Mesenchymal stem cells homing to improve bone healing

Weiping Lin a, Liangliang Xu a, Stefan Zwingenberger b, Emmanuel Gibon c,d, Stuart B. Goodman d,*, Gang Li a,e,f,*

a Department of Orthopaedics and Traumatology, Stem Cells and Regenerative Medicine Laboratory, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong, SAR, China
b Center for Orthopaedics and Traumatology, University Hospital Carl Gustav Carus at Technische Universität Dresden, Dresden, Germany
c Department of Orthopaedic Surgery, Hopital Cochin, APHP, Université Paris 5, Paris, France
d Department of Orthopaedic Surgery, Stanford University Medical Center Outpatient Center, Redwood City, CA 94063, USA
e Key Laboratory for Regenerative Medicine, Ministry of Education, School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, SAR, China
f Lui Che Woo Institute of Innovative Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, SAR, China

Received 13 February 2017; received in revised form 8 March 2017; accepted 9 March 2017
Available online 29 March 2017

Summary Cell therapy continues to attract growing interest as a promising approach to treat a variety of diseases. Mesenchymal stem cells (MSCs) have been one of the most intensely studied candidates for cell therapy. Since the homing capacity of MSCs is an important determinant of effective MSC-based therapy, the enhancement of homing efficiency is essential for optimizing the therapeutic outcome. Furthermore, trafficking of endogenous MSCs to damaged tissues, also referred to as endogenic stem cell homing, and the subsequent participation of MSCs in tissue regeneration are considered to be a natural self-healing response. Therefore, strategies to stimulate and reinforce the mobilisation and homing of MSCs have become a key point in regenerative medicine. The current review focuses on advances in the mechanisms and factors governing trafficking of MSCs, and the relationship between MSC mobilisation and skeletal diseases, providing insights into strategies for their potential translational implications.

* Corresponding authors. Department of Orthopaedic Surgery, Stanford University Medical Center Outpatient Center, Redwood City, CA 94063, USA (S. Goodman); Room 904, 9/F, Li Ka Shing Institute of Health Institute, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, China (G. Li).
E-mail addresses: goodbone@stanford.edu (S.B. Goodman), gangli@cuhk.edu.hk (G. Li).

http://dx.doi.org/10.1016/j.jot.2017.03.002
2214-031X/© 2017 The Authors. Published by Elsevier (Singapore) Pte Ltd on behalf of Chinese Speaking Orthopaedic Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Introduction

Mesenchymal stem cells (MSCs) have been a major research focus in regenerative medicine for several decades. MSC-based translational therapies hold great promise as a novel approach to cure a diverse range of diseases, such as neurological diseases [1], cardiovascular diseases [2,3], wounds [4,5], and various musculoskeletal diseases [6–8]. MSCs are multipotent stromal cells that are capable of differentiating into, and contributing to the regeneration of mesenchymal tissues such as bone, cartilage, fat, tendon, and muscle [9,10]. MSCs express multiple cell surface antigens, such as CD90, CD105, CD73, and CD44, but lack expression of CD45, CD14, CD11b, CD79a, CD19, and HLA-DR [11,12]. MSCs have been successfully isolated from various adult tissues, including bone marrow (BM) [11], adipose tissue [13], and peripheral blood (PB) [14]. MSCs possess powerful immunomodulatory properties and ability for tissue repair. In response to adverse stimuli (e.g., bacterial ligands) or injury, the inflammatory response is activated. MSCs sense these potentially damaging events via surface receptors (e.g., toll-like receptors and the inflammasome) and by alterations in local cytokine and chemokine levels, and then migrate locally and systemically to inflammatory sites. MSCs modulate both innate and adaptive immune responses; biological cues in the local microenvironment determine the activation state of MSCs to become immunosuppressive [15,16]. MSCs not only provide a source of progenitors for cell replacement, but also activate or empower other local cells (such as tissue-resident progenitor or stem cells, endothelial cells, and fibroblasts) to facilitate tissue regeneration via paracrine stimulation [17].

The trafficking of endogenous MSCs to injured tissues, also defined as endogenous stem cell homing, and their subsequent participation in immunomodulation and tissue repair, are considered a natural self-healing response. To take full advantage of the intrinsic regenerative capacity of the body, strategies to stimulate and enhance the mobilisation of endogenous stem cells are of increasing interest. Furthermore, in order to enhance the therapeutic efficiency of exogenous systemically administered stem cells, a clear understanding of the biological concepts underlying stem cell homing is crucial.

