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Review Article

Mesenchymal Stem and Progenitor Cells: Problems, Potential and Promise

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Abstract

Mesenchymal Stem and Progenitor Cells (MSCs) are a heterogeneous population of cells, which can self-renew and differentiate into bone, adipose and cartilage in culture. Initially isolated from bone marrow aspirates 40 years ago, cells with MSC-like features have since been identified in various non-hematopoietic tissues within a putative perivascular niche. Different tissue sources and culture conditions for isolation and expansion of both human and mouse MSCs yield distinct subpopulation of cells with potentially different potency. Despite attempts to characterize the MSC subpopulations from different tissues, the lack of specific phenotypic markers and rigorous potency assays to distinguish MSCs functionally presents a significant challenge in the field. In the clinic, these cells show great potential for skeletal therapy, enhancement of Hematopoietic Stem Cell (HSC) engraftment and have been used extensively for various therapeutic applications based on their ability to repair the damaged micro environment and modulate inflammatory reactions in damaged tissues. However, the need to develop rigorous assays is crucial to better understand MSC mediated repair mechanisms and to further elucidate the potency and safety of MSCs in transforming regenerative medicine.

Keywords: Stem and progenitor cells; HSC; MSC

The Bone Marrow Stroma and MSC Identity

The Bone Marrow (BM) stroma contains a heterogeneous population of cells, including endothelial cells, fibroblasts, adipocytes and osteogenic cells. It was initially thought to function primarily as a structural framework upon which hematopoiesis occurs [1]. Later evidence demonstrated, however, that at least two distinct stem cell populations reside in the bone marrow of many mammalian species: Hematopoietic Stem Cells (HSCs) and Mesenchymal Stem Cells (MSCs), with the latter responsible for the maintenance of the non-hematopoietic bone marrow cells. MSCs, also termed multipotent marrow stromal cells or mesenchymal stromal cells, are a heterogeneous population of plastic-adherent, fibroblast-like cells, which can self-renew and differentiate into bone, adipose and cartilage in culture [2-5].

In the late 1960s, Friedenstein and colleagues established that single cell suspensions of BM aspirates could generate colonies of adherent fibroblast-like cells *in vitro*. These Colony-Forming Unit-Fibroblasts (CFU-Fs) were capable of osteogenic differentiation, and these studies provided the first evidence of a clonogenic precursor for cells of the bone lineage [6]. The CFU-F assay is now widely used as a functional method to quantify the frequency of stromal progenitors present in primary tissue samples [7,8] and may also be used as a means to assess the clonogenicity of culture expanded cells. Functional *in vitro* characterization of the stromal compartment by Dexter in the 1970s then revealed its importance in regulating the proliferation; differentiation and survival of HSCs [1]. CFU-F initiating cells *in vivo* have been shown to be quiescent, existing at a low frequency in human bone marrow [9].

Although MSCs were first described within the bone marrow compartment, cells with MSC-like characteristics have been isolated from a variety of fetal, neonatal and adult tissues, including cord blood, peripheral blood, fetal liver and lung, adipose tissue, compact bone, dental pulp, dermis, human islet, adult brain, skeletal muscle, amniotic fluid, synovium, and the circulatory system [10-18]. Given this broad tissue distribution the focus of much recent research has been upon defining the cellular identity of the MSC as it exists *in vivo*. Mesenchymal cells have been shown to express CD146 and lack CD34, a phenotype which readily identifies pericytes in various tissues.

Pericytes are cells that closely encircle endothelial cells in capillaries and micro vessels in multiple organs [19-26] and are thought to stabilize blood vessels, contribute to tissue homeostasis under physiological conditions, and play an active role in response to focal tissue injury through the release of bioactive molecules with trophic and immunomodulatory properties [25].

