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EDITORIAL

Mesenchymal stem cells: Molecular characteristics and clinical applications

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Abstract

Mesenchymal stem cells (MSCs) are non-hematopoietic stem cells with the capacity to differentiate into tissues of both mesenchymal and non-mesenchymal origin. MSCs can differentiate into osteoblastic, chondrogenic, and adipogenic lineages, although recent studies have demonstrated that MSCs are also able to differentiate into other lineages, including neuronal and cardiomyogenic lineages. Since their original isolation from the bone marrow, MSCs have been successfully harvested from many other tissues. Their ease of isolation and *ex vivo* expansion combined with their immunoprivileged nature has made these cells popular candidates for stem cell therapies. These cells have the potential to alter disease pathophysiology through many modalities including cytokine secretion, capacity to differentiate along various lineages, immune modulation and direct cell-cell interaction with diseased tissue. Here we first review basic features of MSC biology including MSC characteristics in culture, homing mechanisms, differentiation capabilities and immune modulation. We then highlight some *in vivo* and clinical evidence supporting the therapeutic roles

of MSCs and their uses in orthopedic, autoimmune, and ischemic disorders.

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Key words: Mesenchymal stem cells; Bone marrow stem cell; Mesenchymal stromal cell; Autoimmune disease; Cell-based therapy; Autologous transplant; Therapeutic application

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INTRODUCTION

Mesenchymal stem cells (MSCs) are a heterogeneous population of cells with the potential to differentiate into diverse somatic lineages. They were originally described by Friedentstein and colleagues 40 years ago as adherent cells with a fibroblast-like appearance capable of differentiating into osteocytes, chondrocytes, adipocytes, tenocytes and myocytes[1-3]. In recent years MSCs have attracted significant attention for their potential role in elucidating differentiation pathways, promoting tissue engineering and function as gene vectors and immunomodulators in autoimmune diseases.

Stem cells are defined by their ability to remain undifferentiated for a prolonged period while retaining the potential to differentiate along one lineage (unipotent), multiple lineages (multipotent) or into all three germ layers (pluripotent)[4]. While MSCs were initially defined by their ability to differentiate into cells of mesodermal origin, recent studies have provided support for their capacity to differentiate into cells from all three germ layers[5]. In addition, MSCs have other characteristics making them attractive modalities in treating human disease. These cells are described as MHC II negative cells, lacking costimulatory molecules such as CD40, CD80 and CD86, which permits allogenic transplantation without immunosuppression[6]. Furthermore, they can be easily isolated from an autologous source, enabling easy accessibility for therapeutic intent. MSCs can provide therapeutic benefit through the secretion of specific cytokines, *ex vivo* genetic modification and direct cell-cell contact. As such, these cells have been studied for their use in diverse diseases ranging from genetic disorders to tissue ischemia. In this review we summarize the current understanding of MSC biology and conclude by revealing some relevant clinical applications.

SOURCES AND CHARACTERISTICS OF MESENCHYMAL STEM CELLS

After their initial isolation from humans, MSCs have since been successfully harvested from many other species including: mouse, rat, dog and horse[6-14]. They have also been isolated from almost every type of tissue, including: periosteum, brain, liver, bone marrow, adipose, skeletal muscle, amniotic fluid and hair follicle[15-24]. When harvested from the bone marrow, MSCs make up a minute fraction of nucleated cells and account for approximately 0.001%-0.01% of all cells in each aspirate, depending on the technique[25]. However, the therapeutic application of MSCs often requires a large number of cells, which requires *ex vivo* expansion post-harvest. Of note, MSCs have also been isolated from pathologic sites such as joints damaged by rheumatoid arthritis and in such cases they demonstrate characteristic up-regulation of BMP receptors. While cells isolated from various tissues share many similar characteristics, they exhibit minor differences in their expression profile and differentiation potential[26].

Culture and expansion of mesenchymal stem cells in vitro

In order to utilize MSCs in a translational fashion, it is important to expand these cells *ex vivo* in order to obtain sufficient quantities for therapeutic uses. While stem cells are capable of continuous regeneration and expansion throughout an individual's life, they demonstrate limited proliferation and differentiation capacity in an *ex-vivo* setting[27]. Mesenchymal stem cell capacity to expand is highly variable, even amongst two samples from the same patient[27]. While still a source of debate, some studies have also suggested that MSCs have the capacity to undergo malignant transformation *in vitro*[28]. This variability has posed a challenge in comparing data from different groups. Numerous factors including culture parameters such as nutritional level, cell confluence, oxygen level, number of passages and plastic surface quality influence MSC behavior[29]. Vacanti *et al*[30] examined passage number and its effect on MSC characteristics. They compared early (< 5 passages) to late MSCs (> 15 passages) and demonstrated that late MSCs had characteristics associated with cell aging as depicted by actin accumulation and reduced substrate adherence[30]. Furthermore, early MSCs remain pluripotent, while late MSCs had limited differentiation capacity[30]. In addressing this concern, investigators have attempted to study the stem cell niche in hopes of mimicking this environment in an *ex-vivo* setting to allow for more predictable cellular behavior.

