Citation: BoneKEy Reports **2**, Article number: 300 (2013) | doi:10.1038/bonekey.2013.34

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REVIEW

CXCL12/CXCR4 signaling and other recruitment and homing pathways in fracture repair

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Cell recruitment, migration and homing to the fracture site are essential for the inflammatory process, neovascularization, chondrogenesis, osteogenesis and ultimately bone remodeling. Mesenchymal stem cells (MSCs) are required to navigate from local sources such as the periosteum and local bone marrow, and may also be recruited from the circulation and distant bone marrow. While the local recruitment process may involve matrix binding and degradation, systemic recruitment may utilize extravasation, a process used by leukocytes to exit the vasculature. CXCL12 (stromal cell-derived factor-1 (SDF-1)), a member of the CXC family of chemokines, is thought to have an important role in cell migration at the fracture site. However, there are many molecules upregulated in the hematoma and callus that have chemotactic potential not only for inflammatory cells but also for endothelial cells and MSCs. Surprisingly, there is little direct data to support their role in cell homing during bone healing. Current therapeutics for bone regeneration utilize local or systemic stem cell transplantation. More recently, a novel strategy that involves mobilization of large numbers of endogenous stem and progenitor cells from bone marrow into the circulation has been shown to have positive effects on bone healing. A more complete understanding of the molecular mechanisms underlying cell recruitment and homing subsequent to fracture will facilitate the fine-tuning of such strategies for bone.

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Overview of Fracture Repair

Bone has a remarkable capacity for repair and regeneration. The vast majority of fractures heal by indirect (secondary) fracture healing, a process that recapitulates some aspects of bone morphogenesis in that it involves both intramembranous and endochondral bone formation.[1] This type of healing occurs when there is motion between the bone ends and is characterized by the formation of a callus. The fracture environment immediately following injury is highly complex. Rupture of blood vessels, activation of platelets and secretion of tissue factor by endothelial cells result in fibrin polymerization. The resulting hematoma, comprised of a fibrin meshwork and platelet aggregates, provides a solid and stable structure for the initial influx of inflammatory cells, the coagulation cascade resulting in their subsequent activation.[2,3] The hematoma, exposed bone matrix and local periosteum are then the source of an array of inflammatory cytokines, chemokines, growth factors, angiogenic factors and other small molecules like prostaglandins.[2,4,5] These factors are thought to be chemotactic for inflammatory cells, endothelial cells and mesenchymal stem cells (MSCs) and promote angiogenesis, MSC proliferation and differentiation, and ultimately bone healing.[4–6]

Cell Sources for Repair

Recruitment of inflammatory cells, endothelial cells and MSCs is essential for bone healing. Tissue-resident, circulating and bone marrow-derived inflammatory cells are recruited. New blood vessels arise as sprouts from existing vessels located nearby. This process involves migration and proliferation of existing endothelial cells and recruitment of circulating endothelial progenitor cells, and those which reside in the bone marrow.[7,8] Recruitment of MSCs to the fracture site is thought to occur very early in the fracture healing process (by day 1),[4] although the exact source of MSCs is debated.

MSCs likely derive from a combination of sources, including bone marrow, periosteum, blood vessel walls, adjacent soft tissues and peripheral blood[9–12] (**Figure 1**). Periosteum is a rich source of skeletal progenitor cells that can differentiate into chondrogenic and osteogenic cell lineages[13–15] and is

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Received 25 October 2012; accepted 8 February 2013; published online 13 March 2013

