Neural Stem Cells
Gene Therapy
Neurodegenerative Disorders

Schaffer Lab
Chemical Engineering
U.C. Berkeley
Neuronal Protection and Replacement

- Gene delivery for control of adult stem cells
- Gene therapy for neuroprotection: ALS and spinocerebellar ataxia
Neuronal Protection and Replacement

- Gene delivery for control of adult stem cells
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Stem Cell Therapy: In Vitro & In Vivo

Harvest, Grow, and Reimplant
Stem Cell Therapy: In Vitro & In Vivo

Direct Control in the Nervous System by Delivery of Drugs or Genes
Underlying Challenge: How to Control the Stem Cells
Underlying Challenge: Cellular Control

Signals(t)
Adult Neural Stem Cells

- Shown very recently to exist throughout the nervous system

- Significant potential for neural regeneration

- Requires better understanding of signaling mechanisms that regulate these cells’ function
1) Site of active adult neural stem cells
2) Site affected by Alzheimer’s Disease
Propagation of Immature Neural Stem Cells

Blue - nucleus
Green - nestin
Differentiation of Stem Cells into Glia

Blue - nucleus
Green - MBP
Red - GFAP
Differentiation into Neurons

Blue - nucleus

Green - NF200
Goal: Control Neural Stem Cell Behavior

Stem Cell → Progenitor Cell

? → Glia

? → Neurons

Stem Cell → ?
Goal: Control Neural Stem Cell Behavior

Sonic Hedgehog

Stem Cell → Progenitor Cell

Progenitor Cell → Neurons

Progenitor Cell → Glia

?
Cell Behavior is Regulated by External Environmental Signals

Ligands

Receptors

Changes in Gene Expression Patterns

Changes in Cell Behavior
(proliferation, migration, differentiation)
Adult Neural Stem Cells Express Ptc

Blue - nucleus
Green - nestin
Red - Shh receptor (Ptc)
Shh Stimulates Neural Progenitor Proliferation

Relative Cell Number

Day

0 nM
0.25 nM
0.5 nM
1 nM
2.5 nM
5 nM
10 nM
25 nM
50 nM
Adeno-associated Viral Gene Delivery Vehicles

Advantages:
+ Extremely safe
+ Highly efficient
+ Very stable expression
Adeno-associated Viral Gene Delivery Vehicles

Green Fluorescent Protein
AAV-GFP Delivery to the Brain after 1 year

Hippocampus 1 Year

after 1 year
Adeno-associated Viral Gene Delivery Vehicles

Sonic Hedgehog
Adult Neural Stem Cell Proliferation: Control

Green - neurons (NeuN)

Red - mitotic cells (BrdU)
Adult Neural Stem Cell Proliferation: AAV-Shh

Green - neurons (NeuN)
Red - mitotic cells (BrdU)
Shh Triples Neural Stem Cell Proliferation

#BrdU+ cells/section

- AAV-GFP
- AAV-Shh
Shh Triples New Neurons

Neuronal Protection and Replacement

- Gene delivery for control of adult stem cells

- Gene delivery for neuroprotection: 
  ALS and spinocerebellar ataxia
Gene Therapy: Concept and Current Status

Definition:
the delivery of genetic material to an individual’s cells for therapeutic benefit

Recent success:
Hemophilia B using AAV (Avigen)
Heart disease using adenovirus (Coll.Thx.)
Cancer using adenovirus (Onyx)

Challenge:
Need better gene delivery technology
Adeno-associated Viral Gene Delivery Vehicles

Green Fluorescent Protein
AAV Neuroprotection in the Spinal Cord

Fred Gage Lab, Salk Institute
Amyotrophic Lateral Sclerosis

- Progressive disease that selectively kills spinal cord motor neurons (Lou Gherig’s Disease, Stephen Hawkins)

- Fatal within 1-5 years of onset

- Prevalence of 2-3 per 100,000 people

- Causes of disease remain unknown

- 5-10% of cases, inherited in a dominant manner [SOD-1 mutations (Superoxide dismutase-1) 90 mutations known]
AAV Gene Therapy for ALS

Neuroprotective Factor IGF-I
Gene Delivery Protects Motor Neurons

Gene Therapy Significantly Delays Symptoms

Neuronal Protection and Replacement

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Challenge for Dominant Disorders: Blocking Defective Genes

RNA interference can degrade mutated RNA sequences
RNA Interference to Knock Down Mutant Ataxin

Gene delivery can reduce mutant Ataxin expression in cell culture

Miller et al., PNAS (2003)
Gene delivery can be used to control adult neural stem cells in the nervous system for neuron replacement.

Gene therapy can be used to deliver genes for general neuroprotection.

Targeted degradation of defective mRNA.

Animal models of ataxia.

Combining stem cell therapy and gene therapy may prove a powerful approach.
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