

Chapter 1

Not all Stem Cells are Created Equal

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Introduction

In 1867, German pathologist Dr. Julius Friedrich-Cohnheim observed non-hematopoietic stem cells in the bone marrow for the first time [1]. He observed that this new unique population of cells had the capacity to differentiate and travel through the bloodstream to reach injured tissue. This was later confirmed as cell homing by the groundbreaking reports by Friedenstein in the mid-1970s [2]. Mesenchymal stem cells (MSCs) were characterized as adherent cells with a heterogeneous, spindle-shaped appearance and with a marvelous facility to differentiate into multi-lineage mesenchymal cell phenotypes and specialized tissues [3].

MSCs are multipotent, heterogenic stromal cells derived from the mesoderm, one of the three primary germ layers of the embryo, from which muscle (smooth, cardiac, skeletal), connective tissue, serous membranes, bone and cartilage, blood and lymph, among others, are derived [4]. MSCs function to support the parenchyma and have the capacity for multi-lineage differentiation [5,6]. Studies have found that they not only have osteogenic, adipogenic, and chondrogenic differentiation potential, but also the capacity to differentiate into cells derived from the any of the 3 germ layers under precise conditions [7-11].

Mesenchymal stem cells are found throughout the body and a number of different protocols have been established to extract them from different tissues. Due to the vast diversity of methods of cell isolation and culture, universal minimal criteria for MSCs were introduced by

the International Society for Cellular Therapy [6]. First, they should exhibit adherence to plastic. Second, they should have a characteristic surface antigen phenotype expressing CD105, CD73, CD90 and the absence of CD45, CD34, CD14 or CD11b, CD79 α or CD19 and HLA class II. Lastly, the unique capacity for tri-lineage mesenchymal differentiation into osteoblasts, adipocytes and chondroblasts under standard in vitro culture conditions.

Interestingly it appears that cells that fulfill these criteria can be different amongst themselves in terms of function based on the mode of extraction they undergo. Kellner et al isolated stromal cells from several human sources including bone marrow, heart muscle stroma, adipose stroma and liver stroma to compare their morphology, phenotype and differentiating capacities. It was found that while the harvested cells uniformly satisfied the criteria set forth but the International Society for Cellular Therapy, they differed in the nuances of their function based on the source of harvest and isolation method used [12].

Sources and Isolation Methods

MSCs can be found in various sites throughout the body and are amenable to isolation from a number of tissues. The most common sources found in clinical use include adipose and bone marrow. While these are the predominantly investigated tissue types many others have emerged as candidates for isolation [13]. These sources, discussed below, include compact bone, dermis, products

of conception, and dental pulp.

Bone Marrow

With the innovative finding of Friedenstein based on the propensity of MSCs to adhere to plastic flasks led him to discovered adult non-hematopoietic stem cells, also known as mesenchymal stem cells (MSCs). His work has been widely used for the isolation of human BM-MSCs as a reservoir of stem cells that have the ability to differentiate into multiple tissues. His pioneer method, using a fragment of rodent bone and flushing out the bone marrow into plastic culture dishes, and discarding the non-adherent cells, spindle-like cells were isolated based on plastic adherence, has been modified and used to isolate MSCs from multiple tissues and had led to the rising interest in utilizing MSCs cell based therapy [14].

Bone marrow is a complex organ, containing a vast diversity of haematopoietic and non-haematopoietic cell types. The bone marrow primary function is extravascular hematopoiesis, this dynamic process is supported by an extravascular marrow stroma which shelters BM-MSCs and hematopoietic stem cells (HSC), it is composed by a powerful interconnected microenvironment called the “niche”, a specific anatomic location that regulate how stem-cell participate in tissue generation, maintenance and repair, defined by anatomy and function [15]. The BM-MSCs are located lining the endosteal surface of the marrow space, their primary role is to support and main-

tain tissue homeostasis for cell development and specialization to give rise to functional mature cells by producing SCF [16], CXCL12 [17] and fibroblast activation protein (FAP). Actively support blood formation providing structural and physiological support for the expansion of the hematopoietic cells [18,19] MSCs produce a large number of cytokines and extracellular matrix proteins and express cell adhesion molecules, all involved in the regulation of hematopoiesis [20].

