Dr. Charles H. Tator graduated from the faculty of Medicine at the University of Toronto, and trained there in research and neuropathology for which he received MA and PHD degrees. He then completed the Neurosurgery resident training program at the University of Toronto. In 1989, he became Chair of Neurosurgery, at the University of Toronto and Chief of Neurosurgery at the Toronto Western Hospital, and University Health Network. He has trained a large number of neurosurgical residents in the hospital, and many surgeon-scientists in his laboratory. In 1992, he founded ThinkFirst, Canada, a national brain and spinal cord injury foundation aimed at reducing the incidence of catastrophic brain and spinal cord injuries. He has published 321 papers in peer review journals and 85 book chapters, mostly in the field of brain and spinal cord injury. He developed the first acute spinal cord injury (SCI) unit in Canada in 1974 and has performed research on the epidemiology, prevention and treatment of acute SCI. He has examined the role of spinal cord surgery and decompression in both clinical and experimental studies, and identified posttraumatic ischemia and other mechanisms of secondary injury in the pathophysiology of SCI. His acute cord clip compression model was one of the first clinically relevant SCI model in rodents. Currently, he is focused on the use of stem cells for regeneration of the spinal cord after trauma, ischemic or demyelinating disease. He initiated and has held two research chairs at the University of Toronto, the Dan Family Chair in Neurosurgery and the Campeau Family-Charles Tator Chair in Brain and Spinal Cord Research. He is a member of the Order of Canada, and an inductee into the Canadian Medical Hall of Fame. At present, he is a Senior Scientist in the Toronto Western Research Institute and a Professor of Neurosurgery at the University of Toronto. He is the Director of the Canadian Paraplegic Association Spinal Cord Injury Research Laboratory in the Krembil Neuroscience Centre at the Toronto Western Hospital. In 2010, he received the Lifetime Achievement Award, of the Canadian Neurosurgical Society and the Ken Langford Lifetime Award, of the Canadian Paraplegic Association. In 2011, he received the Lifetime Achievement Award of the American Spinal Injury Association, and in 2012 he received the Reeve-Irvine Award for spinal cord injury research.

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Stem Cell Transplantation for Spinal Cord Injury

Charles H. Tator
University of Toronto and Toronto Western Hospital

Back to School
Managing Your Injury to Independence
CPA and Thomson Rogers
Toronto
Sept. 13, 2012
Historical Perspective: It Has Been Extremely Difficult to Develop Effective Strategies to Improve Recovery after SCI because…..

• There are many primary and secondary injury mechanisms that need to be treated
• The loss of tissue is usually extensive, and thus tissue regeneration will be required
• The regeneration of axons will have to extend for long distances to make reconnections
• The axons will have to reach precise targets
• There are serious inhibitory factors eg scar tissue and myelin based inhibitors

Major Questions Requiring Answers-WHICH CELLS????

Neural or Non-Neural Cells which are better for transplantation?

Endogenous vs. Exogenous (transplanted) Neural Stem Cells? - which source is better?

Developmental Age of the Transplanted Cells - Embryonic, Fetal, Neonatal, or Adult?-which is better?

If adult cells, what is best source of cells? Spinal Cord or Brain?

Answer: We do not know, and we need to continue to examine many types of cells in experimental studies and clinical trials.

We prefer ADULT SPINAL CORD DERIVED TRANSPLANTED NEURAL STEM/PROGENITOR CELLS (NSPC)
Neural Stem Cells are present in the Brain and Spinal Cord

- can self-renew and are multipotential for neurons and glia
- In the brain and spinal cord, multipotential stem/progenitor cells reside in the ependymal region around the ventricles and central canal of the spinal cord,
- In amphibians, these cells proliferate and differentiate into neurons and glia to regenerate the injured cord
- In mammals, these cells proliferate in response to injury but have limited regenerative ability
TWO Categories of Strategies

#1 Endogenous Neural Stem Cells.
Stimulate them with growth factors or other agents to regenerate the damaged spinal cord

