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Mesenchymal Stem Cell Therapy for Nonmusculoskeletal Diseases: Emerging Applications

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Mesenchymal stem cells are stem/progenitor cells originated from the mesoderm and can different into multiple cell types of the musculoskeletal system. The vast differentiation potential and the relative ease for culture expansion have established mesenchymal stem cells as the building blocks in cell therapy and tissue engineering applications for a variety of musculoskeletal diseases, including repair of fractures and bone defects, cartilage regeneration, treatment of osteonecrosis of the femoral head, and correction of genetic diseases such as osteogenesis imperfect. However, research in the past decade has revealed differentiation potentials of mesenchymal stem cells beyond lineages of the mesoderm, suggesting broader applications than originally perceived. In this article, we review the recent developments in mesenchymal stem cell research with respect to their emerging properties and applications in nonmusculoskeletal diseases.

Key words: Mesenchymal stem cells; Stem cell therapy; Immunoregulation; Myocardial infarction; Hepatic failure; Ocular disease

MESENCHYMAL STEM CELLS (MSCs)

Nearly 5 decades ago, it was demonstrated that bone marrow transplanted under the renal capsule of mice resulted in the formation of bony tissue (125), suggesting that bone marrow harbored a population of osteogenic precursors distinct from the hematopoietic counterpart. Several years later, Friedenstein et al. first described that spindle-shaped stromal cells could be isolated from the bone marrow based on their potential to adhere to tissue culture plastic (57). These rare fibroblastoid cells formed foci of 2–4 cells. These nonphagocytic adherent cells remained dormant for approximately 60 h and then began to proliferate with high replicative and clonogenic capacity. These cells were distinctly different from the stem cells that give rise to progenies of the hematopoietic lineage. Friedenstein further demonstrated that these cells retained the capacity to form bone tissue upon transplantation into animals even after multiple culture passages in vitro. Subsequently, a numbers of studies further reported that the stromal population isolated based on adherence to tissue culture plastic was multipotential and readily differentiated into osteoblasts, chondroblasts, adipocytes, as well as myoblasts, even after 20–30 population doublings in culture (18,23,29,33,56, 80,133,174,176).

Given the heterogenic nature of the stromal population it is apparent that cells prepared based only on the selective adherence to tissue culture plastic were functionally heterogenous. Friedenstein first reported that cultured stromal cell populations varied in size, morphology, proliferative potential, gene expression, and osteogenic capacities (58). Subsequently, rigorous efforts were devoted to establishing conditions for the isolation and maintenance of purer cell populations with stem cell properties. Approximately 30 years after the pioneering work by Friedenstein, Pittenger et al. described conditions for isolation and culture expansion of a homogenous cell population that today is mostly widely referred to as mesenchymal stem cells (MSCs) (126). In that

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study, homogeneity was defined by uniform expression of a panel of cell surface antigens and complete differentiation into three distinct lineages of the limb-bud mesoderm, namely bone, fat, and cartilage. However, it was found that even such a relatively homogenous cell population was functionally heterogeneous, and that only one third of individual colonies derived from single MSC precursors exhibited trilineage potential. Furthermore, Muraglia et al. showed that of 185 nonimmortalized human MSC clones tested only 30% exhibited trilineage differentiation potential, approximately 50% of the clones displayed osteochondrogenic potential, and the remainder were unipotential (114).

To date, the initial techniques developed by Friedenstein remain the fundamental basis for the isolation of MSCs given its effectiveness. In contrast to the hematopoietic stem cells (HSCs) that express characteristic markers for isolation—CD34—the expression of a unique and definitive surface antigen on MSCs is yet to be identified and, presently, isolation of these cells remains largely dependent on a retrospective approach—isolation of a putative cell population by selective growth on tissue culture substrate, and demonstrate their differentiation potential into at least three lineages of the limb-bud mesoderm (osteo/chondro/adipo). In recent years, a number of approaches have been developed to enrich the frequency of MSCs for isolation. Techniques developed include negative immunoselection (97); cell size-based physical enrichment (128); plating density-based selection (34,141); and selection based on the expression of cell surface markers such as STRO-1, SH-2, SH-3, and SH-4 (64,68,151). Other attempts to generate more homogenous population of MSCs include supplementing the initial plate adhering cultures with different growth factors such as basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor, and transforming growth factor-β1 (TGF-β1) (24, 96,160).

