

Stem Cell Plasticity

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Stem Cells

- Capable of self renewal
- Differentiate into at least one mature cell type

Stem Cell Potency

- ***Totipotent***: Fertilized egg (zygote) and its immediate progeny: Only cells capable of differentiating into any cell type: ***Embryonic stem cells***.
- ***Pluripotent***: Adult (postnatal) stem cells: More limited differentiation ability, organ specific: ***Organ or tissue stem cell*** e. g. HSC → Blood
Oval cells → Liver
Type II pneumocytes → Lung

Challenge of Stem Cell Specificity

Over the past 5 years:

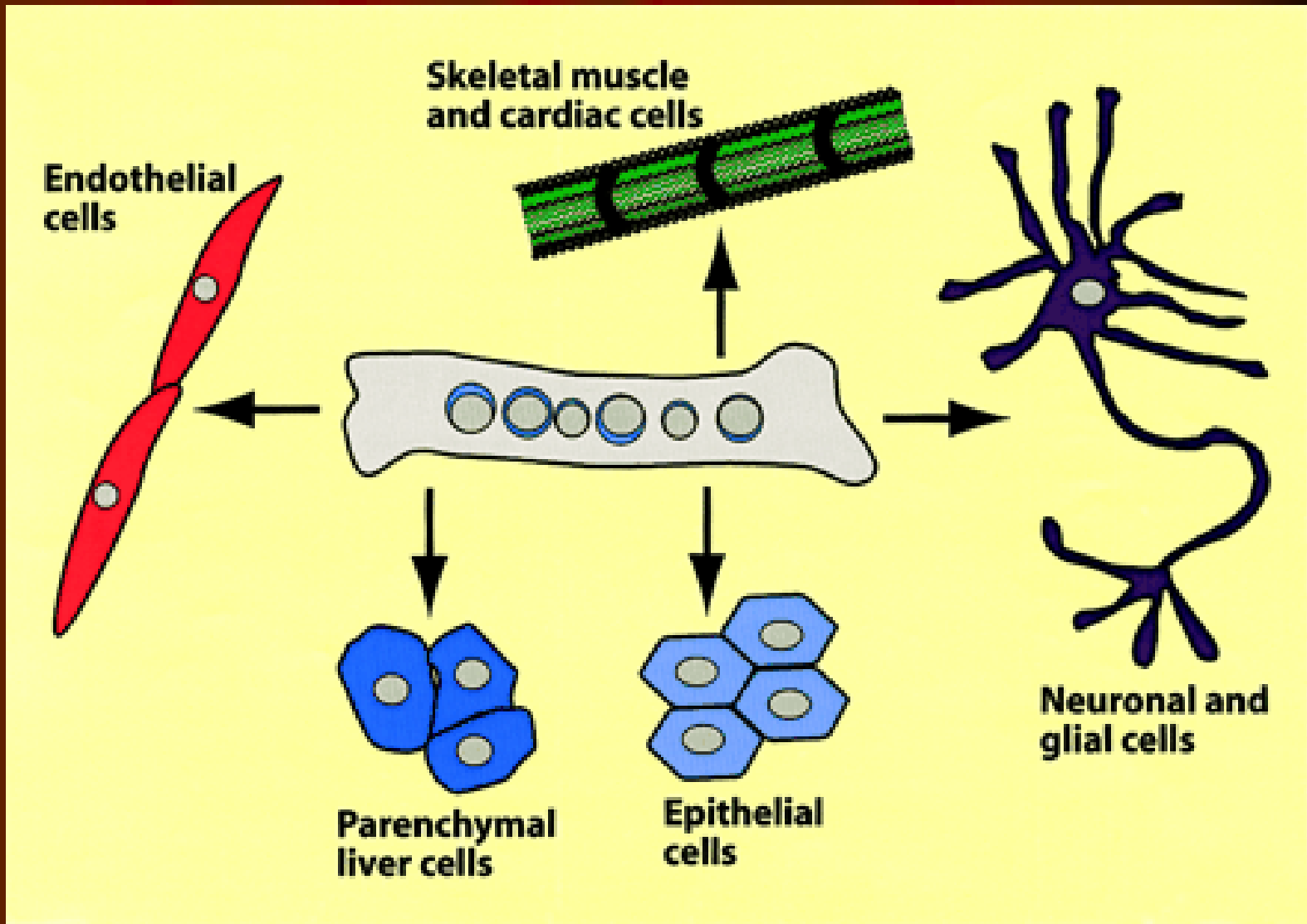
- **BMT → Donor cells differentiated also into muscle cells.**
- **Cells derived from brain and muscle → Hematopoietic reconstitution of lethally irradiated mice**



