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Concise Review: Mesenchymal Stem Cells Derived from Human Pluripotent Cells, an Unlimited and Quality-Controllable Source for Therapeutic Applications

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Key Words. Mesenchymal stem cells • Human pluripotent stem cells • Derivation • Three dimension • Therapy

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ABSTRACT

Despite the long discrepancy over their definition, heterogeneity, and functions, mesenchymal stem cells (MSCs) have proved to be a key player in tissue repair and homeostasis. Generally, somatic tissue-derived MSCs (st-MSCs) are subject to quality variations related to donated samples and biosafety concern for transmission of potential pathogens from the donors. In contrast, human pluripotent stem cells (hPSCs) are unlimited in supply, clear in the biological background, and convenient for quality control, genetic modification, and scale-up production. We, and others, have shown that hPSCs can differentiate in two dimensions or three dimensions to MSCs (ps-MSCs) via embryonic (mesoderm and neural crest) or extraembryonic (trophoblast) cell types under serum-containing or xeno-free and defined conditions. Compared to st-MSCs, ps-MSCs appear less mature, proliferate faster, express lower levels of inflammatory cytokines, and respond less to traditional protocols for st-MSC differentiation to other cell types, especially adipocytes. Nevertheless, ps-MSCs are capable of immune modulation and treatment of an increasing number of animal disease models via mitochondria transfer, paracrine, exosomes, and direct differentiation, and can be potentially used as a universal and endless therapy for clinical application. This review summarizes the progress on ps-MSCs and discusses perspectives and challenges for their potential translation to the clinic. STEM CELLS *2019;37:572–581*

SIGNIFICANCE STATEMENT

This article provides a thorough and timely review of the history, progress, challenges, and perspective of mesenchymal stem cells derived from human pluripotent cells as a promising, quality-controllable, and unlimited source for therapeutic application.

INTRODUCTION

Mesenchymal stem cells (MSCs) have attracted a breadth of interest from the public and academia for their unique biological characteristics, diverse functions, and potential therapeutic values. Because of their regenerative capabilities and immunomodulatory properties [1], MSCs have been used for the treatments of a broad range of degenerative and inflammatory diseases [2]. MSCs are present in almost all connective tissues and often isolated from somatic (including fetal and adult) tissues such as the umbilical cord and blood, amniotic membrane and fluid, placenta, bone marrow, fat, and dental pulp (Fig. 1). MSCs demonstrate high proliferative capabilities and plasticity to differentiate into many somatic cell lineages and can migrate and home to injured and inflamed tissues [3]. They have provided great promise in the treatment of many degenerative, autoimmune, and inflammatory diseases as well as injuries. However, somatic tissue-derived MSCs (st-MSCs) are restricted by limited donation, quality variations, and biosafety concern for possible transmission of pathogens, if any, from the donors [4].

Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), possess the capability to differentiate into any type of somatic cells, including MSCs [5, 6]. Like st-MSCs, hPSC-derived MSCs (ps-MSCs), including MSCs derived from hESCs (hES-MSCs) and human iPSCs (hiPS-MSCs), have shown similar properties such as multilineage differentiation capability and immunomodulatory effects both in vitro and in vivo [7].

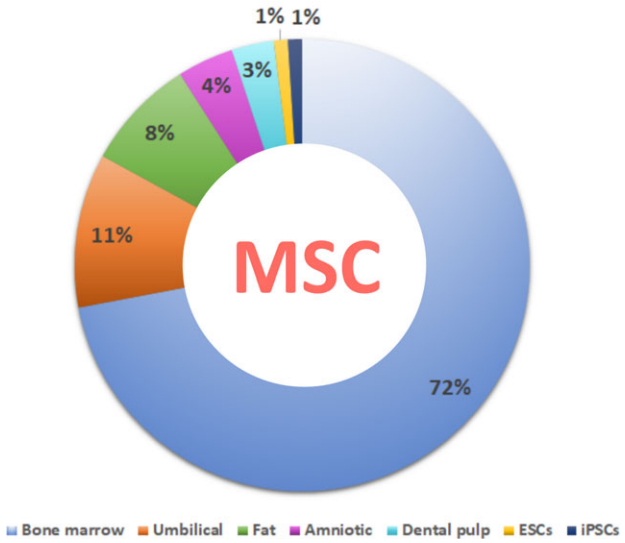


Figure 1. Percentage of publications on MSCs generated from various tissue sources according to PubMed. Abbreviations: ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; MSCs, mesenchymal stem cells.

