Histol Histopathol (2013) 28: 1427-1438
DOI: 10.14670/HH-28.1427

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Signaling molecules and pathways involved in MSC tumor tropism

Ivy AW Ho[1] and Paula YP Lam[1,2,3]

[1]Laboratory of Cancer Gene Therapy, Humphrey Oei Institute of Cancer Research, National Cancer Centre of Singapore, Singapore, [2]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore and [3]Duke-NUS Graduate Medical School, Singapore

Summary. Human bone marrow is a reservoir containing cells with different self-renewal capabilities, such as mesenchymal stem cells (MSC) and hematopoeitic stem cells (HSC). MSC in particular have been increasingly used in preclinical and clinical treatment of tissue regenerative disorder. Understanding the molecular mechanisms underlying MSC homing and mobilization is critical to the design of rational cell therapy approaches. In this review, we will discuss the key molecular mechanisms that govern the homing of MSC to bone marrow, the mobilization of MSC to tumors and injured sites via circulation, and strategies that enhance MSC migration.

Key words: Mesenchymal stem cells, Migration, Tumor tropism

Introduction

Mesenchymal stem cells (MSC), also known as mesenchymal stromal cells, are non-hematopoietic stem cells that can be isolated from various tissues, such as bone marrow, adipose tissue, umbilical cord, cord blood and Wharton's jelly. Thus far, MSC are identified according to the criteria set forth by the International Society for Cell Therapy that described MSC as plastic adherent cells that express CD73, CD90 and CD105; are immunonegative for the monocyte and macrophage marker CD14, hematopoietic markers CD34 and CD45, and the Major Histocompatibility Complex (MHC) class II surface receptor HLA-DR; can be differentiated into osteoblasts, adipocytes and chondrocytes when cultured in defined medium (Dominici et al., 2006). Besides mesodermal lineage-specific differentiation, neuronal and astrocytic-lineage differentiation were also observed (Wislet-Gendebien et al., 2005; Li et al., 2011). The multipotent potential of MSC renders it an excellent cell source for regenerative medicine. MSC have been used in clinical trials for the treatment of myocardial infarction (Behfar et al., 2010; Jorgensen et al., 2010; Trachtenberg et al., 2011), spinal cord injury (Bhanot et al., 2011; Park et al., 2012), cartilage and bone injury (Qi et al., 2012). Moreover, MSC are poorly immunogenic due to low expressions of MHC class I and absence of MSC class II (Barry et al., 2005), rendering it ideal for treating inflammation-associated disorders such as graft-versus-host disease (GVHD) (reviewed by Sato et al. (2010)) and Crohn's disease (Duijvestein et al., 2010; Ciccocioppo et al., 2011). To date, the most impressive effect of MSC in regeneration and tissue repair was observed in the treatment of GVHD after allogeneic transplantation of HSC, whereby infusion of MSC into patients with steroid resistant grade III and IV disease markedly improved clinical response with no side effects or toxicity (Le Blanc et al., 2008; Prasad et al., 2011).

In the laboratory, MSC have been shown to migrate to and stimulate repair of pancreatic islet and renal glomeruli in a model of diabetes in NOD/SCID mice (Lee et al., 2006). In addition, MSC migrated to coronary artery occlusion-induced ischemic brain following intrathecal administration promoted neurological recovery and reduced ischemic damage (Lim et al., 2011). MSC also supported hematopoietic stem cells (HSC) transplantation in the bone marrow microenvironment and hematopoietic recovery after myeloablative therapy (Koc et al., 2000). Because a tumor is known as a "wound that never heals," it was hypothesized that the tumor milieu may be an ideal environment for the engraftment of exogenous MSC. Indeed, MSC were found to specifically home to and

Offprint requests to: Dr. Paula Y.P. Lam, Laboratory of Cancer Gene Therapy, Cellular and Molecular Research Division, Humphrey Oei Institute of Cancer Research, National Cancer Centre of Singapore, 11, Hospital Drive, Singapore 169610. e-mail: cmrlyp@nccs.com.sg

integrate into melanoma and orthotopic glioma when administered systemically (Studeny et al., 2002, 2004; Nakamizo et al., 2005). Although local or systemic transplantation of MSC has proven to be beneficial and suggests a potential for clinical use, the migration of MSC towards the target region is not well elucidated. In this article, we will review the signaling cascade that has been implicated in the mobilization of MSC to and from bone marrow with emphasis on its tumor tropism properties (Fig. 1).

Signals that govern the homing of MSC to the bone marrow versus mobilization into the circulation

MSC mobilization has been postulated to be similar to the migration of leukocytes and HSC. Leukocyte migration is well documented and involves the activation and presentation of selectins on the cell surface, which initiates leukocyte rolling. This is followed by cell adhesion that involves molecules such as vascular cell adhesion molecule (V-CAM) and its interacting protein, very late antigen (VLA)-4, and hyaluronic acid/CD44, followed by transendothelial migration that is facilitated by matrix metalloproteinases (MMP) (Ley et al., 2007; Woodfin et al., 2010; Hess and Allan, 2011). HSC migration involves the interaction of chemokine (C-X-C) receptor (CXCR)-4 with its ligand, stromal cell-derived factor (SDF)-1 (also known as chemokine (C-X-C motif) ligand (CXCL)-12) (Peled et al., 1999; Broxmeyer et al., 2005; Dar et al., 2006), which not only directs migration of HSC, is also implicated in regulating survival and engraftment of the cells. Activation of SDF-1 has been shown to stimulate the migration of stem cells from the bone marrow reservoir into circulation (Dar et al., 2006). Furthermore, SDF-1-induced migration of MSC could be effectively inhibited by CXCR4-specific antagonist AMD 3100 (Ryu et al., 2010; Song et al., 2010; Park et al., 2011). However, only a small fraction of MSC has been shown to express functional cell surface CXCR4 receptors, although intracellular CXCR4 could be detected (Wynn et al., 2004; Brooke et al., 2008). Findings from Ip and

