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Mini review

Mechanisms involved in the therapeutic properties of mesenchymal stem cells

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ABSTRACT

Mesenchymal stem cells (MSCs) have been described as being able to give rise to several quite different mesenchymal cell phenotypes. However, the ability to differentiate is not the only characteristic that makes these cells attractive for therapeutic purposes. The secretion of a broad range of bioactive molecules by MSCs, such as growth factors, cytokines and chemokines, constitutes their most biologically significant role under injury conditions. Understanding this intricate secretory activity as well as the properties of MSCs *in vivo* is central to harnessing their clinical potential. Herein, we identify some of the molecules involved in the paracrine effects of MSCs with a perspective that these cells intrinsically belong to a perivascular niche *in vivo*, and discuss how this knowledge could be advantageously used in clinical applications.

1. Introduction: mesenchymal stem cells

Adult mesenchymal stem cells (MSCs) can be defined as multipotent cells able to differentiate into various types of end-stage, specialized mesenchymal cells such as osteoblasts, chondrocytes, adipocytes, tenocytes and others [1]. Research involving MSCs can be traced back to the 1970s, when A.J. Friedenstein and colleagues described an adherent, non-hematopoietic cell type present in the bone marrow (BM) of different species that could form fibroblastic colonies *in vitro* (reviewed in [2]). These cells were termed fibroblastic colony-forming units (CFU-Fs), and the *in vitro* progeny of CFU-Fs were later found to have the ability to differentiate along osteogenic, adipogenic and chondrogenic pathways *in vitro* and when implanted *in vivo* (reviewed in [3] and [4]).

The introduction of a culture system for the study of hematopoietic stem cells (HSCs) by T.M. Dexter and colleagues [5] facilitated the elucidation of the cell hierarchy of the hematopoietic system. The notion that CFU-F can be found in the "stromal" adherent layer of Dexter-type cultures [6] led to the notion that this cell type is present in the BM stroma, and the term "stromal cell" became popular. The existence of a stromal system, with a stromal stem cells at the top of the hierarchy, was proposed by M. Owen [7]. At nearly the same time, based on an analogy with the hierarchical system described for HSCs, the existence of an MSC at the top of a hierarchy of non-hematopoietic cells in BM was proposed [1]. This proposed non-hematopoietic hierarchy would be able to give rise to cells characteristic of connective tissues, such as bone, cartilage, adipose, tendon, ligament and others, but not stromal components such as endothelial cells or macrophages [1]. Accordingly, plastic-adherent BM cell populations have been operationally defined as MSCs based on their ability to proliferate in culture and to differentiate when maintained under appropriate conditions *in vitro* or when implanted *in vivo* (reviewed in [8]).

The notion that BM contains MSCs has been and still is useful for the purpose of tissue engineering, which relies on the ability of these cells to differentiate into tissue-specific cell types when combined with biomaterials and given proper differentiation stimuli [9]. The same concept has also allowed the suggestion that MSCs are distributed throughout the body, as different research groups, using various organs as cell sources, have derived cells able to proliferate and differentiate into mesenchymal cell types (reviewed in [10]).

2. Conceptual problems regarding MSCs

Currently, there is much debate in the field as to how to refer to the plastic-adherent cells operationally defined as MSCs. The International Society for Cellular Therapy (ISCT) has proposed that human fibroblast-like, plastic-adherent cells be termed "multipotent

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mesenchymal stromal cells," regardless of the tissue from which they are isolated, and that the term "mesenchymal stem cell" be used only for cells that meet clearly stated stem cell criteria [11]. Subsequently, ISCT proposed minimal criteria to define multipotent mesenchymal stromal cells: the cells should be adherent to plastic; positive for CD73, CD90 and CD 105 and negative for CD11b or CD14, CD19 or CD79α, CD34, CD45 and HLA-DR; and differentiate into osteoblasts, adipocytes and chondroblasts *in vitro* [12]. However, these criteria will require some adjustments as research describing MSCs obtained from tissues other than BM advances. Short-term cultured MSCs from human adipose tissue, for example, express CD34 [13,14]. It is likely that suggestions for changes in the nomenclature concerning MSCs will also emerge with the advancement of knowledge regarding MSCs *in vivo*. In this review, we will take advantage of the acronym overlap between "mesenchymal stem cells" and "multipotent stromal cells" and refer to this particular cell population as "MSCs."

