REVIEW

Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration

Jyoti A. Kode[1], Shayanti Mukherjee[1], Mugdha V. Joglekar[2] and Anandwardhan A. Hardikar[2]

[1]Chiplunkar Laboratory, Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Kharghar, Navi Mumbai, India, and [2]Stem Cells and Diabetes Section, Laboratory 12, National Centre for Cell Science, Pune, India

Mesenchymal stem cells (MSC) are multipotent cells that differentiate into osteoblasts, myocytes, chondrocytes and adipocytes as well as insulin-producing cells. The mechanism underlying their in vivo differentiation is not clear and is thought to be caused by spontaneous cell fusion or factors present in the microenvironment. However, their ease of isolation, high 'ex-vivo' expansion potential and ability to differentiate into multiple lineages make them attractive tools for potential use in cell therapy. MSC have been isolated from several tissues, including bone/bone marrow, fat, Wharton's jelly, umbilical cord blood, placenta and pancreas. The 'immunosuppressive' property of human MSC makes them an important candidate for cellular therapy in allogeneic settings. Use of allogeneic MSC for repair of large defects may be an alternative to autologous and allogeneic tissue-grafting procedures. An allogeneic approach would enable MSC to be isolated from any donor, expanded and cryopreserved, providing a readily available source of progenitors for cell replacement therapy. Their immunomodulatory properties have raised the possibility of establishing allogeneic MSC banks for tissue regeneration. These facts are strongly reflected in the current exponential growth in stem cell research in the pharmaceutical and biotechnology communities. Current knowledge regarding the immunobiology and clinical application of MSC needs to be strengthened further to establish MSC as a safe and effective therapeutic tool in regenerative medicine. This paper discusses human MSC with particular reference to the expression of their surface markers, their role as immunomodulators and their multilineage differentiation potential and possible use in tissue regeneration and repair.

Keywords

Cell therapy, immunobiology, immunomodulation, mesenchymal stem cells, tissue regeneration.

Introduction

Mesenchymal stem cells (MSC) have been of interest long before the term 'MSC' came into existence. The repair blastema generated in amputated amphibians, bone callus formations that follow a fracture and mechanism of wound healing have all been of great interest in the study of the extensive expansion and organization of primitive/disorganized cell types, typically seen during tissue repair. In 1867, Cohnheim first identified MSC in bone marrow (BM) as a non-hematopoietic stem cell. In 1901, Marchand discussed tissue repair mechanisms in detail and implicated the role of 'fibrocytes' (fibroblasts) in wound repair. In 1949, experiments carried out by Jackobson and colleagues demonstrated that protection of the spleen during radiation prevents lethal effects of radiation in mice. A follow-up to these studies was the demonstration of the survival benefit offered by spleen or BM cells injected in lethally irradiated mice. These studies led to the development of therapeutic BM transplantation as reviewed in [1]. Friedenstein and others (1966–1976) characterized these cells as BM-derived, clonal, plastic-adherent cells, capable of differentiating into osteoblasts, adipocytes and chondrocytes [2]. These cells were initially referred to as 'marrow stem cells'. The term 'human mesenchymal stem cells' was first used by Caplan in 1991 to describe adherent, marrow-derived homogeneous cells

that proliferate *ex vivo* and can be differentiated to produce multiple connective cell types [3].

MSC reside in diverse host tissues and organs, such as circulating blood, adult and fetal BM, spleen, amniotic fluid, cartilage, muscle tendons, placenta, adipose tissues, fetal tissues, periosteum, synovial fluid, thymus, trabecular bone, dermis, dental pulp and lung [4–6]. MSC have been shown to differentiate not only into osteogenic, chondrogenic and adipogenic lineages but also others such as the mesodermal (myocyte, osteocyte, endothelium, adipocyte, cardiomyocyte), ectodermal (neuronal) and endodermal (hepatic, pancreatic, respiratory epithelium) lineages (Figure 1).

Characterization of MSC

The identification of specific cell-surface proteins is one of the major challenges in characterization of a cell type that defines the kind of heterotypic/homotypic interactions

Table I. Characterization of MSC.

Marker type	Examples
Surface markers	CD13, CD29, CD44, CD73, CD90, CD105, CD106, Stro-1, Sca-1
Cytokine receptors	IL-1R, IL-3R, IL-4R, IL-6R, IL-7R
Extracellular matrix receptors	ICAM-1, ICAM-2, VCAM-1, ALCAM, endoglin, hyaluronate receptor integrins α1, α2, α3, αA, αV, β1, β2, β3, β4
Growth factor receptors	BFGF-R, PDGF-R
Other receptors	Thy-1, IFN-γR, TGF-βR, TNF-R

Commonly used cell surface proteins/receptors that are characteristic markers for isolation and characterization of MSC are shown. Fluorescently tagged antibodies to most of these are commercially available and can be used in a flow cytometry setting.