An extensive study by Crisan and colleagues has attempted to established links between MSCs and pericytes by validating the phenotype of pericytes as $CD146^+$, $NG2^+$, $PDGFR^+$, ALP^+, $CD34^-$, $CD45^-$, vWF^- and $CD144^-$ throughout human fetal and adult organs [26]. It has been reported that Pericytes and MSCs share several common markers such as CD105, CD73, CD90, CD44, CD10 and CD13. Accumulating evidence indicates a perivascular location for these MSC-like cells in all tissues, implying that all MSCs are pericytes [19]. Evidence to the contrary is provided by functional studies investigating the ability of various MSC populations to support endothelial tubular networks on matrigel. Only CD146+CD34- BM derived MSCs and their progeny, not CD146- MSCs displayed a pericyte function despite the progeny of the CD146- MSCs expressing

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CD146 once cultured [27]. These data suggest that pericytes may represent a subpopulation of the total pool of assayable MSCs at least within the bone marrow.

Despite their shared markers and perivascular location *in vivo*, more evidence is required to prove that MSC-like cells in every tissue are derived from or indeed function as pericytes. A recent study by Corselli and colleagues identified a second cell type, adventitial cells (cells lining the outermost layer of all blood vessels except capillaries) as precursor of MSCs, suggesting pericytes are not exclusively precursors of MSCs [28]. Both pericytes and adventitial cells contain subsets of multipotent precursor cells natively expressing mesenchymal markers as well as sharing differentiation potential with mesenchymal cells, indicating these cells as possible *in vivo* counterparts of MSCs obtained in culture from diverse tissues [29-31]. However, the precise link between pericytes/adventitial cells and MSCs remains elusive. Recent studies utilizing co-culture models have begun to examine the relationship between MSC and Endothelial Cells (EC). Short term contact co-cultures combined with an RNA-seq approach revealed a significant upregulation of key angiogenic genes potentially mediated via MSC-secreted cytokines including IL-1β and IL-6 modulating NF-κB signaling [32]. MSCs may also adopt a smooth muscle fate as a result of co-culture with ECs with myocardin, a master regulator of smooth muscle gene expression, shown to be upregulated.

Phenotypic and Functional Properties of Human and Mouse MSCs

Human MSC

As no unique cell surface marker allowing for prospective MSC isolation has been reported, the study and characterization of human MSCs is thus heavily reliant on their ability to adhere to and subsequently proliferate on tissue culture plastic. Culture selection is often used in combination with Ficoll™ separation and/or pre-enrichment using various cocktails of antibodies [33]. Some of the first studies to successfully isolate enriched populations of MSCs from human BM aspirates utilized a STRO-1 monoclonal antibody in conjunction with antibodies against VCAM-1/CD106, [34] and later CD271, [35] D7-Fib [36] and CD49a [37]. Other molecules reportedly co-expressed by $CD271^+$ MSCs include PDGFR-α, HER-2/ErbB2 (CD340) and frizzled-9 (CD349) [38]. However, not all cells expressing these markers are MSCs as flow cytometric isolation using specific phenotypes yields heterogeneous populations varying in their clonogenicity.

Mesenchymal stem cells are also variously reported to express SH2 (CD105), SH3/SH4 (CD73), CD29, CD44, CD90, CD71, CD106, CD166, STRO-1, GD2, and CD146 [4,24,25,34,39-44]. Despite years of research and a large number of reported cell surface markers there is no consensus regarding the MSC phenotype, likely due to the broad variety of potential tissue sources and the differences in cell isolation and cell culture procedures used? In addition, differences in media formulations (FBS, platelet lysates, growth factor combinations), plating density and oxygen tension may affect the gene profile, epigenomic state and phenotype of the mesenchymal population once these cells have been cultured [45]. In an attempt to standardize the definition of an MSC, the International Society for Cellular Therapy (ISCT) proposed the concept of essential minimal criteria for MSCs in culture. The four minimal defining criteria for MSCs are: i) adherence to plastic under standard tissue culture conditions ii) expression of CD105, CD73, CD90 iii) lack of expression of CD45, CD34, CD14/CD11b, CD79a/CD19 and HLA-DR surface molecules and iv) differentiation into adipocytes, osteoblasts and chondroblasts *in vitro* [46].