Another interesting point regarding MSCs is the question as to whether or not cultured MSCs represent homogeneous or heterogeneous populations. A study published in 1999 showed that MSCs obtained by adherence to plastic and cultured in pre-selected lots of fetal bovine serum [15] were nearly homogeneously positive or negative for 48 molecules, which suggests that MSC cultures represent homogeneous populations [16]. Clonal analyses of human MSCs have shown that, at a given moment during cell culture, there are clones able to differentiate into three, two or only one mature cell type assayed, and that the frequency of tripotent clones decreased as the cells were expanded in culture [17]. When murine MSCs were plated at a single cell per well, the frequency of colony-forming clones was found to be nearly 50%, and when some of these clones were expanded and analyzed, they showed phenotypic characteristics similar to those of the parental populations [18]. These results indicate that cultured MSCs are heterogeneous, stem cell-containing populations. Furthermore, since mature cells have been shown to dedifferentiate in culture and express markers considered to be characteristic of MSCs [19], it is possible that mature cell types also contribute to the establishment of MSC cultures and, by extension, to culture heterogeneity. For example, when CD34+CD31+ (endothelial) cells are sorted out of primary cultures derived from the stromal/vascular fraction of human adipose tissue and further cultured under MSC conditions, they lose expression of these markers and display a surface profile comparable to that of MSCs (Fig. 1).

As mentioned above, much of what is known about MSCs derives from experiments involving cultured cells, which makes the task of transposing the *in vitro* results to an *in vivo* context difficult. So far, MSCs have not been unequivocally identified *in vivo*, partly because there is no established consensus on what markers can reliably identify MSCs *in situ*. The use of markers of cultured MSCs to search for these SCs *in vivo* is hampered by the fact that expression of these markers may be spuriously determined by the culture conditions rather than characteristic of MSCs *in situ*. In spite of this drawback, this approach has allowed the suggestion that MSCs are associated with blood vessels *in vivo* [20–23]. And, indeed, earlier and recent reports suggest that pericytes, cells that are located on the abluminal side of blood vessels in close contact with endothelial cells, show great similarity to MSCs *in vitro* [21,24–27] and may behave as tissue-specific SCs *in vivo* [28–36]. The finding that the frequency of fibroblastic colonies observed in CFU-F assays strongly correlates with vascular density is a natural consequence of the association of MSCs with blood vessels [37]. Further support for the view that the perivascular niche is a common stem cell microenvironment for resident MSC-like populations within different vascularized tissues comes from the demonstration that CD146+ cells isolated from the retina (putative pericytes, as described below) display properties similar to those of MSCs isolated from multiple adult and fetal tissues, such as the capacity for differentiation toward adipogenic, osteogenic and chondrogenic lineages ([25]; reviewed in [38]).

The term "pericyte" is used here for the sake of simplification, as many names can be used for these periendothelial cells depending on their anatomical location and whether or not they are embedded in the basement membrane that surrounds blood vessels (e.g., perisinusoidal cells in sinusoids, adventitial reticular cells in BM sinusoids, Ito or stellate cells in the liver, mesangial cells in kidney glomeruli, and so on). The perspective, in light of both physiological and injury circumstances, is that some pericytes are SCs in the tissues from which they originate and correspond to MSCs in connective tissues [10]. This proposition does not imply that pericytes/MSCs from all organs are equivalent; indeed, MSCs obtained from different tissue sources show some differences regarding differentiation potential [18,37] and gene expression profiles [25,39–41]. Differences in gene expression are observable between MSCs obtained from different fetal organs, which suggests that they are already determined in prenatal life (Fig. 2).

In spite of the existence of abundant evidence indicating that the best *in vivo* candidate for the role of MSC in connective tissues is the pericyte, definitive proof that this cell type can self-renew for a lifetime is still lacking. Recently, Sachetti et al. showed that clonal, CD146+ adventitial reticular cells (BM pericytes) are able to organize a hematopoietic environment when implanted in mice after *in vitro* expansion, and do so again when re-transplanted into secondary recipients [42]. However, this finding alone does not provide strong basis for the assumption that the cells used are representative of or behave as stem cells *in vivo*, as they were subjected to culture before implantation and, most importantly, no evidence of self-renewal *in vivo* was presented as the absolute

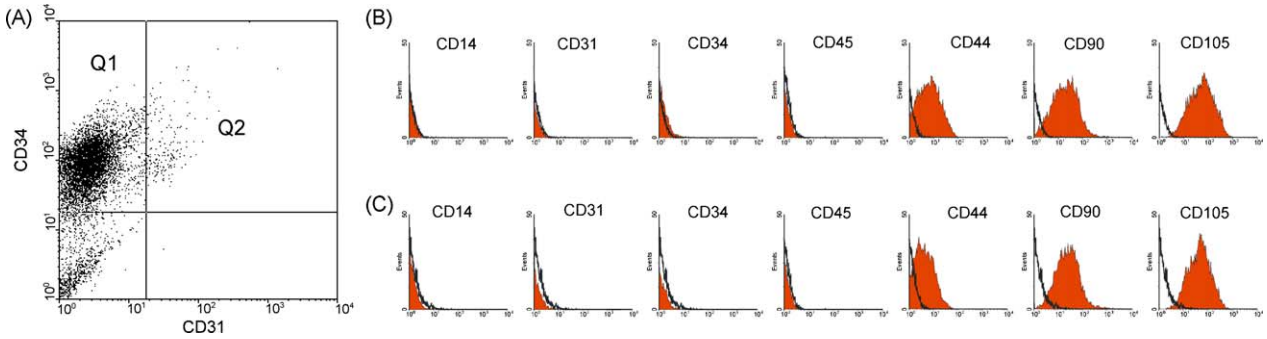

Fig. 1. Loss of expression of CD34 and CD31 by adipose tissue-derived cells. CD34+CD31− cells (Q1, panel A) and CD34+CD31+ cells (Q2, panel A) were sorted out of a 4-day-old primary culture of stromal/vascular cells from human adipose tissue and culture-expanded for an additional 3 and 2 passages, respectively. The surface marker profile exhibited by the progeny of CD34+CD31− cells (panel B) and CD34+CD31+ cells (panel C) is comparable to that commonly attributed to MSCs.

