Homing properties of mesenchymal stromal cells

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Abstract

A large number of clinical trials are underway to assess the therapeutic potential of mesenchymal stromal cells. This is based on preclinical observations that point to the probable efficacy of these cells. Very little is known however about the homing mechanisms of these cells to sites of injury and inflammation. This is important if one is to enhance this process to improve the therapeutic outcome. We have addressed this issue by highlighting what is known and through an analogy with leukocyte homing, and have identified possible areas of future research.

Keywords : cell migration, cell-based therapy, chemokine, homing, mesenchymal stromal cell, tissue injury

Mesenchymal stromal cells (MSCs) can be isolated with relative ease from a variety of tissues and can be used immediately following isolation (as part of the stromal vascular fraction) or expanded in tissue culture. Systemically administered MSCs are an attractive option in the context of regenerative medicine. They have the ability to migrate to and accumulate at sites of injury and inflammation to contribute to the repair and/or replacement of damaged tissue, largely through paracrine signalling or the activation of endogenous progenitor cells [1]. Efforts to enhance MSC homing to sites of injury and inflammation are hampered by a lack of understanding of the mechanisms of MSC recruitment and migration. Improved understanding of how these cells are recruited and how they exert their therapeutic effects will enable and even enhance their use in cell based therapies. In addition, by understanding the recruitment process, regenerative medicine could potentially enhance the body's ability to repair itself by recruiting endogenous stem and/or progenitor cells to sites of injury.

The classical model of leukocyte homing, which relies on interactions with the endothelium, is currently being used as a model to understand MSC trafficking. Leukocyte trafficking is mediated by complementary receptor-ligand pairs on endothelium and leukocytes. Adhesion molecules (selectins), integrins and chemoattractants (including chemokines and cytokines) mediate this effect. Leukocytes respond to activation signals and inflammation-induced cues through alterations in their trafficking molecules, resulting in a co-ordinated sequence of adhesive and signalling events. The leukocyte adhesion cascade involves three main steps: (1) selectin-mediated tethering and rolling; (2) chemokine-triggered activation and integrin-mediated adhesion; and (3) transmigration across the endothelium [2].

Mesenchymal stromal cell homing has been defined as "the arrest of MSCs within the vasculature of a tissue followed by transmigration across the endothelium" [3]. It has been suggested that this process may be mediated by both leukocyte-like and novel mechanisms [4]. There is thus evidence that infused MSCs home in response to inflammation or injury *in vivo* [3]. It is unclear however whether MSCs actively home to tissues using leukocyte-like cell-adhesion and transmigration mechanisms or whether they become passively entrapped in small-diameter blood vessels. Evidence in favour of specific MSC-endothelium interactions comes from integrin blocking and knockout studies that have reported reduced MSC engraftment [5,6]. Further studies in the setting of inflammation are however required to test this hypothesis. Mobilisation of resident MSCs seems to be directed by cytokines and/or

chemokines that are up-regulated under conditions of inflammation, which release MSCs into the circulation and down-regulate the adhesion molecules that retain them in their niche [7].

Functional CC chemokine receptors CCR1, CCR7, and CCR9 and CXC chemokine receptors CXCR4, CXCR5, and CXCR6 have been shown to be expressed on MSCs *in vitro* [8]. The migration of adipose-derived MSCs (AdMSCs) and bone marrow-derived MSCs (BM-MSCs) is believed to occur in response to various chemokines and cytokines. The exact repertoire of these factors that regulates MSC homing has not been defined, although Ponte *et al.* [9] suggest that BM-MSCs are under the control of a large range of growth factor receptor tyrosine kinases and CC and CXC chemokines. The chemotactic activity of chemokines on human AdMSCs appears to be less efficient than the chemotactic activity of growth factors [10]. Mesenchymal stromal cell migration *in vitro* has also been shown to be regulated by stromal derived factor-1 (SDF-1) and its receptor CXCR4 as well as by hepatocyte growth factor/c-Met interactions [11]. However evidence has been provided that SDF-1 pre-conditioning may not increase cell attachment or chemotaxis of BM-MSCs or fresh, unselected BM-mononuclear cells (BM-MNCs) [12]. Additional pre-conditioning strategies thus need to be investigated as possible activators of MSC migration.

The processes employed by exogenously infused or endogenous MSCs to migrate out of the circulation and across the endothelium to sites of injury has not yet been fully described. High-resolution confocal and dynamic microscopy has shown that BM-MSCs preferentially adhere to and migrate across TNF-α-activated endothelium in a vascular cell adhesion molecule-1 (VCAM-1) and G-protein coupled receptor (GPCR) signalling dependent manner [4]. TNF-α is an inflammatory cytokine usually produced by macrophages/monocytes during acute inflammation and stimulates the expression of a range of chemokines and adhesion molecules by endothelial cells. Interestingly, MSCs seem to have the ability to transmigrate between endothelial cells by paracellular diapedesis utilising discrete gaps and intercellular junctions or directly through pores in individual endothelial cells by transcellular diapedesis [4].

