Using Stem Cells to Study the Developmental Biology of Disease: Case Studies in ALS

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Amyotrophic Lateral Sclerosis (ALS)

- Fatal neural-degenerative disease affecting 6 in 100,000.
- Gradual and progressive paralysis culminating in loss of respiratory function.
- Average onset is between 40 and 70 years of age.
- Average survival 3-5 years.
- Most cases are sporadic, 10% are familial.
- 2% of cases are caused by dominant mutations in SOD1.
- No efficacious drugs.

Lou Gehrig's Disease
ALS caused by death of motor neurons
ALS caused by death of motor neurons

But why do they die?

(How do we stop it?)
It’s Complicated!

Activated microglia

Activated astrocyte

Propagation of disease

Diffusion or secretion of toxic factors


Bax

Other degenerative damage

Glutamatergic neuron

Motor neuron

Excitotoxicity

Glu

EAAT2

Intrinsic damage: mitochondria, axonal transport, ER stress, proteasome, etc.

Muscle: no contribution to motor neuron death
Challenges in studying ALS

• Disease-processes are well underway at the time of diagnosis.

• It has not been possible to isolate the affected cell-type for study.

• Although familial forms of disease have allowed animal modeling, it's unclear whether sporadic disease mechanisms are similar.
Challenges in studying ALS

• Disease-processes are well underway at the time of diagnosis.

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• Stem cells and reprogramming provide a potential solution…
Analysis of Diseased (ALS) Human ES Cells

ALS hES cells

Directed differentiation protocols

Motor neurons

Neuronal survival

Cell-autonomy

Target Validation

Control hES cells
Use of Diseased (ALS) Human ES Cells

ALS hES cells

Differentiation

Chemical screening

Control hES cells

Drugs that slow or prevent neural degeneration
Solving the material problem: Directed differentiation of motor neurons from stem cells
Development of Spinal Motor Neurons

Briscoe 2007
Development of Spinal Motor Neurons

Briscoe 2007

Tanabe 1998

Briscoe 2007

Tanabe 1998

Pax6+ Progenitors

Post-Mitotic Neurons

Shh

[low] → MNR2 → Isl1 → HB9

[low] → Lim3 → Chx10 → V2 Interneuron

[low] → MNR2 → Isl2 → Somatic MN

[low] → Lim3 → IsI1

Shh

BMP

RA
Development of Spinal Motor Neurons

Briscoe 2007

Pax6+ Progenitors

Post-Mitotic Neurons

Lupo 2006

Tanabe 1998

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Tanabe 1998

Briscoe 2007 Lupo 2006

Tanabe 1998
Motor neuron directed differentiation
Human Embryoid Bodies Induced for Motor Neurons
HuES Derived Motor Neurons

ISL β-TUBULIN III DNA
Making Disease Specific Stem Cells
Making Disease Specific Stem Cells

• Generate stem cells from existing animal models.

• Genetically manipulate existing human ES cell lines.

• Generate stem cells from “diseased” embryos.

• Reprogram patient cells into stem cells.
ALS studies using mouse ES cells

•Produced Mouse ES cells from the SOD1 mouse model and differentiated them into motor neurons.

SOD1 Transgenic ALS mouse model
ALS studies using mouse ES cells

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E10.5 Hb9::GFP

SOD1 Transgenic
ALS mouse model

Transgenic

GFP
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• The ALS genotype had a time dependent affect on motor neuron number.
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- Cell autonomous effect: inclusion formation.
- Non-cell autonomous effect: glial toxicity.
ALS studies using human ES cells

• Modified existing HUES lines to report on motor neuron differentiation.
• Asked whether human motor neurons are selectively sensitive to the toxic effect of mutant glia.
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Inter-neurons are NOT sensitive to mutant glia
A novel therapeutic target for ALS
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Genes up-regulated in mutant SOD1 glia

Many involved in inflammatory responses
PGD2 induces motor neuron death in the presence of wt glia.
Effect of prostaglandin receptor antagonists on motor neuron survival

BAY-u3405
Antagonist of DP2

GFP+ve hMNs (%)

DMSO (0.1%) BAYu3405 DP2 Antagonist 10 days

10 days
Effect of prostaglandin receptor antagonists on motor neuron survival

Effect of prostaglandin receptor antagonists on motor neuron survival.

- **BAY-u3405**: Antagonist of DP2
- **BW868C**: Antagonist of DP1

**Graphs:**
- **GFP+ve hMNs (%)**
  - wT Glia vs. SOD1G93A Glia
  - DMSO (0.1%)
  - BAYu3405 DP2 Antagonist
  - 10 days

- **GFP+ve hMNs (%)**
  - wT Glia vs. SOD1G93A Glia
  - DMSO (1%)
  - BWA868C DP1 Antagonist
  - 10 days
Are these things true of patient genotypes?

• Mouse models have super-physiological expression of the mutant protein.

• No way to model sporadic forms of disease in which genetic determinants are unknown.
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• Reprogramming approaches provide a potential solution…
Combining Cloning and Stem Cell Potential

Patient → Biopsy

Genetically matched differentiated cells → Nuclear transfer

In vitro model → Patient-specific ES cells
Human Induced Pluripotent Stem Cells

KLF4, SOX2, OCT4, cMYC

Fibroblast → iPS Cell

YAMANAKA-EERING!
Translating findings into real patient genotypes: Production of patient specific iPS cells
Patient Fibroblasts 3 Weeks Post-Transduction

Hundreds to thousands of rapidly growing colonies appear
Are these cells derived from patient fibroblasts?

SOD1 Genotyping

hSOD1

L144F

SOD1 PCR

hSOD1 83 54 28 182

*
Are these cells derived from patient fibroblasts?

Sequencing, Restriction Polymorphism Confirm cell lines are from patients.
A bank of stem cells for studying ALS

• 51 IPS cell lines from 15 individuals: 5 healthy controls, 6 SOD1 cases (D90A, G85R, L144F), 4 sporadic ALS cases.

• Majority of these cell lines have been made without the use of cMYC.
ALS iPS cells can be directed down the spinal motor neuron and glial lineages.
Future Directions

• Determine whether or not patient-derived glia are toxic to motor neurons.

• Investigate cell autonomous characteristics patient-derived human motor neurons.

• Further characterize ramifications of prostaglandin findings in vitro and in vivo.