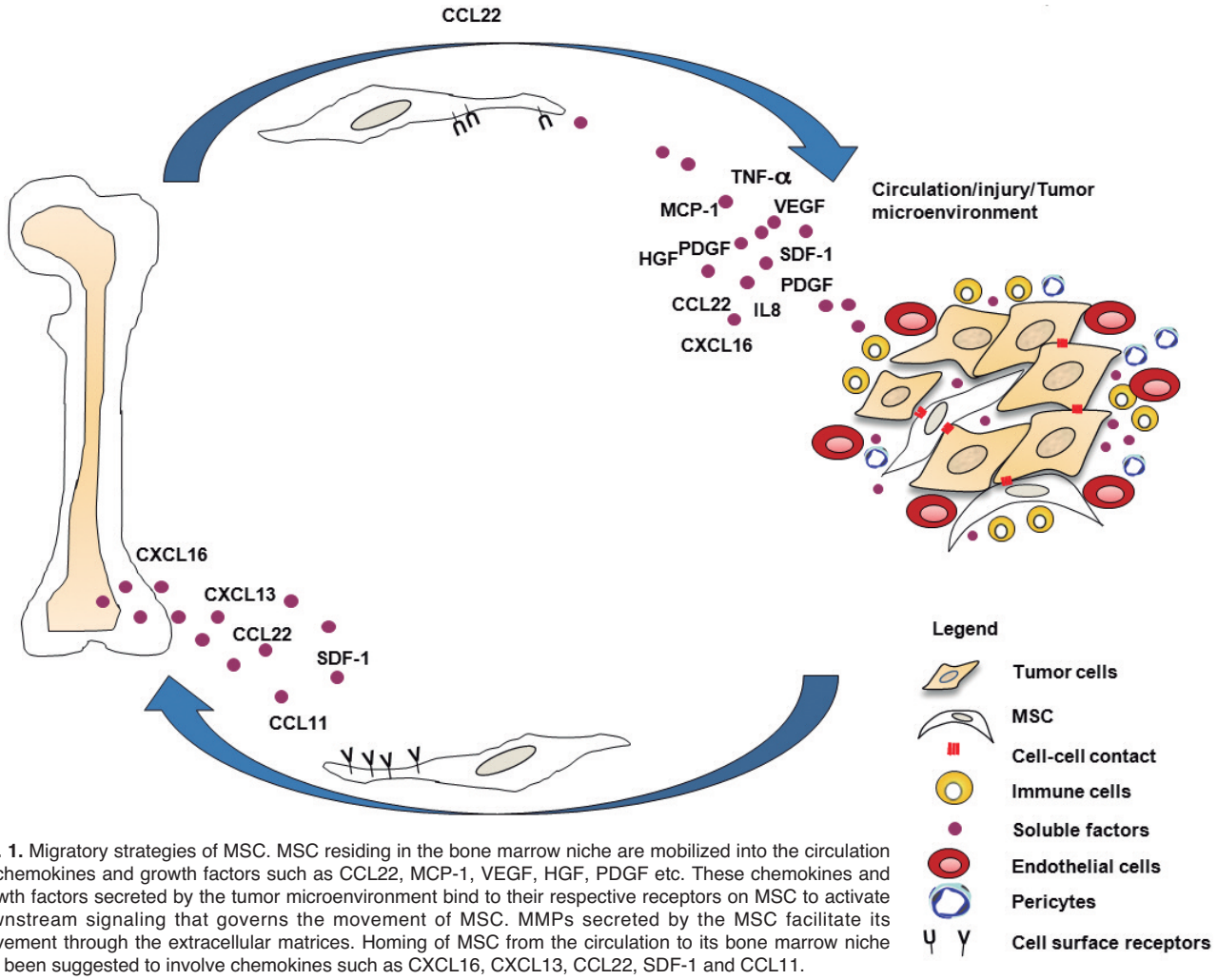

Fig. 1. Migratory strategies of MSC. MSC residing in the bone marrow niche are mobilized into the circulation by chemokines and growth factors such as CCL22, MCP-1, VEGF, HGF, PDGF etc. These chemokines and growth factors secreted by the tumor microenvironment bind to their respective receptors on MSC to activate downstream signaling that governs the movement of MSC. MMPs secreted by the MSC facilitate its movement through the extracellular matrices. Homing of MSC from the circulation to its bone marrow niche has been suggested to involve chemokines such as CXCL16, CXCL13, CCL22, SDF-1 and CCL11.

co-workers suggested that MSC do not require CXCR4 for myocardial migration and engraftment (Ip et al., 2007). At present, it is difficult to conclude the precise role of CXCR4 in MSC migration due to the transient cell surface expression of CXCR4. Even though CXCR4 may be present in the bone marrow (Wynn et al., 2004) and ischemic tissues (Kubo et al., 2009), it is often absent on the surface of culture-expanded MSC, especially during late cell passage (Honczarenko et al., 2006). Thus, further studies are required to delineate the role of CXCR4/SDF1 axis in MSC migration.

Apart from CXCR4, MSC also respond to growth factors, chemokines and cytokines released from injury sites. MSC are known to express a broad range of chemokine receptors including CXCR1, CXCR3, CXCR4, CXCR5, CXCR6, chemokine (C-C motif) receptor (CCR)-1, CCR2, CCR3, CCR4, CCR5, CCR9 and others. Recent results from Middleton lab suggested that chemokines such as CXCL12, CXCL13, CXCL16, chemokine (C-C motif) ligand (CCL)-11 and CCL22 and their receptors can enhance the bidirectional migration of MSC to and from the bone marrow niche (Smith et al., 2012). There is evidence to suggest that specific chemokines are involved in the unidirectional migration of MSC. For example, CXCL16 (ligand for CXCR6) was most effective in the homing of MSC into the bone marrow, while CCL22 (ligand for CCR4) has the strongest chemotactic effect in mobilizing MSC from the bone marrow into the circulation (Smith et al., 2012). CXCL16 are present in most organs and inflammation-associated cancers such as prostate cancer cells, lung carcinoma, hepatocellular carcinoma and colorectal cancer (Darash-Yahana et al., 2009). In normal physiological conditions, CCL22 are expressed in macrophage, monocyte-derived dendritic cells and thymus (Katou et al., 2001). CCL22 expression could also be detected in malignant brain and ovarian cancers, and are found closely associated with infiltrated regulatory T cells (Tregs) (Curiel et al., 2004; Jacobs et al., 2010), suggesting that CCL22/CCR4 signaling may be involved in mobilization of monocytes and T lymphocytes to pathological lesions. Given that CXCL16 and CCL22 are detectable in tumors, it is plausible that the mobilization cues to injury site and tumor are similar to those that direct MSC into the bone marrow.

Mobilization of MSC to tumor

The tumor microenvironment resembles an inflamed site, with a repertoire of macrophages, mast cells, myeloid progenitors and endothelial cells *et cetera* that orchestrate tumor metastasis, growth and neo-vascularization. Changes in the tumor microenvironment will thus affect MSC migration. Solid tumors are often hypoxic as a result of inadequate oxygen supply, and tumors with increased hypoxia are known to express elevated levels of pro-inflammatory mediators, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and monocyte chemoattractant protein (MCP)-1. Most of these factors and cytokines act in a paracrine fashion on MSC to activate signaling pathways involved in cell motility. For instance, recruitment of MSC to breast cancer cells is enhanced via the binding of IL-6 secreted by the tumor cells to the corresponding receptors on the MSC (Rattigan et al., 2010). Likewise, hypoxia upregulates hypoxia-inducible factor (HIF)-1α expression and enhances the promoter activity of hepatocyte growth factor (HGF) (Rosova et al., 2008), TNF-α (Teo et al., 2012) and platelet-derived growth factor (PDGF) (Bos et al., 2005). In this section, we will briefly introduce these factors, their receptors and their role in mediating tumor-specific MSC migration.

TNF-α

TNF-α is a pro-inflammatory cytokine that binds to TNF-Receptor 1 (TNF receptor superfamily member 1A (TNFRSF1A); CD120a; p55TNFR) and TNFR-beta (TNFRSF1B; CD120b; p75TNFR) and is secreted by macrophages (Verma et al., 2012). Furthermore, TNF-α regulates the expression of various cytokines, chemokines, soluble factors and MMPs that may play a role in MSC migration. Pre-treatment of MSC with TNF-α was shown to upregulate HGF (Zhang et al., 2010), CXCR4 (Egea et al., 2011), MMP-2, MT1-MMP and other MMPs (Ries et al., 2007) expression and activity. Increased transcriptions of these proteases are associated with enhanced invasive potential of MSC. TNF-α also increased the expression of cytokines and chemokines such as IL-8, MCP-1 and IL-6 (Lee et al., 2010), which mediate MSC migration through the activation of urokinase plasminogen activator (uPA) and its receptors (uPAR) on human tumor cells (Gutova et al., 2008). TNF-α may also increase the adherence properties of MSC to endothelial cells during extravasation (Segers et al., 2006) by upregulation of cell adhesion molecules such as intercellular cell adhesion protein (ICAM-1, CD54) (Fu et al., 2009), VCAM-1 (Xiao et al., 2012) and neural CAM (N-CAM) (Shi et al., 2012). High resolution imaging techniques showed that the adhesion of MSC to endothelial cells is VCAM dependent; blocking antibodies to VCAM-1 and its ligand VLA-4 inhibited TNF-α-meditated MSC adhesion and transmigration through the endothelial cells (Teo et al., 2012). Other molecules that are known to be upregulated byTNF-α include CCR4, a receptor for CCL22 that has been shown to attract MSC.

HGF/c-Met

HGF, also known as scattered factor, is secreted by stromal fibroblast and smooth muscle cells while its receptor c-Met is expressed on MSC (Neuss et al., 2004). HGF/c-Met signaling has been shown to promote hepatocytes and endothelial cell proliferation, and to regulate the interaction between epithelial and mesenchymal cells during development (Morishita et al.,

1998). Because HGF/c-Met signaling also plays a role in angiogenesis (Morishita et al., 1998), and HGF expression has been shown to be activated by tissue injury, it has been suggested that HGF/c-Met signaling may facilitate wound healing and tissue regeneration through the recruitment of stem cells (Neuss et al., 2004). Indeed, intramuscular injection of MSC overexpressing HGF into dilated myocardium improved ventricular function, enhanced angiogenesis, suppressed fibrosis and increased recruitment of cardiac progenitor cells (Shabbir et al., 2009). Furthermore, Janowska-Wieczorek and colleagues found that MSC from either bone marrow or umbilical cord migrated towards HGF-rich environment (Son et al., 2006), thus confirming the role of HGF/c-Met signaling in MSC migration. The migration of MSC to injured tissues is well established, although not much has been reported regarding its role in MSC migration toward tumors. It was recently found that HGF expression in MSC is upregulated by the pro-inflammatory molecule TNF-α (Zhang et al., 2010). Since tumor cells express a high level of TNF-α, it is reasonable to speculate that MSC will migrate toward the tumor areas in response to HGF stimuli. HGF directed MSC migration was recently shown in a glioma model, whereby elevated HGF expression induced by aminolaevulinic acid-mediated photodynamic therapy (ALA/PDT) facilitated the migration of MSC toward U87 and U251 glioblastoma spheroids (Vogel et al., 2013).