Given the diversity of results produced by the different methodologies employed, there is currently a lack of uniformity on the molecular phenotype of MSCs, and is largely dependent on the method of isolation, conditions of in vitro culture, and on the source of the biological sample. Nevertheless, the generally accepted consensus is that MSCs do not express cell surface markers of the hematopoietic lineages such as CD3 (T-cell receptor), CD11a (lymphocyte function-associated antigen 1), CD14 (monocyte differentiation antigen), CD19, CD38, CD45 (leukocyte common antigen), CD66b (carcinoembryonic antigen-related cell adhesion molecule 8), and glycophorin A, do not express markers of endothelial cells such as CD31 (platelet/endothelial cell adhesion molecule), but express CD29 (integrin-β1), CD44 (hyaluronan receptor), CD73 (ecto-5′-nucleotidase), CD105 (endoglin), CD166 (activated leukocyte cell adhesion molecule) (3,10,15,41,49,52,96,126). In addition to the expression of surface antigens, MSCs should possess differentiation capabilities into at least three of the mesodermal cell lineages, namely osteoblasts, adipocytes, and chondrocytes.

Significant advances have been made with respective to the characterization of MSCs in vitro since the pioneering work of Friedenstein et al. (57). However, there is still a lack of knowledge to the precise anatomical distribution of MSCs in vivo. While bone marrow has been widely accepted to be the primary source of MSCs, numerous groups have reported the successful isolation of MSCs from a variety of tissues, including trabecula bone (155,173), periosteum (136), synovium (42,53), adipose tissue (194,195), muscle (103,186), umbilical cord blood (50,97), umbilical cord (177), placenta (59, 182), amniotic fluid (74,171), scalp tissue (146), and peripheral blood (168,175). A recent study reported that MSCs could be derived from virtually all postnatal organs and tissues including the brain, spleen, liver, kidney, lung, bone marrow, muscle, thymus, and pancreas (38). These findings lend support to a hypothesis that points toward cells of the vessel wall, vascular smooth muscle cells (pericytes), as the cellular identity of clonogenic stromal precursors (148). The hypothesis was postulated based on findings of striking similarities between the stromal precursors and pericytes, including: (i) expression of common surface molecules such as STRO-1 (151), Thy-1 (65), vascular cell adhesion molecule-1 (150), endoglin (109), α1 integrin subunit (44), and MUC-18/CD146 (54,145), and (ii) bone marrow stromal precursors isolated by STRO-1 antibody show sequential expression of cytoskeletal and extracellular matrix proteins that are specific for vascular smooth muscles cells (61, 100).

MSCs AND IMMUNOREGULATION

The immunosuppressive properties of MSCs was only discovered in recent years, but has already generated immense interest to harness these properties for clinical applications. Bartholomew et al. (16) and Di Nicola et al. (45) first demonstrated that MSCs suppressed the proliferation of T lymphocytes induced by cellular or nonspecific mitogenic stimuli, even when MSCs were introduced into culture as late as 5 days after the initiation of mixed lymphocyte reactions (MLRs). Suppression of T-cell proliferation was found to be MSC dose dependent and Di Nicola et al. identified TGF-β1 and hepatocytes growth factor (HGF) produced by MSCs as the key soluble mediators of this effect. Subsequent studies have corroborated the inhibitory effects of MSCs in the presence of a variety of stimulatory molecules including anti-CD3 and anti-CD28 antibodies (87,94,

172). In addition, treatment with interferon-γ (IFN-γ) upregulated the expression of indoleamine 2,3-dioxygenase (IDO) by MSCs, resulting in suppression of T-cell proliferation by depletion of tryptophan in MLRs (111); nitric oxide (NO) has been found to be an important suppressor of Stat5 phosphorylation in T cells in the presence of MSCs and is responsible for the inhibition of T-lymphocyte proliferation (137).

A number of mechanisms have been attributed to the immunosuppressive effect of MSCs. Various studies have shown the unresponsiveness to stimuli can be restored upon removal of MSCs (16,45,81,87,107,108), but another study reported induction of anergy due to cell cycle arrest in G_0/G_1 (62), and yet others have observed the induction of T-cell apoptosis (111,127). MSCs inhibit the formation of cytotoxic T lymphocytes (132) and induce the formation of $CD8^+$ regulatory T cells (Treg) as well as a population of $CD4^+CD25^+$ cells with regulatory phenotype (2,48,107). Albeit a large body of evidence consistently demonstrates the suppressive nature of MSCs on T cells, the detailed molecular mechanism remains unclear. Discrepancies in findings reported by different studies are likely due to differences in the methods of MSC isolation and culture, the specific population of lymphocytes, and the type of stimulant used in MLRs, as well as the complexity of the MLR setup.

MSCs not only interfere directly with the proliferation of T cells but also regulate antigen-presenting cells. MSCs inhibited the generation of immature dendritic cells (DCs) from $CD14^+$ monocytes and $CD34^+$ hematopoietic progenitors in a reversible and dose-dependent manner and that upregulation of markers of DC maturation are reduced in the presence of MSCs, rendering DCs in an immature state (46,76,117,192). Nauta et al. showed that the inhibitory effect is observed in transwell cultures of MSCs and DCs but not with MSC-conditioned media, suggesting an important role for soluble factors and that interaction with DCs is required to induce their expression (117). Interleukin-6, macrophage colony-stimulating factor, and prostaglandin E_2 (PGE_2) produced by MSCs have been implicated to mediate the suppressive effects (2,46,117). In addition, MSCs upregulate the expression of interleukin-10 in DCs, possibly skewing DCs towards a suppressor phenotype (20,66).

