Differential Effects of Neuregulin-1 Isoforms on Myelin Regeneration Following a Spinal Cord Injury

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Myelin is a deterministic component of axon survival, insulating the axon and permitting normal flow of CNS electrical signals that also help maintain axon viability. When a person suffers a spinal cord injury, lesion site scar tissue and inflammatory factors lead to neurological signal interruption between the brain, spinal cord, and the rest of the body. These interruptions in connectivity are the result of demyelination and truncated myelination of surviving axons that limits their ability to function properly. Neuregulin-1 (NRG1) isoforms, and their therapeutic effects on myelin regeneration and gliogenesis, have only recently begun to be studied in depth. Elucidating the effects of NRG1 isoforms has the potential to elicit novel therapeutic modalities in SCI recovery.
Methods

A T9 laminectomy and moderate contusion injury procedure was performed on 8 week old Female C57BL/6 mice. Of two groups, one was remitted for RNA measurements while the other received a partial laminectomy and pump implantation that administered one of three agents for a period of two weeks. During the second week, animals received IP injections of BrdU. Later, animals were sacrificed at either 15 or 52 days post-injury for behavioral testing, quantification of BrdU positive cells and cell phenotype, immunohistochemistry, and EM.

Sagittal cerebral slices (400-µm) were obtained from postnatal day 10 (P10) Sprague Dawley rats. Slices were placed in an organotypic slice culture and maintained for 14 days. Slices were fixed and stained for internodal length measurements in the different treatment groups.
Results

Figure 1. SCI alters NRG1 type I and III gene expression; infused NRG1 perfuses throughout injured spinal cords. A. Lesion epicenter NRG1 type I expression significantly increased 24 hrs and 5 days p.i., but fell significantly by 1 month p.i. NRG1 type III expression was significantly decreased at all time points examined. B. Motor cortex NRG1 type I expression significantly decreased at 24 hrs and 2 weeks p.i. NRG1 type III expression significantly decreased compared to uninjured controls at all time points examined, except at 5 days p.i. Baseline level = 1. C-D. Merged confocal images taken with identical microscope settings confirms (C) no anti-penta his immunoreactivity in aCSF controls and (D) widespread perfusion of 6xHis-tagged NRG1 type III (green) after 3 days of type III infusion. DAPI is blue; 20x magnification.
Results

Figure 2. Infused NRG1 type I and III increase proliferation after SCI. A-C. BrdU+ cells (green) are more abundant at the lesion epicenter in (B) type I- and (C) NRG1 type III- infused animals compared to (A) aCSF-infused controls 15 days p.i. 10x magnification. D. The density of proliferating cells (BrdU+ cells/mm³) was significantly increased with type III infusion at the lesion site, rostrally and caudally 15 days p.i. (P<0.001), while type I infusion increased proliferation only at the lesion epicenter (P<0.05) E. There are no differences in the number of BrdU+ cells 52 days p.i.
Results

Figure 3. Infusion of NRG1 type III affects generation of NG2+ progenitors and oligodendrocytes. 

A-D. At 15 days p.i., NRG1 type III significantly reduced the percentage of BrdU+ cells positive for the progenitor marker, NG2 (A; P<0.05), but did not significantly alter the percentage of recently divided cells positive for the astrocyte marker S100β (B) or for the oligodendrocyte markers Olig2 and CC1 (C). NRG1 type I did not have any significant effect on the percentage of BrdU+ cells acquiring a progenitor (A; NG2), astrocyte (E; S100β) or oligodendrocyte (F; Olig2/CC1) phenotype at 15 or 52 days p.i. compared to controls. D. The density of BrdU+ cells co-localizing with oligodendrocyte markers (Olig2 and CC1) increased with NRG1 type III infusion at 15 days p.i. (P<0.01).
Results

Figure 4. Myelin morphology after NRG1 infusion. A. Compared to controls (black line), g-ratio distribution for the NRG1 type III-infused group (green line) shifted to the left, indicating thicker myelin. The mean g-ratio in NRG1 type III is significantly smaller than control (P<0.001). G-ratio distribution for the NRG1 type I-infused group (grey line) shifted to the right compared to control, indicating thinner myelin. The mean g-ratio in NRG1 type I is significantly larger than control (P<0.001). B. The decrease in g-ratio with NRG type III infusion is maintained at 52 days p.i. (P<0.05), but g-ratios with type I infusion are not different from controls. C. Internode length was measured between CASPR+ paranodes along NF+ purkinje axons. D. Internode lengths were significantly higher in Type I treated slices (P<0.001) and lower in Type III-treated slices (P<0.05) compared to untreated controls, confirming the differential regulation of myelin sheath length between these isoforms.
Discussion

- This study has tested the hypothesis that isoforms of NRG1 in the setting of injury are potent regulators of the oligodendrocyte lineage and subsequent myelin production.

- Gene expression studies revealed decreased NRG1 type III levels following SCI in both the lesion epicenter and motor cortex.

- Provided support for the concept that NRG1 type III regulates the formation of oligodendrocytes following SCI.

- Demonstrated that NRG1 type I and type III have divergent effects on myelin that is produced following injury.
Summary

Injury to the CNS commonly leaves spared axons that exhibit myelin deficits or incomplete remyelination. These studies implicate neuregulin as a potentially important therapeutic targets for altering myelin dynamics following injury. We demonstrate that NRG1 type I and type III have divergent effects on myelin that is produced following injury. In the future we will focus on incorporating data showing the effects NRG1 treatment has on locomotor and autonomic function following a SCI.