Novel view on hematopoietic stem cell mobilization and homing – clinical implications

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Trafficking of Hematopoietic Stem Cells

- Development/Organogenesis
- Physiology – circadian rhythm
- Strenous exercise
- Inflammation
- Tissue/organ injury-induced mobilization (e.g., heart infarct, stroke)
- Hemolytic syndromes (SSA, PNH)
- Pharmacological mobilization (e.g., G-CSF, AMD3100) – HSPC circulating in PB increase up to 100 times.
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Novel view on stem cell retention in BM
Retention of HSCs in the BM-niches

SDF-1
(Stromal derived factor-1)

VCAM-1
(vascular cell adhesion molecule 1)

CXCR4

VLA-4
Retention of hematopoietic stem cells in bone marrow niches is lipid raft dependent.

Examples of lipid raft associated proteins:
- CXCR4
- VLA-4
- CD55 and CD59
BM derived CD34\(^+\) cells – CXCR4 and VLA-4 are lipid raft regulated receptors
Novel view on stem cell mobilization
EDITORIAL

Spotlight series on stem cell mobilization: many hands on the ball, but who is the quarterback?
Retention of HSCs in the BM-niches is an active process.

- SDF-1 – CXCR4
- VCAM-1 – VLA-4 (α4β1)
- AMD3100 - CXCR4 antagonist
- BIO4860 - VLA-4 antagonist

MOBILIZATION
Retention of HSCs in the BM-niches is an active process that counteracts continuous chemottractive gradient of factors present in plasma ("Gravitation field analogy").
Mobilization of Stem Cells

Bone Marrow

Peripheral Blood

BM-Blood Barrier

SDF-1

SDF-1

Conventional Model
SDF-1- CXCR4 axis plays an unquestionable important role in retention of HSPCs in BM however, evidence accumulated that PB plasma SDF-1 level does not always correlate with mobilization of HSPCs

- Kozuka T et al. *Bone Marrow Transplantation* 2003, 31:651–654
- Ratajczak MZ et al. *Leukemia* 2010, 24:976–985

Based on this, not SDF-1 but other factor/s present in plasma are involved in egress of HSPCs from BM into PB
Lack of correlation between SDF-1 level and mobilization of CD34\(^+\) cells

\[ y = -1.3435x + 1115.5 \]

\[ R^2 = 0.0612 \]
SDF-1 level in human and murine plasma is low (< 2 ng/ml) and does not increase significantly during HSPCs mobilization. This physiological concentration of SDF-1 is biologically irrelevant for HSPCs migration.

Ratajczak et al. *Leukemia* 2010, 24, 976.
Sphingosine 1 phosphate


Bone Marrow

Peripheral Blood

25 x higher concentration!
Novel insight into stem cell mobilization-Plasma sphingosine-1-phosphate is a major chemoattractant that directs the egress of hematopoietic stem progenitor cells from the bone marrow and its level in peripheral blood increases during mobilization due to activation of complement cascade/membrane attack complex.

MZ Ratajczak, H Lee, M Wysoczynski, W Wan, W Marlicz, MJ Laughlin, M Kucia, A Janowska-Wieczorek, and J Ratajczak

Ratajczak et al. *Leukemia* 2010, 24, 976-985
Novel insight into stem cell mobilization-Plasma sphingosine-1-phosphate is a major chemoattractant that directs the egress of hematopoietic stem progenitor cells from the bone marrow and its level in peripheral blood increases during mobilization due to activation of complement cascade/membrane attack complex

MZ Ratajczak¹, H Lee¹, M Wysoczynski¹, W Wan¹, W Marlicz², MJ Laughlin³, M Kucia¹, A Janowska-Wieczorek⁴ and J Ratajczak¹

Sphingosine-1-phosphate facilitates trafficking of hematopoietic stem cells and their mobilization by CXCR4 antagonists in mice