MSCs have also been reported to modulate B-cell functions. Proliferation, differentiation, and antibody secretion of B cells that were stimulated with mitogens, anti-CD40 ligand, or with interleukin-4 (IL-4) were shown to be inhibited in the presence of MSCs (7,21, 35,36,43,62). On the other hand, one study reported that MSCs exerted suppressive activity on B cells only in the presence of IFN-γ (86), and a recent study showed that MSCs can promote or inhibit antibody production depending on the level of stimulation on B cells by lipopolysaccharide or viral antigens (131). Although the mechanisms are yet to be fully elucidated, the suppressive effects on B cells appear to be mediated through the production of soluble factors by MSCs in response to paracrine signals from B cells (36).

Experiments have shown that MSCs can suppress IL-2 and IL-15-induced proliferation and IFN-γ production of resting natural killer (NK) cells as well as reduce their cytolytic activity, particularly against human leukocyte antigen (HLA) class I-positive cells but not HLA class I-negative targets (2,86,107,132,154,156); however, IL-2-activated NK cells were still capable of lysing MSCs, but pretreatment of MSCs with IFN-γ to upregulate HLA class I molecules decreased their susceptibility to lysis by NK cells (156). Experiments have shown that PGE_2 and TGF-β secreted by MSCs suppresses the proliferation of NK cells, but effects on cytolytic activity required cell–cell contact (154).

One of the first studies to examine the immunoregulatory activities of MSCs in animal models demonstrated that allogeneic MSC transplantation prolonged the survival of skin grafts in a baboon model (16). In a murine model of bone marrow (BM) transplantation Nauta et al. showed that coinfusion of host MSCs significantly enhanced the long-term engraftment of allogeneic BM, associated with tolerance to host and donor antigens; on the other hand, coinfusion of allogenic MSCs promoted rejection of the graft (116). In murine models of graft-versus-host disease (GVHD) one study showed that coinfusion of MSCs with haploidentical hematopoietic grafts controlled lethal GVHD (184), while another study reported the lack of improvement in the severity of GVHD (158). Recently, Tisato et al. reported that transplantation of MSCs effectively prevented the development of GVHD but had no therapeutic effect if transplanted at onset of the disease (162). Djouad et al. demonstrated that coinfusion of MSCs enhanced tumor formation by allogeneic tumor cells in immunocompetent mice that would otherwise be eliminated by the host immune system (48). In addition, MSCs have also shown to ameliorate disease severity and improve functional recovery in animal models of experimental autoimmune encephalomyelitis (EAE) (189,191), autoimmune enteropathy (122), and collagen-induced arthritis (8); however, another study reported failure to confer any therapeutic benefit (47).

Although further research is required to corroborate the discrepancies, current experimental results suggest an underlying potential advantage of the use of MSCs in immunosuppressive therapy—that MSCs may be able to deliver temporary and localized immunosuppression, thereby minimizing the systemic complications of non-specific immunosuppressants such as the risk of infec-

tion, malignant transformation, and suppression of a graft-versus-tumor effect (77,116). Furthermore, these studies also signify that the use of MSCs in cell therapy may not require autologous cells and those cells from unmatched donors could be well tolerated. A number of case studies and clinical trials have already been conducted to examine the feasibility of MSC treatment in hematological disorders. A patient was treated for myelogenous leukemia with allogeneic MSCs and HSCs from the haploidentical father (99). Donor HSCs engrafted rapidly without the development of acute or chronic GVHD. A patient suffering from end-stage severe idiopathic aplastic anemia, refractory to conventional therapies, received coinfusion of allogeneic MSCs and HSCs (55). Although recovery of hematopoietic tissue was not achieved, interstitial hemorrhage, edema, and adipocytic necrosis all disappeared. In a multicenter trial, 46 patients were coinfused with MSCs and HSCs from HLA-identical sibling donors and demonstrated prompt hematopoietic recovery without any MSC-related adverse events (91). GVHD did not develop in 23 of 46 patients, grade II to IV acute GVHD was observed in 13 of 46 patients, and chronic GVHD was observed in 22 patients. A European Phase I–II study coinfused MSCs with HSCs and found sustained hematopoietic engraftment without any adverse reaction, compared with a graft failure rate of 15% in 47 historic controls (11). In a case study, Le Blanc et al. reported successful treatment of server GVHD with third-party haploidentical MSCs (93) and summarized the outcome of treating 8 patients with steroid-refractory grades III–IV acute GVHD and one patient with chronic GVHD (134). Acute GVHD disappeared in 6 of 8 patients and showed improved survival over patients not treated with MSCs. Furthermore, Le Blanc et al. reported that 55 patients treated for steroid-resistant acute GVHD showed an overall response rate of 69%, and an overall survival rate of 23 of 55 from 2 months to 5 years (92). Additional randomized studies are needed to further explore the therapeutic potential of MSCs for immunological disorders and a search on the Clinical Trials Registry (www.ClinicalTrial.gov) shows a number of Phase I–II trials are currently under way.

