Chapter 1

Stromal Stem Cells: Nature, Biology and Potential Therapeutic Applications

Amira Hassouna, Marwa M. Abd Elgwad and Hoda Fahmy

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77346

Abstract

Stromal cells are connective tissue cells of any organ. Mesenchymal stromal cells (MSCs), are multipotent progenitors, which were first described by Caplan and colleagues in 1991. MSCs hold great potential for regenerative medicine because of their ability for self-renewal and differentiation into tissue-specific cells such as osteoblasts, chondrocytes, and adipocytes. Recent studies indicate that MSCs resemble pericytes and emerge from the peripheral stromal region surrounding blood vessels, thus clarifying their broad regenerative potential in adult tissues. The development of uniform protocols for both preparation and characterization of MSCs, including standardized functional assays for evaluation of their biological potential, are critical factors contributing to their clinical utility. Nowadays, due to the capacity of modulating immunological responses, supporting hematopoiesis and repairing tissues, MSCs have been widely used to treat immune-based disorders, such as Crohn's disease, rheumatoid arthritis, diabetes, and multiple sclerosis. Based on animal experiments and clinical studies, the most successful clinical application of MSCs is in the field of hematological disease.

Keywords: mesenchymal stromal cells, stromal stem cells

1. Introduction

Stem cells have the ability of self-renewal, giving rise to a variety of cell lineages. They constitute a significant paradigm of cell-based therapy for various diseases.

Embryonic and non-embryonic stem cells, are the two principal types of stem cells. Embryonic stem cells (ESCs) originate from the blastocyst's inner cell mass and have the ability for differentiation into cells of all three germ layers. However, teratoma formation and ethical controversy represent an obstacle in their research and clinical application.

On the other side, non-embryonic stem cells, mostly adult stem cells, are fairly specialized and have limited differentiation potential. They can be isolated from various tissues and are currently the most used in regenerative medicine.

Over the last decade, cellular therapy has developed quickly at the level of in vitro and in vivo preclinical research and in clinical trials. Mesenchymal stem cells (MSCs), one type of adult stem cells, have provided a great amount of interest in the field of regenerative medicine due to their unique biological properties [1]. The acronym "MSCs" is restricted to the subset of mesenchymal cells demonstrating stem cell activity by accurate criteria.

1.1. Definition

MSCs are multipotent stromal cells that can differentiate into a variety of cell types, including: osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells) and adipocytes (fat cells which give rise to marrow adipose tissue). MSCs exist in organisms (*in-vivo*) and have been studied as well in tissue culture (*in-vitro*) [2].

1.2. Terminology

Due to the lack of a particularly unique MSCs function, they have been termed 'mesenchymal stem cells' or interchangeably 'mesenchymal stromal cells', 'BM (bone marrow) stromal cells' and 'marrow stromal cells'. MSCs are usually identified by mere plastic adherence and by their morphological appearance, such as the fibroblastoid phenotype. This procedure leads to a diverse population containing both single stem cell-like cells and progenitor cells having various lineage commitment. Compared to hematopoietic stem cells (HSCs), which have been proven to repopulate the bone marrow and give all blood types, and embryonic stem cells (ESCs) which after re-injection into early embryos, were proven to participate in embryonic development of all tissues; MSCs have no established in vivo tests [3].

2. History

In 1970, Friedenstein and colleagues were the first who identified mesenchymal stem cells as colony-forming unit-fibroblasts (CFU-Fs) [4]. Pittenger and colleagues were the first to describe the tri-lineage potential of MSCs [3].

The first clinical trials of MSCs were completed in 1995 when a group of 15 patients were injected with cultured MSCs to test the safety of the treatment [1].

3. Sources

3.1. Bone marrow

BM-MSCs are isolated from bone marrow aspirate. This invasive procedure is painful for the patient with a risk of infection. The commonly used method for the generation of MSCs from bone marrow is density gradient centrifugation [3]. The collected fraction containing mononuclear cells is washed and the cells are seeded on a plastic dish for proliferation.

3.2. Adipose tissue

AT-MSC also termed as adipose-derived stem cells are usually isolated from the biological material generated during liposuction, lipoplasty or lipectomy procedures by enzymatic digestion with collagenase followed by centrifugation and washing [5].

3.3. Peripheral blood

Following a density gradient centrifugation, PB-MSC can be collected from the mononuclear cells' lymphocyte separation fluid fraction. Kassis et al. [6] described another method, which is loading PB-MSC on fibrin microbeads, then separating the cell loaded beads from the mononuclear fraction. This method allows getting enormous amounts of MSCs [6].

Pittenger et al. [3], isolated MSCs from BM by density gradient centrifugation to eliminate unwanted cell types and only 0.001 to 0.01% of the cells isolated from the density interface were identified as mesenchymal stem cells.

