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MESENCHYMAL STEM CELLS: A NOVEL APPROACH IN REGENERATIVE MEDICINE

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ABSTRACT

Mesenchymal stem cells (MSCs) are heterogenous cell population derived from the mesodermal mesenchyme. MSCs reside throughout the adult organism and possess the ability to regenerate cell types of specific origin. These help to maintain tissue homeostasis and are actively involved in repair processes. MSCs are developed therapeutic agents to treat several diseases of integumentary, musculoskeletal, cardiac and nerve tissues. Allogeneic transplants of MSCs do not produce any adverse effect which may be due to their immunetolerance capacity. This review provides an integrated overview of the current concepts of MSCs and their biological perspectives that support different aspect of regenerative medicine.

KEYWORDS: Mesenchymal stem cells, Mesodermal mesenchyme, Allogeneic transplants, Regenerative medicine.

INTRODUCTION

Mesenchymal stem cells (MSCs) reside in almost all postnatal organs and tissues. MSCs lie at the top of the mesenchymal cell hierarchy and progress through discrete stages of differentiation in an orderly manner to give rise to functionally and phenotypically mature tissues, including bones, smooth muscles, tendons and cartilage.[1] *In vivo*, MSCs facilitate the homeostatic balance by migrating along chemoattractant gradients in the stromal extracellular matrix (ECM) and peripheral blood. They secrete bioactive factors and signals at variable concentration in response to local microenvironmental cues which have both

immunomodulatory and trophic properties. The majority of therapeutic approaches rely on an *in vitro* cell expansion phase either in monolayer or on scaffold to produce large cell number prior to implantation. MSCs have several advantages over other contemporary cells and cell lines, such as (i) easy availability, (ii) few ethical concerns, (iii) ability to evade host immune response and (iv) hypoimmunogenic properties. The therapeutic benefits of MSCs are now established in disease models and preliminary data from clinical trials further confirm these claims. [2]

Recently, MSCs have been given a new acronym "medicinal signaling cells" by Calpan. [3] As MSCs are drug stores for sites of injury or inflammation, they are site-regulated, multi-drug delivery vehicles. So, these may serve as modulatory or curative agents for a variety of human maladies. Recent studies suggest that MSCs may not actually be immune privileged. However, no clinical disadvantages have been noticed upon allogenic transplantation till date. This may be due to the fact that MSCs exert therapeutic function through a brief 'hit and run' mechanism. This protects MSCs from immune detection of host immune system. [4] In current context MSCs emerge as a prominent candidate for cell-based therapies, tissue repair and immune modulation which are widely utilized for different regenerative purposes. Till now there are 344 registered MSCs-based clinical trials conducted throughout the world. [5] The present review summarizes some recent advances in understanding cell biology of MSCs and their regenerative potential for different therapeutics.

NATIVITY OF MSCs

MSCs are now known to originate as "pericytes".[6] According to Caplan all MSCs are pericytes but all pericytes are not MSCs. Pericytes are an elusive cell type recognized by their anatomy and position rather than by a precisely defined phenotype. They reside on the abluminal surface of endothelial cells in the microvasculature of every vascularized connective tissue. Probably, these originate during embryogenesis when the immature vascular progenitor cells, *i.e.*, angioblasts migrate from somites to the embryonic dorsal aorta.[7] In many adult tissues, MSCs are closely associated with perivascular niches. However, at what time the pericytes transform into MSCs remains controversial. MSCs are common to all mammals tested to date. MSCs are largely defined retrospectively based on *in vitro* properties such as the capacity to proliferate extensively, form colonies, adhere to tissue culture plastic, and differentiate into mature mesenchymal lineages when induced with appropriate culture conditions.[8]

The presence of MSCs was first hypothesized in late nineteenth century by Cohnheim.[9] A century later, Fridenstein et al. [10] first isolated MSCs from bone marrow (BM) and described its in *situ* characteristics. Apart from BM, these cells can be derived from adipose tissue, periosteum, synovial membrane, synovial fluid (SF), muscles, dermis, deciduous teeth, pericytes, trabecular bone (TB), infrapatellar fat pad, articular cartilages, umbilical cord blood (UCB), peripheral blood[11], physiological products like menstrual blood[12] and urine.[13, 14] MSCs constitute only 0.002% of total stromal cell population. This fraction varies according to the source of derivation.

IN VITRO CULTURE OF MSCs

In vitro culture of MSCs needs a special environment that must mimic *in vivo* niche. The changes in composition of culture medium are a continuous process. This modification is done either in presence and/or absence of fetal calf serum (FCS) or fetal bovine serum (FBS). Nonetheless, due to zoonotic origin, the use of FCS or FBS raises concern when utilized in clinical grade preparations. This substantial risk may be eliminated by using serum-free media formulation. These formulations basically use different cytokines and growth factors, such as basic fibroblast growth factor (b-FGF) and transforming growth factor beta (TGF-ß). Recently, platelet lysate (PL) is used as substitute of FCS or FBS. MSCs are cultured by three-dimensional (3D) culture technique or on various ECM (extracellular matrix) such as gelatin, collagens, laminins, fibronectin, vitronectin and elastin coated culture plates. [15] MSCs are known to undergo phenotypic rearrangements during *ex vivo* manipulations. They usually lose expression of some markers while also acquiring new ones. [16] In *in vitro* culture, MSCs exhibit stem cell marker profiles, clonogenicity, high proliferation capacity, paracrine immunomodulatory effects and multipotent differentiation capacity. However, there are no established universal markers to identify MSCs *in vivo*. *In vitro* cultured MSCs express cluster of designation or classification determinant (CD) molecules such as CD73, CD90 and CD105 and lack the expression of CD 14, CD19, CD31, CD34, CD45 and HLA-DR. According to some authors, MSCs should express embryonic stem (ES) cell markers, such as Oct-4, Rex-1, and Sox-2 for few passages. [17, 18] The expression of ES cell markers by MSCs depend on source of isolation, culture condition and age of culture. Surface marker expression profiles of MSCs are derived from different sources (Table. 1).

Table 1 Expression profile of cell surface markers of MSCs derived from different sources.

