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REVIEW

Homing and migration of mesenchymal stromal cells: How to improve the efficacy of cell therapy?

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Abstract

Mesenchymal stromal cells (MSCs) are currently being investigated for use in a wide variety of clinical applications. For most of these applications, systemic delivery of the cells is preferred. However, this requires the homing and migration of MSCs to a target tissue. Although MSC homing has been described, this process does not appear to be highly efficacious because only a few cells reach the target tissue and remain there after systemic administration. This has been ascribed to low expression levels of homing molecules, the loss of expression of such molecules during expansion, and the heterogeneity of MSCs in cultures and MSC culture protocols. To overcome these limitations, different methods to improve the homing capacity of MSCs have been examined. Here, we review the current understanding of MSC homing, with a particular focus on homing to bone marrow. In addition, we summarize the strategies that have been developed to improve this process. A better understanding of MSC biology, MSC migration and homing mechanisms will allow us to prepare MSCs with optimal homing capacities. The efficacy of therapeutic applications is dependent on efficient delivery of the cells and can, therefore, only benefit from better insights into the homing mechanisms.

Key words: Mesenchymal stromal cells; Homing; Bone marrow; Homing receptors; Extravasation

Core tip: Mesenchymal stromal cells (MSCs) are currently under investigation for use in a variety of clinical applications. In most studies, MSCs are administered systemically. This requires efficient homing and migration of the MSCs to a target tissue. However, the homing mechanisms of MSCs are not completely understood. Moreover, the *in vivo* homing and migration of MSCs does not appear to be highly efficient. Therefore, different methods have been investigated to improve homing. Here, we will review the current knowledge of bone marrow homing of MSCs, as well as the different strategies that might improve the homing capacity of these stem cells.

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INTRODUCTION

Mesenchymal stromal cells (MSCs) are non-haematopoietic cells that were first derived from the bone marrow and described approximately 40 years ago by Friedenstein *et al*[1]. In 2006, the International Society for Cell Therapy defined the minimal criteria to define human MSCs. They must adhere to plastic in culture and differentiate into osteocytes, chondrocytes and adipocytes. Additionally they must express CD105, CD90 and CD73 and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19, and HLA-DR surface molecules[2].

There is great interest in using these cells in a wide variety of clinical domains, such as Neurology, Orthopaedics, Cardiology and Haematology[3-6]. This interest arises from the following MSC characteristics: They have immunomodulatory capacities, they are multipotent and are thus possible effectors for tissue regeneration, and they tend to migrate to sites of tissue injury/inflammation[7-11]. Additionally MSCs might escape immune recognition, although conflicting observations about this particular phenotype have been published. MSCs do not express MHC class Ⅱ antigens, but the expression of these molecules can be upregulated after exposure to inflammatory cytokines or during MSC differentiation[12]. The data from animal studies suggest that MSCs can elicit allogeneic immune responses and be rejected[13-16]. On the other hand, there is also a report of MSCs that overcame this allogeneic immune response due to their immunomodulatory capacities[17]. von Bahr *et al*[18] addressed this issue and published follow-up data of patients treated with MSCs, showing that there was no correlation between the MSC source (donor-derived or third party) and the patients' response to the MSC treatment. The clinical applications of these cells have been extensively studied in Orthopaedics, where MSCs are used to repair large bone defects, and in Haematology for the treatment of graft-*vs*-host disease and support for the engraftment of hematopoietic stem cells[4,6,19]. In recent years, MSCs have been studied as vehicles to deliver anti-cancer treatments because there is evidence that MSCs home to tumour sites. They can be induced to express anti-cancer proteins [*e.g.*, interleukin (IL) 2], to produce pro-drug activating enzymes, which ensures that the active drug will only be localized in the tumour, or to deliver oncolytic viruses[20-23]. For these applications, the homing and persistence of MSCs in the target tissue are desirable[24].

When MSCs are used in clinical applications, different modes of administration are possible: Systemic administration [intravenous (IV) or intra-arterial (IA) injection] or local administration [intracoronary (IC) injection or direct injection into the tissue of interest]. Of these different options, IV injection is the most widely used because it is minimally invasive, the infusions can be readily repeated and the cells will remain close to the oxygen- and nutrient-rich vasculature after extravasation into the target tissue[25]. However, after IV injection, the cells appear to be trapped in the lungs, and thus efficient homing to the target tissues might be compromised. IA administration requires an invasive procedure that has a higher risk of complications than IV. Although IA injections might improve tissue-specific homing compared to IV, there is a concern that microthrombi might occur as a result of trapping large MSCs in the microvasculature. One example is the concern regarding IC injections of MSCs to treat myocardial infarction[26]. Similar concerns have been raised in studies that used MSCs to treat stroke[27,28]. A true local injection of MSCs might require a surgical intervention, such as that used in the repair of bone defects. In this setting, the MSCs are immediately delivered to the target tissue; however, the cells' survival might be compromised due to a lack of oxygen or nutrients[25]. Currently, haematopoietic stem cell transplantation is performed *via* an IV infusion. Intra-bone marrow transplantation is a more complex procedure, but evidence from an animal model suggests that this might improve the outcome of the treatment[29]. Finally, some animal models of systemic administration, such as intracardiac injection, cannot readily be performed in patients.

The systemic infusion of cells for therapeutic applications implies and requires efficient migration and homing to the target site. Although there is ample evidence of MSC homing, this process appears to be inefficient because only a small percentage of the systemically administered MSCs actually reach the target tissue[30]. The mechanisms by which the MSCs migrate and home are not yet clearly understood.

Currently, in Haematology, MSCs are mainly being tested for their ability to control graft-*vs*-host disease and to support haematopoiesis after haematopoietic stem cell transplantation. Chemo- and radio-therapy can damage the haematopoietic niche. MSCs are part of this niche and secrete a number of haematopoietic growth factors. To facilitate the engraftment of haematopoietic stem cells and stimulate blood formation, the MSCs should successfully home to and persist in the bone marrow[31]. In this review, we discuss current knowledge about MSC homing, specifically focusing on bone marrow homing (based on both *in vitro* and *in vivo* data), and we review the efforts that different groups have undertaken to improve the homing efficiency of these cells.