3.4. Umbilical cord blood

As umbilical cord blood contains MSCs, it could serve as an alternative source of MSCs to bone marrow. A novel method to obtain single cell-derived and clonally expanded MSCs that have multilineage differentiation potential, is negative immunoselection and limiting dilution. The immunophenotype of these clonally expanded cells is similar with bone marrow mesenchymal stem cells. These cells can differentiate into bone, cartilage, and fat under suitable induction conditions. These cells were also able to differentiate into neuroglial- and hepatocyte-like cells; therefore, these cells may be more than mesenchymal stem cells due to their ability to differentiate into cell types of all 3 germ layers [7].

4. Characteristics

In 2006, the International Society of Cellular Therapy defined characterization of MSCs by the following three criteria [8]:

1. MSCs must be adherent to plastic under standard tissue culture conditions;

2. Certain cell surface markers must be expressed such as CD73, CD90, and CD105, other markers must not be expressed such as CD45, CD34, CD14, or CD11b, CD79 alpha or CD19 and HLA-DR surface molecules;
3. MSCs must have the capacity to differentiate into osteoblasts, adipocytes, and chondroblasts under in vitro conditions.

MSCs generally have low immunogenicity as they do not express MHC class II or costimulatory molecules. Thus, injection of autologous or allogeneic MSCs has been employed in clinical studies. Allogeneic MSC therapy has the potential to expand MSCs therapy to a larger range of patients [9].

The effects of MSCs are generally achieved through two mechanisms:

1. Differentiation of recruited MSCs into functional cells to replace damaged cells, permitting the treatment of organ damage [9].
2. Response of MSCs to inflammatory cytokines, prepares the microenvironment through production of immune regulatory factors that modulate the progression of inflammation by affecting dendritic cells, B cells, T cells, and macrophages.

Furthermore, MSCs also produce a large amount of cytokines, chemokines, and GFs, which stimulate angiogenesis, prevent apoptosis, block oxidation reactions, promote remodeling of extra cellular matrix, and induce the differentiation of tissue stem cells [10].

In addition, under the effect of signals of cellular damage, known as homing signals, MSCs migrate toward areas of injury. This migration property of MSCs is important in regenerative medicine, where various injection routes are utilized depending on the damaged tissue or organ [11].

5. Morphology

MSCs are defined by a small cell body with a few long and thin cell processes. The nucleus is round and large with a prominent nucleolus, in the midst of finely spread chromatin particles, providing the nucleus a clear appearance. A small amount of rough endoplasmic reticulum, polyribosomes, Golgi apparatus and mitochondria are also present. The adjacent extracellular matrix is populated by a few reticular fibrils however other types of collagen fibrils are absent [12].

6. Differentiation capacity

The identification of specific signaling networks and 'master' regulatory genes that control unique MSCs differentiation lineages represents a major challenge. Obtaining a desired differentiation program, or preventing false differentiation of MSCs, needs ability to modulate biological effectors for effective clinical application, as in tissue engineering and regeneration.

6.1. Chondrogenesis

There is similarity between chondrogenic differentiation of MSCs *in vitro* and of cartilage development *in vivo*. In MSC-derived chondrocytes, the following has been positively characterized: expression markers associated with chondrogenesis; including transcription factors (sox-9, scleraxis) and extracellular matrix (ECM) genes (collagen types II and IX, aggrecan, biglycan, decorin, and cartilage oligomeric matrix protein) [13, 14]. Many helpful signaling molecules, involving many transforming growth factor-β (TGF-β), bone morphogenetic protein (BMP), growth and differentiation factor (GDF) and Wnt ligands, have been recognized through naturally occurring human mutations and molecular genetic studies. Chondrogenesis of MSCs from a variety of mesodermal tissue sources is rapidly stimulated by recombinant proteins and/or adenoviral infection of MSCs with TGF-β1 and TGF-β3, BMP-2, BMP-4, BMP-6, BMP-12, BMP-13, and GDF-5 [14, 15]. Through specific intracellular Smad proteins and major mitogen-activated protein kinase (MAPK) cascades, TGF-βs and BMPs signal provide levels of specificity that are widely studied in MSC differentiation contexts, upon receptor binding [16]. Downstream MAPK signaling and Smad effectors crosstalk has declared that MAPK substrates include chromatin histone acetyltransferases (HATs). Smads recruit HATs which enhance Smad transactivation capability [17].

