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Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis

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ABSTRACT

Mesenchymal stem cells or multipotent stromal cells (MSCs) have initially captured attention in the scientific world because of their differentiation potential into osteoblasts, chondroblasts and adipocytes and possible transdifferentiation into neurons, glial cells and endothelial cells. This broad plasticity was originally hypothesized as the key mechanism of their demonstrated efficacy in numerous animal models of disease as well as in clinical settings. However, there is accumulating evidence suggesting that the beneficial effects of MSCs are predominantly caused by the multitude of bioactive molecules secreted by these remarkable cells. Numerous angiogenic factors, growth factors and cytokines have been discovered in the MSC secretome, all have been demonstrated to alter endothelial cell behavior in vitro and induce angiogenesis in vivo. As a consequence, MSCs have been widely explored as a promising treatment strategy in disorders caused by insufficient angiogenesis such as chronic wounds, stroke and myocardial infarction. In this review, we will summarize into detail the angiogenic factors found in the MSC secretome and their therapeutic mode of action in pathologies caused by limited blood vessel formation. Also the application of MSC as a vehicle to deliver drugs and/or genes in (anti-) angiogenesis will be discussed. Furthermore, the literature describing MSC transdifferentiation into endothelial cells will be evaluated critically.

Contents

Abbreviations: AD-MSCs, adipose-derived mesenchymal stem cells; Ang-1, angiopoietin-1; BM, bone marrow; BM-MSC, bone marrow mesenchymal stem cell; CAM, chorioallantoic membrane; CM, conditioned medium; Cyr61, cysteine-rich, angiogenic inducer 61; EC, endothelial cell; ECM, extracellular matrix; EGF, epidermal growth factor; EGM-2, endothelial growth medium-2; FGF-2, fibroblast growth factor-2; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor 1; IL-6, interleukin-6; ISCT, International Society for Cell Therapy; MCP-1, monocyte chemoattractant protein-1; MI, myocardial infarction; MMP, matrix metalloprotease; MSC, mesenchymal stromal/stem cell; PAD, peripheral artery disease; PDGF, platelet-derived growth factor; PLGF, placental growth factor; SDF-1, stromal cell derived factor-1; TGF-α, transforming growth factor-α; tMCAO, transient middle cerebral artery occlusion; TNF-α, tumor necrosis factor α; tPA, tissue plasminogen activator; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; vWF, von Willebrand Factor; WHO, World Health Organization.

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1. Identification and characterization of mesenchymal stromal/stem cells

Since their discovery in the 1960s, the fascinating nature of mesenchymal stromal/stem cells (MSCs) attracted many scientists and this is reflected by the 26,968 PubMed citations on MSCs at the moment of this writing.

Almost 5 decades ago, the pioneer Friedenstein and his coworkers identified within the bone marrow (BM) a minor subpopulation of cells that had osteogenic potential in vivo (Friedenstein et al., 1966). These cells could be distinguished from other BM cells (such as the hematopoietic cells) by both their rapid adherence to plastic and their fibroblast-like morphology. Since these cells showed to be highly clonogenic as they could efficiently generate single-cell derived colonies, they were designated as colony-forming unit fibroblasts (Friedenstein et al., 1970). The true physiological and biological role for these cells appeared to be support of the hematopoiesis in the bone marrow (Clark & Keating, 1995). In analogy to the hematopoietic stem cell system, Caplan introduced the popular name 'mesenchymal *stem* cell (MSC)' referring to the multipotent differentiation potential of these cells (Goshima et al., 1991). Later on, it was confirmed that the progeny of such a single BM-derived cell was able to differentiate into multiple mesenchymal tissues such as bone, cartilage, adipose and fibrous tissues (Pittenger et al., 1999). Besides this mesodermal differentiation potential, MSCs are also suggested to form myocardial cells (Toma et al., 2002; Kawada et al., 2004), hepatocytes (K. D. Lee et al., 2004), neuron-like and neuronal cells (Kopen et al., 1999; Wislet-Gendebien et al., 2005) under the appropriate induction conditions. In addition to the bone marrow, MSC populations can be isolated from a wide variety of tissues such as (and not limited to) adipose tissue (Zuk et al., 2002), dental pulp (Gronthos et al., 2000), amniotic fluid (Nadri & Soleimani, 2007), umbilical cord blood (Erices et al., 2000) and even breast milk (Patki et al., 2010). A great contribution to the popularity of the MSCs can be attributed to their immune-suppressive profile: Le Blanc et al. (2003) reported that MSCs do not express MHC class II surface markers and no co-stimulatory molecules such as CD40, CD80 and CD86, suggesting that MSCs can be "invisible" for the host immune system and therefore could be clinically applied in allogenic settings.

In spite of their promising and remarkable features, it remains disputable whether MSCs really fulfill all hallmark 'stem cell' characteristics, such as self-renewal and differentiation in vivo, as adequate test systems for these two properties are still lacking (Bianco et al., 2008). Therefore, it is generally considered that the name 'mesenchymal *stromal* cell' (also abbreviated as MSC) is more appropriate than the widely used historical description 'mesenchymal *stem* cell' (Bianco et al., 2008; Prockop & Oh, 2012).