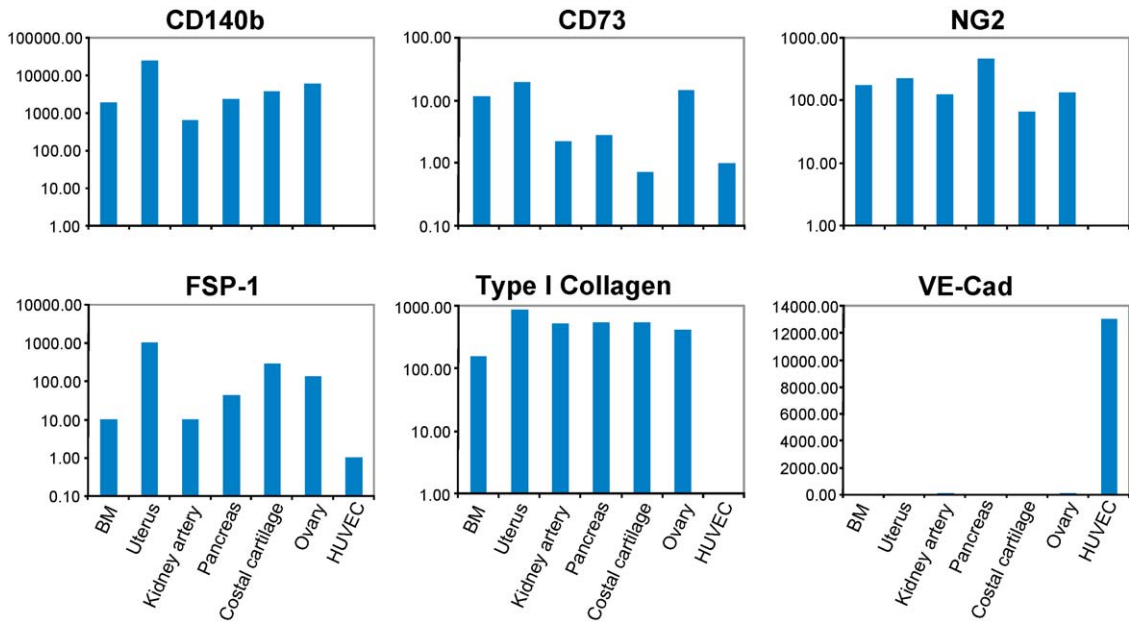

Fig. 2. Comparison between the expression levels of selected genes in MSCs derived from adult bone marrow and various fetal tissues. Transcript levels of CD140b, CD73, nerve/glial antigen 2 (NG2), fibroblast-specific protein 1 (FSP-1), collagen type I and vascular endothelial (VE)-cadherin were evaluated by real time PCR. Cultured MSCs from bone marrow and five fetal tissues including uterus, kidney artery, pancreas, costal cartilage and ovary were analyzed, and human umbilical vein endothelial cells (HUVECs) were used as controls.

numbers of cells present in the recipients were not assessed. Demonstrating self-renewal of putative MSCs *in vivo* is technically difficult because the turnover rate of connective tissues is far lower than that of hematopoietic tissue [43].

3. Conceptual problems aside, MSCs are promising therapeutic tools

Recently, the concept of MSCs discussed above has been broadened to include the secretion of biologically active molecules that exert beneficial effects on other cells [44]. This shifts a paradigm centered on differentiation to a view in which MSCs can be therapeutic even if they do not engraft or differentiate into tissue-specific cells, which significantly increases the range of MSC therapeutic applications. MSC paracrine effects can be divided into trophic ("nurturing"), immunomodulatory, anti-scarring and chemoattractant. The trophic effects of MSCs can be further subdivided into anti-apoptotic, supportive (stimulation of mitosis, proliferation and differentiation of organ-intrinsic precursor or stem cells) and angiogenic. The number of molecules known to mediate the paracrine action of MSCs increases every day; some of these, which may fit more than one category, are discussed in detail below and summarized in Table 1 and contextualized in Fig. 3. The ability to preferentially dock at sites of injured tissues, which may be influenced by several factors (reviewed in [45]), adds to the regenerative properties of MSCs as it increases the likelihood of systemically delivered cells finding the areas where their paracrine effects are most needed, and this is of particular interest for clinical applications.

