Validation of Gene Expression Signatures Employed in Directed Stem Cell Differentiation with Small Molecule Perturbagens

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Cancer Genomics
Summer Undergraduate Research Program
What is a stem cell?

- Potential to differentiate into numerous cell types
- Capacity to self-replicate indefinitely
- When in culture, can remain in an undifferentiated state for many generations
Embryonic stem cells

- Source: Inner cell mass of the pre-implantation blastocyst
  - Blastocyst: 4-5 day embryo

NIH stem cell report, 2001
Embryonic stem cells

NIH Stem Cell Report, 2001

PLURIPOTENT

Gastrula

Zygote

Blastocyst

Ectoderm (external layer)
- Skin cells of epidermis
- Neuron of brain
- Pigment cell

Mesoderm (middle layer)
- Cardiac muscle
- Skeletal muscle cells
- Tubule cell of the kidney
- Red blood cells
- Smooth muscle (in gut)

Endoderm (internal layer)
- Pancreatic cell
- Thyroid cell
- Lung cell (alveolar cell)

Germ cells
- Sperm
- Egg
Potential stem cell applications

- Monitoring developmental biology
- Genetic engineering
- Pharmaceutical testing
- Toxicology
- Therapeutic transplants
  - Chronic heart disease
  - End-stage kidney disease
  - Liver failure
  - Cancer
  - Parkinson’s disease
  - Spinal cord injury
  - Multiple sclerosis
  - Alzheimer’s disease
  - Amyotrophic lateral sclerosis
  - Diabetes
  - Skin grafts
  - Purkinje cell degeneration
  - Duchenne’s muscular dystrophy
  - Osteogenesis imperfecta

NIH stem cell report, 2001
Directed differentiation

Using laboratory techniques:

Unspecialized cell

Small molecule perturbagen

Specialized cell

[determined by gene expression signatures, i.e. expression of marker genes]

http://www.stemcellresearchfoundation.org/index.htm
Microarray Gene Expression Profiling of Embryonic Stem Cells and Dissected Embryonic Tissues for Marker Selection
<table>
<thead>
<tr>
<th>Gene Expression Signatures</th>
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<tbody>
<tr>
<td><strong>Embryonic</strong></td>
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<tr>
<td>Stem cell</td>
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<tr>
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<td>Foxa2</td>
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Project Overview

GOAL: Validate gene signatures in tissue culture

1. Start and maintain a mouse embryonic stem cell (mES) line

2. Treat the mES with the chosen compounds, at various dilutions, for 4 days

3. Determine if differentiation occurred by comparing gene expression signatures
### Treatments to be tested for differentiation

<table>
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<tr>
<th>Treatment</th>
<th>Hypothesized Target Cell</th>
<th>Germ Layer</th>
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<tbody>
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<td>Cardiomyocytes</td>
<td>Mesoderm</td>
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<td>Fluoxetine Hydrochloride</td>
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<td>Mitomycin C</td>
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<td>Reversine</td>
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<td>Mesoderm</td>
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<td>Scriptaid</td>
<td>Induces hemoglobin</td>
<td>Mesoderm</td>
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### DILUTIONS:

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<tr>
<td>H</td>
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</table>
Ligation-mediated amplification (LMA) & Gene expression-based highthroughput (GE-HTS) experimental overview

Treated cells

RT

ligation

Marker | Expression
--- | ---
1 | probe specific tag
2 | primer site
3 | probe specific tag

Courtesy of Cristian Jitianu
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- Cristian Jitianu
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- Bruce Birren