Table 1. Comparison between ps- and st-MSCs

	st-MSCs	ps-MSCs
Quantity	Variable, depending on donors	Reliable and can be derived from a single hPSCs line
Pathogens	Possible and hard to control from the sources	Rare and easy to control from the sources
Cell number	Limited	Unlimited
Purity	Low	High
Procurement	Difficult and painful when derived from bone marrow	Easy and produced from a cell line without pain
Cost	High	Low
Differentiation efficiency	Higher	Lower
Immunomodulatory effects	Similar	Similar
Proliferation	Slower	Faster
Senescence	Faster	Slower
Genome editing	Harder	Easier

Abbreviations: hPSC, human pluripotent stem cell; ps-MSCs, hPSC-derived mesenchymal stem cell; st-MSCs, somatic tissue-derived mesenchymal stem cell.

The unique advantages of ps-MSCs over st-MSCs include unlimited supply, more convenience for quality control, genetic manipulation, and scalable production, lower cost, and higher purity (Table 1). Below, we will introduce the history and latest advancements we and others have achieved in ps-MSC generation and study in vitro and in animal disease models, in comparison with st-MSCs. We will also discuss the perspectives and challenges for research and clinical application of ps-MSCs.

Introduction to MSCs

MSCs are fibroblast-like and plastic-adherent cells. Human MSCs are positive for a group of cell surface markers including CD73, CD44, CD90, and CD105 and negative for the hematopoietic markers such as CD34 and CD45 and Human Leukocyte Antigen-DR isotype (HLA-DR) [1]. MSC can be differentiated into a variety of cell types including chondrocytes, osteocytes, adipocytes [1], neural cells [8], cardiomyocytes [9], and smooth muscle cells [10]. MSCs are likely the only stem cell type that possesses both regenerative and immune-modulatory capabilities. Thus, MSCs have been widely used in the treatment of many degenerative and inflammatory diseases, injuries in animal models, and clinical trials.

Cell replacement and empowerment are the fundamental principles that enable high efficacy in MSC applications. Furthermore, MSCs require no long-term engraftment of the cells [11]. The immunosuppressive capability, the low expression of major histocompatibility (MHC) antigens on MSCs, and the hit-and-done feature grant MHCs an immune privilege status, allowing allogeneic MSC to be used in recipients without the need for MHC match between the donors and the recipients. There have been few reported adverse effects associated with MSC treatment, thus far. These advantages together explain why MSCs are the most widely used stem cell types for therapy. Moreover, more than 700 MSC-based clinical trials have been registered in the U.S. (https://clinicaltrials.gov/) [2].

The cell empowerment capabilities of MSCs are critical for their therapeutic effects [12]. First, MSCs exert antiapoptotic and trophic effects on local cells via paracrine factors such as hepatocyte growth factor (HGF), brain-derived neurotrophic factor (BDNF), transformation growth factor-β (TGFβ), fibroblast growth factor 2 (FGF-2), and exosomes [13–15]. Second, MSCs promote angiogenesis to enhance the survival and engraftment of transplanted cells or tissues such as hPSC-derived hepatocytes, cardiomyocytes, and neural cells [16–18].

The efficacy of MSCs also relies on the activation of the cells by inflammatory cytokines such as tumor necrosis factor (TNF) and interferon-γ (IFNγ) [19]. Activated (or primed) MSCs can inhibit the proliferation, maturation, and differentiation of T and B lymphocytes and increase the ratio of regulatory T (Treg) cells [20, 21]. Conversely, primed MSCs often have reduced or lost regenerative capability. For example, TNF-primed MSCs lose their regeneration potential for osteogenesis and develop a proinflammatory Th1-like phenotype [22, 23], and IFNγ-primed MSCs have reduced cell replacement capability while secreting more anti-inflammatory factors such as indoleamine 2,3-dioxygenase, compared to untreated MSCs [24].

The above studies have been largely conducted on a variety of st-MSCs, especially those from bone marrow, umbilical cord, and adipose. Recently, an increasing number of studies have demonstrated that ps-MSCs possess similar but more consistent therapeutic effects, compared to st-MSCs [25]. For example, by using a mouse model of multiple sclerosis, we have found that multiple hES-MSC lines had consistently superior efficacy over MSCs derived from multiple human bone marrow samples, which exhibited fluctuating effects. It might at least partially be correlated to the lower secretion of the proinflammatory cytokine interleukin-6 (IL6) from the ps-MSCs than the st-MSCs [26]. An increasing number of studies have also demonstrated that hiPS-MSCs have similar phenotypes and share similar immunomodulatory and therapeutic effects as hES-MSCs. The methods for ps-MSC derivation have undergone

Table 2. Representative methods for mesenchymal stem cell derivation from human pluripotent stem cells and testing in animal models or patients