One key difference between the culture environment and the *in vivo* setting is the amount of oxygen to which the stem cells are exposed. Even though oxygen levels amongst various tissues differ greatly, its concentration is

always significantly less than atmospheric oxygen levels[31]. Bone marrow has a characteristically hypoxic environment with oxygen concentrations very similar to ischemic tissue[32]. MSCs that are cultured in hypoxic environments demonstrate greater expansion and differentiation potential. Some studies have suggested that these characteristics may in part relate to the up-regulation of telomerase activity in cells cultured in hypoxic conditions[33]. These observations suggest that MSCs utilize low oxygen environments *in vivo* to proliferate and renew, differentiating only when they approach blood vessels that expose them to higher oxygen tension.

Telomere length is another important factor that regulates differentiation and proliferation capacity. The *in vitro* culture environment causes significant telomere shortening, accounting for the loss of 100 bps per passage as compared to the 17 bps lost per year *in vivo*[34]. Studies have also shown that loss of telomerase activity prevents MSC differentiation, while over-expression of telomerase enables cells to maintain self-renewal and multipotential characteristics over a 3-year culture period[35,36]. Genetically engineered cells designed to over-express telomerase have also shown greater resistance to oxidative stress through the up-regulation of stress response genes[37]. Asymmetric cell division can also cause loss of multipotentiality by diluting stem cells[38]. To address this concern, Lee *et al*[39] successfully utilized the purine nucleoside xanthosine to suppress asymmetric cell division in hepatically-derived stem cells, which led to the retention of their differentiation capacity and inhibition of senescence.

Growth and phenotypic characteristics of mesenchymal stem cells

Kinetic studies support 3 phases in MSC growth: (1) an initial lag phase lasting 3-5 d followed by (2) rapid expansion and finally, and (3) a stationary phase[40]. Transition from the lag phase to rapid expansion is initiated by an increase in secretion of dickkopf-1 (Dkk-1), an inhibitor of the Wnt signaling cascade[40,41]. This results in decreased β-catenin levels, causing decreased cell proliferation[40]. In addition, the interaction between epidermal growth factor receptor-1 (HER-1) and heparin-binding epidermal growth factor (HB-EGF) promotes MSC proliferation and inhibits differentiation in various selective media[42].

To date, no cellular markers or receptors have been found to be unique to MSCs. In order to facilitate a more unified approach to studying MSC biology, the International Society of Cryotherapy has devised three criteria needed to identify MSCs: (1) plastic adherence of the isolated cells in culture; (2) expression of cluster of differentiation (CD) markers such as CD105, CD73, and CD90 in > 95% of the culture with absent expression of markers including CD34, CD45, CD14 or CD11B, CD79A or CD19 and human leukocyte antigen-DR (HLA-DR) in > 95% of the culture; and 3) capacity to differentiate into osteocytes, adipocytes and chondrocytes[43].

In addition, with minor differences in expression patterns from one tissue source to another, all MSCs express embryonic cell markers such as Oct4, Nanog, and stage-specific embryonic antigen-4 (SSEA-4)[44]. Further variation in MSC characteristics has been associated with the age of the donor, with a direct correlation exiting between advanced age and decreased osteogenic potential. This fact may in part contribute to disorders, such as osteoporosis, seen primarily in the aging population[45]. A similar decrease in cell number as a function of age has also been documented in satellite cells[46]. In addition, MSCs isolated from older donors demonstrate lower proliferation potential which may provide an explanation for the reduced healing capacity observed in older patients[47].

Homing features of mesenchymal stem cells

MSCs possess a unique capacity to home toward injured tissue[48]. While the exact mechanism underlying MSC homing is not yet fully understood, some studies have shed light on factors that may govern MSC trafficking. Rochefort *et al*[49] demonstrated a 15-fold increase in the pool of circulating MSCs when rats were placed in hypoxic chamber over a 3-wk period. This increase in circulating cell number was specific to MSCs, while the number of other hematopoietic precursors remained unchanged. Studies suggest that such hypoxic induced cell trafficking may, in part, be a function of a Matrix metalloproteinase (MMP)-dependent pathway[50]. Stromal cell-derive factor-1(SDF-1) also plays a critical role in MSC recruitment and tissue regeneration through its selective expression at sites of injury[51,52]. Ceradini and colleagues demonstrated that recruitment of CXC chemokine receptor-4 (CXCR-4) positive progenitor cells to injured tissue is mediated by hypoxia-inducible factor-1 (HIF-1) which promotes over-expression of SDF-1 in a gradient proportional to tissue hypoxia[32]. Age dependent decline in HIF-1 expression has also been documented and this may contribute to the impaired ability of MSC homing and tissue repair observed in the elderly[53]. A CXCR4-SDF-1 dependent homing mechanism has also been seen with MSC migration toward sites of malignant growth[54,55], selectively targeting the hypoxic environment of cancer stroma. Frount-mediated clustering of chemokine receptor 2 (CCR2) has also been shown to be critical for the reorganization of cytoskeleton during cellular migration[56].