At this time, bone marrow aspiration is the most frequently used procedure to obtain MSCs for clinical regenerative medicine [21]. This includes their use in orthopedic surgery and craniomaxillofacial surgery. Alternative sources for acquisition of bone marrow MSCs have been identified [22]. Because of the multiple sources for MSCs and their common phenotype for characterization, multiple researchers have compared the osteogenic potential of MSCs obtained from different sources versus MSCs from bone marrow. Specifically, they have demonstrated that bone marrow has more ALP activity, mineral deposition and expression of gene related to osteogenesis than adipose derived cells [23]. While studies have demonstrated the “superiority” of bone marrow as the ideal source for osteogenic regeneration authors have argued that these findings may be related to the multiple methods for cell culture and the large number of isolation protocols [24,25]. This has demonstrated divergence in the behavior of MSCs re-

lated with the osteogenic potential differentiation capacity, immunomodulatory capacity and expansion potency [26]. The ideal source and method for isolation is still a source of ongoing investigations.

The use of BM-MSCs as main source of stem cells is well documented and accepted for clinical and for investigational settings [27]. Iliac crest aspiration, the gold standard procedure to obtain BM-MSCs, though, is invasive and painful. An alternative to this procedure is Iliac crest harvest, used commonly in orthopedic surgery for the simultaneous acquisition of bone marrow MSCs and bone graft, is even more invasive and fraught with surgical site complications [28]. While considered the gold standard, the amount of acquired cells is limited because the frequency of MSCs in human BM is low. Further, it has been seen that transplanted bone marrow MSCs are unable to survive the hypoxic environment of the host tissue and their requirement of nutrients and metabolic pathways need to be enhanced to secure the survival of transplanted MSCs in vitro and in vivo [29]. Because they can be isolated from most adult tissues derived from the primitive mesodermal layers investigator have sought alternative tissue for the acquisition of adequate numbers of MSCs such as adipose tissue.

Adipose Tissue

One of the most reliable described sources of MSCs is adipose tissue [30-32]. Like bone, adipose tissue is derived from the mesodermal germ layer and has stromal support properties. Adipose stem cells (ASCs) would modulate the “stem cell niche” of the host, ASCs has an ability to secrete pro-angiogenic growth factors and serve as stromal support [33] MSCs are easily obtained through lipoaspiration and a large number of cells can be obtained. In addition, processed lipoaspirate (PLA) cells show a greater proliferation profile, higher formation of colonies and better survival rates under serum deprivation when compared to BM-MSCs [34,35]. This population of cells has been shown to differentiate into bone adipose and cartilage. While PLA cells can differentiate into multiple mesodermal cells like their BM-MSC counterparts, they have been reported to be inferior compared to BM-MSC in large animal model of orthopedic regeneration [36]. PLA cells satisfy the parameters of MSCs by molecular and biochemical characterization, however, it was found that these cells express different CD markers and gene expression profiles indicating that cell source may play a role in the ultimate function of these cells clinically [36].

Adipose derived MSCs have been used in many clinical and investigational scenarios ranging from facial rejuvenation, to Crohn's disease, to traumatic brain injury and orthopedic reconstruction [37-39]. The poor regeneration of tendons after injury presents a clinical problem

a vast field for investigators to improve therapy strategies. In tissue engineering investigations, equine species are preferred for tendon regenerative studies. Investigators isolated horse bone marrow stem cells (BM-MSCs) that were co-cultured in a transwell system with tendon tissue fragments showing tenogenic differentiation and interactions between co-cultured equine BM-MSC and native tendon tissue. Also tendon-derived stem cells (TSPCs) might have mesenchymal stem cell functions when compared with BM-MSCs; expressing cell markers and ability to osteogenic and adipogenic differentiation but a limited chondrogenic differentiation. But more studies are needed in to control tenogenesis for regenerative medicine [40,41]. One potential use for PLA cells is as carriers for adenoviral gene transfer. For example, they can be genetically modified to over express BMP-2, a recombinant bone morphogenic protein approved by the FDA; these human processed lipoaspirate (HPLA) cells might be effective to promote bone healing [42].