Derivation method	Cell origin	Testing in animal models or patients	Reference
2D, via coculture of hESCs with murine OP9 stromal cells	hESCs	None	[27]
	hESCs	None	[28]
2D, via isolation from hESC-differentiated epithelium	hESCs	None	[29]
2D, via differentiation with initial factors bFGF and PDGF-AB, followed by sorting	hESCs	None	[30]
2D, via differentiation with initial factors bFGF, PDGF-AB, and EGF	hiPSCs	Hind-limb ischemia in SCID mice	[31]
	hiPSCs	Allergic rhinitis patients	[32]
2D, via sorting of single mesenchymal colonies	hiPSCs	Cigarette smoke-exposed rats	[33]
	hiPSCs	Cardiomyopathy in mice	[78] and [82]
	hiPSCs	OVA-induced asthma in mice	[34]
2D, from attached EBs in MSC medium	hESCs	None	[6]
	hESCs	None	[35]
	hiPSCs	Bone defect in NOD/SCID mice	[36]
	hESCs	Hind-limb ischemia in NOD/SCID mice	[37]
2D, via EB formation and then hemangioblast enrichment	hESCs	Lupus and uveitis in mice	[38]
	hESCs	EAE in mice	[26]
2D, via differentiation with SB431542 at initial stage	hESCs and hiPSCs	Colitis in mice	[39]
	hESCs and hiPSCs	None	[40]
	hiPSCs	Tumor model in mice	[41]
	hESCs	Collagen-induced arthritis in mice	[42]
	hESCs	Hypoxia-ischemia in mice	[43]
2D, via direct differentiation in an MSC medium	hESCs	Transplantation in the renal capsule in SCID mice	[25]
	hESCs and hiPSCs	None	[44]
	hESCs	Tumorigenesis assay in NOD/SCID mice	[45]
	hESCs and hiPSCs	None	[46]
	hESCs	Hematopoiesis study in irradiated NSG mice	[47]
	hiPSCs	Ectopic bone formation in nude mouse	[48]
	hiPSCs	Limb ischemia in mice	[49]
	hiPSCs	Skin wound model in rats	[50]
2D, via differentiation on a synthetic polymer in MSC medium	hiPSCs	Craniofacial defect in mice	[51]
2D, via differentiation in an MSC medium followed by sorting	hiPSCs	None	[52]
2D, via a neural crest development	hESCs and hiPSCs	None	[53]
	hESCs and hiPSCs	None	[54]
	hiPSCs	Osteochondral defects in rats	[55]
2D, via differentiation with BMP4 and A83-01 at the initial stage	hESCs and hiPSCs	Colitis and EAE in mice	[56]
2D, via differentiation in a serum-free medium with BMP4 and A83-01 at the initial stage	hESCs and hiPSCs	None	[57]
3D, via differentiation from single cells in a serum-free medium	hESCs	None	[58]
3D, via spheroid differentiation with BMP4 and A83-01 at the initial stage	hESCs and hiPSCs	Colitis in mice	[7]
	hESCs	EAE in monkeys	[59]

Abbreviations: bFGF, basic fibroblast growth factor; EAE, encephalomyelitis; EBs, embryo bodies; EGF, epidermal growth factor; hESCs, human embryonic stem cells; hiPSC, human induced pluripotent stem cells; MSC, mesenchymal stem cell; OVA, ovalbumin; PDGF-AB, platelet-derived growth factor-AB.

remarkable evolutions since the first report more than a decade ago.

Derivation of ps-MSCs in Monolayer

Since the first generation of hESCs and iPSCs, a variety of methods have been reported to differentiate both hPSC types to MSCs (Fig. 1; Table 2). The attempt to differentiate hESC to MSCs was first reported by Barberi et al., in 2005 [27], and confirmed by Trivedi et al., in 2007 [28]. Briefly, hESCs are induced to differentiate toward hemangioblasts via coculture with OP9 cells derived from mouse bone marrow stromal cells. Forty days later, $CD73^+$ cells are selected and continuously cultured in minimum essential medium containing fetal bovine serum (FBS) for 1–2 weeks. The resultant MSCs exhibit typical fibroblast-like morphology and immunological phenotypes for MSCs [27, 28]. Meanwhile, Olivier et al. reported a feeder-free protocol in 2006 to generate MSCs by picking up spontaneously differentiated cells (raclures) from hESC culture and plating the raclures in a serum-containing medium for differentiation for 4 weeks, followed by isolation of MSC-like cells from the culture [29]. This method took 4 weeks in total. A major problem for this method is that many non-MSCs are also present in the culture as the handpicking lacks objective criteria; thus, the efficiency of the method is not satisfactory.