MULTIPOTENCY AND MESENCHYMAL STEM CELL DIFFERENTIATION

MSC potency extends beyond the conventional mesodermal lineages and includes differentiation into liver, kidney, muscle, skin, neural and cardiac cells[57]. The multipotency of MSCs has allowed for significant progress in our understanding of differentiation pathways of various lineages for tissue engineering and therapeutic purposes (Figure 1). Runt-related transcription factor 2 (Runx2) has been considered as a master regulatory gene responsible for early osteogenic differentiation[58]. Runx2 acts synergistically with transforming growth factor-β (TGFβ) to up-regulate expression of interleukin 11 (IL-11), which reduces adipogenesis while promoting chondrocytic and osteoblastic

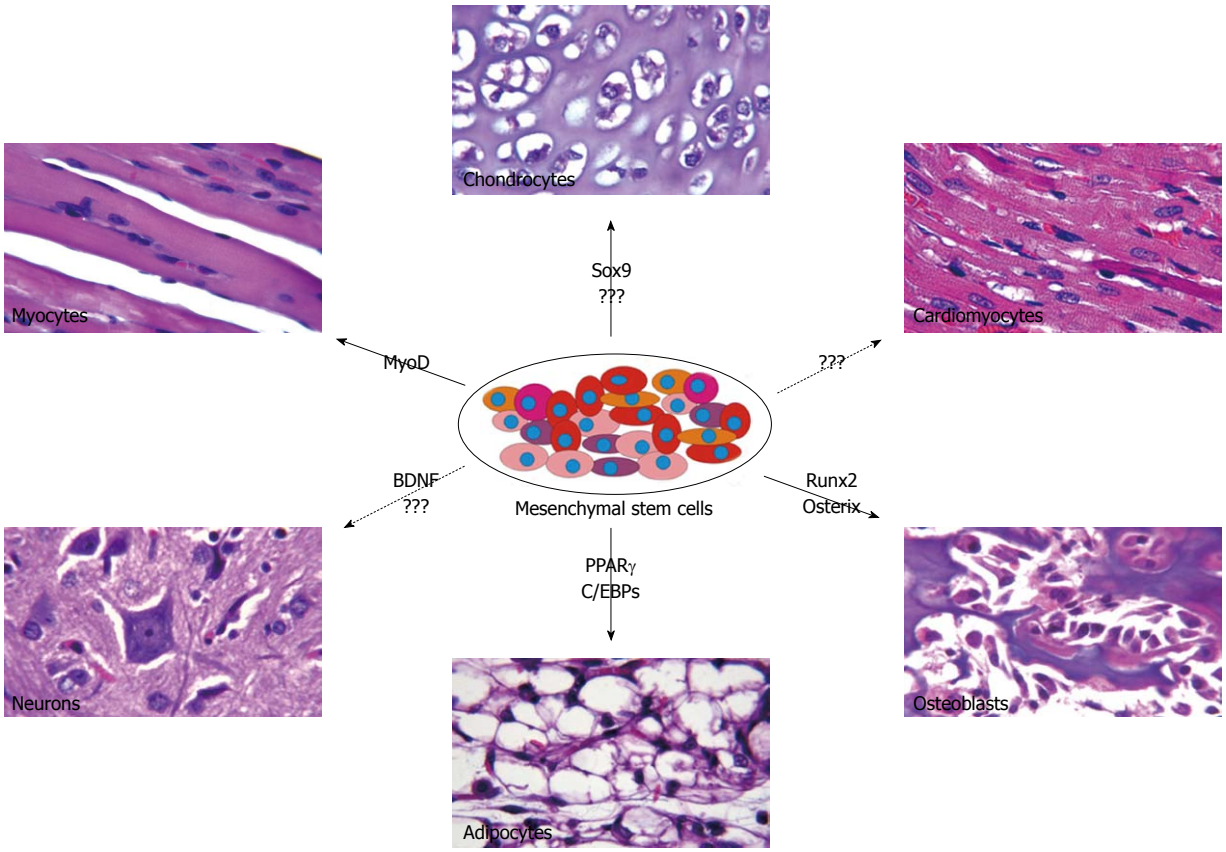

Figure 1 Multipotential differentiation of mesenchymal stem cells. Mesenchymal stem cells (MSCs) are pluripotent progenitor cells with the capacity to differentiate into lineages of all three germ layers. The lineage-specific differentiation is a multi-stage and well-coordinated process controlled by key regulators. Runx2 and Osterix are important master regulators for osteogenic differentiation while peroxisome proliferator-activated receptor γ and CCAAT/enhancer binding protein β are important factors promoting adipogenesis. SRY-box 9 is the main regulator of chondrogenic regulation while MyoD plays a key role in myogenic differentiation. MSCs also have the ability, although to much lesser extent, to differentiate into other lineages, such as neuronal and cardiomyogenic cells (lighter arrows).

