Lecture #2
Identifying & Isolating Stem Cells

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http://courses.engr.illinois.edu/bioe498gu/
Hematopoietic (Blood) Stem Cell Lineage

- Stem cells
- Progenitors
- Mature blood cells

- HSCs
- B cell
- T cell
- Macrophage
- Granulocyte
- Platelets
- Erythrocyte
Why we thought stem cells existed
Example- HSC

• 1945: Fraternal twin cattle that shared a placenta shared for life the blood cell types of both calves
• 1945: Civilian populations in Japan following radiation exposure died from hematopoietic failure. Radiation syndrome in mice could be prevented by injecting spleen cells, or shielding the hind limb with lead.
• 1955: Transplantation of allogeneic marrow could rescue lethally irradiated mice.
• 1961: Clonal colonies in spleen (unique chromosomal markers with retrovirus)

Spleen- Colony Forming Assay (CFU-S)
RGB-color marking of HSC colonies

Weber et al., Nat Med, 2011
HSC Assays

Cobblestone-area forming cell (CAFC) assay
Long-term culture initiating cell (LTC-IC) assay
Spleen colony forming (CFU-S) assay
HSC- Cobblestone Sample
LTC-IC

4.0 Mouse Hematopoietic CFC Assays in MethoCult® Media

4.1 Procedure Diagram

STEP 1
Establish Feeders
Mice: irradiated marrow cells
Human: suitable cell line (e.g., M210B4)

STEP 2
Add Serial Dilutions of Test Cells

- LTC-IC
- OFC
- Mature cell

STEP 3
Maintain Cultures
With weekly 1/2 medium changes
Mice: ≥4 weeks, MyeloCult® M5300
Human: ≥5 weeks, MyeloCult® H5100

STEP 4
Harvest and Plate
Harvest all cells from each well, including adherent and non-adherent cells, and transfer into individual methylcellulose cultures
Mice: 12 days, MethoCult® GF M3434
Human: 14-16 days, MethoCult® H4435 Enriched

STEP 5
Score Dishes
Record growth (≥1 colony) or no growth (no colonies)

STEP 6
Calculate LTC-IC Frequency
Use L-Calc™ - a statistical software program for limiting dilution analysis

Step 1
Prepare Cells
Process mouse cells by:
- ammonium chloride lysis
- density gradient separation
- progenitor cell enrichment with EasySep®, StemSep®, SpinSep® or FACS
Wash cells (e.g., in Iscove’s MDM plus 2% FBS), then count and adjust cell concentration.

Step 2
Add Cells to MethoCult®
Add cells to MethoCult® and vortex. Let tube stand to allow bubbles to dissipate.

Step 3
Plate and Incubate
Dispense cells into pre-tested culture dishes using syringes and blunt-end needle. Incubate mouse cells for 7-14 days in humidified incubator at 37°C and 5% CO₂.

Step 4
Count Colonies
Count and evaluate colony types using inverted microscope and gridded scoring dishes. Alternatively, individual colonies may be plucked for routine staining, PCR, or cytogenetic analysis.
Limiting Dilution Analysis
Poisson Distribution

Expected Number of Occurrences, on Average

(Number of Occurrences)

(probability density)
Limiting Dilution Analysis

\[ F_0 = e^{-m} \quad \text{(Based on Poisson Distribution)} \]

- \( F_0 \), frequency of wells with no colonies
- \( m \), average number of cells per culture
HSC Assays

*in vitro assays*  
HSC  
*in vivo assays*

Cobblestone-area forming cell (CAFC) assay  
Long-term culture initiating cell (LTC-IC) assay  
Spleen colony forming (CFU-S) assay
Retrospective Analysis (genetic markers)

Prospective Isolation (define single cell properties)
Hematopoietic (Blood) Stem Cell Lineage Detailed Version

Hematopoietic Hierarchy

HSC (Long-term Self-renewal)

Multipotent Progenitors:
- CMP
- MEP
- GMP

Oligopotent Progenitors:
- Pro-DC
- Pro-T
- Pro-NK
- Pro-B

Lineage restricted Progenitors:
- Ep
- Mkp

Mature effector cells:
- Erythrocytes
- Platelets
- Granulocytes
- Macrophages
- Dendritic cells
- T-cells
- NK-cells
- B-cells

Shizuru and Weissman
Hematopoietic Stem Cell - Surface Markers (Proteins)

Commonly used:

- Lineage-negative (lin−)
- Side-population
- Rhodamine
- CD34−

Long-term HSC:
- ckit^{hi}
- Sca-1^{hi}
- Thy1.1^{lo}

Short-term HSC:
- Lin−
- c-kit^{+}
- Sca-1^{+}
- Flt3^{+}
- IL7Rα^{−}
- CD150^{+}
- CD11b^{lo}
- Thy1.1^{lo}
- CD34^{+}

Multipotent progenitor:
- Lin−
- c-kit^{+}
- Sca-1^{+}
- Flt3^{+}
- IL7Rα^{−}
- CD150^{+}
- CD11b^{lo}
- Thy1.1^{lo}
- CD34^{+}
Fluorescence Activated Cell Sorting (FACS)