To mimic the developmental procedure and improve the differentiation efficiency, Brown et al. reported MSC derivation from embryo bodies (EBs) formed by hESC. EBs are formed by allowing hESCs to attach to the Petri dish to form a cell cluster in an MSC medium. Fibroblast-like cells sprawling out of the cluster were then collected and expanded. These fibroblast-like

cells proved to be MSCs [6]. The derivation process requires sorting for cells positive for MSC markers such as CD73 and CD105 and several times of passaging to reach the MSCs stage as the EBs contain heterogeneous populations [6, 36]. To avoid the labor-intensive sorting, Kimbrel et al. reported a multistep protocol including EB formation by hESCs, further differentiation of the EBs to hemangioblast, collection of the hemangioblast (suspended cells), and harvesting and expansion of the attached cells as MSCs [26, 38].

Growth factors and small-molecule inhibitors are used to direct the cell fate and induce hPSC differentiation. For example, SB431542 has been reported to augment differentiation of MSCs from hPSCs by blocking the TGFβ signaling that sustains pluripotency [39, 41, 43, 60]. Direct differentiation of hPSCs in an MSC medium has also been used for MSC generation [25, 36, 44, 46, 47]. In 2010, Vodyanik et al. cocultured hESCs with mouse OP9 cells as a mesoderm inducer, isolated single cells depleted of OP9 cells, then treated the single cells with FGF-2, and finally obtained colonies with mesenchymal precursors, which were able to further differentiate to MSCs [58]. This is the first time MSC-oriented differentiation was tracked at the single cells level.

Interestingly, it has been shown that MSCs can also be derived from neural crest cells (NCCs). NCCs are transient structures formed through epithelial-mesenchymal transition and can migrate along the anterior-posterior axis of the body to enter different tissues [61]. Takashima et al. [62] first demonstrated that neuroepithelium gives rise to MSCs in part through a neural crest intermediate stage, although MSCs generated from this pathway are transient and are replaced by MSCs from other sources. Similar findings were reported by Morikawa et al. [63]. Later, Lee et al. [53] and Menendez et al. [64] reported protocols to induce NCCs from hPSCs via activation of WNT signaling and concurrent suppression of Activin/Nodal pathway, which can be further differentiated to MSCs [54, 65].

In addition, extraembryonic tissue chorion, which mainly contains trophoblasts, has also been known to differentiate to MSCs in the placenta [66]. We have previously demonstrated that hESCs can directly differentiate to trophoblast following BMP4 treatment [67]. Based on these, we reasoned that the hESC-derived trophoblast may be directly differentiated to MSCs. To prove this hypothesis, we first differentiated hESCs to trophoblast in the presence of BMP4 (which induces trophoblast differentiation) and A83-01 (which enhances the trophoblast differentiation by blocking mesendoderm differentiation via inhibition of the TGFβ signaling). Then the trophoblasts were further differentiated to MSCs in an FBS-containing medium, and the resultant cells (named T-MSCs) expressed typical MSC markers and were capable of trilineage differentiation and immune modulation [56]. Compared to bone marrow MSCs (BM-MSCs), T-MSCs produce less proinflammatory factors IL6, CXCL10, and CCL2 but express higher PD-L1, an immunosuppressive factor, in response to IFNγ treatment. T-MSCs are efficacious for treatment of mouse models for ulcerative colitis and multiple sclerosis [56].

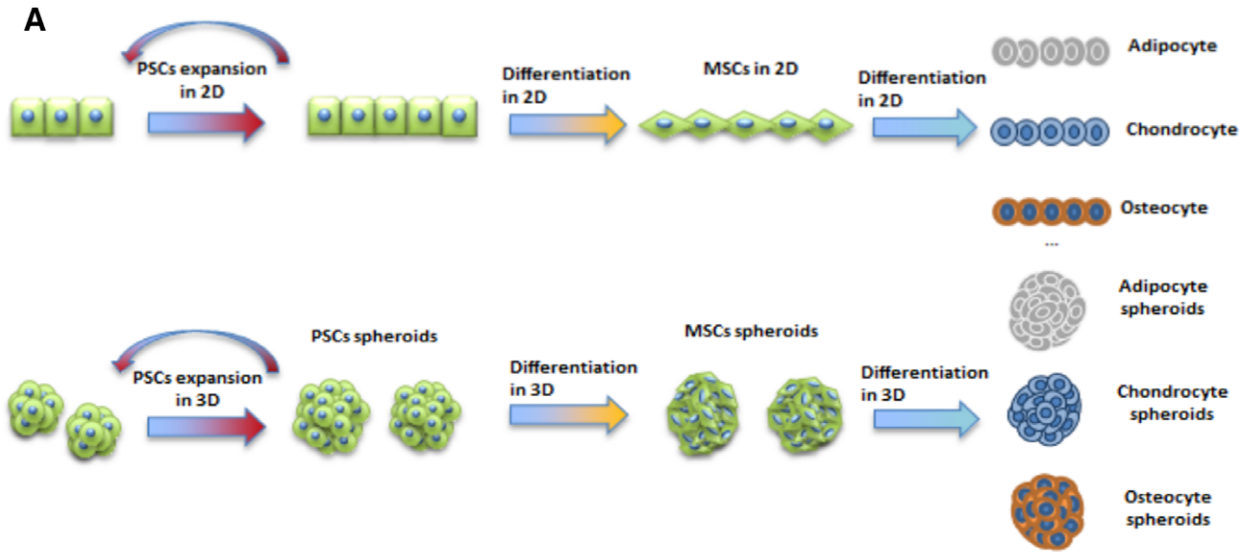
Recently, we have further demonstrated that T-MSCs can be derived in an all-defined system by first differentiating hESCs to trophoblast in the simple and serum-free medium E6 plus BMP4 and A83-01. The resultant trophoblasts can be further differentiated to MSCs by exposure to MesenCult, also a serum-free medium [57]. The T-MSC derivation in the serum-free system takes a bit longer (because of the slower development of CD105) than in a serum-containing medium, but the serum-free derived T-MSCs proliferate faster and produce less inflammatory cytokines IL6 and IL8 than T-MSCs derived in a serum-containing medium [57]. As both hESCs and human iPSCs (hiPSCs) can be derived and expanded in xeno-free and defined systems [68, 69], it is possible to generate medical-grade ps-MSCs for clinical translation. In addition, the combined use of growth factors bFGF, platelet-derived growth factor-AB, and epidermal growth factor has also been reported to induce hPSC differentiation to ps-MSC under xeno-free and defined conditions [30–32].