Sl No	Sources	CD marker expression in derived MSCs
1	BM	$CD44^+$, $CD105^+$, $CD166^+$, $CD28^+$, $CD33^+$, $CD13^+$, HLA class I^+
2	HSCs	$CD34^+$, $CD90^+$
3	Adipose tissues	$CD13^+$, $CD29^+$, $CD44^+$, $CD71^+$, $CD90^+$, CD105/SH2 and $SH3^+$, $STRO\text{-}1^+$
4	Trabecular bone	$CD73^+$, $STRO\text{-}1^+$, $CD105^+$
5	Wharton's Jelly	$CD105^+$,$CD73^+$, $CD90^+$

BIOLOGICAL PROPERTIES OF MSCs

Various studies indicate that *in vitro*-expanded MSCs after transplantation preferentially home to sites of tissue damage. These enhance wound healing, support tissue regeneration and help to restore the tissue specific microenvironment. The fate, migration and commitment of MSCs are regulated by various instructive signals from their immediate vicinity or microenvironment. MSCs invariably express low levels of major 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. [19] Is this contributed by some universally expressed gene of MSCs which can mask immunorejection mechanisms for allogenic grafts? The trophic migration of MSCs are largely influenced by release of biomolecules such as a series of cytokines and signaling molecules those have pleiotropic effects at the site of lesions or injuries. These include interleukins (IL) such as IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15 and growth factors like leukemia inhibitory factor (LIF), granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), stem cell factor (SCF), macrophage colony-stimulating factor (M-CSF) and FMS like tyrosine kinase-3 ligand (flk-3L) implicated mainly in hematopoiesis. They also express cytokine receptors IL-1R, IL-3R, IL-4R, IL-6R and IL-7R. Cytokine-induced MSCs express very high level of several leukocyte chemokines, most notably CXCL9, CXCL10 and CXCL11. These elicit immune-modulatory, anti-inflammatory, antiapoptotic, pro-proliferative effects. MSCs migrate to the damaged area, might interfere with the differentiation, maturation and function of dendritic cells by mediating the soluble factors like IL-6 and macrophage colony-stimulating factor (M-CSF). MSCs strongly inhibit IL-2- induced NK cell proliferation and prevent the induction of effectors functions, such as cytotoxic activity and cytokine production. The important mediators involved in these phenomenons are IL-10, transforming growth factor- ß (TGF-ß), indoleamine 2, 3 dioxygenase (IDO) and prostaglandin E2 (PGE2). However, the entire mechanism is yet to be

elucidated. [20] MSCs are supposed to suppress the activation and proliferation of T and B lymphocytes by restricting their cell division at the G0/G1 phase of the cell cycles. The immunosuppressive ability of MSC is not innate but is rather induced by pro-inflammatory cytokines like IFN-g in combination with tumor necrosis factor (TNF)-a, IL-1a or IL-1b. Another prominent candidate in the mechanism of MSC-mediated immunosuppression is nitric oxide (NO). It is a rapidly diffusing gaseous bioactive molecule. [21] Notably, it induces a remarkable up-regulation in inducible nitric oxide synthase (iNOS) and several leukocyte chemokines. High levels of NO can suppress immune cell function. Therefore, the intensive action of cytokine-induced chemokines and NO is the key to MSC-mediated immunosuppressant.[19] Reports suggest that MSCs express toll-like receptor (TLR) proteins. These play a critical role in immunomodulation mechanisms. Liotta et al. found that TLR3 and TLR4 activation reduce the inhibitory activity of human BM-MSCs on T cell proliferation without influencing IDO activity or PGE2 levels, but down-regulate expression of Jagged1, suggesting that the Notch signaling pathway mediates cell contact-mediated immunosuppression by MSCs.[22] However, Opitz et al. reported that TLR3 and TLR4 engagement enhances the immunosuppressive properties of human BM-MSCs through the indirect induction of IDO1.[23] This comprises many biological molecules (soluble and insoluble) and biomechanical forces. These biochemical and biophysical factors play a pivotal role in determining the efficacy of MSCs differentiation and their contribution to the repair process. The multifunctional aptitude of MSCs makes them an ideal candidate for tissue engineering and regenerative medicine (Fig. 1).

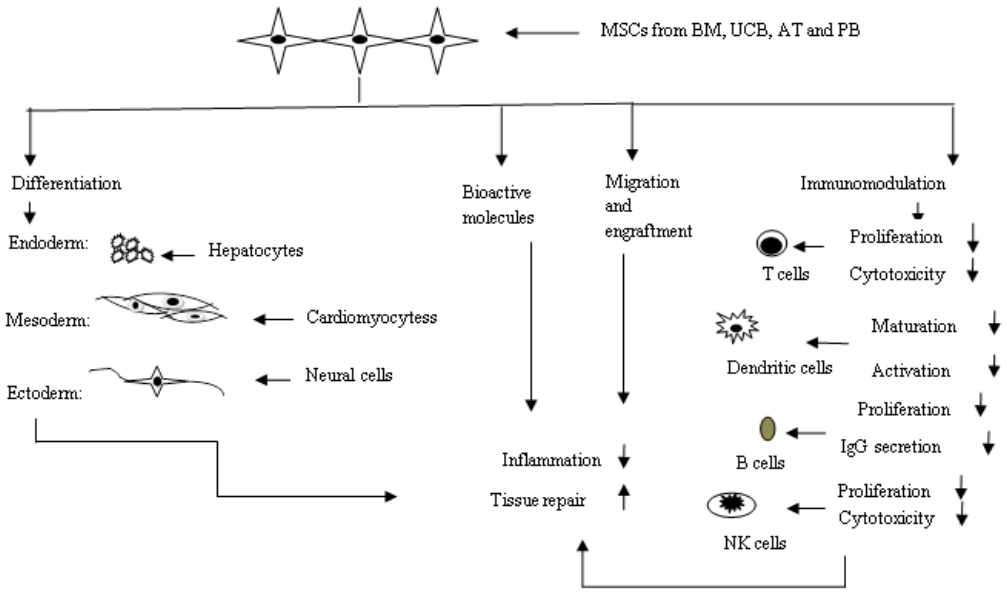

Figure 1 Strategies involve in MSCs mediated regenerative medicine

However, the mechanism of differentiation of MSCs towards various lineages are not yet fully elucidated. Some hypotheses propose that in MSCs, there are some storage genes that could express and adjust their differentiation into varying lineages of cells under different conditions. *In vitro* differentiation of MSCs to a particular phenotype pathway can be controlled by some regulatory genes which induce progenitor cells of a specific lineage. Until last decade, MSCs are particularly focused on for their tri-lineage differentiation potential (osteogenic, adipogenic and chondrogenic lineages). However, recently they were found to cross boundaries from mesodermal to ectodermal and endodermal lineages including neurons, muscle cells, epithelial cells and hepatocytes. Recent development in stem cell research discovered multilineage-differentiating stress enduring (Muse) cells in mesenchymal tissues. [24] These cells are non-tumorigenic having low telomerase activities. Are the biological properties of MSCs contributed due to presence of these Muse cells?

