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REVIEW ARTICLE

Mesenchymal stromal cells for cell therapy: besides supporting hematopoiesis

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Abstract Mesenchymal stromal cells (MSC) have attracted the attention of scientists and clinicians due to their self-renewal, capacity for multipotent differentiation, and immunomodulatory properties. Some essential problems remain to be solved before the clinical application of MSC. Platelet lysate (PL) has recently been used as a substitute for FBS in MSC amplification in vitro to achieve clinically applicable numbers of MSC. In addition to promising trials in regenerative medicine, such as in the treatment of major bone defects and myocardial infarction, MSC have shown therapeutic effect other than direct hematopoiesis support in hematopoietic reconstruction. It has been confirmed that MSC promote hematopoietic cell engraftment and immune recovery after allogeneic hematopoietic stem cell transplantation, probably through the provision of cytokines, matrix proteins, and cell-to-cell contacts. Their suppressive effects on immune cells, including T cells, B cells, NK cells and DC cells, suggest MSCs as a novel therapy for GVHD and other autoimmune disorders. These cells thus present as promising candidates for cellular therapy in the fields of regenerative medicine, allogeneic hematopoietic stem cell transplantation, and autoimmune disorders.

Keywords Mesenchymal stromal cells · Regenerative medicine · Immunomodulatory effect · GVHD · Cell expansion in vitro

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Introduction

Mesenchymal stromal cells (MSC) constitute a population of non-hematopoietic cells in the bone marrow, from which these were identified for the first time. Subsequently, MSC have been identified from various tissues including skin, skeletal muscle, adipose tissue, liver, amniotic fluid, embryonic placenta, umbilical cord blood (UCB), teeth, and other tissues [1–8]. MSC are characterized by self-renewal and multiple differentiation capacity into mesenchymal tissue, including osteocytes, chondrocytes, and adipocytes. There is also evidence that MSC could differentiate into endothelial cells, neural cells, astrocytes, cardiomyocytes, and other cells that are developmentally derived from the endoderm and exoderm [9–14]. So far, there is no specific surface marker available to prospectively identify and isolate MSC. Although stage-specific embryonic antigen-1 (SSEA-1) has been reported to successfully isolate a subset of cells biologically similar to MSC from murine bone marrow, and some molecules such as SSEA-4, STRO-1, CD140b and CD271 have been proposed to be specific surface markers for the enrichment of human MSC [15–20], the practical roles played by these surface markers in experimental and clinical application need to be further evaluated. Recently, a promising

technique called analytical pipeline aimed at detecting surface proteome of MSC has been developed. Surface proteins of intact cells are biotinylated and enriched by cell-impermeable, cleavable sulfo-NHS-SS-biotin, and identified with extremely high confidence by mass spectrometry. The technique provides the basis for better understanding the specific surface markers of MSC [21]. At present, MSC is usually defined as positive for a combination of molecules (CD73, CD90, CD105, CD166, CD44, and CD29) and negative for CD14, CD34, CD31, and CD45 [22, 23]. Characterization of MSC in vitro also relies on its functional property including differentiation capability toward osteoblast, adipocytes, and chondroblasts. MSC have great therapeutical potential in regenerative medicine because of their in vitro differentiation capacity, homing to inflammatory tissues after infusion in vivo [24], and secreting various bioactive molecules [25]. MSC, the most important component of marrow stroma and capable of generating most mature mesenchymal cells in the marrow stroma [26–28], functions in regulating hematopoiesis via providing cytokines, matrix proteins, and cell-to-cell contact [29]. This property leads to the coinfusion of MSC in allogeneic hematopoietic stem cell transplantation (HSCT) aiming to promote hematopoietic cell engraftment and immune recovery. The involvement of MSC in T-cell positive selection in thymus [30] has promoted the study on the interaction between MSC and immune cells. Growing evidence indicate that MSC exhibit immunosuppressive activity on T-cell responses triggered by mitogen, alloantigen, peptide antigen, and CD3/CD28 antibody [31–35]. MSC also inhibit the function of B cells, DC, and NK cells [35–40]. The immunomodulatory effect of MSC has been investigated to potentially treat GVHD and autoimmune diseases, for example, diabetes. This review will outline the advances in the manipulation of MSC in regenerative medicine, HSCT and autoimmune disorders, and the understanding of the roles played by MSC therein.