PDGF/PDGFR signaling

The PDGF family of proteins comprises four different polypeptide chains encoded by different genes; PDGF-A, PDGF-B, PDGF-C and PDGF-D. Dimerization of PDGFs facilitates their binding to their corresponding homo- and hetero-dimeric receptors, PDGFR-α and PDGFR-ß, which in turn activates downstream signaling that includes the activation of phosphoinositide-3-kinase (PI3K), Akt and extracellular signal-regulated kinases (ERK). MSC migration toward tumor has been suggested to be mediated by PDGF-AA, -BB and -AB (Fiedler et al., 2004; Hata et al., 2010) and PDGF-D (Gondi et al., 2010) via the interaction with PDGF receptors expressed on MSC (Hata et al., 2010), and can be abolished upon treatment with PDGFR inhibitor (Beckermann et al., 2008). PDGFR-mediated MSC migration could also involve the interaction between activated PDGFRs with integrin α5ß1 (Veevers-Lowe et al., 2011) or neuropilin (Nrp-1) (Ball et al., 2010a); the complex (PDGFR/α5ß1 and PDGFR/Nrp-1) leads to enhanced PI3K and Akt activity, membrane ruffling, network reorganization and increased cell migration towards PDGF-BB. In fact, PDGFRα+/Lin- bone marrow MSC can be mobilized into the circulation by extracellular high mobility group box 1 (HMGB1) protein secreted by injured tissues (Tamai et al., 2011), confirming the chemotactic effect of recombinant HMGB1 on MSC as observed by Meng et al. (2008). The mechanism of HMGB1-mediated MSC mobilization maybe similar to the recruitment of inflammatory cells to injured tissues. At the injury site, heterocomplex formed between HMGB1 and SDF-1 acts through CXCR4 to enhance the recruitment of monocytes (Schiraldi et al., 2012). Alternatively, HMGB1 interaction with Toll-like receptor 4 may also promote MSC migration (Pevsner-Fischer et al., 2007; Waterman et al., 2010). In a similar fashion, PDGF-BB stimulated MSC migration may involve transforming growth factor (TGF)-ß signaling, which was recently demonstrated to mediate the homing of MSC toward gliomas through its interaction with TGFß-receptor III expressed on MSC (Shinojima et al., 2013). Interaction of other angiogenic molecules that are secreted under hypoxic stress, such as vascular endothelial growth factor (VEGF) (Schichor et al., 2006; Beckermann et al., 2008; Liu et al., 2011) and basic fibroblast growth factor (bFGF) (Langer et al., 2009), with their receptors also regulate MSC migration toward areas of neovascularization (Ritter et al., 2008).

Chemokine signaling

A number of chemokine and cytokine signaling pathways have been associated with migratory activities of MSC. In particular, MCP-1/CCR2, Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES)/CCR5, thymus expressed chemokine (TECK)/CCR9, and SDF-1/CXCR4 have been implicated in tumor mediated migration (as summarized in Table 1).

CCL2, also known as MCP-1, is a 76-amino acids chemokine that mediates the recruitment of monocytes and macrophages during inflammation by binding to CCR2 (Strieter et al., 1996). CCL2 modulates angiogenesis, metastasis, and the establishment of pre-metastatic niche for circulating cancer cells (Ueno et al., 2000; Lu et al., 2006). Human MSC, which expresses CCR2, have been shown to migrate toward MCP-1-containing medium derived from primary cultures of various cancer patient tissues (Dwyer et al., 2007; Huang et al., 2007; Klopp et al., 2007). The role of MCP-1 is exemplified in a mouse model of breast carcinoma treated with low dose irradiation, whereby a high level of MCP-1 expression enhanced the tropism and engraftment of MSC to the tumor region (Dwyer et al., 2007; Klopp et al., 2007). The level of MCP-1 expression not only correlates with the level of MMPs (MT1-MMP/MMP14) and the angiogenic factor thymidine phosphorylase, it also correlates with the extent of breast cancer progression and macrophage accumulation (Saji et al., 2001). The finding that secretion of MCP-1 was enhanced in co-culture of MSC with breast cancer cells when compared with those grown individually (Molloy et al., 2009) suggested that MCP-1/CCR signaling may play a role beyond tumor tropism of MSC, of which the precise function of MSC inside the tumor has yet to be elucidated.

CCL25 is expressed predominantly in the thymus and epithelium of the small intestine, and is known to mediate chemotaxis of T cells through its receptor CCR9 (Papadakis et al., 2001). In some tumor models, the expression and activation of CCR9 has been shown to influence the migration and invasiveness of tumor cells such as melanoma (Amersi et al., 2008), prostate carcinoma (Singh et al., 2004), breast carcinoma (Johnson-Holiday et al., 2011) and ovarian cancers (Johnson et al., 2010). In human MSC, CCR9 is expressed at high levels (~64%) by flow cytometry analysis (Chamberlain et al., 2008). The ligand, CCL25, is expressed in primary human multiple myeloma cells (Xu et al., 2012) and has been shown to act as chemoattractants for MSC and periosteal progenitor cells (Stich et al., 2008; Binger et al., 2009). Targeted knockdown of CCR9 in murine MSC was shown to significantly affect its migratory activity toward multiple myeloma cells when compared with the CCR9 wildtype MSC, although the presence of wildtype MSC has been suggested to favor myeloma growth *in vivo* (Xu et al., 2012).

MMPs in MSC migration

Successful homing of MSC to their target tissue implies that MSC are able to extravasate from the circulation and intravasate into the target tissues, survive during the migration process and finally interact with cells in the target microenvironment. The extravasation and intravasation entail degradation of the extracellular matrix (ECM) that requires the action of proteolytic enzymes such as MMPs, which are zinc-dependent endopeptidases (Ravanti and Kahari, 2000). All MMPs have a common domain structure that includes a propeptide region and a furin cleavage site, a catalytic domain with a zinc-binding region, and a hemopexin-like C-terminal domain. To date, there are 23 MMPs, and these MMPs are classified into several subgroups

Table 1. Molecules involved in tumor tropism of MSC.

Types	Cell surface receptors found on MSC	Primary Ligands	Disease	References
Angiogenic factors	c-Met	HGF	Injured myocardium	Shabbir et al., 2009
	PDGFR/integrin α5ß1/Neuropilin-1	PDGF-AA;		Ball et al., 2010a,b
		PDGF-BB;	Tumor	
		PDGF-D		Fiedler et al., 2004; Klopp et al., 2007
	VEGFR-1	VEGF;	Bone	Fiedler et al., 2005
		Placental growth factor (PlGF)	Tumor	Schichor et al., 2006
	FGFR	Basic fibroblast growth factor	Bone	Fierro et al., 2011
			Spinal cord	Kim et al., 2006
Chemokine/Cytokines	CCR1	CCL2/MIP-1alpha; CCL5/RANTES;	Injured myocardium	Huang et al., 2010
		CCL7/MCP-3; CCL14/HCC-1; CCL15/MIP-1delta; CCL16/HCC-4; CCL23/MPIF-1	Ischemic brain	Wang et al., 2002a,b
	CCR2	CCL2 (MCP-1/MCAF);	Tumor	Kim et al., 2009; Xu et al., 2010
		IL-8;	Tumor + irradiation	Klopp et al., 2007
		GRO-α	Ischemic brain	Wang et al., 2002a,b
			Injured myocardium	Belema-Bedada et al., 2008
			Wound	Walter et al., 2010
	CCR3	CCL3 (MIP-1α)	Bone	Djouad et al., 2007
	CCR4	CCL16 (TARC) & CCL22 (MDC)	Bone	Djouad et al., 2007
	CCR5	CCL5 (RANTES)	Ischemic brain	Wang et al., 2002a,b
			Tumor	Mi et al., 2011
			Wound	Walter et al., 2010
	CCR7	CCL19 (MIP-3,) & CCL21 (6CKine)	Wound	Sasaki et al., 2008
	CCR9	CCL25 (TECK)	Tumor	Xu et al., 2012
	CXCR1	GCP-2; IL8	Tumor	Kim et al., 2011
	CXCR2	GRO-α/ß/γ; ENA-78; GCP-2; NAP-2; IL-8	Tumor	Halpern et al., 2011
	CXCR4	CXCL12 (SDF-1)	Injured myocardium	Huang et al., 2012
			Tumor	Song and Li, 2011
			Bone	Granero-Molto et al., 2009

including collagenases (MMP-1), stromelysin (MMP-3 and MMP-10), matrilysin (MMP-7 and MMP-26), gelatinase (MMP-2 and MMP-9) and membrane type MMP (MT-MMP). One of the main functions of MMPs is to cleave molecules in the ECM and the stromal environment leading to activation of the cleaved molecule in most instances. For example, cleavage of plasminogen by MMP-3, 7, 9 and 12 yield an anti-angiogenic molecule, angiostatin, which is otherwise inactive in the plasminogen state (Chakraborti et al., 2003).