Zhou and coworkers deciphered the molecular mechanism for how hPSCs differentiate to MSCs. First, they demonstrated that the transcription factor MSX2 mediates entry of hPSCs into the mesendoderm by simultaneously suppressing SOX2 and activating NODAL signaling [70]. Then, they further demonstrated that MSX2 can initiate and accelerate MSC specification of hPSCs via NCCs as well as the mesoderm and trophoblast by regulating *TWIST1* and *PRAME* [71]. To reveal the epigenetic landscape during hPSC differentiation to MSCs, Yu et al. conducted an epigenome-wide analysis of hESCs and MSCs. They found that EZH2 declines during the differentiation, and inhibiting EZH2 with GSK126 can direct hESC differentiation toward mesoderm and generate more MSCs by reducing H3K27me3 [72]. Their results suggest that inhibiting EZH2 promotes mesodermal differentiation of hESCs.

Derivation of ps-MSCs in Spheres

All the ps-MSCs derived via the methods above are in two-dimensional (2D) monolayer, although some methods involve EB formation as an intermediate step. Compared to MSCs in monolayer, MSCs in spheroids have demonstrated increased cell viability and angiogenic, anti-inflammatory, and tissue-regenerative capabilities [73]. As an unlimited source for MSCs, hPSCs can be expanded and maintained as spheres in a suspended state without alterations of the karyotype or loss of pluripotency [74, 75]. For example, Otsuji et al. developed a spheroid culture system that maintained hPSCs for more than 60 passages [74]. This system may easily be used for large-scale production of hPSCs in clinical-grade facilities with high efficiency and low cost. Kempf et al. reported a protocol to differentiate hPSC spheroids to cardiomyocytes, by which 40 million predominantly ventricular-like cardiomyocytes can be produced in a 100-ml-scale bioreactor (at up to 85% purity) [76].

We have recently established a three-dimensional (3D) protocol for scalable production of ps-MSCs by directly converting hPSC spheres to MSC spheres within 20 days [7] (Table 2). The ps-MSCs derived in 3D exhibited faster proliferation, slower senescence, antiapoptotic characteristics, and increased differentiation properties, compared to ps-MSCs derived in 2D [7] (Fig. 2). Moreover, 100 ml of hESC spheres cultured in the system was able to generate approximately 7×10^7 MSCs, whereas 35 T75 flasks of hESCs cultured in monolayer were required to generate the same number of MSCs. Thus, the 3D system dramatically reduces the costs for culture medium and other consumables and avoids labor-intense handlings, providing favor for clinical application of ps-MSCs (Fig. 2). This process can be easily scaled up to produce a sufficient number of cells for transplantation in nonhuman primates and human patients for whom usually more than 10^9 cells are needed for each treatment [51].



B **Comparison of MSCs cultured in 2D versus 3D**

Comparison	MSCs in 2D	MSCs in 3D
Scalable production	Hard	Easy
Proliferation	Slow	Fast (following subsequent culture in 2D)
Senescence	Fast	Slow
Tolerance to ambient conditions	Poor	Strong
Immunomodulatory & therapeutic effects	Similar	Similar

Figure 2. **(A):** The sketch of cultivation, derivation, and differentiation of MSCs in 2D and 3D. **(B):** The general comparison of MSCs cultured in 2D versus 3D. Comparison of human PSC-derived mesenchymal stem cell generated in 2D versus 3D. Abbreviations: 2D, two dimensions; 3D, three dimensions; MSC, mesenchymal stem cell; PSC, pluripotent stem cell.