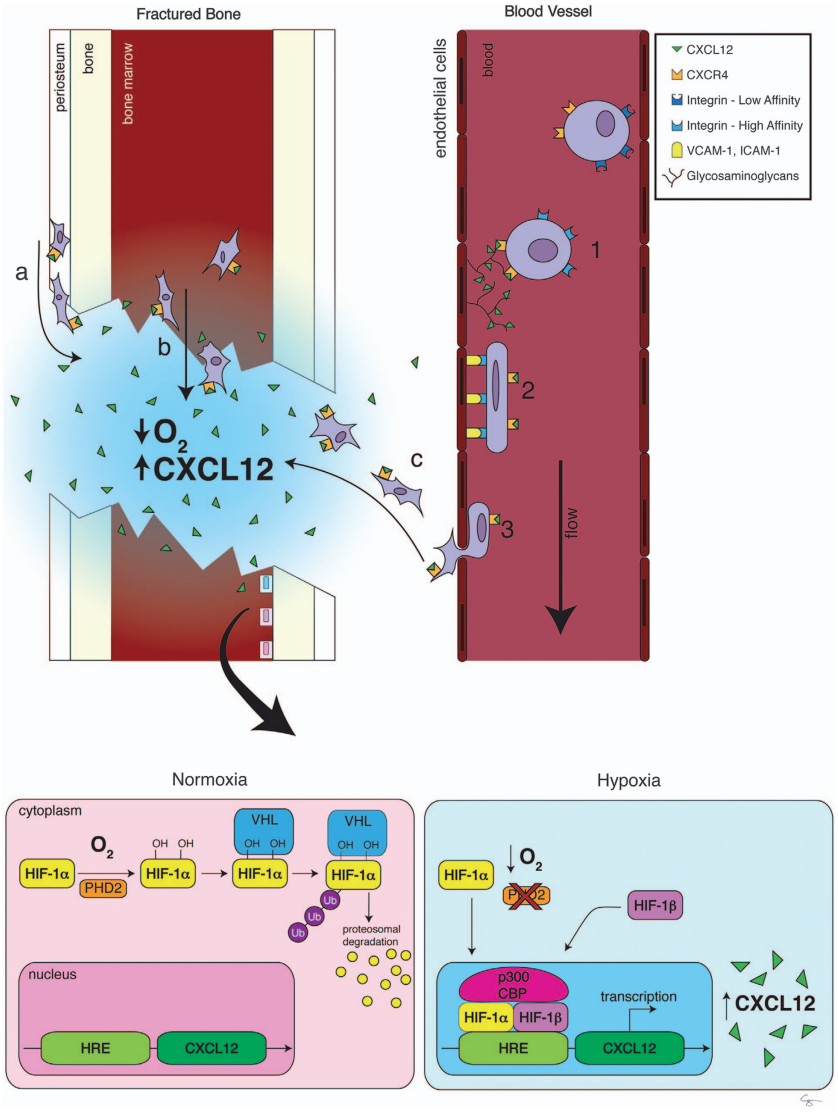

Figure 1 Proposed interaction of hypoxia, CXCL12 production and cell migration at the fracture site. Evidence suggests that the transcription factor HIF-1α may drive the upregulation of CXCL12 production in cells of damaged tissues. Low O_2 levels at the fracture site (indicated by blue shading) reduce the activity of prolylhydroxylase domain protein 2 (PHD2), which would normally hydroxylate HIF-1α, leading to its binding to the von Hippel–Lindau protein (VHL) and subsequent ubiquitination and proteasomal degradation. Instead, HIF-1α levels are stabilized, it migrates into the nucleus and heterodimerizes with HIF-1β. With transcriptional coactivators CREB-binding protein (CBP) and 300-kDa coactivator protein (p300), HIF-1α binds to the hypoxia-responsive element (HRE) on the promoter of the *CXCL12* gene. Increased CXCL12 levels at the site of injury may promote migration of cells, including those from the periosteum (**a**), local bone marrow (**b**) and circulation (**c**) (see text for details). Chemokines like CXCL12 bind to glycosaminoglycans on the surface of endothelial cells, and as a result are presented at high concentrations on the inner wall of the vessel. In this scenario, CXCL12 would engage its receptor CXCR4 on circulating cells and convert cell surface integrins to a high-affinity state (1). ICAM-1 (intercellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1), expressed on the endothelial cell surface, bind to integrins on circulating cells. Cells stop rolling and spread (2), and then migrate through the endothelium towards the chemokine gradient (3). This process of chemokine-mediated cell extravasation from the vasculature has been demonstrated for leukocytes, but it is likely that other circulating cells such as MSCs and EPCs, which express CXCR4, could undergo a similar process of transmigration. This figure was created by Chrisoula Toupadakis (University of California Davis, Davis, CA, USA).

considered to be the major source of skeletal progenitor cells for fracture healing.[16] Indeed, studies have demonstrated that removal of the periosteum has a negative impact on bone healing.[17,18]

