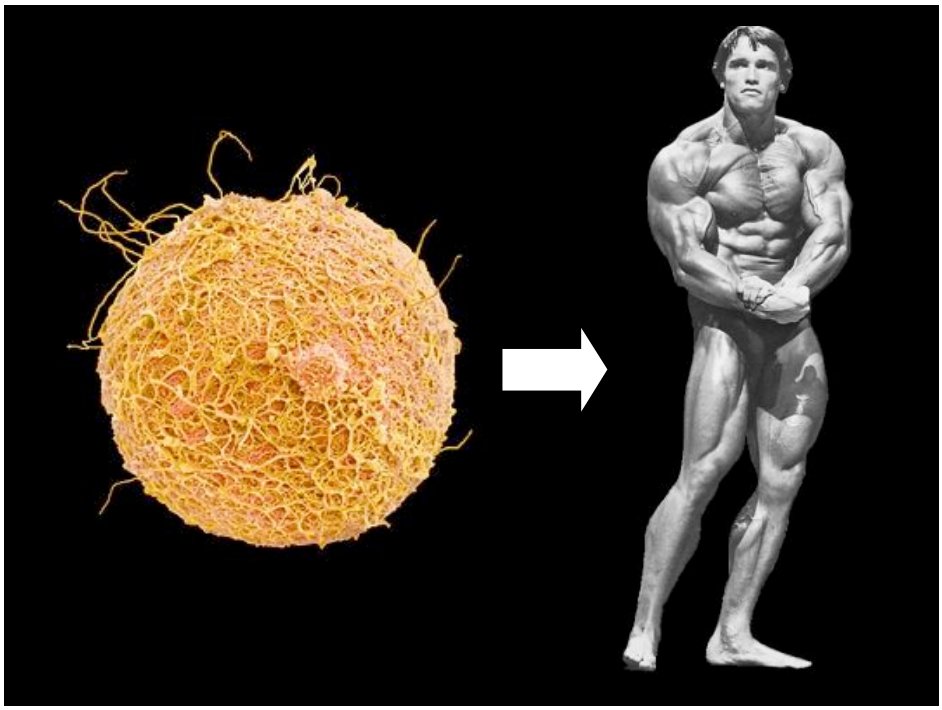
Stem cells: units of development and regeneration

Fernando D. Camargo Ph.D.
Whitehead Fellow
Whitehead Institute for Biomedical Research

Concepts

1. Embryonic vs. adult stem cells
2. Hematopoietic stem cells
3. The stem cell niche
4. Cancer stem cells

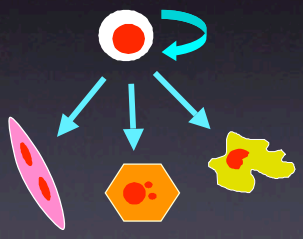


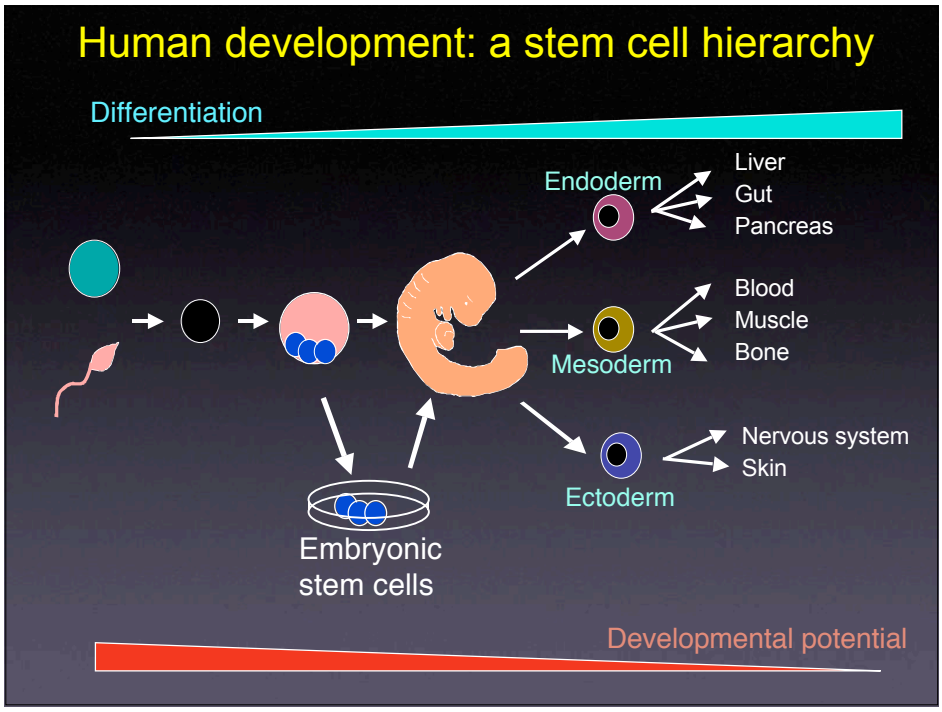
What are stem cells?