The current ISCT criteria are limited to the definition of Human BM-derived MSCs and may not be applicable to MSCs derived from other tissues. The negative markers in the ISCT definition are primarily specific for hematopoietic cells which comprise the vast majority of the marrow and are the major contaminating cells in BM derived stromal cultures which may not be true for MSC cultures derived from other tissues. It may also be possible that MSCs from alternate tissue sources may differ in phenotype from their marrow resident counterparts. For example, Traktuev and colleagues identified a multipotent CD34 + population derived from the adipose stromal vascular fraction which shared pericyte and MSC surface markers [47]. This is supported by a publication referring to the Stromal Vascular Fraction (SVF) of the adipose tissue and the adipose tissue-derived stromal cells as a population identified phenotypically as CD45-CD235a-CD31-CD34+ [48]. The Expression of CD34 by Adipose-derived stromal and Stem Cells (ASC) observed at the time of isolation from primary adipose tissue may then be lost following extensive culture *in vitro*. Further characterization revealed that ASCs expressed markers in common with other mesenchymal stromal/stem cells populations including CD90, CD73, CD105, and CD44 and remained negative for CD45 and CD31. ASC can be distinguished phenotypic ally from BM-derived MSCs by their positivity for CD36 and lack of CD106.

Similar to expression of CD34 on freshly isolated ASC, we and others [33,35,36] have evidence of freshly isolated BM-MSCs expressing low levels of CD45 antigen, which makes the use of CD45 antibody as a negative selection marker for isolating MSCs from fresh BM inefficient. Once in culture, BM-MSCs rapidly lose their CD45 expression, the significance of which is unknown.

The phenotype of MSCs varies not only between tissues but also between species and may also be affected by culture conditions. Differences in cell surface phenotype were reported between populations of human MSCs cultured at 21% O2 as defined by Pittenger et al, [4] and the Marrow Isolated Adult Multilineage Inducible (MIAMI) cells cultured in low oxygen tension as described by D'Ippolito et al [49]. The MIAMI cells were shown to have increased expression of primitive cell markers such as SSEA-4 when cultured at low pO2 compared to the same cells cultured at 21% pO2. A recent study showed that CD146+ Human Umbilical Cord Perivascular Cells (HUPVCs) cultured under hypoxic conditions displayed both increased cell proliferation and colony-forming efficiency however, their differentiation towards the osteogenic lineage was reduced compared to cells cultured in normoxia. It was proposed that hypoxia reduced binding of PPARg to the HIF2a promoter region which ultimately up regulates Oct-4 (a downstream target of HIF2a), a critical gene controlling self-renewal of CD146+ HUPVCs [50]. These studies highlight the importance of standardizing not just a MSC phenotype but also the conditions under which these cells are cultured.

Mouse MSCs

Various phenotypes for mouse MSCs have been proposed, with little or no overlap with human MSC phenotypes. Short and Simmons' extensive study of mouse CFU-F enrichment identified the femoral compact bone as a richer source of progenitor cells than the marrow plug within it. By performing the CFU-F assay on single cell suspensions depleted of hematopoietic cells, this group reported a CFU-F frequency of 2689 ± 58 CFU-F/106 cells in compact bone, compared to 102 ± 80 CFU-F/106 cells in mouse BM [51]. They then used multiparameter Fluorescence-Activated Cell Sorting (FACS) to identify a sub-population of proposed stromal (mesenchymal) precursors with the composite phenotype Lin-CD45-CD31-SCA1$^+$ [52]. However, an alternate population of primitive mesenchymal cells derived from adult mouse BM that express Stage-Specific Embryonic Antigen-1 (SSEA-1) was characterized by Bonnet's group [53]. The SSEA-1$^+$ population demonstrated extensive differentiation potential, forming astrocyte-, endothelial- and hepatocyte-like cells *in vitro*, and was found in the putative mesenchymal compartment *in vivo*, comprising about 0.04% of the total Lin-/CD45-/CD31- fraction of adult mouse BM [53]. In addition, Suire and colleagues defined another phenotype for BM-derived mouse MSCs obtained from enzymatic ally digested marrow plugs at a frequency two orders of magnitude higher than observed in BM harvested by conventional methods [54]. These cells are found in the stromal vascular-fraction and may be isolated prospectively using the composite phenotype Lin-PDGFRαβ$^+$.