- Are all conversions due to HSC plasticity
- It is particularly difficult to verify true conversion of nonhematopoietic to hematopoietic: HSCs could be contaminants reaching via circulation:
Proved to be true

Stem Cell Plasticity

The ability of adult stem cells to cross lineage barriers and adopt the expression profile and functional phenotypes of cells that are unique to other tissues



BMSC Differentiation Potentials

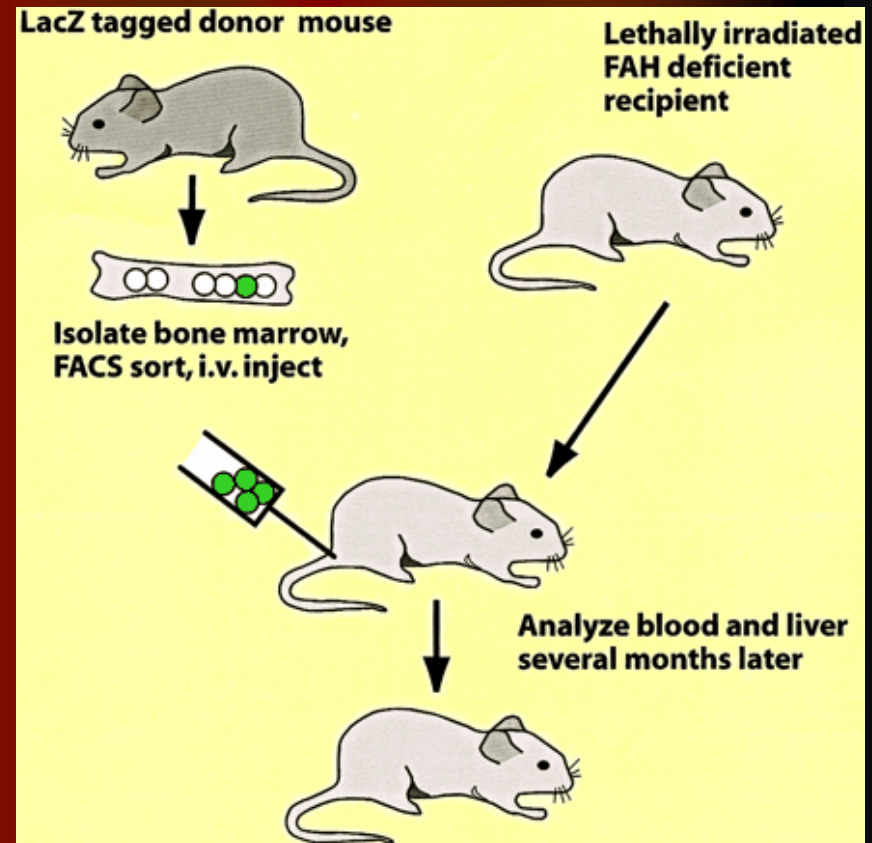
Demonstration of BM Plasticity

Bone Marrow to Liver

- Male to female BMT in Rats, Mice and Human \longrightarrow Hemopoietic reconstitution + Liver cells of donor origin.
- Two requirements:
 - Liver damage
 - Inability of endogenous repair

Bone Marrow to Liver

- lacZ mouse: labeled with β -galactosidase.
- FAH: Mice deficient in fumarylacetoacetate hydrolase leading to progressive liver and kidney failure due to tyrosinemia unless they are treated with NTBC to prevent the breakdown of tyrosine into toxic metabolites.



