proteomics 13:1554–1571.
80. Sze SK, DP de Kleijn, RC Lai, E Khia Way Tan, H Zhao, KS Yeo, TY Low, Q Lian, CN Lee, et al. (2007). Elucidating the secretion proteome of human embryonic stem cell-derived mesenchymal stem cells. Mol Cell Proteomics 6:1680–1689.
81. Lai RC, SS Tan, BJ Teh, SK Sze, F Arslan, DP de Kleijn, A Choo and SK Lim. (2012). Proteolytic potential of the MSC exosome proteome: implications for an exosome-mediated delivery of therapeutic proteasome. Int J Proteomics 2012:971907.
82. Bruno S, C Grange, MC Deregibus, RA Calogero, S Saviozzi, F Collino, L Morando, A Busca, M Falda, et al. (2009). Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. J Am Soc Nephrol 20:1053–1067.
83. Zhu YG, XM Feng, J Abbott, XH Fang, Q Hao, A Monsel, JM Qu, MA Matthay and JW Lee. (2014). Human mesenchymal stem cell microvesicles for treatment of *Escherichia coli* endotoxin-induced acute lung injury in mice. Stem Cells 32:116–125.
84. Ratajczak J, K Miekus, M Kucia, J Zhang, R Reca, P Dvorak and MZ Ratajczak. (2006). Embryonic stem cells-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. Leukemia 20:847–856.
85. Deregibus MC, V Cantaluppi, R Calogero, M Lo Iacono, C Tetta, L Biancone, S Bruno, B Bussolati and G Camussi.