3.1. Homing of cultured MSCs

MSCs are known to migrate or dock preferentially to injured sites when infused in animal models of injury (reviewed in [10]), and this property can be attributed to the expression of growth factor, chemokine and extracellular matrix receptors on the surface of MSCs. In an *in vitro* assay, murine MSCs migrated toward cells isolated from bleomycin-injured mouse lungs, but not toward healthy lung cells [46]. Chemotaxis assays show that cultured MSCs migrate toward different growth factors and chemokines in a dose-dependent fashion *in vitro*, and that chemokine-driven migration is stimulated by the pro-inflammatory cytokine tumor necrosis factor (TNF)-α [47], which indicates that chemoattraction can direct systemically infused MSCs to inflammatory sites. Other mechanisms involved in MSC homing or docking include attachment to endothelium. Endothelial cells become activated under injury conditions, and this state is characterized by the expression of surface molecules that allow docking of circulating cells. Two of these molecules, vascular cell adhesion molecule 1 (VCAM-1, aka CD106) and E-selectin (CD62E), are ligands for the MSC surface molecules integrin α4/β1 (CD49d/CD29) and CD44, respectively [48,49]. Osteopontin, whose expression is upregulated in osteocytes under hypoxic conditions [50], and hyaluronan, whose expression is increased in the kidney after experimentally induced acute renal failure [51], also can retain systemically infused MSCs via binding of CD44, which highlights the importance of this molecule for MSC homing and docking. The irony regarding CD49d and CD44 is that these two molecules are sensitive to the proteolytic action of trypsin [52], the enzyme most widely used to harvest MSCs from culture plates.

3.2. Main soluble factors secreted by MSCs

3.2.1. Anti-apoptosis

In a scenario where MSCs are administered with the aim of treating acute lesions, the first expected effect is the reduction of the extent of cell death, and this is observed in animal models of tissue injury and in co-culture experiments. Togel et al. reported that infused MSCs attach to the renal microvascular circulation and decrease apoptosis of adjacent cells in a model of acute kidney injury [53]. In order to elucidate the factors responsible for the observed renoprotective effect, these authors analyzed the MSC-conditioned medium and verified the presence of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and insulin-like growth factor 1 (IGF-1), factors that

Table 1
Trophic and immunomodulatory factors secreted by cultured MSCs.

Effect	Molecule	Reference(s)
Anti-apoptotic	VEGF	[53,56]
	HGF	[53,56]
	IGF-I	[53]
	Stanniocalcin-1	[55]
	TGF-β	[56]
	bFGF	[56]
	GM-CSF	[56]
Immunomodulatory	PGE-2	[59,63,65,66,68]
	TGF-β	[57,63]
	HGF	[57]
	mpCCL2	[67]
	IDO	[69]
	iNOS	[68]
	HLA-G5	[70–72]
	LIF	[73,74]
Anti-scarring	bFGF	[81]
	HGF	[81]
	Adrenomedullin (?)	[82]
Supportive	SCF	[83,84]
	LIF	[83,84]
	IL-6	[83,84]
	M-CSF	[83,84]
	SDF-1	[85,86]
	Angiopoietin-1	[86]
Angiogenic	bFGF	[89]
	VEGF	[89,90]
	PlGF	[89]
	MCP-1	[89,90]
	IL-6	[90]
	Extracellular matrix molecules	[91]
Chemoattractant	CCL2 (MCP-1)	Reviewed in [10]
	CCL3 (MIP-1α)	
	CCL4 (MIP-1β)	
	CCL5 (RANTES)	
	CCL7 (MCP-3)	
	CCL20 (MIP-3α)	
	CCL26 (eotaxin-3)	
	CX3CL1 (fractalkine)	
	CXCL5 (ENA-78)	
	CXCL11 (i-TAC)	
	CXCL1 (GROα)	
	CXCL2 (GROβ)	
	CXCL8 (IL-8)	
	CCL10 (IP-10)	
	CXCL12 (SDF-1)	

enhance endothelial cell growth and survival. Parekkadan et al. found the presence of these and other anti-apoptotic molecules in MSC-conditioned medium and, interestingly, showed that an MSC-containing bioreactor connected to the bloodstream of rats experimentally subjected to fulminant hepatic failure resulted in the survival of 71% of the animals in contrast to 14% survival in the control group [54]. MSCs reduce apoptosis of UV-irradiated fibroblasts and lung epithelial tumor cells cultured under low pH and hypoxia, and the up-regulation and secretion of stanniocalcin-1 has been found to be at least partially responsible for this anti-apoptotic effect [55]. Also, adipose tissue-derived MSCs have been shown to express HGF, VEGF, transforming growth factor beta (TGF-β), basic fibroblast growth factor (bFGF, aka FGF2) and granulocyte–macrophage colony-stimulating factor (GM-CSF), and the expression of these molecules was found to increase under hypoxic culture conditions; particularly, VEGF upregulation under hypoxia has been shown to be greater than that observed for other factors [56]. Hypoxia takes place in the first stages of tissue injury, and secretion of anti-apoptotic factors by MSCs at this stage minimizes the extent of cell death in the tissues surrounding the injured areas; accordingly, in the latter study, it was further demonstrated that cultured, adipose-derived MSCs reduce necrosis and improve perfusion when injected into mice experimentally subjected to hind limb ischemia [56]. We suggest that this anti-apoptotic activity could serve to limit the field of injury in *in vivo* circumstances.