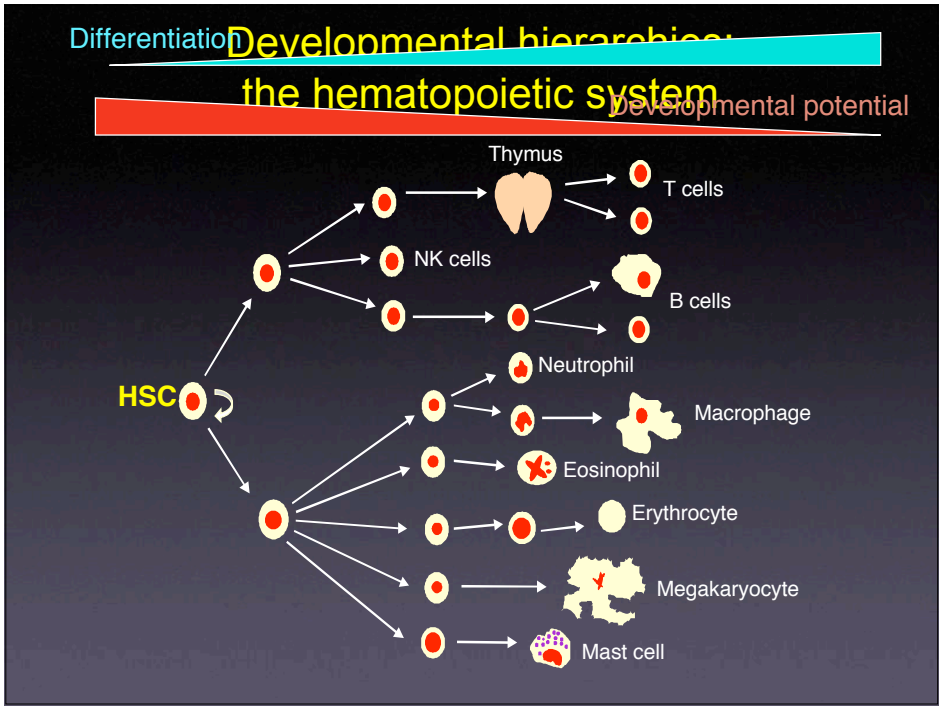
Units of development and regeneration

Why are stem cells special?

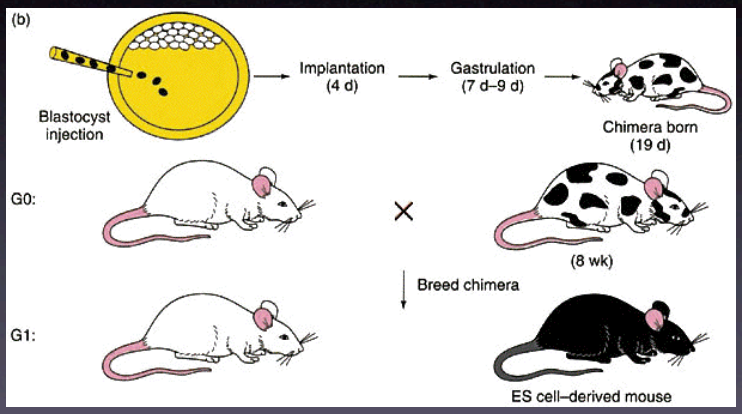
1. They are primitive
2. They can self-renew
3. They can produce differentiated cells



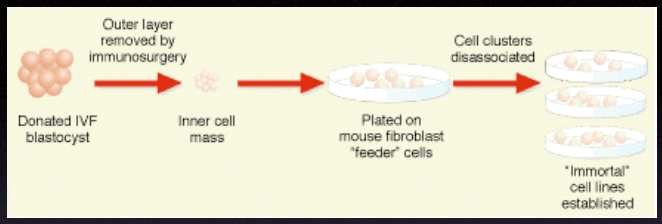

Human development: a stem cell hierarchy
Differentiation
Endoderm
Liver
Gut
Pancreas
Blood
Muscle
Bone
Mesoderm
Nervous system
Skin
Ectoderm
Embryonic
stem cells
Developmental potential

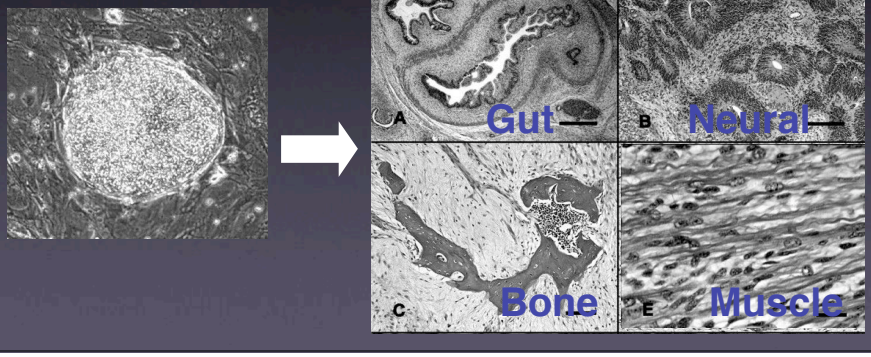

Differentiation
Developmental hierarchies:
the hematopoietic system
Developmental potential
Thymus
T cells
NK cells
B cells
HSC
Neutrophil
Macrophage
Eosinophil
Erythrocyte
Megakaryocyte
Mast cell

Embryonic stem (ES) cells are pluripotential



Human ES cells (Thompson et al, 1998)





Embryonic versus adult

• Isolated from early embryos	• Isolated from adult human tissue
• Can expand indefinitely in culture	• Can not be expanded in culture
• Can give rise to all cell types	• Can only give rise to same tissue

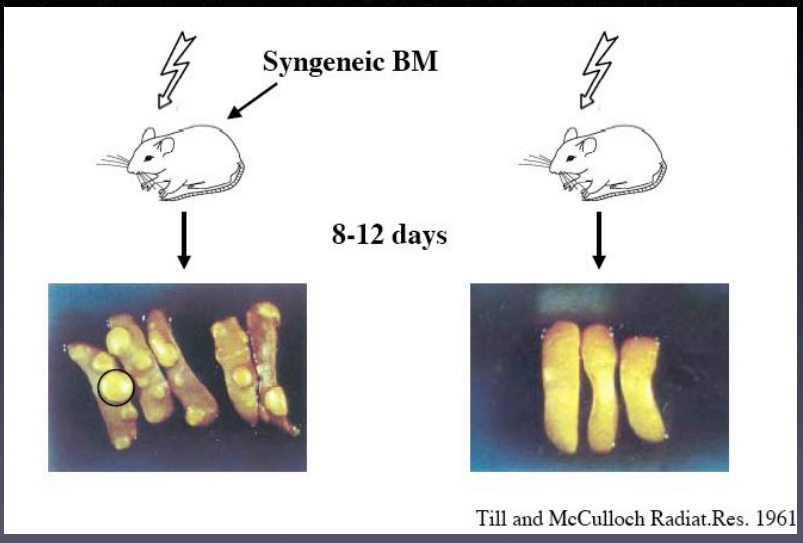
The hematopoietic stem cell (HSC)