It has long been proposed that the cellular and molecular signals of bone injury are highly consistent with embryonic skeletal growth processes, which involve the mobilisation and activation of MSCs. Both tissue-resident and circulating MSCs appear to take part in the processes of bone healing [18]. Immune signals, such as inflammatory mediators and immune cells, trigger the activation and mobilisation of MSCs [19]. Therefore, a better understanding of mechanisms regulating MSC mobilisation and homing may provide novel insights into strategies for successful bone repair. Here, we present a brief summary of the latest findings on the mechanisms and factors regulating MSC trafficking, and the close association between MSC homing and the treatment of musculoskeletal diseases. Our focus is to elucidate the critical role of mobilisation of MSCs in bone healing and provide insights into strategies to accelerate bone healing.

MSCs homing and bone healing

Musculoskeletal diseases remain among the most prevalent and challenging clinical problems, especially for the elderly population. Although simple fractures often heal effectively, the fracture healing process is impaired in 10–20% of fractures, causing nonunion and severe disability [20]. Furthermore, some fractures, such as hip fractures, are threatening injuries with mortality rates of 15–25% [21]. Angiogenesis and osteogenesis are coupled during embryonic skeletal development and bone repair processes, since blood vessels precede the onset of osteogenesis by transporting circulating cells, oxygen, nutrients, and osteogenic signals [22]. Thus, the stimulation of angiogenesis appears to be an important strategy for accelerating fracture healing [23]. Moreover, there is a dynamic homeostatic interplay between bone formation and bone resorption. An imbalance of bone remodelling such that bone formation is not able to compensate for ongoing bone resorption is one of the main mechanisms leading to many bone diseases, such as osteoporosis and nonunion of bone fractures [24,25] (Figure 1).

Healing of fractures is a complex process involving the interplay of osteogenesis and angiogenesis. Natural repair of fractures comprises inflammatory, repair, and remodelling phases. The mobilisation and recruitment of circulating or resident stem cells, and systemically mobilised and recruited MSCs are involved in the fracture healing [19]. The recruitment of BM-MSCs to fracture sites is mainly mediated by the stromal cell-derived factor (SDF)1/CXC chemokine receptor (CXCR) 4 signalling axis [26]. Moreover, MSCs play critical roles in mediating the coupling of bone resorption and formation. In response to osteoclastic bone resorption, active transforming growth factor (TGF)-β released by the bone matrices induces migration and mobilisation of MSCs to the local site of repair, which is essential for coordinating bone remodelling [27]. In addition, transplanted MSCs have been found to stimulate angiogenesis, thereby leading to enhanced bone healing [28]. Conversely, impaired BM-MSCs mobilisation may lead to delayed osteoporotic fracture healing. As the numbers of BM-MSCs and PB-MSCs of ovariectomised mice are significantly lower than those of the mice with sham surgery at 12 hours, 24 hours, and 72 hours after fracture, ovariectomised mice have lower intrinsic capacity for bone regeneration [29]. Therefore, MSC homing augments bone healing mainly by regulating the bone remodelling and angiogenesis processes.
Potential indications of MSCs promoting bone healing

MSCs have been shown to enhance bone regeneration in several preclinical and clinical studies by differentiating directly into bone forming cells and modulating the biological environment by secreting growth factors and anti-inflammatory cytokines [30,31]. Using MSCs expressing firefly luciferase, Granero-Molto et al [32] demonstrated that MSCs migrated (via the CXCR4 receptor) to the fracture site and improved healing by affecting the biomechanical properties and increasing the cartilage and bone content of the callus. MSCs are also a promising tool to treat critical size bone defects. One clinical study with 6–7-years follow-up reported the successful treatment of defects of long bones [33]. Another large study for the treatment of nonunion with in vitro expanded autologous BM-MSCs is currently registered (https://www.clinicaltrials.gov/).