More interestingly, we found that spheroid formation allows MSCs (including st- and ps-MSCs) to tolerate ambient and hypoxic conditions for up to 10 days via a hibernation-like mechanism by reducing cell metabolism and proliferation; similarly, hESC spheres also survive ambient storage for up to 4 days [77]. These findings suggest that any sphere-forming cells may be delivered as spheres in a regular package to anywhere in the world. Thus, the spheroid method can potentially replace the methods based on thermoproof or cryopreserved equipment, remarkably reducing the costs and inconvenience of cell transportation for research and therapy. We further demonstrated that ps-MSC spheres can be directly differentiated to chondrocytes or osteocytes in 3D, which then fill and recellularize demineralized bone matrix as a scaffold for potential therapy of bone defects [7].

Biological Features of ps-MSCs In Vitro

Morphologically, ps-MSCs appear smaller in size and proliferate faster than BM-MSCs [38, 57, 78]. The population-doubling time for hiPS-derived MSCs is shorter than that of BM-MSCs, which is associated with the potassium channel hEAG1. Inhibiting hEAG1 has been reported to reduce the proliferation of hiPS-MSCs [78]. By analyzing the global gene expressing profiles, Barbet et al. demonstrated that hES-MSCs are less mature than BM-MSCs [79]. The medium conditioned by hES-MSCs contained more paracrine factors associated with early embryogenesis and angiogenesis than that by BM-MSCs [80]. It appears that ps-MSCs are less responsive to traditional protocols for trilineage differentiation of st-MSCs [81]. Although hiPS-MSCs are capable of adequate differentiation to osteocytes and chondrocytes, hiPS-MSCs are less efficient to differentiate to adipocytes than BM-MSCs [81].

Fu et al. demonstrated that the inflammatory cytokine interferon-γ (IFNγ) induces less *HLA-DR* expression in hES-MSCs than BM-MSCs because of the lower level of Class II Major Histocompatibility Complex Transactivator (essential for *HLA-DR* expression) in the former than the latter [35]. We also found that hES-MSCs express a lower level of the inflammatory cytokine IL6 than BM-MSCs, which failed to increase even after IFNγ stimulation [26]. In other studies, we showed that IFNγ induces production of both inflammatory cytokines IL6 and IL8 and two chemokines IP-10 (CXCL10) and MCP-1 (CCL2) in hES-MSCs [7, 56].

hiPS-MSCs can also suppress T-cell proliferation, inhibit Th2 differentiation, and promote Treg response in peripheral blood mononucleocytes, dependent on prostaglandin E2 production and cell-cell contact [32]. Like BM-MSCs and hES-MSCs, hiPS-MSCs remarkably inhibit natural killer (NK) cell proliferation and cytotoxicity by downregulating the expression of different activation

markers and ERK1/2 signaling, preventing the formation of immunologic synapses with target cells and, hence, secretion of cytotoxic granules. Interestingly, hiPS-MSCs are more resistant to preactivated NK cells than BM-MSCs [44]. hiPS-MSCs can also inhibit IL-12p70 secretions and CD83 upregulation in dendritic cells and T-cell proliferation induced by dendritic cells [82]. It has been well-known that BM-MSCs possess tumor-tropic effect. Interestingly, such an effect is less prevalent in hiPS-MSCs because of reduced expression of receptors for IL1 and TGFβ, downstream pro-tumor factors, and hyaluronan and its cofactor TSG6 in hiPS-MSCs than BM-MSCs [41].

To compare st-MSCs with ps-MSCs isogenically, Frobel et al. reprogrammed human BM-MSCs into iPSCs and then redifferentiated the iPSCs to MSCs. The resultant iPS-MSCs are similar in general to the primary BM-MSCs. However, they reveal incomplete reacquisition of immunomodulatory function and MSC-specific DNA methylation patterns, especially, those associated with tissue type and aging [83]. This study elegantly demonstrates the similarities and differences between st- and ps-MSCs at the genetic and epigenetic levels.

