Mesenchymal stem cells and it's Characterization

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Abstract

Although acceptable for cell based therapeutic applications, a rigorous understanding of the Mesenchymal Stem Cells (MSC) requires a better definition of what an MSC is. Inspite of the fact that no unique markers are known for MSCs, many attempts have been made to develop a cell-surface antigen profile for the better purification and identification of MSCs, particularly important is whether MSCs isolated from different tissues are identifiable by the same immunophenotype. While the precise identity of MSCs remains a challenge, further understanding of their biological properties will be greatly advanced by analyzing the mechanisms that govern their differentiation potential. The ability of MSCs to differentiate into a variety of connective tissue cell types has rendered them an ideal candidate cell source for clinical tissue regeneration strategies. This review focuses specifically in the context of these advances in characterization of adult stem cells via selection of unique cell surface markers and regulation of lineage- specific differentiation of mesenchymal stem cells.

Keywords: Mesenchymal Stem Cells, Bone Marrow, Cellular Therapy, Immunologic Property.

Abbreviations: MSCs- Mesenchymal stem cells , BM – Bone marrow, ES – Embryonic stem cells, MHC – Major histocompatibility cells, CD – Cluster of differentiation.

Introduction

Mesenchymal stem cells or multipotent stromal cells (MSCs) are adult stem cells which have been isolated from almost every type of connective tissue (da Silva Meirelles et al., 2006). They can self-renew and under appropriate in vitro conditions have the capacity to differentiate into mesodermal, endodermal, and even ectodermal lineages (Lakshmipathy and Verfaillie, 2005). They evoke only minimal immuno-reactivity (Tse et al., 2003) and secrete bioactive factors with anti-inflammatory and immuno-modulatory effects in vivo (Caplan and Dennis, 2006). Hence, MSCs appear to have a remarkable clinical potential in tissue regeneration and immunoregulatory therapeutic applications.

It was Friedenstein and coworkers, in a series of seminal studies in the 1960s and 1970s, who demonstrated that the osteogenic potential, as revealed by heterotopic transplantation of BM cells, was associated with a minor subpopulation of BM cells. These cells were distinguishable from the majority of hematopoietic cells by their rapid adherence to tissue culture vessels and by the fibroblast-like appearance of their progeny in culture, pointing to their origin from the stromal compartment of BM. In addition to establishing BM stroma as the haystack in which to search for the proverbial needle, the work of Friedenstein and coworkers provided a second major breakthrough by showing that seeding of BM cell suspensions at clonal density results in the establishment of discrete colonies initiated by single cells (the colony-forming unit fibroblastic, CFU-Fs (Friedenstein et al., 1966). Combined with the timing of the isolation of human embryonic stem (ES) cells, the term mesenchymal stem cell (MSC), proposed previously as an alternative to "stromal" or "osteogenic" stem cell, gained wide popularity. To date, the best characterized source of MSCs is the bone marrow (BM) and most of the knowledge regarding MSCs is based on BM studies.

MSC have a great appeal for cell therapy and tissue engineering for numerous reasons:
1)   They are relatively easy to procure.

2) They expand rapidly in culture.
3) They show only minor spontaneous differenti- ation during ex vivo expansion.
4) They are multi potential.
5) They form supportive stroma for hematopoiesis.
6) They seem to be largely immunologically inert, paving the way for allogenic and xenogenic transplantation.
7) They are immunosuppressive.
8) They secrete numerous trophic factors which modulate inflammation, remodeling and apoptosis.

In contrast to other cell types that express specific cell surface markers such as hematopoietic cells (CD14, CD34, CD45) (Huss, 2000) and endothelial cells (CD31) (Hristov et al.,2003), the phenotypic identify of MSCs is not unique, sharing features of multiple cell lineages, including mesenchymal, hematopoietic, endothelial, epithelial, and muscle cells (Majumdar et al.,2000). In addition, efforts to characterize phenotypic features of MSCs have been confounded by the fact that MSCs display a variety of morphological characteristics and express various cell lineage-specific antigens that can vary between different preparations and as a function of time in culture (Huss, 2000).

