Hematopoiesis
Hemato – Lymphopoiesis

Hematopoiesis

- Bone Marrow
- Spleen

Lymphopoiesis

- Thymus
- Lymph nodes
- Spleen
- Bone marrow

Cells (erythrocytes, granulocytes, monocytes, platelets) circulate in blood and tissues

Cells (lymphocytes) circulate in lymph/tissues and some of them circulate in blood
Properties of Hematopoietic Stem Cell (HSCs)

- Self-renewal
- Quiescence
- High resistance to radio-chemotherapy and cytostatic drugs
- Characteristic morphology (e.g., large nucleus)
Hematopoietic Stem Cells

- A hematopoietic stem cell (HSC) possesses the ability for self-renewal and may differentiate into any blood cell from both the myeloid and lymphoid lineages.

- During embryogenesis HSC migrate from one to another anatomical site. This developmental journey of the HSC starts with yolk sac (YS) and aorta gonado mesonephros (AGM) region and continues through the fetal liver (FL) to their final destination in the bone marrow (BM).

- HSC, during the differentiation process, give rise to more mature hematopoietic progenitor cells (HPC) that lose the ability for self-renewal; however, they are able to grow colonies containing functional hematopoietic cells.
Origin of HSC

- HSC are among the first stem cells that are specified during embryogenesis from epiblast (primitive ectoderm).
Origin of HSC

- Definitive primitive pre-HSC expand in the YS, and when the developing heart tube begins to propel the first hematopoietic cells in vessels (> E8.25), primitive pre-HSC become detectable in the embryo proper and colonize the luminal surface of the aorta in the so-called aorta-gonado-mesonephros region (AGM).
Stem cells are travellers

- The colonization of BM by HSC does not terminate the developmental journey of HSC. After symmetric division one of a daughter HSC has to leave the BM niche and is released into the circulation in order to find a new niche.

- This mechanism may be responsible for maintaining homeostasis between HSC niches in different areas of BM that is distributed across various bones.
 Trafficking - Immunosurveillance by circulating Stem Cells

Bone Marrow

Blood

Lymph

Tissues Organs

Bone Marrow
Trafficking of Hematopoietic Stem Cells

- Development/Organogenesis
- Physiology – circadian rhythm
- Strenous exercise
- Inflammation
- Tissue/organ injury-induced mobilization (e.g., heart infarct, stroke)
- Pharmacological mobilization (e.g., G-CSF, AMD3100) – HSPC circulating in PB increase up to 100 times.
Classical Pathway

Alternative Pathway

C1q
C2
C4

Factor B

Factor D

C3

C3a, desArg C3a

C5

C5a, desArg C5a

C5b-9

Membrane Attack Complex (MAC)

Promote mobilization

Promote BM retention

Ratajczak et al. *Blood* 2004;93:2071-2078
CXCR4 - SDF-1 Axis

SDF-1

CXCR4

Adhesion
- Fak
- Paxillin
- p130 CAS

PI-3K

MAPK p42/44

Jak, Tyk

Phosphatases
- SHIP 1
- SHIP 2
- CD45

AKT

IκB

NF-κB

NF-κB

Elk-1

STAT

Biological effects
Retention in BM
Homing to BM

CXCR4

Developmental Migration
Retention in BM Homing to BM

CXCR4+ HSCs

SDF-1

SDF-1

SDF-1
Chemotactic assay

Bone Marrow Mononuclear Cells

(-)  SDF-1
Stem Cell Niches

- **Stem cell niche** describes the microenvironment in which stem cells reside and which interacts with stem cells to regulate stem cell fate (quiescence vs. self renewal and differentiation).

- Several factors have been identified that regulate stem cell characteristics within the niche including adhesion molecules, extracellular matrix components, the oxygen tension, growth factors, cytokines, and physiochemical nature of the environment including the pH, ionic strength and metabolites.
Bone Marrow stem cell niches

Humoral
Structural
Paracrine

Hormones
Metabolic
Physical
Neural
Retention of HSCs in the BM-niches is an active process.