between neighboring cells. Growth factor receptors, cell adhesion molecules and other cell-surface markers expressed by MSC are summarized in Table I. MSC express
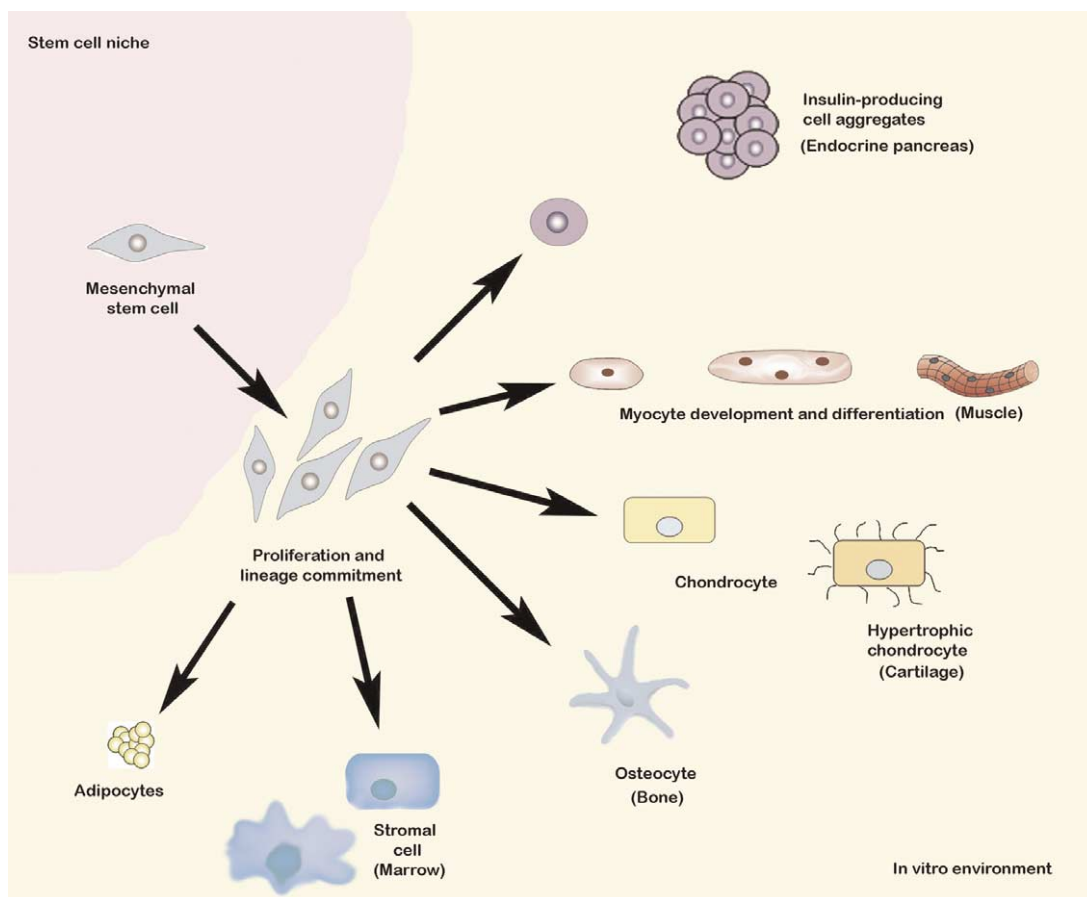

Figure 1. *Differentiation potential of MSC. A pictorial representation demonstrating that MSC isolated from various sources (see text) and expanded* in vitro *largely retain their characteristic markers. These can then be induced to proliferate* in vitro *and induced to differentiate into several lineages following exposure to specific growth/differentiation factors.*

CD44, CD73, CD90 and CD105 receptors while lacking hematopoietic stem cell markers such as CD14, CD31, CD33, CD34 and CD45. Culture-expanded MSC also lack expression of endothelial markers such as von Willebrand factor and P-selectin [6]. MSC show expression of adhesion-related antigens, such as integrins αvβ3 and αvβ5, integrin subunits α4, α5 and β1, intercellular adhesion molecule-1 (ICAM-1) and CD44H, enabling their adherence to extracellular matrix molecules [7]. MSC exhibit low expression of histocompatibility complex (MHC) class I molecules, and are negative for MHC class II antigens. MSC do not express co-stimulatory molecules, such as B7-1, B7-2, CD80, CD86, CD40 and CD40L [8]. MSC produce cytokines, chemokines and growth factors, such as interleukin (IL)-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, leukemia inhibitory factor, granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), stem cell factor, macrophage colony-stimulating factor (M-CSF) and fms-like tyrosine kinase-3 ligand (flk-3L), implicated mainly in hematopoiesis [9,10]. They also express cytokine receptors IL-1R, IL-3R, IL-4R, IL-6R and IL-7R. Cytokine-induced MSC express very high levels of several leukocyte chemokines, most notably CXCL9, CXCL10 and CXCL11. Although no specific cell-surface maker can be assigned to identify MSC explicitly, the array of different cell-surface markers reported provides cues to the signaling interactions that MSC may share with other cell types during cellular expansion and/or differentiation.

Various studies have shown that *in vitro*-expanded MSC preferentially home to sites of tissue damage, where they enhance wound healing, support tissue regeneration and restore the BM microenvironment following damage by myeloablative chemotherapy, or integrate into tumors [11]. This specific migration of MSC is reportedly guided by chemokines; in fact, recent studies have found that MSC express the chemokine receptors CCR1, CCR4, CCR7, CXCR5 and CCR10 [12,13], which may be involved in this process. The characteristics of MSC, as of most stem cells, are known to be regulated by their microenvironment, made up of multiple factors at different concentrations, which interact with these cells either independently or, more likely, in combination. The most studied cytokines include the interleukins, chemokines and interferons. In addition to soluble factors, MSC are known to be affected by intercellular communication. The cytokine/chemokine gene expression profiles of MSC resident in the lung are

distinct from those derived from BM [14]. Several chemokines (IL-8, CXCL1, CXCL2 and CXCL6) and genes associated with angiogenesis (encoding IL-1α, IL-8, Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor-2 (FGF2) and Transforming Growth Factor (TGF)-β2) and hematopoiesis (encoding leukemia inhibitory factor, CSF1 macrophage, CSF2 granulocyte–macrophage and IL-6) have higher expression in BM-derived MSC than MSC from the lung. Increased expression of osteopontin, a marker for osteogenesis and an important factor for hematopoiesis, in BM-derived MSC suggested a role of different tissue environments in determining the functions [14].

Following stimuli, including mechanical injury, inflammation, infection and cancer, MSC are thought to migrate from their niche and engraft to the injured site, leading to tissue repair. The exact signaling events that drive MSC to repair the damage are as yet unknown. This property of MSC has now been applied for their use as therapeutic delivery agents that assist in the repair of damaged tissues [15].