The work of Alexander Friedenstein and his co-workers in the 1960s and 1970s led to the identification of a small population of cells in the bone marrow, now referred to as MSCs, that could adhere to tissue culture plastic and subsequently undergo osteogenic differentiation.[19] Although it is likely that these cells migrate to the site of injury from the local bone marrow, there is also evidence to suggest that they mobilize into peripheral blood and subsequently home to the site of injury. In a parabiotic mouse model, wild-type mice that were surgically linked to donor mice expressing green fluorescent protein (GFP) in non-erythroid tissue underwent a fibular fracture.[9] GFP^+ cells were identified at the fracture site up to 3 weeks after fracture, suggesting that fracture induced the mobilization of cells from the bone marrow of the donor mouse into the peripheral blood that homed to and engrafted into the fracture callus.[9]

Adherent fibroblast-like cells with adipogenic and osteogenic capacity have been detected in very low numbers in the peripheral blood,[20–23] suggesting the existence of a circulating pool of MSC-like cells. In response to tissue trauma, the numbers of bone marrow-derived MSCs and osteogenic

progenitors in peripheral blood are elevated.[9,22,24–27] Likewise, few circulating endothelial progenitor cells could be detected in the peripheral blood under normal conditions;[28,29] however, their numbers are significantly elevated in association with vascular injury, burns and fracture.[30–36] The potential role of systemically mobilized progenitor cells in fracture healing is unclear.

Although their exists a multiplicity of potential sources of MSCs to contribute to fracture healing, both local and systemically derived progenitors are thought to be attracted by the release of potent chemokines at the fracture site and to move down the ensuing chemical gradients.

Recruitment and Homing

Cells migrate toward the damaged tissue along chemical gradients by a process called chemotaxis. Cells derived from the periosteum, bone marrow and soft tissues, which exist close to the fracture site, may simply need to navigate through the local connective tissue, the hematoma and developing granulation tissue, a process that involves cell matrix binding and degradation. Cells recruited from the bloodstream likely undergo a process similar to extravasation, a complex process that has been well described in leukocytes[3,37] (**Figure 1**). Circulating leukocytes constantly survey the endothelial cell walls by slowing down and rolling. In the presence of inflammation, endothelial cells are stimulated to increase the surface expression of adhesion molecules, such as selectins, and integrin ligands, such as VCAM-1 (vascular cell adhesion molecule-1) and ICAM-1 (intercellular adhesion molecule-1). In addition, chemokines produced at the site of injury bind to glycosaminoglycans on the surface of endothelial cells where they accumulate at high concentrations.[3,37] These changes result in leukocyte binding, activation and ultimately transmigration across the vessel wall. MSCs transmigrate through tumor necrosis factor-α-activated endothelium using mechanisms similar to those utilized by leukocytes, in addition to novel mechanisms.[38] It is suggested that while MSCs undergo a process similar to that of leukocyte recruitment, they might utilize a distinct set of adhesion molecules.[39]

The CXCL12/CXCR4 Pathway and Cell Homing

Chemokines are chemotactic cytokines responsible for the establishment of chemical gradients for cell migration. They are small, 8–14 kDa in size and contain four conserved cysteine residues.[40] They are further classified into four subfamilies, CXC, CC, (X)C and CX3C, based on the position of the N-terminal two cysteine residues.[40] Chemokine receptors are G-protein-coupled receptors, classified into the same four subfamilies in accordance with their chemokine ligands.[40] CXCL12 was first identified as a soluble ligand secreted by the bone marrow stromal cells that stimulated the proliferation and growth of B-cell progenitors.[41] It was termed pre-B-cell growth-stimulating factor, later to be known as stromal cell-derived factor-1 (SDF-1).[42] Both CXCR4 and CXCR7 are receptors for CXCL12.[40] Mice with disruption of *CXCL12* or *CXCR4* genes die late in gestation or within an hour of birth.[43–45] Mice exhibited defects in the development of the heart and brain, impaired B-cell lymphopoiesis and bone marrow hematopoiesis, and impaired vascular development.[43–45] Critical roles for CXCL12 and CXCR4 in many aspects of development and organogenesis are now established. In addition, CXCL12/CXCR4 signaling is thought to be a master regulator of stem cell migration.