differentiation[59]. The Runx2 inhibition of adipogenesis is an effect modulated by direct interaction with peroxisome proliferator-activated receptor γ (PPARγ), an adipogenic inducing factor[60]. While Runx2 acts early to promote osteoblastic differentiation, osterix, another important osteogenic inducer, suppresses chondrogenesis and promotes osteoblastic differentiation at a later stage. Low levels of osterix are sufficient to inhibit chondrogenesis while a high expression level is necessary for osteogenic differentiation[61]. Furthermore, *ex vivo* MSCs have successfully differentiated into osteoblasts under exposure to various stimuli including growth factors such as bone morphogenetic proteins (BMPs) and Wnts[1,3,41,62-68], magnetic field stimulation[69] and culture in osteogenic media supplemented by dexamethasone and ascorbate[70,71]. The selective capability to promote osteogenic differentiation has vast clinical implications.

Chondrogenesis is a multi-step cellular event which requires commitment of MSCs, followed by their aggregation and differentiation into chondrocytes[72]. *In-vitro* differentiation of MSCs into a chondrogenic lineage has been studied through exposure to growth factors, co-culture with cartilage or nucleus pulposus and overexpression of specific genes such as SRY-box 9 (Sox9) to promote chondrocytic differentiation[73,74]. Sox9 cooperates with its downstream proteins Sox 5 and Sox6 to promote chondroycte proliferation, maturation and matrix formation[72]. Overexpression of Sox5, Sox 6 and Sox 9 in cultured cells[75] and ectopic expression of Sox9 in mice induce the expression of type Ⅱ collagen[76,77]. Furthermore, TGFβ has been shown to play an important role in chondrogenic differentiation, an effect that is synergistically enhanced when co-administered with BMP-2[73,78]. In addition, previous studies had suggested a lower chondrogenic potential in adipose derived MSCs, a characteristic that was overcome by the up-regulation of TGFβ receptor I expression[79]. The ability to promote chondrogenic differentiation in MSCs has significant clinical implication for treating conditions such as intervertebral disc degeneration[80].

MSC capacity to differentiate into an adipogenic lineage has been extensively studied. PPAR-γ plays a critical role in this process by regulating the function of many adipocyte specific genes[81]. In addition, PPARγ interacts with members of the CCAAT/enhancer binding protein (C/EBP) family to regulate adipogenesis[82]. Cells can also be induced to undergo adipogenesis through exposure to exogenous factors or by culturing them in adipogenic media containing insulin and dexamethasone[62,83,84]. Factors critical in adipogenesis have been viewed as candidates for treatment of obesity and related disorders.

MSCs also possess the ability to differentiate across various lineages when exposed to certain chemical environments. Unlike the mechanism responsible for growth factor induced differentiation, the pathways governing chemical mediated differentiation is poorly understood but is routinely used in the *in-vitro* setting. Adipogenic media consists of dexamethasone, insulin, isobutylmethylxanthine, and indomethacin, which in combination lead to increased expression of PPAR-γ and other adipose specific factors such as lipoprotein lipase[25]. Treatment of MSCs with 5-azacytidine promotes cardiomyocyte differentiation and leads to improved cardiac function in the swine myocardial infarction model[11]. When incubated with nicotinamide and beta-mercaptoethanol, MSCs undergo islet cell differentiation and modulate glucose levels in diabetic rat models[85].

Mesenchymal stem cells as modulators of immune system

The ability of MSCs to modulate immune responses was first described by Bartholomew and colleagues, who demonstrated that injection of allogeneic MSCs prolonged skin graft survival in baboons[86]. Both *in vivo* and *in vitro* studies have provided support for the immunomodulatory role of MSCs. However, the mechanism underlying this immunomodulation is still not fully understood. Interestingly, MSCs have both immune enhancing and suppressing capability. They can promote immune function by serving as antigen presenting cells (APCs) through an autocrine interferon-γ (IFN-γ) dependent pathway. However, when the level of IFN-γ increases above a threshold, it directly inhibits antigen presentation and promotes immune suppression[87]. This narrow window of immune activity suggests that MSCs may provide protection against foreign antigens while limiting damage caused by an exacerbated inflammatory response. IFN-γ promotes MSC induced immune suppression by up-regulating B7-H1, an inhibitory surface molecule on stem cells, suggesting that cell-cell contact is important for immune function of MSCs[88]. While cell-to-cell contact plays a significant role in immune modulation, secretion of soluble factors is also critical to MSCs' immune-regulatory role[89,90].

