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## SDF-1 tells stem cells to mind their P's and Z's

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**Stromal cell-derived factor-1 (SDF-1) is a chemokine with unique functions, including a role in the trafficking of primitive blood precursor cells. A better understanding at a molecular level of how the binding of SDF-1 to its cell surface receptor, CXCR4, elicits specific biological responses in these cells has now been achieved through the identification of PKC- $\zeta$  activation as a common downstream signal. This finding suggests that treatment of a variety of clinical conditions might benefit from the targeting of PKC- $\zeta$  (see the related article beginning on page 168).**

### Overview of the regulation of stem/progenitor cell trafficking

Throughout adult life, the production of blood cells is normally confined to particular sites within bone cavities where a

relatively small number of self-renewing hematopoietic stem cells that turn over slowly are also concentrated (1). In contrast, most of the cells circulating in the blood are highly specialized cells with little or no proliferative potential and a limited lifespan. However, not all hematopoietic stem cells and their primitive progeny are fixed in bone marrow niches. A small proportion of these cells continuously enter the blood and then rapidly return to the marrow (2). The distribution of primitive hematopoietic cells between

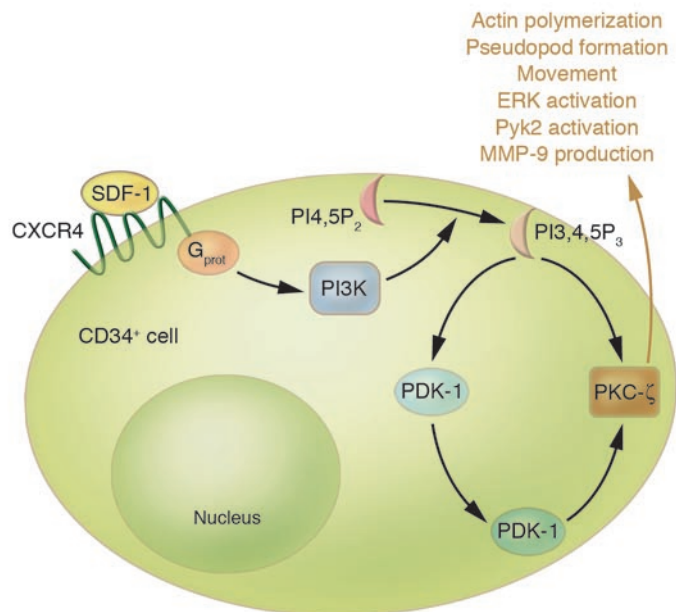
the blood and bone marrow can also vary as a result of many perturbations and disease states. These include a variety of inflammatory conditions, leukemias, myelosuppressive treatments, and the administration of pharmacologic doses of hematopoietic growth factors. All of these conditions involve many physiological changes whose complexity has made it difficult to elucidate the molecular events that regulate the trafficking of primitive hematopoietic cells into and out of the bone marrow.

One fruitful approach came from early analyses of the molecular interactions between marrow stromal cells and primitive hematopoietic cells. This led to the identification of 2 pairs of molecular interactions that are important to the retention of primitive hematopoietic cells in the bone marrow: VCAM-1 with very late antigen-4 (VLA-4) and membrane-bound Steel

**Nonstandard abbreviations used:** PDK-1, phosphoinositide-dependent kinase-1; SDF-1, stromal cell-derived factor-1.

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**Figure 1**

SDF-1 binds to its receptor, CXCR4, on primitive CD34<sup>+</sup> cells and initiates a cascade of downstream signaling events culminating in the directed migration of the cell toward the source of the SDF-1. A critical step in this response is the PI3K-dependent activation of PKC-ζ, a member of the “atypical” subgroup of the PKC superfamily, presumably through the joint activation of PDK-1 (11). PI4,5P<sub>2</sub>, phosphatidylinositol diphosphate; PI3,4,5P<sub>3</sub>, phosphatidylinositol triphosphate.

factor with c-kit (reviewed in ref. 3). An additional role of stromal cell-derived factor-1/CXCR4 (SDF-1/CXCR4) interactions was first suggested by the characterization of primitive hematopoietic cells from mice lacking these genes. Later studies demonstrated the importance of SDF-1/CXCR4 interactions in the chemoattraction of primitive hematopoietic cells in vitro and their marrow homing and mobilization in vivo (reviewed in ref. 4). More recent studies have shown the direct colocalization of primitive hematopoietic cells with SDF-1<sup>+</sup> stromal cells within the bone marrow (5). Additional leads have come from attempts to distinguish between regulatory mechanisms that are intrinsic from those that are extrinsic to the target cells. Examples of mechanisms intrinsic to primitive hematopoietic cells include those affecting the level of expression and activation state of specific receptors and their downstream signaling pathways in addition to their linkages to particular biologic responses; e.g., adhesion to stromal cells, cell polarization, pseudopod formation, and directed motility. Examples of extrinsic mechanisms include the production by other cells of various proteases (e.g., MMP-9 and dipeptidylpeptidase IV/CD26 [DPPIV/CD26]) that can indirectly regulate the concentra-

tion and activity of adhesion molecules and chemoattractants in the marrow environment (reviewed in ref. 6).

Another interesting aspect is the possible role of the cycling status of primitive hematopoietic cells in relation to their retention in the bone marrow. This concept emanates from the observation that primitive normal hematopoietic cells in the S/G<sub>2</sub>/M phases do not appear to enter (or do not survive in) the circulation, even when a significant number of these cells are proliferating in the bone marrow. In contrast, large numbers of neoplastic S/G<sub>2</sub>/M progenitors are consistently found in the circulation of patients with polycythemia vera or chronic myeloid leukemia (7–9). Thus, perturbation of a shared mechanism regulating entry into S phase and into the circulation might be envisaged.