(2007). Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. Blood 110:2440–2448.
86. Valadi H, K Ekström, A Bossios, M Sjöstrand, JJ Lee and JO Lötvall. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9:654–659.
87. Chen TS, RC Lai, MM Lee, AB Choo, CN Lee and SK Lim. (2010). Mesenchymal stem cell secerete microparticles enriched in pre-microRNAs. Nucleic Acids Res 38:215–224.
88. Chen TS and Lim SK. (2013). Measurement of precursor miRNA in exosomes from human ESC-dervied mesenchymal stem cells. Methods Mol Biol 1024:69–86.
89. Koh W, CT Sheng, B Tan, QY Lee, V Kuznetsov, LS Kiang and V Tanavde. (2010). Analysis of deep sequencing microRNA expression profile from human embryonic stem cells derived mesenchymal stem cells reveals possible role of let-7 microRNA family in downstream targeting of hepatic nuclear factor 4 alpha. BMC Genomics 11 (Suppl. 1):S6.
90. Feng Y, W Huang, M Wani, X Yu and M Ashraf. (2014). Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22. PLoS One 9:e88685.
91. Xin H, Y Li, Z Liu, X Wang, X Shang, Y Cui, ZG Zhang and M Chopp. (2013). MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. Stem Cells 31:2737–2746.
92. Xin H, Y Li, B Buller, M Katakowski, Y Zhang, X Wang, X Shang, ZG Zhang and M Chopp. (2012). Exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. Stem Cells 30:1556–1564.
93. Lee JK, SR Park, BK Jung, YK Jeon, YS Lee, MK Kim, YG Kim, JY Jang and CW Kim. (2013). Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating VEGF expression in breast cancer cells. PLoS One 8:e84256.
94. Katakowski M, B Buller, X Zheng, Y Lu, T Rogers, O Osobamiro, W Shu, F Jiang and M Chopp. (2013). Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. Cancer Lett 335:201–204.
95. Joyce N, G Annett, L Wirthlin, S Olson, G Bauer and JA Nolta. (2010). Mesenchymal stem cells for the treatment of neurodegenerative disease. Regen Med 5: 933–946.
96. Ludwig AK and B Giebel. (2012). Exosomes: small vesicles participating in intercellular communication. Int J Biochem Cell Biol 44:11–15.
97. Lee Y, S EI Andaloussi and MJ Wood. (2012). Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum Mol Genet 21(R1):R125–R134.
98. Schorey JS and S Bhatnagar. (2008). Exosome function: from tumor immunology to pathogen biology. Traffic 9:871–881.
99. Candelario KM and DA Steindler. (2014). The role of extracellular vesicles in the progression of neurodegenerative disease and cancer. Trends Mol Med 20:368–374.
100. Lee TH, E D'Asti, N Magnus, K Al-Nedawi, B Meehan and J Rak. (2011). Microvesicles as mediators of intercellular communication in cancer-the emerging science of cellular 'debris'. Semin Immunopathol 33:455–467.
101. Bruno S, F Collino, MC Deregibus, C Grange, C Tetta and G Camussi. (2013). Microvesicles derived from human bone marrow mesenchymal stem cells inhibit tumor growth. Stem Cells Dev 22:758–771.
102. Mizrak A, MF Bolukbasi, GB Ozdener, GJ Brenner, S Madlener, EP Erkan, T Ströbel, XO Breakefield and O Saydam. (2013). Genetically engineered microvesicles carrying suicide mRNA/protein inhibit schwannoma tumor growth. Mol Ther 21:101–108.
103. Burns JS, A Safwat, G Grisendi, M Kassem and M Dominici. (2012). Sarcomas as a mise en abyme of mesenchymal stem cells: exploiting interrelationships for cell mediated anticancer therapy. Cancer Lett 325:1–10.
104. Ono M, N Kosaka, N Tominaga, Y Yoshioka, F Takeshita, RU Takahashi, M Yoshida, H Tsuda, K Tamura and T Ochiya. Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. Sci Signal 7:ra63.
105. Pascucci L, V Coccè, A Bonomi, D Ami, P Ceccarelli, E Ciusani, L Viganò, A Locatelli F Sisto, et al. (2014). Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumor growth: a new approach for drug delivery. J Control Release 192:262–270.
106. Roccaro AM, A Sacco, P Maiso, AK Azab, YT Tai, M Reagan, F Azab, LM Flores, F Campigotto, et al. (2013). BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression. J Clin Invest 123: 1542–1555.
107. Wang M, C Zhao, H Shi, B Zhang, L Zhang, X Zhang, S Wang, X Wu, T Yang, et al (2014). Deregulated microRNAs in gastric cancer tissue-derived mesenchymal stem cells: novel biomarkers and a mechanism for gastric cancer. Br J Cancer 110:1199–1210.
108. Li Y, J Chen, L Wang, M Lu and M Chopp. (2001). Treatment of stroke in rat with intracarotid administration of marrow stromal cells. Neurology 56:1666–1672.
109. Tsai LK, Z Wang, J Munasinghe, Y Leng, P Leeds and DM Chuang. (2011). Mesenchymal stem cells primed with valproate and lithium robustly migrate to infracted regions and facilitate recovery in a stroke model. Stroke 42:2932–2939.
110. Mokarizadeh A, N Delirezh, A Morshedi, G Mosayebi, AA Farshid and K Mardani. (2012). Microvesicles derived from mesenchymal stem cells: potent organelles for induction of tolerogenic signaling. Immunol Lett 147:47–54.
111. Xin H, Y Li, Y Cui, JJ Yang, ZG Zhang and M Chopp. (2013). Systemic administration of exosomes releasesd from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. J Cereb Blood Flow Metab 33:1711–1715.
112. Yang Y, A Otte and R Hass. (2015). Human mesenchymal stroma/stem cells exchange membrane proteins and alter functionality during interaction with different tumor cell lines. Stem Cells Dev 24:1205–1222.
113. Amdur RL, LS Chawla, S Amodeo, PL Kimmel and CE Palant. (2009). Outcomes following diagnosis of acute renal failure in U.S. veterans: focus on acute tubular necrosis. Kidney Int 76:1089–1097.
114. Eirin A, XY Zhu, JD Krier, H Tang, KL Jordan, JP Grande, A Lerman, SC Textor and LO Lerman. (2012). Adipose tissue-derived mesenchymal stem cells improve