Tissue-committed $CXCR4^+$ stem cells could be isolated from bone marrow mononuclear cell populations by chemotaxis towards CXCL12.[46] Bone marrow-derived MSCs express CXCR4 and migrate toward CXCL12 gradients *in vitro*.[47] CXCL12-mediated migration of MSCs and T cells involves activation of a number of signal-transduction pathways, including phosphoinositide 3-kinase/Akt, extracellular signal related kinase and p38 mitogen-activated protein kinase.[48–50] A requirement for changes in intracellular Ca^{2+} has also been implicated in CXCL12-stimulated migration of hematopoietic progenitor cells.[51] It is well documented that CXCL12 is upregulated in damaged tissues, including the brain,[52,53] heart,[54] kidney,[55] skin,[56] bone[57–59] and in irradiated bone marrow.[60] Some of these same studies demonstrated migration of transplanted $CXCR4^+$ stem cell populations to the site of damage.[53,55] Increased CXCL12 expression is considered a key signal to promote the migration of stem and progenitor cells to these tissues to participate in repair and regeneration.[61]

Hypoxia and CXCL12 Expression

Hypoxia at the site of damage, and the expression of the transcription factor hypoxia-inducible factor-1, α-subunit (HIF-1α), may drive the upregulation of CXCL12 in damaged tissues and ultimately regulate the homing of $CXCR4^+$ stem and progenitor cells[62] (**Figure 1**). Under normoxic conditions, HIF-1α undergoes rapid ubiquitination and proteosomal degradation that is dependent on the hydroxylation of proline residues within HIF-1α by the enzyme prolylhydroxylase domain protein 2 (PHD2).[63] Under hypoxic conditions, the activity of PHD2 is reduced and HIF-1α degradation is inhibited; HIF-1α accumulates and binds to its consensus sequence, the hypoxia-responsive element on HIF-1α target genes.[63] HIF-1α has been shown to induce the expression of CXCL12 under hypoxic conditions in human endothelial cells.[62] Since the fracture site is considered hypoxic,[64,65] it is possible that the expression of chemokines such as CXCL12 is regulated by decreased O_2 availability and HIF-1α.

CXCL12 and Bone Regeneration

Evidence suggests that the CXCL12/CXCR4 pathway may have important roles in fracture healing. CXCL12 expression was increased 4 days after induction of a stress fracture in the rat ulna.[57] Plasma levels of CXCL12 were elevated in human patients 2–3 days following osteotomy and application of external fixators for limb lengthening procedures, and remained elevated during the distraction period.[58] In a murine segmental bone graft model, CXCL12 levels were increased in live bone graft 2 days after surgery, with high expression in the periosteum.[59] In a murine model of fracture healing, we identified CXCL12 expression in the fracture callus in hypertrophic cartilage and immature cartilage close to pre-existing cortical bone.[66] Furthermore, CXCL12 staining colocalized with staining for Hypoxyprobe (pimonidazole hydrochloride; Hypoxyprobe, Inc., Burlington, MA, USA) a marker of hypoxic cells.[66] Almost all cells in the callus, including chondrocytes, osteoblasts, osteoclasts and undifferentiated mesenchymal

tissue cells, stained positively for CXCR4.[66] A similar pattern of CXCL12 expression was demonstrated in prehypertrophic and hypertrophic chondrocytes in a murine rib fracture callus.[67]

In 2008, Otsuru *et al.*[68] identified elevated HIF-1 mRNA levels around a bone morphogenetic protein 2 (BMP-2)/collagen pellet implanted in the backs of mice with high expression of CXCL12 in adjacent vascular endothelial cells. CXCL12 levels were high in osteoblasts as new bone formed in the pellet.[68] Furthermore, the migration of tail vein-injected, GFP^+ marrow-derived osteoblast progenitor cells to the pellet, where they contributed to new bone formation, was inhibited by a CXCR4-blocking antibody.[68] Similarly, only $CXCR4^+$ MSCs delivered intravenously were able to home to the site of fracture in a rat model.[69] In another study, wild-type and GFP^+ mice were surgically cojoined as parabiots; transplantation of MSCs overexpressing CXCL12 in a collagen scaffold adjacent to the site of a murine fibular osteotomy in the wild-type mouse increased the recruitment of GFP^+ and GFP^+/alkaline phosphatase-positive cells to the site.[70] New bone formation in a murine femoral bone graft model was inhibited by administration of anti-CXCL12-neutralizing antibody, chemical inhibition of the CXCR4/CXCL12 axis and in mice with genetically reduced CXCL12 and CXCR4 expression.[59] In our murine fracture model, administration of a CXCR4 antagonist, AMD3100, two times daily over the course of healing resulted in significantly reduced callus cartilage volume after 14 days, callus volume and mineralized bone volume at day 42 and reduced expression of genes associated with endochondral bone formation.[66] Taken together, these studies suggest that CXCL12/CXCR4 signaling does have a central role in bone healing by regulating the recruitment of stem and progenitor cells. Furthermore, it is likely that the hypoxic nature of the fracture site[65] and hypoxia in developing tissues such as cartilage[71] contribute to CXCL12 expression.