Another bottleneck in MSC research in the years 1990–2000 was the fact that due to differences and inconsistencies in isolation, expansion and identification methodology between various research groups, it was difficult to compare the results of published MSC studies (Caplan & Correa, 2011; Keating, 2012; Prockop & Oh, 2012). In order to clearly define MSCs, the International Society for Cell Therapy (ISCT) proposed the following minimal criteria for MSCs: MSCs have a fibroblastoid phenotype, adhere to plastic and possess a trilineage mesodermal differentiation capacity towards chondrocytes, adipocytes and osteocytes in vitro. Additional requirements include the expression of the cell surface molecules CD105 (endoglin), CD73 (ecto 5′ nucleotidase) and CD90 (Thy-1) and the absence of the hematopoietic markers CD34, CD45, CD14 (or CD11b), CD79α and the MHC II class cellular receptor HLA-DR (Horwitz et al., 2005). While it is assumed that expression of this broad set of markers defines cells as MSCs, most of these markers are also found on fibroblastic cells from any tissue (Bianco et al., 2008). Although various surface molecules such as CD146 (Sacchetti et al., 2007), STRO-1 (Simmons & Torok-Storb, 1991) and CD271 (Jones et al., 2002) have been used to enrich BM-derived MSCs, the fact that to date there is no single specific unambiguous determinant to designate MSCs in situ, remains a particular struggle (Bianco et al., 2008; Caplan & Correa, 2011; Keating, 2012). On the other hand, it is possible that the ISCT marker profile definition of MSCs is too simplistic and unsuitable for MSCs isolated from other tissues than the BM, as for example MSCs derived from adipose tissue in fact do express CD34 (Traktuev et al., 2008; Keating, 2012). Another drawback is that the expression of these markers may be determined or may even be induced by culture conditions rather than being intrinsic characteristics of MSCs in situ. In mice, even strain differences influence the expression of surface epitopes on BM-MSCs (Peister et al., 2004). As there is still no consensus on the surface marker expression profile of MSCs among leading investigators within the field, new and more advanced molecular tools that help unravel the MSC proteome and secretome could provide a solution to uniformly define MSCs (Keating, 2012).

Traditionally, MSCs are considered to be of 'pure' mesodermal origin as most skeletal bones are derived from the axial and lateral mesoderm (Bianco et al., 2008; Caplan & Correa, 2011). However, in the embryonic development of craniofacial bones and their marrow, the ectodermal neural crest cells are also involved (Hall, 2008). Furthermore, there is accumulating evidence that for almost all organs in the body, an MSC population is present in the perivascular niche associated with blood vessels in vivo. These perivascular cells, principally called pericytes, are located at the abluminal side of blood vessels and communicate with the endothelial cells. Based on immunoselection for CD146, NG2 and the pericyte marker PDGF-Rβ, Crisan et al. purified SC from various tissues such as skeletal muscle, pancreas, adipose tissue and placenta. In addition to their osteogenic, chondrogenic and adipogenic differentiation potentials, all cells appeared to be myogenic in vitro and in vivo, regardless of their origin of skeletal muscle or non-muscle tissues (Crisan et al., 2008). Therefore, it is now speculated that all MSCs indeed are pericytes and that their biological role is to fuel local tissue growth and repair (Caplan & Correa, 2011).

As MSCs display a broad differentiation capacity in vitro, it was originally hypothesized that MSC transplantation would induce tissue repair by replacing the damaged host tissue. Despite their long-lasting therapeutic efficacy in a wide variety of in vivo models and clinical settings (such as bone and cartilage repair, cardiovascular and neurological diseases), the incidence of MSC engraftment remained to be surprisingly low. For example, children with osteogenesis imperfecta who received MSC transplantation showed a significant improvement in bone mineral density and growth velocity, while the population of engrafted MSCs is less than 1% (Horwitz et al., 2002). Another striking example of this low incorporation into the host was demonstrated by Wu et al., who studied the rate of mouse BM-MSC engraftment in a murine wound healing model. By using sex-mismatched GFP + mBM-MSCs, the number of cells engrafted into the wounded skin was found to be 27% at 7 days post-injection and even decreased to only 2.5% at 28 days after administration (Y. Wu et al., 2007). This unexpected low engraftment efficacy implied a major challenge for the field to explain the beneficial effects of the MSCs (as they in most cases only temporarily resided in the host) which was translated into a 'back to the bench' effect. There is now accumulating evidence that the general therapeutic effects of MSC treatment are due to their ability to alter the host microenvironment rather than their capacity to (trans)differentiate and incorporate into the host tissue. The basic mechanisms, other than differentiation, by which MSCs are suggested to affect the host include:

(1) the modulation of the immune system (the 'immunomodulatory effect'),
(2) the secretion of factors that induce tissue repair (the trophic or paracrine effect),
(3) recruitment of endogenous MSCs to the site of injury
(4) possibly transfer of mitochondria or vesicular components containing mRNA, microRNA and proteins (Fig. 1) (Spees et al.,