Safe and effective expansion of MSC

With increasing number of clinical trials regarding MSC application, obtaining a sufficient amount of clinical-grade cellular product (usually more than 1×10^6/kg) by appropriate expansion in vitro remains a critical problem. MSC are usually cultured in vitro in basal medium (such as α-MEM or DMEM) supplemented with 10–20% fetal bovine serum (FBS). Recently, a study showed that DMEM have advantages over IMDM in terms of cell proliferation, differentiation, and stem feature preservation of MSC [41]. One concern about the clinical use of MSC is the possible immune response elicited by infused FBS and its safety. There is a report about FBS-induced arthus-like immune response against xenogenic antigens in patients [42, 43]. Due to its susceptibility to microbial contamination, FBS might lead to the transmission of bacterium and virus or prion diseases, such as bovine spongiform encephalitis to patients [42, 44]. So culture and expansion of MSC without animal serum has become a focus preceding the clinical application of MSC. One of the candidate substitutes for FBS is autologous serum, and human plasma has been demonstrated to be a suitable FBS replacement for the expansion and differentiation of MSC, providing a feasible alternative for tissue engineering with GMP-compatible protocols [45]. MSC can be expanded for a small number of passages using autologous serum without changing their properties [46–48]. However, the amount of autologous serum one patient can provide is limited for large-scale clinical expansion of MSC. Although the protocol, in which the cultures are first expanded in a medium containing FBS and then transferred to autologous human serum, can remove at least 99.99% of all FBS contamination [49]; there are also potential risks, especially for microbial pathogens. Allogeneic human serum is not a selection because the growth of MSC will be arrested in the presence of the serum for unknown reasons [47, 49]. Another expansion protocol has been recently established, in which GMP-produced basic medium LP02 supplemented with 5% of platelet lysate (PL) obtained from human thrombocyte concentrates was used [50]. MSC expanded with PL exhibit higher proliferation activity and less immunogeneic potential compared to cells with FBS. Notably, a normal karyotype can be preserved in MSC with PL-supplemented medium (PLSM) at least for six passages. Subsequent several researches also demonstrated that PL was superior to FBS in the aspects of proliferation potential, colony-forming unit fibroblast frequency, and cell senescence of MSC from various tissues, such as human umbilical cord, UCB, and adipose tissues [51–56]. Further studies are needed to investigate possible functional changes in MSC cultured with PL in comparison with FBS. In a clinical trial, BM-derived MSC, expanded in PLSM from unrelated HLA disparate donors, were infused to treat patients with acute GVHD [57]. Although the response to MSC transfusion was lower than in the previous reports, PL was a promising media supplement candidate for clinically safe and efficient expansion of MSC. Human allogeneic cord blood serum (CBS) is another non-animal serum substitute, with which cultured MSC display higher self-renewal and enhanced osteogeneic potential [58]. As there were few studies that tested the application of CBS, no clear conclusion could be drawn at this time.

Idea medium for safe expansion and application of hMSC in clinical settings is that the medium composition can be completely defined. The effect of a set of growth

