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Biology of the Bone Marrow Stroma

The bone marrow (BM) stroma contains a heterogeneous population of cells, including endothelial cells, fibroblasts, adipocytes and osteogenic cells. The primary function of the bone marrow stroma was originally thought to merely provide a structural framework upon which hematopoiesis occurs.[1] More recently, it has become clear that at least two distinct stem cell populations reside in the bone marrow of many mammalian species: hematopoietic stem cells (HSCs) and a population of stem cells responsible for maintenance of the non-hematopoietic bone marrow elements called mesenchymal stem cells (MSCs). Mesenchymal stem cells, also termed multipotent marrow stromal cells or mesenchymal stromal cells, are a heterogeneous population of plastic-adherent, fibroblast-like cells, which in culture are able to self-renew and differentiate into bone, adipose and cartilage tissue.[2-5] In the late 1960s, Friedenstein and colleagues performed the initial study on BM stromal cells, establishing that single cell suspensions of BM were able to generate colonies of adherent fibroblast-like cells when cultured in vitro. Furthermore, these colony-forming unit - fibroblasts (CFU-F) were shown to have the ability to undergo osteogenic differentiation, providing the first evidence of a clonogenic precursor for cells of the bone lineage.[6] The CFU-F assay is now used by many investigators as a functional method to quantify stromal progenitors, and the rarity of these cells is reflected by the low frequency of CFU-F in human bone marrow.[7,8] Unlike the properties of CFU-F in vitro, CFU-F initiating cells in vivo have been shown to be a quiescent population.[9] Further functional characterization of the stromal compartment by Dexter in the 1970s revealed its importance in regulating the proliferation, differentiation and survival of hematopoietic stem cells in vitro.[1] The morphology and cytochemical characterization of cultured stromal cells was further studied by Friedenstein and Castro-Malaspina in the 1980s, who described these cultured cells as sudan black$^+$, alkaline phosphatase$^+$, esterase$^-$, collagen IV$^+$, and fibronectin$^+$.[6,10]

Isolation of Mesenchymal Stem Cells

MSCs have traditionally been isolated from bone marrow. However, reports have detailed the isolation of cells with MSC characteristics from a variety of tissues including cord blood, peripheral blood, fetal liver and lung, adipose tissue, skeletal muscle, amniotic fluid, synovium and the circulatory system.[11-17] These are all vascularized tissues, and accumulating evidence indicates a perivascular location for these cells, leading to the suggestion that all MSCs are pericytes[18] which closely encircle endothelial cells in capillaries and microvessels in multiple organs.[18-25] An extensive study by Crisan and colleagues has established the links between MSCs and pericytes and validated the phenotype of pericytes as CD146$^+$, NG2$^+$, PDGFR$^+$, ALP$^+$, CD34$^-$, CD45$^-$, vWF$^-$ and CD144$^-$ throughout human fetal and adult organs.[25] Pericytes 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 release of bioactive molecules which have trophic and immunomodulatory properties.[26] The broad variety of tissue sources from which MSCs are isolated, in conjunction with disparate culture conditions, has led to a lack of consensus regarding the phenotype of the MSC. In addition, differences in the media formulations used to culture the cells, the plating density and the oxygen tension may affect the phenotype of the mesenchymal population. Table 1 summarizes the published phenotypes of the following cell populations: Mesenchymal stem cells as defined by Pittenger et al.,[4] Mesodermal Progenitor Cells (MPC) described by Verfaillie's group[27] and Marrow Isolated Adult Multilineage Inducible (MIAMI) cells cultured in low oxygen tension as described by D'Ippolito et al.[28] Despite the variation in reported phenotypes of mesenchymal subpopulations, it is widely accepted that the cultured cells, regardless of the methods employed in their isolation and culture, lack expression of prototypic hematopoietic antigens including CD45, CD34, CD11b and CD14. Mesenchymal stem cells have been reported to express SH2 (CD105), SH3/SH4 (CD73), CD29, CD44, CD90, CD71, CD106, CD166, STRO-1, GD2, and CD146.[4,23,24,29-35] Methodology employed in the isolation and enrichment of human mesenchymal stem cells is heavily reliant on the ability of these cells to adhere to and subsequently proliferate on tissue culture

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plastic. Indeed, culture selection is still widely employed as a means of MSC isolation. Pre-enrichment through cell separation strategies using cocktails of antibodies that deplete the bone marrow of specific cell populations (negative selection)[27,36] or Ficoll™ separation are the most widely used means of MSC isolation. No single, unique marker allowing for MSC isolation has been reported, but rather a range of composite cell surface phenotypes are utilized. Enriched populations of MSCs have been isolated from human BM aspirates using a STRO-1 monoclonal antibody in conjunction with antibodies against VCAM-1/CD106,[29] CD271,[37] D7-Fib[38] and CD49a.[39] A more recent study has also identified molecules co-expressed by CD271$^+$ MSC population including PDGFR-ß, HER-2/ErbB2 (CD340) and frizzled-9 (CD349).[40] These markers are not unique for mesenchymal stem cells, consequently not all cells expressing these markers are mesenchymal stem cells.