Wnts possess double modulatory function in chondrogenesis. In human MSCs, Wnt7a induces chondrogenesis through various TGF-β1–MAPK signaling pathways when it is transiently upregulated, but in case of sustained expression, Wnt7a turns into chondroinhibitory [18]. Wnt3a controls *bmp2* expression [19], providing a feed forward regulatory loop during chondrogenesis. In ATDC5 cells, chondrogenesis is inhibited by Wnt1 through upregulation of the mesodermal basic helix–loop–helix (bHLH) transcription factor, Twist 1 [20], this effect may be through involving negative sequestration of chondrostimulatory factors or direct repression of target genes.

6.2. Osteogenesis

Two bone morphogenic proteins (BMPs), especially BMP-2 and BMP-6, stimulate osteogenesis in MSCs. BMP-2 acts by induction the p300-mediated acetylation of Runx2, a master osteogenic gene, which leads to enhanced Runx2 transactivating capability. Histone deacetylases 4 and 5 stimulate the degradation of Runx2 by deacetylation, through Smurf1, Smurf2 and E3 ubiquitin ligases [21]. The cytokine TNF-α, involved in inflammation-mediated bone degradation, downregulates Runx2protein levels by increasing degradation by Smurf1 and Smurf2. BMPs, Runx2, and histone deacetyltransferases that are responsible for the therapeutic approaches to MSC-based bone tissue engineering, stimulate existing TNF-α based immunotherapy of bone diseases.

Wnts is another important modulator in osteogenesis. Knockout and dosage compensation in Wnt-pathway-related transgenic animals provide the strongest proof that high levels of endogenous Wnts promote osteogenesis, whereas low levels inhibit osteogenesis [22].

The exciting finding of transcriptional mechanisms, suggesting that a global osteogenic gene, *runx2*, and a specific osteogenic homeobox gene, *tbx5*, are responsible for the balance of bone

formation and loss, show two strong models of transcriptional regulation of osteogenesis, and potentially other MSC lineage differentiation programs.

6.3. Adipogenesis

MSC adipogenesis is stimulated by the nuclear hormone receptor peroxisome proliferator-activated receptor γ (PPARγ) but at the same time it represses osteogenesis. Through binding to various ligands, like long-chain fatty acids and thiazolidinedione compounds, PPARγ induces the transactivation and transrepression of PPARγ. The bipotent coregulators TAZ function as a coactivator of Runx2 and as a corepressor of PPARγ, thus promoting osteogenesis while blocking adipogenesis [23]. In general, osteogenic genes are corepressed by a coactivator of adipogenic genes, but the opposite is also possible. This type of cellular efficiency is very likely, allowing that MSCs may be differentiated to both lineages.

Stretch-related mechanoinduction represents another interesting example of exchange between transcriptional cofactors of adipogenesis. If stretch is induced on mouse embryonic lung mesenchymal cells they form myocytes but they form adipocytes if uninduced. This occurs through activation of specific isoforms of tension-induced/−inhibited proteins (TIPs) [24] chromatin-modifying proteins with intrinsic HAT activity that have other distinctive domains such as nuclear receptor-interacting motifs. TIP-1 which is expressed under non-stretch conditions provides a potential mechanistic endpoint for cytoplasmic RhoA-mediated of adipogenesis; induces RhoA signaling which stimulates adipogenesis [25]. Whereas TIP-3 induces myogenesis. These findings propose a molecular model that connects cell morphology mechanical induction cytoskeletal signaling and transcriptional response during MSC adipogenesis induction.

6.4. Myogenesis

The majority of studies of myogenesis in adult stem cells target skeletal muscle-derived stem cells, or satellite cells. The highly successful stimulation of myogenesis from adult stromal MSCs happened after transfection with activated Notch 1. Other studies, mainly target cardiomyogenesis, represented the importance of cell-cell contact in stimulating cardiomyogenesis through co-cultured MSCs and cardiomyocytes, and the stimulation of MSC cardiomyogenesis in a rat intramyocardial infarct model by Jagged 1, a Notch ligand [26].

In normal conditions, the MSCs are present in low numbers, and on induction of myocardial infarction (MI) these cells proliferate rapidly to participate in wound healing, by generation of fibroblasts and myofibroblasts.

After MI, MSCs penetrate the injured tissue by trafficking through the ECM and repairing the cardiac function. This is through production of *HGF* by apoptotic cardiomyocytes, and not by necrotic cardiomyocytes. MSCs are attracted to the apoptotic cell death site by *HGF* receptor MET, which are responsible for activation of a wide range of signaling pathways. Platelets migrate MSCs to the apoptotic cardiac cells by means of the interaction of a nuclear protein with *TLR-4* expressed on MSCs; high mobility group box-1 (*HMGB1*). On activation of platelet, *HMGB1*/*TLR-4* downregulate MET on MSCs, thus, decreasing the recruitment of the cells.

