Using Embryonic Stem Cells as a Model of Human Development

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Stem cells have a unique combination of properties:

1. Capacity for self renewal
2. Capacity for proliferation and differentiation
Adult Stem Cells

Self Renewal

Differentiation

Adult Stem Cells
Organs with Stem Cells in Adults

- Gut
- Liver
- Brain
- Skin, hair
- Hematopoietic Organs
- Muscles
- Male germ cells
- Pancreas (?)

Adult stem cells allow regeneration of some tissues.
Why not rely on adult stem cells for transplantation?

- Little or no self-renewal in vitro
Plasticity of Adult Stem Cells?
Stem Cell Plasticity

• The capacity of stem cells from one system to repopulate others
  – Example: Bone marrow cells giving rise to liver cells after bone marrow transplant

• Requires rigorous (clonal) analysis
  – Shows that a single cell can adopt two fates

• Stem cells can fuse with other cell types
  – Gives the illusion of differentiation to other tissues

Is this a third property of (some) adult stem cells?
The Goal

• To find a source of pluripotent stem cells that can self renew in vitro and differentiate them to many tissues.
The Promise of Stem Cell Research

Drug Development and Toxicity Tests

Experiments to Study Development and Gene Control

Cultured Pluripotent Stem Cells

Tissues/Cells for Therapy

Disease model cell lines
Study degeneration in vitro

Bone Marrow
Nerve Cells
Heart Muscle Cells
Pancreatic Islet Cells

nih.gov/stemcell
Derivation of Human Embryonic Stem Cells

1. **Cultured Blastocyst**
   - Inner cell mass
   - Outer cell mass

2. **Isolated Inner Cell Mass**
   - Cultured inner cell mass

3. **First Plating**
   - Irradiated mouse fibroblast feeder cells
   - Cultured inner cell mass dissociated
   - 9–15 days

4. **Established Cultures**
   - hES cells

5. **Second Plating to Establish Colonies**
   - ...and replated onto new feeder cells
   - 7–10 days
Criteria for hES Cell Lines

• Normal Morphology
• Can be passaged (with normal morphology) several times
• Can be frozen and thawed giving colonies with normal morphology
• Expression of Oct 4, SSEA-3, SSEA-4
• Differentiation to 3 germ layers
• Normal karyotype
Plans for hESC Derivations

Previously made lines:

- NIH Registry lines derived on mouse feeders
- New lines derived under GL conditions on human feeders

New lines at UMN:

- New lines derived from PGD embryos for disease models (trisomy 21, Down syndrome)
- New lines derived under GM conditions for human therapy
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ESC Differentiation is a model of human development

Embryo → Fetal Tissue → Adult tissue

Embryoid body formation → Insulin+ pancreas

Embryonic Stem Cell (ES cell) → Gut endoderm → Pancreas stem cell
ESC Differentiation is a model of human development
Gene expression patterns define β cell development
Pancreatic Development Markers in hESC Differentiation

Expression measured in differentiating EBs without stimulation
If we introduce reporter genes

- **insulin**
- **GFP**
- **pA**

- **pdx1**
- **EYFP**
- **pA**

ES cell → Gut endoderm → Pancreas stem cell → Insulin+ pancreas
We can monitor progress by protein expression.
What if we had PGD embryos?

Genetically normal lines

Differentiation

New lines derived from PGD embryos for disease models

What is the difference?
What if we had PGD embryos?

Genetically normal lines

Differentiation

Can we change this?

New lines derived from PGD embryos for disease models
What if we had PGD embryos?

Genetically normal lines

Aging

New lines derived from PGD embryos for disease models

Can we prevent this?
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Pluripotent Stem Cells for Transplantation

- Establish lines using good tissue practice
  - Donor testing
  - Bovine serum
  - Mouse feeders

- Test in vitro
  - Differentiate
  - Isolate cells of interest
Pluripotent Stem Cells for Transplantation

- **Test efficacy**
  - Animal disease models
  - Engraftment

- **Test Safety**
  - Primate models
  - Tumor formation
  - Viral transmission
Pluripotent Stem Cells for Transplantation

- Derive universal donor hES line
- Bank many different hES lines
- Derive hES lines for individuals (nuclear transfer)
- Induce immunological tolerance
Policy Influencing hESC Research

- Lines derived before Aug 9, 2001 are eligible for federal funding.
- Lines derived after Aug 9, 2001 must be studied without federal funding. Space, salaries, equipment and supplies must be kept separate.
- At UMN, no state funding is allowed.
- Various legislation pending that may make these rules less (or more) restrictive.