- Similar to Flow Cytometry
- Antibody-driven separation
- Greater specificity by sorting with multiple markers

Forward scatter - size
Side scatter - granularity
HSC Sorting

Mouse:
- Long-term repopulation (1/100,000)
- Short-term repopulation (1/1,000-2,000)
Hematopoietic Stem Cell - Surface Markers (Proteins)

Commonly used functional properties:
- ckit^hi
- Sca-1^hi
- Thy1.1^lo
- IL7Rα^-
- CD150^*
- Flt3^-
- Endoglin^*
- Side-population^*
- Rhodamine^lo
- CD34^-

Long-term HSC → Short-term HSC → Multipotent progenitor
Stem Cells Classified by ‘Unique’ Physical Characteristics/Cell Functions

Side-Population;

Efflux of Hoescht dye

Lin et al., Methods Enzym, 2006
Stem Cells Classified by ‘Unique’ Physical Characteristics/Cell Functions

Aldehyde dehydrogenase activity;

- Fluorescent (BODIPY) aminoacetaldehyde (BAAA) enters cell
- ALDH converts to BODIPY aminoacetate (BAA) which is retained in cell
ALDH1 Is a Marker of Normal and Malignant Human Mammary Stem Cells and a Predictor of Poor Clinical Outcome

Christophe Ginestier, Min Hee Hur, Emmanuelle Charafe-Jauffret, Florence Monville, Julie Dutcher, Marty Brown, Jocelyne Jacquemier, Patrice Viens, Celina G. Kleer, Suling Liu, Anne Schott, Dan Hayes, Daniel Birnbaum, Max S. Wicha, and Gabriela Dontu

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Cell Stem Cell 1, 555–567, November 2007
Mammary Cancer Stem Cells

