ISSN 1948-0210 (online)

World Journal of *Stem Cells*

World J Stem Cells 2019 May 26; 11(5): 236-280

Published by Baishideng Publishing Group Inc

WJSC

World Journal of Stem Cells

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Monthly Volume 11 Number 5 May 26, 2019

REVIEW

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World Journal of Stem Cells

Volume 11 Number 5 May 26, 2019

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RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Yun-Xiaojian Wu* **Proofing Editorial Office Director:** *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Stem Cells

ISSN
ISSN 1948-0210 (online)

LAUNCH DATE
December 31, 2009

FREQUENCY
Monthly

PUBLICATION DATE
May 26, 2019

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

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World Journal of Stem Cells

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World J Stem Cells 2019 May 26; 11(5): 270-280

DOI: 10.4252/wjsc.v11.i5.270

ISSN 1948-0210 (online)

REVIEW

Generation of mesenchymal stem-like cells for producing extracellular vesicles

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Author contributions: All authors contributed to manuscript preparation; Kim S contributed towards conception of the ideas and collecting the data; Kim TM contributed towards outlining the structure of the manuscript.

Supported by the Research Resettlement Fund for the New Faculty of Seoul National University, No. 1403-20180039.

Conflict-of-interest statement: No potential conflicts of interest.

Manuscript source: Invited manuscript

Received: February 10, 2019
Peer-review started: February 12, 2019
First decision: March 15, 2019
Revised: April 2, 2019
Accepted: April 19, 2019
Article in press: April 19, 2019

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Abstract

Mesenchymal stem cells (MSCs) are multipotent progenitor cells with therapeutic potential against autoimmune diseases, inflammation, ischemia, and metabolic disorders. Contrary to the previous conceptions, recent studies have revealed that the tissue repair and immunomodulatory functions of MSCs are largely attributed to their secretome, rather than their potential to differentiate into desired cell types. The composition of MSC secretome encompasses cytokines and growth factors, in addition to the cell-derived structures known as extracellular vesicles (EVs). EVs are membrane-enclosed nanoparticles that are capable of delivering biomolecules, and it is now believed that MSC-derived EVs are the major players that induce biological changes in the target tissues. Based on these EVs' characteristics, the potential of EVs derived from MSC (MSC-EV) in terms of tissue regeneration and immune modulation has grown during the last decade. However, the use of MSCs for producing sufficient amount of EVs has not been satisfactory due to limitations in the cell growth and large variations among the donor cell types. In this regard, pluripotent stem cells (PSCs)-derived MSC-like cells, which can be robustly induced and expanded *in vitro*, have emerged as more accessible cell source that can overcome current limitations of using MSCs for EV production. In this review, we have highlighted the methods of generating MSC-like cells from PSCs and their therapeutic outcome in preclinical studies. Finally, we have also discussed future requirements for making this cell-free therapy clinically feasible.

Key words: Mesenchymal stem cells; Extracellular vesicles; Preclinical studies; Exosomes

Published online: May 26, 2019

P-Reviewer: Scuteri A, Miloso M
S-Editor: Dou Y
L-Editor: A
E-Editor: Wu YXJ

Core tip: The therapeutic potential of extracellular vesicles (EVs) from mesenchymal stem cells (MSCs) has recently been reported. However, alternative MSC sources are needed to produce EVs in a quality-controlled manner. Pluripotent stem cells-derived MSC-like cells can be robustly induced *in vitro*, and therefore induced MSC-like cells (iMSCs) have the possibility to become an alternative source for EV production. Herein, we review current data on iMSC generation, and provide key outcomes of their therapeutic functioning in preclinical studies.

Citation: Kim S, Kim TM. Generation of mesenchymal stem-like cells for producing extracellular vesicles. *World J Stem Cells* 2019; 11(5): 270-280
URL: https://www.wjgnet.com/1948-0210/full/v11/i5/270.htm
DOI: https://dx.doi.org/10.4252/wjsc.v11.i5.270