Bone Marrow to Liver

- As few as 50 purified KTLC (Kit+, Thy 1+, Lin -, Sca+) BMSC → animals weaned off NTBC, FAH+ KTLC BMSC engrafted as a renewable supply of functional hepatocytes.
- Only a small number of BMSC →
differentiated into hepatocytes →
Expansion

Bone Marrow to Liver In Human

Male recipients of female liver

&

Female recipients of Male marrow



Contain Y chromosome+ hepatocytes:
Unless fusion has occurred between Y+
marrow and XX hepatocytes, these
CAN ONLY BE MARROW DERIVED

Mechanisms of Homing of BM Cells to Liver or Other Epithelial Cell Differentiation

- **First: To mobilize the cells out of the marrow.**
- **Then: To recruit them to the damaged tissue**
- **The phenotypical stem cell environment is a “niche” in which programming of pluripotent cells depends on the adjacent cells and surrounding soluble and membrane-bound ligands.**
- **Shared factors between BM and injured tissues microenvironment are reported.**

Bone Marrow to Liver

We may be able, in the future, to provide in vivo replacement of diseased tissue without the need for whole organ replacement

Bone Marrow to Heart/Cardiac Muscles: Experimental

- **Study 1:** Mice with experimentally induced myocardial infarction → Intracardiac injection of whole marrow (or Kit+ BM cells) during the initial post infarct period: **Improve the outcome by:**
 1. BM cells → endothelial cells → increased vascularity
 2. BMSC → Cardiac myocytes
 3. Production of cytokines and other factors promote myogenic outcome repair and prevent fibrosis

Bone Marrow to Heart/Cardiac Muscles: Experimental

Study 2: SCF & G-CSF (40 fold ↑ in circulating BMSC pool)

administered ↓ during the
peri infarct ↓ period

Improve cardiac outcome post-infarct

THOUGH: these growth factors may exert ameliorative effects unrelated to stem cells.

YET: It is, any way, clinically beneficial

Bone Marrow to Heart/Cardiac Muscles: In Human

- Female heart to male → Chimerism of cardiac muscles.
- Two phase I studies:
 1. Intracardiac injection of autologous BM cells post- infarct: Safe, improved cardiac function
 2. AC133+ BM cells injected into infarct borders following coronary artery bypass grafting: improved perfusion and cardiac function

Bone Marrow to Heart/Cardiac Muscles: In Human

- **Caution:**

1. Small number of patients.
2. No controls
3. BM to myocyte differentiation cannot be traced in the autologous setting.

- **Hope:**

Studies to assess whether BM administration or mobilization would help in the treatment of ischaemic heart disease are going on.

Bone Marrow to Myocytes

- BM cells injected into leg regenerating after chemical injury

→ 2-5 weeks →

Muscle fibres of donor origin.

- BMT → muscle injury 5 weeks later → 2 – 3 weeks later: Myocytes of donor origin (stem cells recruited from the circulation)

Bone Marrow Stem Cells

- There is **NO** evidence, yet, for the presence of dedicated progenitors of muscle, neuronal or liver cells in the BM.

BUT

- BM is definitely **heterogeneous**: It contains various types of **hematopoietic and nonhematopoietic precursors**