MSC HOMING AND MIGRATION TO BONE MARROW AND OTHER TISSUES

The exact mechanisms used by MSCs to migrate and home to tissues have not been fully elucidated. It is generally assumed that these stem cells follow the same steps that were described for leukocyte homing. In the first step, the cells come into contact with the endothelium by tethering and rolling, resulting in a deceleration of the cells in the blood flow. In the second step, the cells

are activated by G-protein-coupled receptors, followed by integrin-mediated, activation-dependent arrest in the third step. Finally, in the fourth step, the cells transmigrate through the endothelium and the underlying basement membrane[32].

The first studies addressing MSC homing examined the origin of the bone marrow MSCs after allogeneic bone marrow transplantation. Those groups all concluded that the haematopoietic cells were provided by the donor, but the stromal cells were provided by the recipient[33-35]. However, in these studies, the patients received marrow transplants containing only a limited number of MSCs - approximately 1/250000 nucleated cells at 35 years of age - in contrast to the purified MSC product that is used in the majority of clinical trials[36].

Since then, several studies in animal models and patients have shown that MSCs are capable of migrating and homing to a variety of tissues. Early studies of intra-uterine MSC transplantations in animal models showed that donor-derived non-haematopoietic cells were present in the bone marrow, thymus, spleen and liver[37,38]. Devine *et al*[30] and Chapel *et al*[7] performed MSC transplantations in non-human primates and observed MSCs in a variety of tissues, with highest numbers in the gastro-intestinal tract. The percentage of MSCs in the different tissues was estimated between 0.1% and 2.7%[7,30]. Erices *et al*[39] described the homing and survival of human cord blood-derived MSCs in the bone marrow of immunodeficient (nude) mice after systemic infusion[39]. Several studies in patients have also shown MSC homing[40-43].

A few groups have analysed the dynamics of MSC migration after systemic infusion using different techniques. Immediately after infusion, the MSCs are trapped in the lungs, and, subsequently, the cells are cleared from the lungs and distributed to other tissues[44,45]. The cells could be injected intravenously or intra-arterially for systemic infusion. The former is the least invasive method and the easiest to perform; however, as the MSCs were trapped in the lungs, different administration routes were examined. IA injection, which is already more risky because of the arterial puncture, also appears to entail a risk of development of microvascular occlusions called passive entrapment[27,46]. In addition, there have been reports that MSCs have a procoagulant activity[26,47]. A few years ago, a group from the Karolinska Institute reported that MSCs, particularly those that had been subjected to extended passaging and co-culture with activated lymphocytes, exhibited increased prothrombotic capacities; this effect was dose-dependent[47]. Gleeson *et al*[26] reported that MSCs express functionally active tissue factor. When MSCs were injected in the coronary arteries of a porcine myocardial infarction model, it resulted in a decreased coronary flow reserve. This effect could be reversed by the co-administration of heparin, an antithrombin agent[26].

Kyriakou *et al*[48] have studied the factors influencing short-term bone marrow homing of MSCs. The stem cells were observed in the bone marrow, spleen, liver and lungs 24 h after IV injection. It was observed that homing increased in younger animals and after irradiation but decreased with increasing passage numbers of the cells[48]. Several other groups have also shown that MSC homing improves after irradiation[7,8,30,49-52].

MOLECULES INVOLVED IN MSC (BONE MARROW) HOMING

The expression of molecules involved in MSC migration, homing and functionality has been widely studied.

Different molecules are involved/necessary for the different steps in the homing process. The selectins on the endothelium are primarily involved in the first step. For bone marrow homing in particular, the expression of haematopoietic cell E-/L-selectin ligand (HCELL), a specialized glycoform of CD44 on the migrating cell, is very important[53]. Although MSCs express CD44, they do not express HCELL[54].

The G-protein coupled receptors that are involved in the activation step are typically chemokine receptors. It has been extensively demonstrated that the CXCR4-stromal derived factor-1 (SDF-1) axis is critical for bone marrow homing[55]. Both molecules are very physiologically important, as knock-outs are lethal due to bone marrow failure and abnormal heart and brain development[56,57]. The expression of the chemokine receptor CXCR4 on MSCs is controversial. Some groups did not observe expression of the receptor, while other studies demonstrated that CXCR4 was expressed, albeit at low levels on the membrane, which affected migration in response to SDF-1[58-70].

Integrins are important players in the stable activation-dependent arrest in the third step of homing. It has been shown that the inhibition of integrin β1 can abrogate MSC homing[71]. Integrins form dimers that bind to adhesion molecules on the endothelial cells. Integrin α4 and β1 combine to form very late antigen 4 (VLA-4), which interacts with vascular cell adhesion molecule 1 (VCAM-1). It has been shown that the VCAM-1-VLA4 interaction is functionally involved in MSC homing[72,73].

In the final step of diapedesis or transmigration through the endothelial cell layer and the underlying basement membrane, lytic enzymes, such as the matrix metalloproteinases (MMP), are required to cleave the components of the basement membrane. In particular, the gelatinases MMP-2 and MMP-9 have important roles in this step because they preferentially degrade collagen and gelatin, two of the major components of the basement membrane[74,75]. We have shown that MSC migration is regulated by MMP-2 and tissue inhibitor of metalloproteinases 3 (TIMP-3)[76]. Membrane type 1 MMP (MT1-MMP) has also been reported to play a role in MSC migration[63]. MMPs are secreted as pro-enzymes. ProMMP-2 is activated by interactions with MT1-MMP and TIMP-2 and is inhibited by TIMP-1. This explains why the MMP-2, MT1-MMP or TIMP-2 knock-down decreased the invasive capacity of MSCs, and why TIMP-1 knock-down resulted in increased invasion in the study of Ries *et al*[77].