Among the various MMPs, MSC have been shown to secrete MMP-2, MT1-MMP (Ries et al., 2007) and MMP-1 (Ho et al., 2009). Gene knockdown of MMP-2 and MT1-MMP inhibited MSC invasion through the ECM *in vitro* whereas inflammatory cytokines such as TGF-ß, IL-1, and TNF-α upregulate MMP-2 and MT1-MMP-mediated migration (Ries et al., 2007). Wnt-mediated upregulation of MT1-MMP promotes the activation of pro-MMP-2, thus increasing the migratory activities of MSC. By contrast, the ternary complex of pro-MMP-2 with tissue inhibitors of metalloproteinase (TIMP)-2 facilitates the activation of the zymogen by the surface tethered-MT1-MMP. Along the same line, it is reasonable to speculate that MMP-1 synergizes with other MMPs to mobilize MSC because MMP activity is a necessary component of growth factor-mediated cell migration.

In an attempt to determine the molecular pathways involved in the differential migration between the highly migratory and poorly migratory MSC, we performed microarray analysis on representative MSC isolates and found that MMP-1 activity and protein expression was higher in the highly migratory MSC versus those that do not migrate well (Ho et al., 2009). Importantly, the migratory activity can be abolished by RNA interference-mediated knockdown of MMP-1. Results from our laboratory further suggested that MMP-1 mediates MSC migration through its interaction with its cognate receptor, protease activated receptor-1 (PAR-1), because blockade of MMP-1/PAR-1 interaction abolished the migration of both adult (Ho et al., 2009) and fetal (Newman et al., 2011) MSC. Our more recent result suggested that MMP-1 negatively regulates the level of CXCR4 in MSC (unpublished observation), which might provide another reason for the low response of MSC toward SDF-1 and why only a small population of MSC express functional CXCR4 receptors. However, activation of SDF-1/CXCR-4 axis regulates MMP-1 transcription through ERK signaling (Sun et al., 2010), thus suggesting that MMP-1/PAR-1 axis crosstalk with SDF-1/CXCR4 axis to mobilize MSC. Aside from MMP-1, SDF-1/CXCR4 was also shown to interact with other MMPs, such as MMP-2. In the context of lung alveolar epithelial cells, CXCR4 antagonist or knockdown of CXCR4 using small interference RNA markedly reduced cell migration and MMP-2 activities (Ghosh et al., 2012). Also, there is connectivity between MMP-2 and CXCR4 in the homing and engraftment of MSC to tumors. Song et al., showed that MMP-2 expression was elevated after 2 hours exposure to conditioned media from tumor cells and decreased after 24 hours, while the reverse was true for CXCR4 (Song and Li, 2011), demonstrating that MSC migration is a multistep process.

Enhancement of MSC migration

By further understanding the molecules and signaling pathway involved in MSC migration, we can potentially increase the migration activities of MSC through several methods. Genetic modification of MSC to express certain growth factors or chemokine/cytokine receptors on their surface could increase their migration to a specific target. For example, overexpression of CXCR4 in human umbilical cord blood-derived MSC (hUCB-MSC) and bone marrow-derived MSC enhanced its migration towards gliomas (Park et al., 2011) and infarcted heart (Freyman et al., 2006; Yu et al., 2010; Huang et al., 2012). Similarly, genetic modification of poorly migratory MSC with MMP-1 significantly enhanced their migration *in vitro* and *in vivo* (Ho et al., 2009). In the same vein, one could overexpress other receptors such as CCR2 (receptor for MCP-1, IL-8 and GRO-α) and CCR5 (receptor for CCL5; RANTES) to direct MSC migration specifically to ischemic brain, injured myocardium or wound (Table 1).

In addition to genetic modification of MSC, it has been suggested that preconditioning MSC with pro-inflammatory cytokines mentioned in the previous section could increase migration of MSC. Priming MSC with these molecules could lead to global changes in the proteome profile that may impact the expression of ECM-modifying proteins, enzymes etc. In a bilateral murine breast carcinoma mouse model, the hind limb that was irradiated recruited more migratory MSC when compared with the non-irradiated hind limb of the same animal. The enhancement in MSC engraftments seems to have been mediated by higher levels of paracrine factors secreted after irradiation and by an upregulation of CCR2 (Klopp et al., 2007). Along the same line, irradiated LoVo colorectal carcinoma recruited more MSC and had a higher level of MCP-1 expression when compared with the non-irradiated control group (Zielske et al., 2009); while Kim et al showed enhanced recruitment of MSC to the irradiated glioma xenograft through upregulation of IL-8 expression (Kim et al., 2010). Macrophage inhibitory factor (MIF) is a pleiotropic cytokine that induced the expression of pro-inflammatory molecules as well as MMPs (reviewed by (Sanchez-Nino et al., 2013)) and was recently shown to inhibit MSC migration through its interaction with its receptor, CD74 (Barrilleaux et al., 2010; Fischer-Valuck et al., 2010). On the other hand, antagonizing CD74 eliminated its inhibitory effect on MSC migration. Thus, inhibiting CD74 prior to and during MSC infusion may allow accumulation of MSC in the target site. The examples illustrated above highlighted that manipulation

of the inflammatory response may improve MSC mobilization. Alternatively, inhibiting SDF-1/CXCR4 axis using CXCR4 antagonist, AMD3100, in combination with VEGF facilitated egress of MSC from the bone marrow into the circulation (Pitchford et al., 2009; Pitchford and Rankin, 2012). Taken together, the ability to enhance the migration and engraftment of MSC suggested the usefulness of the above mentioned techniques in overcoming the issues of low percentage of engraftment of MSC in the clinics.

Concluding remarks

MSC are increasingly being used in clinical trials worldwide. Although these cells are easily obtainable and expandable, the varied homing efficiency of MSC restricts its therapeutic efficacy. Despite the many factors involved in MSC migration, multiple studies have shown that only a subset of MSC possesses strong migratory properties (Francois et al., 2006; Maijenburg et al., 2010). Thus, it would be ideal if one could isolate the highly migratory subset of MSC based on cell surface markers, a task which is not feasible currently. Furthermore, because molecules that are expressed at the inflamed site resemble those expressed in the tumor, suppression of inflammation may inhibit MSC recruitment and affect the overall therapeutic efficacy (Kidd et al., 2010). Thus, understanding the mechanism of MSC migration will help to develop an ideal delivery protocol in the clinics, including the optimal route of delivery for a particular disease and the dosage of transplanted cells required.

Acknowledgements. IH and PL are jointly supported by the Singapore Ministry of Health's National Medical Research Council (NMRC/1201/2009) and the Singapore Stem Cell Consortium (SSCC/08/013).

References

Amersi F.F., Terando A.M., Goto Y., Scolyer R.A., Thompson J.F., Tran A.N., Faries M.B. Morton D.L. and Hoon D.S. (2008). Activation of CCR9/CCL25 in cutaneous melanoma mediates preferential metastasis to the small intestine. Clin. Cancer Res. 14, 638-645.

Ball S.G., Bayley C., Shuttleworth C.A. and Kielty C.M. (2010a). Neuropilin-1 regulates platelet-derived growth factor receptor signalling in mesenchymal stem cells. Biochem. J. 427, 29-40.

Ball S.G., Shuttleworth C.A. and Kielty C.M. (2010b). Platelet-derived growth factor receptors regulate mesenchymal stem cell fate: implications for neovascularization. Expert Opin. Biol. Ther. 10, 57-71.

Barrilleaux B.L., Fischer-Valuck B.W., Gilliam J.K., Phinney D.G. and O'Connor K.C. (2010). Activation of CD74 inhibits migration of human mesenchymal stem cells. In Vitro Cell Dev. Biol. Anim. 46, 566-572.

Barry F.P., Murphy J.M., English K. and Mahon B.P. (2005). Immunogenicity of adult mesenchymal stem cells: lessons from the fetal allograft. Stem Cells Dev. 14, 252-265.

Beckermann B.M., Kallifatidis G., Groth A., Frommhold D., Apel A., Mattern J., Salnikov A.V., Moldenhauer G., Wagner W., Diehlmann A., Saffrich R., Schubert M., Ho A.D., Giese N., Buchler,M.W., Friess H., Buchler P. and Herr I. (2008). VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. Br. J. Cancer 99, 622-631.

Behfar A., Yamada,S., Crespo-Diaz R., Nesbitt J.J., Rowe L.A., Perez-Terzic C., Gaussin V., Homsy C., Bartunek J. and Terzic A. (2010). Guided cardiopoiesis enhances therapeutic benefit of bone marrow human mesenchymal stem cells in chronic myocardial infarction. J. Am. Coll. Cardiol. 56, 721-734.

Belema-Bedada F., Uchida S., Martire A., Kostin S. and Braun T. (2008). Efficient homing of multipotent adult mesenchymal stem cells depends on FROUNT-mediated clustering of CCR2. Cell Stem Cell. 2, 566-575.

