Teaching Course: Neurotrauma

Challenges to nerve regeneration in humans: The Long Term Denervated Stump

Ahmet Höke MD, PhD, FRCPC
Johns Hopkins University
None relevant to this presentation

This work has been supported by NIH, DoD, Foundation for Peripheral Neuropathy and Dr. Miriam and Sheldon Adelson Medical Research Foundation
Learning Objectives

- To understand cellular mechanisms of poor recovery with proximal nerve injuries in humans
- To compare and contrast regenerative therapy strategies in experimental models versus human nerve regeneration
Challenges in PNS Regeneration

- **Intrinsic determinants of axon regeneration**
  - Slow rate of axonal elongation during regeneration

- **Extrinsic determinants of axon regeneration**
  - Chronic denervation
    - Changes in the pathway (i.e., Schwann cells)
    - Changes in the target (muscle or skin)

- **Common issues**
  - Specificity of target reinnervation
    - Determined through a combination of intrinsic neuronal characteristics, extrinsic pathway properties and finally target
### Acute events after injury: CNS vs PNS

#### Intrinsic Factors

<table>
<thead>
<tr>
<th>CNS</th>
<th>PNS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NEURONS</strong></td>
<td><strong>NEURONS</strong></td>
</tr>
<tr>
<td>Direct damage to neurons at injury site (death)</td>
<td>Very little neuronal death</td>
</tr>
<tr>
<td>Axotomy causes neuronal death depending on proximity to cell body</td>
<td>Axotomy causes neuronal death only when it is very close to cell body</td>
</tr>
<tr>
<td><strong>AXONS</strong></td>
<td><strong>AXONS</strong></td>
</tr>
<tr>
<td>CNS axons fail to form growth cone and form dystrophic end bulbs (microtubule depolymerization)</td>
<td>PNS axons form growth cone within hours (microtubules retain integrity and can bundle)</td>
</tr>
<tr>
<td>~1/3 of axons sprout for ~1mm</td>
<td>PNS axons start to regenerate shortly after injury</td>
</tr>
<tr>
<td>Distal end undergoes inefficient Wallerian degeneration (myelin debris)</td>
<td>Retrograde injury signal</td>
</tr>
<tr>
<td></td>
<td>Distal end undergoes efficient Wallerian degeneration (myelin debris)</td>
</tr>
</tbody>
</table>
Acute events after injury: CNS vs PNS

**Extrinsic Factors**

### CNS

**CELLULAR**
- Quick invasion of epicenter by fibroblast, vascular endothelial cells, and macrophages
- Surviving host cells (astrocytes, OPC, and microglia) surround the epicenter and form a glial scar

**ECM**
- CSPG (NG2, neurocan), fibronectin, laminin
- Myelin derived inhibitors (MAG, Nogo-A, OMgp)
- Guidance molecules (netrin, semaphorin, ephrin and slit families); expression changes after injury

### PNS

**CELLULAR**
- Activation of Schwann cells and intrinsic macrophages
- Invasion of blood-borne macrophages
- Proliferation of blood vessels

**ECM**
- Basal lamina is supportive of regeneration (laminin, COLa4)
- Myelin inhibitors are cleared
Wallerian Degeneration

- Accumulation of intra-axonal organelles
- Granular disintegration of cytoskeleton
- BNB/BBB breakdown
- Glial changes (SC, Oligos)
  - Proliferation vs apoptosis
- Recruitment of macrophages
- Clearance of myelin ovoids
Wallerian Degeneration

- Accumulation of intra-axonal organelles
- Granular disintegration of cytoskeleton
- BNB/BBB breakdown
- Glial changes (SC, Oligos)
  - Proliferation vs apoptosis
- Recruitment of macrophages
- Clearance of myelin ovoids
Axonal regeneration in CNS vs PNS

- Unlike CNS, axons in the PNS can regenerate
- Failure to regenerate in the CNS is NOT due to an innate inability of CNS axons to regenerate
  - PNS environment supports regeneration
    (Aguayo’s experiments)

- However, these observations do not correlate with clinical experience in humans
  - Clinical recovery after nerve trauma is often suboptimal
  - This is primarily due to “chronic denervation” (glial milieu)
Regeneration after nerve trauma

- Sciatic nerve injury: no repair
- Laceration to median nerve: Delayed repair at 10 mo
- Ulnar nerve injury: Immediate repair at day 2

Lyons & Woodhall 1949
Chronically denervated nerves do not support regeneration

- Is this a neuron/axon problem?
  or
- Is there a problem with the Schwann cells?
Effects of Prolonged Axotomy

Fu & Gordon 1995
Impaired motor regeneration following chronic denervation

Sulaiman & Gordon 1997
Chronically denervated SCs lose their ability to support regeneration of axons

- Atrophy of SCs and disappearance of Bands of Büngner
- Decline in expression of growth associated molecules (p75, erbB3) (Hall and Gordon labs) and neurotrophic factors (GDNF) (Hoke lab)
- Increased expression of CSPGs (Muir and Hoke labs)
Chronically denervated SCs lose their ability to support regeneration of axons.