MSCs FOR CARDIOVASCULAR DISEASES

Cardiovascular disease, and the ensuing heart failure, is one of the leading causes of death in many Western countries, with ever-increasing rates of occurrence worldwide. Despite advancements in medical care and treatments, organ transplantation remains the only effective therapeutic intervention for end-stage cardiomyopathies given the inadequate regenerative capacity of the heart. After myocardial infarction (MI), pathological ventricular remodeling ensues and the infarct tissue undergoes progressive thinning, cardiomyocytes lost are replaced by fibrous tissue comprised of nonmyocytic cells, leading to further deterioration of ventricular function. Conventional treatments for cardiomyopathies are targeted at reducing the detrimental manifestations of myocardial damage, but cell-based therapies to regenerate the diseased myocardium are currently under active investigation.

Makino et al. first reported the cardiomyogenic differentiation potential of marrow stromal cells under in vitro culture (110). In the presence of 5-azacytidine cells developed myotube-like structures, expressed myocardial marker genes and proteins, and developed synchronous beating as well as several types of action potentials, including ventricular cell-like action potentials. In addition to induction to by 5-azacytidine, others have reported successful cardiomyogenic differentiation by coculturing with cardiomyocytes (60,130,183), or differentiation in the presence of insulin, dexamethasone, and ascorbic acid (147).

In animal studies, using xenogeneic in utero transplantation techniques, Liechty et al. reported that transplanted human MSCs underwent site-specific differentiation in a number of mesoderm tissue including the heart (102), and our laboratory further demonstrated that transplanted human MSCs contributed to the development of tissues originating from all three germ layers, including the myocardium (31). In studies of direct transplantation of MSCs into the myocardium, Wang et al. reported that labeled donor MSCs expressed sarcomeric myosin heavy chain, organized contractile proteins, incorporated to the host myocardium, and formed gap junction with native cardiomyocytes (178). In another study, Toma et al. injected human MSCs into the myocardium of healthy immunodeficient mice and showed that transplanted donor cells expressed a number of myocardial markers such as desmin, cardiac troponin T, α-actinin, as well as phospholamban, and demonstrated sarcomeric organization (164).

The feasibility of using MSCs for the treatment of MI has been investigated in a variety of preclinical studies. In a model of swine MI, Tomita et al. injected MSCs into the infarct region at 4 weeks after coronary occlusion (166); labeled donor cells were found within the infarct region in islands that had sarcomeres and Z-bands and stained positive for cardiac troponin I. Recipient hearts showed greater capillary density, increased stroke volume, regional perfusion, wall motion, pressure volume, and end-systolic elastance compared with control hearts. MSC transplantation reduced the left ventricular chamber size and greater scar thickness. In another swine study, Shake et al. demonstrated that MSCs transplanted into the infarcted myocardium expressed cardiac troponin T, tropomyosin, myosin heavy chain, and phos-

pholamban, and recipient animals showed improvement in contractile dysfunction and wall thickening (144). Similarly, Amado et al. reported transplantation of MSCs by percutaneous injection catheter in a swine model resulted in long-term engraftment, profound reduction in scar formation, and near-normalization of cardiac function (4).

In murine models, although numerous groups have consistently shown overall improvement in cardiac function by transplantation of MSCs, there have been discrepancies reported with regards to the specific effects of treatment. Berry et al. (19), Hou et al. (71), and Imanishi et al. (72) demonstrated increased myocardial thickness and reduced fibrosis, consistent with those reported by Amado et al. (4) and Tomita et al. (166) in the swine model, while Dai et al. (37) and Olivares et al. (120) reported that no differences were observed between the treatment group and placebo control in the infarct wall size and relative wall thickness. Evidence of a differing degree of differentiation of MSCs into cardiomyocytes has been demonstrated in studies by Berry et al. (19), Dai et al. (37), Olivares et al. (120), and Saito et al. (135); Davani et al. (40) and Grauss et al. (63) reported that transplanted MSCs expressed endothelial cell markers, while Nagaya et al. (115) showed both cardiomyogenic and endothelial differentiation of transplanted cells.