Comparing BM-MSCs and ASCs expanded in hPL-supplemented medium, adipose mesenchymal stem cells exhibited a homogeneous population with similar fibroblast-like morphology, have greater proliferative potential than BM-MSCs, and there are no significant differences in colony efficiency between the two types of cells. BM-MSCs and ASCs revealed very similar expression patterns of surface markers. BM-MSCs possessed higher capacity

toward osteogenic and chondrogenic differentiation compared with AMSCs, while they both have similar adipogenic differentiation potential [43]. MSCs derived from bone marrow and adipose tissue expressed classic MSC marker proteins, but lacked hematopoietic and endothelial markers, but some differences arise regarding the expression of CD90, CD105 and CD106 [44].

Based on the pioneer work of Friedenstein, Caplan and Haynesworth et al. for the isolation of MSCs the work of Robey et al. 1985 isolating bone derived MSCs and the method described by Tuan et al. Has inspired the novel use of compact bone as a source of MSCs [2-4,21,45]. This technique avoids contamination with hematopoietic cells and can be performed intra-operatively, decreasing costs and potentially improving the patient prognosis.

Compact Bone

For the purposes of orthopedic regeneration it seems that the bone would be the most likely place to start but it took until 2002 when Tuan et al. defined the multilineage capacity of trabecular bone cells that compact bone was considered a source [46]. Since then many investigators have demonstrated many methods of isolation and characterization. Most notably, Guo et al proposed a protocol to isolate MSCs from mouse compact bone using collagenase to digest the bone [47]. In his description, the bone marrow was flushed out, the bone cavities were washed, and this was followed by processing with collagenase.

Chips of compact bone were excised into plastic culture dishes with collagenase II. Aspiration of released cells was done, and the bone fragments were washed three times and incubated, changing their medium every 3-4 days. Adherent cells were obtained and harvested by trypsin digestion and passaged; they were then deemed ready to use for experiments.

CB-MSCs are homogeneously positive for mesenchymal markers CD29, CD44 and CD105, and the stem marker Sca-1, and negative for hematopoietic markers CD11b, CD34 and CD45 and for endothelial cell marker CD31 [48]. Like all MSCs, these cells ably differentiate into osteoblast, adipocytes and chondrocytes. Recent studies have shown that for osteogenic differentiation, alkaline phosphatase (ALP) activity increases after 14 days of culture and nodules of mineralization occurred after 4 weeks. For adipogenic differentiation, intracellular Oil-red-0-stained lipids were seen after 2 weeks of culture. In evaluation of chondrogenic differentiation, to identify the development of the cartilage matrix, intracellular sulfated proteoglycan accumulation was assessed by toluidine blue staining due to metachromatic-staining matrix that surrounds the differentiated chondrocytes [8]. Furthermore, their multi-lineage differentiation potential was maintained up to passage 10 [11,47,49].

Nowadays MSCs are being tested in multiple clinical trials in tissue regeneration and in inflammation condi-

tions, but regardless their common mesodermal origin [4] and phenotype, when derived from different tissues express different profiles in their differentiation, proliferation and abilities [50]; also the method for cell culture employed for isolation and preparation seems to alter the lineage differentiation demonstrating distinct features of MSCs in vivo and in vitro [51]. As such, it is important to review the different current state of the art sources of MSCs and understand how different subclasses of MSCs behave when considering which extraction method produces cells with qualities best aligned with the clinical need.