- First stem cell to be isolated and studied
- Most of what we know about stem cells comes from studies with HSCs
- First and only stem cell used in the clinic
- Discovered by Till and McCulloch in 1961 (winners of the 2005 Lasker Award)

The 1960s: stem cells, a hypothetical concept

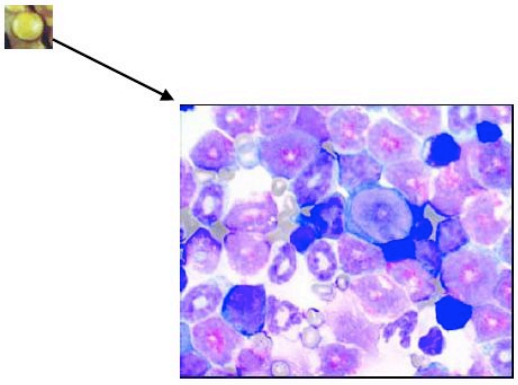
- Several lineages in the blood: lymphoid, myeloid, erythroid. How were these derived? Multipotent progenitor or single lineage progenitor?
- Jacobson: shielding the spleen from total body irradiation or infusion of BM cells provided radioprotection. Cellular or humoral effects?

Till and McCulloch: Those famous bumps



Till and McCulloch Radiat.Res. 1961

Cells within single colonies are heterogeneous
Red cells, platelet progenitors and granulocytes

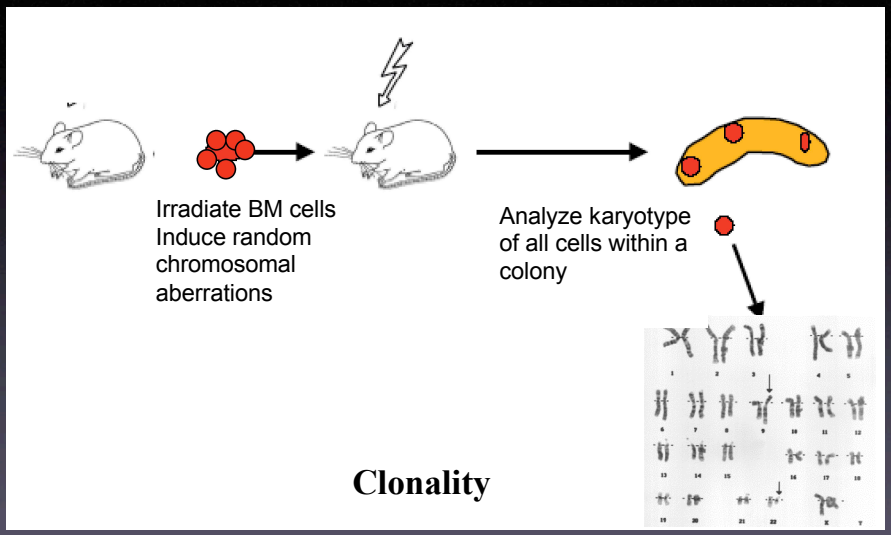


Multipotency

Spherical shape suggested that each nodule was from a single founder cell - maybe the long sought stem cell??

HOW TO PROVE????

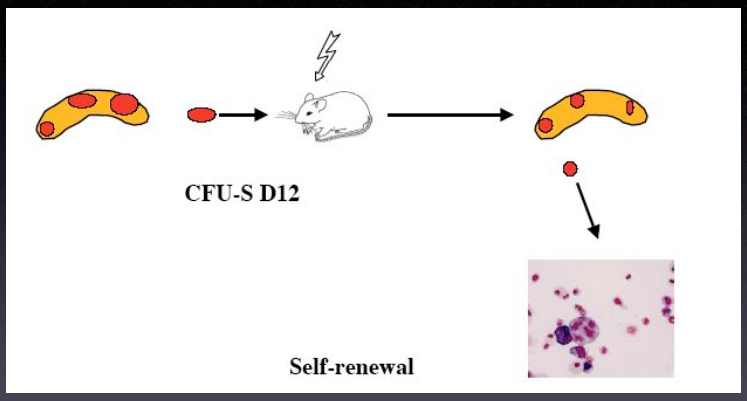
First demonstration of clonality of a stem cell



Clonality

All cell types within one colony (red cells, platelets, granulocytes) had the same chromosomal aberrations!!!!

First demonstration of stem cell self-renewal



A single colony, upon secondary transplantation, gives rise to functionally equivalent colonies

First demonstration of stem cell self-renewal

Criteria established by Till and McCulloch are still used to define a stem cell nowadays:

Clonality, self-renewal, differentiation

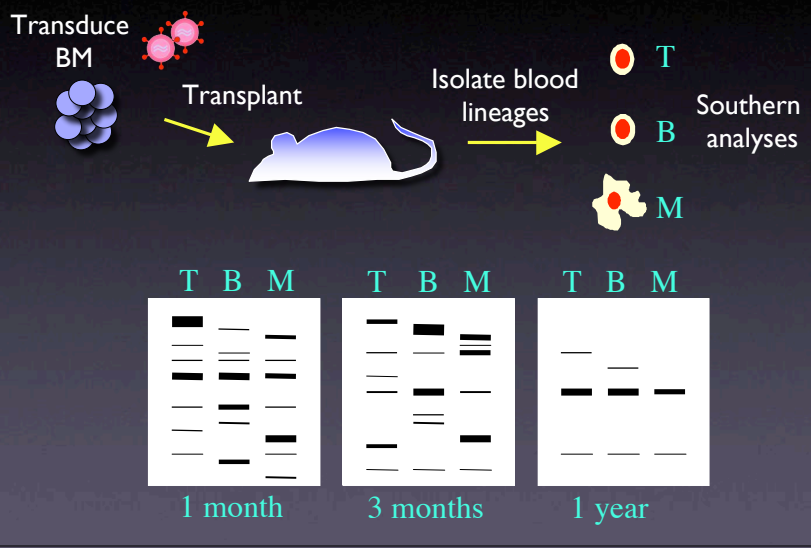
Is there a universal blood stem cell that gave rise to both myeloid and lymphoid cell lineages?