Table 1 gives an overview all of the migration and

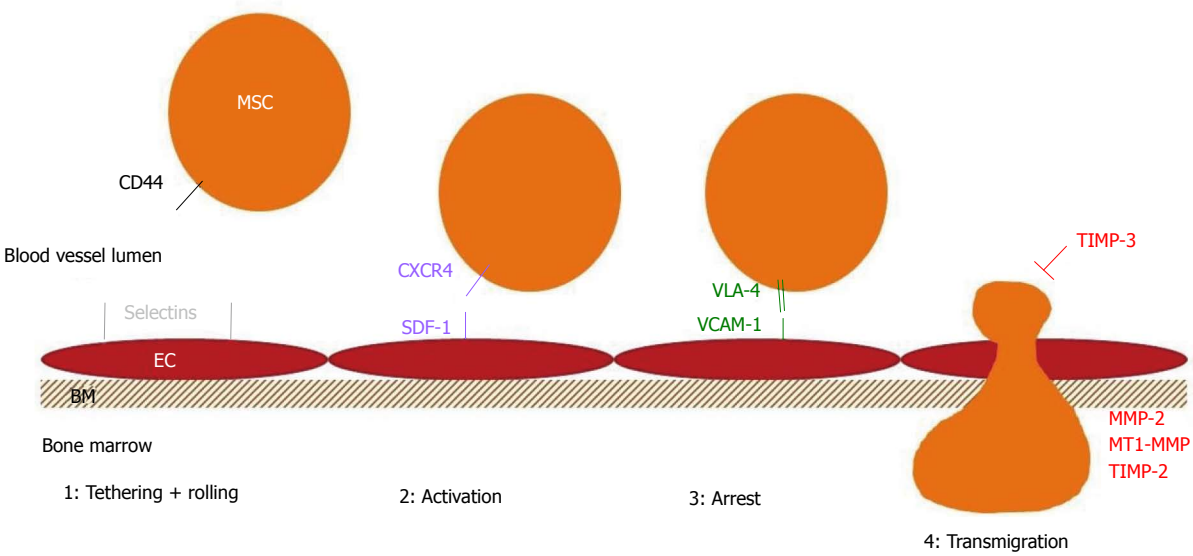

Figure 1 Overview of the homing molecules that are expressed on human mesenchymal stromal cells and known to be involved in the different steps of the bone marrow homing of mesenchymal stromal cells. EC: Endothelial cell; BM: Basement membrane; CD: Cluster of differentiation; SDF-1: Stromal cell derived factor 1; VLA-4: Very late antigen 4; VCAM-1: Vascular cell adhesion molecule 1; TIMP: Tissue inhibitor of metalloproteinases; MMP: Matrix metalloproteinase; MT1-MMP: Membrane type 1 matrix metalloproteinase; MSC: Mesenchymal stromal cell.

homing molecules that are reported to be expressed on human MSCs. Figure 1 shows a schematic overview of the molecules involved in human MSC bone marrow homing.

In addition to the expression of classic homing molecules, different groups have also described the expression of growth factor receptors on MSCs. Several studies have shown that growth factors can also induce MSC migration. For example, platelet-derived growth factor (PDGF) AB and BB can induce MSC migration *in vitro*[68,80,91]. Another growth factor involved in MSC migration is hepatocyte growth factor (HGF), which binds to c-met[63,68,80]. Both PDGF-BB and HGF have been loaded on gels or scaffolds as a means to improve the *in vitro* migration of MSCs[92,93].

HOW CAN WE IMPROVE THE HOMING EFFICIENCY OF MSCs?

Several groups have demonstrated MSC homing and migration, but only a small proportion of systemically administered MSCs actually reaches and remains in the target tissue[30]. Several factors are assumed to be involved. First, the expression of homing molecules on MSCs is limited. For example, the membrane expression of CXCR4, a critical receptor for homing to bone marrow, is very low, and some groups even claim there is no CXCR4 expression at all[58-70]. Another concern is that the MSCs appear to lose the expression of homing molecules during *in vitro* expansion[70,94]. Additionally, there is also heterogeneous expression of homing molecules in MSC cultures and in MSCs derived from different tissues (adipose tissue *vs* bone marrow), which show a different expression profile of homing molecules[95].

Because improving the homing efficiency to and retention of MSCs in a target tissue after systemic administration would improve their therapeutic effects, many groups are investigating methods to achieve this goal. Different strategies have been developed: the mode of administration could be modified, the MSC culture conditions can be adapted to optimize the expression of homing molecules, the cell surface receptors could be engineered to improve homing or the target tissue could be modified to better attract the MSCs. Again, we will mainly focus on the strategies that might improve the bone marrow homing of MSCs. The homing molecules involved in homing to bone marrow can also be of importance in homing to other organs or sites of injury, such as the CXCR4-SDF-1 interaction for homing to the injured myocardium[96]. However, we believe that methods that can upregulate or induce the expression of the homing molecules that are involved in bone marrow homing of MSCs are valuable. They show a potential means for improving bone marrow homing, even though the data supporting/proving this are not yet available. Figure 2 provides an overview of the methods that could be used to improve the bone marrow homing of MSCs.