Immunomodulation

MSC have long been known to be immunomodulatory. The general effects are thought to 'skew' the immune response toward anti-inflammatory/tolerant phenotypes, including the shift from T-helper type 1 (Th1) toward Th2, down-regulation of interferon (IFN)-γ production from natural killer (NK) cells and reduction in the antibody production of B cells. They regulate lymphopoiesis and suppress the immune response. BM MSC participate in the developmental process of both T lymphocytes and B lymphocytes through growth factors, cytokines and adhesion molecules [16]. Moreover, MSC mediate immunoregulatory effects on both innate and adaptive immunity through either indirect soluble factors or direct physical contact [17]. MSC lack MHC Class II indicating immune evasion in the allogeneic setting [18]. MSC have elicited great clinical interest in the form of allogeneic or 'off-the-shelf' products and 'universal donor cells', particularly in regenerative medicine and for induction of tolerance in allogeneic transplantation.

Co-culture of MSC with allogeneic lymphocytes fails to stimulate their proliferation, indicating that these cells are innately non-immunogenic. Furthermore, MSC can inhibit proliferation of lymphocytes, antigen-presenting cells (APC) and NK cells in mixed-lymphocyte reactions

(MLR) [17,19]. The invariably fatal graft-versus-host disease (GvHD) post-organ/BM transplantation is model example of MLR and is frequently unresponsive to various immunosuppressive therapies [20–22]. Current approaches targeting immune molecules show great promise in treating GvHD [23–27]. Most recently, MSC have been shown to be highly effective in the treatment of GvHD in pre-clinical and clinical trials [17]. It has been thought for quite some time that the lack of MHC class II and other classical co-stimulatory molecule expression on MSC makes them less immunogenic [18,28]. However, recent studies have demonstrated that MSC may not be immunoprivileged [29], as thought earlier.

Recent reports indicate that MSC express toll-like receptor (TLR) proteins [30–32], which are critical players in clinically established immunomodulation. The ligation of TLR-3 (which binds double-stranded RNA) and TLR-4 [which binds lipopolysaccharide (LPS) and innate self-antigens) blocks the ability of MSC to inhibit T-cell responses through NOTCH signaling via down-regulation of the expression of Jagged-1 on MSC [31]. Of the many TLR reported, uniquely TLR-3 was found to be primarily mediating the stress migration responses within MSC.

MSC have also been shown to suppress T-cell proliferation and cytokine production, involving mediators such as IL-10, TGF-β, indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2) through as yet unknown mechanisms [33]. The immunosuppressive ability of MSC is not innate but rather is induced by pro-inflammatory cytokines: IFN-γ in combination with tumor necrosis factor (TNF)-α, IL-1α or IL-1β. As MSC are responsive to pro-inflammatory cytokines, it is hypothesized that cytokines produced during an immune response induce chemokine expression by MSC. These chemokines may serve to recruit/mobilize lymphocytes, a key step in MSC-mediated immunosuppression. Another prominent candidate in the mechanism of MSC-mediated immunosuppression is nitric oxide (NO), a rapidly diffusing gaseous and bioactive molecule [34]. The cytokines induce a dramatic up-regulation in inducible nitric oxide synthase (iNOS) and several leukocyte chemokines that may bring immune cells, including T cells, B cells and APC, into close proximity with MSC, where high levels of NO can suppress immune cell function. Therefore, the concerted action of cytokine-induced chemokines and NO is key to MSC-mediated immunosuppression [33]. NK cells are major effector cells of innate immunity and are generally thought to play a fundamental role in antiviral and antitumor responses [35]. MSC strongly inhibit IL-2-induced NK cell proliferation and prevent the induction of effector functions, such as cytotoxic activity and cytokine production [36]. This inhibitory effect was mediated by down-regulation of the expression of the activating NK-surface receptors NKp30, NKp44 and NKG2D and secretion of IDO and PGE2 as key mediators.

Overall, MSC seem to regulate immune responses by reducing the generation/differentiation of dendritic cells, increasing the number of regulatory T cells and suppressing effector T cells through various growth factors, iNOS, heme oxygenase-1, PGE2 and IDO. Also, up-regulation of MHC class II on MSC could lead to down-regulation of NK cell cytotoxicity and proliferation. The possible mechanisms that allow MSC to regulate the immune response are shown in Figure 2.