*Julius G. Juarez,¹ Nadia Harun,¹ Marilyn Thien,¹ Robert Welschinger,¹ Rana Baraz,¹ Aileen Dela Pena,¹ Stuart M. Pitson,² Michael Retting,³ John F. DiPersio,³ Kenneth F. Bradstock,⁴ and Linda J. Benda¹

S1P promotes murine progenitor cell egress and mobilization via S1P₁-mediated ROS signaling and SDF-1 release

Karin Golan,¹ Yaron Vagima,¹ Aya Ludin,¹ Tomer Itkin,¹ Shiri Cohen-Gur,¹ Alexander Kalinkovich,¹ Orit Kollet,¹ Chihwa Kim,² Amir Schajnovitz,¹ Yossi Ovadia,¹ Kfir Lapid,¹ Shoham Shivtiel,¹ Andrew J. Morris,³ Mariusz Z. Ratajczak,²⁴ and Tsvee Lapidot¹
Mechanistic view on stem cell mobilization
Mobilization studies in mice deficient in either C3 or C3a receptor (C3aR) reveal a novel role for complement in retention of hematopoietic stem/progenitor cells in bone marrow

Janina Ratajczak, Ryan Reca, Magda Kucia, Marcin Majka, Daniel J. Allendorf, Jarek T. Baran, Anna Janowska-Wieczorek, Rick A. Wetsel, Gordon D. Ross, and Mariusz Z. Ratajczak
Lectin Pathway

Classical Pathway
- C1q
- C2
- C4

Mbl

Alternative Pathway
- Factor D
- Factor B

C3

C5

C5a, desArg C5a

Mobilization

Membrane Attack Complex (MAC)

C5b-9
Mobilization in Mbl⁻/⁻ mice (G-CSF for 6 days)

![Graphs showing WBC, No. of SKL, No. Of HSC, and No. of CFU-GM x WBC for C57BL/6J and c-MBL-KO mice.](Adamiak et al. ASH 2015 abstract)
Lectin Pathway

Classical Pathway
- C1q
- C2
- C4

Alternative Pathway
- Factor D
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Mbl

C3

C5

C5a, desArg C5a

Mobilization

C5b-9

Membrane Attack Complex (MAC)
Downstream part of complement cascade is required for optimal mobilization of HSPCs.
- Granulocytes and monocytes are required for HSPC mobilization (Pruijt JF et al. PNAS 2002, Christopher MJ et al. J Exp Med 2011), however, the mechanisms by which these cells induce mobilization are not completely understood.

- Bioactive CC anaphylatoxins (C5a and desArgC5a) are potent chemoattractants and activators for granulocytes and monocytes.
Neutrophils and monocytes are first cells that egress from BM

Lee et al. *Leukemia* 2010:24;573-82
Granulocytes are enriched for proteolytic enzymes and are the first cells that egress from BM during mobilization and thus they “pave the way” for HSPC by disintegrating endothelial-BM barrier “ICE BREAKER EFFECT”.

"Granulocyte"

“HSPC”
Activation of myeloid cells induces proteolytic microenvironment.

BM niche

Endothelium

Mobilization activates CC

C5a

Complement Cascade

Mobilization activates CC

MAC (C5b-C9)

Gradient S1P

RBC

S1P
Evidence that a lipolytic enzyme—hematopoietic-specific phospholipase C-β2—promotes mobilization of hematopoietic stem cells by decreasing their lipid raft-mediated bone marrow retention and increasing the promobilizing effects of granulocytes

M Adamiak\textsuperscript{1,4}, A Poniewierska-Baran\textsuperscript{1,4}, S Borkowska\textsuperscript{1}, G Schneider\textsuperscript{1}, A Abdelbaset-Ismail\textsuperscript{1}, M Suszynska\textsuperscript{2}, A Abdel-Latif\textsuperscript{2}, M Kucia\textsuperscript{1,3}, J Ratajczak\textsuperscript{1} and MZ Ratajczak\textsuperscript{1,3}
Induction of lipolytic microenvironment during mobilization of HSPCs – cleavage of GPI-A
Integrity of lipid rafts is perturbed by PLC-β2.