3.2.2. Immunomodulation

The realization that cultured MSCs have immunomodulatory properties comes from experiments where they were shown to directly inhibit the proliferation of αβ T cells *in vitro* [57,58]. More recently, MSCs have been shown to suppress γδ T cells as well [59]. In addition, MSCs have been found to escape cytotoxic T cell-mediated lysis [60]. To date, MSCs are known to affect not only T cells, but also other cells of the immune system. MSCs can inhibit [61] or promote [62] B cell proliferation, suppress NK cell activation [63,64], and modulate the cytokine secretion profile of dendritic cells [65] and macrophages [66]. The ability to interact with dendritic cells and macrophages provides MSCs with extended indirect influence on the immune system. Whereas most of the molecular mechanisms that mediate the MSC suppressive effect on B cells remain unknown, a CCL2 variant resulting from modification of CCL2 by matrix metalloproteinases has been shown to suppress immunoglobulin production by plasma cells, and transplantation of syngeneic MSCs into hemophilic mice with pre-developed anti-human factor VIII (hFVIII) antibodies resulted in a robust decrease in hFVIII-specific IgG levels [67].

Secreted prostaglandin E2 (PGE-2) is a central mediator in many of the effects of MSCs on immune cells, as inhibitors of the synthesis of this molecule diminish MSC-driven anti-proliferative effects on T [59,65,68] and NK cells [63], and PGE-2 is involved in the modulation of the secretory profile of dendritic cells [65] and macrophages [66]. Neutralizing antibodies to TGF-β1 have been reported to impair the immunomodulatory effects of MSCs on T [57] and NK cells [63]. Likewise, neutralizing antibodies to HGF have revealed that this molecule also mediates MSC anti-proliferative effects on T cells [57]. Degradation of tryptophan as a consequence of expression of indoleamine 2,3-dioxygenase (IDO) by MSCs in co-cultures also has been shown to halt T cell proliferation [69], and tryptophan catabolites such as L-kynurenine and picolinic acid have been previously shown to inhibit activation of CD4+ and CD8+ T cells and, to a lesser extent, of NK cells independent of the presence of tryptophan [70].

Sato et al. focused on the expression of inducible nitric oxide synthase (iNOS) by murine MSCs and found that inhibition of this enzyme abolishes MSC anti-proliferative effect on T cells, but failed to obtain the same result with neutralizing antibodies to TGF-β or IDO [68]. It is important to emphasize that this seeming discrepancy between some of the results obtained by Sato et al. and the others shown above may reflect species-specific differences, as all other studies mentioned earlier in this section used human cells. Other molecules that mediate immunomodulatory effects of MSCs include interleukin (IL)-10, human leukocyte antigen G (HLA-G) [70–72] and leukemia inhibitory factor (LIF) [73], the latter playing an important role not only in the suppression of T cell proliferation, but also in the generation and maintenance of regulatory T cells [74].

The consequences of the mechanisms involved in the immunomodulatory effects of cultured MSCs discussed herein can be observed *in vivo*, although in most cases defining the molecules responsible for the observed immunomodulation is hindered by the complexity of the interactions between MSCs and endogenous cells, which may activate the host's intrinsic mechanisms. Le Blanc et al. have shown that MSC infusions are effective in treating cases of severe, steroid-refractory Graft versus Host Disease (GvHD) in humans [75]. In rats, MSC-conditioned medium was found to reduce the migration of adoptive leukocytes to the liver after