Tissue regeneration

Stem cells are being considered as tools for replacing, repairing, regenerating and rejuvenating dead, degenerating or injured cells and tissues. Certain diseases, such as Parkinson's disease, are caused by progressive degeneration of one or more cell types. In such cases, stem cells used as replacement therapy have been proposed to offer 'life-long treatment'. By isolating stem cells, it is believed that new heart cells could be grown to repair damage from heart attacks, or liver cells to treat cirrhosis. Once implanted, stem cells are capable of interacting with the surrounding microenvironment and facilitating the regeneration of the neighboring tissue by secreting certain factors and renewing biologic functions, such as the immune system, or acting trophically to support and rejuvenate host cells. Various *in vitro* and animal studies have determined the feasibility of transplanting MSC. Haynesworth *et al.* [37] successfully isolated and expanded human MSC and demonstrated a reliable *in vivo* bone-forming assay. The next step was to evaluate the safety, feasibility and efficacy of transplanting off-the-shelf MSC for clinical trials [38,39]. *In vivo* models have demonstrated that MSC can engraft into organs like liver, bone, lung and kidney after infusion [40,41]. In a canine model, transplantation of autologous MSC with partially demineralized bone matrix restored bone defects and enhanced bone growth in a non-weight-bearing gap [42]. This combination provided an option for reconstructing bone defects while performing a cementless revision arthroplasty. These properties have led
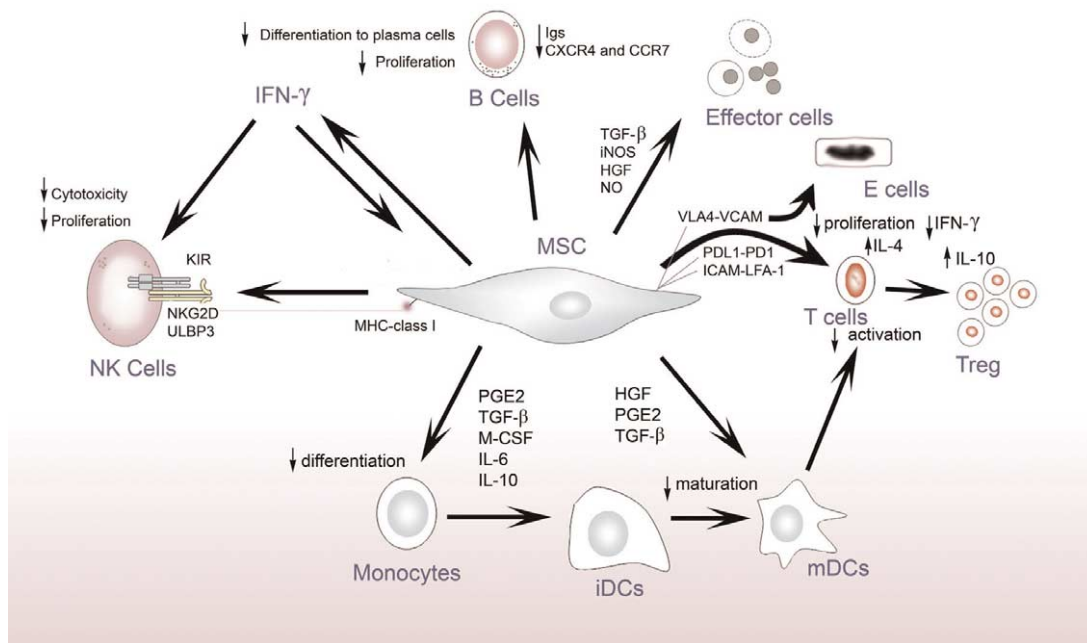
Figure 2. *Immunomodulation by MSC. Immunomodulation by MSC occurs at multiple levels: MSC-induced suppression includes naive and memory T cells. MSC inhibit proliferation and differentiation of B cells into plasma cells, thus reducing antibody formation. MSC suppress NK cell proliferation, IFN-γ production and reduce the cytotoxic potential of NK cells against target cells via several soluble factors. MSC inhibit differentiation of monocytes into immature dendritic cells (iDC) and their further maturation into mature DC (mDC).*

to the suggestion that MSC may have a significant role in tissue repair and regeneration.

Effective stem cell therapy fundamentally rests on the optimized combination of two key elements: the starting cell population and the environment in which the cells are placed. Two different approaches can be envisaged: (i) implanting the tissue-restricted stem cells or progenitors that will differentiate some time after transplantation; and (ii) transplanting differentiated stem cells after *in vitro* manipulation. The choice will depend on our knowledge of the cell system and its niche. Studies should evaluate the differentiation potential of MSC under *in vitro* conditions as well as examine the mechanistic bases for *in vivo* maturation in animals. More studies with strong reporter systems should test the potential of MSC to home and help in tissue regeneration in animal models. This will help further our understanding of the potential of MSC during tissue regeneration in humans.

Ectodermal regeneration

Human MSC derived from the early human embryo can be transformed into epidermal cells *in vitro* and *in vivo* [43]. Given the regenerative, immunomodulatory and immune-privileged properties of MSC, one exciting possible use for these cells is in accelerating wound healing. In a recent pre-clinical study [44], human BM MSC (cultured on a collagen sponge mimicking an artificial dermis layer) were shown to differentiate into dermal tissue upon subcutaneous implantation in immunocompromised mice. Further clinical transplantation of autologous grafts generated from MSC–collagen matrix demonstrated that 18 of 20 patients with treatment-refractory dermatopathies exhibited significant improvement in non-healing wound areas [44]. Injection of autologous biografts composed of autologous skin fibroblasts on biodegradable collagen membrane (Coladerm), in combination with autologous MSC, into the edges of a wound decreased wound size and increased the vascularity of the dermis in diabetic foot wounds [45].

MSC are suggested to have a role in neural regeneration as well. MSC implanted into devitalized muscle grafts support peripheral nerve regeneration to some extent [46]. Intrastriatal transplantation of MSC have promoted functional improvement in murine models of Parkinson's disease [47]. In humans, MSC transdifferentiated into neural stem cells improved the electrical and functional

recovery of two patients with chronic spinal injury [48]. MSC infusion into patients suffering from metachromatic leukodystrophy and Hurler's syndrome demonstrated significant improvement in nerve conduction velocities [49].

Using a mini-pig model, Sonoyama *et al.* [50] transplanted human MSC from apical papilla of tooth as well as periodontal ligament stem cells to generate a root–periodontal complex capable of supporting a porcelain crown, resulting in normal tooth function. This tissue engineering approach led to recovery of tooth strength and appearance.

Mesodermal regeneration

The role of MSC in mesodermal regeneration, especially bones, cartilage, tendons and muscles, is well documented. Repeated endomyocardial transplantation of high doses of allogeneic MSC appeared safe in Yorkshire swine models [51]. Adult human MSC showed persistent engraftment into infarcted rat myocardium and improved cardiac structure and function through the combined effect of myogenesis and angiogenesis [52]. MSC enhanced the survival of existing myocytes in mice through paracrine mechanisms [53]. In murine models, single clonally purified MSC seem to be more beneficial than unpurified transplanted MSC in cardiac repair [54]. Transplantation of MSC combined with erythropoietin treatment in rat models of acute myocardial infarction led to enhancement of capillary density and reduction of infarct size and fibrotic areas, compared with groups that received only MSC [55]. Transplantation of genetically engineered MSC expressing an anti-apoptotic and angiogenic peptide improved cardiac function after myocardial infarction significantly more than MSC alone [56].