Bhanot Y., Rao S., Ghosh D., Balaraju S., Radhika C.R. and Satish Kumar, K. V. (2011). Autologous mesenchymal stem cells in chronic spinal cord injury. Br. J. Neurosurg. 25, 516-522.

Binger T., Stich S., Andreas K., Kaps C., Sezer O., Notter M., Sittinger M. and Ringe J. (2009). Migration potential and gene expression profile of human mesenchymal stem cells induced by CCL25. Exp. Cell Res. 315, 1468-1479.

Bos R., van Diest P.J., de Jong J.S., van der Groep P., van der Valk P. and van der Wall E. (2005). Hypoxia-inducible factor-1alpha is associated with angiogenesis, and expression of bFGF, PDGF-BB, and EGFR in invasive breast cancer. Histopathology 46, 31-36.

Brooke G., Tong H., Levesque J.P. and Atkinson K. (2008). Molecular trafficking mechanisms of multipotent mesenchymal stem cells derived from human bone marrow and placenta. Stem Cells Dev. 17, 929-940.

Broxmeyer H.E., Orschell C.M., Clapp D.W., Hangoc G., Cooper S., Plett P.A., Liles W.C., Li X., Graham-Evans B., Campbell T.B., Calandra G., Bridger G., Dale D.C. and Srour E.F. (2005). Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. J. Exp .Med. 201, 1307-1318.

Chakraborti S., Mandal M., Das S., Mandal A. and Chakraborti T. (2003). Regulation of matrix metalloproteinases: an overview. Mol. Cell Biochem. 253, 269-285.

Chamberlain G., Wright K., Rot A., Ashton B. and Middleton J. (2008). Murine mesenchymal stem cells exhibit a restricted repertoire of functional chemokine receptors: comparison with human. PLoS One 3, e2934.

Ciccocioppo R., Bernardo M.E., Sgarella A., Maccario R., Avanzini M.A., Ubezio C., Minelli A., Alvisi C., Vanoli A., Calliada F., Dionigi P., Perotti C., Locatelli F. and Corazza G.R. (2011). Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. Gut 60, 788-798.

Curiel T.J., Coukos G., Zou L., Alvarez X., Cheng P., Mottram P., Evdemon-Hogan M., Conejo-Garcia J.R., Zhang L., Burow M., Zhu Y., Wei S., Kryczek I., Daniel B., Gordon A., Myers L., Lackner A., Disis M.L., Knutson K.L., Chen L. and Zou, W. (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat. Med. 10, 942-949.

Dar A., Kollet O. and Lapidot T. (2006). Mutual, reciprocal SDF-1/CXCR4 interactions between hematopoietic and bone marrow stromal cells regulate human stem cell migration and development in NOD/SCID chimeric mice. Exp. Hematol. 34, 967-975.

Darash-Yahana M., Gillespie J.W., Hewitt S.M., Chen Y.Y., Maeda S., Stein I., Singh S.P., Bedolla R.B., Peled A., Troyer D.A., Pikarsky E., Karin M. and Farber J.M. (2009). The chemokine CXCL16 and its receptor, CXCR6, as markers and promoters of inflammation-associated cancers. PLoS One 4, e6695.

Djouad F., Delorme B., Maurice M., Bony C., Apparailly F., Louis-Plence P., Canovas F., Charbord P., Noel D. and Jorgensen C. (2007). Microenvironmental changes during differentiation of mesenchymal stem cells towards chondrocytes. Arthritis Res. Ther. 9, R33.

Dominici M., Le Blanc K., Mueller I., Slaper-Cortenbach I., Marini F., Krause D., Deans R., Keating A., Prockop D. and Horwitz E. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8, 315-317.

Duijvestein M., Vos A.C., Roelofs H., Wildenberg M.E., Wendrich B.B., Verspaget H.W., Kooy-Winkelaar E.M., Koning F., Zwaginga J.J., Fidder H.H., Verhaar A.P., Fibbe W.E., van den Brink G.R. and Hommes, D.W. (2010). Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. Gut 59, 1662-1669.

Dwyer R.M., Potter-Beirne S.M., Harrington K.A., Lowery A.J., Hennessy E., Murphy J.M., Barry F.P., O'Brien T. and Kerin M.J. (2007). Monocyte chemotactic protein-1 secreted by primary breast tumors stimulates migration of mesenchymal stem cells. Clin. Cancer Res. 13, 5020-5027.

Egea,V., von Baumgarten L., Schichor C., Berninger B., Popp T., Neth P., Goldbrunner R., Kienast Y., Winkler F., Jochum M. and Ries, C. (2011). TNF-alpha respecifies human mesenchymal stem cells to a neural fate and promotes migration toward experimental glioma. Cell Death Differ. 18, 853-863.

Fiedler J., Etzel N. and Brenner R. (2004). To go or not to go: Migration of human mesenchymal progenitor cells stimulated by isoforms of PDGF. J. Cell. Biochem. 93, 990-998.

Fiedler J., Leucht F., Waltenberger J., Dehio C. and Brenner R.E. (2005). VEGF-A and PlGF-1 stimulate chemotactic migration of human mesenchymal progenitor cells. Biochem. Biophys. Res. Commun. 334, 561-568.

Fierro F.A., Kalomoiris S., Sondergaard C.S. and Nolta J.A. (2011). Effects on proliferation and differentiation of multipotent bone marrow stromal cells engineered to express growth factors for combined cell and gene therapy. Stem Cells 29, 1727-1737.

Fischer-Valuck B.W., Barrilleaux B.L., Phinney D.G., Russell K.C., Prockop D.J. and O'Connor K.C. (2010). Migratory response of mesenchymal stem cells to macrophage migration inhibitory factor and its antagonist as a function of colony-forming efficiency. Biotechnol. Lett. 32, 19-27.

Francois S., Bensidhoum M., Mouiseddine M., Mazurier C., Allenet B., Semont A., Frick J., Sache A., Bouchet S., Thierry D., Gourmelon P., Gorin N.C. and Chapel A. (2006). Local irradiation not only induces homing of human mesenchymal stem cells at exposed sites but promotes their widespread engraftment to multiple organs: a study of their quantitative distribution after irradiation damage. Stem Cells 24, 1020-1029.

Freyman T., Polin G., Osman H., Crary J., Lu M., Cheng L., Palasis M. and Wilensky R.L. (2006). A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. Eur. Heart J. 27, 1114-1122.

Fu X., Han B., Cai S., Lei Y., Sun T. and Sheng Z. (2009). Migration of bone marrow-derived mesenchymal stem cells induced by tumor necrosis factor-alpha and its possible role in wound healing. Wound Repair Regen. 17, 185-191.

Ghosh M.C., Makena P.S., Gorantla V., Sinclair S.E. and Waters C.M. (2012). CXCR4 regulates migration of lung alveolar epithelial cells through activation of Rac1 and matrix metalloproteinase-2. Am. J. Physiol. Lung Cell Mol. Physiol. 302, L846-856.

Gondi C.S., Veeravalli K.K., Gorantla B., Dinh D.H., Fassett D., Klopfenstein J.D., Gujrati M. and Rao J. S. (2010). Human umbilical cord blood stem cells show PDGF-D-dependent glioma cell tropism *in vitro* and *in vivo*. Neuro Oncol. 12, 453-465.

Granero-Molto F., Weis J.A., Miga M.I., Landis B., Myers T.J., O'Rear L., Longobardi L., Jansen E.D., Mortlock D.P. and Spagnoli A. (2009). Regenerative effects of transplanted mesenchymal stem cells in fracture healing. Stem Cells 27, 1887-1898.

Gutova M., Najbauer J., Frank R.T., Kendall S.E., Gevorgyan A., Metz M.Z., Guevorkian M., Edmiston, M., Zhao D., Glackin C.A., Kim S.U. and Aboody K.S. (2008). uPA and uPAR mediate human stem cell tropism to malignant solid tumors. Stem Cells 26, 1406-1413.

Halpern J.L., Kilbarger A. and Lynch C.C. (2011). Mesenchymal stem cells promote mammary cancer cell migration in vitro via the CXCR2 receptor. Cancer Lett. 308, 91-99.

Hata N., Shinojima N., Gumin J., Yong R., Marini F., Andreeff M. and Lang F.F. (2010). Platelet-derived growth factor BB mediates the tropism of human mesenchymal stem cells for malignant gliomas. Neurosurgery 66, 144-156; discussion 156-147.

Hess D.A. and Allan A.L. (2011). Migratory strategies of normal and malignant stem cells. Methods Mol. Biol. 750, 25-44.

Ho I.A., Chan K.Y., Ng W.H., Guo C.M., Hui K.M., Cheang P. and Lam P.Y. (2009). Matrix metalloproteinase 1 is necessary for the migration of human bone marrow-derived mesenchymal stem cells toward human glioma. Stem Cells 27, 1366-1375.