INTRODUCTION

There has been a growing interest in mesenchymal stem cells (MSCs) for clinical applications due to their immunomodulatory, angiogenic, anti-apoptotic, and tissue regenerative properties[1]. Additionally, they are relatively immunologically privileged due to low expression of major histocompatibility complex antigens compared with embryonic stem or induced pluripotent cells (ESC/iPSC), making MSCs readily implantable[2]. Despite their potential for clinical value and safety, as can be seen in the list of ongoing clinical trials, their therapeutic efficiency remains unclear, accompanied by inconsistent clinical outcomes[3,4]. Moreover, the possibility of rejection by allogenic MSCs and tumor formation *in vivo* are other concerns during the MSC therapy[5]. Furthermore, multiple studies on the biodistribution of MSCs demonstrated that the *in vitro*-expanded MSCs generally accumulated in the lungs, and become almost impossible to trace for longer than 3-4 d[6-9]. Thus, an alternative source of MSCs is needed to overcome these hurdles, and enhance therapeutic application.

Generally, it is now believed that the regenerative potential of stem cells is mainly due to its ability to (1) differentiate into specialized cells; (2) stimulate endogenous cells for repair; or (3) produce paracrine factors including growth factors and extracellular vesicles (EVs)[10,11]. Although it was initially believed that MSCs replace the injured cells in the damaged tissues, various studies have reported that they may not primarily fulfill this repair function in a direct, cellular manner[12,13]. In fact, increasing evidence suggest that therapeutic outcome of stem cells are a consequence of the paracrine effects mediated by their secretome[14]. Besides numerous growth factors and cytokines, it is now recognized that MSC-EVs constitute an essential part of the stem cell secretome that harbors functional biomolecules such as DNA, RNA, and lipids[15,16]. Before the identification of MSC-EV as an indispensable player in the MSCs' functioning, an early study by Timmers *et al*[17] showed that conditioned media (CM) from hESC-derived MSCs rescued the myocardial infarct (MI) size, and functionally enhanced the cardiac performance in a porcine model. Most importantly, fractionation study showed that only a sub-fraction within the CM, which had products larger than 1000 kDa (100-220 nm), was effective in such role. Through this study, it was revealed that the therapeutic outcome of paracrine mediator is not caused by the whole secretome from MSCs, but from its specific compartment. Few years later, Lee *et al*[18] showed that intravenously injected exosomes collected from the CM of mouse MSCs significantly attenuated the hypoxic pulmonary hypertension in mice. Furthermore, authors demonstrated that such anti-inflammatory effect of MSC-derived exosomes was due to the inhibition of STAT3 signaling. Since this pioneering work, the therapeutic functions of MSC-derived exosomes and microvesicles have been investigated for a wide range of experiments using various preclinical models[19,20].

Despite the advantages of EVs as an alternative to MSCs, current protocols for preparing sufficient amount of MSCs are suboptimal. For example, the functions and genetic integrity of MSCs could be negatively affected by an extended culture period[21,22]. Moreover, MSCs do not have definitive surface markers due to their innate heterogeneity, and their biological traits largely vary among donor cell types[23,24]. Since pluripotent stem cells (PSCs) can be expanded robustly *in vitro*, induced MSC-like cells (iMSCs) from PSCs are now recognized as an alternative source for producing EVs in a scalable mode[25,26]. Here, we review the current

methods of MSC derivation from PSCs, and give a detailed account of the recent progress in the preclinical studies regarding the therapeutic role of EVs from PSC-derived MSCs in order to facilitate tissue repair and immune modulation.

MSCs: ORIGIN AND BIOLOGICAL TRAITS

MSCs are a population of endogenous stem cells present in almost all adult organs and tissues. They are primarily obtained from bone marrow and adipose tissues. However, other organs including umbilical cord tissue, amniotic fluid, skin, and placenta[27,28] have also been reported to have MSCs. Developmentally, several reports in rodent studies suggest that MSCs are mostly originated from late plate mesoderm and neural crest tissue[1,29]. Functionally, bone marrow MSCs support the stroma structure needed for hematopoiesis, while MSCs from other tissues are known to be involved in the repair process upon injury[11,30]. Most importantly, MSCs are capable of differentiating into multiple cell types such as osteocytes, chondrocytes, adipocytes, hepatocytes, myocytes *etc*[31]. Several studies also show that their regenerative capacity is distinct depending upon their tissue of origin, indicating that other factors involved in their niche formation are vital in rendering them with therapeutic potential[32,33].

