Lecture #5
Stem Cells and Lineages
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
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)
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)

• Later: A single stem cell clone could repopulate one or more hosts, prospective isolation by phenotype, and many more...
What are HSC Features

• Job is NOT to carry out differentiated function, but to produce cells that will
• Small, 6-8 microns
• High nuclear/cytoplasm ratio >> low metabolism
• Multipotent (RBC, WBC, platelet)
• Can repopulate → gene therapy
• Some known markers (CD34+/CD38-/Lin-)
  - 1-3% are CD34+
• 1/10^5 cells in marrow (1/100 progenitor)
Examples of Stem Cells

- Hematopoietic
- Mesenchymal Stem Cells (from human bone marrow)
- Neuronal
- Liver/Skin/Gut
- Muscle (satellite cell)

Adult

- Embryonic Stem Cells - from blastocyst
- Embryonic Germ Cells - derived from primordial germ cells which will later be sperm or eggs
Mesenchymal Stem Cells
In Vitro Differentiation - Mesenchymal Stem Cells


lipid  Coll II  Alk Phos/Ca deposition
Neuronal Stem Cells

Epigenetic means
- bFGF
- EGF
- Astrocyte membranes
- Cell clusters

Genetic means
- v-myc
- SV40 T-antigen

Stem cell

Progenitor cell

Neuroblast

bFGF

Glioblast

? bFGF

Projecting neurons

Interneurons

Type 1A glioblast

Type O2A glioblast

Type 1 astrocyte

Type 2 astrocyte

Oligodendrocyte
Transit Amplifiers

- Stem cells are rare
- Stem cells are slow dividing
- Transit amplifying cells divide rapidly
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.

Morris, Nat Biotech 2004
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.

Morrison and Spradling, 2008
Dynamic Feedback

- **SF1**: Proliferation inhibitor
- **SF2**: Self-renewal inhibitor
- **SF3**: Proliferation stimulator
- **SF4**: Self-renewal stimulator

Zandstra, Mol Sys Biol, 2010
Cell Aging

Adult Somatic Cells

1) they cannot divide indefinitely in culture
   \[\therefore\ NOT\ stem\ cells\]

2) cells from older donors divide less $\Rightarrow$ why?
   
   senescence

---

Fig. 7.21. Human skin fibroblasts are influenced by donor age in their capacity for serial passage in vivo; the statistical analysis indicates that about 30% of the proliferative capacity is lost during the human lifespan, though some very old donors clearly fall into much a younger range. Age accounts for 25% of the variance. Redrawn from Martin et al., 1970.
Cell Growth: Hayflick Limit

Hayflick Limit: 50-70 divisions

Fig. 2.1. Evolution of a cell line. The vertical axis represents total cell growth (assuming no reduction at passage) for a hypothetical cell culture. Total cell number (cell yield) is represented on the Y-axis on a log scale and time in culture, on the X-axis on a linear scale. Although a continuous cell line is depicted as arising at 12½ wk it could, with different cells, arise at any time. Likewise, senescence may occur at any time, but for human diploid fibroblasts it is most likely to occur between 30 and 60 cell doublings or 10 to 20 wk, depending on the doubling time. Terms and definitions used are as in the Glossary. Transformation is explained in more detail in Chapter 15.
Cell Aging: Telomere Shortening Causes Senescence

• TTAGGG repeated hundreds of times at the end of chromosomes
• During normal DNA replication, 50-200 nucleotides fail to be synthesized
• Younger cells have longer telomeres
• “molecular clock”
• Responsible for Hayflick limit \((2^{50})\)
• Some cells have telomerase (ES, cancer, germ line)
Telomere Elongation: Telomerase
Muscle Satellite Cells - Muscle Regeneration

A

Damage → Activation → Proliferation → Differentiation → Migration → Fusion

B

Uninjured → Day 3 → Day 14

Wagers, 2008
The Aging Niche

Parabiotic Mice

- Exposure to young systemic circulation stimulates activation of muscle satellite cells and muscle regeneration
Greater specialization means less plasticity