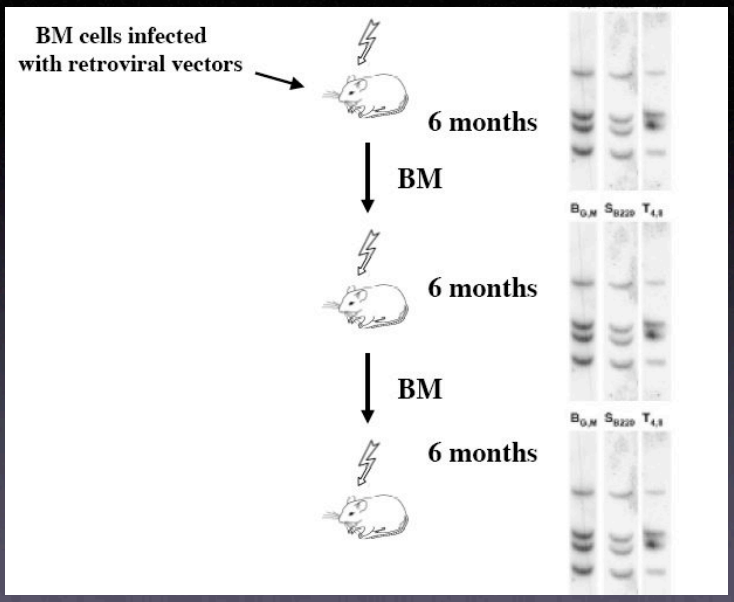
Keller and coworkers (1985)

Infected bone marrow cells with a virus containing neoR gene
- virus insets itself randomly into the bone marrow cell genome
- recombinant bone marrow cells are resistant to neomycin
  - can select the infected cells
- virus is replicated in the same genomic position at each cell division
  - provides each cloned bone marrow cells with a unique marker

They injected the engineered bone marrow cells into an irradiated mouse and analyzed the resulting recolonization of the hematopoietic system

Retroviral marking





Retroviral marking studies: lessons learned

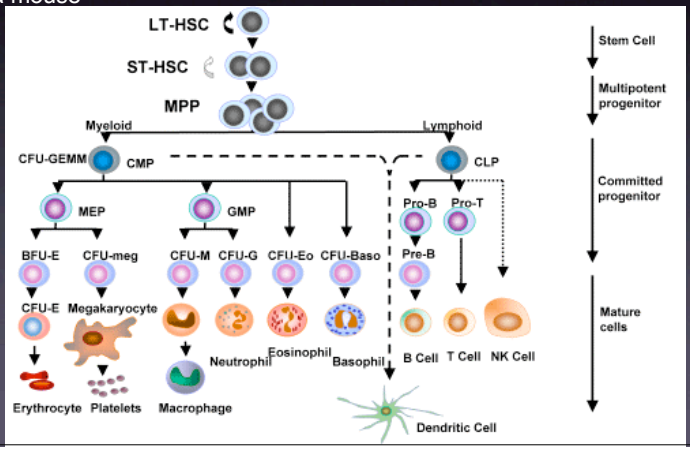
Existance of two types of stem cells : long-term and short-term HSCs

Only a few LT-HSCs are enough to sustain hematopoiesis for life (in primary and secondary recipients)

All LT-HSCs are multipotent, no unilineage HSCs

1990's: Focus on the prospective isolation of HSCs

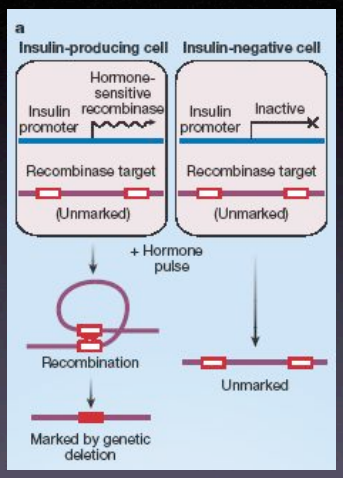
Use of monoclonal antibodies and fluorescence-activated cell sorting (FACS)
-Isolation of homogeneous populations of LT, ST-HSCs, and all committed progenitors
-Single-physically isolated HSCs could reconstitute the entire blood system of a mouse

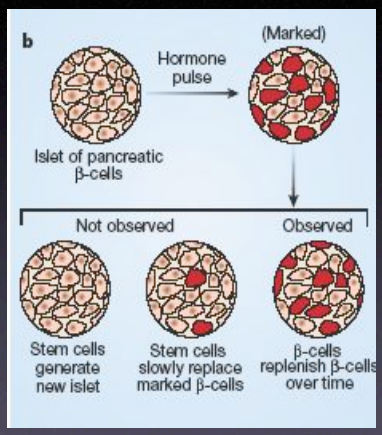


General characteristics of adult stem cells

- Stem cells are very rare
- Only stem cells have the ability to self renew
- Stem cells divide very infrequently

Not all adult tissues are regenerated by stem cells



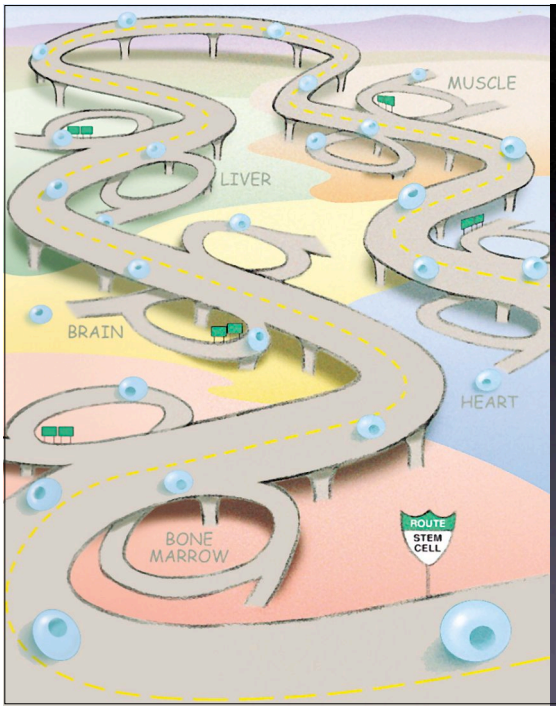


Liver also: are these differentiated cells stem cells?