The underlying mechanism by which MSCs improve myocardial function is unclear. While numerous studies have demonstrated differentiation of MSCs into cardiomyocytes in the infarcted myocardium, the relatively low frequency of such event renders it unlikely to mediate improvements in cardiac function. Likewise, although MSCs have been shown to differentiate into vascular endothelial cell types and increase capillary density in the infarcted area, increase in arteriolar density has not been observed (37,40,149), and perfusion of the infarct as the primary mechanism of therapeutic gain remains controversial. Other studies have suggested the attenuation of postinfarction remodeling and preservation of cardiac function through reduction of apoptosis and increased collagen production as alternative explanations (19,71). The secretion of cytokines and paracrine factors has also been suggested as another plausible explanation. Iso et al. (75) and Kinnaird et al. (84) reported that MSCs express a variety of antiapoptotic and angiogenic/arteriogenic factors, including vascular endothelial growth factor, IL-6, bFGF, placental growth factor, and hepatocytes growth factor; and serum-free MSC-conditioned media significantly prevented cell death of cardiomyocytes and endothelial cells under hypoxia. Paracrine factors secreted by MSCs may also recruit a variety of endogenous stem and progenitor cells to the injury site and contribute to repair of the myocardium. However, it is conceivable that any single mechanism alone may be inadequate for the improvement of cardiac function and that all of the above-mentioned factors act in concert mediating the outcome observed.

While a large number of trials for MI have been performed using bone marrow cells, there has been only one completed study on the use of MSCs post-MI. In this study, 69 patients that underwent emergency angiography or angioplasty were transplanted with either 8 or 10×10^9 autologous MSCs or placebo control into the coronary artery (28). Patients that received MSC transplantation showed improvements in left ventricular ejection fraction, wall movement velocity over the infarcted region, as well as left ventricular end-diastolic volume and end-systolic volume. Additional human studies to evaluate the short- and long-term effect of MSCs for treatment of MI are required and a search on the Clinical Trials Registry (www.ClinicalTrial.gov) shows a number of trials are currently under way.

MSCs FOR LIVER DISEASES

Early studies first suggested that bone marrow harbored cells with hepatogenic potential and may be an alternative source of hepatic progenitors. Petersen et al. reconstituted female rats with BM from male rats then inhibited endogenous hepatocyte proliferation with 2-acetylaminofluorene and, subsequently, induced liver injury in reconstituted animals by treating with carbon tetrachloride (124). Proliferation of hepatic oval cells was induced by liver injury and some oval cells were found to be of male origin, suggesting the BM as an alternative source of hepatic progenitors. In another study, Oh et al. showed that treatment of BM cells with HGF induced detectable levels of albumin and cytokeratin-8/18 transcript by reverse transcriptase-polymerase chain reaction (RT-PCR), suggesting the presence of a subpopulation of cells with hepatic potential in the BM (119). However, the exact population within the BM exhibiting hepatic potential remained unclear.

Schwartz et al. first reported differentiation of a novel population of bone marrow-derived multipotent adult progenitor cells (MAPCs) into hepatocyte-like cells in vitro (140). However, isolation of MAPCs required extensive subculturing of the plate adhering stromal population prior to their emergence and, thus, the existence of MAPCs remains unclear. Our laboratory was first to demonstrate the hepatogenic potential of clonally derived MSCs isolated from human BM and umbilical cord blood (95,97). We designed a two-step serum-free induction protocol that effectively differentiated MSCs into cuboidal epithelial-like cells with almost 100% frequency. Differentiated cells expressed marker genes of the liver not only at the transcription level but also at the protein level, and exhibited a variety of functions characteristic of hepatocytes, including glycogen stor-

age, low-density lipoprotein uptake, urea secretion, and cytochrome P450 enzyme activity. The hepatogenic potential of MSCs has subsequently been confirmed by numerous independent studies (13,70,89,142,152,157,159).

In animal studies, using in utero transplantation techniques, our laboratory and others further demonstrated that transplanted MSCs, in mouse and sheep, contributed to the development of multiple tissues originating from all three germ layers, including the liver (31,139). Sato et al. transplanted human MSCs, $CD34^+$ cells and non-MSC/$CD34^-$ cells into rats treated with a sublethal dose of allylalcohol and showed that donor-derived hepatocytes could only be detected in animals transplanted with MSCs, and that donor-derived hepatocytes did not arise from fusion with endogenous hepatocytes (138). Aurich et al. transplanted MSCs into the livers of immunodeficient mice and found engraftment predominantly in the periportal portion of the liver lobule (9). Consistent with findings reported by Sato et al. (138), transplanted MSCs differentiated into functional hepatocytes in the host liver. Similarly, MSC-derived hepatocytes transplanted into rat liver engrafted predominantly in the periportal region and retained a differentiated hepatic phenotype (143). Chamberlain et al. reported efficient generation of human hepatocytes by intrahepatic transplantation of human MSCs into fetal sheep (25). Intrahepatic injections resulted in widespread distribution of hepatocytes throughout the liver parenchyma while intraperitoneal injections led to a preferential periportal distribution. In another study, Lysy et al. (105) showed that transplantation of MSC-differentiated hepatocyte-like cells into immunodeficient mice resulted in further hepatic maturation in the presence of a liver-inducing microenvironment.