Thereby, gene-knockout or blocking of *TLR-4* on MSCs can produce improved infiltration of MSCs to the damaged tissue, thus, raising the efficacy of MSC-based therapy [27].

In myocardium damage, Stromal cell-derived factor-1α (SDF-1) is a chemokine that mediates the homing of the endogenous MSCs. An intracellular storage of the receptor*CXCR4* present in 80–90% of hMSCs but not expressed in large amounts on the surface. When it is expressed by mRNA nucleofection, Ca^{2+} signaling is stimulated through its ligand *SDF-1α* [28]. However, in dilated cardiomyopathy (DCM), another homing factor of MSCs, monocyte-chemotactic protein-1 (*MCP-1*), has been established because of the presence of chemokine receptor type 2 (CCR2), a *MCP-1* receptor, on the cell surface [29]. Many in-vivo and in-vitro studies have been performed to comprehend the mechanism of MSC recruitment to the site of the damaged tissue, starting the process of repair along with its protective role. For the regenerative process to occur, MSCs either differentiate into beating cardiomyocytes or promote a paracrine effect [30].

7. The immunomodulatory effects of mesenchymal stem cells

Beside cell-to-cell contact, the MSCs secret many factors including EVs and soluble factors modulating the inflammatory response. The main paracrine factors are TGF-β, prostaglandin E2 (PGE2), hepatocyte growth factor (HGF), IL-10, IL-6, indoleamine 2,3-dioxygenase (IDO), nitric oxide (NO), and human leukocyte antigen G (HLA-G). Each of these factors plays an important role in regulation of different target immune cells. Other than such soluble factors, MSCs secrete extracellular vehicles (EVs), lipid bilayers that contain and transport the cytoplasmic components of the MSCs. EV is an inclusive term that has recently been suggested to encompass both exosomes and microvesicles. The immunological potential of MSC EVs in vitro, and the ability of these EVs to attenuate an activated immune system in vivo have been reported [31].

7.1. Natural killer (NK) cells

MSCs are capable of inhibiting proliferation and function of NK cells, mediated by IDO, PGE2, and TGF-β1. Many studies have reported that MSCs only partially inhibit the proliferation of activated NK cells and are susceptible to lysis by activated cells. HLA-G5 inhibits NK cell mediated cytolysis and decreases interferon-gamma (IFN-γ) secretion [32].

7.2. Dendritic cells (DCs)

Dendritic cells are antigen presenting cells that arise from monocytes or CD34+ hematopoietic stem cells. After exposure to antigens, they are turned into mature cells. MSCs impair this differentiation process via PGE2 secretion [33].

7.3. Neutrophils

Chemotaxis attracts neutrophils to the wound site, traversing post capillary venules to lyse pathogens with the granules within phagolysosomes, and then undergo apoptosis. MSCs secrete

IL-10 which inhibit neutrophil invasion into the wound. TNF-stimulated gene/protein-6 (TSG-6) is secreted by MSCs, interacts with protein ligands to inhibit rolling and transendothelial migration of neutrophils. Dyer [34] found that TSG-6 interacts with the glycosaminoglycan binding site of CXCL8 (IL-8), a chemokine produced by macrophages and transported to the surface of the endothelium, impairing neutrophil adhesion and migration.

7.4. Macrophages

Macrophages that arrive at the injury site hours later than neutrophils, are phagocytes that cleanse the wound of matrix and cell debris. They typically classified into two main groups: classically activated macrophages (M1) and alternatively activated macrophages (M2). M1 macrophages generally carry antimicrobial characteristics and stimulate a Th1 type response while M2 macrophages stimulate Th2 type responses. In general, M2 macrophages secrete less proinflammatory cytokines, have high production of anti-inflammatory cytokines such as IL-10, and induce resolution of the inflammatory phase. M2b macrophages show the reverse, as they maintain high levels of inflammatory cytokines [35]. Many studies explain the ability of autologous or allogeneic MSCs to polarize macrophages toward an M2 phenotype in vitro mediated by paracrine mechanisms, enhancing expression of M2 associated macrophage genes. Kim and Hematti [36] have suggested a separate definition for MSC-educated macrophages that secrete high IL-10 and IL-6 and low IL-12 and TNF-α, to call them M2 m, differing them from other subcategories. They suggest the possibility of collecting monocytes through leukapheresis and coculturing these mononuclear cells with allogeneic MSCs to provide MSC-educated macrophages prepared for repair of wounds [36].

7.5. B cells

B lymphocytes produce antibodies on exposure for antigens. MSCs may arrest B cell proliferation in the G0/G1 phase of the cell cycle without enhancing apoptosis [37]. IFN-γ inhibits the proliferation, which is probably mediated by MSC production of IDO. IDO is the first and rate-limiting enzyme of the essential amino acid tryptophan catabolism to kynurenine pathway, producing depletion and therefore halting growth. IFN-γ has IDO inducing effects [38].