MSC expanded in an osteoconductive carrier regenerated a critical segmental defect in the femur of dogs as effectively as autogenous cancellous bone [57]. Transplantation of MSC for restoring bone morphology and repairing bone defects has been demonstrated in various large animal models (reviewed in [19]). Embryonic mesenchymal cells have been shown to differentiate in a chemically defined chondrogenic differentiation medium containing two growth factors [bone morphogenetic protein-2 (BMP-2) and TGF-β3] that promote chondrogenesis [58]. Under these conditions, gene expression of cartilage-, bone- and muscle-specific matrix proteins, including collagen types I, II, III, IX and X, aggrecan, cartilage proteoglycan link protein, cartilage oligomeric protein, chondroitin sulfate-4-S and myf5, was up-regulated, demonstrating the ability of transdifferentiation of MSC to cells of mesodermal lineages. In another study, allogeneic MSC loaded on hydroxyapatite–tricalcium phosphate implants enhanced the repair of a critical-sized segmental defect in a canine femur in the absence of immunosuppressive therapy [59]. No adverse immune response was detected in this model [59].

Endodermal regeneration

Tissue transplantation is the primary treatment for various end-stage hepatic diseases and diabetes. It is hindered by the lack of donor organs and complications associated with rejection and immunosuppression. Although there is increasing evidence suggesting BM as the source of beta cell progenitors, it is now believed that transplanted BM mesenchymal cells may help in inducing regeneration and/or proliferation of resident insulin-producing cells. BM MSC can effectively transdifferentiate into hepatocytes, both *in vitro* and *in vivo*. They also rescue experimental liver failure, bring about liver regeneration and hence offer a potential cell replacement therapy for treatment of liver diseases. Flk1-positive mouse MSC transplanted immediately after exposure to CCl_4, significantly reduced CCl_4-induced liver damage and fibrosis [60]. MSC originating from BM may contribute to the repair of damaged islet vasculature and have been reported to rescue the long-term damage associated with diabetes, such as neuropathy [60], cardiomyopathy [62], nephropathy [63] and diabetic foot [45].

Tissue engineering

MSC-mediated tissue regeneration is a promising approach for replacing and rebuilding diseased and damaged structures in the human body. It was not until the middle of the 1900s, however, that the use of synthetic materials for rebuilding body structures met with widespread and reproducible success. Most of the current success is the result of advances in material sciences and the production of biocompatible materials. The use of MSC in three-dimensional (3-D) scaffolds is limited by the need for an ideal scaffold. It is known that native extracellular matrix (ECM) not only offers physical support for cells but also provides a substrate with specific ligands for cell adhesion and migration and regulates cellular proliferation and function by providing various growth factors. It is reasonable to expect that an ECM-mimicking tissue-engineered

scaffold will play a similar role in promoting tissue regeneration *in vitro* as native ECM does *in vivo*. The ideal scaffold is expected to be biocompatible and biodegradable and should mimic the structure and biologic function of native ECM as much as possible. Besides biocompatibility of its chemical composition and physical structure, it should offer the necessary environment for growth and/or differentiation of MSC. Additionally, the presence of specific molecules that promote cell signaling, proliferation and differentiation on scaffolds would ensure enhancement of tissue regeneration. Hosseinkhani *et al.* [64] demonstrated that the angiogenesis induced by the controlled release of basic-fibroblast growth factor (bFGF) from bFGF-incorporated nanofibre scaffolds plays an important role in creating an environment suitable for the survival and activity of transplanted cells for further applications in tissue regeneration. BMP-2 is a cytokine present in bone extract that induces ectopic bone formation [65,66]. This growth factor has been associated with ceramics in many forms, combined with different ceramics by surface adsorption, as an addition before setting, and injected or incorporated in a carrier material [e.g. poly-(lactic-*co*-glycolic acid) (PLGA), gelatine, and collagen sponge] combined with the ceramic [67]. These have resulted in improved bone formation, attributed to BMP-2 activity.

The method of replacing a body part or filling a void post-surgery or radiation-induced lesions, comprises obtaining a non-diseased cell sample from a patient's BM, isolating MSC and loading them on a 3-D scaffold. The MSC loaded on a 3-D scaffold are allowed to interact with the damaged area, wherein they regenerate and replace the missing body part or void and fuse with the adjacent tissues. MSC-engineered hydroxyapatite used to fill a patient's bone cavity after tumor curettage demonstrated healing potential without adverse reactions [68]. PLGA, one of the few synthetic materials that have been approved by the FDA for clinical applications, has been investigated widely in conventional form to repair whole-thickness cartilage defects [69] and regenerate the gap defect of Achilles tendon [70] with MSC and has shown fairly good results *in vivo*. Chondrocytes have shown proliferation and ECM synthesis on PLGA scaffolds in *in vitro* culture. PLGA co-polymers with improved topographical features have also been used for various applications. Scaffolds fabricated with PLGA co-polymer and gelatin [71] or collagen [72] have demonstrated excellent biocompatibil-

ity, suitable mechanical properties and sustained-release characteristics, which makes them potential artificial nerve scaffolds for use in allogenic grafts. In one study, PLGA co-polymerized with gelatin–chondroitin–hyaluronate significantly augmented the proliferation of MSC and GAG synthesis, indicating its potential in cartilage repair [73].

Clinical trials in cell therapy

MSC are currently being evaluated in various pre-clinical and clinical studies and offer significant potential as a novel cellular therapy for tissue regeneration and repair. A number of studies in animal models of cardiac injury, stroke and ischemic renal injury have demonstrated the clinical potential of MSC in tissue regeneration and repair [74]. A few human studies have demonstrated that application of culture-expanded autologous and allogenic MSC to BM transplantations leads to homing of MSC and rejuvenation of the BM stroma of chemotherapy/radiation-treated patients. The mechanism governing all of these cases seems to be the same: the MSC secrete bioactive factors that inhibit scarring and apoptosis, and induce angiogenesis and proliferation of tissue-intrinsic stem or progenitor cells. This complex, multifaceted activity caused by the secretory activity of MSC is referred to as 'trophic activity', as distinctive from the capacity of MSC to differentiate [74]. Various biotechnologic companies have developed patented formulations (Table II) of adult stem cells and evaluated safety in phase II/III clinical trials for treatment of acute GvHD, hematologic malignancies and Crohn's disease. Similar formulations are proposed with potential for treatment of type 1 diabetes, cardiac failure, liver failure, cirrhosis, long bone defects, non-healing wounds/burns, etc., and will soon be seen in clinical trials. Their focus is to progress through the clinical trials and international regulatory processes necessary to commercialize the technology in as short a time frame as possible.