Modification of the mode of administration

In vivo studies have repeatedly shown that MSCs are trapped in the lung after intravenous injection. When mice were treated with a vasodilator prior to MSC infusion, there was a clear decrease in the number of trapped MSCs in the lungs and a significant increase in MSC homing to the marrow of the long bones[44]. Yukawa *et al*[97] transplanted MSCs in combination with heparin treatment and found that this strategy also significantly decreased MSC trapping

Table 1 Overview of the homing molecules expressed on human mesenchymal stromal cells

Group	Molecule	Source	Transcript	Protein	Functional assay
Chemokine receptors	CCR1[70,77-82]	BM[70,77-79,81] WJ[79] AT[80] PB[82]	Yes[70,77,79,80]	Yes[70,77-82]	*In vitro* migration[70,77,78,80], *in vivo* tail vein injection in mice for tissue distribution[77]
	CCR2[68,78,81,82]	BM[68,78,81,82]	Yes[68,82]	Yes[68,78,81,82]	*In vitro* migration[68,78,82]
	CCR3[68,78,81-83]	BM[68,78,81,83] PB[82]	Yes[68]	Yes[68,78,81,82,83]	*In vitro* migration[68,78]
	CCR4[68,77,78,82]	BM[68,77,78,82]	Yes[68,77,82]	Yes[68,77,78] No[82]	*In vitro* migration[68,77,78,82], *in vivo* tail vein injection in mice for tissue distribution[77]
	CCR5[68,78,81-83]	BM[68,78,81,83] PB[82]	Yes[68]	Yes[68,78,81,82]	*In vitro* migration[68,78]
	CCR6[78,81,83]	BM[78,81,83]	Yes[82]	Yes[78,82]	*In vitro* migration[78]
	CCR7[70,78,80-83]	BM[70,78,81,83] AT[80] PB[82]	Yes[70,80,83]	Yes[70,78,80-83]	*In vitro* migration[70,78,83]
	CCR8[78,82,83]	BM[78,83] PB[82]	Yes[82]	Yes[78,82,83]	*In vitro* migration[78]
	CCR9[70,78,81-83]	BM[70,78,81,83] PB[82]	Yes[70,83]	Yes[70,78,81-83]	*In vitro* migration[70,78]
	CCR10[77,78,81,83]	BM[77,78,81,83]	Yes[77,83]	Yes[77,78,81]	*In vitro* migration[77,78], *in vivo* tail vein injection in mice for tissue distribution[77]
	CXCR1[78,81,82,84]	CB[84] BM[78,81,82] PB[82]	Yes[83,84]	Yes[78,81,82,84]	*In vitro* migration[78,83,84], *in vivo* injection in brain[84]
	CXCR2[62,78,81-83]	BM[62,78,81,83] PB[82]	Yes[62,83]	Yes[62,78,81-83]	*In vitro* migration[62,78,83], *in vivo* lung metastasis model[62]
	CXCR3[78,81-83]	BM[78,81,83] PB[82]	Yes[83]	Yes[78,81-83]	*In vitro* migration[78]
	CXCR4[60,62,65,66,68,70,76,78,80-83,85,90]	BM[60,62,68,70,76,78,81,83,85] CB[65,85,90] Foetal BM[66] AT[80] PB[82]	Yes[60,62,66,68,70,76,80,83,85]	Yes[62,65,66,68,70,76,78,80-83,85,90]	*In vitro* migration[60,62,65,66,68,70,76,78,80,83,85,90], *in vivo* lung metastasis model[62], tail vein injection in sublethally irradiated mice[66]
	CXCR5[68,70,77-83]	BM[68,70,77,78,81,83] WJ[79] AT[80] PB[82]	Yes[68,70,77,79,80,83]	Yes[68,70,77-83]	*In vitro* migration[68,70,77,78,80], *in vivo* tail vein injection in mice for tissue distribution[77]
	CXCR6[70,78,80-83]	BM[70,78,81,83] AT[80] PB[82]	Yes[70,80,83]	Yes[70,78,80-83]	*In vitro* migration[70,78,80]
	CXCR7[60,82]	BM[60] PB[82]	Yes[60]	Yes[82]	*In vitro* migration[60]
	CX3CR[82]	BM[82] PB[82]	Yes[82]	Yes[82]	
	XCR[82,82]	BM[82] PB[82]	Yes[82]	Yes[82]	
Adhesion molecules	VCAM-1[74,85,86]	BM[74,85,86] CB[86] AT[86]	Yes[85]	Yes[85,86]	*In vitro* migration[74]
	ICAM-2[85]	BM[85]	Yes[85]	Yes[85]	
	CD62[11,17,54,86-89]	BM[11,54,86-89] CB[17,86,87,89] AT[86-89] Skin[87]		Yes[11,17,54,86-89]	*In vivo* homing in a mouse model[54]
	LFA-3[85]	BM[85]	Yes[85]	Yes[85]	
	Integrin α1[11,85,87]	BM[11,85,87] CB[87] AT[87] Skin[87]	Yes[85]	Yes[11,85,87]	
	Integrin α2[85]	BM[85]	Yes[85]	Yes[85]	
	Integrin α3[11,85]	BM[11,85]	Yes[85]	Yes[11,85]	
	Integrin α5[11,85]	BM[11,85]	Yes[85]	Yes[11,85]	
	Integrin α6[85]	BM[85]	Yes[85]	Yes[85]	