Honczarenko M., Le Y., Swierkowski M., Ghiran I., Glodek A.M. and Silberstein L. (2006). Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors. Stem Cells 24, 1030-1041.

Huang B., Lei Z., Zhao J., Gong W., Liu J., Chen Z., Liu Y., Li D., Yuan Y., Zhang G.M. and Feng Z.H. (2007). CCL2/CCR2 pathway mediates recruitment of myeloid suppressor cells to cancers. Cancer Lett. 252, 86-92.

Huang W., Zhang D., Millard R.W., Wang T., Zhao T., Fan G.C., Ashraf A., Xu M., Ashraf M. and Wang Y. (2010). Gene manipulated peritoneal cell patch repairs infarcted myocardium. J Mol. Cell Cardiol. 48, 702-712.

Huang W., Wang T., Zhang D., Zhao T., Dai B., Ashraf A., Wang X., Xu M., Millard R.W., Fan G.C., Ashraf M., Yu X.Y. and Wang Y. (2012). Mesenchymal stem cells overexpressing CXCR4 attenuate remodeling of postmyocardial infarction by releasing matrix metalloproteinase-9. Stem Cells Dev. 21, 778-789.

Ip J.E., Wu Y., Huang J., Zhang L., Pratt R.E. and Dzau V.J. (2007). Mesenchymal stem cells use integrin beta1 not CXC chemokine receptor 4 for myocardial migration and engraftment. Mol. Biol. Cell. 18, 2873-2882.

Jacobs J.F., Idema A.J., Bol K.F., Grotenhuis J.A., de Vries I.J., Wesseling P. and Adema G.J. (2010). Prognostic significance and mechanism of Treg infiltration in human brain tumors. J. Neuroimmunol. 225, 195-199.

Johnson E.L., Singh R., Singh S., Johnson-Holiday C.M., Grizzle W.E., Partridge E.E. and Lillard J.W. Jr (2010). CCL25-CCR9 interaction modulates ovarian cancer cell migration, metalloproteinase

expression, and invasion. World J. Surg. Oncol. 8, 62.

Johnson-Holiday C., Singh R., Johnson E., Singh S., Stockard C.R., Grizzle W.E. and Lillard J.W. Jr (2011). CCL25 mediates migration, invasion and matrix metalloproteinase expression by breast cancer cells in a CCR9-dependent fashion. Int. J. Oncol. 38, 1279-1285.

Jorgensen E., Baldazzi F., Ripa R.S., Friis T., Wang Y., Helqvist S. and Kastrup J. (2010). Instent neointimal hyperplasia after percutaneous intervention for ST-elevation myocardial infarction and treatment with granulocyte-colony stimulating factor. Results from the stem cells in myocardial infarction (STEMMI) trial. Int. J. Cardiol. 139, 269-275.

Katou F., Ohtani H., Nakayama T., Ono K., Matsushima K., Saaristo A., Nagura H. Yoshie O. and Motegi K. (2001). Macrophage-derived chemokine (MDC/CCL22) and CCR4 are involved in the formation of T lymphocyte-dendritic cell clusters in human inflamed skin and secondary lymphoid tissue. Am. J. Pathol. 158, 1263-1270.

Kidd S., Caldwell L., Dietrich M., Samudio I., Spaeth E.L., Watson K., Shi Y., Abbruzzese J., Konopleva, M., Andreeff M. and Marini F.C. (2010). Mesenchymal stromal cells alone or expressing interferon-beta suppress pancreatic tumors in vivo, an effect countered by anti-inflammatory treatment. Cytotherapy 12, 615-625.

Kim K.N., Oh S.H., Lee K.H. and Yoon D.H. (2006). Effect of human mesenchymal stem cell transplantation combined with growth factor infusion in the repair of injured spinal cord. Acta Neurochir. (Suppl. 99), 133-136.

Kim D.S., Kim J.H., Lee J.K., Choi S.J., Kim J.S., Jeun S.S., Oh W., Yang Y.S. and Chang J.W. (2009). Overexpression of CXC chemokine receptors is required for the superior glioma-tracking property of umbilical cord blood-derived mesenchymal stem cells. Stem Cells Dev. 18, 511-519.

Kim S.M., Oh J.H., Park S.A., Ryu C.H., Lim J.Y., Kim D.S., Chang J.W., Oh W. and Jeun S.S. (2010). Irradiation enhances the tumor tropism and therapeutic potential of tumor necrosis factor-related apoptosis-inducing ligand-secreting human umbilical cord blood-derived mesenchymal stem cells in glioma therapy. Stem Cells 28, 2217-2228.

Kim S.M., Kim D.S., Jeong C.H., Kim D.H., Kim J.H., Jeon H.B., Kwon S.J., Jeun S.S., Yang Y.S., Oh W. and Chang J.W. (2011). CXC chemokine receptor 1 enhances the ability of human umbilical cord blood-derived mesenchymal stem cells to migrate toward gliomas. Biochem. Biophys. Res. Commun. 407, 741-746.

Klopp A.H., Spaeth E.L., Dembinski J.L., Woodward W.A., Munshi A., Meyn R.E., Cox J.D., Andreeff M. and Marini F.C. (2007). Tumor irradiation increases the recruitment of circulating mesenchymal stem cells into the tumor microenvironment. Cancer Res. 67, 11687-11695.

Koc O.N., Gerson S.L., Cooper B.W., Dyhouse S.M., Haynesworth S.E., Caplan A.I. and Lazarus H.M. (2000). Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J. Clin. Oncol. 18, 307-316.

Kubo M., Li T.S., Kamota T., Ohshima M., Qin S.L. and Hamano K. (2009). Increased expression of CXCR4 and integrin alphaM in hypoxia-preconditioned cells contributes to improved cell retention and angiogenic potency. J. Cell Physiol. 220, 508-514.

Langer H.F., Stellos K., Steingen C., Froihofer A., Schonberger T., Kramer B., Bigalke B., May A.E., Seizer P., Muller I., Gieseke F., Siegel-Axel D., Meuth S.G., Schmidt A., Wendel H.P., Muller I., Bloch W. and Gawaz M. (2009). Platelet derived bFGF mediates vascular integrative mechanisms of mesenchymal stem cells in vitro. J. Mol. Cell Cardiol. 47, 315-325.

Le Blanc K., Frassoni F., Ball L., Locatelli F., Roelofs H., Lewis I., Lanino E., Sundberg B., Bernardo M.E., Remberger M., Dini G., Egeler R.M., Bacigalupo A., Fibbe, W. and Ringden, O. (2008). Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 371, 1579-1586.

Lee R.H., Seo M.J., Reger R.L., Spees J.L., Pulin A.A., Olson S.D. and Prockop D.J. (2006). Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. Proc. Natl. Acad. Sci. USA 103, 17438-17443.

Lee M.J., Kim J., Kim M.Y., Bae Y.S., Ryu S.H., Lee T.G. and Kim J. H. (2010). Proteomic analysis of tumor necrosis factor-alpha-induced secretome of human adipose tissue-derived mesenchymal stem cells. J. Proteome Res. 9, 1754-1762.

Ley K., Laudanna C., Cybulsky M.I. and Nourshargh S. (2007). Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat. Rev. Immunol. 7, 678-689.

Li J.M., Zhu H., Lu S., Liu Y., Li Q., Ravenscroft P., Xu Y.F., Huang L., Ma C.M., Bezard E., Zhao R.C., Wang R.Z. and Qin C. (2011). Migration and differentiation of human mesenchymal stem cells in the normal rat brain. Neurol. Res. 33, 84-92.

Lim J.Y., Jeong C.H., Jun J.A., Kim S.M., Ryu C.H., Hou Y., Oh W., Chang J.W. and Jeun S.S. (2011). Therapeutic effects of human umbilical cord blood-derived mesenchymal stem cells after intrathecal administration by lumbar puncture in a rat model of cerebral ischemia. Stem Cell Res. Ther. 2, 38.

Liu L., Yu Q., Lin J., Lai X., Cao W., Du K., Wang Y., Wu K., Hu Y., Zhang L., Xiao H., Duan Y. and Huang H. (2011). Hypoxia-inducible factor-1alpha is essential for hypoxia-induced mesenchymal stem cell mobilization into the peripheral blood. Stem Cells Dev. 20, 1961-1971.

Lu Y., Cai Z., Galson D.L. Xiao, G. Liu Y., George D.E., Melhem M.F., Yao Z. and Zhang J. (2006). Monocyte chemotactic protein-1 (MCP-1) acts as a paracrine and autocrine factor for prostate cancer growth and invasion. Prostate 66, 1311-1318.