and attachment factors has been identified by Aguilar et al., who found that basic fibroblast growth factor (bFGF) is critical and works synergistically with transforming growth factor (TGF)-beta1 to allow significant cell expansion of hMSC. Ascorbic acid, hydrocortisone, and fetuin are also found to be important growth and attachment factors. They have formulated a medium (PPRF-msc6), consisting of key recombinant and serum-derived components [59]. Using recombinant human platelet-derived growth factor-BB (PDGF-BB), bFGF, and TGF-beta1 supplemented serum-free medium, Chase et al. [60] obtained extensive propagation of MSC with retained phenotypic differentiation and colony-forming unit potential while there was no change in the global gene expression. Panserin 401 is a specifically developed medium for serum-free cell cultivation. When bone marrow MSC were cultured in serum-free media DMEM or Panserin 401, there was no obvious proliferation. Only when Panserin 401 was supplemented with both 2% FBS and growth factors (as well as with 10% FBS), the best proliferation was obtained, and multipotency wad maintained [61]. One study tested the effect of serum-deprived culture conditions on the survival and replication of MSC, and found a significant upregulation of prosurvival and angiogenic factors including VEGF-A, ANGPTs, IGF-1, and HGF. The cells have the potential to differentiate into endothelial-like cells [62].

Another concern about the clinical use of MSC is their potential malignant transformation. The high proliferative capacity in vitro of MSC probably renders the cells with more chances to acquire gene mutation, which may finally lead to malignant transformation. Although it has been demonstrated that mouse MSC subjected to extensive passaging in vitro were susceptible to malignant transformation evidenced by cytogenetic aberration and sarcoma development in vivo [63–66], the results of studies on the transformation of human MSC are controversial. Human MSC have been demonstrated to exhibit more resistance to transformation without genomic instability in vitro and no tumor was induced after infusion for long-term in vivo [64, 67–69]. In a study, MSC derived from human adipose tissue exhibited transformation evidenced by up-regulation of myc, repression of p16, and acquisition of telomerase activity, and carcinoma was generated in mice [70]. However, these results are contrary to the following experiments suggesting that MSC malignant transformation probably resulted from contamination with another tumor epithelial cell line [71]. One recent study showed that donor-dependent aneuploidy was detected in human MSC in vitro regardless of culture conditions, but transformation did not occur when MSC were tested in mice for in vivo tumorigenesis [72]. More preclinical and clinical trials are needed to further and better understand the possibility of human MSC transformation and its mechanisms.

Preservation

Expansion of MSC in vitro to sufficient numbers meeting clinical requirement needs different lengths of time, based on cell sources, isolation, and culture protocols. It is worth weighing the benefits and risks between culturing MSC over a long time and treating patients with other alternative methods. Hence, cell cryopreservation remains a critical issue that should be addressed currently for increasing MSC application in clinical trials. Several studies have suggested that when using DMSO as cytoprotectant, MSC could be frozen with a slow drop in temperature (1°C/min) and finally stored at −196°C in liquid nitrogen without changing its proliferation and differentiation capabilities after thawing at 37°C [73–77]. In a recent study, human adipose tissue-derived MSC were cryopreserved in xeno-free and chemically defined medium supplemented with 5% DMSO, and cell membrane integrity, cell recovery, repopulation, and functionality were not influenced in comparison to conventional cryopreservation method using FBS [78]. So, the elimination of FBS and the reduced addition of DMSO in this chemically defined medium are good for further standardization of cryopreservation protocol. Using penetrating ethylene glycol (EG, MW 62 Da) as basic cryoprotectant represents another novel approach for cryopreservation of MSC without any adverse effects on cell proliferation and differentiation [79]. It is necessary to formulate a standard operating protocol for MSC preservation before its extensive application in regenerative medicine and tissue engineering.

Sources

MSC are originally isolated from bone marrow, which represents the main source of MSC. MSC approximately forms 0.001–0.01% of nucleated marrow cells. Due to the number and quality decline with aging of donors and invasive procurement of bone marrow, more attention has been paid to search for alternative MSC sources. It is demonstrated that MSC can be identified and isolated from all the aforementioned tissues, including adipose tissue and human UCB (hUCB). Low frequency and inconsistency in successful isolation of MSC in hUCB are probably major obstacles limiting its potential clinical application. In a recent study, the rate of success in isolating MSC from hUCB was raised to 90% when cord blood volume was $\geq$90 ml and the interval time between collection and management was $\leq$2 h [80]. Immunophenotype, proliferation, and differentiation of MSC may vary among different sources. For example, MSC derived from adipose tissue initially expressed surface antigen CD34, which disappeared with expansion in vitro. Significant difference