ROLE OF MSCS IN REGENERATIVE MEDICINE

Effective stem cell therapy fundamentally rests on the optimized combination of two key elements namely the starting cell population and the environment in which the cells are placed. Two different holistic approaches are followed such as (i) implanting the naïve stem cells or tissue-restricted stem cells or progenitors that differentiate some time after transplantation and (ii) transplanting differentiated stem cells after *in vitro* manipulation in the concerned area of damage. The choice largely depends on our knowledge of the cell system and its niche and experts involved in entire process. Studies on the effects of direct injection of MSCs without any surgical procedures and design of biocompatible and biodegradable scaffold have shown promising results to treat many diseases (Fig. 2).

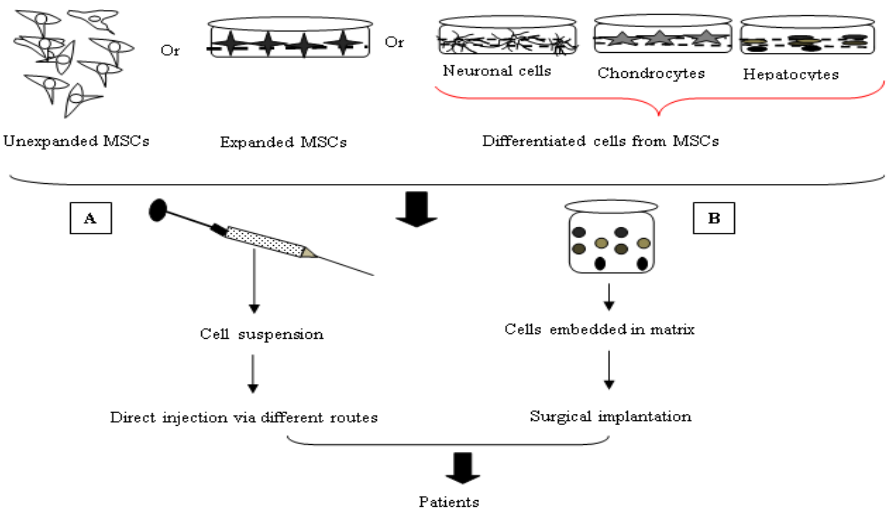

Figure 2 Delivery of MSCs. (A): Direct injection of MSCs. (B): Embedded inside scaffold

NEUROLOGICAL DISORDERS

The dogma that cells of the central nervous system (CNS) can never regenerate has been challenged in the last decade. MSCs activate endogenous restorative responses in injured brain, which include angiogenesis, neurogenesis, and synaptogenesis. [25] The neurogenic and neuroprotective effect of MSCs to treat neurodegenerative disorders are facilitated by secretion of trophic molecules. These include brain-derived neurotrophic factors (BDNF), nerve growth factor (NGF) and insulin-like growth factor-1 (IGF-1). MSCs are transplanted into the injured brain via intracerebral or intrathecal injections. MSCs have been considered as a promising therapeutic strategy to treat spinal cord injury, stroke, Parkinson's disease (PD), autoimmune encephalomyelitis (EAE), amyotrophic lateral sclerosis and multiple system atrophy (MSA).

PD is characterized by the selective loss of dopaminergic neurons in the substantia nigra. MSCs show high reproducibility to differentiate into functional dopaminergic neurons. The clinical features of PD were initially described almost two centuries ago by James Parkinson in 1817. [26] It was well demonstrated in rat model that graft of adult mesemchymal stem cells reduces behavioral effects induced by 6-hydroxydopamine lesion. This partially restores the dopaminergic markers and vesicular striatal pool of dopamine. So transplantation of MSCs might be a restorative therapy in Parkinson's disease. [27] A human pilot study was performed in PD patients by autologous naive BMSCs transplantation. The result indicated certain a degree of amelioration of symptoms with no sign of tumor formation. Nonetheless, BMSCs have certain limitations. They do not survive *in vivo* for a long time, and thus, the trophic effects gradually decrease. Generally, naive BMSCs do not differentiate spontaneously *in vivo* after transplantation. Even if they differentiate, the ratio of differentiated cells would be extremely low. So it is wiser to administer immature or dopamine producing neurons differentiated from MSCs for PD treatment. Readers are suggested to refer to the reviews by Khoo et al. [28] and Kitada et al. [29] to understand the role of MSCs-based therapies for Parkinson's disease in detail.

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease. This disease degenerates motor neurons (MNs) in the primary motor cortex, brainstem, and spinal cord, leading to muscle atrophy, paralysis and death due to respiratory failure within 2-5 years. Currently, there is no cure for ALS. ALS is largely a sporadic disease. However, approximately 5-10% of patients diagnosed with familial history of ALS have an inherited

ALS form of the disease, which shares nearly identical clinical and histopathologic hallmarks with sporadic ALS. [30] Point mutations with amino acid substitutions G37R, G85R and G93A may be genetic cause of ALS. Mechanisms implicated in this multifactorial progress process of ALS include glutamate excitotoxicity, oxidative damage, cytoskeletal abnormalities, endoplasmic reticulum stress from abnormal cellular protein products, mitochondrial dysfunction, abnormal microglial and astrocyte function and impaired neurotrophic support. In several animal studies, MSCs have been shown to effectively ameliorate the clinical and pathological features of ALS. [31] Zhao and his colleagues administered hMSCs into presymptomatic irradiated G93A mice to estimate the efficacy. [32] The result clearly indicates that MSCs survived over 20 weeks in the recipient mice. They integrated into the parenchyma of both the brain and spinal cord. The transplanted mice had both delayed onset and slower disease progression with an increased lifespan when compared to control (untreated mice). In another study, nine patients received intraspinal injections of autologous MSCs. It shows dampened inflammation and reduced motor neuron loss and functional impairment.