MSCs are capable of interacting with various cell lines responsible for mounting an immune reaction. They secrete soluble factors that arrest B-cells in the G0/G1 phase, inhibit B-cell differentiation, and impair B-cell chemotaxis[89]. They modulate monocyte function in a contact independent process through IL1-β secretion, which promotes MSC TGFβ1 expression, leading to inhibition of alloreactive T-cells and the down-regulation of activation markers such as CD25, CD38 and CD 69[91]. Furthermore, they modulate function of dendritic cells, NK cells and T-cells[92-95].

MSC–mediated T-cell suppression is a complex process with many inconsistent reports found in the literature. Nitric oxide (NO) has been shown to play an important role in this process. Sato *et al*[96] showed that MSCs produce NO in the presence of CD4+ and CD8+ T cells, resulting in the suppression of Stat5 phosphorylation and of T-cell proliferation. This suppression was reversed when either prostaglandin or NO synthase (NOS) was inhibited, leading to T-cell proliferation[96]. In addition, Ren and colleagues successfully showed that the concomitant presence of IFN-γ and either tumor necrosis factor-α (TNF-α), IL1-α or IL1-β was sufficient to promote MSC immunosuppression[97]. Their effect was mediated by the increased expression of chemokines and inducible nitric oxide synthase (iNOS) by MSCs, suggesting that pro-inflammatory cytokines are essential in promoting this immune function[97]. MSCs' ability to inhibit allogenic T-cell has also been shown to be partly dependent on indoleamine 2,3-dioxygenase (IDO) mediated tryptophan catabolism[98]. A better understanding of the complex pathways responsible for the immunomodulatory role of MSCs will enable more effective therapeutic applications for immune diseases refractory to today's treatment protocols.

THERAPEUTIC POTENTIALS OF MESENCHYMAL STEM CELLS

Effectiveness of mesenchymal stem cell administration has been studied in various *in vivo* disease models (Table 1), some of which will be mentioned in this review. In addition, we will describe some of the currently reported uses of MSC therapy in the clinical setting (Table 2) and highlight their effectiveness.

Orthopaedic applications: Critical size bone defects

The ability of MSCs to differentiate into osteoblasts, tenocytes and chondrocytes has attracted interest for their use in the orthopedic setting. One such application is the treatment of non-unions or critical size bone defects. Incomplete post-fracture healing of bone longer than 6 mo is referred to as non-union and varies in incidence from 5%-20% depending on fracture type[98-100]. Currently, the mainstay of therapy for critical defects is autologous bone-grafting, which has disadvantages including limited availability and significant donor site morbidity. Stem cell therapy provides an alternative approach enabling bone formation locally at defect sites. MSCs loaded onto a porous ceramic cylinder provide significant healing potential in critical size defects in the canine model[101]. Cells modified to express osteoinductive factors also demonstrate enhanced bone healing at defect sites[102]. Our group has recently demonstrated that BMP-9 is one of the most osteogenic factors in promoting ectopic bone formation from MSCs[68]. Clinical trials also support the therapeutic benefit of MSC therapy in enhancing bone healing.

Quarto *et al*[103] have utilized autologous MSCs loaded onto macroporous hydroxyapatite scaffolds to demonstrate full limb functional recovery in a significantly shorter period than for traditional bone grafting. MSC-engineered bone demonstrated significant durability when used for treatment of critical size defects in long bones[104]. The therapeutic efficacy of MSC promotion of bone healing is directly correlated with the number of progenitor cells available in the graft[105]. The unique capability of MSCs to

Table 1 Examples of *in vivo* applications of mesenchymal stem cells

Animal	Disease model	Outcome
Baboon	Skin graft transplantation	Prolonged skin graft survival[169]
Mouse	GvHD	Prevention of GvHD[124,170]
	EAE	Prevention of EAE development and improved functional recovery[129, 171]
	CIA	Improved arthritic symptoms[172]
	NOD	Prevent T-cell mediated beta-cell destruction[173]
	SLE	Improved symptoms, serological markers and renal function[137]
	Lung injury	Decreased severity of endotoxin induced lung injury and improved survival[174]
Rat	Glomerulonephritis	Accelerate glomerular healing[175]
	Critical size defect	Improved healing and function[176]
	Experimental colitis	Improved survival and healing[177]
	Dilated rat cardiomyopathy	Improved cardiac function[178]
	Heart transplantation	Participate in tissue repair by giving rise to myofibroblasts and cardiomyocytes[179]
	Myocardial infarction	Improved cardiac function and survival[143]
	Cerebral ischemia	Reduced gross lesion volume and improved functional recovery[151]

CIA: Collagen induced arthritis; EAE: Experimental autoimmune encephalomyelitis; GvHD: Graft-versus-Host-Disease; NOD: Non-obese diabetic; SLE: Systemic lupus erythematosus.

promote bone healing combined with their ease of isolation provides a more effective therapy with less morbidity for patients suffering from critical size bone defects.