Novel evidence that crosstalk between the complement, coagulation and fibrinolysis proteolytic cascades is involved in mobilization of hematopoietic stem/progenitor cells (HSPCs)

S Borkowska¹,², M Suszynska¹,², K Mierzejewska¹,², A Ismail¹,², M Budkowska¹,², D Salata¹,², B Dolegowska¹,², M Kucia¹,², J Ratajczak¹,² and MZ Ratajczak¹,²
Lectin Pathway

Classical Pathway

- C1q
- C2
- C4

Alternative Pathway

- Factor D
- Factor B

Mbl

C3

C5

C5a, desArg C5a

Mobilization

C5b-9

Membrane Attack Complex (MAC)
Lectin Pathway

Classical Pathway

- C1q

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Alternative Pathway

- Factor D

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Mbl

Membrane Attack Complex (MAC)

C5

Mobilization

C5b-9

C5a,

desArg C5a

Thrombin

Plasmin
Activation of coagulation cascade (CoaC)

Activation of complement cascade (ComC)

Activation of Fibrinolytic cascade (FibC)

Classical pathway C1q

Alternative pathway C2Fb

Thrombin

Plasmin

C5 convertase

Convertase “like” activity

MAC C5b-C9

Release of S1P from erythrocytes.

Induction of proteolytic environment in BM. Egresses of granulocytes and monocytes.
Evidence of a Pivotal Role for the Distal Part of the Complement Cascade in the Diurnal Release of Hematopoietic Stem Cells Into Peripheral Blood

Sylwia Borkowska,*† Malwina Suszynska,* Janina Ratajczak,* and Mariusz Z. Ratajczak*‡
Deep Sleep
Hypoxia:
- Lactic acidosis
- Release of free radicals (ROS)

Borkowska et al. *Cell Transplantation* 2015
Identification of Heme Oxygenase 1 (HO-1) as a Novel Negative Regulator of Mobilization of Hematopoietic Stem/Progenitor Cells

Marcin Wysoczynski · Janina Ratajczak · Daniel Pedziwiatr · Gregg Rokosh · Roberto Bolli · Mariusz Z. Ratajczak
HO-1-deficient mice are easy mobilizers

A

i.

ii.

iii.

B

i.

ii.

iii.
Novel view on stem cell homing
Challenging question

What are a major players involved in homing of hematopoietic stem cells?
- HSPCs may home to the BM also in SDF-1-CXCR4 axis independent manner

- CXCR4−/− fetal liver HSPCs may home to BM in an SDF-1- independent manner (Immunity 1999, 10:463-471)

- Homing of murine HSPCs made refractory to SDF-1 by incubation and co- injection with a CXCR4 receptor antagonist is normal or only mildly reduced (Science 2004, 305:1000)

- HSPCs in which CXCR4 has been knocked down by means of an SDF-1 intrakine strategy also engraft in lethally irradiated recipients (Blood 2000, 96: 2074-2080).
SDF-1

Myeloablative conditioning for transplantation

Homing factors for HSPCs

⇓ SDF-1

HSPCs
Bone Marrow

Gradient of Chemotactic/Homing Factors

S1P

SDF-1

ATP, C1P, Ca2+

Peripheral Blood

Endothelial Barrier

Homing

HSPC

Adamiak et al. *Oncotarget* 2015, 6, 18819-18828.
Evidence for the involvement of sphingosine-1-phosphate in the homing and engraftment of hematopoietic stem cells to bone marrow

Mateusz Adamiak¹, Sylwia Borkowska¹, Marcin Wysoczynski², Malwina Suszynska¹, Magda Kucia¹,³, Gregg Rokosh², Ahmed Abdel-Latif¹, Janina Ratajczak¹, Mariusz Z. Ratajczak¹,³

Adamiak et al. Oncotarget 2015, 6, 18819-18828.
S1P promotes engraftment of HSPCs

Adamiak et al. *Oncotarget* 2015, 6, 18819-18828.
Priming effect of LL-37 on low doses of SDF-1

