Cellular Therapies for Neurological Disorders: Repair Shop or Drug Store?

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Molecular and Cellular Mechanisms involved in CNS repair

• **Brain Plasticity** - Axonal branching and synaptogenesis from recruitment of “non-damaged” neuronal pathways (e.g. cortical maps)

• **Inflammation Driven Processes** - Shift in mode from tissue damaging events to tissue repair events: neurotrophic support from inflammatory cells

• **Exogenous application or Endogenous Activation of Adult Neural Stem/Precursor Cells** - Cell replacement, remyelination, neuroprotection, angiogenesis
Cell-Based Therapies for Spinal Cord Injury

- Development of Appropriate Cell Type (e.g. ES cells, iPS cells, human bone marrow MSCs, adult neuroprogenitor or committed cells such as Schwann cells or olfactory ensheathing cells?)

- Cell Delivery Route (direct, intraventricular, intravenous)

- Better Understanding of Neurotrophic Factor Secretion and Induction by Endogenous Upregulation by Transplanted Cells

- Clear Demonstration of Structural Changes Associated with Functional Recovery after Cell Transplantation (sprouting, new circuit formation, remyelination, reduction of neuronal loss, inflammation and secondary cell death etc.)

- More Clear Understanding of Homing Mechanisms after Systemic Cell Delivery (Constitutive cell expression of cell adhesion molecules (CAMs) such as CD44, alpha integrins and functional chemokine receptors (e.g. CCR1-5, CXCR4) which are used by immune cells to invade inflamed CNS.

- Safety Issues
Cellular Transplantation as a Tool for Spinal Cord Repair

Repair Objectives:

- Remyelination
- Axon Regeneration/Sprouting
- Neuroprotection
- Neovascularization/microvessel repair
Ethidium Bromide

Cell Injection

X-Irradiation

+ 3 Days

NG2 CONT

NG2 X-RT
Myelin-forming Cells Transplanted into the Demyelinated Spinal Cord leads to Extensive Remyelination

Sasaki et al., J. Neurosci 2004
Human Schwann cell transplants into spinal cord

Evans blue Extravasation in the Spinal Cord in a rat with Experimental Autoimmune Encephalomyelitis (EAE)
MOG/IFA+VEGF injection at 1wk

Scale bars = 100mm (A-F), 4mm (I), 2mm (G, H)
Endogenous Remyelination after Focal EAE Induction (3 weeks post-lesion)
Cellular Transplantaton as a Tool for Spinal Cord Repair

Repair Objectives:

• Remyelination

• Axonal Regeneration/Sprouting

• Neuroprotection
One month later

A

Surgical cut

Degenerating nerve fiber

Nerve cell body in sensory ganglion

B

One month later

Pig olfactory ensheathing cells

Bipolar electrode

Regenerated nerve fiber
Olfactory ensheathing cells (OECs)

Special features of OECs:
- Localization in PNS and CNS
- Morphology *in situ*
- Association with ORN axons known to enter the CNS
- Gene expression *in vitro* (array studies) but no..

Common features of OECs and Schwann cells:
- ...cell type-specific marker
- Neural crest origin
- Morphology *in vitro*
- Growth factor responsiveness *in vitro*
- Regenerative effects after transplantation

OEC

Vincent et al. (2005)

Field et al. (2003)
OECs transplanted into transected spinal cord

Sasaki et al. 2006, J. Neurosci
OECs Form Cellular “Tunnels” and Peripheral Myelin in the Dorsal Hemi-transected Spinal Cord

A

Rostral

R13

14 mm

R2

3 mm

2 mm

Stim

Transection site

Transplant sites

Caudal

R1

L4

L5

B

Transection alone

R1

2 mm

R2

3 mm

0.5 mV

1 msec

C

Transection with OEC transplant

R1

2 mm

R2

3 mm

R3

4 mm

R4

5 mm

0.2 mV

1 msec
OECs Transplanted into Transected Spinal Cord Form Cellular Bridges through which Axons Can Regenerate Across the Lesion
Unique Migratory Properties of OECs as compared to Schwann Cells
Cells transplanted *before* spinal cord injury can mobilize and repair *after* injury: Implications for pre-treatment of progressive neural degenerative diseases.

1. X-RT at day 0
2. OECs injected at Day 7,
3. Demyelinated lesion induced at Day 21,
4. Histology at 1 month later

Sasaki et al., GLIA 2010
Malformed fetuses of cynomolgus monkeys exposed to thalidomide on days 26–28 of gestation.