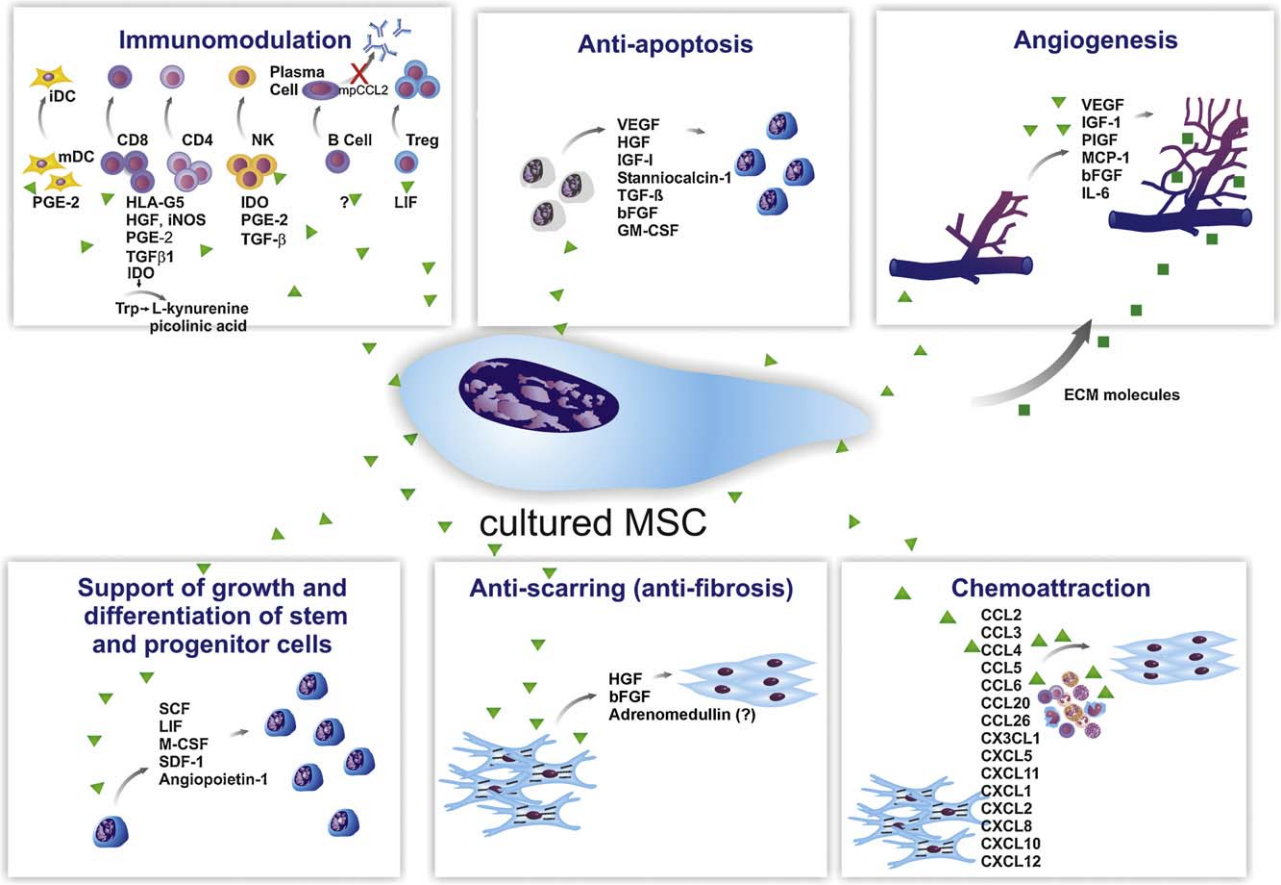

Fig. 3. Paracrine effects of cultured MSCs. The secretion of a broad range of bioactive molecules is now believed to be the main mechanism by which MSCs achieve their therapeutic effect and it can be divided into six main categories: immunomodulation, anti-apoptosis, angiogenesis, support of the growth and differentiation of local stem and progenitor cells, anti-scarring and chemoattraction. Although the number of molecules known to mediate the paracrine action of MSCs increases every day, several factors that have been shown to be secreted by cultured MSC are depicted here for illustrative purposes. The immunomodulatory effects of MSCs consist of inhibition of the proliferation of CD8+ and CD4+ T lymphocytes and natural killer (NK) cells, suppression of immunoglobulin production by plasma cells, inhibition of maturation of dendritic cells (DCs) and stimulation of the proliferation of regulatory T cells. The secretion of PGE-2, HLA-G5, HGF, iNOS, IDO, TGF-β, LIF and IL-10 contributes to this effect. MSCs also limit apoptosis, and the principal bioactive molecules responsible for this are VEGF, HGF, IGF-I, stanniocalcin-1, TGF-β and GM-CSF. In addition, MSCs stimulate local angiogenesis by secretion of extracellular matrix molecules, VEGF, IGF-1, PlGF, MCP-1, bFGF and IL-6, and also stimulate mitosis of tissue-intrinsic progenitor or stem cells by secretion of SCF, LIF, M-CSF, SDF-1 and angiopoietin-1. Moreover, HGF and bFGF (and, possibly, adrenomedullin) produced by MSCs contribute to inhibition of scarring caused by ischemia. Finally, a group of at least 15 chemokines produced by MSCs can elicit leukocyte migration to the injured area, which is important in normal tissue maintenance.

experimentally induced fulminant hepatic failure [54]. In mice subjected to bleomycin-induced lung injury, levels of mRNA for the pro-inflammatory cytokines IL-1β, IL-2 and interferon-γ (IFN-γ) are reduced after systemic MSC administration [46]. In a murine model of lipopolysaccharide-induced acute lung injury, the expression of genes coding for the pro-inflammatory cytokines TNF-α, IL-1α and IL-1β decreased 8.6-, 3.6- and 3.2-fold, respectively, after systemic MSC infusion; conversely, IL-1 receptor antagonist expression showed a 2.2-fold increase [76].

