Stem Cell Bioengineering
BIOE 498/598

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Online Syllabus
http://courses.engr.illinois.edu/bioe498gu/

(Also: http://courses.engr.illinois.edu/bioe598gu/)
Stem Cells

A new twist on an old favorite...
Stem Cells

The ‘Old’ with the ‘New’

Mature Field:
• Discovery of hematopoietic stem cells (1960s)
• First bone marrow transplant (1950s)
• Pluripotent stem cells (1980s mouse, 1990s human)

Rapid Evolution:
• Controlled differentiation protocols
• Adult tissue-specific stem cells
• Reprogramming
Nobel Prize in Physiology & Medicine (2012)
Stem Cell Language

• Potency:
  - Totipotent (can produce all cell types)
    • Example: fertilized egg
  - Pluripotent (can produce most, but not all cell types)
    • Example: Inner Cell Mass (Embryonic Stem Cells)
  - Multipotent (can give rise to cells of a particular function)
    • Example: adult stem cells
  - Unipotent (can produce 1 cell type)

• Origin of Cells:
  - Germ (egg or sperm precursors in adult)
  - Somatic (cell other than egg or sperm)
Wild-type donor of enucleated eggs  Albino parents of nucleus donor
Wild-type donor of enucleated eggs  Albino parents of nucleus donor
Cloning in Animals

Wild-type donor of enucleated eggs

Albino parents of nucleus donor

J. Gurdon
Cloning in Animals

Cloning is based on...

cell reprogramming to a pluripotent state

J. Gurdon, Nat Med, 2009
Cloning in Mammals

Cloning of Dolly (1996)

1. Egg cell from ovary
2. Nucleus removed
3. Cells fused
4. Grown in culture
5. Implanted in uterus of a third sheep
6. Embryonic development

Lamb ("Dolly") genetically identical to mammary cell donor
Embryonic and Adult Stem Cells

THE SUPPLY CHAIN
Embryonic and adult stem cells as a source of new tissue.

Egg → Zygote → Blastocyst → Gastrula

Sperm

Embryonic stem cells

Self renewal

Differentiation signals

Endoderm | Mesoderm | Ectoderm

Somatic stem cells

Self renewal

Differentiation signals

Cell types restricted to organ source

Any cell type?
Stem Cells - Definition

• It is not terminally differentiated (not at the end of a differentiation pathway)

• NIH definition:
  • 1) Cells that have the ability to divide for indefinite periods in vivo/in culture
  • 2) Give rise to specialized cells

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1. asymmetric division: sister cells born different

2. symmetric division: sister cells become different as result of influences acting on them after their birth
Derivation of Human ES cells

Embryonic Stem Cell Lines
Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro,
Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall,
Jeffrey M. Jones

www.sciencemag.org  SCIENCE  VOL 282  6 NOVEMBER 1998
Derivation of Human ES cells

ES cell proliferation

Fig. 3. Expression of cell surface markers by H9 cells. Scale bar, 100 μm. (A) Alkaline phosphatase. (B) SSEA-1. Undifferentiated cells failed to stain for SSEA-1 (large colony, left). Occasional colonies consisted of non-stained, central, undifferentiated cells surrounded by a margin of stained, differentiated, epithelial cells (small colony, right). (C) SSEA-3. Some small colonies stained uniformly for SSEA-3 (colony left of center), but most colonies contained a mixture of weakly stained cells and a majority of non-stained cells (colony right of center). (D) SSEA-4. (E) TRA-1-60. (F) TRA-1-81. Similar results were obtained for cell lines H1, H7, H13, and H14.

Human ES cell colony
Gary Smith, U. of Michigan

Phenotypic Markers
(Thomson et al., Science, 1998)
ES cell proliferation

Human ES cell colony
Gary Smith, U. of Michigan

Karyotyping
In-Vitro Differentiation

Embryoid Bodies

Testing ES Potency In Vivo: Teratomas

- Gut
- Bone

Neural
Cartilage

- Tumors
- Can’t control organization

Thomson et al
5. **Injection of ES cells into blastocysts**

The targeted ES cells are injected into blastocysts... where they mix and form a mosaic with the cells of the inner cell mass from which the embryo develops.

The injected blastocysts are implanted into a surrogate mother where they develop into mosaic embryos.

6. **Birth and breeding of mosaic mice**

The mosaic mice mate with normal mice to produce both gene targeted and normal offspring.