Embryonic versus adult

- Isolated from early embryos
- Can expand indefinitely in culture
- Can give rise to all cell types

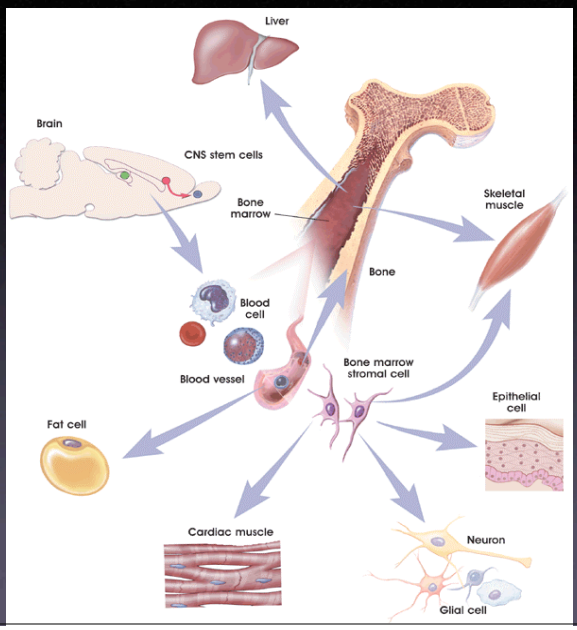
- Isolated from adult human tissue
- Can not be expanded in culture
- Can only give rise to same tissue



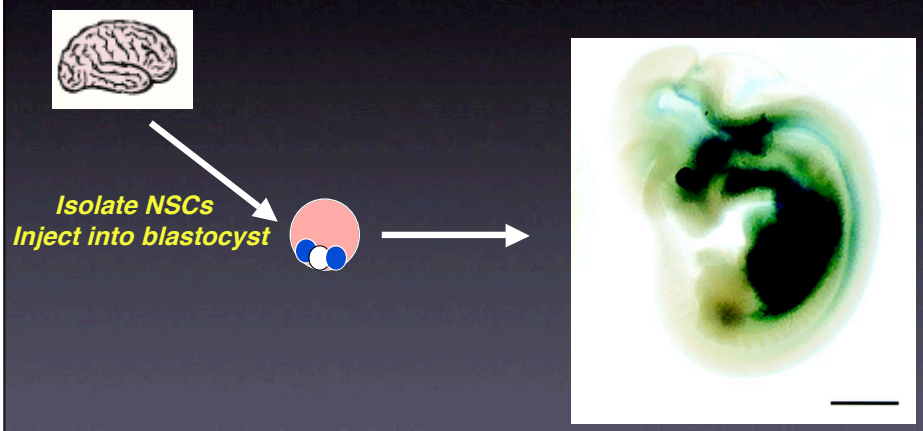
Stem Cell Plasticity

From Blau et al. (*Cell*, 2000)

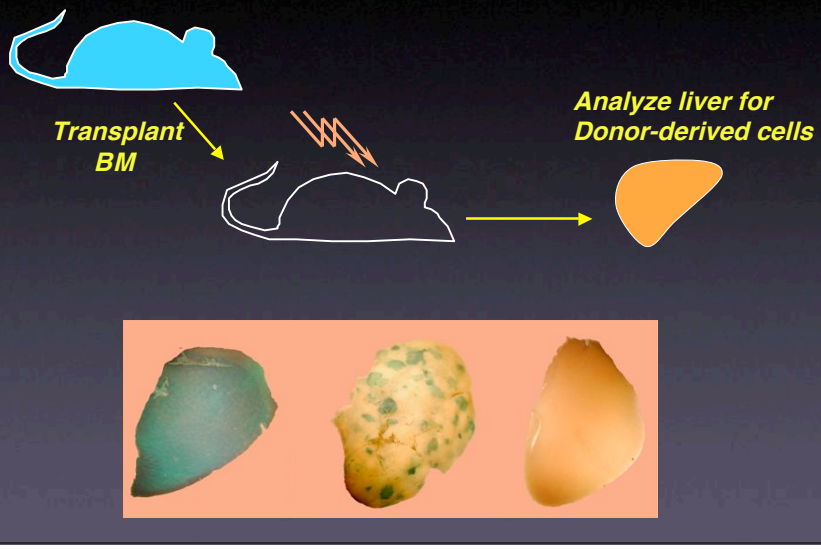
Can adult stem cells learn new tricks?



Adult neural stem cells can contribute to all three germ layers



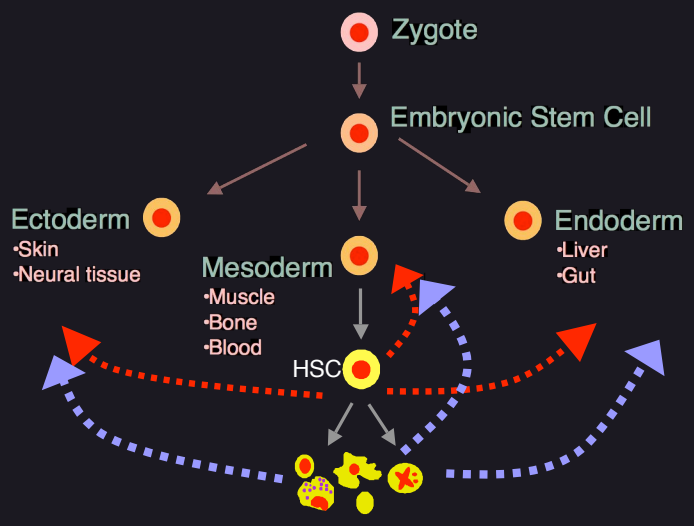
Transplanted HSCs can generate liver cells



Potential therapeutic implications of "plasticity"

- Transplantation of bone marrow for muscular dystrophies
- Direct injection of bone marrow stem cells for cardiac regeneration
- Bone marrow injections for neurodegenerative diseases