MSCs AS A TOOL FOR CELL THERAPY

Compared with ESCs and iPSCs, utilization of MSCs is now regarded as the most potent stem cell resource because they are multipotent, immunologically tolerable, free from ethical concerns, and have low possibility of teratoma formation[34,35]. Functionally, their potential has been exploited for treatment of many degenerative, neurologic, and ischemic diseases. Besides, MSCs hold great promise for cell based therapeutics in inflammatory and autoimmune diseases, owing to their innate immunomodulatory ability[2,36]. However, the use of MSCs for clinical purposes could be restricted by several factors. Most of all, *in vitro* expansion of MSCs over an extended period is complex due to replicative senescence, related with their decreased functionality[37]. In addition, developing a standardized method for quality control of various tissue and donor cells having high variability is difficult. Accordingly, other alternatives are considered necessary to obtain more stable and reliable source of MSCs, and recent studies have shown that MSC-like cells can be generated from PSCs *in vitro* using various experimental protocols (Figure 1).

PSC-DERIVED MSCs

So far, a diverse range of protocols have been developed to derive MSCs from PSCs. An early study of *in vitro* generation of MSCs was conducted by co-culturing of hESCs and mouse bone marrow stroma cell line OP9. Following this, the MSC-specific surface marker CD73-positive cells were sorted[38]. Upon further culturing, the cells exhibited typical fibroblast-like shape and expressed the surface marker for MSCs. They also exhibited multilineage differentiation potential towards connective tissues (cartilage, bone, fat, and skeletal muscles). One year later, a study was published wherein a feeder-free strategy was used to derive functional MSCs from hESC[39]. This research group attempted to mechanically pick up spontaneously differentiated cells in the middle or at the outward edges of hESC colonies, and then cultured the cells in serum-containing DMEM (Dulbecco's modified Eagle's medium) for a minimum period of 4 wk to obtain thick epithelial-like cells. The MSCs were then isolated by enzymatic dissociation and cultured and passaged to obtain a monolayer of fibroblast-like cells, bearing the capacity to differentiate into osteocytes and adipocytes. A more directed method to obtain MSCs was used by first subjecting hESCs to differentiation *via* embryoid body (EB) formation in a low-attachment plate, followed by culturing the EBs in a gelatin-coated plate[40]. After subpassaging, homogenous fibroblast-like cells that subsequently differentiated into osteoblasts and adipocytes were obtained. Lian *et al*[41] described a more specific protocol to derive MSCs from hESCs by culturing trypsinized hESCs with basic fibroblast growth factor (bFGF) and platelet-derived growth factor AB under feeder-free conditions, without using animal-derived products. Within two weeks, $CD105^{+}CD24^{-}$ cells (5% of the total cell population) were sorted, where CD105 and CD24 were used for selecting MSCs and ESCs, respectively. These cells also differentiated into osteocytes, adipocytes, and chondrocytes under standard differentiation protocols. Based on this procedure, iPSC-derived MSCs were also derived under differentiation conditions in the same media