Maijenburg M.W., Noort W.A., Kleijer M., Kompier C.J., Weijer K., van Buul J.D., van der Schoot C.E. and Voermans C. (2010). Cell cycle and tissue of origin contribute to the migratory behaviour of human fetal and adult mesenchymal stromal cells. Br. J. Haematol. 148, 428-440.

Meng E., Guo Z., Wang H., Jin J., Wang J., Wang H., Wu C. and Wang L. (2008). High mobility group box 1 protein inhibits the proliferation of human mesenchymal stem cells and promotes their migration and differentiation along osteoblastic pathway. Stem Cells Dev. 17, 805-813.

Mi Z., Bhattacharya S.D., Kim V.M., Guo H., Talbot L.J. and Kuo P.C. (2011). Osteopontin promotes CCL5-mesenchymal stromal cell-mediated breast cancer metastasis. Carcinogenesis 32, 477-487.

Molloy A.P., Martin F.T., Dwyer R.M., Griffin T.P., Murphy M., Barry F.P., O'Brien T. and Kerin M.J. (2009). Mesenchymal stem cell secretion of chemokines during differentiation into osteoblasts, and their potential role in mediating interactions with breast cancer cells. Int. J. Cancer 124, 326-332.

Morishita R., Nakamura S., Hayashi S., Aoki M., Matsushita H., Tomita N., Yamamoto K., Moriguchi, A. Higaki J. and Ogihara T. (1998).

Contribution of a vascular modulator, hepatocyte growth factor (HGF), to the pathogenesis of cardiovascular disease. J. Atheroscler. Thromb. 4, 128-134.

Nakamizo A., Marini F., Amano T., Khan A., Studeny M., Gumin J., Chen J., Hentschel S., Vecil G., Dembinski J., Andreeff M. and Lang F.F. (2005). Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. Cancer Res. 65, 3307-3318.

Neuss S., Becher, E. Woltje M., Tietze L. and Jahnen-Dechent, W. (2004). Functional expression of HGF and HGF receptor/c-met in adult human mesenchymal stem cells suggests a role in cell mobilization, tissue repair, and wound healing. Stem Cells 22, 405-414.

Newman J.P., Toh X.Y., Chan J., Endaya B. and Lam P. (2011). Migration of human fetal bone marrow-derived mesenchymal stem cells: Possible involvement of GPCR and MMPs. J. Stem Cell Res. Ther. 2011, (special issue):5.

Papadakis K.A., Prehn J., Moreno S.T., Cheng L., Kouroumalis E.A., Deem R., Breaverman T., Ponath P.D., Andrew D.P. Green P.H., Hodge M.R., Binder S.W. and Targan S.R. (2001). CCR9-positive lymphocytes and thymus-expressed chemokine distinguish small bowel from colonic Crohn's disease. Gastroenterology. 121, 246-254.

Park S.A., Ryu C.H., Kim S.M., Lim J.Y., Park S.I., Jeong C.H., Jun J.A., Oh J.H., Park S.H., Oh W. and Jeun S.S. (2011). CXCR4-transfected human umbilical cord blood-derived mesenchymal stem cells exhibit enhanced migratory capacity toward gliomas. Int. J. Oncol. 38, 97-103.

Park J.H., Kim D.Y., Sung I.Y., Choi G.H., Jeon M.H., Kim K.K. and Jeon S.R. (2012). Long-term results of spinal cord injury therapy using mesenchymal stem cells derived from bone marrow in humans. Neurosurgery 70, 1238-1247; discussion 1247.

Peled A., Petit I., Kollet, O. Magid M., Ponomaryov T., Byk T., Nagler A., Ben-Hur H., Many A., Shultz L., Lider O., Alon R., Zipori D. and Lapidot T. (1999). Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science 283, 845-848.

Pevsner-Fischer M., Morad V., Cohen-Sfady M., Rousso-Noori L., Zanin-Zhorov A., Cohen S., Cohen I.R. and Zipori D. (2007). Toll-like receptors and their ligands control mesenchymal stem cell functions. Blood 109, 1422-1432.

Pitchford S.C. and Rankin S.M. (2012). Combinatorial stem cell mobilization in animal models. Methods Mol. Biol. 904, 139-154.

Pitchford S.C., Furze R.C., Jones C.P., Wengner A.M. and Rankin S.M. (2009). Differential mobilization of subsets of progenitor cells from the bone marrow. Cell Stem Cell 4, 62-72.

Prasad V.K., Lucas K.G., Kleiner G.I., Talano J.A., Jacobsohn D., Broadwater G., Monroy R. and Kurtzberg J. (2011). Efficacy and safety of *ex vivo* cultured adult human mesenchymal stem cells (Prochymal) in pediatric patients with severe refractory acute graft-versus-host disease in a compassionate use study. Biol. Blood Marrow Transplant. 17, 534-541.

Qi Y., Feng G. and Yan W. (2012). Mesenchymal stem cell-based treatment for cartilage defects in osteoarthritis. Mol. Biol. Rep. 39, 5683-5689.

Rattigan Y., Hsu J.M., Mishra P.J., Glod J. and Banerjee D. (2010). Interleukin 6 mediated recruitment of mesenchymal stem cells to the hypoxic tumor milieu. Exp. Cell Res. 316, 3417-3424.

Ravanti L. and Kahari V.M. (2000). Matrix metalloproteinases in wound repair (review). Int. J. Mol. Med. 6, 391-407.

Ries C., Egea V., Karow M., Kolb H., Jochum M. and Neth P. (2007). MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines. Blood 109, 4055-4063.

Ritter E., Perry A., Yu J., Wang T., Tang L. and Bieberich E. (2008). Breast cancer cell-derived fibroblast growth factor 2 and vascular endothelial growth factor are chemoattractants for bone marrow stromal stem cells. Ann. Surg. 247, 310-314.

Rosova I., Dao M., Capoccia B., Link D. and Nolta J. A. (2008). Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. Stem Cells 26, 2173-2182.

Ryu C.H., Park S.A., Kim S.M., Lim J.Y., Jeong C.H., Jun J.A., Oh J.H., Park S.H., Oh W.I. and Jeun S.S. (2010). Migration of human umbilical cord blood mesenchymal stem cells mediated by stromal cell-derived factor-1/CXCR4 axis via Akt, ERK, and p38 signal transduction pathways. Biochem. Biophys. Res. Commun. 398, 105-110.

Saji H., Koike M., Yamori T., Saji S., Seiki M., Matsushima K. and Toi M. (2001). Significant correlation of monocyte chemoattractant protein-1 expression with neovascularization and progression of breast carcinoma. Cancer 92, 1085-1091.

Sanchez-Nino M.D., Sanz A.B., Ruiz-Andres O., Poveda, J., Izquierdo M.C., Selgas R., Egido J. and Ortiz A. (2013). MIF, CD74 and other partners in kidney disease: Tales of a promiscuous couple. Cytokine Growth Factor Rev. 24, 23-40.

Sasaki M., Abe R., Fujita Y., Ando S., Inokuma D. and Shimizu H. (2008). Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. J. Immunol. 180, 2581-2587.

Sato K., Ozaki K., Mori M., Muroi K. and Ozawa K. (2010). Mesenchymal stromal cells for graft-versus-host disease: basic aspects and clinical outcomes. J. Clin. Exp. Hematop. 50, 79-89.

Schichor C., Birnbaum T., Etminan N., Schnell O., Grau S., Miebach S., Aboody K., Padovan C., Straube, A., Tonn J.C. and Goldbrunner R. (2006). Vascular endothelial growth factor A contributes to glioma-induced migration of human marrow stromal cells (hMSC). Exp. Neurol. 199, 301-310.

Schiraldi M., Raucci A., Munoz L.M., Livoti E., Celona B., Venereau E., Apuzzo T., De Marchis F., Pedotti M., Bachi A., Thelen M., Varani L., Mellado M., Proudfoot A., Bianchi M.E. and Uguccioni, M. (2012). HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4. J. Exp. Med. 209, 551-563.

Segers V.F., Van Riet I., Andries L.J., Lemmens K., Demolder M.J., De Becker A.J., Kockx M.M. and De Keulenaer G.W. (2006). Mesenchymal stem cell adhesion to cardiac microvascular endothelium: activators and mechanisms. Am. J. Physiol. Heart Circ. Physiol. 290, H1370-1377.

Shabbir A., Zisa D., Suzuki G. and Lee T. (2009). Heart failure therapy mediated by the trophic activities of bone marrow mesenchymal stem cells: a noninvasive therapeutic regimen. Am. J. Physiol. Heart Circ. Physiol. 296, H1888-1897.

Shi Y., Xia Y.Y., Wang L., Liu R., Khoo K.S. and Feng Z.W. (2012). Neural cell adhesion molecule modulates mesenchymal stromal cell migration via activation of MAPK/ERK signaling. Exp. Cell Res. 318, 2257-2267.