Eg. hematopoietic stem cell does not generally turn into neuron
Embryonic Stem Cells

- Cells in Inner Cell Mass of blastocyst are pluripotent
- 1981 Evans and Kaufman showed that ES cells from early embryo could be grown in vitro for many generations
  - Required LIF (Leukemia Inhibitory Factor), lose pluripotency without it
  - Upon reimplantation into another embryo could form all embryonic tissues except trophoectoderm
- Thus ‘self-renew’ without loss in potency

In-Class
Exercise/Discussion
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
Fig. 1. Derivation of the H9 cell line. (A) Inner cell mass–derived cells attached to mouse embryonic fibroblast feeder layer after 8 days of culture, 24 hours before first dissociation. Scale bar, 100 μm. (B) H9 colony. Scale bar, 100 μm. (C) H9 cells. Scale bar, 50 μm. (D) Differentiated H9 cells, cultured for 5 days in the absence of mouse embryonic fibroblasts, but in the presence of human LIF (20 ng/ml; Sigma). Scale bar, 100 μm.
Fig. 2. Telomerase expression by human ES cell lines. MEF, irradiated mouse embryonic fibroblasts used as a feeder layer for the cells in lanes 4 to 18; 293, adenovirus-transformed kidney epithelial cell line 293; MDA, breast cancer cell line MDA; TSR8, quantitation control template. Telomerase activity was measured with the TRAPEZE Telomerase Detection Kit (Oncor, Gaithersburg, Maryland). The ES cell lines were analyzed at passages 10 to 13. About 2000 cells were assayed for each telomeric repeat amplification protocol assay, and 800 cell equivalents were loaded in each well of a 12.5% non-denaturing polyacrylamide gel. Reactions were done in triplicate with the third sample of each triplet heat inactivated for 10 to 15 min at 85°C before reaction to test for telomerase heat sensitivity (lanes 6, 9, 12, 15, 18, 21, 24, and 27). A 36-base pair internal control for amplification efficiency and quantitative analysis was run for each reaction as indicated by the arrowhead. Data were analyzed with the Storm 840 Scanner and ImageQuant package (Molecular Dynamics). Telomerase activity in the human ES cell lines ranged from 3.8 to 5.9 times that observed in the immortal human cell line MDA on a per cell basis.
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.
Fig. 4. Teratomas formed by the human ES cell lines in SCID-beige mice. Human ES cells after 4 to 5 months of culture (passages 14 to 16) from about 50% confluent six-well plates were injected into the rear leg muscles of 4-week-old male SCID-beige mice (two or more mice per cell line). Seven to eight weeks after injection, the resulting teratomas were examined histologically. (A) Gutlike structures. Cell line H9. Scale bar, 400 µm. (B) Rosettes of neural epithelium. Cell line H14. Scale bar, 200 µm. (C) Bone. Cell line H14. Scale bar, 100 µm. (D) Cartilage. Cell line H9. Scale bar, 100 µm. (E) Striated muscle. Cell line H13. Scale bar, 25 µm. (F) Tubules interspersed with structures resembling fetal glomeruli. Cell line H9. Scale bar, 100 µm.
Testing ES Potency In Vivo: Teratomas

Can be formed by subcutaneous injection of ES cells into a immune-deficient mouse
Testing ES Potency In Vivo: Teratomas

- Gut
- Bone
- Muscle

*Neural
*Cartilage

- Tumors
- Can’t control organization

Thomson et al
In-Vitro Differentiation

Chimeric Mice

(A) ES cells growing in tissue culture
- Altered version of target gene constructed by genetic engineering
- Introduce a DNA fragment containing altered gene into many cells
- Let each ES cell grow to form a colony

(B) Pregnant mouse
- Isolate early embryo
- Inject ES cells into early embryo
- Early embryo formed partly from ES cells
- Introduce early embryo into pseudopregnant mouse

ES cells with one copy of target gene replaced by mutant gene

Figure 10-36 part 1 of Essential Cell Biology 3/e (© Garland Science 2010)