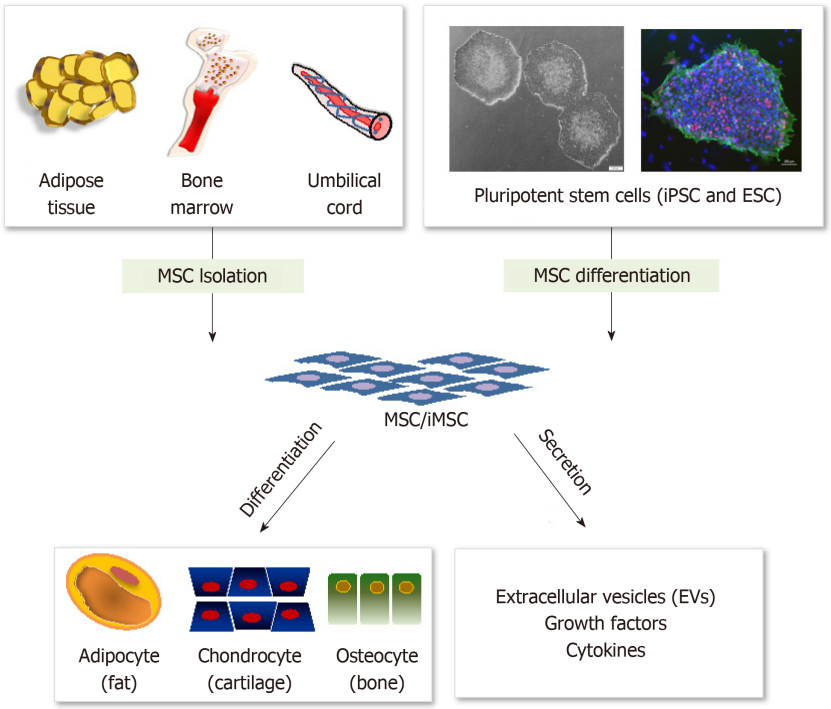

Figure 1 Multifaceted origin and application of mesenchymal stem cells. Mesenchymal stem cells (MSCs) can be isolated directly from various donor tissue types including adipose tissue, bone marrow or umbilical cord. MSC-like cells (induced MSC; iMSC) can be induced from pluripotent stem cells (iPSC, induced pluripotent stem cell). MSC/iMSC can be differentiated to multiple cells types including adipocytes, chondrocytes or osteocytes. The secreted products from MSC/iMSC such as extracellular vesicles, growth factors and cytokines also play essential role in their regenerative function. MSC: Mesenchymal stem cell; iMSC: Induced mesenchymal stem cell; ESC: Embryonic stem cell.

supplemented with additional factors including epidermal growth factor[42]. After being intramuscularly transplanted into the critical limb ischemic model in severe combined immunodeficient mice, the iPSC-MSCs alleviated the progression of severe hind-limb ischemia and enhanced vessel regeneration. MSC-like cells were also obtained using collagen type I as a matrix[43] since this has been known to promote differentiation of MSCs through integrin-mediated signaling[44,45]. In addition, a small molecule-induced protocol was introduced to generate MSCs from hESCs/hiPSCs by inhibiting pathways required for maintenance of pluripotency. Chen *et al*[46] cultured ESCs/iPSCs in serum-free medium containing the transforming growth factor pathway inhibitor (SB431542) for 10 d followed by subsequent culture in conventional MSC medium. By utilizing these serum- or coculture- free methods, it was possible to obtain uniform MSCs from pluripotent cells in a robust and clinically compliant way. Overall, these literatures described various methods for generating iMSCs from PSCs, which are from different origin, and it should be noted that those iMSCs may have heterogenous characteristics and functions such as epigenetic profile, the contents of secretome, and the ability in immune regulation and injury recovery. Thus, it is of critically importance to clearly define the biological characteristics of newly established iMSCs to standardize their usages.

COMPARISON OF MSCs AND iMSCs

In relation to the PSC-derived MSCs, Billing *et al*[47] compared the protein and RNA contents between ESC-derived MSC (ESC-MSC) and bone marrow-derived MSC (BM-MSC) using proteomics and RNA sequencing. GO term analysis showed that several biological processes including ECM organization, vesicle-mediated transport were enriched when comparing BM-MSC to ESC-MSC. In contrast, pathways involved in cell cycle and nuclear division were enriched in ESC-MSC. Further enrichment analysis in terms of development showed that the development of neuron, axon, stem cell, and embryo was enriched in ESC-MSC, whereas the vasculature development was comparatively more enriched in BM-MSC[47]. Another study demonstrated that ESC-MSCs had a greater impact than the BM-MSCs in reducing the progression of

multiple sclerosis using an experimental autoimmune encephalomyelitis model[48], and such therapeutic role may be owed to the EVs produced from ESC-MSC.

Despite the high potential of the PSC-derived MSCs for clinical uses, the biological characteristics such as differentiation potential differ from those originating from bone marrow or adipose tissue. For example, an early study showed that the mesodermal sarcomeric genes (*i.e.*, MYH2, SOX2, TNNI1, ACTA1, and GATA4) were expressed in hES-MSCs, while they were minimally expressed in the hMSCs. This indicates that hES-MSCs have more primitive characteristics than hMSCs, supporting the notion that hES-MSCs are a distinct, less-characterized cell population in a transitional state of development[49]. It would be important to explore whether hES-MSCs' primitiveness affects the therapeutic potential of their secretome, including EVs. Years later, Diederichs *et al*[50] compared the gene expression and differentiation potential between iMSCs obtained from BM-MSC and the original BM-MSCs from the same donor, and found that iMSCs displayed an MSC-like morphology and MSC-related surface marker expression. Most importantly, the trilineage differentiation potential of iMSCs was not as efficient as that of BM-MSCs. This implicates the necessity to develop a distinguishing criteria for the basic cellular traits of iMSCs, rather than following the conventional definition suggested by the International Society for Cellular Therapy[51]. Collectively, it is evident that PSC-derived MSCs are a unique cell source having different characteristics most likely lesser differentiated than the adult derived MSCs.