Orthopedic applications: Cartilage regeneration

Traditionally, joint cartilage defects were managed through local injection of autologous chondrocyte suspensions expanded *in vitro*[106]. Currently, research is underway to delineate the role of MSCs in cartilage repair. Wakitani *et al*[107] utilized MSCs to treat full-thickness articular cartilage defects in a rabbit model. The MSC treated group demonstrated total repair of subchondral bone 2 wk after implantation. MSC repair of full-thickness cartilage defects was found to be superior to tissue repair by chondrocytes, fibroblasts or human umbilical cord blood (hUCB) stem cells in relation to cell arrangement, subchondral bone remodeling, and integration with surrounding cartilage[108].

In comparison to animal studies, there are limited reports outlining the role of MSCs in promoting cartilage repair in the human clinical setting. Two patients with full thickness articular patellar defects experienced significant improvement in symptoms post autologous MSC transplantation with fibrocartilage repair[109]. The same group reported a similar therapeutic benefit in three different patients with nine defects in five knees[110]. In an another case report, autologous bone marrow transplantation in an athlete with a full-thickness articular cartilage defect of the femoral condyle led to significant improvement in symptoms and the resumption of previous activities[111]. Improvements in osteoarthritic knees treated by MSCs were also noted *via* histology and arthroscopy[112]. While these results are promising, our ability to utilize MSC therapy routinely for common clinical applications is limited by our incomplete understanding of their specific role and function.

Mesenchymal stem cells therapy in metabolic bone disease

Allogenic MSC transplantation has also shown to be beneficial in treating genetic bone disorders such as osteogenesis imperfecta and hypophosphatasia. Osteogenesis imperfecta (OI) is characterized by skeletal fragility and connective tissue alterations. The defect usually results from alteration in the production of type I collagen by osteoblasts, which manifests clinically as short stature, skeletal deformities and low density bones that are prone to fracture. Traditionally, this disorder had been treated through pharmacological means to enhance bone mass and decrease bone resorption[113,114]. However, the immunoprivileged nature of MSCs combined with their ability to undergo osteoblastic differentiation has made these cells highly favorable candidates for treatment of genetic disorders such as OI.

In vivo murine models of OI have demonstrated selective incorporation of MSCs in bony tissue with subsequently reduced fracture rates and increased bone strength[115]. In addition, these engrafted cells demonstrate higher efficiency in bone matrix formation[116]. Clinically, MSC therapy has led to enhanced growth and increased mineral content post-allogenic MSC transplantation[117,118]. Successful in utero-transplantation of MSCs has also been reported in a fetus with severe OI who after birth demonstrated regular growth and psychomotor development[119].

Hypophosphatasia, another genetic disorder of mesenchymal origin, is caused by a mutation in tissue non-specific alkaline phosphatase (TNALP). This syndrome has a highly variable clinical presentation, ranging from still birth without bone mineralization to early loss of teeth in the absence of skeletal symptoms. Limited clinical studies have demonstrated that cellular therapy utilizing allogenic MSCs will allow donor cells to engraft in the skeletal microenvironment, express sufficient TNALP and improve bone mineralization[120]. An 8-mo old suffering from severe hypophosphatasia with poor prognosis experienced significant clinical improvement 3 mo after receiving an allogenic bone marrow cell transplant from a haplo-identical sibling. At last follow up, the patient at age six years was ambulatory without significant clinical symptoms[121]. Another 8-mo old female diagnosed with severe hypophosphatasia also experienced significant symptomatic and clinical im-

Table 2 Examples of the therapeutic applications of mesenchymal stem cells in humans

Indications	Source	Rout of administration	Outcome
Myocardial infarction	Allogenic BM	IV	Increased GSS and EF[148]
	Autologous BM	Intracoronary	Improved LVF[149]
Cartilage defect	Autologous BM	Direct site transplantation	Improved clinical symptom and coverage of defect[110]
Osteogenesis imperfecta	Allogenic BM	IV	Growth acceleration[117]
	Fetal MSC	Intrauterine transplantation	Osteoblastic differentiation and reduced fracture[119]
Critical size defect	Autologous BM	Scaffold loaded	Faster full recovery of limb function than bone graft[103]
MLD and hurler syndrome	Allogenic BM	IV	Improved nerve conduction velocity in MLD patient and increased bone mineral density[167]
Severe idiopathic aplastic anemia	Allogenic BM	IV	Improved stroma[168]
Crohn's disease	Adipose derived stem cell	Intralesional	Improved fistula and quality of life[136]

BM: Bon marrow; EF: Ejection fraction; GSS: Global strain score; IV: Intravenous; LVF: Left ventricular failure; MSC: Mesenchymal stem cell; MLD: Metachromatic leukodystrophy.

provement after allogenic MSC transplantation. At eight years, the patient now suffers from only a mild form of hypophosphatasia[120]. These promising outcomes open the door to new therapeutic modalities for diseases that have shown minimal response to current management protocols.