in the expression of multiple molecules, including PODXL, CD36, CD49f, CD106, and CD146, between MSC from adipose and bone marrow has also been demonstrated [81]. MSC from cord blood exhibited higher proliferation rate in vitro, and was more readily induced to differentiate into chondrocytes than those from adipose and bone marrow [80]. MSC from bone marrow showed higher capacity for osteogenic differentiation compared to those from adipose tissue. However, MSC from adipose tissue was more prone to adipogenic differentiation [81, 82]. So, appropriate option of the origin of MSC should be made based on experimental and clinical application to achieve a satisfactory effect.

Application of MSC in regenerative medicine

MSC-based tissue engineering in the treatment of major bone defect

The well-defined property and well-established culture protocol of differentiation along osteogeneic lineage of MSC in vitro have made MSC promising candidate cells for engineered tissue to treat bone defect. Large-area bone defect is usually repaired by scarring and complicated by nonunion. So far, no effective approach has been established. To date, there are various preclinical and clinical trials that try to treat the disease with MSC. There is evidence that the number and function of MSC are reduced in severe bone fractures, which finally lead to nonunion [83, 84]. MSC combined with scaffold, for example hydroxyapatite (HA)/tri-calcium phosphate, has significantly promoted bone union after transplantation in several experimental animal models [85–89]. PL seemed to be an optimal culturing medium due to the enhanced osteogeneic differentiation of MSC cultured in PL compared to those in FBS [90]. In humans, three patients with loss of 4.0–7.0 cm bone segment were transplanted with MSC-seeded macroporous hydroxyapatite scaffolds. Abundant callus formation along the implants and good integration at the interfaces with the host bones were revealed by radiographs and computed tomographic scans by the second month after surgery [91]. In another study, four patients with large bone diaphysis defects were transplanted with porous hydroxyapatite (HA) ceramic scaffolds seeded with MSC derived from autologous bone marrow, and complete fusion between the implant and the host bone occurred 5–7 months after surgery. No major complications occurred in the early or late postoperative periods. In all patients at the last follow-up (at least 6–7 years postsurgery), a good integration of the implants was maintained, indicating long-term durability of bone regeneration achieved by a bone engineering approach [92]. A combination of gene therapy and cell therapy for treatment of this disease has attracted much more attention from scientists. In a study, lentiviral-mediated expression of alpha5 integrin (ITGA5) in human MSC showed higher bone repair potential compared to MSC alone when mixed with coral/hydroxyapatite particles and transplanted into the critical size long-bone defect in nude mice, providing a novel therapy that uses MSC for bone regeneration [93]. Although possessing strong osteogeneic differentiation potential in vitro, MSC engraftment and differentiated osteopoietic cell in vivo are very low in most experimental studies. In addition to direct differentiation to osteogenic cells, MSC can generate bone tissue via an endochondral program (endochondral ossification), which might be another mechanism for promoting bone repair [94]. The beneficial effects of MSC on bone repair and its underlying mechanisms need further in-depth research.

MSC infusion for the treatment of myocardial infarction

Acute myocardial infarction (MI) is characterized by a massive loss of cardiomyocytes due to the disruption of blood supply, which results in cell death and is usually ensued by heart failure. Current clinical therapies include thrombolytic therapy, bypass surgery, and percutaneous coronary intervention (PCI), which have limited effect in preventing the progression of heart failure in MI survivors but reduce MI-related mortality. Cardiomyocyte regeneration becomes the main goal of recovering heart function in patients with MI. MSC have previously been demonstrated to be capable of differentiating into cardiomyocytes in vitro [9, 95]. On the basis of this fact, it is hypothesized that MSC may promote cardiomyocyte regeneration in vivo and hold promise in the treatment of MI. Transplantation of MSC by intracoronary, transendocardial, intramyocardial, or intravenous methods have shown positive results in increasing blood supply, reducing infarct size, decreasing arrhythmias, and improving left ventricular function in animal models of MI [96–103]. Intracoronary injection appears to lead to retention of more therapeutic cells in infracted areas compared with other methods [104]. The mechanisms underlying the beneficial effects of transplanted MSC remain elusive. However, it is indicated that the paracrine property of MSC contributes to the antiapoptotic effect on cardiomyocytes and enhances angiogenesis, thus improving whole heart function after MI. However, direct transdifferentiation of MSC into cardiomyocytes seems to play a small role [97, 98, 105–109]. Gene-modified MSC overexpressing neuropeptide Y (NPY) [110], PGI_2 [111], HGF, VEGF [112], or GSK-3β [113] aimed at promoting MSC differentiation or angiogenesis have more advantages over naïve MSC in MI