#2 Transplanted Neural Stem Cells.
Harvest them, grow them in culture and then transplant them into the injured cord
Two Potential Strategies Involving Neural Stem/Progenitor Cells for Repair of the Injured Spinal Cord in Patients

1. **Manipulation** of **Endogenous** stem cells.
   That is those already present in the spinal cord

2. **Transplantation** of **Exogenous** stem cells
   harvested from somewhere else in the body or from another person or species

Rat Clip Compression Injury - 1 Hour

Several Rat Models Produce Injuries Similar to Humans
Stem/Progenitor Cells in the Brain and Spinal Cord

- Neural stem/progenitor cells can self-renew and are multipotential for neurons and glia
- In the spinal cord, stem/progenitor cells reside in the ependymal region around the central canal
- In amphibians, these cells proliferate and differentiate into neurons and glia to regenerate the injured cord
- In mammals, these cells proliferate in response to injury but have limited regenerative ability
- Would increasing the number of stem cells by stimulation or transplantation improve regeneration?
Ependymal Cells in the Adult Mammalian Spinal Cord

1. Normal ependymal cells - proliferate with a labelling index of 1-2%.

2. After injury ependymal cells proliferate, migrate, and act as stem cells.
SCI Causes Marked Proliferation of Stem/Progenitor Cells

Double Label (BrdU/Nestin)

Stem/Progenitor Expansion
Transplanted Stem Cells are better than Endogenous Stem Cells

- We spent 6 years, from 1998-2004 trying to encourage endogenous NSPCs to repair the spinal cord, but achieved minimal success. To do this we had to massively stimulate the injured spinal cord with mitogens and growth factors (EGF and FGF2).
- We got minimal functional recovery and tissue repair.
- We were concerned about the growth factors causing tumor formation.

TRANSPANTATION TRIALS and “EXPERIMENTS” IN HUMANS with SCI

- Many recent trials in patients, mostly Phase 1 uncontrolled, small number of patients, many without published reports.
- Many non-trial “Experiments” in humans.
- “Stem Cell Tourism”
- Many types of cells have been transplanted into humans with SCI in several countries.
## Transplantation Trials in Humans with SCI – Recent All Phase 1 and many not reported

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Name of Study, Authors and Reference</th>
<th>Countries</th>
<th>Year of Published Report if any</th>
<th>No. of Patients</th>
<th>Neurologic Result</th>
<th>Other Results and Comments</th>
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<tbody>
<tr>
<td>Fetal Porcine Stem Cells – into cord</td>
<td>Dream Study (80)</td>
<td>USA</td>
<td>2007</td>
<td>26</td>
<td>初步</td>
<td>继续招募</td>
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<tr>
<td>Autologous Activated Macrophages – into cord</td>
<td>Perot Study</td>
<td>Israel</td>
<td>2005</td>
<td>6</td>
<td>改善</td>
<td>第二阶段试验进展2006</td>
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<tr>
<td>Peripheral Nerve Transplants</td>
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<td></td>
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<tr>
<td>1. Peripheral Nerve Grafts – cord to cord</td>
<td>Cheng</td>
<td>Taiwan</td>
<td>2004</td>
<td>1</td>
<td>改善</td>
<td>继续招募</td>
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<tr>
<td>2. Peripheral Nerve Grafts – cord to cord</td>
<td>Barros</td>
<td>Brazil</td>
<td>2003</td>
<td>8</td>
<td>无改善</td>
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<td>3. Peripheral Nerve Grafts – cord to nerve</td>
<td>Bonelli</td>
<td>Italy</td>
<td>2003, 2008</td>
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## Transplantation Trials in Humans with SCI – Cont’d