Mesenchymal stem cell therapy for immune associated conditions

Autoimmune diseases have great clinical implications and are associated with significant patient morbidity. MSCs have been shown to inhibit immune response against minor histocompatibility antigens[122]. Animal models of Graft-versus-host disease (GVHD) have suggested that bone marrow or adipose-derived MSCs have the same immunosuppressive effect and lead to significant symptomatic improvement[123,124]. This immunomodulatory role was utilized to manage a patient with treatment-resistant grade Ⅳ GVHD who subsequently demonstrated significant clinical improvement[125]. In a phase Ⅱ clinical trial on patients with steroid-resistant, severe, acute GVHD, MSC treatment led to lower transplantation related mortality and higher 2-year survival post-MSC transplantation[126].

Multiple sclerosis (MS) is a devastating disease with limited effective treatment protocols at present[127]. Given the minimal success in treatment of this disease, research has focused on identifying the therapeutic potential of MSC therapy in enhancing patients' quality of life. Experimental Autoimmune Encephalomyelitis (EAE) is a murine model of MS, which enables study of the pathogenesis of this disease[128]. *In vivo* MSC treatment leads to T-cell anergy in the secondary lymphoid tissue in the absence of T-cell apoptosis[129]. Over expression of human ciliary neurotrophic factor (CNTF) enhances the therapeutic benefit of MSCs in EAE by inhibiting inflammation and stimulating oligodendrogenesis[129]. MSC transplantation also inhibits Th1 inflammatory response and promotes expression of anti-inflammatory cytokines in EAE mice[130]. There are limited clinical reports on the therapeutic role of MSC therapy in patients with MS; however, one pilot study demonstrated improvement in sensory, pyramidal and cerebral function in 6/10 patients without any side effects[131].

Joint destruction in rheumatoid arthritis (RA) is a T-cell mediated disease that results in significant patient morbidity. Collagen induced arthritis (CIA) is an animal model of RA that is widely used to delineate the pathogenesis of this disease[132]. MSC therapy leads to significant cytokine dependent improvement in arthritic symptoms in transplanted animals[133]. MSCs are able to prevent immune destruction of joint architecture by inhibiting collagen-reactive T-cells even after differentiation into chondrocytes[134]. To date no clinical trials have reported the role of MSC transplantation in patients suffering from RA.

In patients undergoing renal transplantation, administration of donor MSCs lead to a dose dependent immune suppression by inhibiting proliferation in alloreactive T-cells[135]. Furthermore, the therapeutic role of MSC has also been investigated in patients with Crohn's disease. In a phase Ⅱ clinical trial of patients with Crohn's disease and complex perianal fistulas, MSC therapy lead to enhanced fistula healing and increased the quality of life in treated patients[136]. Therapeutic role of MSCs has also been documented in patients with systemic lupus erythematosus (SLE)[137]. While the above mentioned applications of MSC therapy are promising, significant strides in our understanding is needed prior to utilization of such therapy as a routine method of managing autoimmune diseases.

Mesenchymal stem cell therapy for ischemic injury: Heart diseases

MSCs' propensity to migrate toward ischemic tissue through a CXCR4-SDF-1 dependent pathway and their ability to proliferate and differentiate into different cell types has made them attractive modalities for treatment of ischemic injuries such as myocardial infarction and cerebral ischemia. Heart failure constitutes a significant medical problem that is associated with reduced quality of life, lower life expectancy and increased medical costs[138]. Current management is focused on symptomatic improvement but a means of reversing the changes associated with heart disease is still lacking. However, stem cell biology and regenerative medicine have introduced a unique method of replacing damaged cardiac tissue with healthy cells, as *in vitro* studies have successfully demonstrated MSC differen-

tiation into cardiomyocytes[139,140].

MSC therapy post myocardial infarction (MI) can improve left ventricular function[141,142]. Miyahara *et al*[143] demonstrated that, in addition to improving cardiac function, MSC transplantation significantly enhanced survival rate in post-MI rats. Even though MSCs demonstrate the capacity to differentiate into cardiomyocytes, this ability is limited. Their beneficial effect has been attributed at least in part to their propensity for cytokine secretion. These angiogenic promoting factors protect the surrounding cells in the ischemic tissue[144] in a paracrine manner and can be synergistically enhanced when cells are genetically altered to express angiogenic and pro-survival factors[145-147]. In a randomized double-blinded study, patients receiving IV infusion of MSCs post-MI demonstrated significant enhancement in cardiovascular function[148]. Similar therapeutic benefit was reported in patients receiving intracoronary MSC administration compared to placebo[149]. The ability of MSCs to differentiate into cardiomyocytes, combined with their capacity to secrete cytokines that enhance tissue repair, will enable increased survival and quality of life by improving cardiac repair and function after myocardial infarction.