treatment. In humans, the first report was of a randomized study of 69 patients with MI [114]. The patients with intracoronary infusion of autologous bone marrow-derived MSC exhibited improved left ventricular function and increased EF at 3 months compared with controls. Adverse effects related to MSC infusion did not occur. Another randomized, double-blind, placebo-controlled phase I study on intravenous infusion of allogeneic MSC in 53 patients with MI has been recently reported [115]. The patients transplanted with MSC intravenously showed no cellular therapy-related complications. Increased left ventricular function, decreased cardiac arrhythmias, and improved pulmonary function were attained in patients with MSC infusion. At the current stage, the optimal MSC source, transplantation methods, dosing, and timing still need to be further optimized.

MSC enhances the reconstruction of hematopoiesis

Allogeneic HSCT is an effective therapeutical modality for hematological or nonhematological disorders considering the extreme difficulties in finding gene-matched donors. However, conditioning regimens such as chemotherapy before HSCT usually damage the host marrow stroma and lead to graft failure, which is a life-threatening complication after HSCT [116]. Moreover, immunologic resistance and limited number of HSC are also the reasons for graft failure. MSC, the main component of the bone marrow microenvironment, have been shown to regulate hematopoiesis by mechanisms of secreting bioactive molecules and by cell–cell contact [29]. Coinfusion of MSC has shown to facilitate the engraftment of $CD34^+$ hematopoietic cells derived from peripheral blood or UCB in several animal models [117–123]. Our laboratory has previously isolated a novel population of adherent fibroblast-like cells from hUCB $CD34^+$ cells, and called it hUCB-derived stromal cells (hUCBDSC) [124]. A series of surface markers positive on hUCBDSC include CD29, CD31, CD44, CD45, CD50, CD68, CD106, fibronectin (Fn), laminin (Lm), and collagen IV, but not CD34. Compared with MSC from bone marrow, hUCBDSC can synthesize and secrete higher level of TPO and lower level of granulocyte-macrophage colony stimulating factor (GM-CSF) and stem cell factor (SCF). In vitro coculturing tests showed that hUCBDSC have the ability to promote $CD34^+$ cell expansion and the formation of colony-forming unit (CFU). When mice were cotransplanted with haploidentical hematopoietic cells and hUCBDSC, the engraftment of hematopoietic cells was faster than in HSC-only control [125]. Recently, MSC in combination with SCF, TPO, and FGF-1 showed higher capacity of hematopoiesis enhancement compared to MSC alone, evidenced by more CFU formation in vitro, and rapid hematopoietic reconstitution in mice [126]. It seems that compared with bone marrow, MSC derived from adipose could lead to faster proliferation and differentiation of hematopoietic progenitors in vitro and higher production of immature human hematopoietic progenitors and CD45(+) cells in mice [127]. The mechanisms underlying the supportive roles of MSC on hematopoietic reconstitution in vivo remain unclear. In fact, the number of MSC distributed into the bone marrow after infusion is limited [128], suggesting that considerable engraftment of MSC may not be indispensable for its effects [129]. It is possible that secreted cytokines by MSC mainly mediate the engraftment improvement of hematopoietic cells. Infusion of in vitro expanded autologous MSC in patients has been demonstrated to be safe with no adverse events and ectopic tissue formation [130, 131]. Subsequent clinical trials indicate that cotransplantation of MSC with HSC contributes to rapid hematopoietic recovery in patients, although the source and number of MSC and HSC and patient conditions were different [132–136]. A recent phase I–II clinical trial enrolled fifteen pediatric patients with high-risk acute leukemia [137]. Eight patients were transplanted with single unit UCB and MSC from haploidentical parental donors. No serious MSC-related adverse events occurred. All eight evaluable patients achieved neutrophil engraftment at a median of 19 days. Probability of platelet engraftment was 75%, at a median of 53 days. Although the result is inspiring, further double-blind and randomized controlled clinical trials are needed to confirm the effect of MSC and optimal cell source, dosing, and timing.