Clinical application in gene therapy

Over the past two decades, the ability to transfer genes into MSC and the ability of MSC to migrate to a lesion has raised hopes for using gene therapy-based approaches to provide long-term therapeutic impacts, as reviewed in [19] and [75]. Transplantation of IL-7 gene-engineered MSC into lethally irradiated mice led to a significant increase in thymopoiesis, enhanced immune reconstitution and protected the host from GvHD [76]. MSC transfected with

Table II. Details of cell-based therapies carried out using MSC.

Prochymal adult human MSC for treatment of moderate-to-severe Crohn's disease	Crohn's disease, BM-derived allogeneic MSC (Prochymal)	Osiris Therapeutics, USA	Active, not recruiting
Follow-up study to evaluate the safety of Prochymal for the treatment of GvHD patients	GvHD, BM-derived allogeneic MSC	MD Anderson Cancer Center and Osiris Therapeutics, USA	Active, not recruiting
OTI-010 for GvHD prophylaxis in treating patients who are undergoing donor peripheral stem cell transplantation for hematologic malignancies	Hematologic malignancies, BM-derived autologous MSC	Johnson Comprehensive Cancer Center and National Cancer Institute, USA	Active, not recruiting
MSC infusion as prevention for graft rejection and GvHD	Hematologic malignancies, BM-derived donor MSC	University Hospital of Liege, Belgium	Recruiting
MSC infusion as treatment for steroid-resistant acute GvHD or poor graft function	GvHD, BM-derived allogeneic MCS	University Hospital of Liege and Katholieke, Universiteit Leuven, Belgium,	Recruiting
Evaluation of the role of MSC in the treatment of GvHD	GvHD, BM-derived donor MCS	Christian Medical College, India	Recruiting
Treatment of refractory GvHD by the Infusion of expanded *in vitro* allogeneic MSC	GvHD, BM-derived allogeneic MSC	University of Salamanca, Spain	Recruiting
Donor MSC infusion in treating patients with acute or chronic GvHD After undergoing a donor stem cell transplant	GvHD, BM-derived donor MSC	Case Comprehensive Cancer Center and National Cancer Institute, USA	Recruiting
Evaluation of Prochymal adult human stem cells for treatment-resistant moderate-to-severe Crohn's disease	Crohn's disease, BM-derived allogeneic MSC (Prochymal)	Osiris Therapeutics, USA	Recruiting
Prochymal infusion for the treatment of steroid-refractory acute GvHD	BM-derived allogeneic MSC (Prochymal)	MD Anderson Cancer Center and Osiris Therapeutics, USA	Recruiting
Efficacy and safety of Prochymal infusion in combination with corticosteroids for the treatment of newly diagnosed acute GvHD	GvHD, BM-derived allogeneic MSC (Prochymal)	Osiris Therapeutics, USA	Recruiting
Prochymal adult human MSC for treatment of moderate-to-severe Crohn's disease	GvHD, BM-derived allogeneic MSC (Prochymal)	Osiris Therapeutics, USA	Completed, clinicaltrials.gov identifier NCT00294112
Safety and efficacy of Prochymal for the salvage of treatment-refractory acute GvHD patients	GvHD, BM-derived allogeneic MSC (Prochymal)	Osiris Therapeutics, USA	Completed
Marrow mesenchymal cell therapy for osteogenesis imperfecta: a pilot study	Transplantation of BM-derived cells for treatment of osteogenesis imperfecta	St Jude Children's Research Hospital, USA	Completed, clinicaltrials.gov identifier NCT00187018

Some of the key registered clinical trials using MSC are listed. More information is available of individual studies on clinicaltrials.gov.

hHCN4 genes by lentiviral transfection was successful in developing biologic cardiac pacemaker cells *in vitro* [77]. In a murine model, MSC transfected *ex vivo* with the hepatocyte growth factor gene were more therapeutically efficient than MSC alone in protecting brain tissues from acute ischemic damage in midcerebral artery occlusion [78]. MSC transduced with the brain-derived neurotrophic factor gene further enhanced the protective efficacy against ischemic damage [79]. Transplantation of genetically engineered MSC expressing an anti-apoptotic and angiogenic peptide improved cardiac function after myocardial infarction significantly more than MSC or gene therapy alone [56]. MSC transduced with the BMP-2 gene when injected directly in articular fractures with both

bone/cartilage injury exhibited successful repair in both bone and cartilage *in vivo*, which was caused by augmentation of the BMP-2 delivered by MSC at the repair site [80].

MSC can target tumor cells and have been suggested as a possible approach for the delivery of therapeutic agents. This property could also be used to track cancers and target and selectively kill tumor cells by incorporating an anti-tumor gene into MSC (reviewed in [19]). Genetically modified MSC expressing the vascular endothelial growth factor receptor (tsFlk-1) gene can inhibit growth of Burkitt's lymphoma in a murine model [81]. MSC engineered to express IFN-β constitutively when used as cellular vehicles has demonstrated a significant increase in antitumor NK cell activity along with a reduction in lung metastasis of TRAMP-C2 prostate cancer cells in an immunocompetent mouse therapy model [82].

Commercialization

Osiris Therapeutics Inc (Columbia, Maryland, USA; www.osiristx.com, accessed on 12 June 2009) has undertaken clinical trials of allogenic hMSC for treatment of Crohn's disease, cardiac disorders and diabetes. The proposed product (Prochymal) contains adult MSC isolated from normal healthy adult volunteers. These cells are free of any animal contaminant and have been shown to be universally compatible in pre-clinical animal studies. The pre-clinical studies demonstrated that MSC follow inflammatory signals and migrate to various areas of the body to facilitate tissue repair and interact with immune cells (to reduce inflammation). Phase I results suggest that matched hMSC are well tolerated and phase II studies have provided the dosage data. The multicenter phase III studies are currently in progress and are expected to be out by 2010. So far, the company has tested these MSC in more than 400 patients without any complications or major risk indications (http://www.osiristx.com/clinical_trials.php/; accessed on 13 June 2009).