Therapeutic Effects of ps-MSCs In Vivo

An increasing number of studies have demonstrated the therapeutic effects of ps-MSCs on experimental animal disease models. Intrafemoral injection of hES-MSCs supports hematopoiesis but does not suppress immune functions in immunocompromised NSG mice; however, i.v. injection of hES-MSCs or BM-MSCs failed to home to the bone marrow of the mice [47]. In addition, ps-MSCs exhibit osteogenic potential by forming functional ectopic bone to aid in bone regeneration [36, 48]. Transplanted ps-MSCs remarkably attenuate severe hind-limb ischemia in mice via direct differentiation and paracrine mechanisms and provide a stronger therapeutic effect than BM-MSCs on vascular and muscle regeneration [31]. Moreover, ps-MSCs reduce the symptoms in mice with a variety of inflammatory and autoimmune disease such as allergy airway inflammation [45], lupus, uveitis, collagen-induced arthritis [42], and inflammatory bowel disease (IBD) [39]. ps-MSCs improve clinical abnormalities in IBD by promoting intestinal epithelial cell proliferation, increasing $Lgr5^+$ intestinal stem cells, and stimulating intestinal angiogenesis [84]. We have demonstrated that hES-MSCs outperform BM-MSCs in reducing clinical symptoms and preventing neural demyelination in a murine encephalomyelitis model by inhibiting Th17 differentiation and increasing Treg ratio [26]. Consistently, Hawkins et al. demonstrated that hES-MSCs secreted more anti-inflammatory cytokines and exerted higher neuroprotective effects than amniotic fluid MSCs in a mouse model of brain ischemia [43]. The NCC-derived MSCs were also reported to exhibit chondrogenic potential to repair osteochondral defects in vivo possibly via participation in regeneration of hyaline cartilaginous tissue [55]. A subpopulation of NCCs derived from hiPSCs was found to promote peripheral nerve regeneration in a murine model of massive peripheral nerve defect [85].

Like st-MSCs, ps-MSCs can exert therapeutic effects via paracrine. ps-MSCs secret anti-inflammation and antiapoptosis factors such as GDF-15, MIF, and EMAP-II, attenuating doxorubicin-induced cardiomyopathy [86]. Lian and coworkers also found that hiPS-MSCs attenuate the inflammation and reverse the imbalance between apoptosis and proliferation through the paracrine of stem cell factor in airway cells with cigarette smoke-induced injury [87]. Using mouse models, they found that Rap1-mediated NFκB activity regulates the paracrine capacity of MSCs in repair of infarcted heart [88], and Rap1 deficiency impairs the immunosuppressive potency of MSCs in allograft rejection of heart transplantation [89].

In addition, Lian and coworkers demonstrated that hiPS-MSCs can connect with target cells through microtubes to transfer mitochondria. They demonstrated that, compared with BM-MSCs, hiPS-MSCs following transplantation into a mouse model of anthracycline-induced cardiomyopathy resulted in more human mitochondrial retention and bioenergetic preservation in heart tissue because of the higher intrinsic Rho GTPase 1 (MIRO1) expression and responsiveness to TNFα-induced nanotube (TNT) formation [90]. TNF/NFκB/TMFαIP2 signaling pathway and connexin-43 are involved in the TNT formation for mitochondrial transfer [34, 90]. Mitochondrial transfer of hiPS-MSCs effectively protects corneal epithelial cells from mitochondrial damage [91]. Rodriguez and coworkers demonstrated that MSCs can sense mitochondria released from damaged cells as danger signals to activate their rescue properties, thus acting as both sensors and rescuers of the tissue injury [92, 93].

Secretion of exosomes is another way for ps-MSCs to exert therapeutic effects. hiPS-MSCs have stronger exosome secretion than synovial membrane-derived MSCs and gained better efficacy on a collagenase-induced osteoarthritis in a mouse model [94]. Exosomes of hiPS-MSCs promote the collagen synthesis and angiogenesis in wound healing [50]. Hu et al. showed that the exosome secreted from hiPS-MSCs attenuates the syndrome of limb ischemia in mice [49].

However, numerous studies have demonstrated that a high ratio of implanted MSCs fails to survive, which severely compromises the regenerative potential of the implant [95]. Spheroid delivery may improve the efficacy of cell-based therapy, making them more feasible in the clinic by reducing the number of cells required for similar or improved therapeutic outcomes. As MSC spheres can be delivered under ambient conditions for up to 10 days without loss of cell viability [77], we tested whether MSC spheres could be directly used (without the need for dissociation) to treat animal disease models. Interestingly, hES-MSC spheres can be directly injected i.p. to treat mouse models of ulcerative colitis [7, 77].

Moreover, hES-MSC spheres reduced symptoms and brain lesions in a monkey model of multiple sclerosis following i.t. injection into the central nervous system; and the cerebral-spinal fluid from the diseased monkeys promoted hES-MSCs to transdifferentiate to neurons [59]. These results suggest that cell sphere-based therapy may be administered directly from the shelf to patients without the need for cell dissociation, which can markedly increase the convenience and perhaps also the efficacy of cell-based therapy.