#### How does SDF-1 mediate its effects on primitive hematopoietic cells?

SDF-1 (also called CXCL12) is a member of the large chemokine family but differs from most other family members in its reactivity with a single high-affinity receptor, CXCR4. Strategies that upregulate CXCR4 expression on primitive hematopoietic cells enhance the ability of these cells to engraft transplanted recipients. Conversely, strate-

gies that reduce SDF-1-mediated activation of CXCR4 on primitive hematopoietic cells inhibit this function (6). One mechanism of SDF-1 action may be a simple gradient effect governing the probability of CXCR4-positive cell adhesion to endothelial and/or stromal cells inside the bone marrow. However, there is also increasing evidence that downstream signaling events triggered by SDF-1 binding are critical to the ability of primitive hematopoietic cells to migrate toward an SDF-1 gradient and adhere to stroma (6).

CXCR4 is a member of the superfamily of pertussis toxin-sensitive G-protein-coupled serpentine receptors that activate PI3K and hence the production of specific types of membrane lipids. The generation of these lipids, in turn, promotes the phosphoinositide-dependent kinase-1-dependent (PDK-1-dependent) stimulation of multiple downstream signaling cascades, including those regulated by protein kinase B/Akt and various PKC isozymes (10, 11). The particular intracellular pathways that lead to the arrest of primitive hematopoietic cells in the microvasculature of the bone marrow and then their migration into and subsequent retention within the bone marrow cavity (or vice versa) have remained unclear. In a recent study, the migration of SDF-1-treated primitive (CD34<sup>+</sup>) human bone marrow cells was confirmed to be dependent on a PI3K-activated PKC pathway and associated with the downstream phosphorylation of various focal adhesion proteins and the adaptor molecules Crk and Crk-L, whereas none of these were found to be dependent on the concomitant activation of ERK1 and ERK2 also elicited (12).

In an article published in this issue of the *JCI*, Petit and colleagues now clarify the next step in this complex signaling process. Specifically, they identify PKC-ζ as the PKC isoform responsible for multiple aspects of the chemoattractant response of primitive human hematopoietic cells obtained from cord blood (13). This conclusion is derived from an elegant series of experiments in which both chemical and specific small peptide inhibitors of different PKC isoforms were used to analyze the mechanism by which SDF-1 mediates its effects on progenitor cell chemotaxis, adhesion to stromal cells, survival, actin polymerization, pseudopod formation, ERK and Pyk2 activation, and stimulation of MMP-9 gene expression in vitro, as well as engraftment and mobilization in vivo. The results indicate that SDF-1 effects on many of these responses are channeled through a PKC-ζ-activation step.



In addition, the authors showed that the proliferative response of a human B-lineage leukemic cell line to SDF-1 is PKC- $\zeta$  dependent. It remains to be seen how this relates to the mechanism by which SDF-1 influences the cycling status of primitive normal cells, since it appears that the result can range from induced quiescence (14, 15) to enhanced turnover (16), depending on the type of progenitor being assessed, the context of its exposure, and/or the concentration of SDF-1 present.

### Clinical implications

As pointed out by Petit et al. (13), pinpointing the molecular signaling mechanisms that mediate SDF-1 effects on primitive hematopoietic cells and leukemic cells may have important implications for future therapies. The present findings certainly introduce the possibility of considering new agents for improving stem cell mobilization regimes. More speculative is the concept of exploiting small molecule inhibitors of PKC- $\zeta$  to interfere with SDF-1-promoted metastases. Since the initial report of a role of SDF-1 in breast cancer metastases (17), evidence that this pathway is hijacked in numerous other tumors has been obtained (18). The study by Petit et al. (13) has thus also set the stage for

examining a new approach to the treatment of disseminating malignant cells.

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## Mac the knife? Macrophages — the double-edged sword of hepatic fibrosis

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**Progression of hepatic fibrosis requires sustained inflammation leading to activation of stellate cells into a fibrogenic and proliferative cell type, whereas regression is associated with stellate cell apoptosis. The contribution of hepatic macrophages to these events has been largely overlooked. However, a study in this issue of the JCI demonstrates that macrophages play pivotal but divergent roles, favoring ECM accumulation during ongoing injury but enhancing matrix degradation during recovery (see the related article beginning on page 56). These findings underscore the potential importance of hepatic macrophages in regulating both stellate cell biology and ECM degradation during regression of hepatic fibrosis.**

**Nonstandard abbreviations used:** CCl<sub>4</sub>, carbon tetrachloride; TIMP-1, tissue inhibitor of metalloproteinase-1; TRAIL, TNF-related apoptosis-inducing ligand.

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Kupffer cells, the resident macrophage population of the liver, have in recent years lost their leading position in the pecking order of cell types known to contribute to hepatic injury and repair. Long recognized for their activity in liver inflammation, they had been increasingly overlooked while a stellate cell-

centric view of hepatic fibrosis had replaced the earlier focus on macrophages (Figure 1). Symbolic of this demotion, a biannual meeting initially convened as the International Kupffer Cell Symposium changed its name in 1990 to the International Symposium on Cells of the Hepatic Sinusoid (1). This evolution has been understandable because recent studies have established the activated hepatic stellate cell and its myofibroblast counterpart as the major sources of ECM in both experimental and human liver disease (2). As a result, a comprehensive picture of hepatic stellate cell activation in liver injury has emerged, resulting in exciting new prospects for targeting a range of growth factors, receptors, and intracellular mediators in the treatment of hepatic fibrosis (3).