The cortical fraction of the bone shelters a homogeneous population of cells with distinct capacities. Recent investigations looking for MSCs sources, found that cortical bone is a rich reservoir of MSCs [52]. Comparing bone marrow and adipose MSCs with cortical bone mesenchymal stem cells (CB-MSCs) demonstrated that although they had a similar phenotype and shared lineage, some differences were present. First the osteogenic potential assessed in vitro suggested that the cortical fraction of bone is a superior source of osteoprogenitor cells to use in orthopedic regeneration for healing and repair of bone, second CB-MSCs exhibited higher activity of alkaline phosphatase (ALP) following osteogenic induction after 14 days and third greater calcium deposition [53]. Previous data have definitely shown that human MSCs derived from compact bone exhibit phenotypes similar to those of their counterparts from bone marrow [54]. Given that the

ideal source of MSCs may be the tissues they are all ultimately there to recreate, work has been done to identify and characterize MSCs from those sources.

Dental Pulp

Yet another source of MSCs is human dental pulp. First, human teeth are obtained as discarded medical waste under current good tissue practice standards. After extraction, the teeth are submerged in a sterile transport solution and transported on ice to the laboratory where they are incubated, digested, and cultured to obtain dental-pulp derived MSCs (DPSCs). These cells then undergo evaluation for the minimal criteria to be designated as MSCs. DPSCs potentially can be used for clinical banking for regenerative applications as well as for regeneration of tooth-specific structures [55]. Their potential medical uses embrace regeneration and repair of multiple mesenchymal tissues, and in tissue engineering alone or with biomaterials [55,56]. One innovative use for DPSCs are biobanks to store biological samples for current and future medical research and clinical applications [57,58].

Dermis

Adult human skin-derived precursors (SKPs) that can differentiate into both neural and mesodermal progeny were described by Toma et al. As an alternative to embryonic stem cells for therapeutic purposes. Isolated and cultured from human foreskin, dermal cells underwent clonal analysis, immunocytochemistry, PCR and karyotyping to

identify the SKP subpopulation. These cells differentiated into neural and mesodermal cell types demonstrating that they are a potential source of multipotent adult human cell precursors [59].

Products of Conception

With age, the abundance and differentiating capacity of MSCs diminishes significantly [60]. As such, the hunt for different sources has included products of conception, such as amniotic fluid (AF), amniotic membranes, umbilical cord blood (UCB), and placenta [2,61-67]. Rich sources of MSCs have been found in first- and second-trimester fetal tissues. However, the use of fetal tissues involves legal and ethical restrictions. Amniotic fluid (AF) was found to contain abundant stem cells showing properties of MSCs [68]. Particularly, second-trimester AF has a stunning amount of heterogeneous fetal MSCs [26,68]. Unfortunately amniocentesis is a high-risk procedure. The placenta is an alternative source of MSCs and the greatest advantage is its availability. When compared with BM-MSCs, placental MSCs exhibit higher expansion potency. In addition, fetal membranes were found to contain MSCs and further studies in tissue engineering may use these approach in to repair fetal membrane rupture [26].

Immunology of MSCs

MSCs continue to emerge as cells with versatile immune properties. However, it is difficult to assign MSCs as immune suppressor or immune enhancer cells. The emerging data indicate that the properties of MSCs might depend on the surrounding microenvironment. To be specific, cells and soluble factors within a tissue microenvironment license the MSCs for the final function. Thus, during inflammation the MSCs could exert immune suppressor functions and as the mediators subside the outcome could be immune enhancer.

The early studies show MSCs as third party cells exerting veto functions and inhibitor of T-cell proliferation [69,70]. These early studies established that the suppressive effects of MSCs were independent of the expression of the major histocompatibility complex antigens [71]. A keen observation from these early studies was the different immune effects, depending on the dosages of MSCs. At high doses, the MSCs inhibited mitogen-activated stimulation whereas low dose as stimulatory. The data acquired since these studies might explain these differences. The use of mitogen would cause overt inflammation and this might require large number of MSCs for licensing into immune suppressor cells. The data also provided insights of the immune enhancing effects of MSCs.