A distinct subset of mouse perivascular cells (nestin+) was found to regulate murine Hematopoietic stem cell maintenance as ablation of this nestin+ MSCs cells significantly reduced the number and homing ability of HSCs [55]. A direct role for perivascular cells in regulating HSCs was examined using a SCF knock-in mouse model by selectively silencing c-kit ligand expression in Leptin-receptor positive cells surrounding BM blood vessels which significantly reduced the frequency of long-term reconstitution of HSCs [56].

Like many other cell types, [57-61] mouse MSCs demonstrate enhanced proliferation and optimal clonogenicity when cultured in a hypoxic environment (Brenton Short, unpublished data) [52]. It has also been reported that increased proliferation of rat bone marrow-derived MSCs at 5% oxygen is most likely due to increased expression of Hypoxia Inducible Factor (HIF), which in turn upregulates genes involved in metabolism, cell proliferation and survival [61,62]. Culture in low oxygen conditions thus appears to be a critical factor in the *in vitro* expansion of mouse mesenchymal cells.

MSC: A question of definition within non hematopoietic tissues

As well as lacking a consensus phenotype by which to identify putative MSCs, the inability to phenotypically distinguish between MSCs of differing potency either from primary tissues or in culture hinders the ability to identify more primitive from mature cells and precludes the analysis of different subpopulation of MSCs in culture. All cultured MSCs are the progeny of the rare cells responsible for generating CFU-F in the primary culture and as such likely posess a wide range of proliferative and differentiative capabilities when analyzed at the clonal level. This heterogeneity is in large part masked by the nature of the culture conditions; the cells capable of more rapid division maintain the cultures and are selected for over time such that a population of passaged MSCs may be more oligoclonal than a true representation of the possible cellular diversity.

Accordingly, the phenotypes used to isolate MSC from primary tissues do not necessarily predict MSC function particularly when related to their differentiation potential. In culture, CD73, CD105 and CD90 continued to be highly expressed on human BM-derived MSCs from early to late passages (P3 to P9) during which time the same cell population showed significant reduction in their differentiation potential after Passage 5, particularly when cultured in serum-containing medium (unpublished data).Researchers have also reported poor correlation between the broad differentiation potential of the MSCs *in vitro* and the function of these cells *in vivo*. For example, Adipose derived MSCs were reported to exhibit inferior osteogenic potential to that of Bone marrow MSCs *in vitro*, however the *in vivo* studies were more controversial [37]. Similarly, MSC populations derived from umbilical veins [63] and dermis (Daniel Blashki and Brenton Short, unpublished data) failed to generate ossified tissue when transplanted into an *in vivo* bone forming model, whereas their BM derived counterparts displayed robust osteogenic potential.

It is possible that MSC populations derived from different primary tissues may have intrinsic differences in their capacity to differentiate into the various mesenchymal lineages despite exhibiting identical phenotypes *in vitro*. One possible explanation for this was provided by Ackema and Charite' who investigated whether Hox genes, master regulators of regional specification and organ development, may play a role in the tissue-specific properties of MSC [64]. Hox gene expression profiles were generated from clonal CFU-F populations derived from various non-hematopoietic tissues including lung and thymus as well as BM from the sternum, forelimbs and hind limbs, and the relatedness of these profiles analyzed using hierarchical cluster analysis. This analysis revealed that CFU-F have heterogeneous Hox signatures that are highly specific for their anatomical origin, and that this topographic Hox specificity is maintained during differentiation. Similarly Sági and colleagues showed that not only were Hox genes differentially expressed, but MSC maintained expression of a subset of transcription factors characteristic of the tissue from which they were isolated, with thymus-derived cells expressing Tbx5 and Pitx2, spleen derived cells Tlx1 and Nkx2.5, femoral BM cells Pitx1 and aortic wall derived MSCs expressing En2 [65]. These data suggest that Hox proteins and other transcription factors play a role in specifying the cellular identity of an MSC and may affect the biological properties of tissue specific populations of MSC long after the removal of these cells from their tissue of origin. This innate cellular identity may have implications in the clinical use of MSC should cells from various tissues have different immunomodulatory or differentiative function depending on their origins. More rigorous investigation of the properties of MSC from various tissues is needed to further elucidate the differences in potency of cells which, despite appearing phenotypically indistinguishable, may in fact be functionally very different.