Conversely, the MSC effects on the immune system may not be strictly suppressive. For example, low numbers of MSCs can render dendritic cells prone to promoting T cell activation whereas high numbers of MSCs are required to cause the opposite effect [77]. Low doses of IFN-γ allow MSCs to express class II major histocompatibility complex (MHC) molecules and behave as antigen-presenting cells [78]; on the other hand, higher doses of IFN-γ lead to a decrease in the surface levels of class II MHC molecules [78] and secretion of anti-inflammatory factors [79]. Furthermore, MSCs support B cell expansion and differentiation [62]. These findings indicate that the behavior of MSCs toward the immune system is context-sensitive.

We have recently proposed a model in which pericytes play an active role during tissue repair [10]. According to our model, focal injury leads to local cell death; inflammatory cells migrate into the damaged area; pericytes become activated (i.e., undergo a change in gene expression and become proliferative) and secrete different bioactive molecules that act in concert to resolve the lesion. It is likely that, at the early steps of this process, these pericyte-derived cells provide a supportive effect on immune cells. Consistent with this view, cultured retinal pericytes have been shown to express pro-inflammatory molecules that are chemoattractant to inflammatory cells, namely eotaxin (aka CCL11), granulocyte colony-stimulating factor (G-CSF), IL-8 and regulated upon activation, normal T cell expressed and secreted (RANTES, aka CCL5), when insulted by exposure to a high glucose concentration [80]. In the same study, exposure of pericytes to the pro-inflammatory molecules TNF-α or IL-1β for 24 h resulted in an increase in the expression of many molecules known to be chemoattractive and stimulatory for different immune cells, including IL-1β, IL-6, IL-7, IL-12, IL-16, IL-1 receptor antagonist (IL-1ra), TNF-α, TNF-β, epithelial neutrophil-activating protein 78 (ENA-78, aka CXCL5), eotaxin, IL-8 (aka CXCL8), monocyte chemoattractant protein 1 (MCP-1, aka CCL2), macrophage inflammatory protein (MIP)-1α (aka CCL3), MIP-1β (aka CCL4), RANTES, intercellular adhesion molecule-1 (ICAM-1), VCAM-1, G-CSF, GM-CSF, growth hormone, stem cell factor (SCF), $VEGF_{165}$, bFGF, thyroid-stimulating hormone (TSH), CD40 and CD40 ligand. This suggests that pericytes respond to inflammatory cells at the early stages of wound healing and

provide physiological support for the subsequent steps of the immune response. However, as the local environment undergoes changes during the healing process, it is likely that the expression profile of pericytes changes with time. For example, perivascular cells have been shown to proliferate and secrete HGF only 3 days after induction of an ischemic lesion in murine adipose tissue ([81]; see below). As the pericyte changes, it becomes an MSC-like cell that inhibits the immunosurveillance of the injury site and prevents the initiation of autoimmune events.

3.2.3. Anti-scarring

Although the anti-fibrotic effects of cultured MSCs have been demonstrated in different animal models, the molecular mechanisms behind this effect are not yet fully understood. Based on studies done to date, in most cases MSC administration is effective only if it takes place before the establishment of massive fibrosis (reviewed in [8]). Recently, bFGF and HGF have been shown to be involved in the prevention of fibrosis in a murine model of ischemia-reperfusion of adipose tissue [81]. In that study, human and murine BM-derived MSCs, human adipose-derived stem cells and dermal fibroblasts were found to express HGF in response to stimulation with bFGF, an effect that could be blocked by a c-Jun N-terminal kinase (JNK) inhibitor. Administration of either a neutralizing antibody against bFGF or a JNK inhibitor to the injured adipose tissue resulted in impaired proliferation of CD34+CD31-stromal/perivascular cells (viewed here as MSCs) and a reduction of severe post-lesion fibrogenesis; on the other hand, a neutralizing antibody against HGF did not stop proliferation of CD34+CD31-stromal/perivascular cells, and this resulted in marked fibrogenesis. These results reveal that, in a situation of tissue injury, perivascular cells become proliferative and secrete HGF, which in turn mediates anti-fibrotic (and immunomodulatory) effects. Administration of culture-expanded MSCs to prevent fibrosis can, thus, be viewed as a way to augment local production of HGF (and probably other anti-scarring factors) in cases where fibrosis is to be avoided. Lastly, in a rat model of global heart failure, it was shown that transplanted MSCs decreased cardiac fibrosis; the secretion of adrenomedullin appears to be one of the factors which mediate this anti-fibrotic effect [82].

3.2.4. Support to the growth and differentiation of local stem and progenitor cells

Cultured MSCs support hematopoiesis *in vitro*, and this ability involves the constitutive secretion of soluble factors such as SCF, LIF, IL-6, and macrophage colony-stimulating factor (M-CSF); in addition, hematopoietic support can be further augmented by IL-1α-induced secretion of G-CSF and GM-CSF [83,84]. *In vivo*, adventitial reticular cells, which are putative MSCs *in vivo* [42], sustain the hematopoietic stem cell pool through the secretion of stromal-derived factor 1 (SDF-1, aka CXCL12) [85], which suggests that the hematopoietic support observed *in vitro* mimics some aspects of that observed under physiological conditions *in vivo*. In a situation of ischemic injury in murine brain, cells in blood vessels were shown to express SDF-1 and angiopoietin-1, which recruited and supported neural precursors [86]. Expression of SDF-1 and angiopoietin-1 is characteristic of pericytes [87,88], which indicates that these cells were responsible for the observed recruitment and support of neural progenitors.

