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The SDF-1–CXCR4 signaling pathway: a molecular hub modulating neo-angiogenesis

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Pro-angiogenic bone marrow (BM) cells include subsets of hematopoietic cells that provide vascular support and endothelial progenitor cells (EPCs), which under certain permissive conditions could differentiate into functional vascular cells. Recent evidence demonstrates that the chemokine stromal-cell derived factor-1 (SDF-1, also known as CXCL12) has a major role in the recruitment and retention of CXCR4$^+$ BM cells to the neo-angiogenic niches supporting revascularization of ischemic tissue and tumor growth. However, the precise mechanism by which activation of CXCR4 modulates neo-angiogenesis is not clear. SDF-1 not only promotes revascularization by engaging with CXCR4 expressed on the vascular cells but also supports mobilization of pro-angiogenic CXCR4$^+$VEGFR1$^+$ hematopoietic cells, thereby accelerating revascularization of ischemic organs. Here, we attempt to define the multiple functions of the SDF-1–CXCR4 signaling pathway in the regulation of neo-vascularization during acute ischemia and tumor growth. In particular, we introduce the concept that, by modulating plasma SDF-1 levels, the CXCR4 antagonist AMD3100 acutely promotes, while chronic AMD3100 treatment inhibits, mobilization of pro-angiogenic cells. We will also discuss strategies to modulate the mobilization of essential subsets of BM cells that participate in neo-angiogenesis, setting up the stage for enhancing revascularization or targeting tumor vessels by exploiting CXCR4 agonists and antagonists, respectively.

Contribution of pro-angiogenic bone marrow cells to adult vascularization

The majority of the adult vasculature consists of mature, stable, non-leaky vessels invested by perivascular cells. Perivascular cells, which comprise pericytes, smooth muscle cells, or specialized hematopoietic cells, stabilize neo-vessels by releasing angiogenic factors or providing physical support. However, following injury or trauma, regeneration of blood vessels (neo-angiogenesis) is required to revascularize the wounded tissue. Injured tissues release pro-angiogenic factors that support assembly of the neo-vessels. However the precise identities of pro-angiogenic factors that recruit hematopoietic cells to stabilize the newly formed vessels are unknown.

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Similarly, neo-angiogenesis is also essential for growth and progression of malignant tumors, which require nutrients and oxygen to overcome hypoxia and for tumor metastasis. Dysfunctional and leaky blood vessels contribute to the development of pathological conditions, such as retinopathies, atherosclerosis and rheumatoid arthritis. Generation of new blood vessels could be initiated from surrounding existing vessels (sprouting), and, under certain conditions, recruitment of endothelial progenitor cells (EPCs) could also contribute to vessel formation (adult vasculogenesis). Circulating EPCs can originate from the bone marrow (BM) [1], or also from other organs [2]. It is well established that EPCs are recruited in conjunction with pro-angiogenic hematopoietic cells and participate in driving neo-angiogenesis. The nature and exact functions of these mobilized BM-derived cells have been under intense scrutiny and are the subject of recent reviews [3,4]. Recruitment of EPCs and supporting cells contributes to the acceleration of ischemic revascularization and augments neo-angiogenesis in specific tumors, including lymphomas [5,6].

The chemokine stromal cell derived factor-1 (SDF-1, also known as CXCL12) is a constitutively expressed and inducible chemokine that regulates multiple physiological processes, including embryonic development and organ homeostasis [7]. This pleiotropic chemokine is expressed in several organs including lung, liver, skin and BM. The cognate receptor for SDF-1, CXCR4 (also known as CD184), is widely and constitutively expressed by numerous tissues, including hematopoietic and endothelial cells. CXCR4$^+$ pro-angiogenic cells are composed of immature and mature hematopoietic cells, EPCs, and smooth muscle cell (SMC) progenitors, which all have direct or indirect pro-angiogenic properties. Accumulating evidence derived from mice deficient in SDF-1 and CXCR4, in addition to *in vitro* biochemical and migration studies, have set forth the concept that the SDF-1–CXCR4 pathway has a crucial role in modulating the trafficking and proper engraftment of hematopoietic stem cell (HSCs) and reconstitution of hematopoiesis [7]. The crucial roles of SDF-1 and CXCR4 in embryonic vasculogenesis are demonstrated by the blood vessel abnormalities manifested in SDF-1$^{-/-}$ and CXCR4$^{-/-}$mice [8]. Indeed, the expression of SDF-1 in a large number of tumors and injured tissues strongly suggests that activation of CXCR4 participates in promoting neo-angiogenesis (Table 1). In the past few years, numerous studies have

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Table 1. Increased expression of SDF-1 in tumors and pathological conditions[a]

Cancers		Pathological conditions	
Tumor cells	**Tumor stromal cells**	**Vasculature**	**Injured tissues**
Ovarian cancer	Breast cancer fibroblasts	MS blood vessels	Myocardium infarct
Neuroblastoma	ICC fibroblasts	Systemic sclerosis endothelial cells	Diabetic retinopathy
Breast cancer		RA endothelium	Injured liver
Prostate cancer		Diabetic retinopathy	Irradiated BM
Rhabdomyosarcoma		Asthmatic endothelium	Ischemic brain
Melanoma		Vascular intima	Ischemic kidney
Lung cancer		Atherosclerotic plaque	RA synovium
AML			Systemic sclerosis
See Ref. [24]			

[a]Abbreviations: AML, acute myelogenous leukemia; ICC, intrahepatic cholangiocarcinoma; MS, multiple sclerosis; RA: rheumatoid arthritis.

focused on studying the role of SDF-1 in neo-vascularization models, suggesting a central role for this chemokine in triggering and supporting organ repair and tumor development. However, the precise mechanisms by which SDF-1 exerts its pro-angiogenic effects are not fully elucidated. In addition, it is not known how the CXCR4 antagonist, AMD3100, promotes or inhibits mobilization of pro-angiogenic hematopoietic cells.