Some insight into potential methods to distinguish between phenotypically identical cells was provided by a recent publication examining the physical characteristics of MSCs. The authors

identified a set of biophysical markers predictive of a multi-potent MSC subpopulation in cultures derived from fetal and adult BM. The three biophysical markers including small cell diameter, low cell stiffness and high nuclear membrane fluctuations together were able to identify multipotent stem cells from committed osteochondral progenitors [66]. It is possible that a combination of biophysical traits can be used in conjunction with standard Immunophenotypic analysis to give further insight into MSC biology and may lead to reproducible methodologies to separate primitive cells from committed and differentiated cells within the same cultures.

Assays to define MSCs

Unlike the HSC field where multiple rigorous assays are available (eg.NOD-SCID, LTC-IC, CFC) to analyze the different function and maturation stage of distinct stem and progenitor cells of HSCs, the MSCs field is lacking stringent assays to demonstrate self-renewal and multipotency derived from single cells and stemness properties through serial transplantation experiments *in vivo*. Most researchers currently characterize a heterogonous pool of MSCs derived from high plating density cultures which do not accurately reflect cell behavior at a clonal level.

More stringent studies utilizing clonally derived MSC populations may enable the identification of sub-populations of cells better able to maintain the characteristics required of a stem cell population. Similarly these potency assays could help increase the efficacy and safety of cell therapies utilizing large numbers of culture expanded cells.

Generation of gene and protein expression databases of MSCs from diverse MSCs tissue, donor and culture conditions may provide knowledge of the heterogeneity of MSC subpopulations (FDA Voice, 2014). Transplantations of heterotopic ossicles can serve as an assay to show the intrinsic capacity of cells to generate specific tissue in animal reflecting the functional capacity of MSCs *in vivo* [60]. Should serial transplantation of MSCs derived from a primary ossicle prove to be feasible and reproducible, the question of MSC self-renewal may finally be answered. The use of animal models is essential to assess the efficacy of MSCs *in vivo* and together with rigorous *in vitro* assays the true nature and function of the MSC may finally be unraveled.

Therapeutic Potential of MSCs

MSC in the Clinic: bench to bedside

It is still poorly understood how mesenchymal cells repair damaged tissues *in vivo*. Recent evidence suggests that repair is achieved using paracrine factors released by mesenchymal cells, rather than by the Trans differentiation of mesenchymal cells into specific tissue cell types. Paracrine secretion by MSCs has been reported to support tissue repair by promoting neovascularization and increasing angiogenesis. For example, exosome purified from culture medium conditioned by human ESC-derived MSCs was recently identified as the active compound for reducing infarct size in pig and mouse models of myocardial ischemia [68]. Other ongoing studies examining the efficacy of transplanted mesenchymal cells in animal models of myocardial infarction [69], lung injury [70], kidney damage [71] and neurological diseases [72] may provide further insight into mechanisms underlying MSC-mediated tissue repair. Understanding the biology and the role of different mesenchymal cell subpopulations in tissue repair will be key to determining their potential for various therapeutic applications.