Gene targeted mice – called "knockout mice" when the targeted gene is inactivated

© The Nobel Committee for Physiology or Medicine   Illustration: Annika Röhl
Nobel Prize in Physiology & Medicine (2012)
Reprogramming of Somatic Cells

- Somatic Cell Nuclear Transfer, i.e. Cloning

- ‘Therapeutic Cloning’ vs. ‘Reproductive Cloning’
Reprogramming of Somatic Cells

• Induced Pluripotent Stem Cells (iPS)

• Discovery by S. Yamanaka

S Yamanaka, Nature, 2009
iPS cells: therapeutic trajectories

# Adult Stem Cells - Tissue Homeostasis

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Stem Cell Location</th>
<th>Niche Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissues with constant turnover</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematopoietic system</td>
<td>Bone marrow</td>
<td>Macrophages*, T(_{Rec}) cells*, osteoblasts, adipocytes, nestin* MSCs, CAR cells, glia</td>
</tr>
<tr>
<td>Intestine</td>
<td>Fast-cycling: base of crypt</td>
<td>Paneth cells*, mesenchymal cells</td>
</tr>
<tr>
<td>Interfollicular epidermis</td>
<td>Basal layer of epidermis</td>
<td>Dermal fibroblasts</td>
</tr>
<tr>
<td>Hair follicle</td>
<td>Bulge</td>
<td>K6* bulge*, dermal papilla, adipocyte precursor cells, subcutaneous fat, dermal fibroblasts</td>
</tr>
<tr>
<td>Tissues with low or no turnover</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>Subventricular zone, subgranular zone</td>
<td>Ependymal cells, vasculature</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Between the basement membrane and the muscle fibres</td>
<td>Myofibres* (?)</td>
</tr>
</tbody>
</table>
The Stem Cell Niche

- Specific anatomical location in adult tissue

- Microenvironment maintains balance between self-renewal and differentiation

- Interactions between stem cells, niche cells, extracellular matrix, and secreted factors.
Epidermal (Bulge) Stem Cells

Blanpain et al., Cell, 2004
Intestinal Stem Cells
(intestinal structures in culture)

Single Cell (‘Clonal’) Assays

Lutolf et al., Integr Biol, 2009
Intestinal Stem Cells (from single cell)

Cancer Stem Cells
Stem Cells

A new twist on an old favorite...
Stem Cell *Engineering*

Gene Expression, Protein Interactions
- Microarray, RNA-seq
- Mass Spectrometry, RNAi screens
- Mathematical Analysis
- RNAi Arrays & Barcoded Screens
- Microfluidics, Single Cell Analysis

Stem Cell Genetic Programs
- microRNAs, Epigenome
  - RNA-seq, Deep Sequencing
  - Bisulfite-seq, ChIP-seq
  - Computational Analysis
  - Affinity Purified miRNA-mRNA
  - Microfluidics, Single Cell Analysis

Gene Regulatory Networks
- ChIP-chip, ChIP-PET, ChIP-seq
- Computational Analysis
- Reprogramming
- Microenvironmental Connections
- Synthetic Biology

Underhill GH, Wiley Inter Rev, 2012
Stem Cell *Engineering*

Stem Cell Microenvironments

- Mechanical
- Autocrine Soluble Factor
- Cell Surface Ligands
- Oxygen/pH
- Paracrine Soluble Factors
- Niche Cell
- Niche Cell
- ECM
- Electrical

- Micropatterning
- High-Throughput Arrays
- Bioreactors/Microfluidics
- 3D Biomaterials
- Computational Models

Underhill GH, Wiley Inter Rev, 2012
Course Overview

• Assignments and Dates
  – Homeworks/Group paper presentation
  – Midterm #1 Mon 2/28
  – Midterm #2 Mon 4/18
  – Project group presentations 4/25, 4/27, 5/2
  – Project group papers due 5/11

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  – ** Additional assignments for 598 students

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Course Overview

• Grading (*498 students)
  – Homeworks/Paper presentation: 27%
  – Midterm #1: 26%
  – Midterm #2: 26%
  – Project presentation: 10%
  – Project paper: 11%
Course Overview

• Grading (*598 students)
  – Homeworks/Paper presentation: 25%
  – Midterm #1: 24%
  – Midterm #2: 24%
  – Project presentation: 10%
  – Project paper: 10%
  – Additional 598 assignments: 7%
Course Overview

• Please do not disturb others during lecture
  – If you must use laptops in class, make sure they are for note taking only
• No cheating, copying, plagiarism, etc.
  – I take academic integrity very seriously
  – All group members will be held responsible for any plagiarism in the project work that is turned in
• Please let me know if you have any questions, concerns, problems, etc.