The overall frequencies of donor MSC-derived hepatocytes in animal studies have been found to be relatively low, although, in one study, frequencies up to $12.5 \pm 3.5\%$ were reported (25). These findings raised questions of whether MSCs possess sufficient therapeutic benefit for the treatment of liver diseases. To evaluate the therapeutic potential of MSCs for liver diseases our laboratory induced fulminant hepatic failure (FHF) in nonobese diabetic-severe combined immunodeficient (NOD-SCID) mice by administration of a single lethal dosage of carbon tetrachloride (CCl_4), and transplanted with a single injection of either human BM-derived MSCs or MSC-differentiated hepatocyte-like cells (MDHs), via intrasplenic and intravenous routes (88). Transplantation of both MDHs and MSCs effectively rescued recipient animals from FHF compared with 100% lethality in the control group not transplanted with cells. Under the optimal combination of parameters for cell therapy all recipient animals were rescued from FHF. Rescued animals showed complete recovery of liver functions as well as regeneration of the liver parenchyma. While transplanted cells effectively engrafted in the liver of recipient animals, only a relatively small proportion of transplanted cells differentiated into functional hepatocytes, consistent with previous reports, and rescue of FHF was mediated via antioxidative and paracrine effects of donor cells. In another study, injection of MSC-derived molecules partially reversed FHF in rats, suggesting the paracrine-mediated rescue of FHF (123).

In experimental models of liver fibrosis, fibrosis was induced in rats by multiple intraperitoneal injections of CCl_4 or dimethylnitrosamine and MSCs were transplanted to assess the therapeutic efficacy. Near-normalization of liver functions, restoration of albumin production, and significant reduction in liver fibrosis were found in animals treated with MSC transplantation compared with the control group (1,12,121,193). In a recent study, cocultured MSCs and MSC-conditioned media were found to inhibit proliferation of hepatoma cells lines in vitro, prolong the latent period of tumor formation, as well as decrease the size of the tumor formed in SCID mice (129). These findings demonstrate the potential application of MSCs in the treatment of liver diseases and warrants clinical studies to further assess the feasibility. In the only Phase I clinical trial reported to date, four patients with decompensated liver cirrhosis were transplanted with a mean of 31.73×10^6 autologous MSCs through the peripheral vein (112). No adverse side effects were observed with MSC transplantation and the model for end-stage liver disease (MELD) scores of patients 1 and 4 improved by 4 and 3 points, respectively, by the end of the 12-month follow-up period. In one patient, the MELD score was improved at 6 months posttransplantation, but returned to pretransplant level by the end of the follow-up. Liver volumes increased in 3 of 4 patients, and the quality of life of all 4 patients was improved. Based on the encouraging outcomes of this study, a Phase II trial for the treatment of decompensated liver cirrhosis by transplantation of MSCs, as well as a Phase I trial for the treatment of homozygous familial hypercholesterolemia by transplantation of MSC-derived hepatocytes has been initiated by the same group. In addition, a Phase I–II study for the treatment of end-stage liver disease with MSC-derived hepatic progenitors is also under way.

MSCs FOR OCULAR DISEASES

Age-related macular degeneration (ARMD), retinitis pigmentosa, glaucoma, corneal opacity, and many other ocular diseases can lead to blindness. Irreversible degenerative retinopathy involving dysfunction of retinal pigment epithelium (RPE) and photoreceptor cells are the pathomechanisms of ARMD as well as degenerative op-

tic neuropathy, which involves dysfunction of retinal ganglion cells (RGCs) and results in glaucoma.

The retina, developed from neuroectoderm, is composed of sensory retina and RPE. There are several types of neural cells in sensory retina such as RGCs, bipolar cells, horizontal cells, amacrine cells, Müller cells, and photoreceptor cells. Among these cells photoreceptor cells, rods and cones are the most highly specialized neuroepithelial cells. Foveola, the small central area of macula, is made up entirely of cones, which is primarily responsible for visual acuity, color sensation, and contrast sensitivity. Rods are responsible for darkness adaptation and are widely distributed over the entire retina except foveola. Light-activated rhodopsin in rods, as well as the functional equivalent opsin in cones, initiates the phototransduction of photoreceptor cells.