In recent years, over 400 clinical trials worldwide have used MSCs to treat various diseases (www.clinicaltrials.gov) [74]. Most trials are currently in Phase I/II for various diseases such as heart disease, diabetes, cancer, bone/cartilage, neurological and immune-related disorders. MSCs are attractive candidates for cell therapy, being: 1) easy to isolate and expand in culture, 2) able to home to sites of inflammation 3) able to differentiate into multiple mesodermal cell types, 4) immunomodulatory, 5) low in immunogenicity, 6) able to confer cytoprotection by secreting a broad spectrum of cytokines and growth factors.

MSC homing

MSCs are able to migrate to site of inflammation under diverse pathological conditions which makes these cells attractive for therapy. Homing of MSCs to injured or inflamed tissues depends on many signals including growth factors, chemokines and interleukins secreted by damaged cells and the microenvironment. Chemokines such as CCR2, CCR3, and CCR4 have been implicated in supporting MSC migration. For example murine MSCs could home to lung and reduce inflammation in lung tissue of mice exposed to bleomycin in and improve fibrotic effects in response to injury [70]. Transplanted adipose tissue derived MSCs- were able to migrate to injured muscle tissue and ameliorate muscular dystrophy in MDX mice [75].

A recent study by Park and colleagues investigated the chemo tactic response of BM-MSCs toward chemokines in an *in vivo* model of articular cartilage repair [76]. MSC migration was increased *in vitro* by MIP-3a and IL-8 and *in vivo* IL-8- and MIP-3-containing scaffolds enhanced tissue regeneration of an ostechondral defect site through increased recruitment of MSCs to the transplanted scaffolds. MSCs were also shown to express CCR2, CCR4, CCR6, CXCR1, and CXCR2 and to upregulate expression of these molecules following treatment with pro-inflammatoy cytokines, suggesting that not only do MSC themselves migrate to damaged tissues but they may also play a key role in attracting other immune cells.

Differentiation potential of MSCs

It has been extensively documented that under specific stimuli, cultured MSCs can differentiate into mesodermal cell types (e.g. adipocytes, osteoblasts, chondrocytes). However, accumulating evidence has revealed that the tissue of origin impacts the differentiation potential of derived MSCs [67,77]. The stem cell niche could thus be a determining factor for stem cell relf-renewal and lineage differentiation potential and moreover, the same perivascular niche in different tissues may impart different characteristics on cells extracted from a particular tissue. MSC-like cells derived from synovium were shown to be superior for chondrogenic potential compared to MSCs derived from bone marrow [78]. This information is particularly relevant when designing a therapy to stimulate resident progenitors for repair in articular cartilage. Isolation of MSCs from a particular tissue can yield distinct subsets of MSC populations with diverse differentiation potential. A recent study by Harrington and colleagues examined the differentiation potential of clonally derived MSC populations from Dental Pulp (DP) and BM [79]. All BM derived MSC clones were shown to be capable of generating osteogenic, adipogenic and chondrogenic lineages whereas DP

derived clones were all capable of osteogenesis but were restricted in their chondrogenic and adipogenic capacity. Another study comparing BM-MSCs to DP- MSCs also reported less differentiation ability into the adipogenic lineage but stronger differentiation into osteogenic lineage of DP–MSCs compared to BM-MSCs [80], a finding supported by a recent study showing DP derived MSC to be superior to both adipose and BM MSC in their osteogenic differentiation capacity [81].

The apparent ability of MSCs to give rise to cells of multiple germ layers, however, must be examined with care, as undifferentiated mesenchymal cells from human adipose tissue, skin, periodontal ligament and dental pulp tissues have been shown to spontaneously express neural [82] as well as smooth muscle cell markers in culture [83].

The effect of tissue-specific origin of MSCs on their differentiation potential into specific lineages thus may need to be taken into consideration for cell therapy applications.