Mesoblast Limited (www.mesoblast.com; accessed on 12 June 2009) is an Australian biotechnology company committed to the development of novel treatments for orthopedic conditions. Mesoblast's high margin business model, allogeneic or off-the-shelf products, is to develop clinical products using allogeneic or off-the-shelf adult stem cells. Consequently, Mesoblast's cells obtained from a single donor can be used to treat thousands of unrelated patients. This results in an efficient, highly reproducible

product, with low manufacturing costs that can generate high margins akin to pharmaceutical sales. Equally as important, such off-the-shelf products will be available at hospitals for immediate use by orthopedic surgeons when acute trauma or other injury needs rapid treatment.

Mesoblast Ltd has invested up to US$17 million in the USA-based sister company Angioblast Systems Inc. (New York, NY, USA; www.angioblast.com; accessed on 13 June 2009). In alliance with Abbott, a global healthcare company (www.abbott.com; accessed on 13 June 2009 an investment of US$5 million), Angioblast has entered into development and commercialization of catheter-based therapy for heart failure.

Cytori Therapeutics (San Diego, CA, USA; accessed on 13 June 2009; www.cytoritx.com) has successfully started marketing a system, Celution 800, that provides a patient's own adipose-derived tissue-derived stem cells at the bedside. The Celution system is a closed device that circumvents the need for a good manufacturing practice (GMP) clean room for cellular expansion/manipulation and allows easy purification of stem cells at the point of care. The devices are being sold into the growing international reconstructive surgery market for so-called cell-enhanced reconstruction. Radiation-related tissue injury, poor wound healing and deep skin ulcers are among the serious effects of cancer therapy and may also occur from exposure to nuclear materials. Currently, patients with these injuries have few options for treatment. Nagasaki University, which is a global strategic center for radiation health risk control (www-sdc.med.-nagasaki-u.ac.jp/gcoe/index.html), has invested up to US$15 million in grants to develop Cytori's regenerative medicine technology as a standard of care for patients with injuries resulting from radiation exposure.

Software companies are also exploiting market opportunities in stem cell therapeutics by building software [83] that helps ensure that quality and regulatory compliance standards are met and integrated with established procedural protocols, monitoring manufacturing processes and documenting lot-to-lot traceability. StemLab software also provides all the tools needed to comply with GMP and regulatory requirements for documentation of identity, purity and potency of products (www.stemsoft.com). This software was introduced to meet the needs of researchers and clinicians involved in stem-cell based processing, manipulation and therapies.

Stem Cell bioprocessing is being developed in the UK in partnership with system engineers and experts on bioreactors and cell encapsulation technologies for the successful transfer of the laboratory-based practice of stem cell and tissue culture to the clinic as therapeutics. It aims to have products that are cost-effective, rapid in outcome, robust, reliable and reproducible.

Stem cell market trends

The stem cell marketplace is one of the dynamic areas in life science research today. Unlike many other emerging themes in biotechnology, stem cells are an established research area that is experiencing significant growth. A recently published stem cell market survey (http://www.selectbiosciences.com/marketreports/) shows that most stem cell research is conducted in academic and university communities (Figure 3A). Considering the ethical and intellectual property issues surrounding stem cells, pharmaceutical and biotechnology communities are in wait-and-watch mode. This survey also covered the types of stem cells used for research and found significant usage of MSC in the global stem cell marketplace (Figure 3B). The types of markers currently used to characterize MSC were also surveyed (Figure 3C). However, this report did not note specially categorized therapeutic usage or revenue generation by these cells. Nonetheless, these market analyzes are important as they seek to characterize and focus technology and product development in an important area of life sciences. Investors are optimistic on stem cell products that have application in the treatment of diseases that cannot be cured by non-cell-based products. The global market for stem cell therapies is expected to be US $32 billion by 2013 (www.biospectrumasia.com). India is expected to have a market share of about US $540 million with an annual growth rate of 15%.

Challenges

Some guidelines on the widespread clinical exploitation of MSC are being formulated by the International Society for Cellular Therapy (http://www.celltherapysociety.org/). One major problem is the lack of a commercial GMP-licensed MSC product. There are no generally accepted assays of the potency of MSC, and the optimal route of MSC delivery must be defined for individual indications. The best MSC source, its purity and the optimal dose remain to be specified. Purity, also defined as the identification and presence of 'contaminating' cell popula-

tions, is critical because the degree of contamination may affect both the biologic effects observed and the potential side-effects. Quantification of all of these factors will be required to obtain a reproducible and consistent cell preparation that can potentially be used in clinical studies [84]. Safety issues are a concern, although injection of syngeneic, allogeneic and xenogeneic MSC into immunocompetent mice is tolerated without apparent side-effects. Despite the heterogeneous nature of stromal progenitor cell populations, a consensus concerning the definition of MSC and GMP protocols is evolving.