	Integrin αv[85]	BM[85]	Yes[85]	Yes[85]	
	Integrin β1[11,86-88]	BM[11,86-88] CB[86,87] AT[86-88] Skin[87]		Yes[11,86-88]	
	Integrin β3[85]	BM[85]	Yes[85]	Yes[85]	
	Integrin β4[85]	BM[85]	Yes[85]	Yes[85]	
	ALCAM[17,87]	BM[87] AT[87] CB[17,87]		Yes[17]	
Proteases	MMP-1[90]	BM[90]	Yes[90]	Yes[90]	*In vitro* migration[90]
	MMP-2[65,68,74,76,77,85,90]	BM[68,76,77,85,90] CB[85]	Yes[68,76,77,85,90]	Yes[65,68,76,77,85,90]	*In vitro* migration[65,68,74,76,77,85,90]
	MMP-13[68,90]	BM[68,90]	Yes[68,90]	Yes[68,90]	*In vitro* migration[68,90]
	MT1-MMP[68,77,85]	BM[68,77,85] CB[85]	Yes[68,77, 85]	Yes[68,77,85]	*In vitro* migration[68,77,85]
	TIMP-1[68,77,90]	BM[68,77,90]	Yes[68,77,90]	Yes[68,77,90]	*In vitro* migration[68,77,90]
	TIMP-2[68,90]	BM[68,77,90]	Yes[68,77,90]	Yes[68,77,90]	*In vitro* migration[68,77,90]
	TIMP-3[76]	BM[76]	Yes[76]	Yes[76]	*In vitro* migration[76]
Growth factor receptors	c-met (HGF-R)[68,80,85]	BM[68,85] CB[85] AT[80]	Yes[68,80, 85]	Yes[68,85] No[80]	*In vitro* migration[85,68]
	PDGFRα[68,80,87]	BM[68,87] AT[80,87] CB[87] Skin[87]	Yes[68,80]	Yes[68,80,87]	*In vitro* migration[68,80]
	PDGFRβ[68,80,87]	BM[68,87] AT[80,87] CB[87] Skin[87]	Yes[68,80]	Yes[68,80,87]	*In vitro* migration[68,80]
	FGF-R1[80]	AT[80]	Yes[80]	Yes[80]	*In vitro* migration[80]
	FGF-R2[68]	BM[68]	Yes[68]	Yes[68]	*In vitro* migration[68]
	EGF-R[68,78]	BM[68,78] AT[80]	Yes[68,80]	Yes[68,80]	*In vitro* migration[68,80]
	IGF-R1[68]	BM[68]	Yes[68]	Yes[68]	*In vitro* migration[68]
	TIE-2[68]	BM[68]	Yes[68]	Yes[68]	*In vitro* migration[68]
	TGFRB2[80]	AT[80]	Yes[80]	Yes[80]	*In vitro* migration[80]
	TNFRSF1A[80]	AT[80]	Yes[80]	Yes[80]	*In vitro* migration[80]

BM: Bone marrow; CB: Cord blood; AT: Adipose tissue; WJ: Wharton's Jelly; VCAM: Vascular cell adhesion molecule; ICAM: Intercellular adhesion molecule; CD: Cluster of differentiation; LFA: Lymphocyte function associated antigen; ALCAM: Activated leukocyte cell adhesion molecule; MMP: Matrix metalloproteinase; TIMP: Tissue inhibitor of metalloproteinase; HGF: Hepatocyte growth factor; PDGFR: Platelet-derived growth factor receptor; FGF-R: Fibroblast growth factor receptor; EGF-R: Epidermal growth factor receptor; IGF-R: Insulin-like growth factor receptor; TIE: Tyrosine kinase with immunoglobulin-like and EGF-like domains; TGFR: Transforming growth factor receptor; TNFRSF: Tumour necrosis factor receptor superfamily.

in the lungs.

Pretreatment or priming of MSCs in culture or modifying the MSC culture conditions

Because MSCs appear to downregulate homing molecule expression during expansion, many groups are investigating different ways to induce or upregulate the expression of important homing molecules.

Much effort has been focused on increasing CXCR4 expression on the membrane. One way to achieve this is by adding cytokines or cytokine cocktails to the culture medium during expansion. Shi *et al*[66] showed that exposure to a combination of flt3 ligand, stem cell factor (SCF), IL 3, IL 6 and hepatocyte growth factor (HGF) increased both the intracellular and membrane expression of CXCR4 on cultured MSCs. More of the pretreated cells migrated towards an SDF-1 gradient, and there was no effect of the pretreatment on the function of the MSCs in supporting haematopoiesis. *In vivo* homing experiments where MSCs were intravenously injected into sublethally irradiated mice revealed a significant increase in bone marrow homing after the cytokine treatment[66]. Other molecules that have been shown to increase CXCR4 expression are insulin-like growth factor 1 (IGF-1), tumour necrosis factor α (TNFα), IL 1β, interferon γ (IFNγ)[68,98-100]. CXCR4 expression could also be upregulated by treating cultured MSCs with glycogen synthase kinase 3β (GSK-3β) inhibitors, resulting in an improved *in vitro* migration capacity, without affecting cell viability[101]. Exposure to complement 1q (C1q) has been shown to increase MSC migration towards SDF-1, although there was no significant increase in CXCR4 expression. Therefore, it was postulated that C1q exposure increases the MSCs' ability to sense SDF-1 gradients[65].

Treatments with GSK-3β inhibitors and C1q also increase MMP expression in MSCs, which are important for the degradation of the basement membrane during extravasation[60,101]. A combination of the haematopoietic growth factors erythropoietin (EPO) and granulocyte colony stimulating factor (G-CSF) has also been reported