SDF-1
(Stromal derived factor-1)

VCAM-1
(vascular cell adhesion molecule 1)

CXCR4

VLA-4
Retention of HSCs in the BM-niches is an active process.

SDF-1  
(Stromal derived factor-1)

VCAM-1  
(vascular cell adhesion molecule 1)

CXCR4

VLA-4
Stem cell mobilization and homing are two opposite processes

- Egress of HSCs from bone marrow into peripheral blood is called **mobilization**. HSCs mobilized from bone marrow into peripheral blood are employed for transplantation.

- Reverse phenomenon is called **homing**. Homing of HSCs from peripheral blood into bone marrow proceeds engraftment.
Dogma of SDF-1 balance in homing/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”).

Ratajczak et al. *Leukemia* 2010, 24, 976.
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").

Ratajczak et al. *Leukemia* 2010, 24, 976.
Bone Marrow Examination
Hematopoietic Growth Factors
Kit Ligand

- Growth factors for many type of cells
- KL or c-kit receptor deficiency – anemia,
- Activates early steps of hematopoiesis
- Activates mast cells
Erythropoietin

- Secreted by kidneys
- Regulated by hypoxia
- Required for red cell development
Trombopoietin

- Secreted by liver, kidney and spleen
- Secretion regulated by blood platelet number
- Stimulates megakaryopoiesis and thrombopoiesis
- 20% of blood platelets may be still produced in n TpO deficient mice
G-CSF
Granulocyte colony stimulating factor

- Stimulates myelopoiesis (granulocyto-monopoiesis)
- Employed to mobilize HSCs from bone marrow into peripheral blood
Progenitor Cell Assays
Clonogeneic Assays

Cytokines + Growth Factors

Colonies
Kit ligand (KL), Interleukin-1, Interleukin-3, Angiopoietin-1, Trombopoietin

Kit ligand (KL)
Interleukin-3 (IL-3)
Erythropoietin (EpO)
GM-CSF
Colony Forming Unit of Mixed Lineages (CFU-GEEM)
Kit ligand (KL), Interleukin-1, Interleukin-3, Angiopoietin-1, Trombopoietin

HSC

CFU-Mix

Kit ligand (KL)
Erythropoietin

BFU-E

Erythrocytes
Burst Forming Unit of Erythroblasts (BFU-E)
HSC

Kit ligand (KL), Interleukin-1, Interleukin-3, Angiopoietin-1, Trombopoietin

CFU-Mix

Kit Ligand (KL)

Thrombopoietin

Megakaryopoiesis

CFU-Meg

Megakaryocytes/Platelets
Colony Forming Unit of Megakaryocytes (CFU-Meg)
Kit ligand (KL), Interleukin-1, Interleukin-3, Angiopoietin-1, Trombopoietin

CFU-Mix

Kit ligand (KL)
Interleukin-3 (IL-3)
GM-CSF

CFU-GM

Granulocyto/Macrophagopoiesis
Colony Forming Unit of Granulocyte-Macrophages (CFU-GM)
HSC

Kit ligand (KL), Interleukin-1, Interleukin-3, Angiopoietin-1, Trombopoietin

CFU-Mix

Kit ligand (KL)
Interleukin-3 (IL-3)

GM-CSF

Granulocytes

CFU-G

G-CSF

CFU-GM

Monocytes

CFU-M

M-CSF

Eozynophils

CFU-Eos

IL-5

Bazophils

CFU-Baso

IL-4
HSC

Kit ligand (KL), Interleukin-1 (IL-1), Interleukin-3 (IL-3), Angiopoetin-1, trombopoetin

IL-7

T lymphocytes

B lymphocytes

NK Cells
Stem Cell Markers
HSCs Receptors

- CD34
- CD133
- C-KIT
- CXCR4
HSCs Metabolic Markers

Hoe33342

Pyronin Y

Rhodamine 123

Stem Cell properties

Hoe 33341 low
Pyronin Y low
Rh 123 dim
Hematopoietic Stem Cell Phenotype
Phenotype of human HSCs

- CD34$^+$ CD133$^+$ CXCR4$^+$ c-kit$^+$ Lin$^-$ CD45$^+$
- Hoechst33342$^{\text{low}}$, Rhodamin123$^{\text{low}}$ and Pyronin Y$^{\text{low}}$
HSCs Isolation Strategies
Magnetic separation of HSPC MACS beads

1. Label the cells of interest with MACS MicroBeads in a short incubation step.

2. Pass the mixture of labeled and unlabeled cells over a separation column placed in the magnetic field of a MACS separator. Collect the flow trough as the non-magnetic fraction.

