Hematopoiesis: Stem Cells

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Overview

• Hematopoiesis - development from stem cell
• Definitions / type / identification of stem cells
• The stem cell environment - isolation - culture
• HSC self renewal
• HSC signaling pathways - gene expression
• HSC during development
• Cancer and the HSC
• HSC plasticity
The Hematopoietic Lineages

Myeloid Lineage:
- Pluripotent Stem Cell
- CFU-Blast
- SCF
- IL-3
- GM-CSF
- G-CSF
- EPO
- BFU-E
- CFU-GM
- CFU-E
- Megakaryocyte
- Erythroblast
- Platelet
- Neutrophil
- Monocyte
- Macrophage
- Eosinophil
- Basophil
- Plasma Cell
- T Lymphocyte
- NK Cell

Lymphoid Lineage:
- Pluripotent Stem Cell
- CFU-GEMM
- SCF
- IL-3
- GM-CSF
- G-CSF
- Megakaryocyte
- Erythroblast
- Platelet
- Neutrophil
- Monocyte
- Macrophage
- Eosinophil
- Basophil
- Plasma Cell
- T Lymphocyte
- NK Cell

Lineage restricted (committed) stem cells
Differentiating cells
Differentiated cells
Definition of a Stem Cell

• They can proliferate extremely well
• They are self-renewing
• They can differentiate into several (at least 2) different cell-types
• They can reconstitute tissues after injury
Different Types of Stem Cells

• Totipotent stem cells: Has the ability to generate an entire organism. Only embryonic stem cells are able to do this.

• Multipotent stem cells: Has the ability to differentiate into several different cell types, for example the hematopoietic stem cell.
How to Determine Whether a Cell is a Stem-cell or Not

Inject the cells (bone-marrow) intravenously into the irradiated mouse.

CFU-S: colony-Forming-Units in the Spleen
- Granulocyte
- Erythrocytes
- Lymphocytes etc.
How to Determine Whether a Cell is a Stem-Cell or Not

1st mouse alive after cell injected
2nd mouse alive after cell injected from 1st mouse
3rd mouse alive after cell injected from 2nd mouse
4th mouse dies after cell injected from 3rd mouse

Original cells were not real stem cells

But how long must a HSC live for?
Self Renewal

Self renewing cells

Cells mostly quiescent, only enter cell cycle to replace self

Differentiating cells

Cells enter the cell cycle, with an increasing propensity to become more committed to a particular lineage

Fully differentiated cells exit the cell cycle
Niche cells (green) underlying a basement membrane signal to stem cells (red) to block differentiation and regulate division. When a lineage mechanism prevails (lower mitotic cell), the stem cell divides such that one daughter retains its connections to the niche, while the other (yellow) becomes untethered and begins to differentiate. When a population mechanism prevails (upper mitotic cell), stem cell division may be either symmetric (shown) or asymmetric (not shown), as determined by local factors. ECM, extracellular matrix.
Bone Marrow Progenitor Environment

- Macrophage
- Erythroblast area
- Lymphoblast
- Erythroblast
- Neutrophil
- Myeloid area
- Megacaryocyte
- Arterial capillary
- Fat cell
- Simus
- Adventitial cell
- Endothelial cell
- Lateral longitudinal v. cir.
- Erythrocyte
Bone Marrow Culture Systems

Short term: Fibroblasts (feeder cells) make M-CSF + bone marrow cells
colony growth for about 10-14 days

Long term: bone-marrow stromal cells (feeder cells)
- Dexter cultures
  - Myeloid lineage (erythroid cells)
- Whitlock-Witte cultures
  - B-lymphocytic lineage
Differentiation of pluripotent stem cells into differentiated derivatives.