The bone marrow-expressed antimicrobial cationic peptide LL-37 enhances the responsiveness of hematopoietic stem progenitor cells to an SDF-1 gradient and accelerates their engraftment after transplantation

W Wu¹, CH Kim¹, R Liu¹, M Kucia¹ ², W Marlicz², N Greco³, J Ratajczak¹ ², MJ Laughlin⁴ and MZ Ratajczak¹ ²
Myeloablative conditioning for transplantation

Homing factors for HSPCs

SDF-1-CXCR4 axis priming factors

\[ \downarrow \text{SDF-1} \]

C3a
LL-37
\( \beta_2 \)-defensin

HSPCs

HSPCs
Optimal SDF-1-CXCR4 receptor signaling is lipid raft dependent

CXCR4

SDF-1

INCORPORATION INTO A MEMBRANE LIPID RAFT

DESENSITIZATION

SENSITIZATION

C3a
LL-37
β2-defensin

SIGNALING

Optimal SDF-1-CXCR4 receptor signaling is lipid raft dependent

- SDF-1
- CXCR4
- SENSITIZATION
- DESENSITIZATION
- C3a
- LL-37
- β2-defensin
- INCORPORATION INTO A MEMBRANE LIPID RAFT
- SIGNALING

**Priming effect of LL-37 on low doses of SDF-1**

*Original Article*

The bone marrow-expressed antimicrobial cationic peptide LL-37 enhances the responsiveness of hematopoietic stem progenitor cells to an SDF-1 gradient and accelerates their engraftment after transplantation.

W Wu¹, CH Kim¹, R Liu¹, M Kucia¹,², W Marlicz², N Greco³, J Ratajczak¹,², MJ Laughlin⁴, and MZ Ratajczak¹,²

*Wu et al. Leukemia* 2012, 26, 736–745
Mild Heat Treatment Primes Human CD34+ Cord Blood Cells for Migration Towards SDF-1α and Enhances Engraftment in an NSG Mouse Model

Maegan L. Capitano, Giao Hangoc, Scott Cooper, and Hal E. Broxmeyer

Key words. Hematopoietic stem cells • hematopoietic progenitor cells • SDF-1/CXCL12 • chemotaxis • lipid rafts • cord blood transplantation
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“Cell Transplantation”

Downregulation of heme oxygenase 1 (HO-1) activity in hematopoietic cells enhances their engraftment after transplantation

Mateusz Adamiak¹, Joseph B. Moore IV², John Zhao², Ahmed Abdelbaset-Ismail¹,
Kamil Grubczak³, Sylwia Borkowska¹,⁴, Marcin Wysoczynski² & Mariusz Z. Ratajczak¹,⁵.
Inhibition of HO-1 accelerates engraftment

A MODULATION OF MEMBRANE LIPID RAFTS

- priming factors
- mild heat exposure
- Lipid raft
- CXCR4 in lipid raft

B MODULATION OF HOMING MOLECULES

- Upregulation of CXCR4
- PGE₂
- VPA
- PSGL-1
- ExMV
  (CXCR4 transfer)
- fucosylation
- SDF-1
- DPP/CD26

C METABOLICAL MODIFICATION OF HSCs

- EPHOSS
- HO-I
- GSK3β

D ENHANCING CHEMOTACTIC ACTIVITY OF CHEMOTRACTANTS

- S1P
- S1PR1
- S1PR2
- LPP-1-3
- JTE-013
- SDF-1
- DPP/CD26

Conclusions

Innate immunity including complement cascade, granulocytes and monocytes are major orchestrators of stem cell mobilization and mobilization is triggered by activation of mannann lectin binding pathway.

Evidence accumulates that in addition to SDF-1 also other chemoattractants (S1P, ATP, UTP, H\textsuperscript{+}, Ca\textsuperscript{2+}) and mechanisms (e.g., priming effect) are involved in homing of HSPCs.

Lipid rafts are essential for retention of HSPCs in BM. Their disintegration occurs during mobilization and reassembly is important in homing process.

HO-1 is a novel identified important negative regulator of HSPCs trafficking.
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