7.6. T cells

Inhibitory effects of T cell proliferation by MSCs are mediated by both cell-to-cell contact and soluble factors. T cell proliferation was suppressed by TGF-β1 and HGF [39]. MSCs secrete PGE2 which prevents differentiation of CD4+ T cells into Th17 cells. MSCs also release IDO and enhance secretion of IL-10, which also inhibit cell proliferation [40].

8. Isolation and culturing

All MSCs, despite the protocol used for isolation, characterization and expansion, show the minimum criteria suggested by International Society for Cellular Therapy.

hMSCs are isolated based on their adherence ability to plastic surfaces, however, this method leads to the formation of a diversity of cells (stem cells as well as their progenitor cells) [41]. Considered as the best cell source, Bone marrow-derived MSCs (BM-MSCs) are taken as a standard to compare MSCs from other sources.

To ensure the success of the usage of these cells as a dependable source for regenerative medicine, a complete procedure should be established for MSCs isolation, characterization and expansion [42]. Contrary to bone marrow, MSCs from other tissues can be easily collected through non-invasive methods and their proliferation could be sustained up to many passages. Ficoll density gradient method with small modifications is utilized for isolation of MSCs from bone marrow, peripheral blood and synovial fluid [13] and seeded into culture plates. During isolation of MSCs from bone marrow, some hematopoietic cells also adhere to the plastic plate but they are washed away during sub-culturing, leaving only adherent fibroblast like cells [43]. MSCs from various tissue sources (adipose, dental, endometrium, foreskin, placenta, Wharton's Jelly) were isolated after digestion with collagenase and then cultured at varying densities [42]. Novel marrow filter device is recently explored as an efficient method for isolation of BM-MSCs [16], avoiding the risk of external contamination and saving time. Following their isolation from different sources, MSCs were cultured in condition media such as Dulbecco's modified Eagle's media (DMEM), DMEM-F12, DMEM-LG, DMED-HG, αMEM and RPMI (Roswell Park Memorial Institute medium) [44]. The primary culture medium was supplemented with fetal calf serum (FCS), new-born calf serum (NBCS) or10% FBS [45]. Besides the culture media and supplementation, the oxygen concentration is very important in the expansion and proliferation of MSCs [46]. It is also documented when cultured in DMEM culture with low glucose enriched with growth factors like fibroblast growth factor (FGF), epidermal growth factor (EGF) and B27 also leads to MSCs expansion [47]. But most commonly DMEM with 10% FBS is vastly employed in culturing and expanding MSCs *in vitro*, on the other hand, the use of exogenous FBS is highly debated.

8.1. Expression of cell surface markers

One of the essential characteristics of hMSCs is expression of specific set of cell surface markers. According to the International Society for Cellular Therapy standard criteria, MSCs are positive for CD73, D90, CD105 but negative for CD14, CD34, CD45 and HLA-DR [8]. MSCs can be isolated from various human tissues, which express cell surface markers mentioned above along with positive expression of CD29, CD44, CD146, CD140 b specific to tissue origin. The expression of CD34, which is a negative marker, is still controversial [48]. Stage-specific embryonic antigen (SSEA)-4 [49], stromal precursor antigen-1 (Stro-1) and CD146 are reported as are stemness markers for MSCs [50]. MSCs isolated from the human amniotic fluid express HLA-ABC [major histocompatibility complex class I (MHC I)], CD29, CD44, CD90, CD105, as well as SH2 (Src homology 2), SH3 (Src homology 3) and SH4 (Src homology 4). On the other hand, they lack the expression of HLA-DR (MHC II) [51]. Stro-1, a stemness marker for MSCs, is reported positive in dental and bone marrow MSCs, while reported negative in human adipose-derived MSCs (AD-MSCs) [52].

8.2. Msc niche

Schofield 1978 first introduced a stem cell 'niche' term [52]. The niche consists of the elements surrounding the stem cells in their naïve state including the non-stem cells as well as ECM and soluble molecules found in that locale. The above factors act together to maintain the stem cells in their undifferentiated state. Differentiation of the stem cells needs certain signals which must find their way into the niche for the regeneration or repopulation of a tissue.

8.3. Cellular components

The expression of α-smooth muscle actin (α SMA) in MSCs from all tissue types tested, is the basis of a perivascular nature of the MSC niche [53] and the immunohistochemical localization of CD45−/CD31−/Sca-1+/Thy-1+ cells to perivascular sites [54]. These cells also expressed α SMA and some even expressed 3G5, a pericyte-associated cell-surface marker. Doherty et al. [55] suggested that pericytes are in fact MSCs, because their differentiation into osteoblasts, chondrocytes, and adipocytes. MSCs have easy access to all tissues and participate in healing of many different tissues due to their presence in perivascular niches throughout the body.