Immunomodulatory effect of MSC

Initially, MSC are identified to express HLA-I but not HLA-II, and exhibit low immunogenicity. Later, MSC were confirmed to be implicated in suppressing proliferation and influencing effector functions of cells from both the innate and adaptive immune system including natural killer cells, monocytes, macrophages, dendritic cells, B cells, and T cells (Table 1). It has been demonstrated that MSC has the capability to inhibit the activation and proliferation of T lymphocytes in vitro stimulated by mitogens [32, 138], alloantigens [139, 140], as well as CD3 and CD28 antibodies [141] in a dose-dependent manner. Several soluble factors have been proposed to mediate the suppressive effect of MSC including transforming growth factor-β, hepatocyte growth factor, prostaglandin E2 (PGE2), indoleamine 2,3-deoxygenase, insulin-like protein, NO, heme-oxygenase-1(HO), leukemia inhibitory factor, programmed death-ligand1, jagged-1, B7-H1, IL-10, HLA-G galectin-1, and adenosine depending on the stimuli [32, 139, 142–154].

Table 1 The immunomodulatory effects of MSC on immune cells and their mechanisms

	Stimulator	Immunomodulatory effects	Mechanisms	References
T cells	PHA, alloantigen, CD3 and CD28 antibodies	Inhibition of T-cell proliferation; Induction of $CD4^+CD25^+Foxp3^+$ T cells (Tregs); downregulation of Th1-type cytokine secretion; upregulation of Th2-type cytokine secretion	TGF-β, HGF, PGE2, IDO, ILP, NO, HO, LIF, IL-10, HLA-G, programmed death-ligand1, jagged-1, B7-H1, galectin-1 and adenosine; activating complement; cell–cell contact	[32, 37, 138–158]
B cells	Anti-Ig antibodies, soluble CD40 ligand cytokines	Inhibition of B cell proliferation, differentiation and antibody production. Downregulation of CXCR-4, CXCR-5 and CCR7	PGE2, cell-cell contact	[36, 160, 161]
DC cells	GM-CSF, IL-4, TNF-α	Inhibition of monocyte and HSC-derived DC differentiation, maturation, T-cell stimulation function; inhibition of TNF-α release by native myeloid DC and promotion of IL-10 release by native plasmacytoid DC; induction of regulatory DC	IL-6, M-CSF, PGE-2, IL-10 cell–cell contact	[39, 139, 162–166]
NK cells	IL-2, IL-15	Inhibition of NK cell proliferation and cytotoxicity; downregulation of IFN-γ and TNF-α secretion	PGE2, TGF-β, IDO, HLA-G5, cell–cell contact	[37, 38, 138, 158, 167, 168]

However, the requirement of cell-to-cell contact also cannot be excluded [155]. In a recent study, complement-activating properties of MSC were also shown to be probably involved in its immunosuppressive effects on T cells in vitro [156]. Further studies showed that MSC could skew type1 T-cell response toward type2 T-cell response [139, 157]. $CD4^+CD25^+Foxp3^+$ cells (Tregs) were also induced when cocultured with MSC in vitro [139, 158]. MSC can also strongly inhibit differentiation of naive $CD4^+$ T cells into T helper (Th) 17 cells and induce a functionally Treg cell phenotype in fully differentiated Th17 cells [159].