The immune suppressor functions of MSCs were validated in vivo in which MSCs from a third party were transplanted with hematopoietic stem cells [72]. This third party MSCs were shown to suppress graft versus host disease (GvHD) [73]. Current studies can explain why the authors were able to use MSCs to alleviate the inflammatory response of bone marrow transplantation. The MSCs were placed in an inflammatory milieu where they could become immune suppressor cells. At this time, the fate of MSCs when the inflammation subsides is unclear. This is an important issue, not only for bone marrow transplantation but other inflammatory diseases. This issue is important because if the MSCs survive they will be able to integrate into tissues and respond to the environment to differentiate into cell types that may be foreign to the organ. These are relevant problems that have not been studied but are required for the safe use of MSCs in patients.

Due to the vast literature on the immune suppressive effects of MSCs, the immune enhancing effects have been overlooked. However, there are more reports on MSCs as immune enhancers [74,75]. MSCs can produce cytokines and express their receptors, indicating the ability of cytokines to autostimulate the MSCs or to act in paracrine manner where the MSCs interact with each other or with other cells in the microenvironment [76]. MSCs can act as antigen-presenting cells [77].

MSCs can suppress T-cell activation by transitioning them into G0/G1 phases, through a complex process [69,78]. During the licensing process of MSCs, the levels of indoleamine-pyrrole 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2) and the expression of other immune checkpoint molecules such as programmed death 1 (PD-1) are increased [79,80]. MSCs do not always act as immune suppressor cells. The level of the co-stimulatory molecules on MSCs is undetectable, indicating that despite the immune enhancing property of MSCs, this could hinder the ability of MSCs to fully stimulate T-cells [81]. Thus, it is proposed that MSCs could lead to T-cell anergy [81]. Indeed, MSCs added together with low antigens to peripheral blood mononuclear cells of allergic asthma patients have been shown to induce an anergic response to the antigen [82].

MSCs can secrete cytokines to influence immune functions, and to induce the differentiation of T-cells. The cytokines produced by MSCs include hepatocyte growth factor (HGF), IL-6, IL-10, IFN γ and TGF- β 1 [83,84]. Thus, it is not a surprise that MSCs can suppress the differentiation of T-cells into TH1 and TH17 types with enhanced TH2 and regulatory T-cell (Treg) differentiation [85]. Similar to its role on T-cells, MSCs can also arrest B-cells in G0/G1 phase and blunt B-cell chemotaxis [86]. In general, the mechanism by which MSCs suppress inflammation is an ongoing process of investigation. This is the same for B-cells that may undergo cell arrest though the

PD-1 pathway or from factors produced by MSCs [79,86]. In line with the immune-enhancing effects of MSCs, these cells can stimulate B-cell proliferation and differentiation [87].

T- and B-cells are not the only immune cells that can be affected by MSCs. These stem cells can produce IDO and PGE2 to suppress the proliferation and function of natural killer (NK) cells [88,89]. This could occur by MSC-mediated decrease in the activating receptors, NKG2D, Nkp30 and Nkp44 to reduce the production of needed cytokines [88]. MSCs also inhibit dendritic cell differentiation and maturation, through the production of PGE2 [90].

Macrophages can differentiate into subtypes that exert pro- and anti-inflammatory functions. Depending on cues from the microenvironment, MSCs can induce the differentiation of macrophages into anti-inflammatory M2 macrophage [91,92]. The transition of macrophages into immune suppressor type has been linked to the production of IDO, the activation of PGE2 pathways, and the production of cytokines such as IL-10 [93].