Here, we describe the potential mechanisms by which activation of the SDF-1–CXCR4 pathway supports recruitment of the pro-angiogenic cells from the BM and other organs, thereby facilitating the formation of stable vasculature during ischemic revascularization and tumor growth. Moreover, we set forth a novel concept whereby AMD3100, through modulation of plasma SDF-1 levels, acutely promotes, whereas chronic treatment blocks, mobilization of $CXCR4^{+}VEGFR1^{+}$ cells. Taken together, accumulating evidence suggests that the magnitude of SDF-1 plasma level is the key regulator of recruitment of pro-angiogenic cells and extent of revascularization.

Expression of SDF-1 in hypoxic tissues

Chemokine production at sites in demand of rapid vascularization is crucial for recruiting circulating cells that are essential for neo-angiogenesis. SDF-1 is upregulated in damaged tissues, such as the liver [9], arteries [10] or irradiated BM [11] (Table 1). Indeed, hypoxic and/or apoptotic conditions are the trigger to induce expression of cytokines and chemokines. Ceradini *et al.* have demonstrated that SDF-1 expression in ischemic sites is directly correlated with the amplitude of hypoxia [12]. Signaling activated by hypoxia leading to SDF-1 upregulation involves the recruitment of integrin-linked kinase and hypoxia-inducible factor-1 (HIF-1) [12,13]. Increased expression of SDF-1 in human and rodent models of infarcted myocardium has been confirmed by several studies [14–17]. SDF-1 expression was increased as early as 1h after induction of hypoxia in the myocardium or hindlimbs, suggesting a role in the initiation of tissue repair and revascularization [13,18,19]. Taken together, these data suggest that induction of SDF-1 by hypoxia provides an effective means to recruit circulating cells into neo-angiogenic niches.

A myriad of other cytokines are also upregulated in damaged ischemic tissues, including vascular endothelial growth factor-A (VEGF-A), or hematopoietic cytokines, such as erythropoietin (EPO), Kit-ligand (KitL, also known as stem cell factor), thrombopoietin (TPO), granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) [20]. Notably, enforced VEGF-A elevation in the liver induces SDF-1 expression [21]. Similarly, hematopoietic cytokines increase SDF-1 secretion, thereby augmenting neo-angiogenic processes [20]. Therefore, induction of SDF-1 expression seems to have a major role in initiating revascularization of the ischemic injured tissues. In support of these findings, SDF-1 is expressed on endothelial cells and pericytes of hypoxic, injured or pathological tissues, including injured carotid arteries and atherosclerotic plaques (Table 1) [22]. Finally, EPCs themselves express and secrete SDF-1 together with VEGF-A [23]. Mimicking injured normal tissues, both main components of the tumor microenvironment, namely tumor cells and tumor stroma cells, also secrete SDF-1. A large variety of tumor cells express SDF-1 (recently reviewed in Ref. [24]) (Table 1). Moreover, tumor stromal fibroblasts, but not normal fibroblasts, elaborate SDF-1, which in turn promotes tumor growth and angiogenesis [25,26] (Table 1). Finally, SDF-1 expression by tumor vasculature has been reported in brain tumors [27,28].

Several studies have demonstrated that SDF-1 expression is sufficient to induce cell mobilization, enhancing angiogenesis and tissue recovery. Enforced release of SDF-1 locally in ischemic tissue leads to increased EPC mobilization and induction of neo-angiogenesis. Of note, those effects are abrogated in the absence of injury, suggesting that other signals emitted by damaged tissue are required for the incorporation of EPCs in the vascular niche within tissues [29,30]. Moreover, implantation of SDF-1-overexpressing cardiac fibroblasts leads to significant revascularization and cardiac functions [14]. Altogether, SDF-1 seems to be a key molecule released by ischemic injured tissues into the circulation that regulates the recruitment of pro-angiogenic cells to the injured and tumoral tissues.

SDF-1 is the primary chemokine that supports mobilization of pro-angiogenic cells

The main SDF-1 receptor, CXCR4, is widely expressed on BM cells, which endows SDF-1 with the capacity to affect a broad range of effector cells. $VEGFR2^{+}$, $CXCR4^{+}$ and $c\text{-}Kit^{+}$ cells represent specific subsets of pro-angiogenic and vascular cells with overlapping phenotypes that are mobilized by their respective ligands: VEGF-A, SDF-1 and KitL. The precise roles of each of these ligands in BM cell mobilization are still under investigation. Mobilization of specific subpopulations is likely to be regulated by tight