Cadherins, MSCs transmembrane proteins responsible for cell–cell adhesion, polarity, differentiation, migration [18], interact with Wnts, which implicates in the biology of other stem cell niches [56].

8.4. Soluble components

The nature of bone marrow milieu is hypoxic. Comparison of human MSCs proliferative capacity was better maintained in the former when cultured in hypoxic versus normoxic conditions (2 and 20% oxygen). Additionally, hypoxia has doubled the number of existing CFU-Fs, as well as enhanced the expression of *rex-1and oct-4*; genes which are expressed by embryonic stem cells and are crucial in maintaining 'stemness'. Therefore, through increasing the plasticity and the proliferative capacity of MSCs, hypoxia is considered to have a double effect. However, the mechanism of action of hypoxia on MSCs is still unknown, although there is a possibility through the *oct-4* upregulation by the transcription factor hypoxia-induced factor-2α (HIF-2α) [57].

The effect of proteins secreted in the MSC niche is unexplained. The cell types studied have either induced differentiation or had no effect on MSCs. Finding soluble proteins permitting proliferation while inhibiting MSC differentiation would be ideal for simulating the niche and for MSCs expansion *ex vivo*.

8.5. Extracellular matrix components

However, ECM alone can regulate MSC differentiation, with potential applications for tissue engineering, no specific matrix components have been isolated to maintain MSCs in their naïve state, as a niche matrix would do. For example, osteoblasts on titanium scaffolds leave ECM after decellularization increasing osteogenesis markers, such as alkaline phosphatase and calcium deposition, in MSCs [58]. The ability to design artificial matrices that can resemble

the tissue microenvironment *in vivo* and control the appropriate differentiation of stem cells is a promising approach to therapeutic applications. Molecular information on ECM–MSC interactions, involving integrins, which involved in niche biology in other systems [59], is clearly needed.

9. Applications

9.1. Human mesenchymal stem cells and chronic diseases

MSCs are promising cell source for treatment of autoimmune, degenerative and inflammatory diseases due to the homing ability, multilineage potential, secretion of anti-inflammatory molecules and immunoregulatory effects. MSCs role in treating chronic diseases have been extensively studied in animal disease model.

9.2. Amyotrophic lateral sclerosis

MSCs are capable of differentiating into neurons [60]. An acid sphingomyelinase mouse model was used to conduct the first MSCs transplantation for neurodegenerative disorders. After MSCs injection, an amelioration in the overall survivability of the mouse and a decrease in disease abnormalities were detected [61]. Based on this study, a new study was performed in order to ensure the MSC transplantation efficiency in a neurodegenerative disease that leads to motor neurons degeneration and muscle function distortion, Amyotrophic lateral sclerosis (ALS) [61]. MSCs were isolated from the bone marrow and then reinjected into the spinal cord of the same patients, followed by MRI at 3 and 6 months for MSCs tracking. Results did not reveal any abnormal cells proliferation or structural changes in the spinal cord. However, mild adverse effects occurred which were reversed in few weeks duration e.g. intercostal pain irradiation and leg sensory dysesthesia. In another study, genetically modified AD-MSCs were made to express GDNF to be transplanted in a rat model of ALS, an increased number of neuromuscular connections and an improved pathological phenotype were observed [62].

9.3. Parkinson's disease

Parkinson's disease (PD), a neurodegenerative disorder, characterized by significant loss of dopaminergic neurons. After MSCs transplantation in PD mice model, tyrosine hydroxylase level increased [63]. MSCs participate to neuroprotection by secretion of trophic factors like vascular endothelial growth factor (VEGF), EGF, FGF-2, neurotrophin-3 (NT3), HGF and BDNF without differentiating into neurocytes [64]. Genetically modified hMSCs are used to induce the secretions of specific factors or to increase the dopamine (DA) cell differentiation. BM-MSCs transduction with lentivirus carrying LMX1a gene, resulted in cells which were similar to mesodiencephalic neurons with high DA cell differentiation [65]. Experiments were performed on Parkinson diseased rat, the research group from the university hospital of Tubingen in Germany administered BM-MSCs nasally to treat neurodegenerative patients. MSCs were found in different brain regions after 4.5 months of administration. They have

been found in the cerebral cortex, olfactory lobe, hippocampus and brain stem, suggesting that MSCs could successfully survive and proliferate *in vivo* [66]. Moreover, this type of administration was observed to increase the level of tyrosine hydroxylase and decrease the toxin 6-hydroxydopamine in the ipsilateral striatum and substantia nigra lesions. This novel MSCs administration route could change the face of MSCs transplantation in future.