Bone repair

Devine et al. first showed that cultured mesenchymal cells could home to the bone marrow in non-human primates [84]. One study has also shown that culture-expanded MSCs can persist and contribute to de novo bone formation *in vivo*. Eight weeks after MSCs were placed into a porous cylinder and implanted into a rat femur, the implant-containing defect healed completely [85,86]. However, culture-expanded MSCs are unable to home to osteogenic sites. Two different methods for overcoming this challenge have been explored: 1) peptidomimetic ligands for ß1 integrin on the MSC surface, coupled to a bisphosphonate to facilitate migration of transfused MSCs to the bone surface [87], and 2) RNAi against Ckip-1, a negative regulator of osteogenesis that targets RunX2 for degradation [88], to bone surfaces using AspSerSer6 liposomal targeting moieties . The latter method was the first to provide a means of facilitating bone formation without concomitant osteoclast activation and bone resorption. These two methodologies may provide alternate and complementary strategies for enhancing bone formation in a clinical setting by facilitating both the homing of transplanted MSCs to osteogenic sites, and by enhancing the osteogenesis of resident osteogenic lineage cells at bone forming surfaces.

Cultured allogeneic human mesenchymal cells have also been used in clinical trials for the treatment of children suffering from osteogenesis imperfect [89]. The first year after MSC engraftment, patients showed improvement as measured by reduced incidence of bone breakages, effects that however declined with time. The decline could have been caused by senescence of the culture-expanded cells or by terminal differentiation during cell culture and passaging [90], which may be related to epigenetic changes of mesenchymal cells during prolonged culture [91]. It is also possible that achieving sustained and measurable improvement in a systemic disease affecting bone, a tissue in which cell and tissue turnover is low compared to most soft tissues, requires repeated infusions of large numbers of cells. Recent innovative cell targeting strategies may enable improvements in the efficacy of MSCs in treating skeletal disorders.

Cellular allografts containing MSCs have been used in high risk foot and ankle surgery for bone reconstruction purposes, resulting in improved healing and interval to partial weight bearing [92]. A serial transplantation experiment using fluorescently tagged MSCs showed MSCs were able to localize to areas of bone injury regardless of their administration route [93].

Immunomodulatory effect of MSCs

One property of MSC that greatly increases their value in a clinical setting is their ability to modulate immune responses. The immunosuppressive activity of MSCs is poorly understood but recent reports provide some mechanistic insights into key regulatory molecules. Programmed death-ligand 1 (PD-L1)/CD274 also known as B7 Homolog 1 (B7-H1) has been shown to be expressed in cultured MSCs and is strongly upregulated following IFN-ϒ stimulation. Combination therapy using rapamycin and MSCs induced immune tolerance to allografts, but monoclonal antibodies against B7-H1 were shown to abrogate this tolerance leading to allograft rejection [94]. The immunomodulatory effects of MSC were show to be mediated in part through upregulation of regulatory immune cells including CD4+CD25+FoxP3+ T cells and tolorogenic dendritic cells and a decrease in alloantibody levels.

MSC expressed B7H1 may also induce the apoptosis of activated T-cells as co-culture of CD4+CD25- T cells with MSCs resulted in significant upregulation of Programmed cell Death-1 receptor (PD-1) on activated T cells [95]. Similar results were reported by Chinnadurai who further examined the role of IFN-ϒ in the 'licensing' of MSCs to inhibit the proliferation of activated T cells [96]. Both MSCs and IFN-ϒ licensed MSCs inhibited T-cell proliferation, however only IFN-ϒ licensed MSCs significantly inhibited Th1 cytokine (IFN-ϒ, TNFα and IL-2) production as well as T-cell degranulation. This IFN-ϒ licensed MSC inhibitory effect on T cells is thought to be dependent on Indoleamine 2,3-Dioxygenase (IDO), however Chinnadurai showed that MSC IDO catalytic function is dispensible with regard to MSC driven T-cell inhibition and identified the B7-H1 PD1 pathways as essential effectors in blocking T-cell function. Further complexity was also suggested by a recent report that IFN-ϒ treatment of MSC upregulated HLA-DR /Class II MHC after 48 hours and MSCs ability to inhibit T cells through B7-H1 was dependent upon the presence of HLA-DR [97].