Recent studies suggest that CXCL12 administration to the site of bone damage may have potential therapeutic benefits. In a murine fracture model, a single injection of CXCL12 immediately after fracture elevated the expression of genes associated with endochondral ossification and induced changes in callus histology, which suggests accelerated healing.[72] In a murine model of high-speed distraction osteogenesis, where the bone fragments are distracted faster than normal, resulting in impaired callus formation, local injection of CXCL12 every other day rescued callus formation, increased the number of resident bone marrow endothelial and endothelial progenitor cells, and improved vascularization.[73]

It is of note that CXCL12 has also been shown to induce the chemotactic recruitment of human osteoclast precursors, promote their differentiation into osteoclasts and regulate their activity and survival.[74,75] Osteoclast activity is essential for remodeling of woven bone in the hard callus,[76] although the interplay of CXCL12 and osteoclasts in the fracture environment has yet to be explored.

Role of CXCL12 in Bone and Cartilage Development

Several studies suggest a pivotal role for CXCL12 signaling in bone development, a process that is recapitulated in many aspects during fracture healing. In a study of developing mouse bones, CXCL12 was expressed in prehypertrophic and hypertrophic chondrocytes of the growth plate.[67] Compared with wild-type embryonic mice, $SDF^{-/-}$ mice had significantly shorter humeri and smaller hypertrophic and calcification zones in the growth plate.[67] In contrast, another study reported low-level expression of CXCL12 in the growth plate and high expression of CXCL12 in bone marrow adjacent to the hypertrophic zone of the growth plate.[77] However, CXCR4 was strongly expressed in the hypertrophic zone and CXCL12 treatment stimulated chondrocyte hypertrophy.[77] In another study, CXCL12 was expressed at the site of the future periosteum early in development (E14) with increased expression in endosteal osteoblasts and chondrocytes in the hypertrophic zone at E18.[78] At birth, CXCL12 expression decreased on the periosteal surface but increased on endosteal marrow surfaces.[78] CXCL12 has been shown to regulate BMP-2-stimulated osteogenic differentiation[79] and CXCR4 regulates osteoblast development in postnatal bone.[80] As such, CXCL12 signaling may have roles in fracture healing that extend beyond cell recruitment, including direct effects on MSC proliferation, and differentiation into cells of the chondrogenic and osteogenic lineages.

Manipulation of CXCL12/CXCR4 Interactions in the Bone Marrow to Mobilize Stem and Progenitor Cell Populations

It is well recognized that CXCL12 production by endosteal osteoblasts, endothelial cells and reticular cells is critical for retention of HSCs in the bone marrow[60,81,82] (**Figure 2a**). Low O_2 levels at the endosteal surface are thought to potentiate CXCL12 production by these bone marrow niche cells.[83] Tissue damage, in particular ischemic tissue damage including fracture, results in mobilization of stem and progenitor cells from their bone marrow niche into the peripheral blood.[9,31,32,34] For example, hindlimb ischemia induced mobilization of murine bone marrow derived $c\text{-}kit^+$ stem cells into the peripheral blood at 6 h.[84] At this same time point, CXCL12 levels were increased in ischemic muscle, significantly increased in the plasma and significantly downregulated in the bone marrow.[84] It is likely that ischemic tissue damage results in increased CXCL12 levels at the fracture site driven by decreased O_2 (**Figure 2b**). Increased levels of CXCL12 at the fracture site coupled with increased plasma CXCL12 levels, and potentially decreased bone marrow levels, could create a CXCL12 chemotactic gradient that results in stem and progenitor cell mobilization into peripheral blood and homing to the fracture site (**Figure 2b**). Although there is evidence for fracture-induced stem cell mobilization,[9,26,31,32,36] increased CXCL12 after stress fracture site[57] and in plasma following osteotomy,[58] the effects of fracture on bone marrow levels of CXCL12 are as yet unknown.