MSCs trafficking

When potentially injurious situations occur, MSCs will be recruited and mobilised into damaged bone via local mechanisms and the peripheral circulation. The specific factors that lead to tissue-specific homing of MSCs are still under debate. MSC homing is defined as the arrest of MSCs within the vasculature of a tissue followed by transmigration across the endothelium. MSC migration appears to be a multistep process, which is mainly mediated by homing receptors, endothermal co-receptors, and chemotactic cytokines. Among these, SDF-1/CXCR4 signalling axis has been demonstrated to be vital for MSC homing [40]. In addition, the monocyte chemoattractant proteins (MCPs) have also been demonstrated to regulate MSC migration. MCPs attract cells by activating their cognate receptor, CCR2, which is expressed on monocyte surfaces [41,42]. Thus, the MCP/CCR2 pathway is also
involved in recruiting MSCs to inflammatory sites [43]. Shino-hara et al [44] used a parabiosis model with green fluorescent protein (GFP)MSCs [44]. MSCs were also engineered to express SDF-1 or MCP-3 or remained naïve. Parabiosis mice were allocated into five different groups. A fibular osteotomy was performed on the GFP− mouse 4 weeks after parabiosis and the homing of GFP+MSCs investigated. Consistently, the authors found more GFP+ cells in SDF-1 and MCP-3 groups. Furthermore, in order to prove the contribution of recruited GFP+MSCs to the fracture callus, the authors colocalised GFP expression and alkaline-phosphatase-positive (AP+) cells using immunohistochemistry. They showed that the fraction of AP+ and GFP+ was significantly higher in the callus of both the SDF-1 and MCP-3 groups. Using the same parabiosis model, Otsuru et al [45] found similar results. In addition, hepatocyte growth factor (HGF)/c-met signalling has also been found taking part in mobilising human MSCs [46]. Takai et al [47] first reported the expression of HGF and the cognate receptor c-met in human BM stromal cells, which is required for haematopoiesis. HGF is a multifunctional cytokine involved in many biological processes [48−50]. Studies have further demonstrated that HGF also functions as a strong chemotactic signal to mobilise and attract MSCs for tissue repair by interacting with c-met [46,48].

There may exist various subpopulations of MSCs with varying homing capacities as MSCs are heterogeneous, thus they possess higher homing capacity [51]. Moreover, freshly isolated MSCs have been shown to display enhanced homing ability compared to their culture-expanded counterparts [52,53]. Homing receptors, such as CXCR4, which have been upregulated in the BM and in ischaemic tissues, are usually absent on the surface of culture-expanded MSCs [52,53]. As MSCs have been proven to gain or lose certain surface markers during culture [54], which might influence their homing capability, the passage number of MSCs used for cellular therapy is an important determinant. Furthermore, MSCs treated with a cocktail of cytokines [HGF, stem cell factor, Flt-3 ligand, interleukin (IL)-3, and IL-6] in culture expressed higher levels of CXCR4 and possessed enhanced homing capacity [52]. Three-dimensional culture mimicking the in vivo niche may be an important research direction for maintaining the homing ability of MSCs during long-term ex vivo culture (e.g., culture in hydrogels under hypoxic conditions).

However, the signals that regulate stem cell mobilisation are often weakened or impaired because the function of SDF-1 is short lived. A recent study has demonstrated that low-intensity pulsed ultrasound promotes fracture healing by stimulating MSCs homing via upregulation of local and serum SDF-1 levels [55]. Prolongation of the expression of SDF-1 may be an important strategy for improving MSC homing and bone healing. Therefore, in pathological settings, particularly during the late phases of certain musculoskeletal diseases, restoration of the impaired SDF-1/CXCR4 signalling axis may be crucial for restoring and maintaining MSC homing capacity.

### Inflammation and MSCs homing

Inflammation is a cellular response that occurs during tissue injury, which is characterised by increased vascular permeability, recruitment of inflammatory cells, release of inflammatory mediators, and turnover of matrices. It is generally believed that inflammation is an important regulator for bone regeneration, which initiates the repair cascade [56]. Following bone injury, initial inflammatory response occurs, macrophages infiltrate into sites of injury, which is vital for endochondral ossification [57]. After macrophage recruitment, lymphocytes (e.g., T lymphocytes) migrate into the fracture callus and initiate the adaptive immune response [58]. Concomitantly, large numbers of proinflammatory cytokines [such as IL-1β, IL-6, and tumour necrosis factor (TNF)-α] are released [17,56,59]. When MSCs sense the immune signals, they will be activated, mobilised, and recruited into inflammatory sites, thereby facilitating tissue regeneration.

Immune cells and MSCs may share common signalling pathways regulating cell migration. The recruitment of inflammatory cells and MSCs requires interactions of multiple adhesion molecules expressed on the migrating cells and their cognate ligands expressed on vascular endothelium. One of the most important adhesion molecules is monocyte chemoattractant protein (MCP)-1, which is produced predominantly by macrophages and endothelial cells. Increased expression level of MCP-1 stimulates macrophage infiltration [60]. MCP-1/CCR2 interaction also enhances MSCs adhesion and migration [43]. Moreover, CD44 appears to be another important adhesion molecule [61]. The CD44−hyaluronic acid (HA) interaction is crucial for activated T-cell extravasation into sites of inflammation. Furthermore, CD44−HA interaction also enhances MSC adhesion and motility. Platelet-derived growth factor facilitates MSC migration by elevating CD44 expression level [62].