Mesenchymal stem cell therapy for ischemic injury: Cerebral ischemia

MSCs have the capacity to differentiate into neuron-like cells in the presence of epidermal growth factor (EGF) or bone-derived neurotrophic factor (BDNF)[150]. In addition, they have the ability to promote angiogenesis and tissue repair, which are key functions that play a beneficial role post-ischemic injury. In a rat model of middle cerebral occlusion, MSC administration led to significant reduction in gross lesion volume and improved functional recovery. These symptomatic improvements were more pronounced when MSCs where administered in a large dose immediately post ischemia as compared to temporally dispersed dosing[151]. MSC efficacy in this setting may rely upon their secretion of time-appropriate cytokines, as suggested by Omori *et al*[151]. MSCs also have the capacity to enhance proliferation of endogenous neural stem cells and protect newborn cells from the deleterious environment found at sites of ischemia.

In mice with embolic middle cerebral artery occlusion, MSCs successfully migrate, survive and differentiate into neuron-like cells after transplantation into the striatum, thus enhancing functional recovery[152]. *In vivo* studies have suggested that MSC therapy leads to early restoration of cerebral blood flow and blood brain barrier integrity post ischemic injury, which may play a part in their beneficial therapeutic role[153]. MSCs also facilitate axonal sprouting and remyelination after stroke[154,155]. While there are many modalities of MSC administration, intravenous transplantation is superior to striatal delivery when measured by long-term functional recovery in animal stroke models[156].

In a clinical trial, autologous transplantation of MSCs in 30 patients with middle cerbral artery (MCA) infarcts and severe neurological deficits resulted in significant improvement in the treated group without any adverse reactions after serial follow-up evaluations[157]. The safety associated with studies such as these set the stage for a more comprehensive exploration of the potential role of MSCs in humans. Hopefully, in the near future MSC therapy will lead to enhanced quality of life for patients whose everyday activities have been compromised by cerebral ischemia.

Mesenchymal stem cells and tumor microenvironment

As previously mentioned, MSCs have the propensity to migrate to hypoxic environments such as ischemic tissue and tumor stroma[158]. Furthermore, the high local concentration of paracrine growth factors, including IL-8, transforming growth factor-ss1 (TGF-ss1) and vascular endothelial growth factor (VEGF) found at sites of tumor formation, selectively recruits MSCs to sites of tumor growth[159]. Such selective migration, combined with the capacity to act as vectors for gene therapy, has suggested that MSCs could be used to provide localized anti-neoplastic treatments while minimizing systemic side effects. Studeny *et al*[160] utilized adenoviral transfected MSCs expressing INF-β to successfully suppress tumor growth and increase survival in a mouse xenograft model. Furthermore, inducible TRAIL-expressing MSCs have been shown to successfully suppress metastatic disease in the murine model[161]. Moreover, Khakoo *et al*[158] illustrated that MSCs can also exert their anti-neoplastic effect through direct cell-to-cell contact, which inhibits Akt activation in Kaposi sarcoma cells. This effect was abrogated using neutralizing antibody against E-cadherin[158]. MSCs help to slow tumor proliferation by promoting apoptosis and cell cycle arrest in G0/G1phase[162]. In addition, MSCs can directly enhance the immune response against cancer cells by taking up and presenting tumor associated antigens to enhance the CD8 mediated anti-tumor response[163].

While many studies have described MSCs' anti-neoplastic characteristics, others have raised concern regarding ability of MSCs to promote tumor formation. MSCs may serve as important contributors to the cancer stroma, promoting cell survival and metastasis[164]. The active recruitment of MSCs to the site of tumor formation, combined with their pro-angiogenic characteristics, has suggested that these cells are capable of enhancing tumor growth[165]. Furthermore, MSCs have been found to act as precursors of cancers such as gastric carcinoma through their selective recruitment to sites of chronic inflammation followed by their ability to undergo metaplasia and subsequently dysplasia to become cancerous cells[166]. It's hoped that improvement in our understanding of MSCs and their role in oncogenesis will enable more insight into cancer formation and also allow more innovative therapeutic approaches.

CONCLUSION

The characteristics of MSCs have attracted the attention of many researchers hoping to utilize the unique properties of these cells for diverse therapeutic applications. With their ability to differentiate into multiple lineages, migrate toward injured tissue, and propensity to secrete

factors identified to be important in tissue repair, it is easy to imagine how a reduction in MSC number or functional capacity can result in impaired healing, as seen in the aging population. As our understanding of mesenchymal stem cell biology and other fields such as molecular genetics improves, it may be possible to genetically alter these "rescue cells" and enhance their capacity to heal common clinical diseases. In addition, their ability to modulate immune responses and specific homing capacity towards sites of malignant growth makes MSCs attractive cellular vehicles for the treatment of autoimmune disease and solid cancers. Their ease of accessibility combined with their immune-privileged nature makes these cells great therapeutic tools. While MSC therapy is very promising, the lack of unique phenotypic markers and inefficient extraction are factors currently limiting their use. With further advancement in our understanding of their biology and behavior during *ex vivo* expansion these cells will play an important role in managing disorders that lack effective treatment.

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