Biosafety Concerns of ps-MSCs In Vivo

As MSCs possess a unique capability of homing to the stroma of various primary and metastatic cancers, they have been used as a cargo to deliver anticancer agents for cancer therapy [96]. However, we cannot neglect the downside of the

tumor-tropic effects of MSCs. It has been well documented that exogenous st-MSCs can promote, rather than inhibit, cancers in many circumstances. Conversely, endogenous MSCs as reactive stromal cells can also promote the growth and metastasis of cancers [97]. Like BM-MSCs, hiPS-MSCs can home to cancers but are much less tumor-tropic than BM-MSCs. This is probably because of the lower expression of receptors for IL1 and TGFβ, downstream protumor factors, and hyaluronan and its cofactor TSG6 in hiPS-MSCs than BM-MSCs [41]. As hPSCs can form teratomas in immunocompromised mice [98], any residual hPSCs, if ever left among ps-MSCs, can potentially form teratomas in immunosuppressed patients. Thus, there are greater biosafety concerns for ps-MSCs than st-MSCs over their therapeutic application.

Although no tumor formation was found in immunocompromised mice treated with hES-MSCs [99], great efforts have been made to reduce the biosafety concerns of hPSCs in general. For example, the herpes simplex virus thymidine kinase (*HSV-TK*) or *caspase-9* was inserted into the genome of hPSCs. Thus, hPSCs and their progeny that harbor the suicide gene can be removed from a recipient animal following injection with ganciclovir (a substrate for HSV-TK) or AP1903 (an inducer of caspase-9 dimerization) [100].

Conclusion

Given the great progress in both basic and therapeutic studies on ps-MSCs, many outstanding questions and challenges have yet to be addressed.

1. Compared to st-MSCs, ps-MSCs appear immature and somehow different in phenotype, gene expression profile, proliferation speed, senescence development, and immune response to some inflammatory cytokines. What are the intrinsic and extrinsic mechanisms behind the differences? How do various source tissue types and epigenetic marks affect the biological features of MSCs during their derivation and maintenance? If ps-MSCs are classified as a subtype of MSCs, should we modify the current minimal standards for MSCs?
2. It has been difficult to identify endogenous MSCs because of the nature of heterogeneity and lack of biologically relevant and uniformed markers for the cell type. Using stringent in vivo differentiation assays and transcriptome analysis, Sacchetti et al. demonstrated that human cell populations from different anatomical sources, regarded as "MSCs" based on these criteria and assumptions, actually differ widely in their transcriptomic signature and in vivo differentiation potential, although they share the capacity to guide the assembly of functional microvessels [101]. This study supports the view that rather than a uniform class of "MSCs," different mesoderm derivatives include distinct classes of tissue-specific committed progenitors, possibly of different developmental origin. Based on this, it is interesting to test whether ps- and st-MSCs represent "MSCs" from different origins in vivo.
3. Although hiPS-MSCs are shown to have less tumor-tropic effects than BM-MSCs, ps-MSCs are derived from pluripotent cells and less responsive to differentiation cues than st-MSCs. Would the immature status indicates that ps-MSCs have a higher risk to transform and develop a tumor than st-MSCs? To address this question, it is necessary to conduct longer and more systematic and stringent studies in larger and immune competent animal species with autologous and allogeneic MSCs, compared to most studies documented in the literature.
4. What is the optimal route for MSC delivery to recipients with a specific disease? How do transplanted ps-MSCs (in single cells or spheres) interact with various cell types in vivo? Could they also home to some specific tissues and organs like st-MSCs? How long can they function and survive? How are they removed from the body?
5. Although hPSC spheres can be directly differentiated to MSC spheres and ps-MSCs can be derived in serum-free and defined conditions, how should these protocols be combined, optimized, and scaled up for clinical-grade production of ps-MSCs in large quantity? How to conduct quality control and quality assurance on ps-MSCs at various passages?

The research on MSCs is a highly disputable field in terms of their definition, identity, biological functions, and clinical efficacy. In addition, ps-MSCs are comparably new to st-MSCs in this big family. Thus, ps-MSCs have brought, and will certainly bring more, questions to be addressed and solved. As ps-MSCs are derived from pluripotent cells, any residual pluripotent cells because of incomplete differentiation might cause benign tumors such as teratoma. Stricter standards should be used for ps-MSCs than st-MSCs to assure their purity and quality when advancing ps-MSCs to the clinic. This review has summarized the history and progress we and others have made in deriving and studying of ps-MSCs versus st-MSCs in vitro and in vivo. Through this article, we hope to stimulate further valuable discussions and in-depth studies.

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Author Contributions

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Disclosure of Potential Conflicts of Interest

R.-H.X. declared patent holder and stock interests. The other authors indicated no potential conflicts of interest.

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