Huntington's disease (HD) is a fatal autosomal dominant neurodegenerative disorder which usually occurs due to the mutation of gene located on short arm of chromosome 4. The mutations in the HTT gene result loss of functional neuron especially from the cerebral cortex and striatum region. The experiment conducted on rat model had demonstrated that transplanted MSCs were able to survive and differentiate into neurons in the affected striatal region. MSCs also reduce motor malfunctioning and degeneration of striatum in these Huntington rats. The detailed determination of factors which MSCs express and under what conditions, is the key to understand the innate capacity of MSCs to influence neural cell growth, survival and neurite extension and regeneration. Transplanted MSCs promote endogenous neuronal growth, decrease apoptosis and regulate inflammation, primarily through the use of secreted factors. The specific subpopulations of hMSCs express brain-derived neurotrophic factor (BDNF) and β-nerve growth factor (β-NGF) but not neurotrophin-3 and -4. [33] These factors are neuroregulatory in nature and induce survival and neurite outgrowth. Dickkopf (Dkk)-1, a Wnt antagonist is another candidate for MSC-mediated neuritogenesis. In addition to neurotrophic factors, the extracellular matrix molecules produced by MSCs have also been demonstrated to support neural cell attachment, growth and axonal extension. Using MSCs, approximately 23 clinical trials of neurological

disorders are complete to date. [34] The mechanisms by which MSCs show their neuroprotective and neurorestorative effects are shown (Fig. 3).

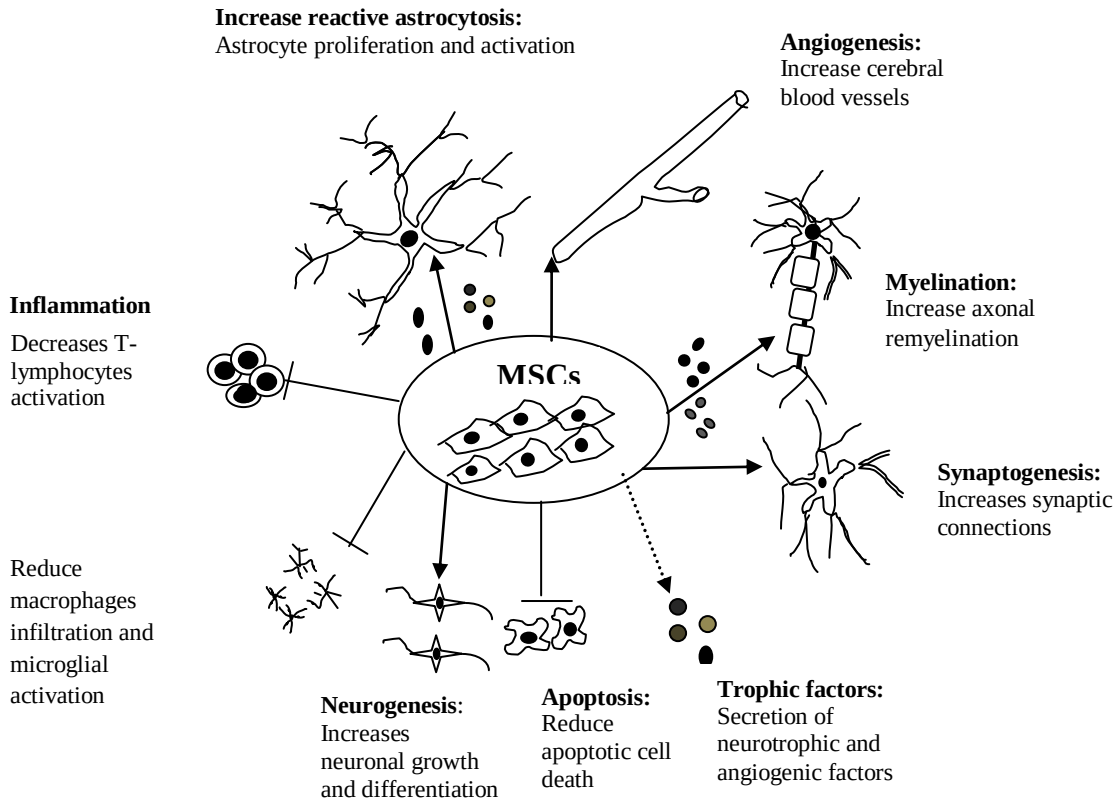

Figure 3 Potential neuroprotective and neurorestorative effects of MSCs mediated via paracrine signaling

BONE AND CARDIOVASCULAR DISEASES

The dynamic events like osteogenesis and adipogenesis start when mesenchymal cells are committed to form either osteocytes or adipocytes [35]. It is noteworthy that there exists inverse correlation between osteogenic and adipogenic lineage commitment and differentiation that highly depend on a variety of signaling and transcription factors (Fig. 4). It is clearly seen that differentiation towards an osteoblast phenotype occurs at the expense of an adipocytic phenotype. This balance is tightly regulated by numerous intersecting signaling pathways that converge on the regulation of two main transcription factors such as peroxisome proliferator-activated receptor-γ (PPARγ) and Runt-related transcription factor 2 (Runx2). [36] These are regarded as the master regulators of adipogenesis and osteogenesis. The signaling pathways governed by proosteogenic/antiadipogenic stimuli are β-catenin dependent Wingless (Wnt) signaling, Hedgehog signaling and NELL-1 signaling. The

signaling pathways which exhibit more context-dependent effects on adipogenic and osteogenic differentiation which are bone morphogenic proteins (BMPs) signaling, insulin growth factor (IGF) signaling and Notch signaling. They convey both proosteogenic and proadipogenic effects. The expression\of Runt related transcription factors 2, Distal-less homeobox 5 (Dlx5) and osterix (Osx) are crucial for osteoblast differentiation. Runx2 has been demonstrated to upregulate osteoblast-related genes such as collagen A1 (ColIA1), alkaline phosphatase (ALP), ß-glycerophosphatase (BGLAP) and osteocalcin (OCN). MSCs convey a particular set of chemokine receptors, such as CCR1, CCR7, CCR9 and CXCR4-6. Chemokines (e.g., CXCL12) that are bound by these surface receptors initiate cellular response-specific chemotaxis events and ß-actin filament reorganization.