Bone Marrow Cells

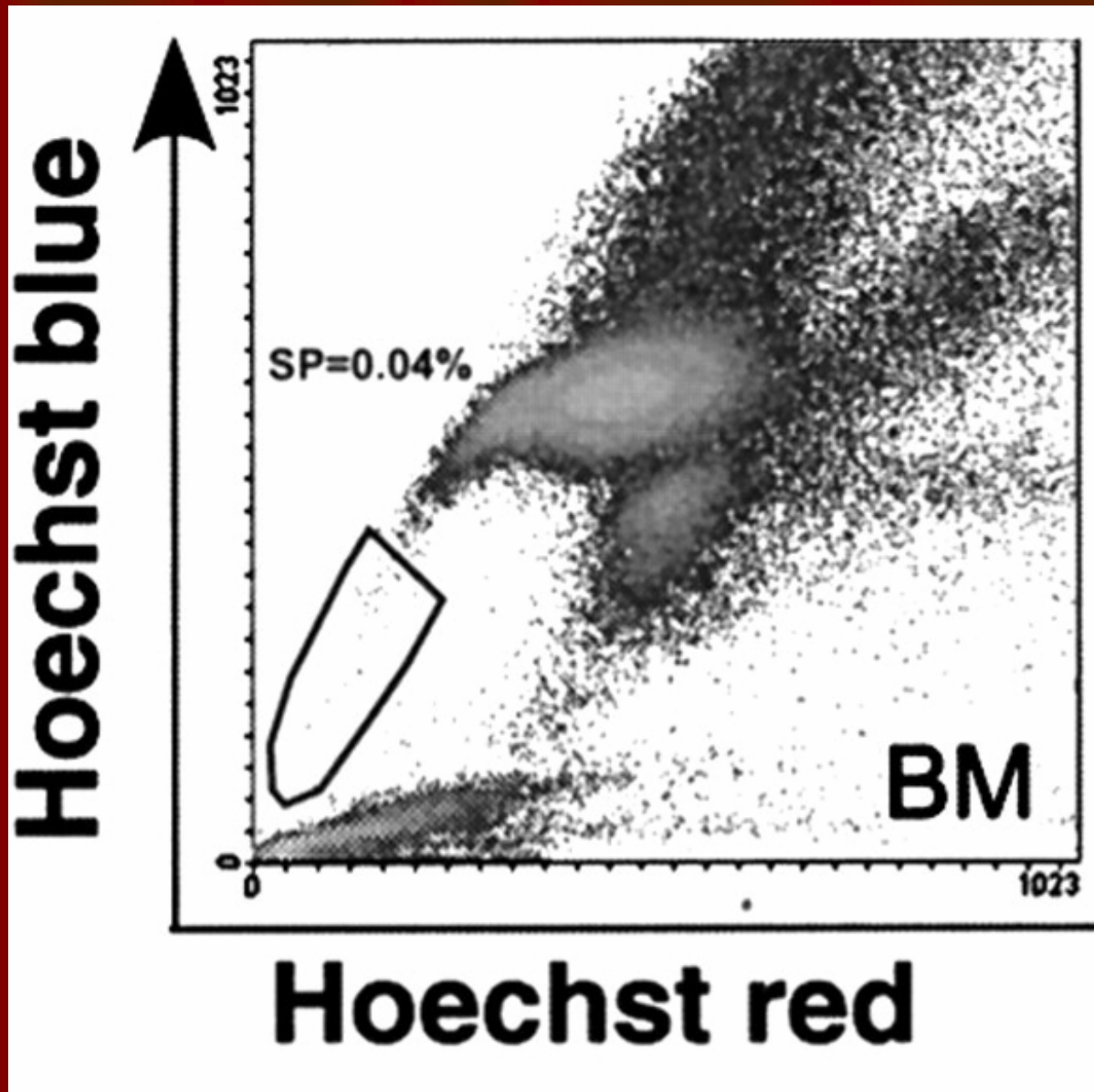
- **HSC: Hematopoietic stem cells**
- **MSC: Mesenchymal stem cells / Marrow stromal cells.**
- **Angiogenic precursor: Endothelial progenitor cells or angioblasts**

Hematopoietic stem cells

- The only true assay is the ability to reconstitute hematopoietic system of myeloablated host:
- Lin-, Rhodamine^{low}, Hoechst^{low}, CD34+, Sca+, Kit+, Thy+.
- In human: CD34+, CD38- is enriched for HSC

Side Population (SP) Cells

- Unique ability to extrude Hoechst dye
- By Flow Cytometry: They fall within a separate population that is to the “side” of the rest of the cells on a dot plot emission data in the blue vs red spectrum.
- SP cells are present in other tissues including skeletal muscles:
 - ? Tissue specific stem cells
 - ? BM derived SP cells lodged within these tissues