DIRECT REPROGRAMMING OF SOMATIC CELLS INTO MSCs

Since the impending risk of tumorigeneicity of PSC-derived MSCs can be a major limitation for their therapeutic use, a better strategy for producing immunologically safe MSCs would be direct reprogramming of terminally differentiated somatic cells. Using a cocktail comprising various cell signaling inhibitors (SP600125, SB202190, Go6983, Y-27632, PD0325901, and CHIR99021, which are the inhibitors of JNK, p38, PKC, ROCK, ERK1/2, GSK3β, respectively) and growth factors (TGF-β1, bFGF, and leukemia inhibitory factor), adult dermal fibroblasts were converted into MSC-like cells within 6 d[52]. Functionally, these cells can readily be expanded for eight or more passages, and differentiated into three mesenchymal tissues such as osteocytes, adipocytes, and chondrocytes. Notably, these cells succeeded in attenuating the LPS-mediated lung injury to a degree comparable to bone marrow-derived MSCs, indicating that MSC-like cells having anti-inflammatory functions can be generated from fibroblasts within weeks. An earlier report by Meng *et al*[53] also demonstrated that $CD34^+$ cells from cord blood or adult peripheral blood could be trans-differentiated to MSC-like cells by OCT4 overexpression in the presence of GSK3 inhibitor. They found that higher level of OCT4 plays a central role in the reprogramming process and the self-renewal of iMSCs, and that OCT4 expression should be down regulated to allow iMSCs to gain differentiation potential.

PRECLINICAL STUDIES FOR EVALUATING iMSC-EV

So far, the therapeutic function of iMSC-EV in preclinical studies has been demonstrated in tissue repair models. The recent progress of the preclinical evaluations on iMSC-EVs is listed in Table 1. The earliest evidence of the therapeutic role of iMSC-EV was shown in a mice model of hind limb ischemia[54]. MSCs were derived from iPSCs by culturing the latter in low glucose DMEM supplemented with 10% FBS for several passages, and exosomes were produced from the medium conditioned for 48 h. The detailed procedure of concentration and purification of EVs is described in Table 1. With this protocol, iMSC-exo was able to enhance the vessel density and blood perfusion in the ischemic limb. Moreover, iMSC-exo was shown to stimulate the expression of angiogenic genes, and enhance endothelial cell migration and proliferation. The regenerative potential of iMSC-EV was subsequently demonstrated using cutaneous wound healing model[55]. Topical administration of iMSC-EV on the wound site resulted in recovery, as shown by the enhanced epithelialization and reduced the wound size. In addition, iMSC-exo induced increased neovascularization and maturation of vessels. *In vitro*, iMSC-exo treatment stimulated the migration and growth of dermal fibroblasts and HUVEC (human umbilical vein endothelial cell), and Collagen and Elastin protein secretions were increased. We also recently found that the proliferation of skin epithelial cell was more significant after iMSC-exo treatment compared to those treated with exosomes

from Wharton's jelly tissue derived MSCs, and that such effect was due to the activation of ERK1/2 signaling in skin cells[56]. The trophic role of iMSC-exo was also revealed in a rodent models of hepatic ischemia/reperfusion[57,58]. In one approach in rat model, iMSC-exo had been injected into inferior vena cava immediately after reperfusion following an ischemic period (60 min), and improved hepatic histology including reduced necrosis and sinusoidal congestion were observed. Moreover, the levels of liver injury markers (ALT and AST) and inflammatory proteins (TNF-α and HMGB1) were reduced by iMSC-exo treatment. Finally, decreased levels of apoptotic markers (Caspase3 and Bax) and increased level of anti-oxidant proteins (GSH, GSH-Px, SOD) were observed[57].