Aldefluor+
Tumor cells present

Aldefluor-
No tumor, only ‘loading’ gel & residual other cells

Ginestier et al., Cell Stem Cell, 2007
Cancer Stem Cells

Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell

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Cancer Stem Cells

## Cancer Stem Cells

<table>
<thead>
<tr>
<th>Tumor</th>
<th>CSC phenotype</th>
<th>% Tumor cells with CSC immunophenotype</th>
<th>Tumors engrafting using sorted CSCs (1st Tx)</th>
<th>Min. CSCs to engraft (1st Tx)</th>
<th>Serial Tx performed</th>
<th>Min. CSCs or bulk tumor cells for serial Tx (2nd Tx)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>CD34^+CD38^-</td>
<td>0.1–1</td>
<td>n/a</td>
<td>2×10^5</td>
<td>No</td>
<td>—</td>
<td>20.</td>
</tr>
<tr>
<td>AML</td>
<td>CD34^+CD38^-</td>
<td>0.02–2</td>
<td>15/18</td>
<td>5×10^3</td>
<td>Yes</td>
<td>2×10^5–2×10^6 Bulk</td>
<td>19.</td>
</tr>
<tr>
<td>AML</td>
<td>CD34^+CD38^-</td>
<td>n/a</td>
<td>9/9</td>
<td>10^4</td>
<td>Yes</td>
<td>10^5 CSCs</td>
<td>69.</td>
</tr>
<tr>
<td>Breast</td>
<td>CD44^+/CD24^-/low</td>
<td>11–35</td>
<td>9/9</td>
<td>100</td>
<td>Yes</td>
<td>200 CSCs</td>
<td>30.</td>
</tr>
<tr>
<td>Medullo-blastoma</td>
<td>CD133^+</td>
<td>6–21</td>
<td>3/3</td>
<td>10^4</td>
<td>No</td>
<td>—</td>
<td>44.</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>CD133^+</td>
<td>19–29</td>
<td>4/4</td>
<td>100</td>
<td>Yes</td>
<td>10^5 CSCs</td>
<td>44.</td>
</tr>
<tr>
<td>HNSCC</td>
<td>CD44^+</td>
<td>0.1–42.7</td>
<td>13/25</td>
<td>5×10^3</td>
<td>Yes</td>
<td>2.5×10^4 CSCs</td>
<td>38.</td>
</tr>
<tr>
<td>Colon</td>
<td>CD133^+</td>
<td>1.8–24.5</td>
<td>11/17</td>
<td>100</td>
<td>Yes</td>
<td>10^5 CSCs</td>
<td>47.</td>
</tr>
<tr>
<td>Colon</td>
<td>CD133^+</td>
<td>0.7–6.1</td>
<td>12/18</td>
<td>1,500</td>
<td>Yes</td>
<td>n/a</td>
<td>48.</td>
</tr>
<tr>
<td>Colon</td>
<td>CD133^+</td>
<td>0.3–3.0</td>
<td>n/a</td>
<td>2.5–5×10^1</td>
<td>Yes</td>
<td>n/a</td>
<td>48,49</td>
</tr>
<tr>
<td>Colon</td>
<td>ESAhi/CD44^+</td>
<td>0.03–38.0</td>
<td>6/6</td>
<td>200</td>
<td>No</td>
<td>—</td>
<td>35.</td>
</tr>
<tr>
<td>Colon</td>
<td>ESAhi/CD44^+/CD166^+</td>
<td>1.2–16.0</td>
<td>3/3</td>
<td>150</td>
<td>Yes</td>
<td>500 CSCs</td>
<td>48.</td>
</tr>
<tr>
<td>Melanoma</td>
<td>ABCB5^+</td>
<td>1.6–20.4</td>
<td>4/7</td>
<td>10^5</td>
<td>Yes</td>
<td>10^4 CSCs</td>
<td>53.</td>
</tr>
<tr>
<td>Prostate</td>
<td>CD44^+</td>
<td>0.1–20.0</td>
<td>2/2</td>
<td>10^5</td>
<td>No</td>
<td>—</td>
<td>129.</td>
</tr>
<tr>
<td>Pancreas</td>
<td>CD44^+CD24^-ESA^+</td>
<td>0.2–0.8</td>
<td>10/10^1</td>
<td>100</td>
<td>Yes</td>
<td>n/a</td>
<td>36.</td>
</tr>
<tr>
<td>Pancreas</td>
<td>CD133^+</td>
<td>0.68–3.21</td>
<td>11/11</td>
<td>500</td>
<td>Yes</td>
<td>500 CSCs</td>
<td>50.</td>
</tr>
<tr>
<td>Lung (non-small cell)</td>
<td>CD133^+</td>
<td>0.32–22</td>
<td>n/a</td>
<td>1×10^4</td>
<td>No</td>
<td>—</td>
<td>51.</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>CD44^+CD90^+</td>
<td>0.74–6.2</td>
<td>13/13</td>
<td>5,000</td>
<td>Yes</td>
<td>n/a</td>
<td>39.</td>
</tr>
<tr>
<td>B-ALL</td>
<td>CD34^+CD10^-</td>
<td>4.0–12.0</td>
<td>6/6</td>
<td>7×10^4</td>
<td>Yes</td>
<td>10^5 Bulk</td>
<td>61.</td>
</tr>
<tr>
<td>B-ALL</td>
<td>CD34^+CD19^-</td>
<td>2.0–4.0</td>
<td>5/5</td>
<td>5×10^4</td>
<td>Yes</td>
<td>10^5 Bulk</td>
<td>61.</td>
</tr>
<tr>
<td>B-ALL (BCR-ABL^+ ETv6/RUNX1^+</td>
<td>CD34^+CD38^-CD19^-</td>
<td>0.17–4.51</td>
<td>8/10</td>
<td>5.5×10^3–9×10^4</td>
<td>Yes</td>
<td>n/a</td>
<td>46.</td>
</tr>
<tr>
<td>T-ALL</td>
<td>CD34^+CD4^-</td>
<td>5.0–15.0</td>
<td>6/7</td>
<td>10^4</td>
<td>Yes</td>
<td>3×10^4 Bulk</td>
<td>62.</td>
</tr>
<tr>
<td>T-ALL</td>
<td>CD34^+CD7^-</td>
<td>6.0–14.0</td>
<td>5/5</td>
<td>4×10^3</td>
<td>Yes</td>
<td>4×10^4 Bulk</td>
<td>62.</td>
</tr>
<tr>
<td>Myeloma</td>
<td>CD138^-CD34^-</td>
<td>n/a</td>
<td>1/1</td>
<td>1×10^6</td>
<td>No</td>
<td>—</td>
<td>64.</td>
</tr>
</tbody>
</table>

Abbreviations: 1st, primary; 2nd, secondary; AML, acute myeloid leukemia; B-ALL, B-cell lymphoblastic leukemia; CSC, cancer stem cell; HNSCC, head-and-neck squamous cell carcinoma; Max, maximum; Min, minimum; n/a, not available; T-ALL, T-cell lymphoblastic leukemia; Tx, transplant.

Lineage marker positive cells were also excluded. Tertiary and/or quaternary transplants also performed in addition to secondary transplants. Transplants were performed using CSCs purified from primary xenografts. P. Dalerba, personal communication.
Mesenchymal Stem Cells
In Vitro Differentiation - Mesenchymal Stem Cells


Controls:
Fibroblasts (Not MSCs)

Adipo  Chondro  Osteo

A  B  C

D  E  F

G  H  I

J  K  L

M  N  O

Lipid  Coll II  Alk Phos/Ca deposition
Mesenchymal Stem Cells
(from human bone marrow)

Procedure:
• Density cell fractionation
• Adherence and proliferation on cell culture plastic

Pittenger et al., Science, 1999
Mesenchymal Stem Cells
(from human bone marrow)

Procedure:
- Density cell fractionation
- Adherence and proliferation on cell culture plastic

Modified procedures:
- Magnetic column separation (mouse needs this due to more contamination of hematopoietic cells)
- Modifications for distinct tissue sources
Mesenchymal Stem Cells

Mesenchymal Stem Cells
(Role in the bone marrow...)

Neurogenesis in the adult human hippocampus

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