CXCR4 antagonists such as AMD3100 rapidly mobilize hematopoietic progenitor cells into the peripheral blood in both humans and mice,[85] as a result of disruption to CXCL12/CXCR4 interactions in the bone marrow (**Figure 2c**). Interestingly, there is also evidence to suggest that AMD3100 induces mobilization of endothelial progenitor cells and MSCs into the peripheral blood.[86–88] The ability of molecules such as AMD3100 to mobilize large numbers of stem and progenitor cells into the peripheral blood has been utilized in a radical new approach to enhance bone healing. It is proposed that these mobilized cells will home to the site of injury to participate in bone regeneration. For example, 15 daily injections of AMD3100 significantly enhanced murine calvarial defect healing at 8 weeks with

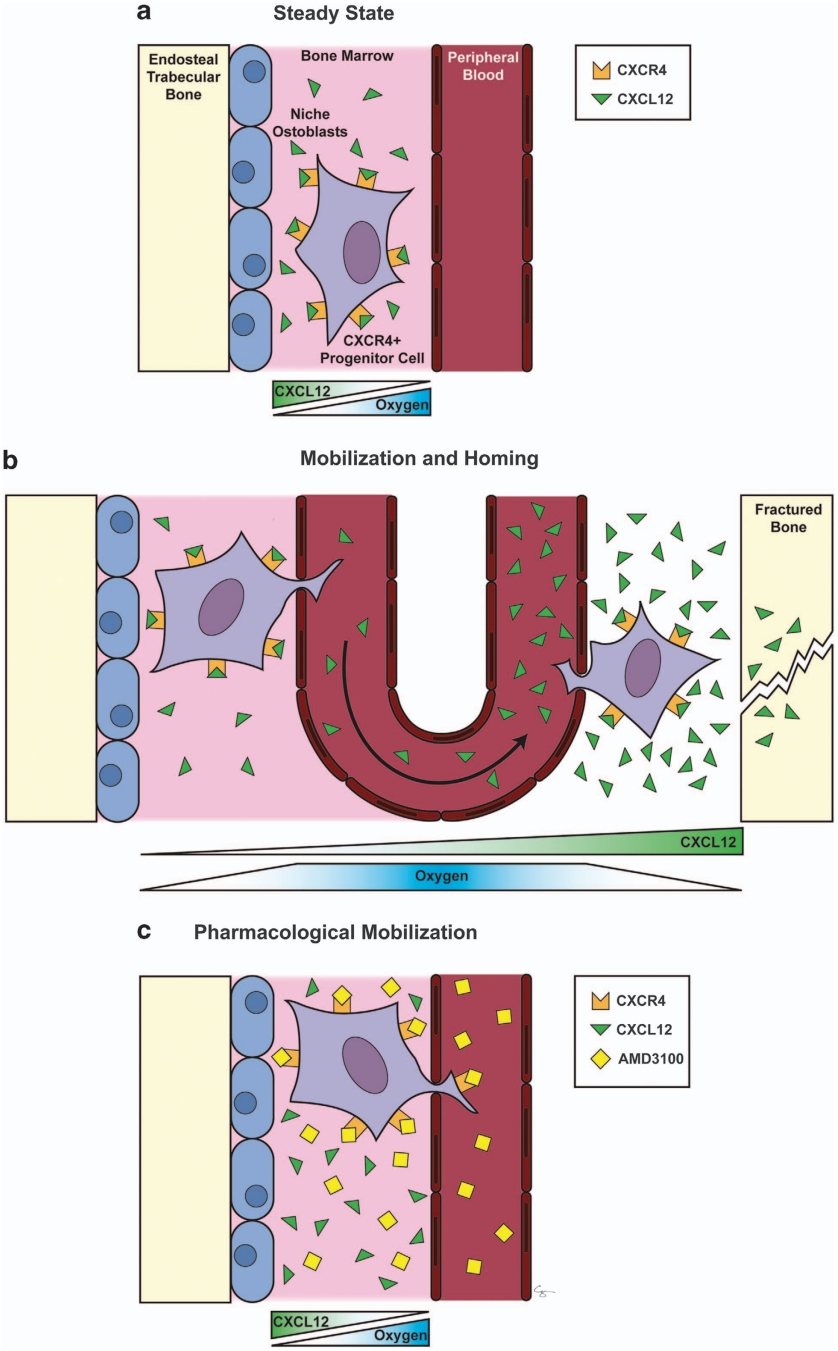

Figure 2 CXCL12/CXCR4 interactions in the bone marrow. (**a**) CXCL12 production by endosteal osteoblasts (shown), endothelial cells and reticular cells (not shown) is critical for the retention of $CXCR4^+$ stem and progenitor cells in the bone marrow. Low O_2 levels at the endosteal wall, which is at a distance from marrow sinusoids, are thought to drive CXCL12 production in these cells. (**b**) High levels of CXCL12 produced at the site of bone damage may result in the establishment of a chemotactic gradient between the fracture, blood plasma and bone marrow. It is possible that CXCL12 production at the fracture site is driven by low O_2 levels. $CXCR4^+$ stem and progenitor cells are mobilized from the bone marrow into the peripheral blood and migrate down the CXCL12 chemotactic gradient. At the fracture site, cells must undergo extravasation through the vascular wall. (**c**) Molecules that interfere with CXCL12/CXCR4 binding mobilize $CXCR4^+$ cells into the peripheral blood. AMD3100, a CXCR4 antagonist, causes rapid mobilization of hematopoietic stem cells, MSCs and endothelial progenitor cells. Pharmacological mobilization of large numbers of stem and progenitor cells into the peripheral blood may be an effective therapeutic for bone regeneration. This figure was created by Chrisoula Toupadakis (University of California Davis, Davis, CA, USA).

increased neovascularization of regenerating tissue observed a early as 1 week.[89] AMD3100, administered one time after murine bone marrow ablation surgery, significantly enhanced intramedullary trabecular bone regeneration 3 weeks later.[87] AMD3100 improved healing of a murine tibial defect after 8 weeks, but only when administered in combination with insulin-like growth factor-1.[90] In our own studies of murine femoral fracture healing, mice injected for 3 days following injury with AMD3100 had a significantly greater total callus volume at day 21 after surgery compared with mice injected with saline