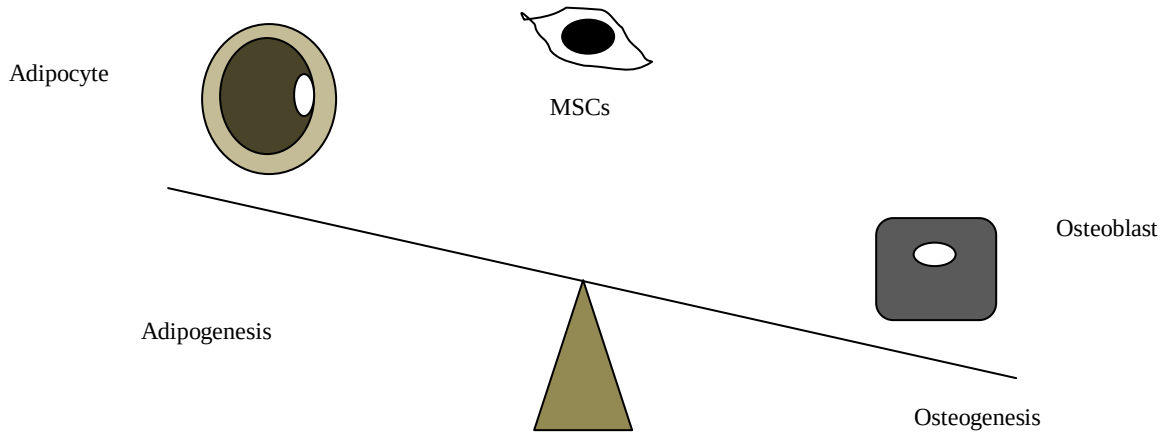

Figure 4 Inverse relationship between osteogenic and adipogenic programming

Chondrogenesis is a process which is important for the creation of chondrocytes both during embryogenesis as well as in adult life (e.g., during skeletal tissue repair). The process begins with the aggregation and condensation of loose mesenchyme. Factors such as BMPs are known to play critical roles in the compaction of mesenchymal cells and the shaping of the condensations. This leads to the establishment of various types of cartilage, including hyaline, fibrous and elastic cartilage. Hyaline cartilage is the most prominent and most susceptible to both normal and pathologic forms of stress of the limb and trunk skeleton. Intracellular signaling cascades involved in MSCs mediated chondrogenesis are mitogen-activated protein (MAP) kinases, p38, ERK-1, TGF-βs and JNK pathways. [37] These have been shown to activate cartilage-specific gene expression. The limited self-healing capacity of cartilage and non availability of effective pharmacotherapies, globally increase the incidence of diseases associated with articular cartilage and bone. The relative decline in

bone formation with age is influenced by many factors such as altered stoichiometry of matrix components, increased matrix degradation processes, decreased osteoblast function and increased apoptosis of more mature osteoblasts. These result in the non-union of fracture or autoimmune diseases like rheumatoid arthritis, metabolic bone disorders or genetic diseases like osteogenesis imperfecta and hypophosphatasia.

Approximately, 5-10% of the 7.9 million fractures sustained annually in the United States fail to achieve bony union. [38] MSCs in combination with osteoconductive scaffold or osteoinductive protein are used as tools in bone and cartilage regeneration. Infusion of MSCs has both direct and indirect effect on *in vivo* system. Transplanted MSCs trigger patients' own regenerative mechanism by activating CXCR4 expression at fracture site or sites. Per-cutaneous autologous bone marrow grafting healed fracture nonunion effectively. In a study marrow was aspirated from both anterior iliac crests, concentrated on a cell separator and then injected into 60 non-infected atrophic non-unions of the tibia. A positive correlation was noted between the volumes of mineralized callus and the number of cell infused. High union rate was obtained in 43/60. Seven patients did not respond well and union was not achieved because the concentration and the total number of stem cells injected were significantly lower than in patients with osseous union (p=0.001 and p<.01). [39] A report by Raulo et al. [40] demonstrates that the administration of cultured MSCs healed a patient with non-union having long-standing bacterial infection. Transplanted MSCs secrete multiple factors those generate osteogenic signals involved in cascades of molecular events facilitating bone union. Further infused MSCs direct inflammation and antimicrobial activity and promoting cell migration during epithelial remodeling in patients. [41] Osteoarthritis (OA) is the most prevalent joint disorder resulting in cartilage destruction, subchondral sclerosis, osteophyte formation, and synovitis. It is associated with joint pain, stiffness, swelling, crepitus, and disability in the elderly. [42] Arthritis is the most common source of disability among adults in the United States. According to a survey (2003), the disease afflicted 50 million Americans and this number is expected to increase to 67 million by 2030. [43] MSCs alteration seems to be involved in this disease-specific pathology. Chondrocyte implantation (ACI) has been used for treatment of osteoarticular lesions. Administration of MSCs would help preserve joint integrity in a variety of knee OA models which include horse, sheep, rat, mouse, rabbit and guinea pig. The fundamental work of therapeutic intervention of MSCs was done by Murphy et al. [44] in a goat model of post-traumatic OA. They observed that joints which receive subsequent injection of autologous MSCs show improvement of meniscal and

cartilage regeneration in comparison to control. This may be due to the fact that administration of MSCs induce alternation in cell signaling milieu. These events are coordinated by complex network of transcription factors, cofactors and signaling intermediates from numerous pathways. MSCs may orchestrate the reparative response by stimulating production of signaling molecules like TGF-ß3, TGF-ß1, insulin-like growth factor (IGF), fibroblastic growth factor-2 (FGF2), corticosteroids and interleukins. Through paracrine signaling and direct cell to cell contact, the exchange of these chemical factors has been found to promote ECM development and subsequently increase Type II collagen production by the host. The convective transport of growth factors facilitate by physical loading may increase synergistic interactions. *In vitro* cultured MSCs were embedded in a collagen gel and delivered to the knee joint of 24 knee osteoarthritis (OA) patients. Good functional recovery was observed after forty-two weeks of transplantation, the defects were covered with white soft tissue, in which metachromasia was observed in almost all areas of the sampled tissue and hyaline cartilage-like tissue was partially observed. Although the clinical improvement was not significantly different, the arthroscopic and histological grading score were better in the cell-transplanted group than in the cell-free control group. [45] A review by Kristjánsson and Honsawek [42] deciphers stem cell-based approaches for treatment with patients suffering from knee osteoarthritis in detail.