Based on the osteogenic potential of MSCs, the reparative function of iMSC-exo has also been tested in a rat model of osteoporosis. In this report, it was shown that the bone marrow MSCs from osteoporotic rats, when treated with iMSC-exo, gained better ability to proliferate and differentiate into osteoblasts. Moreover, transplantation of β-TCP (Beta-tricalcium phosphate) scaffolds lyophilized with iMSC-exosomes facilitated bone regeneration as well as vessel formation *via* a calvarial injury model[59]. The potential of iMSC-exo was also evaluated in steroid-induced osteonecrosis model in rats. A reduced bone loss and augmented vessel density was found in the femoral head after iMSC-exo was intramuscularly injected. *In vitro* assays revealed that iMSC-exo enhanced the growth, migration, and vessel formation of HUVECs, and that PI3K/AKT signaling was activated *via* iMSC-exo[60]. A more recent study compared the therapeutic efficacy of exosomes produced from iMSC (iMSC-exo) and synovial membrane MSCs (SMMSC-exo) in a collagen-induced osteoarthritis mice model. Notably, iMSC-exo showed enhanced therapeutic effect over SMMSC-exo. Also, iMSC-exo was more effective in enhancing the migration and growth of chondrocytes compared to SMMSC-exo[61]. Collectively, these preclinical and *in vitro* studies indicate that iMSC-exo carry the potential to promote tissue regeneration in skin wounds, blood vessels, bones, liver, and articular chondrocytes.

THE DISTINCT CONTENTS OF iMSC-EVs AND THEIR FUTURE APPLICATION

Although the potential use of iMSC-EV has been demonstrated in various animal disease models, this cell-free therapeutic strategy should be further optimized and standardized to become clinically feasible. The molecular profile of EVs should be critically characterized to understand their biological function, since they are the end-product of the bioprocessing of conditioned medium from MSC culture[62]. At the same time, the analysis of the EV-secreting cells is also important, because the molecular profile of cell-derived EVs is directly affected by the biology of the secreting cells[14]. Several in-depth studies have been conducted to identify the difference of microRNAs between MSC-EV and MSCs. Shao *et al*[63] demonstrated that EVs collected from the cultured rat bone marrow-derived MSCs (MSC-EV) had a similar expression profile of microRNAs against MSCs, suggesting that MSC-EV represents a functional moiety of the MSCs. On the other hand, the same group also identified several microRNAs that were expressed differentially between MSC-EV and MSCs. Another study showed that the small RNA content of EVs derived from MSCs is higher than that from parental MSCs, and additionally, the expression levels of miRNA-155 and miRNA-146, which are functional regulators of inflammation, had been altered by inflammatory priming of the cells[64]. This suggests that the biological contents of MSC-EV and MSCs may be dissimilar, and it should be noted that standardization of parameters in the contents, function, and bioprocessing of EVs is needed for their specific usage. Another issue that should be addressed upon using iMSCs is their distinct functional and epigenetical characteristics. It has been recently reported that iMSCs are less immunomodulatory than isogenic MSCs, as shown by reduced activity on inhibiting T cell replication. Also, re-acquisition of tissue- or age-specific DNA methylation did not occur, indicating that the therapeutic role of iMSCs should be thoroughly assessed[65].

Another critical point among others is improving the yield of iMSC-EV, since the conventional cell culture system is not suitable for acquiring large amount of EVs. In this respect, three-dimensional scaffold with native or synthetic biomaterial can be generated to mimic the distinct niche to increase the amount of EVs generated from iMSCs[66,67]. Another approach to gain a sizable amount of EVs is to use larger amount of cell culture using bioreactors, *e.g*., microcarrier/spinner or hollow-fiber system[68-70]. Due to the innate tumorigenic potential of PSCs, an unknown risk is the chance of delivering tumorigenic EVs to the recipient tissue. Although the oncogenic potential can be significantly reduced by using iMSC-EV compared to iMSC itself, an optimized

Table 1 Preclinical studies on the therapeutic effect of human extracellular vesicles derived from mesenchymal stem-like cells