9.4. Alzheimer disease

Alzheimer disease (AD), one of the commonest neurodegenerative diseases, characterized by symptoms as intellectual disabilities, dementia and memory loss. Till present, no treatment was established to slow down or stop the progression of AD [67]. Researchers use stem cell therapy in AD animal model aiming to decrease the neuropathological deficits. Mostly by activating the alternate microglia, increasing the expression of Aβ-degradation enzymes and decreasing the expression of pro-inflammatory cytokines, that the human AD-MSCs modulate the inflammatory environment [68]. Furthermore, MSCs modulate the inflammatory environment of AD and inadequacy of regulatory T-cells (Tregs) and they could modulate microglia activation [69]. Shin et al. [70] demonstrated that human UCB-MSCs increase the neuronal survival and stimulate Tregs which control microglia activation in AD mice model. Most recently, it was confirmed that MSCs stimulates the cell autophagy pathway, causing increased neuronal survivability and clearing of the amyloid plaque both *in vivo* and *in vitro* [70].

9.5. Autoimmune diseases

MSCs have the ability of regulating immune responses, thus it can treat immune disorders. Other hMSCs can be used for autoimmune diseases treatment, after revealing that human BM-MSCs are able to protect hematopoietic precursors from inflammatory damage [71].

9.6. Rheumatoid arthritis

Rheumatoid arthritis (RA), a joint inflammatory disease resulting from loss of immunological self-tolerance. The use of MSCs in animal models' studies, were successful in slowing disease progression and enhancing the disease recovery. Beside its anti-inflammatory function, IL-10 is an important factor in the activation of Tregs that controls self-reactive T-cells and motivates peripheral tolerance *in vivo* [72]. Similar effects were produced by human BM-MSCs in the collagen-induced arthritis model in DBA/1 mice [73]. These studies suggest that the improvement of the RA pathogenesis in DBA/1 mice model in case of using MSCs, can be caused by activating Treg cells as well as suppressing the production of inflammatory cytokines. However, MSCs were only effective when administered at the onset of disease, in case of adjuvant-induced and spontaneous arthritis model, which suggests that MSCs lost their immunoregulatory properties when exposed to inflammatory microenvironment [74].

9.7. Type 1 diabetes

Type 1 diabetes, an autoimmune disease caused by the destruction of β-cells due the production of auto antibody directed against these cells. As a result, there is decrease in the insulin

production to a level which is failed to control the blood glucose. It has been proved that MSCs can differentiate into insulin producing cells and have the capacity to regulate the immunomodulatory effects [75]. Zulewski et al. [76] isolated Nestin positive cells from rat pancreatic islets which differentiated into pancreatic endocrine cells. Nestin positive cells were isolated from human pancreas and transplanted to diabetic nonobese diabetic/severe combined immunodeficiency (NOD-SCID) mice, which improved hyperglycemic condition [77]. However, these studies were found controversial and it was suggested that besides pancreatic tissues, other tissues can be used as an alternative for MSCs isolation to treat type 1 diabetes. Human BM-MSCs can be differentiated efficiently into pancreatic endocrine cells *in vitro* as well as *in vivo* [78]. There is an option for the use of UCB-MSCs as insulin producing cells. UCB-MSCs were similar to human ESCs, following similar steps producing the differentiated β-cells [79]. Unsal et al. [80] showed that transplantation of MSCs together with islets cells into streptozotocin treated diabetic rat model improve the survival rate of engrafted islets.

9.8. Cardiovascular diseases

Cardiac cells transplantation is a novel strategy for myocardial repair, which is currently applied in animal models. Although MSCs are a good source for cardiomyocytes differentiation, it was found that *in vitro* differentiation is effective only from young cell sources and *in vivo* differentiation of cardiomyocytes is very rare [81]. MSCs, which have differentiated into cardiomyocytes under the effect of cocktail of growth factors [82], were used in treatment of left ventricular heart failure and MI [83]. The systematic injection of BM-MSCs into the infarcted myocardium of rodent models partially produced recompensation [84]. Katritsis et al. [85] reported improvement in myocardial contractibility when autologous MSCs were transplanted with endothelial progenitor cells. Despite the fact that MSCs are proven to be effective in MI and related problems, still the ability of the heart to retain cells is low; only 10% cell retention after 4 h of cells injection and 1% after 24 h [86]. Roura et al. [87] recorded that UCB-MSCs proliferated and then differentiated into endothelial lineage, were retained for several weeks when injected in acute MI mice. Transplantation of UCB-MSCs into myocardial infarction animal model along with fibronectin-immobilized polycaprolactone nanofibers were found very effective [88].

