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The secretome of mesenchymal stromal cells: Role of extracellular vesicles in immunomodulation

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ABSTRACT

Mesenchymal stromal cells (MSCs) inhibit natural killer cell proliferation and activity, suppress T lymphocyte proliferation, dendritic cells maturation and B lymphocyte proliferation and activation. Moreover, MSCs may induce regulatory T cells. Several factors released from MSCs have been implicated in their immune-modulatory properties. These include soluble factors such as interleukins 6 and 10, prostaglandin E2, hepatocyte growth factor, indoleamine 2,3-dioxygenase, nitric oxide, transforming growth factor β1, human leukocyte antigen and extracellular vesicles. These vesicles released from cells have been characterized as a new mechanism of cell-to-cell communication and emerged as mediators of the MSC-immune-modulatory effects. In this review we focused our attention on the extracellular vesicles as paracrine mediators of MSC immune-modulation.

1. Introduction

Mesenchymal stromal cells (MSCs) can alter the behavior of both adaptive and innate immune cells, to induce a more anti-inflammatory or tolerant phenotype. In particular, MSCs may inhibit natural killer cell (NK) proliferation and activity, suppress T lymphocyte proliferation, dendritic cells (DCs) maturation and B lymphocyte proliferation and activation. Moreover, MSCs may induce regulatory T cell (Treg) expansion [1]. These immunomodulatory effects of MSCs are mediated by an anti-proliferative action, which is dependent both on cell-to-cell contact and on secreted factors. Several studies identified soluble mediators involved the MSC-immune-modulatory effects, such as interleukins (IL) 6 and 10, prostaglandin E2 (PGE2), hepatocyte growth factor (HGF), indoleamine 2,3-dioxygenase (IDO), nitric oxide (NO), transforming growth factor (TGF)-β1 and human leukocyte antigen G (HLA-G) (Table 1).

Recent studies indicated that besides soluble factors, MSCs secrete also extracellular vesicles (EVs) that may be involved in their immune-modulatory actions. EVs are composed of a lipid bilayer enclosing cytoplasmic components. In particular, EVs have been shown to deliver protein, lipid and nucleic acid content from one cell to another, thus contributing to the intercellular communication processes. By delivering their bio-active constituents EVs derived from MSCs (EV-MSCs) have been shown to mimic the pro-regenerative effect of the cells of origin in different experimental animal models [2]. Moreover, recently it has also been shown that EV-MSCs retain the immune-modulatory properties of MSCs.

2. Main paracrine factors involved in the immunomodulatory effects of MSCs

MSCs may suppress T-lymphocyte proliferation and functions both *in vitro* and *in vivo* [3–8]. Cell-to-cell contact is not a mandatory requirement for suppressing T-cell proliferation. MSCs produce soluble factors, including TGF-β1 and HGF, able to mediate suppression of T-cell proliferation. In fact, when TGF-β1 and HGF blocking antibodies are added in co-culture experiments of T cells/MSCs, the T cell proliferation is fully restored. These data indicate that TGF-β1 and HGF produced by MSCs work together, as T cell proliferation is more efficiently restored when both antibodies are used simultaneously [5]. MSCs can also inhibit T cell proliferation by increasing IL-10 and IDO secretion [9]. IDO is the rate-limiting enzyme involved in the catabolism of the essential amino acid tryptophan which is critical in T cell proliferation. Moreover, when co-cultured with T cells, MSCs are able to produce high concentration of PGE2 that is synthesized from arachidonic acid *via* the action of the constitutive cyclooxygenase-1 (COX-1) or the inducible COX-2 enzymes [3]. PGE2 is involved in the immunomodulatory effect

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Table 1
Main paracrine factors produced by MSCs involved in immunomodulatory effects.

Growth factors produced by MSCs	Target cells	References
TGF-β1	T cells	[5]
	NKs	[3]
	Treg	[16]
HGF	T cells	[5]
IL-10	T cells	[9]
IDO	T cells	[9]
	NKs	[3,11]
PGE-2	T cells	[3]
	DCs	[14]
	NKs	[3,11]
	Treg	[17]
NO	T cells	[10]
IL-6	DCs	[13]
HLA-G5	NKs	[17]
	Treg	[17]

of MSCs, since the inhibitors of PGE2 production diminish MSC-mediated immune-modulation *in vitro* [3]. Also nitric oxide (NO) mediates the immune-suppressive effect of MSCs on T cell proliferation and cytokine production [10].

IDO, PGE2 and TGF-β1 are also mediators of MSC inhibition of NK functions [3]. MSCs reduce proliferation and function of NKs and the presence of specific inhibitors of both IDO and PGE2 completely restores NK proliferation [11].

MSCs are also able to interfere with the maturation of DCs [12]. In particular, MSCs secrete IL-6 that is involved in the reversion of the maturation of DCs to an immature phenotype [13]. When PGE2 synthesis is inhibited in MSCs, DC differentiation and function are restored [14].