revascularization outcomes to restore renal function in swine atherosclerotic renal artery stenosis. Stem Cells 30:1030–1041.
115. Zhu XY, V Urbieta-Caceres, JD Krier, SC Textor, A Lerman and LO Lerman. (2013). Mesenchymal stem cells and endothelial progenitor cells decrease renal injury in experimental swine renal artery stenosis through different mechanisms. Stem Cells 31:117–125.
116. Tögel F, K Weiss, Y Yang, Z Hu, P Zhang and C Westenfelder. (2007). Vasculotropic, paracrine actions of infused mesenchymal stem cells are important to the recovery from acute kidney injury. Am J Physiol Renal Physiol 292:1626–1635.
117. Bi B, R Schmitt, M Israilova, H Nishio and LG Cantley. (2007). Stromal cells protect against acute tubular injury via an endocrine effect. J Am Soc Nephrol 18:2486–2496.
118. Gatti S, S Bruno, MC Deregibus, A Sordi, V Cantaluppi, C Tetta and G Camussi. (2011). Microvesicle derived from human adult mesenchymal stem cells protect against ischemia-reperfusion-induced acute and chronic kidney injury. Nephrol Dial Transplant 26:1474–1483.
119. Lindoso RS, F Collino, S Bruno, DS Araujo, JF Sant'Anna, C Tetta, P Provero, PJ Quesenberry, A Vieyra, M Einicker-Lamas and G Camussi. (2014). Extracellular vesicles released from mesenchymal stromal cells modulate miRNA in renal tubular cells and inhibit ATP depletion injury. Stem Cells Dev 23:1809–1819.
120. Tomasoni S, L Longaretti, C Rota, M Morigi, S Conti, E Gotti, C Capelli, M Introna, G Remuzzi and A Benigni. (2013). Transfer of growth factor receptor mRNA via exosomes unravels the regenerative effect of mesenchymal stem cells. Stem Cells Dev 22:772–780.
121. Kunter U, S Rong, P Boor, F Eitner, G Müller-Newen, Z Djuric, CR van Roeyen, A Konieczny, T Ostendorf, et al. (2007). Mesenchymal stem cells prevent progressive experimental renal failure but maldifferentiate into glomerular adipocytes. J Am Soc Nephrol 18:1754–1764.
122. Karp JM and GS Leng Teo. (2009). Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell 4:206–216.
123. Duttenhoefer F, R Lara de Freitas, T Meury, M Loibl, LM Benneker, RG Richards, M Alini and S Verrier. (2013). 3D scaffolds co-seeded with human endothelial progenitor and mesenchymal stem cells: evidence of prevascularisation within 7 days. Eur Cell Mater 26: 49–64.
124. Zhang Y, M Chopp, Y Meng, M Katakowski, H Xin, A Mahmood and Y Xiong. (2015). Effect of exosomes derived from multipotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brains injury. J Neurosurg 122:856–867.
125. Zhuang X, X Xiang, W Grizzle, D Sun, S Zhang, RC Axtell, S Ju, J Mu, L Zhang, et al. (2011). Treatment of brain inflammatory disease by delivery exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. Mol Ther 19:1769–1779.
126. Chevillet JR, Q Kang, IK Ruf, HA Briggs, LN Vojtech, SM Hughes, HH Cheng, JD Arroyo, EK Meredith, et al. (2014). Quantitative and stoichiometric analysis of the microRNA content of exosomes. Proc Natl Acad Sci U S A 111:14888–14893.
127. de Jong R, GP van Hout, JH Houtgraaf, K Kazemi, C Wallrapp, A Lewis, G Pasterkamp, IE Hoefer and HJ Duckers. (2014). Intracoronary infusion of encapsulated glucagon-like peptidlle-1-eluting mesenchyma stem cells preserves left ventricular function in a porcine model of acute myocardial infarction. Circ Cardiovasc Interv 7:673–683.
128. Yang JX, N Zhang, HW Wang, P Gao, QP Yang and QP Wen. (2015). CXCR4 overexpression in mesenchymal stem cells facilitates treatment of acute lung injury in rats. J Biol Chem 290:1994–2006.
129. Maguire CA, L Balaj, S Sivaraman, MH Crommentuijn, M Ericsson, L Mincheva-Nilsson, V Baranov, D Gianni, BA Tannous, et al. (2012). Microvesicle-associated AAV vector as a novel gene delivery system. Mol Ther 20:960–971.

Address correspondence to:
Dr. Bing Jiang
Department of Ophthalmology
The Second Xiangya Hospital
Central South University
No. 139 Remin Middle Road
Changsha 410011
China

E-mail: bingj82@126.com

Received for publication August 29, 2015
Accepted after revision November 16, 2015
Prepublished on Liebert Instant Online November 17, 2015