Animal	Disease model	Methods for generating mesenchymal stem-like cells (iMSCs)	Extracellular vesicle preparation		Route, time, and dose	Main outcome	Ref.
			Production	Isolation			
Mice	Hindlimb ischemia	Culture of iPSCs with DMEM with FBS (10%) and L-Glutamine (2 mmol) for 14 d (P1) followed by subsequent passaging (up to P4)	Culture with MesenGro® at 80% confluency for 48 h	UC followed by purification by concentration (Amicon®) and additional UC using sucrose (30%) density	Four injections of iMSC-exosome (200 μg) into the quadriceps muscle after 24 h of femoral artery excision	Decrease of ischemic injury with the increase of vessel density and blood perfusion	[54]
Rat	Dorsal skin wound	Same as ref[54] but not clear of the cell passages	Culture with MesenGro® at 80% confluency for 48 h	UC followed by purification by concentration with filter (100 kDa MWCO)	Four injections of iMSC-exosome (160 μg) around the wound sites	(1) Reduction of scar width, with an accelerated epithelialization and increased collagen maturity; (2) Enhanced vessel formation and maturation	[55]
Rat	Partial hepatic ischemia (60 min)	Same as ref[54] but not clear of the passaging time	Same as ref[55]	Same as ref[55]	iMSC-exosome (600 μg) was injected *via* inferior vena cava right after reperfusion	(1) Reduction in hepatocyte necrosis and sinusoidal congestion; (2) Reduced serum level of TNF-α, IL-6, and HMGB 1; (3) Reduced level of apoptotic markers (caspase-3, glutathione peroxidase, superoxide dismutase)	[57]
Mice	Partial hepatic ischemia (60 min)	Same as ref[55]	Same as ref[55] except that DMEM was used for exosome production	ExoQuik® precipitation	iMSC-exosome (2.5×10^{12} particles) were injected *via* the inferior vena cava right after declamping	(1) Reduction in ALT, AST, and hepatocyte necrosis; (2) Reduced sinusoidal congestion with lower pathology score	[58]
Rat	Osteoporosis induced by ovariectomy	Same as ref[55]	Same as ref[55] except that MGro-500® was used for exosome production	Same as ref[55]	Transplantation of β-TCP scaffolds lyophilized with iMSC-exosomes (100 or 200 μg) into the scalp incision	Increase in the bone regeneration and vessel formation as shown by micro-CT, microfil perfusion, and morphological and IHC analyses	[59]
Rat	Steriod-induced osteonecrosis of femoral head	Same as ref[54]	Same as ref[55]	Same as ref[55]	100 μL of iMSC-exosome (1×10^{10}/mL or 1×10^{11}/mL) was I.V. injected before each methylprednisolone injection (once per week for 3 wk)	Inhibition of bone loss and increase of vessel density in the femoral head as shown by micro-CT	[60]

Mice	Collagenase-induced osteoarthritis	Same as ref[55]	Same as ref[55]	UC followed by purification by concentration with filter (100 kDa MWCO) for two repeats	Intra-articular injection of 8 μL iMSC-exosome (1.0×10^{10}/ml) for three times (7, 14, 21 d after induction) and analyzed at day 28	Reduction in the osteonecrotic change as shown by the pathology of macro-morphology (tibial plateus), histology, and IHC	[61]

ALT: Alanine aminotransferase; AST: Aspartate transaminase; β-TCP: Beta-tricalcium phosphate; DMEM: Dulbecco's modified Eagle medium; FBS: Fetal bovine serum; HMGB1: High mobility group box 1; IHC: Immunohistochemistry; iPSCs: Induced pluripotent stem cells; Micro-CT: micro-computed tomography; MWCO: Molecular weight cut-off; UC: Ultracentrifugation; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α; iMSC: Induced mesenchymal stem cell.

protocol is still ardently needed to exclude PSCs within the differentiated iMSC population.

Since functional studies of EVs in animal and human are in the early phase, determining the proper formulation, optimal administration route, and dosage are other hindrances. More comprehensive studies on ADME (absorption, distribution, metabolism, and excretion) of EV will be needed using large, immune-competent disease model animals. Lastly, it should be noted that iMSCs are a population of heterogenous cell types, even within the same passage. Thus, formulation of a standardized protocol for producing clinical-quality EVs is needed to assure their quantity and function.

CONCLUSION

The outcome of recent preclinical and *in vitro* studies suggests that induced MSC-like cells can possibly overcome the limitations of current uses of MSCs for EV production. However, the protocol for inducing PSCs into MSCs should be further refined so that the potentially oncogenic EVs are not produced from less- or un-differentiated PSCs. In addition, a scalable and quality-controllable method based on native/engineered scaffolds, and innovative bioreactor systems should be developed to make this unique cell-free therapy feasible for clinical use.

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