MSCs can also induce the generation of CD4+CD25 high T cells with a regulatory phenotype (Foxp3+) (Treg) in mitogen stimulated cultures of peripheral blood mononuclear cells [3,15]. MSCs produce and secrete TGF-β1 and PGE2 that contribute to the expansion of Treg [16]. The secretion of HLA-G5 also contributes to the capacity of MSCs to expand the CD4+CD25 high Foxp3+ Treg cells [17]. HLA-G5 contributes first to the suppression of allogeneic T-cell proliferation and then to the expansion of Treg cells as shown by blocking experiments *using* neutralizing anti-HLA-G antibody. Moreover, soluble HLA-G5 is able to inhibit both NK cell-mediated cytolysis and interferon-gamma secretion (IFN-γ) [17].

MSCs by secreting PGE2 prevent also the differentiation of CD4+ T cells into Th17 cells and inhibit *in vitro* the function of Th17 cells [18].

MSCs have also been shown to inhibit the proliferation without inducing apoptosis of B cells [19]. In addition, MSCs inhibit B-cell differentiation with consequent impairment of IgM, IgG, and IgA production [19]. CXCR4, CXCR5, and CCR7 B-cell expression, as well as chemotaxis to CXCL12, the CXCR4 ligand, and CXCL13, the CXCR5 ligand, are significantly down-regulated by MSCs, suggesting that MSCs affect chemotactic properties of B cells [19]. Another study indicates that MSCs are able to inhibit the proliferation of B cells only in the presence of IFN-γ that causes the production of IDO [20].

3. Role of the EVs in immuno-regulation

Recent studies have shown the involvement of EVs released from MSCs in their immune-modulatory properties. Cell-derived EVs are generally classified according to their size and intracellular origin. Exosomes are vesicles of 40–100 nm size and derived from the multi-vesicular bodies, the late endosomal compartment. Exosomes are secreted *via* fusion of multi-vesicular bodies with the plasma membrane [21]. Microvesicles, also known as shedding vesicles, are a heterogeneous population of vesicles directly derived from budding of the cell membrane [21,22]. It is difficult to distinguish between exosomes and microvesicles because of the overlapping characteristics and the lack of discriminating markers. Since cells concomitantly released exosomes and microvesicles, the inclusive term of EVs has been suggested [23].

EVs may contribute to cell-to-cell communication through different mechanisms. EVs express on their membranes specific receptors that can interact with ligands on target cells inducing functional changes. EVs may alter the fate of the recipients cells after their internalization by delivering transcriptional factors able to modulate the phenotype and/or behavior [24]. Moreover, EVs carry functional RNAs, and may act as vehicle for genetic exchange between cells [25–28]. For example, EVs released from embryonic stem cells may reprogram hematopoietic progenitors by delivery of mRNA and proteins [25] and EVs generated from endothelial progenitor cells can activate an angiogenic program in quiescent endothelial cells by an horizontal transfer of mRNA [27].

Depending on the cells of origin, EVs can exert immune-stimulatory or immune-suppressive activities [29,30]. Macrophages infected with Mycobacterium release EVs containing pathogen-derived pro-inflammatory molecules, inducing the secretion of pro-inflammatory cytokines [31]. EVs secreted by DCs are able to induce humoral responses against antigens processed by DCs before EVs purification, leading to strong protection against infection [32,33]. DC-derived EVs can shuttle both antigenic materials and MHC–peptide complexes, that can be presented to T lymphocytes. Alternatively, EVs can transfer the MHC complex and the antigen to neighboring DCs, that may present antigen to T cells [34]. Moreover, DC-derived EVs may induce activation of CD8+ T lymphocytes by themselves [35,36] or after incubation with DCs expressing allogenic MHC I [37,38].

EVs derived from tumors can stimulate DCs to initiate the immune response, but on the other hand, they have immunosuppressive properties favoring tumor cell escape from immune surveillance [39,40]. In particular, EVs from cancer cell lines or from tumor patients are able to induce *in vitro* T cell apoptosis *via* Fas ligand [41,42], to promote differentiation into Treg cells and to reduce CD8+ cells proliferation [43]. Moreover, tumor-EVs are able to decrease NK cell cytotoxicity [44,45] and to impair DC differentiation [46].

Intriguing, EVs produced by placenta are involved in feto-maternal tolerance [47]. Placenta secreted EVs express NKG2D ligands and are able to reduce cytotoxicity of NKs and CD8 cells [48].

4. Characterization of EVs derived from MSCs

EVs derived from MSCs express surface molecules that are characteristic of the cells of origin, such as CD29, CD73, CD44, and CD105 [49]. Moreover, EV-MSCs express some specific exosomal markers, such as CD107 [50,51], CD63, CD9 and CD81 [52,53].

Proteomic analyses indicated that MSC-EVs contain cytoplasmatic proteins associated with intracellular vesicle biogenesis and trafficking (RAB protein family), and proteins associated with self-renewal and differentiation (TGF-β, MAPK, PPAR, etc.) [54]. Treatment of EV-producing cells with specific growth factors can change the phenotype and the protein content of EVs. In particular, adipose derived stem cells (ASCs) treated with platelet derived growth factor produced EVs with enhanced angiogenic activity [55]. The stronger angiogenic potential is due to the different protein content, in particular to the presence of stem cell factor and its ligand, c-kit, inside. In contrast, when cultured with basic fibroblast growth factor ASC-EVs show a decreased expression of angiogenic factors and an up-regulation of an antiangiogenic miRNA result in a reduced angiogenesis both *in vitro* and *in vivo* [56].

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