(unpublished observations). Although AMD3100-induced cell mobilization appears to have positive effects on bone healing, disruption of CXCL12/CXCR4 signaling is likely to disrupt cell homing to the site of damage. While this strategy to enhance healing is pursued, it will be important to take potential negative effects on cell homing into consideration when considering dosage, timing or alternate mobilization strategies.

Other Recruitment and Homing Pathways

There are a large number of potential molecules upregulated at the fracture site during the healing cascade that are good candidates for potentiating stem cell homing to the site of injury. The major growth factors, cytokines and chemokines identified at the fracture site are noted in **Table 1**, along with references to their potential to induce migration of MSCs and endothelial-type cells. Studies that either decrease or increase the concentration of these factors during bone healing show that they have a significant impact on the fracture healing process. However, their potential role as chemotactic agents during healing is largely unknown, as it is difficult to clearly separate effects on cell recruitment from significant effects on cell proliferation, differentiation and angiogenesis. Surprisingly, there is sparse information regarding the expression of specific chemokines (CXC, CC, (X)C and CX3C families) during fracture healing, with the exception of CXCL12. Increased expression of CCL2 (monocyte chemotactic protein-1) and CCL3 (macrophage inflammatory protein-1α) were described in fractured bone from human osteoporotic patients undergoing hip arthroplasty.[91] CCL5 (regulated and normal T-cell expressed and secreted) was detected at high levels in bone samples from human vertebral compression fractures, although the normal levels in healthy controls are not known.[92] While it is not clear that CCL7 (monocyte chemotactic protein-3) levels have been detected at the site of bone injury, CCL7 has been used to enhance homing of osteogenic cells to the fracture site.[70] Experiments that utilize the parabiotic mouse model or inject labeled stem cells have the potential to give the most direct evidence regarding the roles of specific molecules in stem cell homing. While these studies are few and far between, most have focused on a role for CXCL12 in cell homing as described above.[68–70]

Summary

Recruitment of endogenous stem and progenitor cells is essential for bone healing. There are likely many chemotactic signals that initiate the migration of these cells from both local

Table 1 Potential chemotactic signaling molecules identified at the site of fracture, stress fracture or vertebral compression fracture were derived from the following reviews and articles[5,57,92–94]