MSCs can alter the migration of neutrophils to the regions of inflammation and infection [94,95]. This may be due to MSCs producing cytokines that are contrary to cell migration. Indeed, IL-10 and TGF- β secreted by MSCs reduce E-selectins on the endothelial cells to minimize the extravasation of neutrophils [95]. On the other hand, MSCs secrete IL-6, IL-8 and MIF to activate neutrophils [96,97]. This paragraph shows the dual properties of

MSCs as immune suppressor and enhancer cells.

Based on the above discussion, MSCs are attractive cells for the treatment of inflammation. Their ability to be used across allogeneic barrier makes them easily available for delivery to patients. However, MSCs need to be carefully evaluated for their behavior in varied microenvironment. A major question for the field is the fate of MSCs after these cells have subsided the desired inflammation such as the potential use in allergic asthma and rhinitis [82,98].

Clinical Applications

The immense variety of cell types into which MSCs can differentiate makes them an exciting potential therapy for a variety of conditions. Their therapeutic potential stems from that can regenerate injured tissue, restore its function, avoid host rejection and exert immunomodulation is a promising idea for the potential use of the MSCs capacities, leading investigators to think that MSCs can be suitable in cell-based therapy and change the medical world as we know it [99,100].

Orthopaedics

Compact bone-derived MSCs (CB-MSCs) were shown to not only to tolerate a low-oxygen and nutrient-depleted environment, but that this environment augmented their osteogenic potential. The increased and persistent biosynthetic activity of the CB-MSCs under hypoxic conditions

would be highly advantageous for purposes of orthopedic tissue engineering compared with other sources of MSCs. One potential mechanism for the differences in osteogenesis seen could be differential activation of hypoxia response pathway. Hypoxia-inducible factor (HIF) is the major regulator of cellular hypoxic responses and is upregulated in MSCs [49]. Compared to BM-MSCs, CB-MSCs exhibit superior growth rates, reducing the number of mice needed to obtain cell quota in culture, which is an important ethical and economic consideration [11].

Procedures such as spinal fusions, laminectomies, and traumatic orthopedic reconstruction would be ideal for harvesting exposed bone tissue for cell isolation and potential re-implantation, the patient would be spared from additional procedures. Without the need of additional procedures for acquisition of tissue because these pieces that were previously thought of a surgical waste, house a population of cells with the potential for dramatic augmentation of bony regeneration.

CB-MSCs were found to express higher level of SSP1, suggesting more mature osteoblastogenesis even compared with BM-MSCs [49,52]. In addition, the higher activity of ALP in CB-MSCs might also suggest their tendency for an osteoblast lineage. At molecular level, in prior studies these cells showed a marked upregulation in *Alp*, *Runx2*, and *Spp1* expression. Specifically, *Alp* and *Runx2* both are crucial in the regulation of osteoblastogenesis, and *Bglap* and *Sppl* are involved with a more mature differentiation.

Their potential to maintain regenerative capacity under hypoxic conditions has also been tested. Hypoxic pre-conditioning is capable of increasing the regenerative capacity of MSCs by increasing hypoxic inducible factor 1 alpha, C-X-C chemokine receptor type 4 (CXCR-4), stromal derived factor 1, and overall mobility [101-103]. These properties lead to their improved therapeutic potential [103]. While preconditioning is important, the hostile hypoxic and inflamed environment these cells are transplanted into may not provide sufficient nutrients to support this enhanced metabolic activity and thus investigators have looked into the regenerative potential of compact bone MSCs in hypoxic conditions to understand if they will not only survive but thrive in these conditions. Fernandez-Moure et al. demonstrated that when cultured long term under hypoxic conditions, MSCs derived from the compact bone not only were able to maintain the regenerative capacity with persistent upregulation of alkaline phosphatase and calcium deposition but were able to increase this production over the course of a month [53]. This demonstrated that this source may be an ideal source of cells for orthopedic regeneration where revascularization of the implanted tissue or injury may not occur for days or weeks.