Cultured EC, ES and EG cells can be induced to differentiate into a wide variety of differentiated derivatives in culture including pancreatic islet cells, blood cells, muscle cells and nerve cells. Differentiation can be induced by withdrawal of leukaemia inhibitory factor (LIF), separation of stem cells from feeder cells, or by growth of stem cell colonies in suspension culture to form embryoid bodies, which upon dissociation can be plated to yield differentiating cells.
Bone Marrow Culture Systems

Important actors involved in these culture systems:

Soluble factors: Colony-stimulating factors (CSF)
Growth factors (cytokines, nuclear hormones)

Contact factors: Extracellular matrix
Hyaluronic acid
Collagen IV
Perlecan
Tenascin
Laminins

Cell contacts
Cytokine Receptor Signaling

[Diagram showing signaling pathways involving cytokines, Tyk2, JAK, STAT3, mTOR, MAPK, and transcription.]
Gene Expression in HSC

- Expression profiling of human B cell development. Hierarchical cluster analysis identified a gene expression pattern that clearly separated five consecutive B-cell populations isolated by cell sorting from human bone marrow.

- Total cellular RNA was extracted from five purified subpopulations, and the mRNA was amplified by in vitro transcription. The RNA samples were hybridized to Lymphochip cDNA microarrays interrogating 15,132 cDNA clones representing 7,399 known or uncharacterized genes.
HSC Survival Signaling
Hematopoiesis During Life

Prenatal

Birth

Postnatal

Foetal Months

Age in years

Cellularity (%)

Yolk sack

Bone Marrow

Vertebra

Spleen

Liver

Sternum

Femur

Rib

Tibia

Definitive hematopoiesis

Primitive hematopoiesis

E12
Blood Islands and the AGM

(a) Blood islands
(b) Yolk sac, Liver, AGM, Dorsal aortae, PAS (ParaAortic space), AGM (Aortic Gonads Mesonephros)
Stem Cell Migration During Development
Self-renewal during stem cell development and leukaemic transformation.

Because of their high level of self-renewal, stem cells are particularly good targets of leukaemic transformation. Unlike normal haematopoiesis, where signalling pathways that have been proposed to regulate self-renewal are tightly regulated (top), during transformation of stem cells, the same mechanisms may be dysregulated to allow uncontrolled self-renewal (middle). Furthermore, if the transformation event occurs in progenitor cells, it must endow the progenitor cell with the self-renewal properties of a stem cell, because these progenitors would otherwise differentiate (bottom).
Stem Cell Plasticity
Summary-1

• The HSC is capable of forming all the lineage through divisions that result in progressively more committed progenitors which eventually fully differentiate.
• Many types of stem cells; toti- and multi-potent.
• Serial transplantation used to identify the HSC as a functional biological unit. HSC must self renew.
• HSC resides in a particular environment which is important for maintaining its stem cell ability. Pluripotent and progenitors cells also have specific microenvironments which nurture their development.
• Various culture systems used to study HSC in vitro, all require feeder/stromal cells (cell-cell and cell-matrix factors) and cytokines to promote survival proliferation and prevent differentiation. Removing feeder cells, cytokines or addition of other cytokines promotes HSC differentiation. The HSC is a condition requiring intrinsic and external stimuli.
• The various cytokines, eg LIF, which maintain the HSC do so by promoting the transcription of genes that maintain the HSC condition. The main signaling pathway involved JAK family tyrosine kinases (JAK3 and Tyk2) phosphorylating the latent cytoplasmic transcription factors STAT3, which dimerise causing exposure of NLS, promoting nuclear translocation to activate specific gene transcription.

• The gene expression profile of the HSC is similar to that of committed progenitors but wildly different to that of fully committed and differentiating end cells.

• Other signals are also transmitted by cytokines to directly promote the survival (anti-apoptosis) of the HSC, mainly through the activation of a serine/threonine kinase Akt which phosphorylates pro-apoptotic proteins rendering them inactive, thus preventing cells death.
The HSC moves through different organs during development - yolk sac, AGM (Aortic Gonad Mesonephros), foetal liver, bone marrow.

Cancer cells display properties of SC in their ability to self renew, this is at the expense of differentiation resulting in fewer normal end cells. Leukaemia treatments can inhibit proliferation/survival or promote differentiation of the transformed cells.

When “pushed”, HSC like other SC show plasticity, they can show true stem like properties when but in particular environments/conditions.