Recent studies have suggested that immune signals have a direct influence on MSC migration. Some proinflammatory cytokines, such as interferon-γ and TNF-α, increased the production of matrix metalloproteinases (MMPs) in MSCs, thereby enhancing the capacity of MSCs to migrate through the extracellular matrix [63]. Preincubation with TNF-α has been shown to enhance the sensitivity and migration of MSCs toward chemokines. These chemokines include SDF-1, RANTES, and macrophage-derived chemokine [64]. Some anti-inflammatory mediators themselves are chemotactic cytokines that attract MSCs. For example, IL-6 secreted by active contractile muscle cells during short intensive exercise, which is associated with an anti-inflammatory response, stimulates migration and recruitment of MSCs [65,66]. Therefore, the local and systemic inflammatory state may have an important role in triggering the migration and homing of MSCs.

### Hypoxia and MSC homing

The local oxygen level is another important factor governing MSC mobilisation and migration. MSCs reside in a complex microenvironment or so-called niche in vivo. The components of the niche include local oxygen tension, extracellular matrices (ECMs), and other stromal cells [67−69]. Bone regeneration attempts to recapitulate the normal skeletal development during embryogenesis. In pathological situations such as fracture, blood supply is usually disrupted and hypoxic microenvironments occur.
The hypoxia-inducible factors (HIFs) are key regulators of cellular adaptive response to hypoxia for adult and embryonic organisms, regulating the expression of numerous genes affecting cell survival and trafficking, angiogenesis, and cell metabolism in adverse conditions [70–72]. Both HIF-α and HIF-β subunits exist as isoforms. HIF-α subunits are regulated by a multistep process, including changes in activity, abundance, mitochondrial RNA splicing, and subcellular localization. HIFs mainly consist of HIF-1, HIF-2, and HIF-3 [73]. HIF-1α levels are regulated by proteolysis through an oxygen-sensitive mechanism. Under normoxic conditions, HIF-1α undergoes prolyl hydroxylation and is ligated by von Hippel–Lindau protein, an E3 ubiquitin ligase, and degraded by the proteasome finally. Under hypoxia, HIF-1α prolyl hydroxylation and degradation is suppressed, and HIF-1α accumulates in the nucleus where it forms a dimer with the HIF-1β subunit. The dimer then forms a transcriptional complex with coactivator p300, regulating the expression of > 60 downstream target genes, including VEGF, SDF-1, and CXCR4 [71,72,74–77]. The HIF pathway plays important roles in skeletal development. The HIF-1α pathway is also activated during the process of bone repair, which is required for angiogenesis and bone healing [78]. Therefore, the HIF/vascular endothelial growth factor signalling pathway may be another important therapeutic target for successful bone healing (Figure 2).

Recent studies have indicated that hypoxia contributes to MSC mobilisation and homing. Transiently hypoxic microenvironments (such as injured tissue or tumour) may represent the stem cell niche to some extent, in which HIF-1α stabilisation and activation of SDF-1 and CXCR4 occur, thereby facilitating the recruitment and homing of CXCR4-positive stem cells to damaged tissues. MSCs were found in circulating blood of nonstimulated rats; the circulating MSC pool was consistently and dramatically increased by almost 15-fold when the rats were exposed to chronic hypoxia [79]. Furthermore, hypoxic preconditioning enhances the migration ability and therapeutic efficacy of human MSCs [80]. Moreover, the state of tumour-induced hypoxia, which often perpetuates the inflammatory state, induces numerous angiogenic and inflammatory mediators that can stimulate MSC migration towards tumours [81]. Both SDF-1 and CXCR4 have been implicated in tumour cell metastasis [82]. Goldstein has shown endogenous human BM-MSC migration from a physiological bone environment to tumours based on tumour-derived TGF-β1, increasing their bone metastasis frequency consequently [83]. Thus, hypoxia and inflammation attract MSCs to tumours. In conclusion, HIF1α-induced SDF-1 expression stimulates the migration and homing of circulating CXCR4-positive MSCs to injured tissues [71].

MMPs also play critical roles in the transendothelial migration of MSCs. MMPs function mainly by stimulating the degradation of ECMs around MSCs. In particular, MMP-2 and MMP-9 participate in MSC migration through degradation of collagen and gelatin [84,85]. Furthermore, the expression level of MMPs in MSCs is increased by hypoxia [86].