Mouse CFU-F enrichment has been extensively studied by Short and Simmons who identified the femoral compact bone itself as a richer source of progenitor cells than the marrow plug within it. This group reported the frequency of CFU-F in mouse compact bone as 2689 ± 58 CFU-F/10^6 cells compared to 102 ± 80 CFU-F/10^6 cells in mouse BM.[41] By using a number of physical and enzymatic treatments to generate a single cell suspension, followed by depletion of hematopoietic cell lineages, this group significantly enriched for CFU-F. Further cell separation based upon multiparameter fluorescence-activated cell sorting (FACS) identified a population of proposed stromal (mesenchymal) mouse precursors with the composite phenotype Lin$^-$CD45$^-$CD31$^-$SCA1$^+$.[42] A recent study by Bonnet's group[43] has identified and characterized an alternate population of primitive mesenchymal cells derived from the adult mouse bone marrow, based upon their expression of the stage-specific embryonic antigen-1 (SSEA-1). The SSEA-1$^+$ population was shown to have extensive differentiation potential, forming astrocyte-, endothelial- and hepatocyte-like cells in vitro. Moreover, they identified the SSEA-1$^+$ population in the putative mesenchymal compartment in vivo, which comprised about 0.04% of the total Lin$^-$/CD45$^-$/CD31$^-$ fraction of the adult mouse BM.[43]

The Effect of Oxygen Concentration on MSC Culture

Mouse mesenchymal stem cells have been shown to be highly sensitive to atmospheric oxygen tension, as a hypoxic environment facilitates optimal clonogenicity and cell proliferation (Brenton Short, unpublished data). Many cell types have also been shown to have enhanced proliferation when cultured with lowered atmospheric oxygen tension, including rat central nervous system (CNS) stem cells, adult mouse skeletal muscle satellite cells, human CD34$^+$ marrow progenitor stem cells and rat mesenchymal progenitor cells.[44-48] In rat bone marrow-derived mesenchymal progenitor cells, it was reported that the increased cell proliferation at 5% oxygen was most likely due to increased expression of hypoxia inducible

TABLE 1: Expression of Various Cell Surface Markers on Three Distinct Cell Populations, all of which demonstrate Multilineage Differentiation Potential

MARKER	MESENCHYMAL STEM CELLS	MPC	MIAMI CELLS
CD4	-ve	nd	nd
CD9	+ve	nd	nd
CD10	+ve	-ve	+ve
CD11a	-ve	nd	nd
CD13	+ve	+ve	nd
CD14	-ve	nd	nd
CD15	-ve	nd	nd
CD18	-ve	nd	nd
CD25	-ve	nd	nd
CD29	+ve	nd	+ve
CD31	-ve	-ve	nd
CD34	-ve	-ve	-ve
CD36	nd	-ve	-ve
CD38	nd	-ve	nd
CD44	+ve	+ve (low)	+ve
CD45	-ve	-ve	-ve
CD49a	+ve	nd	nd
CD49b	+ve	+ve	nd
CD49c	+ve	nd	nd
CD49d	-ve	nd	nd
CD49e	+ve	nd	+ve
CD50	-ve	-ve	nd
CD51	+ve	nd	nd
CD54	+ve	nd	-ve
CD56	nd	nd	-ve
CD58	+ve	nd	nd
CD61	+ve	nd	nd
CD62e	-ve	-ve	nd
CD62L	+ve	nd	nd
CD62p	-ve	nd	nd
CD63	nd	nd	+ve
CD71	+ve	nd	nd
CD73 (SH3 and SH4)	+ve	nd	nd
CD81	nd	nd	+ve
CD90	+ve	+ve (low)	+ve
CD102	+ve	nd	nd
CD103	nd	nd	+ve
CD104	+ve	nd	nd
CD105 (SH2)	+ve	nd	nd
CD106	+ve	-ve	nd
CD109	nd	nd	-ve
CD113	nd	nd	nd
CD117	-ve	-ve	-ve
CD119	+ve	nd	nd
CD120a	+ve	nd	nd
CD120b	+ve	nd	nd
CD121	+ve	nd	nd
CD122	nd	nd	+ve
CD123	+ve	nd	nd
CD124	+ve	nd	nd
CD126	+ve	nd	nd
CD127	+ve	nd	nd
CD140a	+ve	nd	nd
CD164	nd	nd	+ve
CD166	+ve	nd	nd
HLA class 1	+ve	-ve	-ve
HLA-DR	-ve	-ve	-ve
LNGFR	+ve	nd	nd
H1P12	nd	-ve	nd
Beta 2 microglobulin	nd	+ve (low)	nd
KDR	nd	+ve (low)	+ve (low)
Flt1	nd	+ve (low)	+ve (low)
Fibroblast surface antigen	nd	-ve	nd

nd = not described; +ve = positive; -ve = negative.

factor (HIF) which in turn upregulates genes involved in metabolism, cell proliferation and survival.[48,49] Culture in low oxygen conditions appears to be a critical factor in the culture-expansion of mouse mesenchymal cells.