The study by Teo *et al.* [4] on MSC transmigration was performed under static conditions. In contrast, Chamberlain *et al.* considered shear stress in their investigations on the effect of chemokines during MSC transmigration [13]. They found that although murine BM-MSCs were unable to interact with murine aortic endothelial cells in the presence of continuous flow *in vitro*, these cells adhered to and

crawled on the endothelial surface when the flow was stopped for a short period and then reinitiated. The authors postulated that the lack of rolling of MSCs "is probably related to the lack of expression of L-selectin and the P- and E-selectin counterligands glycoprotein ligand-1 (PSGL-1) and sialyl Lewis X carbohydrates, reflecting the finding that MSCs are unable to bind functionally to constructs of P- and E-selectin". Interruption of flow is believed to have enabled chemokine presentation and firm adhesion of the MSCs to the endothelium. The chemokines CXCL9, CXCL16, CCL20 and CCL25 significantly enhanced transendothelial migration. It was also found that following adhesion and transmigration of MSCs across the endothelium, gene expression was altered and chemokine receptors (CXCR3, CXCR6, CCR6 and CCR9) were down-regulated.

An interesting question that still needs to be answered is how MSCs decelerate within the vasculature during the extravasation process. Chamberlain *et al.* postulated two different mechanisms [13]. Firstly, passive homing where the large size of MSCs reduces their velocity due to physical interactions with narrow capillaries leading to arrest and passive entrapment. A second mechanism involves active homing, in which MSCs actively tether to and roll on the activated vasculature leading to arrest and firm adhesion. The results from Chamberlain *et al.* indicate that MSCs are likely to utilise the first mechanism [13]. The MSCs were able to adhere firmly without leukocyte-like rolling prior to arrest. They just had to be slowed or be stationary to enable chemokine presentation and firm adhesion prior to crawling on and spreading over the endothelium. In contrast, the study by *Teo et al.* supports the existence of an active mechanism of MSC arrest at inflamed sites [14]. These authors found that platelets and possibly neutrophils play a significant role in regulating MSC trafficking and suggested that this may be as a result of secondary adhesive interactions [14].

Many inconsistencies still exist regarding the conditions under which MSCs are cultured. Since gene expression is significantly altered in tissue culture, it is imperative to clearly indicate the culture conditions used prior to performing homing experiments as this may have a significant impact on MSC function. De Becker and colleagues found that high culture confluence increased the production of the natural MMP-inhibitor TIMP-3 which decreased MSC transendothelial migration [15]. Passage number may also play a role as MSCs have been shown to gain and lose expression of certain cell surface markers during culture [3]. A small increase in passage number from passage 3 to passage 5 resulted in altered cell adhesion characteristics. At higher passage numbers an increased rate of attachment to fibronectin and decreased rate of chemotactic migration was observed [12]. The efficiency of homing

also seems to decline *in vitro* with prolonged culturing [15]. Since MSCs are purported to exist in hypoxic conditions *in vivo* and since tissue damage at ischemic sites occurs in a setting of low oxygen tension, it may be important to maintain these cells under conditions of hypoxia during culture and homing experiments *in vitro* as this may influence MSC motility and potency [16].

The use of *in vitro* models to study MSC transmigration cannot fully recapitulate the physiological setting in which MSC extravasation occurs *in vivo*. Generic inflammatory cues such as those induced by TNF-α cannot fully mimic the inflammatory response [4,12]. There is thus a need to take into account the influence of blood flow induced shear stress when designing therapeutic strategies in which MSCs are to be administered via the circulation. Understanding how the cells will engraft *in vivo* will require appropriate *in vitro* mimicking of the environmental conditions of target physiological sites. A change in future studies away from an intense focus on precisely defining the identity of MSCs using surface markers, to rather defining the functional characteristics of these cells in physiological and pathological settings, will almost certainly enhance their future clinical application.

It will be important to sequentially examine the whole process of MSC homing, beginning with MSC activation and mobilisation, transmigration across vascular endothelium and further migration into the parenchyma of inflamed and/or injured tissue. This will need to be studied in well-designed experiments *in vitro* as well as in appropriate models *in vivo* in order to be able to understand the mechanisms that regulate MSC homing at a molecular level. An in-depth understanding of these parameters will contribute to our understanding of the therapeutic potential of these cells and may lead to improvements in their clinical application.

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