Many cytokines or growth factors, such as vascular endothelial growth factor-A [87] and basic fibroblast growth factor [88], increase MSC migration. Mobilising MSCs can also be achieved by administering cytokines such as granulocyte-colony stimulating factor (G-CSF), SDF-1, and stem cell factor [89,90]. G-CSF is the most commonly used mobilising agent. However, a minority of healthy donors could hardly respond to administration of G-CSF [91]. Therefore, a careful search for more general and effective stem-cell-mobilising agent is imperative (Figure 3).

**Safety concerns of systemic MSC therapy**

The main cytokine that has proven to be important to the recruitment of MSCs is SDF-1. In addition to its critical role in facilitating tissue regeneration, SDF-1 is known to be secreted by tumours and is in clinical use as tumour marker [92]. No previous reports have described SDF-1-induced malignancy, but this potential adverse event cannot be excluded yet. Additionally, there is some evidence that MSC therapy might promote cancer recurrence in tumour-bearing animals [93], but there is no report that MSCs lead to tumour formation in humans. Due to the potential tumour-promoting risk in tumour patients, it would be

![Figure 2](image-url)  
**Figure 2**  
Hypoxia-inducible factor (HIF)-1-dependent signalling pathways regulating bone healing. Once bone injury or hypoxia happens, HIF-1α activation and stabilisation occur. Vascular endothelial growth factor (VEGF), stromal cell-derived factor (SDF)-1, and CXC chemokine receptor (CXCR) 4 are directly positively regulated by HIF-1α. Increased expression of VEGF, SDF-1, and CXCR4 stimulates mesenchymal stem cell (MSC) homing. VEGF is critical for angiogenesis. Improved MSC homing is involved in both osteogenesis and proangiogenesis, which is vital for bone healing.
prudent to screen patients for the presence of pre-existing malignancy before they receive MSC therapy or a therapy with systemic MSC mobilisation.

Conclusion

The recruitment and homing of MSCs are essential for bone healing. MSC mobilisation accelerates bone healing mainly by stimulating angiogenesis and coordinating bone remodelling. SDF-1/CXCR4 and HIF-1α signalling pathways play critical roles in MSC homing and bone healing. Furthermore, factors such as CD44, hypoxia, immune signals, and different cytokines are crucial for MSC migration. In pathological settings, MSC homing is often impaired due to decreased expression of SDF-1. Therefore, the restoration and normalization of signalling pathways of impaired tissue due to injury may be an important strategy for augmenting bone regeneration. To stimulate and reinforce MSC homing is promising for the future translational medicine.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgements

The work was partially supported by grants from National Natural Science Foundation of China (81371946 and 81374568); Hong Kong Government Research Grant Council, General Research Fund (470813, 14119115 and 9054014) and grants from China Shenzhen City Science and Technology Bureau (GJHZ20140419120051680 and JCYJ20150630165236960). This study was also supported in part by SMART program, Lui Che Woo Institute of Innovative Medicine, The Chinese University of Hong Kong and the research was made possible by resources donated by Lui Che Woo Foundation Limited.

References

[1] Deng J, Petersen BE, Steindler DA, Jorgensen ML, Laywell ED. Mesenchymal stem cells spontaneously express neural proteins in culture and are neurogenic after transplantation. Stem Cells 2006;24:1054–64.
[2] Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 2002;105:93–8.
[3] Duran JM, Makarewich CA, Sharp TE, Starosta T, Zhu F, Hoffman NE, et al. Bone-derived stem cells repair the heart after myocardial infarction through transdifferentiation and paracrine signaling mechanisms. Circ Res 2013;113:539–52.
[4] Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 2007;25:2648–59.
[5] Wang Y, Sun Y, Yang XY, Ji SZ, Han S, Xia ZF. Mobilised bone marrow-derived cells accelerate wound healing. Int Wound J 2013;10:473–9.
[6] Mendicino M, Bailey AM, Wonnacott K, Puri RK, Bauer SR. MSC-based product characterization for clinical trials: an FDA perspective. Cell Stem Cell 2014;14:141–5.
[7] Pountos I, Jones E, Tzioupi C, McGonagle D, Giannoudis PV. Growing bone and cartilage: the role of mesenchymal stem cells. J Bone Joint Surgery Br 2006;88:421–6.
[8] Harris MT, Butler DL, Boivin GP, Floer JD, Schantz EJ, Wenstrup RJ. Mesenchymal stem cells used for rabbit tendon repair can form ectopic bone and express alkaline phosphatase activity in constructs. J Orthop Res 2004;22:998–1003.
[9] Yuehua Jiang BNJ, Reinhardt RL, Schwartz RE, Keenek CD, Ortiz-Gonzalez XR, Morayma Reyes TL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41–9.
[10] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.
[11] Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41–9.
[12] Lv FJ, Tian RS, Cheung KA, Leung VY. Concise review: the surface markers and identity of human mesenchymal stem cells. Stem Cells 2014;32:1408–19.
[13] Eririn A, Zhu XY, Krier JD, Tang H, Jordan KL, Grande JP, et al. Adipose tissue-derived mesenchymal stem cells improve revascularization outcomes to restore renal function in swine atherosclerotic renal artery stenosis. Stem Cells 2012;30:1030–41.