A number of early studies have shown the neurogenic potential of MSCs. Kopen et al. (85) injected murine marrow stromal cells into the lateral ventricle of neonatal mice and demonstrated that those multipotential mesenchymal progenitors from bone marrow can adopt neural cell fates in vivo, evidenced by positive glial fibrillary acidic protein and neurofilament staining in donor cells. Subsequently, reports of in vitro differentiation of MSCs into neuroglial cells emerged (83,101,179,180). Although others have suggested an artifact basis of such findings (118), recent studies have demonstrated that functional cells, including dopaminergic neurons and Schwann cells, can be derived from MSCs (17,22,78, 153,167,169,170).

Kicic et al. first reported that $CD90^+$ rat MSCs could be induced in vitro to express photoreceptor-specific markers (rhodopsin) and photoreceptor-specific proteins (opsin and recoverin) in the presence of taurine, activin A, or EGF (82). Upon transplantation into the retina of nonpathological rats, green fluorescent protein (GFP)- or BrdU-labeled MSCs were found to integrate into the retina, forming structures similar to the photoreceptor layer, and expressed a photoreceptor-specific marker. In another study, Tomita et al. demonstrated that MSCs can be induced to express neuronal and retina-specific markers under in vitro culture conditions (165). When cocultured with explanted retina from rhodopsin-knockout ($rho^{-/-}$) mice, MSCs migrated into the explants and expressed neurofilament 200, glial fibrillary acidic protein, protein kinase C-alpha, and recoverin. However, the expression of a photoreceptor marker, rhodopsin, was not detected.

Dysfunction of RPE cells also plays a crucial role in retinal degeneration. RPE cells physiologically support normal sensory retina function by multiple mechanisms, such as vitamin A metabolism, maintenance of the outer blood–retina barrier, phagocytosis of the photoreceptor outer segments, absorption of light, heat exchange, and active transport of materials in and out of the RPE. Arnhold et al. reported that coculturing with RPE cells induced differentiation of MSCs into RPE-like cells (6). At 2 months posttransplantation into subretinal space of Wistar rats or dystrophic Royal College of Surgeons (RCS) rats, GFP-expressing MSCs were found integrated within the RPE layer and expressed the epithelial marker cytokeratin, as well as the tight junction marker ZO-1. Transplantation of MSCs further preserved several rows of photoreceptor cells in the dystrophic retina of RCS rats compared with control animals. Similar results were found when MSCs were injected into the subretinal space of $rho^{-/-}$ mice, indicating that MSCs possess the capacity to slow down photoreceptor degeneration in $rho^{-/-}$ mice and RCS rats (5).

In addition to direct differentiation, paracrine effects of MSCs have also been suggested. Inoue et al. demonstrated that conditioned medium of mouse BM MSCs delayed apoptosis of mouse photoreceptor cells, suggesting that secreted factors from MSCs primarily contributed to the survival of photoreceptor cells (73). Subretinal injection of mouse MSCs into RGC rat retina not only preserved photoreceptor cells but also improved retinal function measured by electroretinogram. In another study, Lund et al. compared the efficacy of four human-derived cell types including umbilical tissue-derived MSCs (hUTC), placenta-derived MSCs (hPTC), bone marrow-derived MSCs (hMSC), and dermal fibroblasts (hADF) in preserving photoreceptor integrity and visual functions after injection into the subretinal space of RGC rat early in the progress of degeneration (104). It was found that hUTC and hMSC significantly reduced the degree of functional deterioration in electroretinogram responses, spatial acuity, and luminance threshold test. Histological examination demonstrated that hUTC gave larger areas of photoreceptor rescue than hMSC. However, authors reported lack of evidence to support differentiation of donor cells into neurons. Higher concentration of several neurotropic factors (IL-6, bFGF, and brain-derived neurotrophic factor) secreted by hUTC may be the possible mechanism contributing to the better prognosis after transplantation.

Glaucoma

Glaucoma is a progressive optic neuropathy associated with visual field defect resulting from nerve fiber loss. The axons of the RGCs bend to become parallel to the inner surface of the retina, where they form the nerve fiber layer and the optic nerve. Notably, retinal nerve fibers, the axon of RGCs, from the temporal retina follow an arcuate course around the macula to enter the superior and inferior poles of the optic disc are most sensitive to intraocular pressure (IOP) elevation. Prevention of RGC death and promotion of its axon growth

may improve the prognosis of glaucoma. In an experimental model of glaucoma in rats by ligating the episcleral vein, it was demonstrated that intravitreous injection of rat bone marrow stromal cells rescued the RGCs from death induced by the elevated IOP (188). GFP-labeled donor cells were mostly present along with the inner limiting membrane and expressed various trophic factors, such as bFGF and ciliary neurotrophic factor, at 2 or 4 weeks posttransplantation. In a recent study, Yu et al. showed that MSCs improved RGC survival and neurite outgrowth by upregulation of synaptophsin, microtubule associated protein-2, and TGF-β expression in RGCs while coculturing rat bone marrow MSCs and rat RGCs in vitro (187). Hence, the percentage of neural-like MSCs increased progressively within the first week after coculture. Thrombospondin-1, a multifactorial extracellular matrix protein critical for formation of neural connections during development, was the key factor produced by MSCs that contributed RGC survival and neurite outgrowth.