MSC (stromal)/migration		*Endothelial cell/EPC migration*
Growth factors/morphogens		
TGF-β	Human BM-MSCs[95]	Human cerebral microvascular endothelial cells[96]
PDGF	Human BM-MSC;[47,97–99] rabbit BM-MSCs;[99] human adipose MSCs[100]	Rat BM-EPCs;[101] human cerebral microvascular endothelial cells[96]
FGF	Rabbit MSC;[99] human BM-MSCs;[102] human adipose MSCs[100]	Rat BM-EPCs;[101] human cerebral microvascular endothelial cells[96]
IGF	Human BM-MSCs;[47,103] rabbit BM-MSCs;[99]human adipose MSCs[100]	Human endothelial cell (ECV304)[104]
GDF-5	—	Bovine aortic endothelial cells[105]
VEGF	Human BM-MSCs[98,106]	HUVEC;[107] human microvascular endothelial cells;[108] human cerebral microvascular endothelial cells[96]
Angiopoietin 1	—	HUVEC;[107,109] EPCs[109]
Angiopoietin 2	—	EPCs[109]
BMP-2	Human BM-MSCs[110]	Human microvascular endothelial cells;[108] HUVEC[111]
BMP-4	Human BM-MSCs[98,110]	Human microvascular endothelial cells[112]
BMP-6	—	Murine intraembryonic endothelial cells[113]
BMP-7	Human BM-MSCs[98,114]	—
Chemokines		
CXCL12 (SDF-1)	Human BM-MSCs;[47,115,116] human adipose MSCs;[100] human periosteal progenitor cells[117]	Human retinal endothelial cells;[118] HUVEC;[119] human peripheral blood EPCs[120]
CCL2 (MCP-1)	Rat BM-MSC;[121] human adipose-MSCs;[100] human BM-MSCs;[116] human periosteal progenitor cells[117]	HUVEC[122,123]
CCL3 (MIP1α)	Human BM-MSCs[115,116]	—
CCL5 (RANTES)	Human BM-MSCs[47,116]	Murine BM-EPCs[124]
CCL7 (MCP-3)	Rat BM-MSCs[125]	Human circulating angiogenic cells[126]
Cytokines		
IL-1	Human BM-MSCs[127]	Human peripheral blood-EPCs[128]
IL-6	Human BM-MSCs[102,129]	Human cerebral endothelial cells[130]
TNF-α	Human adipose MSCs;[100] human BM-MSCs;[131] human muscle-derived stem cells[132]	Bovine pulmonary artery endothelial cells[133]

Abbreviations: BM, bone marrow; BMP, bone morphogenetic protein; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; EPC, endothelial progenitor cell; FGF, fibroblast growth factor; GDF, growth/differentiation factor; HUVEC, human umbilical vein endothelial cell; IGF, insulin-like growth factor; IL, interleukin; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; MSC, mesenchymal stem (stromal) cell; PDGF, platelet-derived growth factor; RANTES, regulated and normal T-cell expressed and secreted; SDF, stromal cell-derived factor; TGF, tumor growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor. Increased expression of CCL2 and CCL3 was described in fractured bone from human osteoporotic patients undergoing hip arthroplasty.[91] CCL7 has been used to enhance homing of osteogenic cells to the fracture site.[70] In addition, many of these factors have clear and critical roles in the recruitment of mature cells of hematopoietic origin and hematopoietic stem and progenitor cells (including osteoclast and their precursors) that coordinate the inflammatory response at the fracture site. This literature is not reported here.

and systemic sources. While data suggest that CXCL12 upregulation at the site of damage may have a significant role in recruitment, very little is known regarding other candidate molecules. Transplantation of large numbers of stem and progenitor cells to augment the natural healing process holds significant promise for musculoskeletal regenerative medicine, especially in circumstances where healing is impaired. Cells are commonly transplanted locally with or without scaffolds, or systemically into the peripheral blood. Targeting chemotactic pathways to maximize both endogenous and/or transplanted cell recruitment could be a highly effective strategy to promote healing in slow or non-healing fractures and bone defects.

While systemic recruitment of stem and progenitor cells is controversial, data support the idea that both endothelial cells and cells with osteogenic potential are increased in the peripheral blood following fracture. The exact nature of these cells, how and when they are mobilized subsequent to bone damage, recruitment mechanisms and what role they might play in bone regeneration warrants further investigation. Strategies to enhance mobilization of endogenous cell populations, and increase circulating stem and progenitor cell number, appear to have positive effects on bone healing. However, a more complete understanding of the molecular mechanisms underlying mobilization and homing in response to fracture is required to develop the most effective therapeutics.

Conflict of Interest

The author declares no conflict of interest.

Acknowledgements

We received support from the AO Foundation (Project S-10-62Y), NIH (NIAMS R21 AR061604) and a private grant from Dick and Carolyn Randall.

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