MSCs can inhibit proliferation of B cells stimulated by anti-Ig antibodies, soluble CD40 ligand, or cytokines via cell-to-cell contact or soluble factors such as PGE2 in most studies. The differentiation, antibody production, and chemokine receptor expression (such as CXCR-4, CXCR-5 and CCR7) of B cells are also reduced by MSC [36, 160, 161].

Monocyte and hematopoietic stem cell-derived DC differentiation and maturation are impaired in the presence of MSC [39, 139, 162–164]. The function of T cell stimulated by DC cells is also suppressed by MSC. In addition, MSC can inhibit the release of TNF-α by native myeloid DC and promote IL-10 secretion by native plasmacytoid DC, respectively [39, 139, 162–164]. MSC can induce the generation of regulatory DC, which has the ability to suppress the proliferation of T cells. Two aspects may be involved in the mechanisms underlying the effect of MSC on DC cells: cell-to-cell contact and soluble factors, such as IL-6, M-CSF, PGE2, and IL-10 [39, 139, 163–166].

IL-2 or IL-15 stimulated NK cell proliferation is also impaired by MSC. It seems that MSC could inhibit cytotoxicity of IL-2-activated rather than freshly isolated NK cells [37]. MSC exhibits inhibitory effect on proinflammatory cytokine release including IFN-γ and TNF-α by NK cells. Cell-to-cell contact and soluble factors such as PGE2, TGF-β, IDO, and HLA-G5 may contribute to the suppressive effect of MSC on NK cells [37, 38, 138, 158, 167, 168]. In general, the immunosuppressive property has made MSC attractive in the treatment of immune disorders.

Potential application of MSC in GVHD and other immune-related disease

Graft-versus-host disease (GVHD), a major cause of morbidity and mortality after HSCT, is characterized by recognition and proliferation of alloreactive donor T cells and subsequent attacking host target tissues and organs such as skin, gut, and liver. Standard therapeutic approaches for treatment of GVHD include conventional immunosuppressive drugs and/or T-cell depletion of the graft. However, 30–70% of recipients still suffer from GVHD after HSCT [169, 170]. In fact, T-cell depletion increases the incidence of graft failure, tumor relapse, and opportunistic infections [171]. Based on the previous findings that MSC have the capacity to regulate immune response in vitro, it has been proposed that MSC infusion may benefit the treatment of GVHD. In the following experiments, MSC were demonstrated to significantly attenuate the incidence and severity of GVHD after cotransplantation in most animal models. [121, 172–180]. Suppressing T-cell proliferation and activation, promoting T-cell traffic to secondary lymphoid organs, changing cytokine microenvironment in peripheral blood (decreased IFN-γ and increased IL-10