(A) The fetus of maternal monkey given thalidomide at 15 mg/kg-d exhibiting brachydactyly in the paw, micromelia in the hindlimb, hyperflexion, ectrodactyly and brachydactyly in the foot and curled tail. (B) The fetus of maternal monkey given thalidomide at 20 mg/kg-d exhibiting amelia in the fore- and hindlimb and bent tail. Emma et al. Reproductive Toxicology; 2010 b
NHP surgery in VA facility
Kocsis and Sasaki
ENDOGENOUS REMYELINATION IS DELAYED IN THE NHP AS COMPARED TO THE RAT

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<th>Monkey</th>
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<td>EB after 3wk</td>
<td>EB after 6mon</td>
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OECs from Transgenic Pigs Expressing human 1,2-fucosyltransferase gene Remyelinate Demyelinated NHP Spinal Cord Axons

Radtke et al
FASEB (2004)
Transplantation of Human Embryonic Stem Cell Derived Oligodendrocyte Progenitor cells (OPCs) into the Demyelinated NHP Spinal Cord
hESC-Derived Cellular Therapies

• Derived by Dr. James Thomson at University of Wisconsin in 1998
  From excess IVF blastocyst in accordance with ethical guidelines
    ▪ Informed consent
    ▪ Anonymous donors

• Immortal: Repeated derivations not required

• Pluripotent: Able to form all somatic cell types

• Suitable for clinical use:
  NIH and MRC approved
  Fully qualified for human use
GRNOPC1

- Cryopreserved Allogeneic Cell Population
- Differentiated from Human Embryonic Stem Cells
- Characterized Composition of Oligodendrocyte Progenitor Cells

Stages of GRNOPC1 differentiation

Progenitor population

In vitro differentiation
Human ES-derived GRNOPC1 Remyelinate the NHP Spinal cord
Cellular Transplantation as a Tool for Spinal Cord Repair

Repair Objectives:

- Axonal Regeneration
- Remyelination
- Neuroprotection
- Neovascularization/microvessel repair
PKH26-labeled hMSCs and BDNF-hMSCs \textit{in vitro}

Immunohistochemical Characterization of hMSCs *in vivo*
Intravenous Infusion of MSCs Reduce SCI Lesion Volume

Osaka et al., 2010 BR
Intravenous infusion of MSCs improves motor function after contusive SCI
The Blood Spinal Cord Barrier Opens After SCI and Remains Open for Months

1 mo post-SCI

3 mo post-SCI
Intravenous delivery of human Mesenchymal Stem Cells (hMSCs) reduces lesion volume in a rat stroke model (MCAo)
Occlusion of the MCA (M1) in the NHP as a Model for Stroke

Sasaki et al. PloS 2011
ahMSC Therapy for Stroke Patients

Aspiration → IV injection → Clinical evaluation

Cell Processing Center
- Selection
- Expansion for 2 weeks
- Culture
- Safety testing
- Storage
- Cryopreserved
- Thawed

Trends in Molecular Medicine Honmou et al., 2012
Case 6

Honmou et al. BRAIN. 2011 1341;790-807
POSSIBLE MECHANISMS FOR FUNCTIONAL IMPROVEMENT IN SCI FOLLOWING CELL TRANSPLANTATION

- Directed axonal regeneration
- Axonal sprouting and synaptic plasticity (polysynaptic?)
- Remyelination of regenerated and spared axons
- Neuroprotection
  - Local (spared axons and neurons)
  - Remote (preservation of projection neurons)
- Angiogenesis (Neovascularization)
- Blood brain (SC) barrier stabilization
Yale University and VA (Neurology and Neurobiology)

Masanori Sasaki, MD, PhD (PVA awardee)
Karen Lankford, PhD
Christine Radtke MD, PhD (University of Hannover)
Edgardo Arroyo, Ph.D.
Kewei Yu, MD, PhD
Hajime Tokuno, MD
Eleni Makakis, PhD
Robert Brown, MD
Takashi Mutsushita, MD

Sapporo Medical University Collaboration
Osamu Honmou, MD, PhD and many colleagues
Spinal cord hemi-section at C6 in the nonhuman primate to study the potential therapeutic effects of intravenous MSCs

• **Kocsis-video-NHP.wmv**
5-HT staining for Serotonergic fibers

Sasaki et al. J Neurosci, 2009