A novel mechanism for MSC induced immunosuppression was recently proposed by Obermajer and colleagues who showed that cells of the Th17 type, predominantly associated with the rejection of allogeneic solid organ grafts, can be directly converted into a regulatory T cell (Treg) type [98]. The induction of Tregs was preceded by development of a CD11b (hi)Gr1(int) myeloid-derived immunosuppressive cell mediated Th17 response. They identified retinoic acid receptor-related orphan receptor γ as a common factor in the differentiation of Treg and Th17 cells. Treatment of enriched IL-17A (+) cells from MSC-primed allograft mouse recipients with the immunosuppressant mycophenolate mofetil reduced IL-17A production and increased the Foxp3 (+) Treg cell fraction. The identification of specific subset of T cells, IL-17A(+) Foxp3(+) double-positive and ex-IL-17-producing IL-17A(neg)Foxp3(+) in this paper argues for direct conversion as the mechanism for MSC-mediated immunotolerance. This proposed mechamism where MSCs –induced myeloid –derived immunosuppressive cells act as mediator for immunetolerance without complete immunosuppression may have significant implication for therapeutic application.

The importance of species variations related to immunosuppression mechanisms by MSCs are sometimes overlooked. For example, Immunosuppression by human -derived MSCs is mediated by Indoleamine 2,3-Dioxygenase (IDO), whereas mouse MSCs is mediated by nitric oxide [99]. When the expression of IDO and inducible nitric oxide synthase (iNOS) were examined in human and mouse MSCs after stimulation with their respective inflammatory cytokines, human MSCs expressed extremely high levels of IDO, and very low levels of iNOS, whereas mouse MSCs expressed abundant iNOS and very little IDO. Thus, studies of MSC-mediated immunomodulation in mice may not be informative in the setting of human disease.

HSC engraftment: MSCs appear to be extremely sensitive to chemical and radiation-induced damage, and remain at a significantly lower frequency after exposure [100]. Transplantation may perturb the ability of MSCs to regulate hematopoietic cells, which would explain the slow and skewed recovery of many immune cell populations [101].

Co-transplanting MSCs with HSCs could thus enhance long-term HSC engraftment, as has been demonstrated by in utero co-transplantation of fetal sheep with human bone marrow stromal cells and human HSCs [102]. MSCs may also prevent the onset of immune cell-induced Graft-Versus-Host Disease (GVHD) following transplantation [103], as cultured MSCs do not express MHC-class II antigens on their cell surface and can suppress a primary mixed lymphocyte reaction. When MSCs cultured in both FBS- and platelet lysate-based media were given to patients with chronic and acute GVHD, half of the patients responded positively, with pediatric patients faring best [104]. In one clinical trial, the infusion of MSCs into 8 patients with steroid-refractory grade III - IV acute GVHD even resulted in the complete disappearance of GVHD in 6 of 8 patients [105].

Despite the extensive use of MSCs in clinics and in many ongoing clinical trials, there is a lack of long term safety data examining the use of MSCs in humans. It has been reported that MSCs may variously exert an anti- or pro-tumor growth effect depending on the tumor type and its microenvironment [10-108]. Tumor formation in patients receiving MSCs has not been reported to date; however, the risk of potential tumorigenicity when MSCs are used in therapy was recently discussed [107]. Two possible scenarios include 1. Malignant transformation of the MSCs occurs (possibly as a result of extensive proliferation *in vitro* and resultant accumulation of genetic pertubations) 2. The immunesuppressive effect of MSCs which could enhance the growth of existing malignant cells of a patient. A third potential area of concern is the risk of MSCs contributing to neo-angiogenesis in developing tumors thus promoting enhanced tumor growth. The absence of a suitable *in vivo* model system which can completely rule out the risk of tumor formation is of concern.

Despite the challenges facing the field, the potential of MSCs to transform regenerative medicine is undeniable. From repair of skeletal maladies, to the treatment of GVHD, to their efficacy in abrogating the severity of myocardial infarcts, MSCs may one day be able to treat a broad range of debilitating conditions. Through rigorous studies and the development of novel assays this potential may soon be realized.

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