Lecture #13
Transplantation & Drug Screening

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http://courses.engr.illinois.edu/bioe498gu/
The Promise of Stem Cell Research

Identify drug targets and test potential therapeutics

Toxicity testing

Understanding prevention and treatment of birth defects

Study cell differentiation

Ectoderm
Neuron
Blood cells

Mesoderm
Liver cell

Endoderm

Tissues/Cells for Transplantation
Case Study: Duchenne Muscular Dystrophy

Dystrophin

Actin-binding region

α helix

Linking proteins

Actin filaments

Extracellular matrix

Dystrophin-associated glycoprotein complex

Sarcolemma

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

Identify promyogenic chemicals

Mouse satellite cells

forskolin

Ex vivo expansion

Human IPSCs

BIO + forskolin

Skeletal muscle differentiation

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

Intact Zebrafish (In Vivo)

A

myf5-GFP

mylz2-mCherry

bright field

11ss

32 hpf

48 hpf

Embryonic Tissues (Ex Vivo)

B

myf5-GFP

mylz2-mCherry

bright field

basic media

basic media + bFGF

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
Category I

DMSO
TCPOBOP
CA-074-Me

Category II

Tyrphostin AG 879
Ro 41-1049
SB 415286

Category III

cAMP activator

GSK3 inhibitor

DMSO
kenpaullone
SB 415286
SB 216763

C

cAMP activator

forskolin
E-64-D
MDL28170

calpain inhibitor
Induction of Satellite Cell Proliferation

A

Freshly isolated satellite cells
Cultured DMSO-treated
Cultured forskolin-treated

Beta-1 integrin
CXCR4

C

Satellite cell isolation
β-acin GFP mouse

IM Transplantation
mIX 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

Fresh DMSO Forskolin

P = 0.0002
P = 0.001
P = 0.007

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

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3 iPS Lines

Negative Controls

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

myf5-GFP; mylz2-mCherry zebrafish embryos

Disassociate embryos

Aliquot to chemical plate

Identify promyogenic chemicals

Mouse satellite cells

Mouse

forskolin

Ex vivo expansion

BIO + forskolin + bFGF

Skeletal muscle differentiation

No Genetic Manipulation

Xu et al., Cell, 2013
Fate Therapeutics

http://fatetherapeautics.com/
Group Project

• Groups (4-5 students) will select a topic from the list given and write a 7-10 page paper that addresses a current limitation (either basic science or translational) in the stem cell field.

• Presentation in class

• Written paper will include the following sections (1.5 line spacing, 1 in margins):
  - Background
  - Advances & Limitations (description of specific research paper)
  - Project Plan Overview
  - Experimental Design/Methods
  - Expected Results & Next Steps
  + References (not counted in 7-10 pages)
  + Description of group member contributions
Presentations

• ~18 min presentation (~15 slides)
  – Contribution from each group member
  – 6 min questions/discussion
  – Powerpoint format

  – Title Slide
  – Background (2 slides)
  – Advances & Limitations- Example Paper (4 slides)
  – Project Plan Overview (1 slide)
  – Experimental Design/Methods (4 slides)
  – Expected Results & Next Steps (3 slides)
  – Summary and Conclusion (1 slide)
Dates

• Project Presentations (4/27, 5/2, 5/4- in class)
  Refer to syllabus for day/order
  Peer feedback***
• Paper Due (5/12, 5pm)

***Attendance required at presentations
Midterm #2- Wednesday 4/20