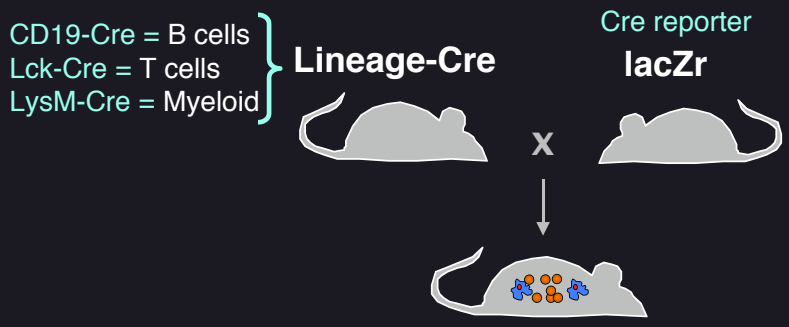
What is the mechanism for plasticity?



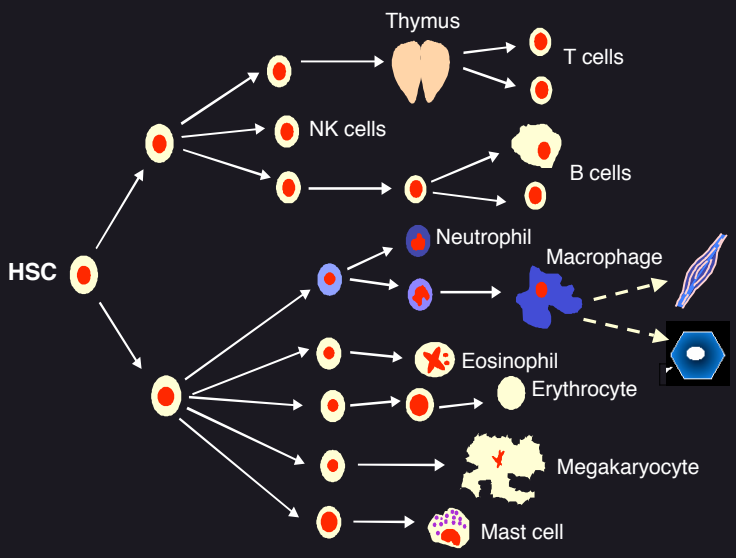
Reevaluating the role of HSCs in plasticity

- Muscle and liver engraftment require previous BM and blood engraftment by donor HSCs.
- HSCs directly injected into the liver or muscle do not engraft.
- Non-hematopoietic engraftment by a single or hundreds of HSCs is identical.
- Experimental models of plasticity require severe injuries, which result in extensive recruitment of inflammatory cells.

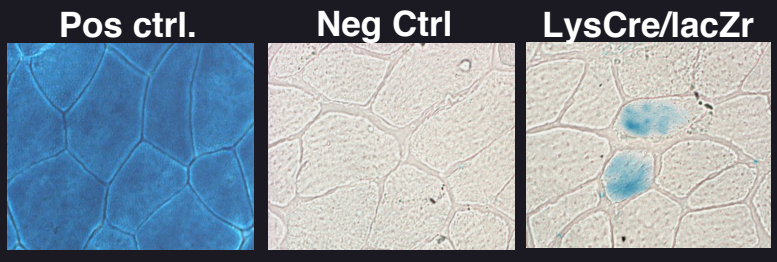
Marking mature hematopoietic cells in vivo



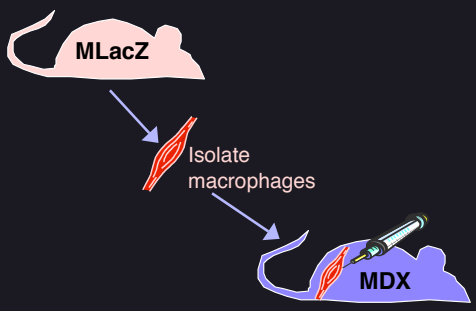
Lineage tracing in mice



Do differentiated myeloid cells contribute to muscle?



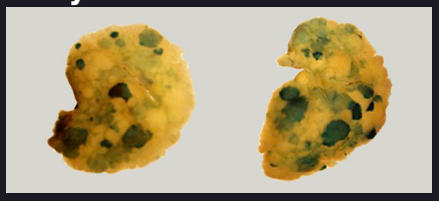
Purified macrophages fuse with myofibers



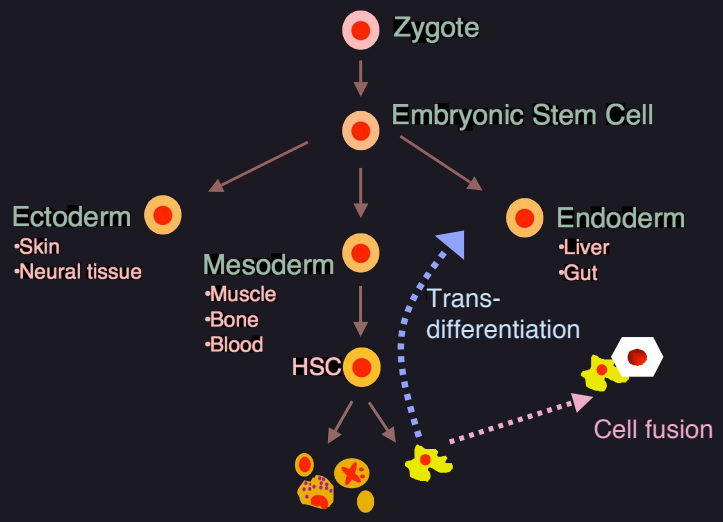
Myeloid cells regenerate liver

Pos. R26→FAH Wild type

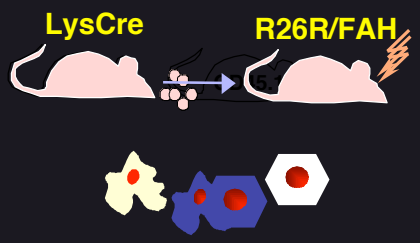
LysCre/R26R→*FAH*



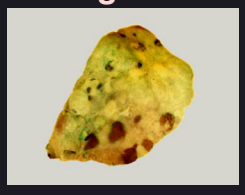
What is the mechanism for plasticity?