International Society for Cellular Therapies (ISCT)

The MSC's derived from different tissues are immuno-phenotypically identical with only minor variations. The current consensus states that MSC do not express hematopoetic lineage markers. Based on the minimal criteria defined by International society for cellular therapies, (Dominici et al.,2006) MSC must express CD 105, CD 73 and CD 90 and lack expression of CD 45, CD 34, CD 14 or CD11b, CD 79 alpha or CD 19. The lack of knowledge of exclusive positive markers for MSC hampers the discrimination from other cell types, especially endothelial cells and fibroblasts. Furthermore MSC's are plastic adherent and should in vitro exert at least tri-lineage differentiation potential, ie. towards the adipogenic, osteogenic and chondrogenic lineages. In addition MSC may trans-differentiate in to other lineages as well. Any variation in isolation protocols and characterization strategies may result in the isolation and expansion of different sub-population of cells, or may change the characteristics of the cells. Given that MSC used in clinical trials are produced using a variety of different protocols, the results may not be interpretable or reproducible. An essential requirement

therefore is that all steps of MSC manufacturing from starting material up to potency testing in the intended indications have to be highly standardized to assure a required and reproducible cellular quality and potency.

Surface markers of MSC

Coating the surface of every cell in the body are specialized proteins, called receptors that have the capability of selectively binding or adhering to other "signaling" molecules. There are many different types of receptors that differ in their structure and affinity for the signaling molecules. Normally, cells use these receptors and the molecules that bind to them as a way of communicating with other cells and to carry out their proper functions in the body. These same cell surface receptors are the stem cell markers.

Positive markers: As part of the minimal criteria proposed by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy to define MSCs (Dominici et al.,2006), cells must be positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR. The identification of a definitive marker allowing the prospective isolation of MSCs from fresh tissue would be of the utmost importance. Stro-1 is by far the best-known MSC marker is not exclusive to these cells and is lost during culture (Kolf et al., 2007), so that it is not a general MSC marker. The cell population negative for Stro-1 is not capable of forming colonies (that is, it does not contain CFU-Fs). Recent studies have shown that stage-specific embryonic antigen 1 (SSEA-1) and SSEA-4 are markers for primitive mesenchymal cells in murine and human bone marrow (Anjos-Afonso et al.,2007; Gang et al.,2007). CD106, or VCAM-1 (vascular cell adhesion molecule-1), is likely to be functional in MSCs because it is involved in cell adhesion, chemotaxis, and signal transduction, and has been implicated in rheumatoid arthritis (Carter and Wiks, 2001). Fluorescence-activated cell sorting of human bone marrow mononuclear cells showed the expression of CD49b, CD105, CD90, CD73, CD130, CD146, CD200, and V/5 integrin and the expression of CD73, CD146. But CD200 was found to be down regulated during differentiation (Gang et al., 2007).

Negative markers: There is a consensus that MSCs do not express CD11b (an immune cell marker) (Pittenger et al.1999), glycophorin-A (an erythroid lineage marker) and CD45 (a marker of all hematopoietic cells) (Colter et al., 2001). CD34, the

primitive hematopoietic stem cell (HSC) marker, is rarely expressed in MSCs, although it is positive in mice (Honczarenko *et al*., 2006). CD31 (expressed on endothelial and hematopoietic cells) (Pittenger *et al*.1999) and CD117 (a hematopoietic stem/progenitor cell marker) (da Silva Meirelles *et al*., 2006) are almost always absent from MSCs. Currently, the thorn in the side of the MSC biologist is the lack of a definitive positive marker for MSCs; there is a myriad of reported positive markers, with each research group using a different subset of markers. Without a definitive marker, *in vivo* studies on cell lineage and niche are difficult.