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<tr>
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<th>Other Results and Comments</th>
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<tbody>
<tr>
<td>Human Ectopic Ensheathing Cells (fetal and adult) – into cord</td>
<td>Huang et al, Force et al, Babcock et al (160)</td>
<td>China, Australia, Russia, Portugal</td>
<td>2003, 2005, 2006</td>
<td>171</td>
<td>Improvement</td>
<td>Continuing to recruit</td>
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<td>Blood Derived Stem Cells – ALS Intrathecal – SCI – Intrathecal</td>
<td>Janmohamed et al</td>
<td>USA</td>
<td>2001</td>
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<td>Schwann Cells</td>
<td>Zhu et al, Fang et al</td>
<td>China</td>
<td>2001</td>
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<th>Other Results and Comments</th>
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<tr>
<td>Human Fetal Spinal Cord - syringomyelia</td>
<td>Falci et al</td>
<td>Sweden</td>
<td>1997</td>
<td>1</td>
<td>No further deterioration</td>
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<td>Wirth et al</td>
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<td>Ultra-Attractive</td>
<td>Cathala Study / Bio-Axone</td>
<td>USA, Canada</td>
<td>2000</td>
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<td>Some good neurological</td>
<td>Completed - some Patients improved</td>
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<td>Anti-Nogo-A Antibody</td>
<td>Neurona Study</td>
<td>Europe</td>
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<td>Human Bone Marrow</td>
<td>Park et al</td>
<td>South Korea</td>
<td>2005</td>
<td>5</td>
<td>Improvement</td>
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<td>Neural or Hematopoietic Cells</td>
<td>Zhang et al</td>
<td>China</td>
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<td></td>
<td>Bryukhovetskiy</td>
<td>Czech Republic</td>
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<td>NSPC Human Embryonic Stem Cells</td>
<td>Geri et al (Keremel, USA)</td>
<td>USA</td>
<td>2008</td>
<td>10</td>
<td>Some improvement</td>
<td>Began 2009 Planning Stage</td>
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<td>NSPC Human Fetal Brain Cells</td>
<td>Stem Cells Inc.</td>
<td>USA</td>
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<tr>
<td>Electrical Stimulation</td>
<td>Shapiro et al</td>
<td>USA</td>
<td>2005</td>
<td>10</td>
<td>Some improvement</td>
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<tr>
<td></td>
<td>Xu, Liu</td>
<td>China</td>
<td></td>
<td></td>
<td></td>
<td>Continuing to recruit</td>
</tr>
</tbody>
</table>

### Human Neural Stem Cells: Two Recent/Current Trials in Humans

**Human Embryonic Stem Cells for SCI in USA and Canada**
- **Embryonic** and therefore fear of producing tumors.
- **Geron, Inc.** used human embryonic stem cells (hESC), differentiated toward oligo lineage by Hans Keirstead, and in Jan. 2009 the FDA gave permission to start a trial in human SCI in US centres. Trial began in 2010.
- Transplanted in five ACUTE patients up to 14 days after SCI
- 2011 trial discontinued-ran out of money!

**Human Fetal Stem Cells for SCI in Switzerland**
- **Fetal** source was challenged because of ethical concerns (still).
- **StemCells Inc.** completed a transplant trial of human fetal brain stem cells programmed to differentiate into oligos in 6 children with Batten’s Disease.
- Trial started in 2011 in Zurich in SUBACUTE and CHRONIC CASES 3-12 months after injury. So far 3 thoracic ASIA A patients have received transplants.
My Choice for Spinal Cord Repair-
Neural Stem/Progenitor Cells (NSPC) from the
Adult Spinal Cord

- Multipotential and have inherent ability to divide
- Transplanted rather than endogenous
- Rodent now, humans-pending
- Adult cells- no ethical concerns and do not cause cancer (versus embryonic)
- From spinal cord because they differentiate preferentially toward oligos without needing extra growth factors
- With differentiating factors such as C-AMP we can generate neuronally enriched NSPC

Why Spinal Cord Source of Cells Rather than Brain?

- After several years, we and several other labs have not established that one is superior to the other, and both have shown good results.

- Examples: Karimi et al 2008 in the Fehlings lab and other labs have shown good results with brain-derived neural stem cells.

- The Tator lab and others have shown good results with spinal cord-derived neural stem cells - see Parr et al 2008.
Highlights from Our Recent Studies with Transplanted Adult Rat Spinal Cord-Derived NSPCs for Spinal Cord Repair in the Rat-to show the potential of these cells


Parr AM, Kulbatski I, Zahir T, Wang X, Yue C, Keating A, Tator CH.