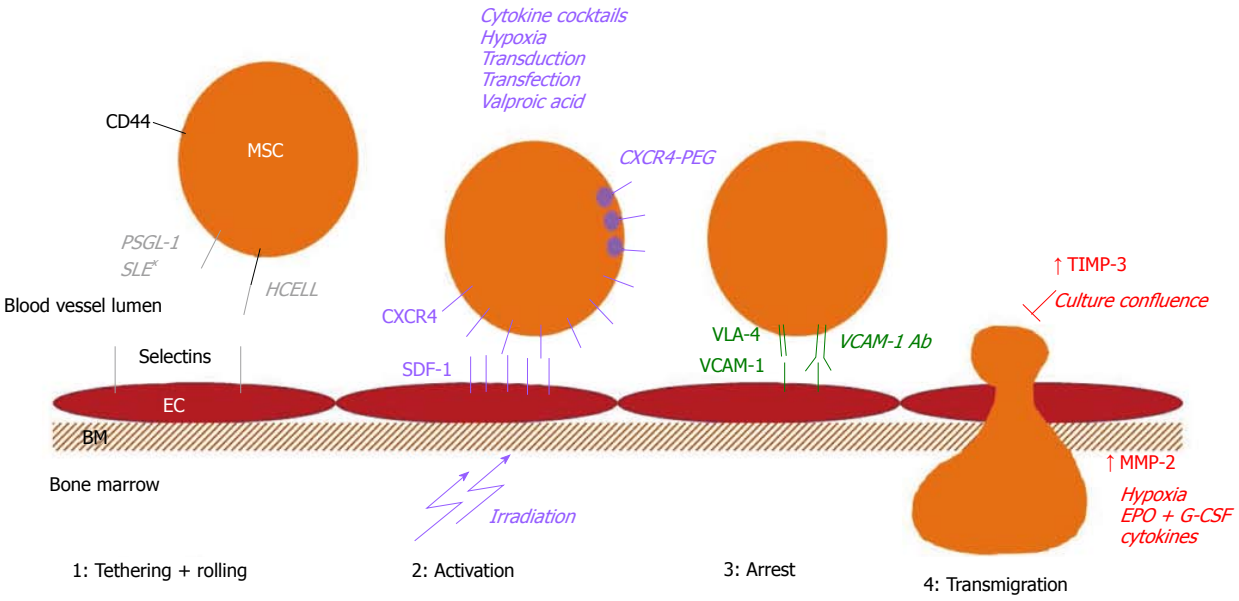

Figure 2 Schematic overview of the different strategies that can be used to improve homing in the different steps of mesenchymal stromal cell migration. CD: Cluster of differentiation; EC: Endothelial cell; BM: Basement membrane; HCELL: Hematopoietic cell E-/L-selectin ligand; PSGL-1: P-selectin glycoprotein ligand-1; SLEX: Sialyl Lewis X; SDF-1: Stromal cell derived factor 1; VLA-4: Very late antigen 4; VCAM-1: Vascular cell adhesion molecule 1; Ab: Antibody; TIMP: Tissue inhibitor of metalloproteinases; MMP: Matrix metalloproteinase; EPO: Erythropoietin; G-CSF: Granulocyte colony stimulating factor; MSC: Mesenchymal stromal cell.

to increase MMP-2 expression in MSCs and improve their motility[102].

There is also evidence that the epigenetic modulation induced by a short-term exposure to valproic acid results in increased expression of CXCR4 and MMP-2 in cultured MSCs and an increase in their migration towards SDF-1. There was no impact of this priming on the differentiation capacity of the cells[103].

Another approach that is under investigation is culturing MSCs under hypoxic conditions. Several groups have shown that these conditions result in increased CXCR4 expression and an improvement in MSC migration both *in vitro* and *in vivo*. This effect of hypoxia not only appears after short-term exposure but also in response to continuous culture in hypoxic conditions[104-108]. The increase in CXCR4 expression is reported to be regulated by an increase in hypoxia inducible factor (HIF) 1α[108]. Hypoxia also leads to differential expression of MMPs. For example, a decrease in MMP-2 secretion and an increase in MT1-MMP secretion and activity has been described in MSCs cultured under hypoxic conditions[104]. However, one could be concerned that culturing MSCs under hypoxia might change their behaviour. Valorani *et al*[109] reported that adipose tissue-derived MSCs cultured under hypoxic conditions exhibited an increased adipogenic or osteogenic differentiation capacity[109]. Crowder *et al*[110] reported that concurrent exposure to extreme hypoxia (0.5%) and a carcinogenic metal (nickel) induces carcinogenic changes in late passage MSCs. They did not observe these changes in early passage control cells[110].

A simpler modification of culture conditions is to maintain lower confluence. Our group found that MSCs that were cultured to complete confluence had a lower migration capacity than MSCs maintained at a low confluence. The cells cultured at higher confluence secrete more TIMP-3, an inhibitor of MMPs, which decreases migration compared to the MSCs cultured at low confluence[76].

Finally, MSCs are a heterogeneous cell population, and a particular subset of MSCs might have better homing abilities. MSCs were separated based on their expression of Stro1 and cultured further; these cells exhibited different migration capacities in NOD/SCID transplantation experiments. The amount of Stro1- MSCs was higher than the amount of Stro1+ MSCs in the target tissues of the mice, such as the bone marrow and spleen, after systemic administration *via* the retro-orbital plexus[111].

Genetic modifications

As already mentioned, MSCs express low levels of CXCR4, if any at all[58,59]. Because the CXCR4-SDF1 axis is important for bone marrow homing[20,112], many groups have designed transfection or transduction experiments in which CXCR4 expression plasmids are either nonvirally or virally introduced into the cells. Viral transduction is the most efficient method for obtaining high and stable expression levels in the target cells. CXCR4 overexpression resulted in improved MSC homing to the bone marrow after intracardiac injection into a NOD/SCID transplant model[112]. In a similar model, the overexpression of integrin α4, a subunit of VLA4 that interacts with VCAM-1, also resulted in increased bone marrow homing[113]. However, there are some draw-backs to this technique. Most importantly, there is the concern that the use of viral vectors to introduce the plasmid DNA poses a risk of insertional oncogenesis. Techniques for site-directed integration have been developed to circumvent

this problem[114]. Moreover, there is also a risk of adverse immune reactions and the production costs are high[115].

Different modes of non-viral transfection of plasmid DNA have been developed. One group overexpressed CXCR4 in MSCs using mRNA nucleofection. They obtained 90% expression of the surface receptor, but cell viability was only 62% and no increase in MSC homing could be observed[96]. Another group investigated the feasibility of inserting a short interfering RNA in MSCs using ultrasound and microbubbles to promote survival. A significant knock-down of the target (PTEN) could be obtained, but the cells were damaged after the manipulation[116].

Different modes of chemical, non-viral transfection have been studied, including the use of lipid agents. Although these techniques are easier to scale up and less expensive than viral transduction, they come with a price. The transfection efficiencies are significantly lower because approximately 35% of the MSCs express the transfected protein compared to over 90% of the cells after viral transduction[20].

Cell surface engineering

A method to improve homing efficiency of MSCs that has garnered interest in recent years is cell surface engineering, *i.e.*, a transient modification of the cell surface. Because transmigration through the activated endothelium takes 1-2 h, these transient alterations can be instrumental in improving MSC homing[117]. It has been shown that these modifications do not impact cell viability, proliferation, adhesion or differentiation[118-121]. For cell surface engineering, most groups focus on improving the first step of the homing process, tethering and rolling, by modulating the expression of adhesion molecules[54,118,120,121]. Since the first publications, many groups have developed different techniques for the cell surface modifications of MSCs.