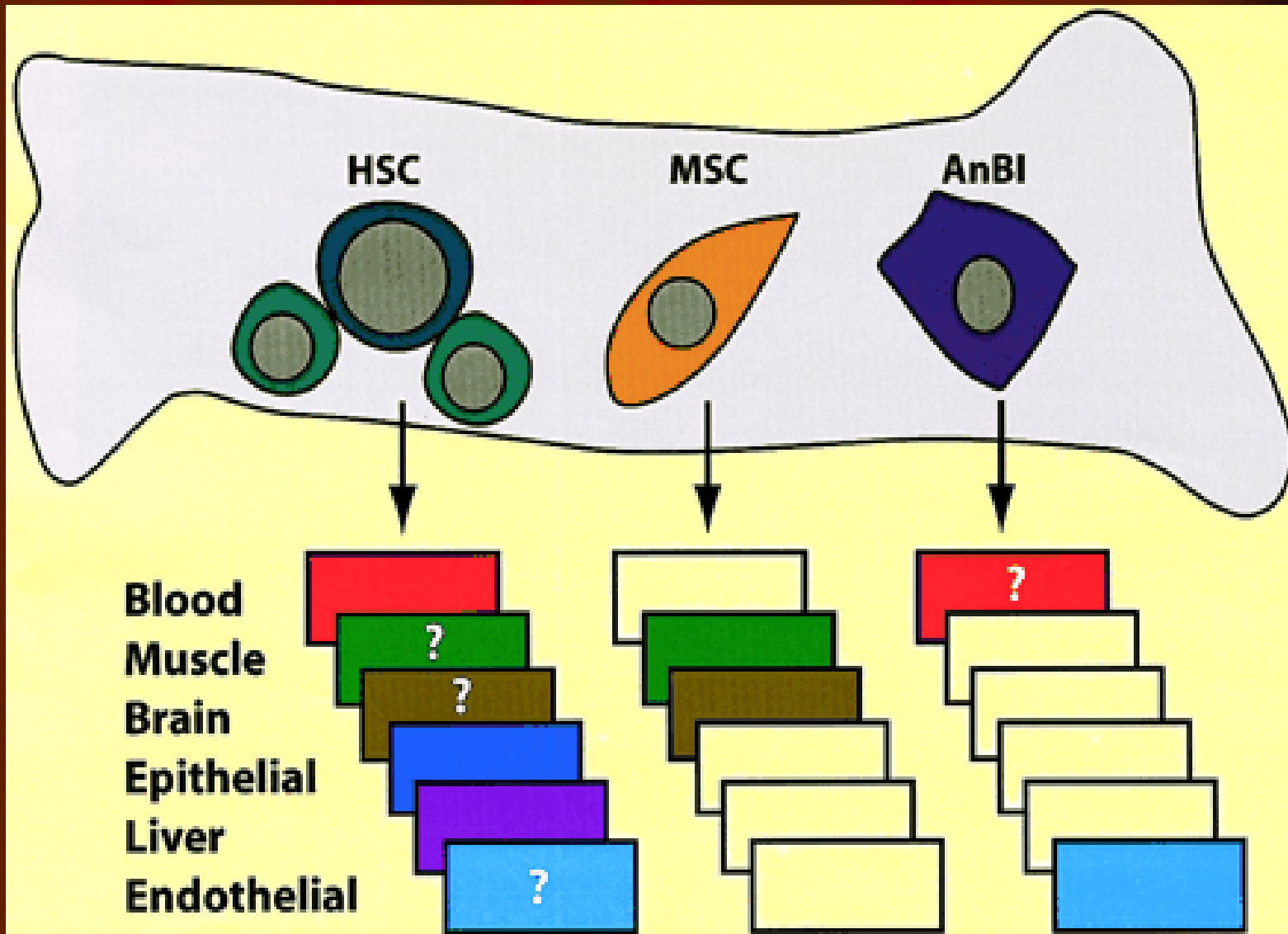
Side Population Cells

Angiogenic Precursor

- Also called endothelial progenitor cells or Angioblasts:
- In human: CD34+, AC133+, Kit^{high}, FLK-1+, Tie-2+
- Found in circulation & mobilized by G-CSF

Mesenchymal Stem Cells

- Grow in adherent layer.
- Lack CD45.
- Has a finite life span.
- Can differentiate into: Adipocytes, Chondrocytes, Osteocytes & muscle cells, Cardiomyocytes, Neurone like cells and (in culture) to astrocytes and possibly to neurons (in neonatal mouse brains).



Differentiation Potential of BMSC

Requirements (Gold Standard) to indicate True Conversion of Hematopoietic to Nonhematopoietic:

1. Show that BM cells purified on the basis of established surface markers
→ in irradiated mice →
Hematopoietic + nonhematopoietic reconstitution.