Cornea

Keratopathy is another cause of blindness. In severe corneal injury, corneal limbus and epithelial cell loss, inflammation, neovascularization, and conjunctivalization may lead to subsequent loss of vision. In a rabbit model of corneal injury, Ye et al. systemically transplanted DiI-labeled MSCs via the ear vein at 24 h post-alkali-induced corneal burn (185). It was found that transplanted MSCs engrafted in the injured cornea and promoted wound healing. In another study, MSCs expanded on amniotic membrane were transplanted into rat corneas 7 days after chemical burns (106). Reconstruction of the damaged cornea was evidenced by slit lamp evaluation and optokinetic head-tracking test. However, it suggested that therapeutic effect of the transplantation may be associated with the inhibition of inflammation and angiogenesis rather than differentiation of MSCs.

The retina and the cornea are relatively immunoprivileged tissues, thus making it an attractive target for cell-based therapies. The immunoregulatory properties, together with the potential to differentiate into neural epithelial cell lineages and paracrine effects, MSCs represent a promising candidate in the treatment of ocular diseases. Thus far, preclinical studies have shown encouraging results for MSCs in the treatment of degenerative retinal disorder and acute corneal injury, and warrants further investigations in clinical studies.

MSCs FOR OTHER DISEASES

Preclinical studies of neurological diseases have shown that transplanted MSCs not only can traverse to sites of injury but improve neurologic functions in rat models of ischemic brain injury, Parkinson's disease, stroke, intracerebral hemorrhage, and spinal cord injury (27,32,67, 69,190). Moreover, in a clinical study, 5 stroke patients transplanted with autologous MSCs showed improvements in Barthel index and modified Rankin scores compared with the 25 control patients that did not receive cell transplantation (14). In another case study, transplantation of umbilical cord blood-derived MSCs improved sensory perception and mobility in a patient suffering from spinal cord injury (79).

Therapeutic benefits of MSCs have also been implicated in renal failures, improving functional recovery in animal models of acute and ischemia injury, although the mechanism of improvement appears to be independent of direct differentiation of transplanted cells (90, 113,163). Recent emerging data also suggest potential pancreatic application of MSCs. Treatment of MSCs with either pancreatic extract or defined culture formulations in vitro induced formation of clusters resembling islet of Langerhans that expressed insulin, glucagon, pancreatic polypeptide, and somatostatin (30,161). Upon infusion into mice, MSC-derived epithelial cell clusters further matured into functional cells that secreted human c-peptide in response to glucose (39), and improved hyperglycemia as well as glucose tolerance in murine models of streptozotocin-induced diabetes (26,181). Transplantation of undifferentiated MSCs has also shown to improve disease symptoms and the repair of pancreatic islets (51,98). The pancreatic differentiation potential together with the immunoregulatory potential of MSCs warrants further investigation into the treatment of type 1 diabetes mellitus, and two Phase I–II clinical trials are currently being conducted.

CONCLUSION AND OUTLOOK

The global escalation in major debilitating diseases that currently lack adequate treatments, such as cardiovascular disease and diabetes, mount a significant burden not only financially, but also socially. A large collection of literature in the recent years has demonstrated the therapeutic promise of MSCs and the vast potential to be harnessed. The potency of MSCs beyond the fundamental definition as progenitors of the musculoskeletal system not only has been demonstrated in a variety of studies, but numerous clinical investigations have also underlined the safety of autologous and allogeneic MSC transplantation.

The accessibility of MSCs from a wide range of somatic tissues, the relative ease for culture amplification, together with the comprehensive potentials, make these cells an ideal candidate for a multiplicity of cell therapy applications. Furthermore, its value in the areas of tissue engineering, and as vehicles for gene therapy and tumor-targeting delivery systems are additional potentials to be explored. However, the evolvement of MSC transplantation into widespread therapeutic modalities will depend

on more detailed investigations, involving large-scale clinical trials with randomized controls, and critical evaluation of key parameters that constitute successful cell therapies, namely (i) cell type(s) to transplant, (ii) cell dose and dosing regimen, (iii) route of administration, (iv) timing of transplantation, and (v) functional evaluation. In parallel, further efforts in basic research are required to better understand the biology of MSCs and to elucidate the molecular mechanisms mediating therapeutic benefit in a vast array of diseases, and to delineate the comprehensive therapeutic potential of these cells.

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