Osteogenesis imperfecta (OI) is a heterogeneous group of inherited disorders of connective tissue. This is characterized by bone fragility and other connective tissues malfunction. The genetic defect responsible for OI results in abnormal type I collagen production by osteoblasts. This leads to osteopenia, multiple fractures, severe bony deformities and considerably shortened stature. Would transplant of MSCs or marrow stromal cells attenuate or possibly correct genetic disorders of bone, cartilage, muscle, and other connective tissues? The initial investigation of allogeneic bone marrow transplantation (BMT) to treat 3 children suffering from OI was undertaken by Horwitz et al. [46] New bone formation was recorded in trabecular tissues after three months of osteoblasts engraftment. There was a mass improvement in total body bone mineral content that ranges from 21 to 29 g in all patients compared with predicted values of 0 to 4 g for healthy children with similar changes in weight, marked increases in growth velocity and reduced frequency of fractures. This investigation demonstrates that MSCs by allogeneic BMT can migrate to bone in children with OI and then gives rise to osteoblasts. However, how far is it possible to cure the disease

from gene level??? What is the optimal cellular dose? Whether a single injection is sufficient or multiple injections might be required for satisfactory results?

Hypophosphatasia is a rare, heritable, metabolic bone disease. It occurs due to deficient activity of tissue-nonspecific alkaline phosphatase (TNSALP). The infantile form features severe rickets often cause death in the first year of life from respiratory complications. BM transplantation was done in an 8-month-old girl suffering from infantile HPP. [47] The case was followed up after four months of transplantation. Improved skeletal mineralization was observed. After 7 years of transplantation, the patient was active and grew with mild clinical symptoms of HPP. Osteoporosis is a bone related metabolic disorder. In this disorder there is imbalance in between osteoclastogenesis and osteoblastogenesis. This balance shifts in favor of osteoclasts, and bone resorption exceeds bone formation. MSCs activity and their microenvironment may be disturbed in such conditions of bone related problems. The bone loss leads to fragile bones and the persons are susceptible to concurrent fractures. Administration of MSCs primarily derived from the periosteum, endosteum, and marrow cavity (allogenic or autologous) is supposed to elevate the endogenous MSCs of osteoporosis patients and contribute to increase bone mass.

CARDIOVASCULAR DISEASES

Cardiac fibroblasts are actively involved during the remodeling process. Cardiac fibroblasts show excellent cell–to-cell communiqué by cell to cell interactions through mechanical, chemical (autocrine and/or paracrine) and electrophysiological means to alter gene and protein expression, cellular processes, synthesis and degradation of the extracellular matrix. All these processes synchronize together and ultimately result in cardiac function. Following cardiac injuries, there are dramatic shifts in the various cardiac cell populations. These affect cell to cell and cell to extracellular matrix interactions and ultimately impair cardiac function. Cardiovascular disease (CVD) is the leading cause of death; worldwide. According to the World Health Organization (WHO), an estimate of 17.3 million people died from CVDs in 2008 and by 2030, the number of death is estimated to reach almost 23.6 million. In the United States alone, there are 7.1 million survivors of myocardial infarction (MI) and 4.9 million people live with congestive heart failure (CHF). [48] In MI significant loss of cardiac cells occur with formation of scar tissue. After an acute MI, the heart has limited capacity for self-renewal and undergoes remodeling with resulting depressed left ventricular (LV) function. MI further decreases the divisional ability of cardioblasts.

Numerous preclinical studies applying hMSCs to infarcted myocardium have revealed their positive effects such as reduction in scar size, improved cardiac contractility and increased tissue perfusion. Transplanted MSCs not only differentiate into cardiomyocytes and vascular cells, but also secrete adequate amounts of growth factors and cytokines which may mediate endogenous regeneration via activation of resident cardiac stem cells and other stem cells, as well as induce neovascularization, anti-inflammation, anti-apoptosis, anti-remodelling and cardiac contractility in paracrine manner. It has also been postulated that the anti-arrhythmic and cardiac nerve sprouting potential of MSCs may contribute to their beneficial effects in cardiac repair. Human heart is physiologically similar to swine heart. [49] In the swine model, Shake et al. [50] reported that cardiomyocyte like cells show differentiation after two weeks of intramyocardial implantation of MSCs. This therapy results in significant improvement of contractile dysfunction and wall thinning. Similarly, the investigation was carried out for the first time to see the outcomes of intracoronary injection of autologous BM-MSCs in sixty-nine acute MI patients. [51] When the cases were followed up, after 3 months there was marked improvement in cardiac functions. Augmentation of myocardial perfusion, LV ejection fraction and LV chamber dimensions were marked in MSCs-treated patients in comparison with the control.

A pilot study was conducted by Mohyeddin-Bonab et al.[52] to estimate safety and feasibility of MSCs therapy in cardiac repair. They administered MSCs @ ($2.1–9.1 \times 10^6$ cells) into eight patients with old MI. The administration was done either by coronary intervention or by direct epicardial injection.

They observed that transplantation of *ex vivo* expanded bone marrow derived MSCs able to improve the cardiac function without causing serious adverse effects. MSCs help to repair cardiac damage by activating TGF-β signaling pathways. TGF-β may act in an autocrine manner to promote differentiation of MSCs into smooth muscle cell-like phenotypes. TGF-β may play an important role in monocyte recruitment in the healing infarct, promoting granulation tissue formation and regulate many events associated with infarct healing. The possible mechanisms of cardiac repairs execute by MSCs (Fig. 5).