3.2.5. Angiogenesis

Angiogenic support provided by MSCs can be considered one more supportive effect as discussed above, and it is treated separately here because re-establishment of blood supply is fundamental for recovery of damaged tissues. The pro-angiogenic effect of MSCs has been demonstrated in a murine model of hind limb ischemia [89]. In that study, the authors detected bFGF, VEGF, placental growth factor (PlGF), and MCP-1 in MSC-conditioned medium, and also verified the presence of bFGF and VEGF around the infused cells *in situ* after local administration. Hung et al. also demonstrated that MSC-conditioned medium contains high amounts of angiogenic and anti-apoptotic factors such as IL-6, VEGF and MCP-1, which inhibit the death of endothelial cells cultured under hypoxic conditions and promote the formation of capillary-like structures in an *in vitro* assay [90]. Recently, some populations of BM-derived MSCs have been shown to support the formation of vessel-like structures by endothelial cells *in vitro* in a medium devoid of the angiogenic factors VEGF-A, bFGF and IGF-1; in this system, MSCs provide, in addition to soluble angiogenic factors, extracellular matrix components that serve as a substrate for endothelial cells [91]. Lastly, the transition of MSCs back to pericytes situated on newly formed vessels serves to stabilize the forming vasculature both *in vitro* [91] and *in vivo* [92].

3.2.6. Chemoattraction

Cultured MSCs secrete a variety of chemoattractant molecules, which include CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), CCL7 (MCP-3), CCL20 (MIP-3α), CCL26 (eotaxin-3), CX3CL1 (fractalkine), CXCL5 (ENA-78), CXCL11 (i-TAC), CXCL1 (GROα), CXCL12 (SDF-1), CXCL8 (IL-8), CXCL2 (GROβ) and CXCL10 (IP-10) (reviewed in [10]). Target cells for these include monocytes, eosinophils, neutrophils, basophils, memory and naïve T cells, B cells, NK cells, dendritic cells and hematopoietic and endothelial progenitors [93]. Although these molecules are constitutively expressed by cultured MSCs, with some discrepancies between studies most probably reflecting different culture conditions, it is likely that the pattern of chemokine expression by MSCs is modified by exposure to other cell types, particularly immune cells.

4. Consequences for the clinic

The overview discussed herein indicates that MSCs are promising tools for the treatment of different types of conditions, because they secrete a multitude of bioactive molecules that ultimately lead to reformation of tissues at sites of injury. In many instances, the time required to isolate and culture-expand MSCs to increase cell numbers precludes the immediate application of autologous MSCs to cases of acute injury, e.g., ischemic lesions. Given that long-term engraftment is not necessarily required for MSCs to exert most of their therapeutic effects, this hurdle could be overcome by banking third-party MSCs so that they are readily available when necessary. Since MSCs are present in different tissues due to their association with blood vessels, every piece of vascularized tissue could be used as a source of MSCs. Considering the data showing that MSCs from different tissues are not identical, further studies comparing MSCs obtained from different tissue sources are necessary to determine if MSCs could be more effective for specific applications as a function of their site of origin.

The alternative to the use of cultured MSCs is the isolation of fresh MSCs, which in our view are pericytes; however, there are no data available to determine if pericytes and cultured MSCs share the same properties. Also, it is possible that the frequency of bona fide MSCs *in vivo* is low, which again points to *ex vivo* expansion. The Cell Therapy Laboratory at National Institute of Science and Technology for Stem Cells and Cell Therapy (Ribeirão Preto, Brazil) currently produces good manufacturing practice (GMP)-grade MSCs to experimentally treat GvHD patients. One of the problems with the culture technology currently used (serial passaging in plastic flasks) is that it is inefficient albeit effective. To minimize this difficulty, the scale-up of MSC production in bioreactors is under development in this Institute and elsewhere.

In spite of the fact that some of the mechanisms involved in the therapeutic effects of MSCs are known today, further research

focusing on this topic is required to devise strategies aimed at increasing the efficiency of therapies not only for GvHD but also for other conditions. Pre-treatment of cultured MSCs with soluble factors such as IFN-γ, for example, could enhance the treatment of certain immunological diseases. The efficiency of MSC-based therapies could also be increased by subjecting cultured MSCs to hypoxic conditions before administration to patients. Screening for enzymes that can efficiently harvest cultured MSCs while preserving molecules responsible for docking systemically infused cells at injured sites may also prove valuable for therapeutic purposes. Lastly, the targeting of MSCs to precise locations of injury to deliver optimized doses of cells is the next technological frontier to be overcome.

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