[14] Wan C, He Q, Li G. Allogenic peripheral blood derived mesenchymal stem cells (MSCs) enhance bone regeneration in rabbit ulna critical-sized bone defect model. J orthop Res 2006;24:610–8.

[15] Ren G, Zhang L, Zhao X, Xu G, Zhan Y, Roberts AJ, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2008;2:141–50.

[16] Chen X, Armstrong MA, Li G. Mesenchymal stem cells in immunoregulation. Immunol Cell Biol 2006;84:413–21.

[17] Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol 2014;15:1009–16.

[18] Bruder SP, Fink DJ, Caplan AI. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. J Cell Biochem 1994;56:283–94.

[19] Shirley D, Marsh D, Jordan G, McQuaid S, Li G. Concepts of fracture union, delayed union, and nonunion. Clin Orthop Relat Res 1998;322:30–5.

[20] Marsh D. Skeletal remodeling in health and disease. Nat Med 2005;11:939–45.

[21] Schipani E, Maes C, Carmeliet G, Semenza GL. Regulation of osteogenesis by factors from the bone marrow stromal cell niche. J Cell Biochem 1994;56:283–94.

[22] Todeschi MR, El Backly R, Capelli C, Daga A, Patrone E, Introna M, et al. Transplanted umbilical cord mesenchymal stem cells into sites of musculoskeletal repair. J Orthop Res 2011;29:1013–21.

[23] Granero-Molto F, Weis JA, Miga MI, Landis B, Myers TJ, O’Rear L, et al. Regenerative effects of transplanted mesenchymal stem cells in fracture healing. Stem Cells 2009;27:1887–98.

[24] Huang Z, Ma T, Ren PG, Smith RL, Goodman SB. Effects of orthopedic polymer particles on chemotaxis of macrophages and mesenchymal stem cells. J Biomed Mater Res A 2010;94:1236–9.

[25] Shi Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol 2014;15:1009–16.

[26] Bruder SP, Fink DJ, Caplan AI. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. J Cell Biochem 1994;56:283–94.

[27] Tang Y, Wu X, Lei W, Pang L, Wan C, Shi Z, et al. TGF-beta1-stimulated reporter gene expression is increased by SDF-1alpha mediated stem cell recruitment. Tissue Eng A 2006;12:801–9.

[28] Schipani E, Maes C, Carmeliet G, Semenza GL. Regulation of osteogenesis-angiogenesis coupling by HIFs and VEGF. J Bone Miner Res 2009;24:1347–53.

[29] Glowacki J. Angiogenesis in fracture repair. Clin Orthop Relat Res 1998;338:80–91.

[30] Zaidi M. Skeletal remodeling in health and disease. Nat Med 2005;11:939–45.

[31] Hill PA. Bone remodelling. Br J Orthodont 1998;25:101–7.

[32] Kitaori T, Ito H, Schwarz EM, Tsutsumi R, Yoshitomi H, Oishi S, et al. Stromal cell-derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. Arth Rheum 2009;60:813–23.

[33] Wang L, Li Y, Chen J, Gautam SC, Zhang Z, Lu M, et al. Ischemic cerebral tissue and MCP-1 enhance rat bone marrow stromal cell migration in interface culture. Exp Hematol 2002;30:831–6.

[34] Shinohara K, Greenfield S, Pan H, Vasanji A, Kumagai K, Midura RJ, et al. Stromal cell-derived factor-1 and monocyte chemotactic protein 1 receptors reveal alternative splicing of the carboxyl-terminal tails. Proc Natl Acad Sci U S A 1994;91:2752–6.

[35] Sui B, Hu C, Zhang X, Zhao P, He T, Zhou C, et al. Allogeneic mesenchymal stem cell therapy promotes osteoblastogenesis and prevents glucocorticoid-induced osteoporosis. Stem Cells Transl Med 2016;5:1238–46.

[36] Neuss S, Becher E, Woltje M, Tietze L, Jahnen-Dechent W. Efficient homing of multipotent adult mesenchymal stem cells depends on FROUNT-mediated clustering of CCR2. Cell Stem Cell 2008;2:566–75.