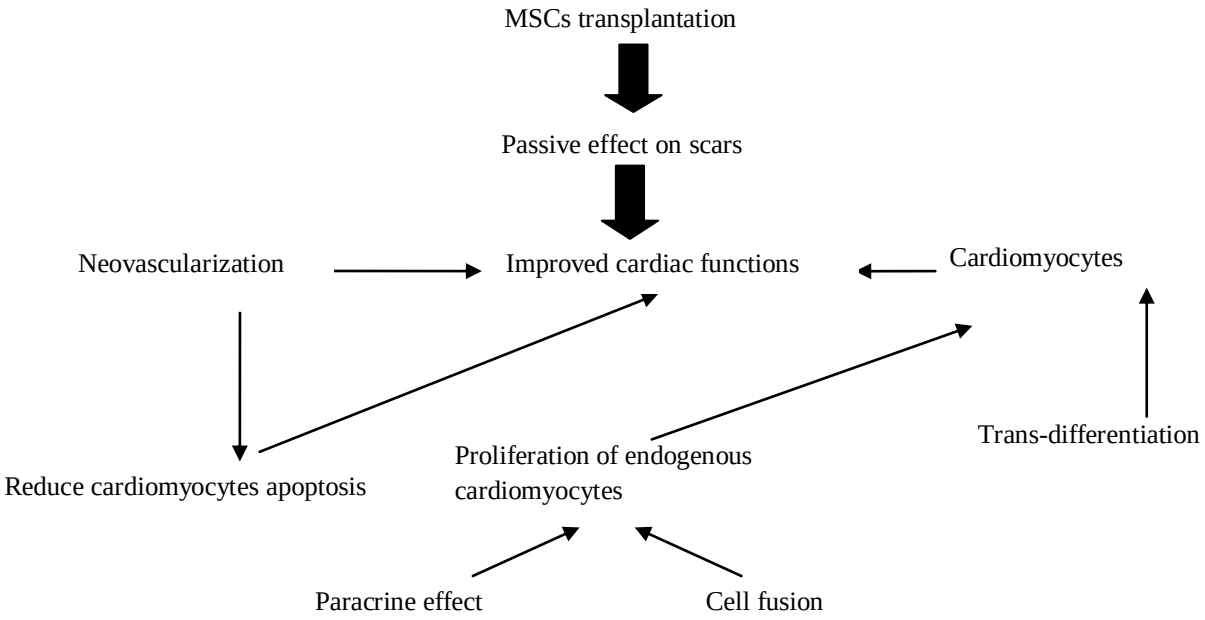

Figure 5 MSCs mediated cardiac repair

LIVER RELATED DISORDERS

Adult liver exhibits the remarkable ability to "regenerate" following surgical resection or toxic liver injuries. This restoration of hepatic tissue homeostasis occurs through rapid and partially synchronous proliferation of adult mature hepatocytes. Many chronic diseases like liver cirrhosis, hepatitis and liver failure are most prevalent worldwide. Only liver cirrhosis causes more than one million deaths in 2010 globally. Till now, liver transplantation is the only alternative. The recent MSCs based transplantation strategy is a promising approach to treat liver dysfunctions. Under *in vitro* conditions, transformation of MSCs to hepatocytes is a critical task, as it involves a change from a fibroblast like morphology to the polygonal shape. [53] To date, four primary strategies have been developed to induce MSCs into hepatocytes such as the addition of chemical compounds and cytokines, genetic, adjustment of the microenvironment and alteration of the physical parameters used for culturing MSCs. It is important to understand the intrinsic and extrinsic factors that influence the hepatic differentiation of MSCs (Fig. 6).

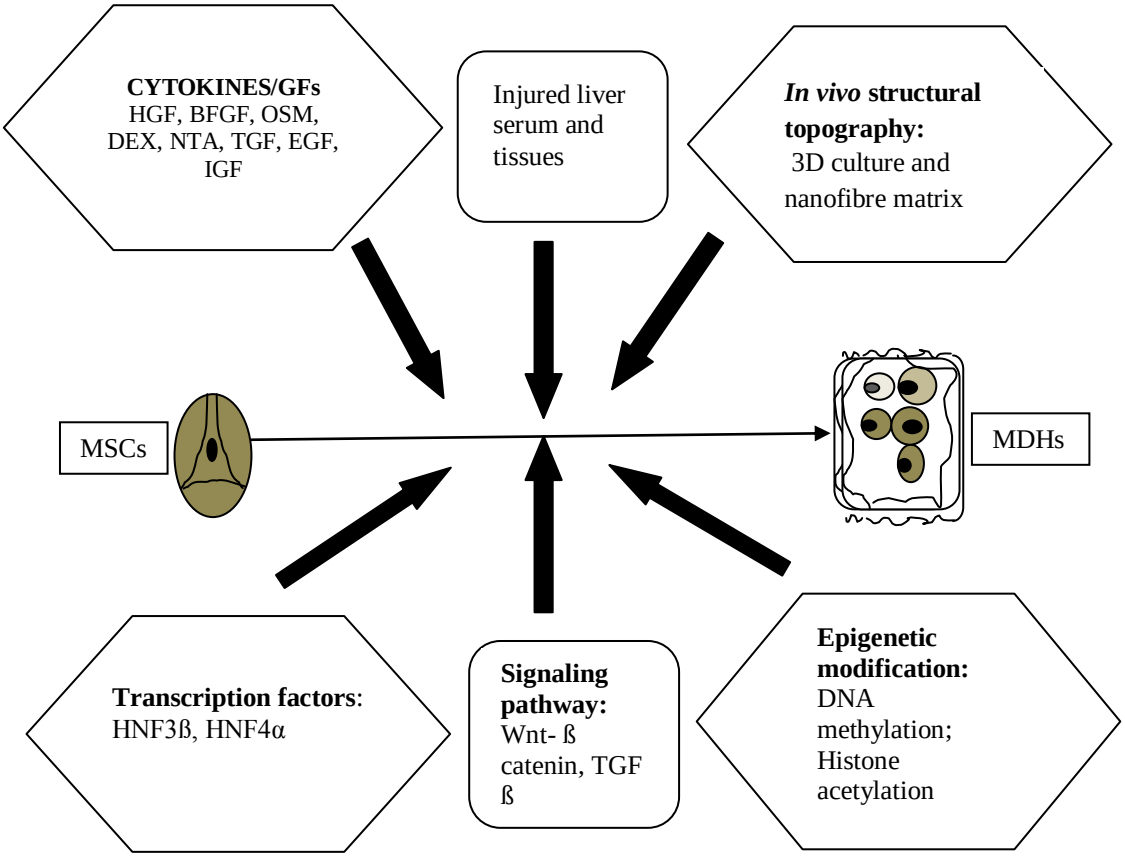

Figure 6 Modulation of MSCs differentiation into hepatocytes: The modulation of MSCs differentiation into MDHs can be induced by various factors. Extracellular (stimulating) factors: here we mainly discuss the roles of cytokines, growth factors, ECM cues and the physical parameters of culture