Shinojima N., Hossain A., Takezaki T., Fueyo-Margareto J., Gumin J., Gao F., Nwajei F., Marini F.C., Andreeff M., Kuratsu J.I. and Lang F.F. (2013). TGF-beta mediates homing of bone marrow-derived

human mesenchymal stem cells to glioma stem cells. Cancer Res. 73, 2333-2344.

Singh S., Singh U.P., Stiles J.K., Grizzle W.E. and Lillard J.W. Jr (2004). Expression and functional role of CCR9 in prostate cancer cell migration and invasion. Clin. Cancer Res. 10, 8743-8750.

Smith H., Whittall C., Weksler B. and Middleton J. (2012). Chemokines stimulate bidirectional migration of human mesenchymal stem cells across bone marrow endothelial cells. Stem Cells Dev. 21, 476-486.

Son B.R., Marquez-Curtis L.A., Kucia M., Wysoczynski M., Turner A.R., Ratajczak J., Ratajczak M.Z. and Janowska-Wieczorek A. (2006). Migration of bone marrow and cord blood mesenchymal stem cells in vitro is regulated by stromal-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes and involves matrix metalloproteinases. Stem Cells 24, 1254-1264.

Song C. and Li G. (2011). CXCR4 and matrix metalloproteinase-2 are involved in mesenchymal stromal cell homing and engraftment to tumors. Cytotherapy 13, 549-561.

Song J.S., Kang C.M., Kang H.H., Yoon H.K., Kim Y.K., Kim K.H., Moon H.S. and Park S.H. (2010). Inhibitory effect of CXC chemokine receptor 4 antagonist AMD3100 on bleomycin induced murine pulmonary fibrosis. Exp. Mol. Med. 42, 465-472.

Stich S., Loch A., Leinhase I., Neumann K., Kaps C., Sittinger M. and Ringe J. (2008). Human periosteum-derived progenitor cells express distinct chemokine receptors and migrate upon stimulation with CCL2, CCL25, CXCL8, CXCL12, and CXCL13. Eur. J. Cell Biol. 87, 365-376.

Strieter R.M., Standiford T.J., Huffnagle G.B., Colletti L.M., Lukacs N.W. and Kunkel S.L. (1996). "The good, the bad, and the ugly." The role of chemokines in models of human disease. J. Immunol. 156, 3583-3586.

Studeny M., Marini F.C., Champlin R.E., Zompetta C., Fidler I.J. and Andreeff M. (2002). Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. Cancer Res. 62, 3603-3608.

Studeny M., Marini F.C., Dembinski J.L., Zompetta C., Cabreira-Hansen M., Bekele B.N., Champlin R.E. and Andreeff M. (2004). Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents. J. Natl. Cancer Inst. 96, 1593-1603.

Sun X., Wei L., Chen Q. and Terek R.M. (2010). CXCR4/SDF1 mediate hypoxia induced chondrosarcoma cell invasion through ERK signaling and increased MMP1 expression. Mol. Cancer. 9, 17.

Tamai K., Yamazaki T., Chino T., Ishii M., Otsuru S., Kikuchi Y., Iinuma S., Saga K., Nimura K., Shimbo T., Umegaki N., Katayama I., Miyazaki J., Takeda J., McGrath J.A., Uitto J. and Kaneda, Y. (2011). PDGFRalpha-positive cells in bone marrow are mobilized by high mobility group box 1 (HMGB1) to regenerate injured epithelia. Proc. Natl. Acad. Sci. USA 108, 6609-6614.

Teo G.S., Ankrum J.A., Martinelli R., Boetto S.E., Simms K., Sciuto T.E., Dvorak A.M., Karp J.M. and Carman C.V. (2012). Mesenchymal stem cells transmigrate between and directly through tumor necrosis factor-alpha-activated endothelial cells via both leukocyte-like and novel mechanisms. Stem Cells 30, 2472-2486.

Trachtenberg B., Velazquez D.L., Williams A.R., McNiece I., Fishman J., Nguyen K., Rouy D., Altman P., Schwarz R., Mendizabal A., Oskouei B., Byrnes J., Soto V., Tracy M., Zambrano J.P., Heldman A.W. and Hare J.M. (2011). Rationale and design of the transendocardial injection of autologous human cells (bone marrow or mesenchymal) in Chronic ischemic left ventricular dysfunction and heart failure secondary to myocardial infarction (TAC-HFT) trial: A randomized, double-blind, placebo-controlled study of safety and efficacy. Am. Heart J. 161, 487-493.

Ueno T., Toi M., Saji H., Muta M., Bando H., Kuroi K., Koike M., Inadera H. and Matsushima K. (2000). Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer. Clin. Cancer Res. 6, 3282-3289.

Veevers-Lowe J., Ball S.G., Shuttleworth A. and Kielty C.M. (2011). Mesenchymal stem cell migration is regulated by fibronectin through alpha5beta1-integrin-mediated activation of PDGFR-beta and potentiation of growth factor signals. J. Cell Sci. 124, 1288-1300.

Verma P.K., Bala M., Kumar N. and Singh B. (2012). Therapeutic potential of natural products from terrestrial plants as TNF-alpha antagonist. Curr. Top Med. Chem. 12, 1422-1435.

Vogel S., Peters C., Etminan N., Borger V., Schimanski A., Sabel M.C. and Sorg R.V. (2013). Migration of mesenchymal stem cells towards glioblastoma cells depends on hepatocyte-growth factor and is enhanced by aminolaevulinic acid-mediated photodynamic treatment. Biochem. Biophys. Res. Commun. 431, 428-432.

Walter M.N., Wright K.T., Fuller H.R., MacNeil S. and Johnson W.E. (2010). Mesenchymal stem cell-conditioned medium accelerates skin wound healing: an in vitro study of fibroblast and keratinocyte scratch assays. Exp. Cell Res. 316, 1271-1281.

Wang L., Li Y., Chen J., Gautam S.C., Zhang Z., Lu M. and Chopp M. (2002a). Ischemic cerebral tissue and MCP-1 enhance rat bone marrow stromal cell migration in interface culture. Exp. Hematol. 30, 831-836.

Wang L., Li Y., Chen X., Chen J., Gautam S.C., Xu Y. and Chopp M. (2002b). MCP-1, MIP-1, IL-8 and ischemic cerebral tissue enhance human bone marrow stromal cell migration in interface culture. Hematology 7, 113-117.

Waterman R.S., Tomchuck S.L., Henkle S.L. and Betancourt A.M. (2010). A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. PLoS One 5, e10088.

Wislet-Gendebien S., Wautier F., Leprince P. and Rogister B. (2005). Astrocytic and neuronal fate of mesenchymal stem cells expressing nestin. Brain Res. Bull. 68, 95-102.

Woodfin A., Voisin M.B. and Nourshargh S. (2010). Recent developments and complexities in neutrophil transmigration. Curr. Opin. Hematol. 17, 9-17.

Wynn R.F., Hart C.A., Corradi-Perini C., O'Neill L., Evans C.A., Wraith J.E., Fairbairn L.J. and Bellantuono I. (2004). A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. Blood 104, 2643-2645.

Xiao Q., Wang S.K., Tian H., Xin L., Zou Z.G., Hu Y.L., Chang C.M., Wang X.Y., Yin Q.S. Zhang X.H. and Wang L.Y. (2012). TNF-alpha increases bone marrow mesenchymal stem cell migration to ischemic tissues. Cell Biochem. Biophys. 62, 409-414.

Xu F., Shi J., Yu B., Ni W., Wu X. and Gu Z. (2010). Chemokines mediate mesenchymal stem cell migration toward gliomas *in vitro*. Oncol. Rep. 23, 1561-1567.

Xu S., Menu E., De Becker A., Van Camp,B., Vanderkerken K. and Van Riet I. (2012). Bone marrow-derived mesenchymal stromal cells are attracted by multiple myeloma cell-produced chemokine CCL25 and favor myeloma cell growth in vitro and in vivo. Stem Cells 30, 266-

279.
Yu J., Li M., Qu Z., Yan D., Li D. and Ruan Q. (2010). SDF-1/CXCR4-mediated migration of transplanted bone marrow stromal cells toward areas of heart myocardial infarction through activation of PI3K/Akt. J. Cardiovasc. Pharmacol. 55, 496-505.
Zhang A., Wang Y., Ye Z., Xie H., Zhou L. and Zheng S. (2010). Mechanism of TNF-alpha-induced migration and hepatocyte growth factor production in human mesenchymal stem cells. J. Cell Biochem. 111, 469-475.
Zielske S.P., Livant D.L. and Lawrence T.S. (2009). Radiation increases invasion of gene-modified mesenchymal stem cells into tumors. Int. J. Radiat. Oncol. Biol. Phys. 75, 843-853.

Accepted July 2, 2013