[37] Wang L, Li Y, Chen J, Gautam SC, Zhang Z, Lu M, et al. Hepatocyte growth factor effects on mesenchymal stem cells. Stem Cells 2003;21:1887–98.

[38] Pieper CF, Mautalen C, et al. Zoledronic acid and clinical revascularization outcomes to restore renal function in swine atherosclerotic renal artery stenosis. Stem Cells 2012;30:801.  

[39] Poliarinen J, Lin TH, Nabeshima A, Jamsen E, Lu L, Nathan K, et al. Mesenchymal stem cells in the aseptic loosening of total joint replacements. J Biomed Mater Res A 2017;105:1195–207.

[40] Belema-Bedada F, Uchida S, Martire A, Kostin S, Braun T. Functional expression of HGF and HGF receptor/c-met in adult human mesenchymal stem cells suggests a role in cell immunoregulation. Immunol Cell Biol 2006;84:413–21.

[41] Neuss S, Becher E, Woltje M, Tietze L, Jahnen-Dechent W. 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 2004;22:405–14.

[42] Takai K, Hara J, Matsumoto K, Hosoi G, Osugi Y, Tawa A, et al. Hepatocyte growth factor is constitutively produced by human bone marrow stromal cells and indirectly promotes hematopoiesis. Blood 1997;89:1560–5.

[43] Pozzi G, Mirielli M, Costi P, Antonucci D, Sala M, Gnocichi V, et al. Hepatocyte growth factor effects on mesenchymal stem cells: proliferation, migration, and differentiation. Stem Cells 2006;24:23–33.
Matsumoto K, Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. J Biochem 1996;119:591–600.

Okunishi K, Doi M, Nakagome K, Tanaka R, Mizuno S, Matsumoto K, et al. A novel role of hepatocyte growth factor as an immune regulator through suppressing dendritic cell function. J immunol 2005;175:4745–53.

Wynn RF, Hart CA, Corradi-Perini C, O’Neill L, Evans CA, Wraith JE, et al. A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. Blood 2004;104:2643–5.

Shi M, Li J, Liao L, Chen B, Li B, Chen L, et al. Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: role in homing efficiency in NOD/SCID mice. Haematologica 2007;92:897–904.

Rombouts WJ, Ploemacher RE. Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. Leukemia 2003;17:160–70.

Jones E, McGonagle D. Human bone marrow mesenchymal stem cells in vivo. Rheumatology 2008;47:126–31.

Wei FY, Leung KS, Li G, Qin J, Chow SK, Huang S, et al. Low intensity pulsed ultrasound enhanced mesenchymal stem cell recruitment through stromal derived factor-1 signaling in fracture healing. PLoS One 2014;9:e87222.

Claes L, Recknagel S, Ignatius A. Fracture healing under healthy and inflammatory conditions. Nat Rev Rheumatol 2012;8:133–43.

Xing Z, Lu C, Hu D, Yu YY, Wang X, Colnot C, et al. Multiple roles for CCR2 during fracture healing. Dis Model Mech 2010;3:451–8.

Andrew JG, Andrew SM, Freemont AJ, Marsh DR. Inflammatory cells in normal human fracture healing. Acta Orthopaed Scand 1994;65:462–6.

Gerstenfeld LC, Cullinane DM, Barnes GL, Graves DT, Einhorn TA. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. J Cell Biochem 2003;88:873–84.

Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest 2006;116:1494–505.

Pure E, Cuff CA. A crucial role for CD44 in inflammation. Trends Mol Med 2001;7:213–21.

Zhu H, Mitsushashi N, Klein A, Barsky LW, Weinberg K, Barr ML, et al. The role of the hyaluronan receptor CD44 in mesenchymal stem cell migration in the extracellular matrix. Stem Cells 2006;24:928–35.

Hemeda H, Jakob M, Ludwig AK, Giebel B, Lang S, Brandau S. Interferon-gamma and tumor necrosis factor-alpha differentially affect cytokine expression and migration properties of mesenchymal stem cells. Stem Cells Dev 2010;19:693–706.

Pointe AL, Marais E, Gallay N, Langonne A, Delorme B, Herault O, et al. The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. Stem Cells 2007;25:1737–45.

Schmidt A, Bierwirth S, Weber S, Platen P, Schinkothe T, Bloch W. Short intensive exercise increases the migratory activity of mesenchymal stem cells. Br J Sports Med 2009;43:195–8.

Wong MM, Fish EN. Chemokines: attractive mediators of the immune response. Semin Immunol 2003;15:5–14.

Fuchs E, Tumbar T, Guasch G. Socializing with the neighbors: stem cells and their niche. Cell 2004;116:769–78.