Methods - 
Bone Marrow Stem Cells versus Neural Stem Cells

• Acute extradural clip compression of spinal cord - T8
  - 27g injury- a moderate injury

• Bone Marrow Stem cells (transpl day 0), Neural Stem Cells (transpl day 9) were injected 1mm rostral and caudal to the injury site (200,000 cells). Control-culture medium
  12 week survival
• Daily cyclosporine to prevent rejection

---

**Adult Rat Spinal Cord Stem Cells**

SFP adult rat spinal cord neurospheres
Day 7 in culture P3
Phase 40x

**DIFFERENTIATION**
After plating on Matrigel, removing growth factors, and adding 1% FBS

- **NESTIN+ 40x**
- **NEURONS MAP2+ 40x**
- **ASTROCYTES GFAP+ 40x**
- **OLIGODENDROCYTES O4+ 40x**
Transplantation of Stem Cells

- Transplantation of eGFP adult rat spinal cord
- Stem/precursor cells at 7 days, rostral and caudal to SCI site

NSPCs Differentiated into Oligodendrocytes and Astrocytes

Cell Fate of SC-NSPCs

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>% double labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nestin</td>
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</tr>
<tr>
<td>Astrocytes</td>
<td>1</td>
</tr>
<tr>
<td>Oligodendrocytes</td>
<td>45</td>
</tr>
<tr>
<td>Neurons</td>
<td>1</td>
</tr>
</tbody>
</table>

Images show differentiated cell types: Astrocytes, Oligodendrocytes.
Transplantation of Neural Stem Cells Resulted in Functional Improvement (red line)

(A) Locomotor performance (B) Motor subscore (C) Ladderwalk
significant improvement in rats receiving Neural Stem Cells from the Spinal Cord

Conclusions of this Experiment—Cell Survival and Differentiation

- Spinal Cord derived neural stem cells had better survival and produced better recovery:
  - when rats received rostral and caudal injections
  - when cells were injected at 9 days after injury

- Neural stem cells differentiated mainly into oligodendrocytes

- Bone Marrow stem cells - better survival and reduced cavitation
  - filled the cavity with collagen and fibronectin
  - BUT did not express neural markers
Conclusions-Functional Analysis

• **Spinal cord stem cells produced early functional improvement**

• After 27g injury there was some preserved tissue and therefore, allowed neuroprotective effect to be demonstrated

• Bone Marrow stem cells caused a trend towards improved cell survival of the neural stem cells when transplanted as a scaffold, but did not produce functional improvement

Beneficial Mechanisms of Transplanted Stem/Progenitor Cells in CNS Injury

• **Replacement** of damaged neuronal or glial cells promote recovery through regeneration, such as axonal regeneration **YES!!!**

• **Remyelination** by transplanted cells or host cells
  – by oligodendrocytes, or Schwann cells **YES!!!**

• **Neuroprotection** – increased host cell/axon survival, reduction of demyelination **YES!!!**

• **Creation of a favorable environment** – proliferation of endogenous cells
  - creation of cellular bridges and guidance for regeneration
  - counteract glial scar or other inhibitors
  - expression of growth factors or cytokines for neuroprotection or axonal regeneration

• **Vascular effects** – restoration of blood flow by angiogenesis
  - repair of blood brain barrier, reduction of edema
Future Strategies to Enhance Effectiveness

A. Strategies to enhance stem cell survival:
   1. Fibrin Scaffold to hold the stem cells
   2. Pre-differentiation of stem cells in vitro
   3. Guidance channels for the stem cells

Future Strategies

B. Strategies to enhance axonal regeneration:
   1. Anti-Nogo-A
   2. Chondroitinase-abc
   3. Cyclic-AMP to enhance production of neurons from the stem cells
More Work is needed on the Stem Cell Strategies

• Endogenous versus transplanted
• Source and viability of cells

• More Pre-Clinical trials
• More Well-Organized Scientifically Sound Clinical Trials

Thank You