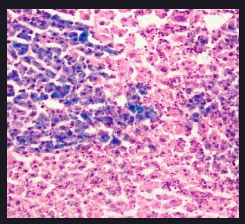


BM-derived hepatocytes arise through fusion of myeloid cells



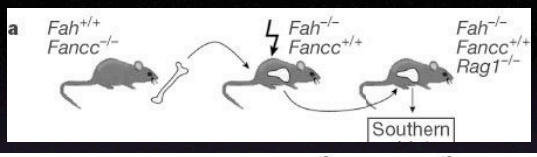
X-gal

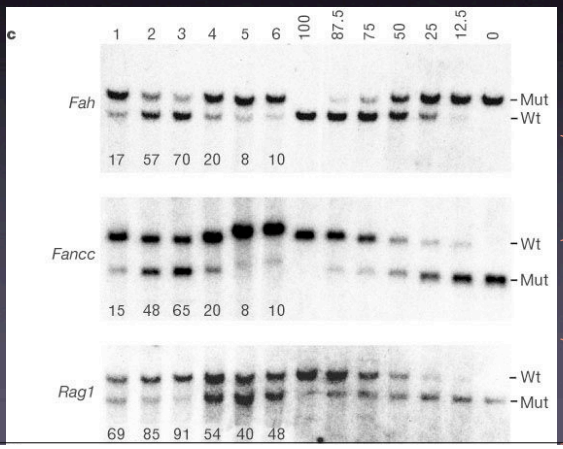




Donor	Recipient	#of lacZ^{pos} nodules
Rosa26 (n=5)	FAH	279 ± 38
LysM-Cre (n=2)	FAH/R26R	239 ± 43

Cell fusion underlies plasticity



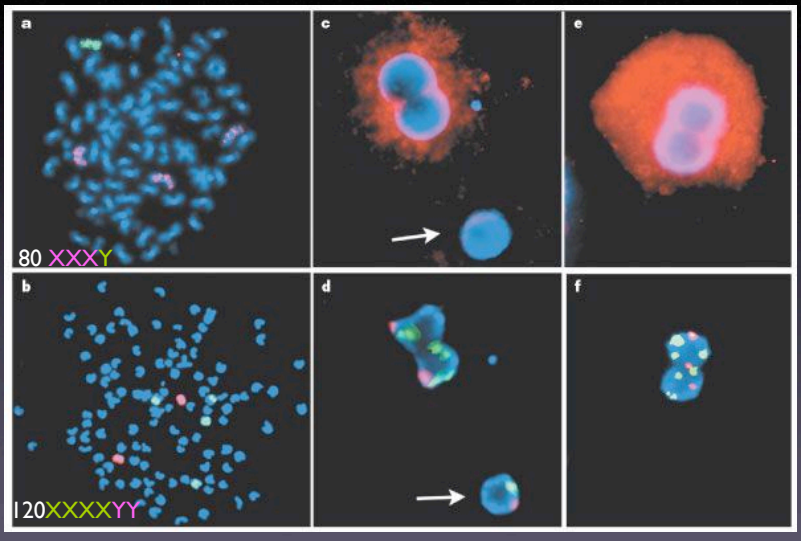


BM-derived engraftment

Total engraftment

Cell fusion underlies plasticity

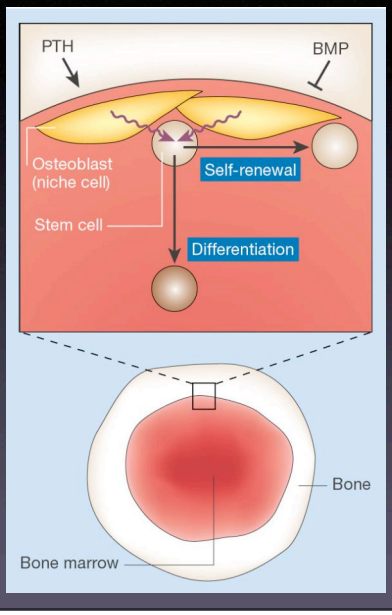
XY into XX transplants

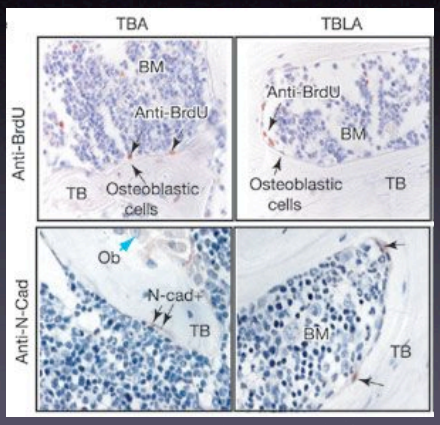


Lessons learned

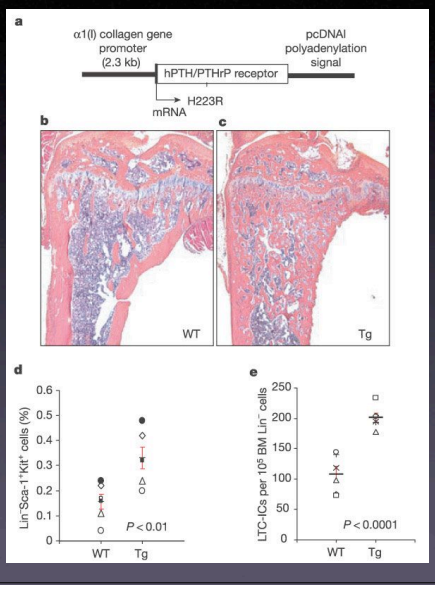
- HSCs do not “transdifferentiate”
- Mature myeloid cells are the direct mediators of plasticity.
- Macrophages randomly fuse with other cell types.