Requirements (Gold Standard) to indicate True Conversion of Hematopoietic to Nonhematopoietic:

- 2. Show that this can be produced by a single cell or alternatively prove clonal reconstitution of both types of tissues.**
- 3. The BM derived nonhemopoietic cells should be capable of differentiation and function.**

Plasticity of a Single BM-Derived Stem Cell

Enriched Lin- quiescent BM cells →
Transplanted into lethally irradiated mice → 2 days later donor cells collected from BM → **Single cell** (by limited dilution) transplanted into secondary recipients

Plasticity of a Single BM-Derived Stem Cell

In the secondary recipients  1/6 reconstituted their hematopoietic system with donor cells detected in alveoli, oesophagus, stomach, small & large bowel, skin, bile ducts and hepatocytes. Lung type II pneumocytes were shown to be functional expressing mRNA
(Krause et al, Cell 2001; 105: 369)

Possible Mechanisms for Plasticity

Role of Tissue Injury

- All the studies involved models of **TISSUE INJURY** to induce homing and differentiation of BMSCs.
- Tissue damage likely creates a favorable environment for the crossing of lineage barriers.

Possible Mechanisms for Plasticity

Role of Tissue Injury

Apoptosis and/or necrosis →

Create cytokine milieu / extracellular matrix characteristics →

A microenvironment suitable for or requesting tissue regeneration.

? Similar to the situation requesting and supporting continuous cell production in the normal BM microenvironment

Possible Mechanisms for Plasticity:

A. Direct and Indirect Differentiation

- 1. Presence of highly pluripotent, not yet committed, cells in the BM.**

- 2. Committed hematopoietic cells that can transdifferentiate: It is the ability of one committed cell type to change its gene expression pattern to that of a completely different cell type:**

Possible Mechanisms for Plasticity: Transdifferentiation

- a. Direct transition in the gene expression.
- b. Indirect: Dedifferentiation and then maturation down an alternative pathway.

Possible Mechanisms for Plasticity:

B. Fusion

- BM-derived cell + nonhematopoietic cell \longrightarrow heterokaryon: converting the gene expression pattern of the original BM cell to that of the fusion pattern e.g. Fibroblast + Myoblast \longrightarrow Expression of muscle specific RNA by the fibroblast nuclei.
- Evidence for fusion: **Controversial**

Possible Mechanisms for Plasticity:

B. Fusion

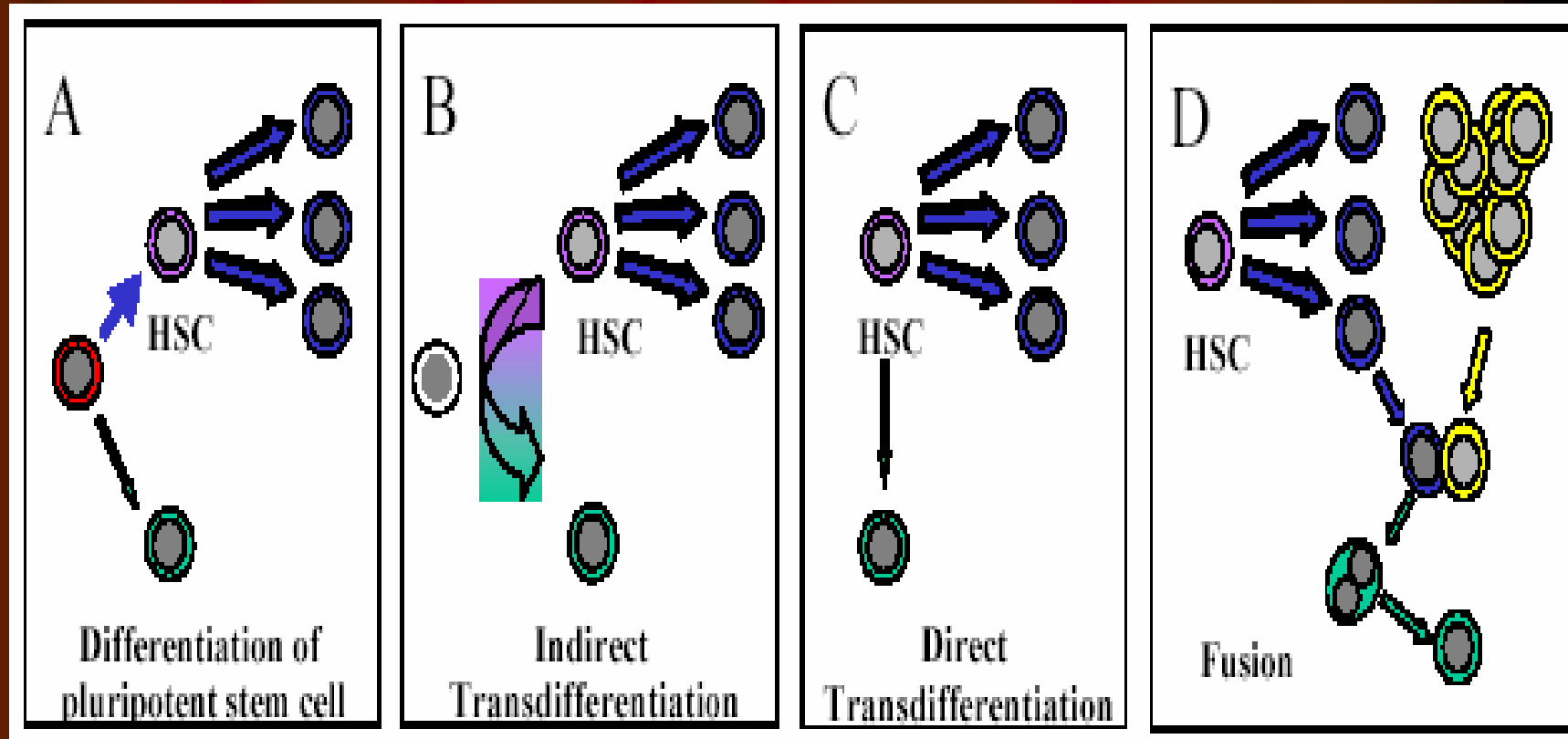
? Magnitude of contribution to plasticity:

- Fusion may be naturally occurring phenomenon or an abnormal response to intense selective pressure for circulating BMSCs to fuse with epithelia of multiple tissues.

Possible Mechanisms for Plasticity:

B. Fusion

- **HOPE:** Healthy and functioning cells: Great physiological significance
- **CONCERN:** High potential for malignancy



Proposed Mechanisms for Adult Stem Cell Plasticity

Stem Cell Plasticity

Extreme Views

- **Artifact**
- **Universally Adult Stem Cell:**
Various types of stem cells residing in the BM, brain, heart, muscles represent different states of a progenitor cell whose phenotype is defined by its local environment: a **universal adult stem cell in different coats.**

Universal Adult Stem Cell

The stem cells can move from one tissue to another via the circulation and are more plastic in early than in more differentiating stage.

Universal Adult Stem Cell

- According to this model: Differentiated cells: Transdifferentiation → Stem cells → Other differentiated cells. e.g. Myocytes → proliferating stem cells in amphibians (Brookes, Science 1997;276:81) and Pancreas → Liver cells (Shem & Slack, Nat Cell Biol 2002; 2:879)

Potential Clinical Applications

Pluripotent Marrow cells could be used in the following ways:

1. Transplantation of normal autologous cells.
2. Enhancement and/or mobilization of endogenous marrow derived stem cells.
3. Transplantation of gene modified autologous marrow cells.
4. Transplantation of allogeneic BM cells

Potential Clinical Applications

- Engraftment of BMSCs as epithelial cells is likely to be of most benefit in response to tissue damage e.g. infarction or toxins.
- Endogenous BMSCs may normally play a role in repair of low level injury.
- Administration of exogenous BMSCs or mobilization of endogenous may be advantageous with severe injury.

Potential Clinical Applications

- For treatment of genetic diseases, transplantation of genetically modified autologous marrow would avoid the risk of allogeneic BMT e.g. SS and glycogen storage disease.
- BMSC transplanted into irradiated syngeneic host can engraft as lung epithelia and astroglia.

Stem Cell Plasticity

?

- Is tissue injury necessary for differentiation into other tissue cells.
- Mechanisms by which it occurs.
- Which BM subpopulations are capable of this differentiation.
- Potential clinical applications

Stem Cell Plasticity

The Future

- **Optimize & standardize experiments:**
 - The **donor cell source** must be indicated & the specific cell populations analyzed.
 - **Tissue damage** models need to be optimized.
 - Improve **detection methods** so that cell Source, Phenotype and Function can be assessed unequivocally.
- **Clinical applications can occur concurrently.**