The Potential of MSCs in Therapy

In recent years there has been increased interest in mesenchymal stem cells and their potential utility in both tissue engineering and repair. Animal studies have provided a useful tool for defining a number of diverse potential applications for MSCs. Devine et al. demonstrated that cultured mesenchymal cells could home to the bone marrow in non-human primates.[50] The ability of culture-expanded human mesenchymal cells to contribute to the functional repair of a skeletal defect has also been examined using a variety of animal models. MSCs repaired an 8 mm defect in a rat femur when placed into a porous cylinder which was then implanted into the bone. After 8 weeks, the defect containing the MSC-loaded implant completely healed, while the defects containing cylinders filled with control cell populations failed to heal successfully.[51,52] These studies indicate that culture-expanded MSCs are able to both persist and contribute to de novo bone formation in vivo.

Studies in sheep have addressed the possibility of using mesenchymal cells to enhance engraftment of transplanted hematopoietic cells. In utero co-transplantation of fetal sheep with human bone marrow stromal cells along with human HSCs enhanced long-term engraftment of human HSCs.[53] Transplantation of unprocessed whole bone marrow cells has been shown to restore microenvironmental function, suggesting that unprocessed bone marrow contains stromal precursors as well as hematopoietic precursors that contribute to hematopoietic regeneration following transplantation. Studies by Gallotto et al. demonstrate that these microenvironmental precursor cells, as measured by the CFU-F assay, are susceptible to damage following chemotherapy or radiation and they remain at a significantly reduced frequency for a considerable amount of time following transplantation.

This decrease in CFU-F number suggests that MSCs may be extremely sensitive to chemical and radiation-induced damage.[54] Given that osteoblasts are a crucial component of the HSC niche, it is likely that transplanted MSCs serve to reconstitute the hematopoietic microenvironment. Thus, the MSC function of regulating hematopoietic cells may be perturbed after transplantation, which may partly explain the slow and skewed recovery of many immune cell populations following transplantation.[55] Mesenchymal cells also exhibit immunomodulatory and anti-proliferative effects on T cells, an attractive feature for cell therapy. Cultured MSCs do not express MHC-class II antigens on their cell surface and they suppress a primary mixed lymphocyte reaction, which has prompted the question as to whether these cells can suppress ongoing immune cell-induced reactions in humans and hence be used for the treatment of graft-versus-host disease (GVHD).[56] Mesenchymal stem cells have been used in clinical trials to treat steroid-resistant acute GVHD. Infusion of MSCs into 8 patients with steroid-refractory grade III - IV acute GVHD resulted in the complete disappearance of GVHD in 6 of 8 patients.[57] Cultured allogeneic human mesenchymal cells have been used in clinical trials for the treatment of children suffering from osteogenesis imperfecta, and the results have demonstrated that MSCs could engraft in these children.[58] Patients showed improvement, as measured by reduced incidence of bone breakages, for about one year, however the beneficial effects declined with time. The decline could be caused by senescence of the culture-expanded cells or by terminal differentiation through the course of cell culture and passaging,[59] which may be related to epigenetic changes of mesenchymal cells during prolonged culture.[60] Several studies have provided evidence of a strong correlation between age and the proliferative potential exhibited by MSCs in vitro, with decreasing progenitor proliferation associated with increasing age.[7,8] Thus, the progenitor pool may be depleted following extensive proliferation. A decline in MSC function may therefore be important in several disease states[61,62] and may also explain the decrease in bone mass observed in the elderly.

Differentiation Potential of MSCs

Cultured mesenchymal cells have been shown to exhibit some unique properties that challenge the dogma that stem cells derived from adult tissue produce only the cell lineages characteristic of tissues in which they reside. Studies by Verfaillie's group have demonstrated the ability of cultured Multipotent Adult Progenitor Cells (MAPC) to differentiate into skeletal cells, cardiomyocytes, endothelial cells, smooth muscle and neural cells.[63] The apparent ability of MSCs to give rise to cells of multiple germ layers, however, must be examined cautiously, as undifferentiated mesenchymal cells have been shown to spontaneously express neural markers[64] as well as markers of smooth muscle cells. The mechanism by which mesenchymal cells repair damaged tissues in vivo is still poorly understood. However, recent evidence suggests that repair is likely achieved through paracrine factors released by mesenchymal cells rather than transdifferentiation of mesenchymal cells into specific tissue cell types.[65] Studies examining the efficacy of transplanted mesenchymal cells in animal models of myocardial infarction,[66] lung injury,[67] kidney damage[68] and neurological diseases[69] are ongoing, and may provide further insights into mechanisms underlying MSC-mediated tissue repair. It will be important to further characterize the biology of different subpopulations of mesenchymal cells and to better understand their role in tissue repair to determine the potential of mesenchymal cells in various cell therapy applications.

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