Potential negative effects of MSC

Detrimental effects of MSC have been reported [85–87]. In contrast to mouse and human embryonic stem (ES) cells, BM-derived undifferentiated MSC do not pose a risk of producing teratomas that contain derivatives of all three germ layers. However, they can be a source of carcinoma-associated fibroblasts (CAF), which may contribute to the promotion of tumor growth, invasiveness and metastasis, possibly through sustained expression of chemokines and/or by harboring cancer-promoting mutations. CAF may participate in altering the drug response of tumors *in vivo* [85,88]. MSC, which have very high levels of asparaginase expression, can protect leukemic cells from asparaginase cytotoxicity by providing increased concentrations of asparagine in the leukemic cell microenvironment [88]. It is suggested that MSC may have the ability to form a cancer stem cell niche in which tumor cells preserve their self-renewal ability and potential to sustain malignant processes, especially in epithelial tumors [86,89]. A better understanding of the interplay between different BM-derived cell types and the tumor cells within the tumor microenvironment may prove to be important in manipulating tumor stroma and thus the development of strategies for improved tumor therapy. Furthermore, it is thought that the clinical use of MSC in the context of malignant conditions should be managed with extreme caution. In some reports, no karyotypic changes were detected by cytogenetic analysis in BM MSC at passages 2–10. However, aberrant karyotypic changes (monosomy and translocations) have been observed in long-term MSC cultures (11–14 passages) at a frequency of 1.5–6% in two of seven human cases [90]. One study reported the development of sarcomas in mice injected with *ex vivo*-expanded MSC [91]. Although the growth characteristics of human and murine MSC are not identical
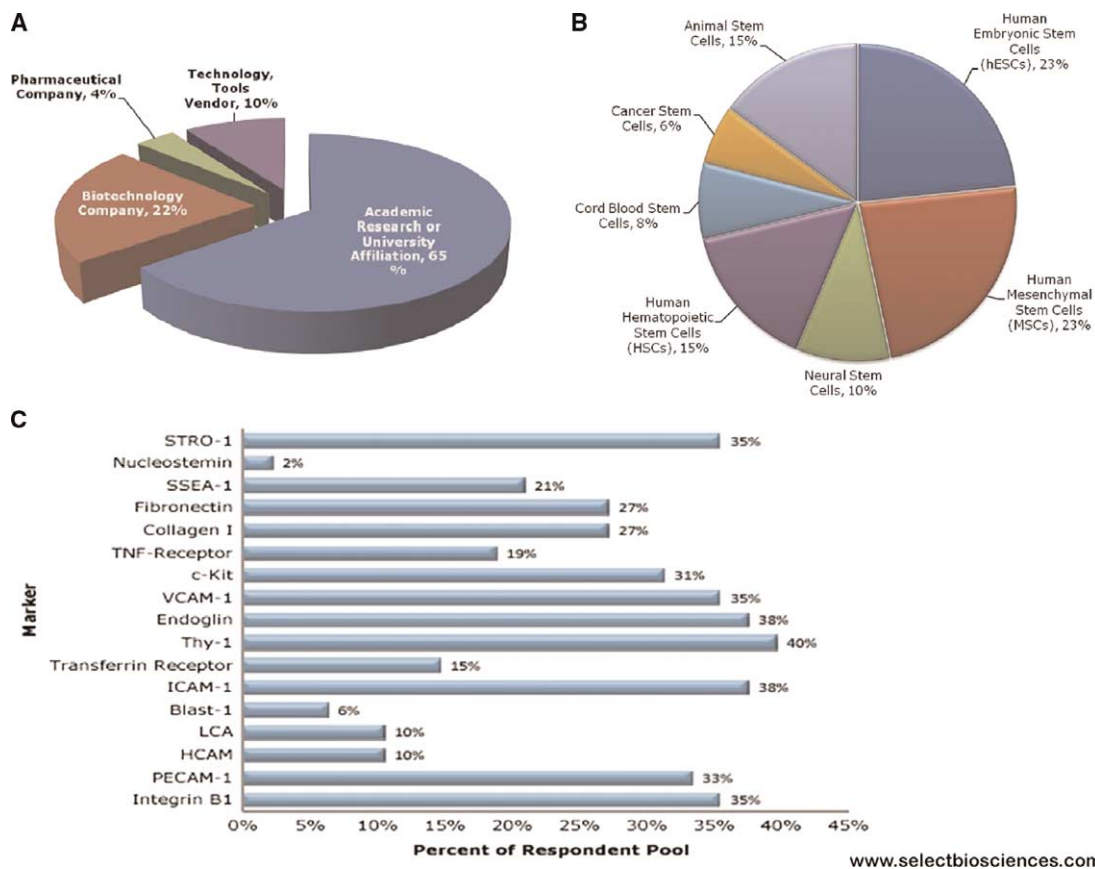
Figure 3. Present and potential therapeutic endpoints for MSC. (A) Recent market survey exhibiting distribution in academic/university communities and pharmaceutical/biotechnology communities. (B) A depiction of the survey of stem cells used for research in the global marketplace. (C) The survey on the type of markers. http://www.selectbiosciences.com/marketreports/StemCellsRegenMed08.aspx. Panels in this figure were obtained with due permission from Select Biosciences, UK.

and murine cells are more prone to undergo immortalization and transformation in culture than human cells, these observations underscore the requirement for cytogenetic monitoring of human MSC in clinical protocols.

Conclusions

An allogeneic approach would enable MSC to be isolated from any donor, expanded and cryopreserved, providing a readily available source of cells for tissue engineering. These facts are strongly reflected in the current exponential growth in stem cell research in the pharmaceutical and biotechnology communities. With expanding knowledge of induced pluripotent stem (iPS) cells, immunomodulation may be easier to achieve using patients' own somatic cells. For instance, obtaining a skin biopsy to establish a pluripotent cell population that is not immune-rejected by the patient seems to be achievable. However, the ability to

differentiate these cells efficiently into terminally differentiated cell types that would retain these properties on transplantation *in situ* still remains a major task in successful cell replacement therapies. Moreover, the approach of stem cell therapeutics, using adult stem cells, eliminates the additional risk factor of teratoma formation and immune rejection that ES cell treatments face. Based on studies conducted to date, MSC therapy is thought to provide a safer approach for the treatment of neurodegenerative diseases, cardiac failures, burns, bone defects, etc., compared with the potential offered by ES cells. However, the current knowledge of the immunobiology and clinical application of MSC needs to be strengthened by carrying out systematic studies in mice, large animal models and then pilot human studies. This may pave the way to faster commercialization of MSC-based products compared with ES or iPS cell-based therapy.

Acknowledgements

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