3. Remove the separation column from the magnet and flush out the retained cells as the positively selected cells.
FACS sorter
Hematopoietic Transplants
Bone Marrow Transplant
Mobilized Peripheral Blood Transplant
Umbilical Cord Blood Transplant
Stem cell homing
Dogma of SDF-1 balance in homing/mobilization

- Bone Marrow: SDF-1
- Blood: SDF-1
Dogma of SDF-1 balance in homing/mobilization
- HSPCs may home to the BM also in SDF-1-CXCR4 axis independent manner

- CXCR4<sup>-/-</sup> 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).
Conditioning for transplantation induces proteolytic environment in BM that affects SDF-1 level

Conditioning for transplantation activates complement cascade in BM microenvironment
SDF-1 as peptide is degraded in proteolytic microenvironment

SDF-1 expression decreases in BM conditioned for transplantation

Kim et al. *Leukemia* 2012, 26, 106–116
C1P and S1P level in BM 24 hours after conditioning for transplant (MAS Spectrophotometer data)

\[ \text{C1P level (fold change)} \]

control  
irradiation  

\[ \text{S1P level (fold change)} \]

control  
irradiation  

\[ p=0.022 \]

\[ p=0.124 \]

Kim et al. *Leukemia* 2012, 26, 106–116
Myeloablative conditioning for transplantation

Chemotactic factors for HSPCs

↓ SDF-1
↑ S1P
↑ C1P
↑ ATP
↑ UTP

HSPCs
Tug of War

BM-Blood Barrier

S1P  C1P  ATP  SDF-1  homing
Myeloablative conditioning for transplantation

Chemotactic factors for HSPCs

SDF-1-CXCR4 axis priming factors

\[ \text{C}3\text{a, } \text{desArgC}3\text{a, LL-37, } \beta2\text{-defensin} \]

\[ \downarrow \text{SDF-1} \]
Priming effect of CAMPs on low doses of SDF-1

SDF-1 (low dose) + priming agent (e.g., C3a, LL-37, β2-defensin)

Wu et al. Leukemia 2012, 26, 736–745
Optimal SDF-1-mCXCR4 receptor signaling is lipid raft dependent
Optimal SDF-1-mCXCR4 receptor signaling is lipid raft dependent.
Ex vivo priming by CAMP (e.g., C3a)

Priming effect of CAMPs on low doses of SDF-1

Wu et al. *Leukemia* 2012, 26, 736–745
Human BM- and UCB-derived CD34+ cells primed with C3a engraft faster in immunodeficient mice

Current strategies to accelerate hematopoietic reconstitution after transplantation:

- Transplantation with greater numbers of HSCs
- *Ex vivo* expansion of harvested HSCs before transplant.

However, unfortunately the number of HSPCs available for allogeneic or autologous transplantation could be low (e.g., umbilical cord blood, poor mobilizers) and current strategies to expand HSC *ex vivo* are usually inefficient.

- Priming of HSCs in their responsiveness to SDF-1 gradient
Clinical trial - “Stem Cell Priming to Enhance Engraftment”

Treatment Schema

-6 -5 -4 -3 -2 -1 0 1 2 7 14 21 28

Cyclophosphamide 50 mg/kg
Fludarabine 40 mg/m²
C3A Frag Primed UCB unit
UCB Unit
Chimerism
Monitoring Toxicity
Stem cell mobilization
Mobilized Peripheral Blood Transplant
Retention of HSCs in the BM-niches is an active process that counteracts continuous chemottractive gradient of factors present in plasma ("Gravitation field analogy").
Dogma of SDF-1 balance in homing/mobilization
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

