Regulation of Hematopoietic Stem Cells and their Bone Marrow Niches by the Coagulation System*

* Via PAR-1 & CXCR4 upregulation, SDF-1 secretion and EPCR shedding.

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Hosts: Drs Tessa Kerre & Marc Andre
The chemokine SDF-1 (CXCL12), is expressed by CXCR4+ endosteal human bone lining osteoblasts (a) and by endothelial and reticular cells (b,c). High levels of SDF-1 are expressed by human and murine CXCR4+ Adventitial Reticular Cells, which are Nestin+ and form a niche for CXCR4+ hematopoietic stem cells.

SDF-1 and its major receptor CXCR4 are essential for murine stem cell quiescence in the bone marrow!

How are SDF-1/CXCR4 interactions in the BM regulated?

Ponomaryov T. et al, JCI. 2000
BM stromal niches* inhibit stem cell migration & development via adhesion interactions, and are dynamic, in order to allow blood cell production on demand (our hypothesis).

A. Stem cell retention

B. Stem cell migration & development.

A. SDF-1 mediated anchorage via adhesion interactions, induces CXCR4+ stem cell retention in a quiescent, non-motile mode, preserving their developmental potential.

B. These anchoring adhesion interactions need to be altered in order for stem cells to replenish the blood with new cells, suggesting that the niches are dynamic, producing leukocytes on demand.

* BM stromal cells inhibit differentiation of hematopoietic progenitor cells, via adhesion interactions, while maintaining and promoting their proliferation potential. Zipori D. et al, Exp Hem 1980.

Are the coagulation factor Thrombin and its major receptor PAR-1 involved in stem cell motility?
Thrombin induces rapid HSPC mobilization in mice.

A. PBL HSPC

B. MMP-9

What is the status of PAR-1 and CXCR4 in the BM?
Thrombin augments PAR-1 and CXCR4 expression on BM HSPC

A. BM PAR-1/HSPC

B. FACS plot

C. BM CXCR4/HSPC

What is the status of SDF-1?

Gur-Cohen et al.,
Rapid HSPC mobilization was preceded by a dramatic decrease of SDF-1 in BM stromal cells

Does PAR-1 activation by thrombin in-vivo enhance SDF-1 secretion from rare Nestin+ MSC?

Gur-Cohen et al.,
Rare, Adv. Reticular Nestin+ MSC express PAR-1.

A. Nestin MSC  PAR-1  Merge

B. PAR-1 on Nestin+ MSC

Gur-Cohen et al.,
Rare Nestin+ MSC functionally express PAR-1 and release SDF-1 in response to thrombin stimulation.
BM CD45+/EPCR\textsuperscript{high} LT-HSC express PAR-1 and circulating primitive HSPC in the blood and spleen lack EPCR\textsuperscript{high} expression

Long term repopulating hematopoietic stem cells (HSC) in murine BM highly express endothelial protein C receptor (EPCR\textsuperscript{high}) (Balazs & Mulligan et al Blood 2006; Kent & Eaves et al Blood 2009)

A. BM EPCR on CD45+ cells

B. EPCR on SKL
In-vivo thrombin stimulation induces rapid \( \text{EPCR}^{\text{high}} \) shedding on BM LT-HSC

A.

B.

What is the underline mechanism of thrombin-induced EPCR shedding from HSC?

Gur-Cohen et al.,
Thrombin-induced rapid HSPC mobilization is dependent on the downstream p38 MAPK and eNOS signaling pathway.
Thrombin stimulation increases eNOS phosphorylation on BM HSPC

A.

B.

Gur-Cohen et al.,
Thrombin induced HSPC mobilization is dependent on e-NOS signaling

Will thrombin treatment induce EPCR\textsuperscript{high} shedding from HSC?

Gur-Cohen et al.,
Thrombin fails to induce EPCR$^{\text{high}}$ shedding in eNOS deficient mice

A.

Will NO (nitric oxide) stimulation induce HSPC mobilization via EPCR shedding?

Gur-Cohen et al.,
NO is involved in HSPC mobilization and EPCR$^{\text{high}}$ shedding

Yes, injection of the NO donor SNAP induces rapid EPCR$^{\text{high}}$ shedding and HSPC mobilization within 1 hour of stimulation.

Gur-Cohen et al.
The involvement of PAR-1 in G-CSF–induced HSPC mobilization

Will inhibition of G-CSF mobilization induce EPCR\textsuperscript{high} cell accumulation in the BM?

Gur-Cohen et al.,
The involvement of PAR-1 in G-CSF–induced HSPC mobilization

Yes, co-administration of G-CSF with NAC or PAR-1 antagonist increased the levels of BM EPCR^{high} HSC and reduced HSPC mobilization.

The antioxidant NAC inhibits G-CSF induced mobilization (Tesio & Lapidot et al Blood 2011)

A.

B.

Gur-Cohen et al.,
Thrombin precursor prothrombin is expressed in the endosteum region of the BM

A. Pro-Thrombin, Nuclei, Merge

B. pro-Thrombin, Thrombin

Initiation of thrombin generation

Tissue Factor (TF)

Gur-Cohen et al.,
In vitro, immature osteoclasts exhibited increased TF expression

A. in-vitro stimulation on TBM cells with RANKL and M-CSF

B. TF mRNA expression (fold change)

Gur-Cohen et al., Is TF expressed in the murine BM?
TF clusters are located in the trabecular-rich area of the femoral metaphysis in murine bone tips.
TF clusters are adjacent to TRAP+ mature osteoclasts in the femoral metaphysis.

Gur-Cohen et al.,
LPS systemically upregulates TF expression by BM hematopoietic cells

Gur-Cohen et al.,
Increased expression, numbers and size of TF expressing clusters in bone metaphysis of LPS treated mice

A. PBS

B. LPS
1. TF expressed by immature OC can initiate thrombin generation via the formation of the “prothrombinase complex”.
2. Thrombin activates PAR-1 on HSPC/HSC and initiate eNOS phosphorylation and NO upregulation which subsequently induce CXCR4 upregulation and EPCR shedding.
3. Thrombin activates PAR-1 on BM stromal cells, including Nestin+ MSC and initiates SDF-1 secretion.
4. Release of HSC from their BM microenvironment anchorage, leads to their mobilization to the circulation.

Gur-Cohen et al.
Thrombin augments CXCR4 expression on human CD34+ HSPC in a chimeric mouse model

A. Is PAR-1 signaling also important for G-CSF-induced human CD34+ HSPC mobilization?
Thrombin induces rapid HSPC mobilization of human CD34+ HSPC in a chimeric mouse model
The involvement of PAR-1 signaling in G-CSF–induced human HSPC mobilization in a chimeric mouse model

PAR-1 gene expression was found to be 3.6-fold higher in mobilized CD34+ cells than in BM CD34+ cells. Steidl U & Haas R, Ann N Y Acad Sci. 2003

A. WBC

B. PB human CD34+

C. Spleen morphology
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Than you for your Attention!

The Koffler Accelerator
Weizmann Institute of Science
Thrombin stimulation enhances BM-MNC spontaneous and SDF-1-induced migration (A), while PAR-1 inhibition preferentially impairs progenitor migration toward SDF-1 (B).

A. Enhanced migration

B. BM Lin⁻/c-Kit⁺ reduced migration

C. BM Lin⁻/c-Kit⁺ progenitor migration

Migration of progenitor cells to a gradient of SDF-1 in a transwell assay:

Gur-Cohen et al.,
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