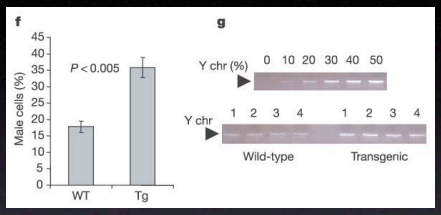
The hematopoietic stem cell niche





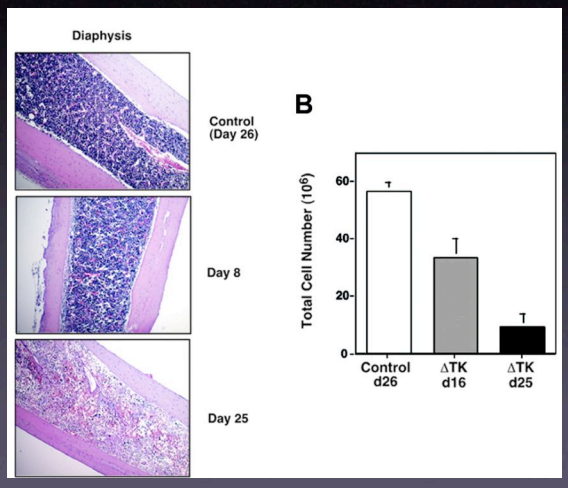
The hematopoietic stem cell niche





The hematopoietic stem cell niche

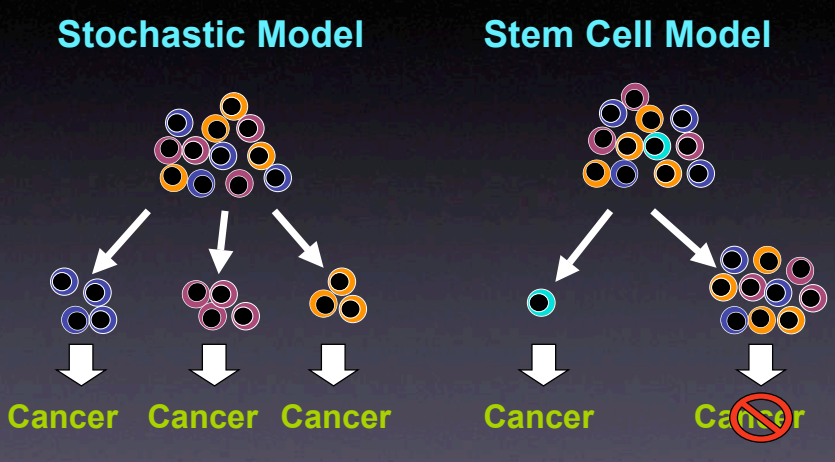
Osteoblast-specific promoter drives HSV-TK gene



Cancer as a stem cell disease?

- 1855, "Embryonal-rest" hypohesis (R. Virchow)
- Low clonogenic ability of tumors in vitro
- Most tumors are morphologically heterogeneous

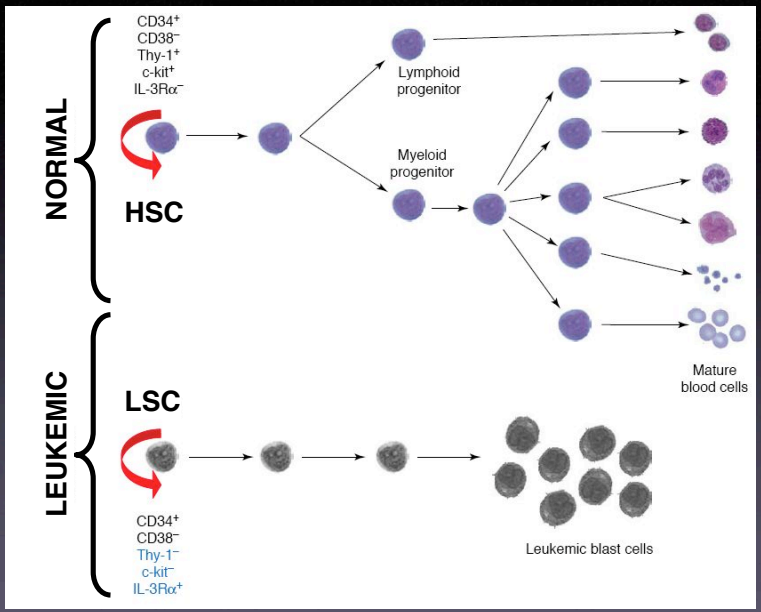
Models of cancer cell growth



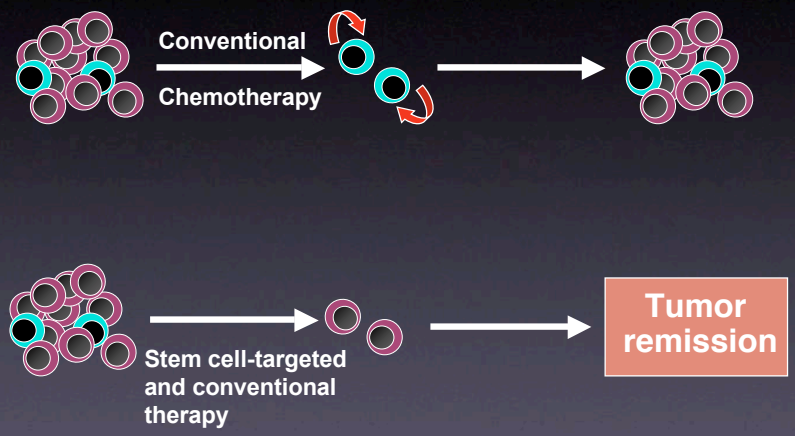
Experimental evidence of cancer stem cells

- Acute myeloid leukemia: $CD34^{+}$ $CD38^{neg}$ cells are tumor initiating (only 0.1% of total tumor cells)
- Breast cancer: $CD44^{+}CD24^{neg}$ (2% of all tumor cells)
- Brain cancer: $CD133^{+}$ (~20% of all tumor cells)

Cancer as a stem cell disease



Targeting tumor stem cells



How do we target cancer stem cells?

Need to know how self-renewal is regulated:

What are the **genes** that tell stem cells to be stem cells?