CB-MSCs are suitable candidates to replace bone marrow as the preferred cell source in selected situations of orthopedic tissue engineering and regenerative medicine. The choice of cell source must be based on the in-

tended surgical application and accessibility of patient tissue [104].

Autoimmune Disease

In addition to their role in regeneration, immunomodulatory properties of MSCs have also demonstrated immunomodulatory properties both in vitro and in vivo [105]. Nemeth et al. demonstrated that MSCs had effect in cell to cell communication when in direct contact with macrophages, causing secretion of prostaglandin E2 to increase production of interleukin-10 (IL-10), a potent anti-inflammatory cytokine [95].

Although this mechanism of action is only partially understood, it suggests a potential novel cell-based therapy for diverse conditions caused by immune dysregulation. Amid the clinical applications and trials, the compelling therapeutic aims include acute graft-versus-host disease one of the major causes of post-transplant mortality by increasing the traffic of T lymphocytes and reducing their migration to target tissues [104,106-111].

There have experimental attempts into treatment of a plethora of autoimmune disease including autoimmune encephalomyelitis (EAE), an autoimmune inflammatory disease model resembling multiple sclerosis (MS), rheumatoid arthritis, systemic lupus erythematosus (SLE), Crohn's disease, and amyotrophic lateral sclerosis (ALS) [99,112-118].

Solid Organ Transplantation

The transplantation of solid organs like liver, pancreas, and kidney represents a clinical dilemma where there is greater demand than supply. Thus, clinicians and researchers have sought alternative strategies to manipulate tissues to prevent the permanent failure of that organ, restore its function, or regenerate the organ through the acquisition of its MSC population. Diabetes mellitus is a highly prevalent disease and a large source of healthcare utilization in the United States [119]. The pathophysiology of DM is the relatively low or absent production of insulin by pancreatic β -cells. MSCs are an attractive alternative as a potential therapy and the pancreas itself is a source of isolated MSCs [120]. Multiple studies have used mice and human fetal pancreatic tissue to obtain fibroblast-like cells [121]. These pancreatic MSCs demonstrated potential to differentiate into insulin producing islet-like cell aggregates, potentially restoring the insulin-producing β -cells [122].

Clinical studies have been performed to evaluate the clinical feasibility and efficacy of MSC therapy for an array of hepatic pathologies including cirrhosis, this condition represents a progressive and irreversible hepatic fibrosis, the standard treatment for decompensated liver cirrhosis is transplantation, meaning a long waiting list, high cost and hazardous complications. The transplantation of MSCs can be an alternative treatment for liver cirrhosis. In a phase 1 trial, was shown that autologous MSC

transplant were safe and feasible as a potential treatment improving liver function test and MELD scores of some patients, suggesting the beneficial effects of MSC transplantation [123,124].

Kidney

Chronic kidney disease (CKD) based on the latest KDOKI guidelines is one of the most disabling conditions that with required either transplant or a permanent dialysis access nowadays. Multiple causes including high blood pressure and type II diabetes contribute to the higher rates of end stage kidney disease. Unfortunately there are not enough donor organs to meet this growing need. MSCs has a great potential in kidney transplantation. Preclinical and early clinical results have shown positive results promoting operational tolerance and in animal studies, chronic kidney disease rats injected with MSCs have an increased expression of VEGF and Tie-2 suggesting that there is a pathway related to vascular protection induced by MSCs, which is a protective factor against glomerular injury [125,126].

Dermal/Wound Healing

There is evidence that MSCs can be induced to differentiate into epithelial phenotypes [127]. Some studies suggest that these cells may be engineered to serve as functional skin grafts. The use of hematopoietic cell transplantation (HCT) has shown benefits in the treatment of skin disorders, unexpected MSCs were found within the

HCT graft and they could be responsible for this improvement. It was demonstrated that treating murine MSCs with TGF β (15 ng/mL) and TNF α (30 ng/mL) for 48 hours induces an 8-fold increase in Col7a1 expression and a significant increase in secretion of C7 protein, and that the effects of these cytokines are both time and concentration dependent. This cytokine treatment also promotes a 4-fold increase in Tsg-6 expression, a gene whose product is associated with improved wound-healing and immunosuppressive features [128,129].