levels), and downregulating DC migration to lymph nodes may all contribute to the suppressive effect of MSC on GVHD in vivo [172–174, 177–181]. In our laboratory, hUCBDSC have also been demonstrated to constitutively express HLA-I, but not HLA-II and other costimulators such as CD80, CD86, CD40, and CD40L. hUCBDSC suppress xeogenic T-cell reaction stimulated by PHA and DC possibly via the induction of $CD4^+$ Treg and reversion of mature DC to immature [182]. When hUCBDSC were infused into acute GVHD mouse model after haploidentical stem cell transplantation, clinical and histopathologic scores of GVHD were significantly reduced. The expression levels of immune molecules including MHC-I, II, CD80 and CD86 on splenic $CD11c^+$ DC were dramatically decreased at 1w, 2w, 3w, and 4w posttransplantation. The proportion of $CD4^+$ Treg in splenic $CD4^+$ T cells was also significantly increased. So, the protective effect of hUCBDSC against GVHD in mice is probably ascribed to the induction of $CD4^+$ Treg and the postponed DC maturation [183], which is in accordance with in vitro results [182]. The interaction between hUCBDSC and human immune cells remains to be explored further. However, some individual studies report that the severity of GVHD could not be ameliorated by the infusion of MSC [179, 184, 185]. The possible reasons for this discrepancy may include MSC sources, isolation and expansion methods, cell number, and timing of HSCT. However, clinical application of MSC has generated more beneficial effects on the prevention and treatment of GVHD. The first report was that a 9-year-old patient with severe treatment-resistant grade IV acute GVHD completely recovered after transplantation with haploidentical MSC [186]. In another study, MSC (median 1.0×10^6/kg) were given to eight patients with steroid-refractory grades III–IV GVHD. As a result, the acute GVHD disappeared in six of eight patients, and the survival rate was significantly higher than controls [187]. MSC from unrelated HLA disparate donors were expanded in PL-containing medium, and then delivered to 13 patients with steroid-refractory aGVHD (median 0.9×10^6/kg). The overall response (OR) 28 days after MSC infusion was 54% [57]. It is possible that the responses and results may vary with patient characteristics and MSC regimen. Although promising in prophylaxis and treatment of GVHD, one adverse effect of MSC transplantation is that it probably decreases graft versus leukemia (GVL) effect in HSCT. In a prospective randomized clinical trial [188], patients with hematological malignancies were transplanted HLA-matched HSC with or without MSC (median 3.4×10^5/kg). The incidence of grade II–IV GVHD in patients cotransplanted with MSC was significantly reduced compared with patients transplanted HSC alone (10 vs. 53%). However, relapse occurred in 60% patients with MSC cotransplantation, significantly higher than patients with HSC alone (20%). The results indicate that MSC probably decreases the GVL effect, which leads to the increased rate of leukemia relapse. More large randomized controlled clinical trials are needed to assess the benefits and risks (such as malignant transformation, supporting tumor growth and ectopic formation) of MSC, optimal source, dosing, and timing.

Type 1 diabetes is characterized by damaged β cells in the pancreas, attacked by specific T cells and subsequent decreased insulin level and metabolism disorder. Although exogenous insulin represents the current major therapy for type 1 diabetes, the probability of diabetic complication in patients is high due to the lack of physiological oscillation in insulin secretion and high glucose level [189]. An ideal approach for the treatment of type 1 diabetes should address β cell deficit and immune response. The capability of differentiation into insulin-positive cells [190, 191] and immunomodulatory effect of MSC raise its possible therapeutical application targeting type 1 diabetes. MSC application has been confirmed to increase the level of blood insulin and decrease blood glucose in animal models, in which streptozotocin was used to damage the pancreas [192–195]. Because less evidence supports the transdifferentiation of MSC into insulin-secreting cells in vivo, it is proposed that the soluble cytokines might mediate the beneficial effects of MSC rather than transdifferentiation. As data about MSC in clinical application for the disease were limited, a solid conclusion cannot be drawn.

Conclusions and future directions

In summary, due to their multi-lineage differentiation potential, secreting multiple biomolecules and immunomodulatory properties, MSC have become attractive candidates for cell therapy in the field of regenerative medicine, hematology, and immunology. MSC have been used to treat osteogenesis imperfecta, vascular diseases, and neurological disorders besides the aforementioned diseases. MSC also appear to have therapeutical potential in the treatment of autoimmune disorders. Clinical trials have tested the roles played by MSC in Crohn's disease and systemic lupus erythematosus. The effects of MSC in vivo appear to be more linked with its paracrine function rather than transdifferentiation. Development of tracking technique may help observe MSC distribution and better understand the roles played by MSC in vivo. Safe and effective expansion, malignant transformation monitoring, optimal cell source, application method, and dosing are urgent issues that need to be addressed. So, clinical application of MSC should be considered cautiously at present. Large clinical trials are necessary to determine the effect of MSC. In the near future, gene-modified MSC is

expected to play much more powerful roles in experimental and clinical application, because the selection of therapeutic gene(s) can be accomplished in a disease- or molecule-specific way aiming to more effectively target clinical settings.

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