A seminal paper in this field was published in 2008, when Sackstein *et al*[54] reported that they had converted the native CD44, which is readily expressed on MSCs, into the haematopoietic cell E-selectin/L-selectin ligand (HCELL) glycoform *ex vivo*[54]. E-selectin plays a key role in haematopoietic stem cell (HSC) homing to the bone marrow; however, MSCs do not express P-selectin glycoprotein ligand-1 (PSGL-1) or HCELL, the two E-selectin ligands that are required for HSC bone marrow homing, thus impairing their homing capacity to the bone marrow[54,122]. MSCs natively express CD44. In this study, Sackstein *et al*[54] were able to alter sialofucosylation *ex vivo* and transform CD44 into the HCELL glycoform. This treatment had no effect on the viability or phenotype of the cells. *In vivo* homing experiments that injected MSCs into the tail veins of NOD/SCID mice showed that the HCELL+ MSCs homed to the bone marrow, even in the absence of CXCR4, in contrast to the unmanipulated MSCs[54].

Sialyl Lewis X (SLE^X) is the active site of PSGL-1. Therefore, introducing this molecule into the MSC cell membrane should also lead to improved MSC homing. Sarkar *et al*[118] used biotinylated microvesicles to modify the MSCs. When the vesicles were brought into contact with the MSCs, they integrated into the cell membrane, thus generating biotinylated MSCs. Using a streptavidin linker, biotinylated SLE^X could be immobilized on the cell surface. The accessibility of the lipids integrated in the cell membrane was assessed and the researchers found they could still be detected after 4 h, but the intensity had already decreased to 50% compared with that at 0 h. After 8 h, all signals were lost, confirming that the modification is indeed transient. *In vitro* tests showed that the SLE^X-expressing MSCs exhibited improved adhesion under shear stress compared to the sham-treated MSCs[118].

Cheng *et al*[120] described a rapid (30 min) procedure to conjugate peptide K, an E-selectin binding peptide, to the MSC membrane. The MSC viability and proliferation rates were normal after engineering and their differentiation capacity was also maintained. In an *in vitro* model of inflamed endothelium, they subsequently demonstrated that the engineered MSCs adhered better than the control MSCs under shear stress[120].

Lo *et al*[121] described yet another engineering method to improve MSC binding to selectins and facilitate tethering and rolling. The first 19 amino acids of PSGL-1 (Fc19) were combined with an IgG tail and with an SLE^X glycan to engineer a pan-selectin-binding ligand. Tests in flow chambers showed that these MSCs were indeed capable of adhesion under shear stresses[121].

However, adhesion molecules are not the sole targets of the cell surface engineers. There is also interest in conjugating antibodies to the cell surface. Protein painting is a technique that binds antibodies to the cell surface. First, the palmitated proteins acting as docking stations for the antibodies are integrated into the cell membrane, and, subsequently, antibodies can be bound to the cell without losing affinity and with no impact on the viability and differentiation potential of the engineered cells[123]. One example using this technique is the binding of intercellular adhesion molecule (ICAM)-1 antibodies to MSCs, which increased the binding of these cells to endothelial cells[124]. This same protein painting technique has been applied to express VCAM-1 antibodies on MSCs, resulting in improved homing. In this study, the target tissues were the mesenteric lymph nodes and the colon. However, this technique might also be applied to improve homing to other organs, such as the bone marrow, because VCAM-1 is implicated in the bone marrow homing of MSCs[125].

Recently, a method was also described in which recombinant CXCR4 is bound to the cell surface of MSCs using lipid-PEG. In a one-step mixture procedure, recombinant CXCR4 could be transiently expressed on MSCs, leading to migration towards SDF-1 in a concentration-dependent manner[119].

Modification of the target tissue

Finally, MSC migration and homing can be influenced by modifying the target tissue. In early homing studies, it was

already shown that altering the target tissue by irradiation increases MSC homing[7,8]. After chemo- and radio-therapy, there are increased levels of SDF-1 in the bone marrow, thus increasing its attraction for HSCs and MSCs[126]. There are also reports of manipulating MSC migration with ultrasound or magnetic or electric fields[127-129]. However, these techniques do not appear to be very practical and they need adequate expression of homing molecules. For example, application of electrical fields could induce heat and electrochemical products near the electrodes. On the other hand, ultrasound-guided delivery might be more challenging in deep organs. Finally, homing directed by a magnetic field might require the implantation of a magnet in or near the tissue of interest[127-129].

Caveats in modifying homing molecules

In animal models and clinical studies, only limited engraftment or no engraftment at all is often observed, raising the question of whether tissue-specific homing is required for the therapeutic effect of MSCs[30,42]. A study on the use of systemically administered MSCs for the treatment of stroke in an animal model also showed very limited migration of MSCs to the tissue of interest, the brain. However, the researchers found that MSC homing to the spleen was important and correlated with a reduced infarct size and peri-infarct inflammation. They propose that MSCs exert a beneficial effect by abrogating secondary, inflammation-related cell death[130]. These data show that tissue-specific MSC homing is important, even though the target tissue is not the brain, as one would expect in a stroke model. Fernández-García *et al*[131] performed cotransplantation studies with MSCs and HSCs and found that cotransplantation improves short- and long-term haematopoietic reconstitution. This was the result of MSC and HSC interactions, and they propose that MSCs act as carriers that facilitate HSC homing to the bone marrow[131].