10. Cryopreservation and banking

From all the previous studies, it becomes clear that the use of hMSCs in clinical field will increase in future. For clinical applications, a large number of MSCs in an 'off the shelf' format is required. For this purpose, cryopreservation and banking are necessary to be established. This will allow unique opportunities to improve the potential uses of these cells in research and clinical applications. Keeping in mind its use in future clinical and therapeutic applications, there is a need to ensure the safety and efficacy of these cells while cryopreserving and banking. Cryopreservation media should be optimal so uniform change in temperature during freezing and thawing, long-term storage in liquid nitrogen and employed freezing device are the main factors to consider.

In the cryopreservation media in which cells can maintain their stem cells abilities for long time, the cells require a source of their nutrients as the animal base reagent, like FBS, but previous studies have showed that there is difficulty in removing animal proteins from the hMSCs and that may elicit adverse reactions in the patients who receive these cells for treatment [89]. Therefore, a serum-free media is alternative for the cryopreservation of MSCs and it was successfully used [90]. Lately, instead of using FBS, human albumin and neuropeptide were used. It was observed that MSCs maintained their proliferation potential and cell survival in the culture conditions. Moreover, cryoprotective agents (CPAs) are found to be required for the cryopreservation media to prevent any freezing damage to cells. A large number of CPAs are available [91], DMSO is the commonest CPAs agent used in cryopreservation of MSCs. However, DMSO toxicity to humans and animals hinders its usage in MSCs freezing for clinical applications. Due to these complications, it is necessary to use an alternate CPA. There are many methods along with the introduction of automated cells washing for the removal of DMSO from the frozen thawed cells [92].

The second important factor in cryopreservation of MSCs is the freezing temperature rate. The optimum rate for MSCs preservation is slow freezing at the rate of 1°C/min is [93]. For the purpose of maintaining the rate of temperature during cryopreservation, controlled rate freezers (CRFs) are suitable for regulating the temperature. These CRFs can be programmed to determine the exact temperature the sample is experiencing during freezing [94]. Despite of these advantage, these CRFs do not apply a uniform temperature to all vials during large-scale MSCs banking [95], therefore, the development of advanced CRFs is mandatory for large-scale banking. Lately, Praxair Inc. created advanced CRF, providing unidirectional flow of cryogen to each sample. The safe and efficient cryopreservation as well as the regulatory guidelines are important for large-scale MSCs banking. In the U.S.A., Food and Drug Administration (FDA) is responsible for supervising MSCs based cell therapy products, while in Europe it is the European Medicines Agency that is responsible.

11. Summary and conclusion

Mesenchymal stem cells (MSCs) are plastic-adherent, fibroblast-like, multipotent cells found in the human body having the ability to differentiate into different cell types including osteoblasts, adipocytes and chondrocytes. They are normally present in the umbilical cord, adipose tissue, bone marrow but can also be resident in other tissues and are recruited to sites of wound healing as well as growing tumors.

MSCs are a promising candidate for cell-based tissue regeneration that can potentially revolutionize the current pharmaceutical landscape. The extracellular matrix (ECM), adjacent cells and different types of cytokines and growth factors forming MSC niche microenvironment, are critical for their lineage differentiation. Standardized protocols for cell culture, differentiation, expansion and cryopreservation need to be in place. These factors in combination with safely preconditioned and genetically modified MSCs may pave the way for the development of an effective cellular therapy for countless human immune disorders.

Recently, research and basic knowledge of these cells has fast-tracked, both from fundamental and translational perspectives. There have been important discoveries about the available variety of tissue sources. In addition, novel abilities such as immune-modulation together with improved delivery to the selected optimal tissue site has been discovered. However, the molecular fingerprint of MSCs in these contexts remains imprecise and inadequate. Consequently, without this crucial knowledge the progress is difficult in order to determine with precision the MSCs practical developmental potentials.

Overall, the unavoidable propaganda fluctuation that continued for more than 40 years of work on BMSCs did not reduce the novel biological flavor of these cells. Concurrently functioning as stem cells and as cells providing the microenvironment for other stem cells, BMSCs incorporate properties of the "seed" and "soil." As expectations linked to BMSC plasticity are diminishing, these unique properties of BMSCs challenge both biology and medicine in a quite remarkable fashion.

Author details

Amira Hassouna[1,2]*, Marwa M. Abd Elgwad[1] and Hoda Fahmy[3]

*Address all correspondence to: amira_hassouna@yahoo.co.uk

1 Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Egypt

2 Faculty of Health and Environmental Sciences, School of Interprofessional Health Studies, Auckland University of Technology (AUT University), New Zealand

3 Faculty of Medical and Health Sciences, School of Pharmacy, University of Auckland, New Zealand

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