Numerous studies have confirmed the therapeutic effects of MSCs on hepatic fibrosis, cirrhosis and other liver diseases. [54, 55] In these studies they are either used direct MSCs infusion or mesenchymal derived hepatocytes to treat different liver disorders. These differentiated mesenchymal derived hepatocytes (MDHs) should show functional characteristics like glycogen storage, detoxification and lipid metabolism after *in vivo* transplantation However, hepatocyte pre-differentiated MSCs were more effective as compared to their undifferentiated precursors during therapeutic applications. Transplanted hepatocytes mainly engraft in the periportal regions of the liver lobule and acquire the gene expression pattern of periportal hepatocytes. [56] Hepatic microenvironment governs the differentiation state of transplanted cells. MSC-derived hepatocyte-like cells, both after intrasplenic and portal administration, were found in the periportal areas of the liver lobule where they featured typical characteristics of periportal hepatocytes 10 weeks post-implantation. Recent studies indicate that intrasplenic transplantation of human BMMSCs into the carbon tetrachloride (CCl_4) injured livers of SCID mice enhanced host liver

parenchyma, exhibited typical hepatocyte morphology, and formed a three-dimensional architecture. [57] Preexisting cirrhosis usually leads to an inadequate and delayed regeneration of the future liver remnant (FLR) after portal vein embolization (PVE). After transplantation of autologous BMMSCs, they differentiate into hepatocytes and promote future liver remnant regeneration after portal vein embolization in cirrhotic liver. [58] Transplanted MSCs reduce cirrhosis by up-regulating hepatoinducing gene expression of VEGF, HGF, IL-10 and matrix metalloproteinase. The effect of BMMSCs derived hepatocyte like cells (HLCs) was improved when they were pretreated with a combination, of dynamic cultured scaffold and growth factors and then transplanted into CCl4 injured mice. This strategy increased their survival rate, liver function, engraftment into the host liver, and further hepatic differentiation. [59] These results clearly indicate that MSCs showed therapeutic effects including repair of the damaged hepatocytes, intracellular glycogen restoration and resolution of fibrosis. MSCs derived from different sources were able to improve liver functions and promote liver regeneration after therapeutic intervention. Direct transplantation of ADMSCs is an effective treatment for acute liver failure (ALF). [60] Recently it was reported that MSCs derived from human menstrual blood (MenSC) when cultured in hepatocyte inducing medium more preferentially generate hepatocyte-like cells (HLCs).[12] These cultured HLCs have cuboidal morphology and exhibit hepatocyte-specific marker genes including albumin (ALB), α-fetoprotein (AFP), cytokeratin 18/19 (CK18/19) and cytochrome P450 1A1/3A4 (CYP1A1/3A4). MenSC-derived HLC after transplantation could restore the serum ALB level and significantly suppress transaminase activity of liver injury animals. MenSCs may serve as another source for hepatocyte generation to treat liver specific diseases.

MSCs IN AGING

All the morphological and physiological changes occur during the complex biological process “aging”. The relation between aging and MSCs is complex and unique. It includes the effect of aging on MSCs and the contribution of MSCs to delay the aging response. However, aging affects MSCs potential and consequently impairs tissue homeostasis and organ function. In particular, over time, the potential of BMSCs and other ASCs decreases in rodents, monkeys and humans. The hypothesis proposes that there is an irreversible loss of the effective regenerative MSCs pool in aging and some clinical situations like lipodystrophic syndromes, progeria. [61] The strategy for the treatment of aging and age-related disorders could be the use of “younger” allogeneic mesenchymal progenitor cells. By far, it is well known that administration of MSCs from young donors to elderly patients is able to restore

their MSCs deficiency and to treat age related problems like osteoporosis, cardiovascular diseases and wound healing. How do MSCs delay aging responses? It is experimentally verified that telomerized MSCs cells exhibited extended life span and maintained a robust bone-forming ability when transplanted *in vivo* in immunodeficient mice. Telomerase activation of hMSCs is a potential strategy for obtaining a large number of biologically competent cells for clinical use. [62] However, it is difficult to know when the cell is aged and due to what factors. So after monitoring cell aging conditional or intermittent activation of the hTERT gene is more appropriate approach to avoid aging.

PROBLEMS AND PERSPECTIVES OF THERAPEUTIC APPLICATIONS OF MSCs

The mechanisms of MSCs therapy are poorly understood. The size of MSCs dramatically increases in culture and become around 20 μm in diameter and also the expression of adhesion molecules is strongly up regulated. The size of MSCs is greatly affected by culture medium and also due to their plastic adherent nature. Since this diameter is larger than the size of pulmonary micro-capillaries, after intravenous infusion of MSCs the majority of the cells are trapped in the lung. The infused MSCs have short survival time of about 24 h. It suggests that MSCs rapidly pass on their effect to resident cells, which may subsequently mediate the immunomodulatory and regenerative effect induced by MSCs administration. [63] But how efficiently the transfers of cellular characters occur? MSCs have successfully completed phase III clinical trials for the treatment of graft-versus-host-disease, Crohns disease (Prochymal®, Osiris Therapeutics) and perianal fistula (Ontaril®, Cellerix). After the approval of European Directive 2001/83/CE, MSCs-based therapies are now considered as drugs for advanced medicinal therapy (AMT). Controversial reports are available regarding role of MSCs in tumor metastasis.

CONCLUSION

The long-term safety of MSC-based therapies is not well established and continues to be one major limitation to clinical translation. More broadly, only a small minority of clinical trials has employed rigorous designs that include prospective randomization. Further studies need to find a suitable source of derivation, optimized doses for cellular transplantation and reliable system to enhance the longevity of engrafted MSCs. Moreover, regular clinical follow-up of patients, proper data management are major current requisites to estimate the effectiveness of MSCs based therapy. Collaborative efforts from stem cell biologists,

nanotechnocrats, plant biotechnologists, physicians, industry and regulatory agencies may make this royal regenerative element into continuum clinical care.

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