Manipulating stem cells, such as MSCs, to improve their homing capacities might not only change their migratory capacities but also have other consequences. For example, Liu *et al*[132] claim that the CXCR4-SDF-1 axis plays an important role in MSC survival because MSCs pretreated with SDF-1 exhibited significantly improved survival and proliferation. These effects could be partially inhibited by AMD3100, an inhibitor of CXCR4[132]. The pretreatment of MSCs with cytokines also revealed some conflicting observations. In a recently published paper, Kavanagh *et al*[133] report that licensing murine MSCs with inflammatory cytokines does not improve homing to the injured gut in an ischaemia/reperfusion model in their hands. More importantly, they found that while the untreated MSCs improved tissue perfusion, this effect was abrogated with the pretreated MSCs[133]. However, another group reported positive effects of pretreatment on the biological functions of the MSCs. Szabó *et al*[134] found that licensing murine MSCs with pro-inflammatory cytokines resulted in a significant reduction in the variability in immunosuppressive capacities of these MSCs. This reduction in variability was due to an increased immunosuppression of clones that were poor inhibitors of T-cell proliferation prior to licensing[134].

The pretreatment of MSCs with different factors or conditions, *e.g.*, hypoxia and inflammatory cytokines, could also modify their response to these treatments. Naaldijk *et al*[135] found that the oxygen concentration (normoxia *vs* hypoxia) alters the response of rat and human AT MSCs. They also found that the migration of MSCs isolated from older donors (rat and human) was not significantly impaired compared with the MSCs from young donors[135]. In contrast to this last finding, Choudery described that MSCs from aged mice exhibit diminished effectiveness and increased expression of apoptotic and senescent genes[136].

In this review, we have described different techniques for improving MSC homing and the expression of homing molecules on MSCs. Importantly, however, the expression of homing molecules and the resulting migration, homing and biological functions of MSCs might easily be altered unintentionally. Currently, many different protocols are used to expand MSCs for *in vitro*, animal and clinical studies. These variables can have a major impact on the expression of the homing molecules and the biological functions of MSCs; we will briefly discuss this below.

MSCs were first isolated from bone marrow. Since then, MSCs have been isolated from a wide variety of tissues, including adipose tissue (AT), umbilical cord blood (CB), Wharton's jelly (WJ), *etc*.[59,79,80,82]. Several groups have reported differences in the expression of homing molecules in human MSCs isolated from different sources; these are listed in Table 1. Additionally, the MSCs derived from different sources also exhibit differences in their biological functions. For example, AT MSCs might have better immunosuppressive capacities than bone marrow MSCs[95]. On the other hand, bone marrow MSCs appear to be the only MSCs that are capable of forming a haematopoietic niche that can support human haematopoietic tissue in an *in vivo* model[87].

When using MSCs for organ-specific treatments, one might choose to induce differentiation *in vitro* before transplantation. However, *in vitro* differentiation might not always result in a clinical benefit during MSC therapy. In a study using human CB MSCs in a mouse model for liver disease, the researchers found that hepatic differentiated MSCs performed worse than the undifferentiated MSCs. The differentiated MSCs showed decreased expression of the homing molecules and decreased *in vivo* migration after IV infusion. Additionally, their immunosuppressive capacity was decreased and the expression of HLA DR was increased, thus increasing their immunogenicity[137]. Ullah *et al*[138] also found that chondrogenic differentiated human MSCs exhibited a significantly reduced *in vitro* migration capacity than undifferentiated MSCs. However, CCR9 expression and *in vitro* migration to its ligand, CCL25, were retained in the differentiated MSCs[138].

Many parameters in MSC cultures vary between different research groups, including seeding density, number of passages, basal medium, and growth supplements [foetal bovine serum (FBS) *vs* platelet lysate (PL)]. All of these

factors might have an important impact on MSC function and migration. For example, Cholewa *et al*[139] found that PL increased MSC proliferation and increased the number of population doublings before senescence compared to FBS. However, they also showed that seeding MSCs at lower densities selected a highly migratory MSC population[139]. There are also reports of MSCs losing their migratory capacity and/or expression of homing molecules after *ex vivo* expansion[48,94]. After culture, MSCs are harvested with trypsin to detach them for passaging. Chamberlain *et al*[140] reported that the cell surface expression of chemokine receptors was decreased when the cells were detached with trypsin.

Future research directions

As described above, there is currently substantial variability in the isolation and expansion protocols for MSCs. Research on MSC homing and migration would clearly benefit from standardized MSC expansion protocols. What appears to be a rather minor aspect of the expansion protocol might have a significant impact on MSC function and/or migration. Thus, standardizing MSC expansion protocols would minimize unintentional modifications of the homing molecules. Of course, different culture conditions should be compared to create an optimal expansion protocol. Once this protocol is defined, it will also be easier to evaluate therapeutic efficacy of MSCs in clinical settings. It may be that different clinical applications require different expansion protocols to obtain the desired therapeutic effect.

We summarized the strategies for improving MSC homing. Many of these methods have not yet been validated *in vivo*. Before they can be translated to the clinic, the techniques with the most promising results should be first validated using *in vivo* homing models. In these experiments, the migration of engineered MSCs should be compared with the migration of untreated cells, and the therapeutic efficacy of the treated MSCs can also be assessed in animal disease models.

Although MSCs are widely studied and used in many clinical trials in a variety of clinical domains, little is known about the exact mechanisms by which MSCs exert certain therapeutic effects and their homing to certain tissues. Further studies would benefit from a better understanding of MSC biology. Understanding whether and where MSC migration or homing is necessary can help to define the optimal expansion protocols.

Finally, when transitioning to clinical trials, all conditions should be strictly defined, and, ideally, randomized controlled trials would be designed.

CONCLUSION

MSCs are interesting effector cells that can be used in a variety of therapeutic applications. Systemic administration is often the preferred route of delivery. However, this approach requires that adequate numbers of MSCs migrate and home to the target tissue(s). MSCs do not express many homing receptors, which impairs their migration capacity and hampers their therapeutic efficacy. Studies are ongoing and are needed to further elucidate the MSC homing mechanisms. A better understanding of MSC homing, as well as the factors influencing this process, will allow researchers to optimize the migration capacities of these stem cells and their therapeutic effects in a target tissue.

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