MSCs have prolonged skin graft survival in animal studies altering the lymphocyte reactivity to allogenic target cells and tissues showing potential immunoregulatory features that can be used in tissue regeneration [105].

Other

New techniques for processing MSCs and new clinical applications of MSCs are being constantly introduced. In the realm of tumor inhibition, The potential of MSCs from heart stroma to inhibit proliferation of tumor cells is being explored [12,130]. Amniotic MSCs have been explored as new ideas for targeted ovarian cancer therapy [131,132]. Animal models have also explored the role of MSCs in treating chronic obstructive pulmonary disease (COPD) [133,134]. Recent human trials have shown MSCs to be promising in a multitude of ways. Other ways MSCs are being studied are for their potential in ameliorating heart failure in LVAD recipients, myocardial infarction, and

stroke [135-138]. MSCs have demonstrated a capacity that might repair injured organs including the central nervous system and peripheral nerve injury [139-141].

Challenges and Future Directions

The heterogeneity of MSCs represents a challenge when attempting to generalize findings from different investigators, and the different outcomes that occur when derived from different sources, the differences in culture conditions plays a critical impact in their behavior and performance in translational applications [50,52]. The pathway of how MSCs affect and react with host tissues is unknown, being a living therapy that can replicate, migrate, undergo cell cycle, differentiate, alter their phenotype and interact with the host microenvironment, important changes regarding their therapeutic potential and efficacy could be altered [99].

Translating these in vitro approaches to in vivo models, and animal studies into human clinical trials, has produced unexpected results leading to new paradigms in the scientific and clinical community. One of the potential adverse outcomes that have been poorly elucidated is ectopicity. Transplantation of bone marrow mesenchymal stem cells (BM-MSC) is promising for tendon regeneration, but after the injection of BM-MSC, bone tissue formation was seen in the tendon tissue repair in rabbits [142].

While the mechanisms through which MSCs impact host biology are largely known from animal studies, their impact on human biology and physiology has only been characterized functionally. Currently there are 546 studies in ClinicalTrials.gov mentioning “Mesenchymal stem cells”, and hopefully future results might more fully elucidate therapeutic potential of MSCs. As such, after recent clinical trials involving systemic infusion therapy, in which only a small percentage of the infused MSC (<1%) reached the target sites, concerns emerged regarding the fate of MSCs following treatment [143]. Other sites of cell deposition were seen in capillaries within the liver, spleen and lung capillary beds. The long-term effects of these retained cells, whether beneficial or deleterious, have yet to be determined. Future methods of engineering MSCs to have more selective homing to target tissues, promoting their transmigration and longevity may enhance their bio-availability, thus boost the efficacy and cost-efficiency of such therapy [99,144,145].

Conclusions

Over the past several decades the promising qualities of MSCs have been explored vastly. Our deepened understanding of their innate ability to maintain tissue micro-environments and homeostasis and their multi-lineage progenitor capacity [45] can bring tangible alternatives for the treatment of an extensive number of medical conditions [146-148]. Further study into their regenerative and immunologic qualities may reveal new modalities for

tissue engineering. The use of MSCs has exploded since their discovery many decades ago. Yet, the more we learn the more we realize that the delicate interplay of the MSC and its microenvironment is not one dimensional but multidimensional. Further, the source originates from also impacts their ultimate function. This field, which originated from the observations of a unique population of cells adhering to plastic has blossomed into therapeutic potentials to cure some of most debilitating diseases. More work and efforts, though, need to be made in order to continue this trajectory and fully realize the potential MSCs hold for human health and the treatment of previously untreatable diseases.

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