Multilineage Differentiation Potential

The differentiation potential of MSCs into bone, cartilage and fat has been described and characterized by multiple laboratories (Barry *et al.,*2001; Muraglia *et al*., 2000; Pittenger *et al*.,1999). Osteogenic activation requires the presence of glycerol phosphate, ascorbic acid-2-phosphate, dexamethasone and fetal bovine serum .When cultured in monolayer in the presence of these supplements the cells acquire an osteoblastic morphology with upregulation of alkaline phosphatase activity and deposition of a calcium rich mineralized extracellular matrix.

Chondrogenic differentiation occurs when MSCs are grown under conditions that include (1) a three-dimensional culture format, (2) a serum-free nutrient medium and (3) the addition of a member of the TGF- super family . When these conditions are met the cells rapidly lose their fibroblastic morphology and begin to initiate expression of a number of cartilage specific extracellular matrix components. MSCs cultured in monolayer in the presence of isobutylmethylxanthine become adipocytes with the production of large lipid-filled vacuoles . Adipogenic differentiation of MSCs is induced by the nuclear receptor and transcription factor, peroxisome proliferator-activated receptor-gamma (PPAR-) as well as fatty acid synthetase.

5-Azacytidine induction of myogenesis was reported by Taylor and Jones for embryonic and adult cells (Taylor & Jones, 1982) and by Wakitani et al. for rat stromal cells (Wakitani, *et al*., 1995). Phinney et al.1999 found that exposure of mouse MSCs to amphotericin B, but not 5-azacytidine, resulted in the formation of multinucleated fibers resembling myotubes (Phinney and Prockop, 2007). Treatment of MSCs with isobutylmethylxanthine and dibutyryl cyclic AMP induced expression of early markers of neuronal differentiation (Deng *et al*., 2001). The

potential role of MSCs in blood vessel formation has also been evaluated. Enhanced neovasculari-genesis has been associated with regeneration of infracted myocardium by bone marrow derived stem cells (Orlic *et al*., 2001).

Immune privileged properties of Mesen-chymal stem cells

Stem cells are envisioned to be a major source for cell based therapies. Survival of transplanted cells correlates with the number of differences in major histocompatibility (MHC) antigens between donor and recipient that trigger T-cell responses and rejection of cells with disparate MHC profiles (Janeway *et al.*, 1999). The expression of MHC antigens on   tissues determines the outcome of alloantigen-specific T-cell responses in vitro and in vivo (Janeway *et al.*, 1999). Although most mammalian cells express MHC class I antigens, expression of MHC class II molecules are more restricted (Viret and Janeway, 1999).

Recently, unique immunologic properties of MSCs have been described, such as their poor immunogenicity in vitro and in vivo (Ryan *et al.*, 2005), inhibition of the proliferation and cytotoxicity of natural killer (NK) cells (Spaggiari *et al.*, 2008), inhibit the function of mature, naive and memory T-cells whereas increase the proportion of regulatory T-cells (di Nicola *et al.*, 2002), and suppression of B cell proliferation and differentiation (Corcione *et al.*, 2006). In addition MSC have been shown to be decreasing the tumor necrosis factor secretion and increase IL-10 secretion from dendritic cells (DC) , block the antigen-presenting cells (APC) maturation and these immature APC, in turn, secrete immuno-suppressive cytokine IL-10 (Oh *et al*.,2008). To date, the mechanism of MSC-mediated immuno-suppression has not been fully explained. Taking advantage of their immune privileges, MSCs promise tremendous therapeutic potential.

Choice of stem cells for therapy

Both embryonic and adult stem cells have its own pros and cons. Embryonic stem cells are pluripotent (blastocyst stage embryos) or totipotent (early stage embryos) in nature and contains the genetic blue print of each and every type of body cells, so as margin of safety will be more while going for therapeutics, but cannot say impeccable as it's proneness to immune rejection and teratoma formation (Verma O P., 2010). Cuncurrenlty mesenchymal stem cells are easy to culture and large number of cells will be available for therapeutics purpose even though they

are multipotent , pre-specialized cells and are more prone to DNA mutations when compared to embryonic stem cells (Serakinci *et al.*,2004).

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