Estrada JC, Albo C, Benguria A, Dopazo A, Lopez-Romero P, Carrera-Quintanar L, et al. Culture of human mesenchymal stem cells at low oxygen tension improves growth and genetic stability by activating glycolysis. Cell Death Differ 2012;19:743–55.

Mohyeldin A, Garzon-Muvdi T, Quinones-Hinojosa A. Oxygen in stem cell biology: a critical component of the stem cell niche. Cell Stem Cell 2010;7:150–61.

Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell 2012;148:399–408.

Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinnan ME, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004;10:858–64.

Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3:721–32.

Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med 2003;9:677–84.

Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 1996;16:4604–13.

Kallio PJ, Okamoto K, O’Brien S, Carrero P, Makino Y, Tanaka H, et al. Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1alpha. EMBO J 1998;17:6573–86.

Saakko P, Nevalainen T, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitination complex by O2-regulated prolyl hydroxylation. Science 2001;292:468–72.

Liu H, Xue W, Ge G, Luo X, Li Y, Xiang H, et al. Hypoxic preconditioning advances CXCR4 and CXCR7 expression by activating HIF-1alpha in MSCs. Biochem Biophys Res Comm 2010;401:509–15.

Wan C, Gilbert SR, Wang Y, Cao X, Shen X, Ramaswamy G, et al. Activation of the hypoxia-inducible factor-1alpha pathway accelerates bone regeneration. Proc Natl Acad Sci USA 2008;105:686–91.

Rochefort GY, Delorme B, Lopez A, Herault O, Bonnet P, Charbord P, et al. Multipotential mesenchymal stem cells are mobilized into peripheral blood by hypoxia. Stem Cells 2006;24:2202–8.

Rosova I, Mao D, Capoccia B, Link D, Nolta JA. Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. Stem Cells 2008;26:2173–82.

Spaeth E, Klopp A, Dembski J, Andreeff M, Marini F. Inflammation and tumor microenvironments: defining the migratory itineraries of mesenchymal stem cells. Gene Ther 2008;15:730–8.

Muller A, Homey B, Soto H, Ge N, Catron D, Buchanen ME, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50–6.

Goldstein RH, Reagan MR, Anderson K, Kaplan DL, Rosenblatt M. Human bone marrow-derived MSCs can home to orthotopic breast cancer tumors and promote bone metastasis. Cancer Res 2010;70:10044–50.

Steininger C, Brenig F, Baumgartner L, Schmidt J, Schmidt A, Bloch W. Characterization of key mechanisms in transmigration and invasion of mesenchymal stem cells. J Mol Cell Cardiol 2008;44:1072–84.

De Becker A, Van Hummelen P, Bakkus M, Vande Broek I, De Wever J, De Wael M, et al. Migration of culture-expanded human mesenchymal stem cells through bone marrow endothelium is regulated by matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-3. Haematologica 2007;92:540–9.

Ben-Yosef Y, Lahat N, Shapiro S, Bitterman H, Miller A. Regulation of endothelial matrix metalloproteinase-2 by hypoxia/reoxygenation. Circ Res 2002;90:784–91.

Schichor C, Birnbaum T, Etminan N, Schnell O, Grau S, Miebach S, et al. Vascular endothelial growth factor A
contributes to glioma-induced migration of human marrow stromal cells (hMSC). Exp Neurol 2006;199:301–10.

[88] Schmidt A, Ladage D, Schinkothe T, Klausmann U, Ulrichs C, Klinz FJ, et al. Basic fibroblast growth factor controls migration in human mesenchymal stem cells. Stem Cells 2006;24:1750–8.

[89] Deng J, Zou ZM, Zhou TL, Su YP, Ai GP, Wang JP, et al. Bone marrow mesenchymal stem cells can be mobilized into peripheral blood by G-CSF in vivo and integrate into traumatically injured cerebral tissue. Neurol Sci 2011;32:641–51.

[90] Lapidot T, Petit I. Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. Exp Hematol 2002;30:973–81.

[91] Fu S, Liesveld J. Mobilization of hematopoietic stem cells. Blood Rev 2000;14:205–18.

[92] Sun X, Cheng G, Hao M, Zheng J, Zhou X, Zhang J, et al. CXCL12/CXCR4/CXCR7 chemokine axis and cancer progression. Cancer Metast Rev 2010;29:709–22.

[93] Paino F, La Noce M, Di Nucci D, Nicoletti GF, Salzillo R, De Rosa A, et al. Human adipose stem cell differentiation is highly affected by cancer cells both in vitro and in vivo: implication for autologous fat grafting. Cell Death Dis 2017;8:e2568.