**Nerve regeneration during critical period**
- Schwann cells maintain basal lamina tubes
- Axons regenerate
- Local protein synthesis at growth cones
- Target muscle accepts regenerating axons

**Failed regeneration after delay**
- Schwann cell tubes deteriorate: mechanism unknown
- Apoptosis occurs in some neurons after axotomy
- Axons are unable to regenerate through unsupportive distal nerve
- Atrophied target muscle prevents reinnervation

*Nature Neurol Reviews 2013*
Chronically denervated SCs lose their ability to support regeneration of axons

Acute denervation and regeneration

Chronic denervation and regeneration
Regenerative challenges in CNS and PNS

- Glial scar
- Neuronal death
- Demyelination of spared axons

- Regeneration is not the same as developmental growth:
  - Distance to regenerate – embryo vs adult
Impact of slow rate of axonal elongation on nerve regeneration in humans
Can we alter the rate of axonal elongation? - PNS

- Conditioning lesion
  - Impact on peripheral regeneration
  - Impact on central regeneration
- Electrical stimulation (Gordon & Brushart)
  - Provide pathway specificity without enhancing rate of axonal elongation
- ATF-3 over expression (Woolf)
  - Effect on peripheral but not central regeneration
- Hsp27 over expression (Woolf)
  - Effect on sensory and motor regeneration

(Seifert et al. 2007)
Can we alter the rate of axonal elongation? - PNS

- Unbiased screen for compounds that increased outgrowth in SC explants and DRG neurons

- D-cycloserine
  - A weak NMDA agonist

(Wang et al. Unpublished)
Can we alter the rate of axonal elongation? - CNS

- Phosphatase and tensin homologue (PTEN)
  - Mammalian target of rapamycin (mTOR)
- Suppressor of cytokine signaling 3 (SOCS3)
  - Janus kinase/signal transducers and activators of transcription (JAK/STAT)
- Optic nerve model of CNS regeneration:
  - Adult retinal ganglion cells (RGCs)

(Park et al. 2008 and Smith et al. 2009)
Can we alter the rate of axonal elongation? – CNS vs PNS

- Combined PTEN/SOCS3 deletion
  - Concurrent activation of mTOR and JAK/STAT pathways in neurons

- But no effect on PNS regeneration!
  - Intrinsic mechanisms of CNS vs PNS regeneration are likely to be different
  - Nevertheless, local inhibition of PTEN at site of regeneration accelerates PNS regeneration (Christie et al. 2010)
Gaps in our knowledge and future directions – ongoing studies

- Chronic denervation in the:
  - Pathway: Schwann cells
  - Target: muscle, skin
- Can we reactivate atrophied SCs or prevent atrophy?
  - Role of neuregulin-1 type III
- Do we need to replace atrophied SCs? Can we do it?
  - Human ESC or iPS generated Neural Crest Stem Cells as a source
Chronic denervation in the SCs

Rat sciatic nerve denervation 1d – 6 mo

Transcription Factors

Apoptosis Related

Cell-cycle proteins

(Wright et al. Unpublished)
Replacement of chronically denervated Schwann cells

Aligned nanofibers enhance efficiency of SC generation from hiPSCs and hESCs

(Ren et al. In Press)
Replacement of chronically denervated Schwann cells

- hESC-derived Schwann cells enhance regeneration in chronically denervated nerves

- But they do not survive

(Mi et al. Unpublished)
Replacement of chronically denervated Schwann cells

- hESC-derived Schwann cells enhance regeneration in chronically denervated nerves
- Increased secretion of NTFs

(Metal Unpublished)
Chronically denervated target: muscle

Even if we solve the issue of chronic denervation in Schwann cells, we still need to focus on changes at the NMJ and muscle.

100-500 days of denervation

(Gordon et al. J Neurosci 2011)
Challenges in translating to humans

- Do we have the appropriate biomarkers, tools to assess regeneration?
  - Nerve regeneration in humans is slow!!!
    - Issue of distance and rate of nerve regeneration in humans
  - We need validated biomarkers that can predict successful outcome before full recovery takes place
    - Novel human experimental assays
    - Imaging?
Challenges in translating to humans

- Regeneration is not same as development
- We need to make it faster and better
Challenges in translating to humans

- Do we have the appropriate biomarkers, tools to assess regeneration?
  - Nerve regeneration in humans is slow!!!
    - Issue of distance and rate of nerve regeneration in humans
  - We need validated biomarkers that can predict successful outcome before full recovery takes place
    - Novel human experimental assays
    - Imaging?
Biomarkers and tools to assess nerve regeneration

- Clinical trials in peripheral nerve injuries are costly:
  - Nerve regeneration in humans is slow
- Novel models of assessing nerve regeneration in humans
- Novel imaging techniques:
  - Diffusion Tensor Imaging

Thanks

Johns Hopkins

• Ruifa Mi
• Weiran Chen
• Jing Zhou
• Kimberly Ostrow
• Jae-Sung Park
• Shuo Wang
• Zola Zhou
• Nicole Reed
• Erika Timar
• Mitali Ray
• Megan Wright
• Jami Scheib
• Chris Cashman
• Katelyn Donaldson

University of Calgary

• Doug Zochodne

University of Alberta

• Tessa Gordon

UCLA

• Dan Geschwind
• Giovanni Coppola

Funding: NINDS, Packard Center for ALS Research, Dr. Miriam and Sheldon Adelson Foundation for Medical Research, Merkin Family Foundation, Foundation for Peripheral Neuropathy
References

**Books**

**Reviews**
- Experimental Neurology Special Issue: “Regeneration in the Peripheral Nervous System” Volume 223, Issue 1, pp. 1-250 (May 2010)