- Cecyn KZ et al. Transfus Apher Sci 2009, 40:159
- Ratajczak MZ et al. Leukemia 2010, 24:976–985

Based on this, not SDF-1 but other factors present in plasma are involved in egress of HSPCs from BM into PB
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 HSPCs

- **Mobilizing agents induce proteolytic microenvironment in BM that attenuates SDF-1-CXCR4 interactions**


  **Disruption of the CXCR4/CXCL12 chemotactic interaction during hematopoietic stem cell mobilization induced by GCSF or cyclophosphamide**

  Jean-Pierre Lévesque,¹ Jean Hendy,¹ Yasushi Takamatsu,² Paul J. Simmons,¹ and Linda J. Bendl²

- **Mobilizing agents activate complement cascade in BM microenvironment**

  (Blood. 2004;103:2071-2078)

  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
Mobilization in C5^-/- mice

- Reca et al. Stem Cells 2007; 25, 3093.
- Ratajczak et al. Leukemia 2010, 24, 976.
Classical Pathway

- NA-Ig-dependent
- C1q
- C2

Alternative Pathway

- Factor D
- Factor B

C3

C5

C5a, desArgC5a

C5b-9

Membrane Attack Complex (MAC)

Promote mobilization

Ratajczak et al. *Blood* 2004;93:2071-2078
Bioactive CC anaphylatoxins (C5a and desArg C5a) are potent chemoattractants and activators for granulocytes and monocytes.

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 were not completely understood.

I. Source of proteases
   (e.g., Cathepsin G, Elastase, MMPs …)

II. Mechanical “ice breaker” effect
    (Granulocytes are the first cells that egress from bone marrow)
Neutrophils and monocytes are first cells that egress from BM

Lee et al. *Leukemia* 2010:24;573-82
“Ice Breaker” effect

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”.
C5a chemoattracts into peripheral blood granulocytes and monocytes but does not chemoattract hematopoietic stem/progenitor cells……...

Question - what chemoattracts hematopoietic stem/progenitor cells (HSPCs) into peripheral blood?
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.
HSPCs chemotaxis results to plasma from mobilized blood indicate that SDF-1 is not a major chemoattractant present in blood.
HSPCs chemotaxis results to plasma from mobilized blood indicate that SDF-1 is not a major chemoattractant present in blood.
Sphingosine 1 phosphate


Bone Marrow

Peripheral Blood

Erythrocytes

25 x higher concentration!
Classical Pathway

Alternative Pathway

Lectin Pathway

NA-Ig-dependent

C1q
C2
C4

Factor D
Factor B

C3

C5

C5b-9
Membrane Attack Complex (MAC)

Lytic MAC
Sublytic MAC

Ratajczak et al. *Blood* 2004;93:2071-2078
C5b-9 (MAC) releases S1P from erythrocytes

The **membrane attack complex C5b-9 (MAC)** forms transmembrane channels. These channels disrupt the phospholipid bilayer of target cells, leading to cell lysis and death.

Ratajczak et al. *Leukemia* 2010, 24, 976.
S1P is major chemoattractant for HSPCs and its concentration in murine plasma increases during mobilization.

Ratajczak et al. *Leukemia* 2010, 24, 976.
Mobilization of HSPCs is impaired in S1P kinase 1 (Sphk1) deficient animals

Golan et al. *Blood* 2012; 119: 2478-2488
Mobilization of HSPCs is impaired in S1P receptor type 1 (S1P$_1$)- deficient mice.

Tug of War

BM - Blood Barrier

SDF-1

BM-Blood Barrier

PB

SDF-1

mobilization

S1P
C1P
SDF-1
BM-Blood Barrier

Bone Marrow

Peripheral Blood

Conventional Model

Proposed Model

SDF-1

SDF-1

SDF-1

S1P
Activation of myeloid cells induces proteolytic microenvironment.

1. C5a

2. Proteases

3. C5a

4. S1P gradient

5. HSPC

BM niche

Endothelium

Complement Cascade

MAC (C5b-C9)

S1P

RBC

BM sinusoids
Questions ?