Lecture #12
Transplantation & Drug Screening

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http://courses.engr.illinois.edu/bioe498gu/
Case Study: Duchenne Muscular Dystrophy

Van Deutekomr, Nat Rev Genet, 2003

www.vetmed.vt.edu
Gene Therapy Using iPS Cells

- iPS reprogramming of knockout mouse cells
- Introduce utrophin gene (gene correction)
- Directed differentiation with Dox-inducible Pax3

Filareto et al., Nature, 2013
Gene Therapy Using iPS Cells

Filareto et al., Nature, 2013
Gene Therapy Using iPS Cells

f  Corrected myogenic precursors  Corrected myotubes

g  μUTRN  μUTRN DAPI  μUTRN  μUTRN DAPI

Filareto et al., Nature, 2013
Transplantation Back into Animal Model

- Expression of utrophin
- Differentiation of progenitor cells in vivo
- No tumor formation

Filareto et al., Nature, 2013
Replenishment of Satellite Cell Compartment

Filareto et al., Nature, 2013
Could the Pax3 directed differentiation step pose a problem for further translation?

Are there alternative ways to enhance the efficiency of engraftment, and of differentiation?

Screening of factors (small molecule drugs)
Zebrafish...Mouse...Human

myf5-GFP; mylz2-mCherry
zebrafish embryos

Disassociate embryos

Aliquot to chemical plate

Mouse
satellite cells

forskolin

Ex vivo expansion

Mouse

Identify promyogenic
chemicals

Human
IPSCs

Human

BIO + forskolin

Skeletal muscle differentiation

Xu et al., Cell, 2013
Zebrafish with Fluorescent Reporters for Skeletal Muscle

<table>
<thead>
<tr>
<th>Intact Zebrafish (In Vivo)</th>
<th>Embryonic Tissues (Ex Vivo)</th>
</tr>
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<tbody>
<tr>
<td><strong>A</strong></td>
<td><strong>B</strong></td>
</tr>
<tr>
<td><em>myf5-GFP</em></td>
<td><em>myf5-GFP</em></td>
</tr>
<tr>
<td><em>mylz2-mCherry</em></td>
<td><em>mylz2-mCherry</em></td>
</tr>
<tr>
<td>bright field</td>
<td>bright field</td>
</tr>
</tbody>
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11ss  
32 hpf  
48 hpf

Xu et al., Cell, 2013
High-Throughput Screen

A
myf5-GFP; mylz2-mCherry embryos at the oblong stage → Disassociated blastomere cells → Aliquot cells to the chemical plate → Image by Celigo cytometer

B

<table>
<thead>
<tr>
<th>Category I</th>
<th>Category II</th>
<th>Category III</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>Tyrphostin AG 679</td>
<td>cAMP activator</td>
</tr>
<tr>
<td>TCPOBOP</td>
<td>Ro 41-1049</td>
<td>forskolin</td>
</tr>
<tr>
<td>CA-074-Me</td>
<td>SB 415286</td>
<td>E-64-D</td>
</tr>
</tbody>
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C

<table>
<thead>
<tr>
<th>GSK3 inhibitor</th>
<th>calpain inhibitor</th>
</tr>
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<tbody>
<tr>
<td>DMSO</td>
<td>forskolin</td>
</tr>
<tr>
<td>Kenpaullone</td>
<td>E-64-D</td>
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<tr>
<td>SB 415286</td>
<td>MDL28170</td>
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<tr>
<td>SB 216783</td>
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</tbody>
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Xu et al., Cell, 2013
Induction of Satellite Cell Proliferation

A

Freshly isolated satellite cells  Cultured DMSO-treated  Cultured forskolin treated

Beta 1 integrin

CXCR4

C

Satellite cell isolation  IM Transplantation  CDTX

β-actin GFP mouse

Culture for 5 days
Forskolin/DMSO treatment

E

6000 Freshly isolated satellite cells  Cultured DMSO-treated cells (expanded from 6000 cells)  Cultured forskolin-treated cells (expanded from 6000 cells)

G

Number of GFP+ fibers per cross-section

P = 0.0002

P = 0.001

P = 0.007

Fresh  DMSO  Forskolin

Xu et al., Cell, 2013
Cocktail of (i) forskolin, (ii) GSK3 inhibitor (BIO), and (iii) bFGF increases muscle differentiation of iPS cells

Xu et al., Cell, 2013
Improved Differentiation of Pluripotent Stem Cells Towards Muscle Lineage-Engraftment In Vivo

Xu et al., Cell, 2013
Methods for Expansion and Differentiation

No Genetic Manipulation

myf5-GFP; mylz2-mCherry
zebrafish embryos

Disassociate embryos

Aliquot to chemical plate

Mouse satellite cells

Ex vivo expansion

Identify promyogenic chemicals

Mouse

forskolin

BIO + forskolin + bFGF

Ex vivo expansion

Skeletal muscle differentiation

Human IPSCs

Xu et al., Cell, 2013
Stem Cell Companies

- Cellular Dynamics: [https://cellulardynamics.com/](https://cellulardynamics.com/)
- Fate Therapeutics: [http://fatetherapeutics.com/](http://fatetherapeutics.com/)
- Excellthera: [http://excellthera.ca/](http://excellthera.ca/)
- Stemgent: [https://www.stemgent.com/](https://www.stemgent.com/)
Midterm #2- Tuesday 4/18- Class Period

Review Session: Thursday 4/13 6pm (Tonight), DCL 1265
Things to Review

- Approximately 1/3 multiple choice, 2/3 short answer
- ~5 main topics (lecture + paper discussion)
- 2 Homeworks
- 5 paper presentations
- In-class exercises/discussions (e.g. questions I posed during lecture period)

Topics Areas of Particular Emphasis:
- Role of stem cell niches in stem/progenitor expansion & differentiation
- Types of microenvironmental signals, methods for systematically testing signals
- Approaches for optimizing stem cell protocols
- Methods for testing effects of mechanical forces on stem cells
- Mechanisms of mechanical sensing in stem cells
- Examples of microfabrication and high-throughput technologies
- Challenges related to scale-up, and commonly used strategies
- Overall design of transplantation and drug screening approaches