FINAL NARRATIVE REPORT

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Grant title: Functional analysis of erbB2 signaling during myelination
Grant number: 06-3059-SCR-E-O
Grant period: 06/15/2006 - 06/30/2010

Date of submission of the report: August 10, 2010

1. Original aims of the project

The objective of study was to determine the therapeutic potential of Neuregulin (Nrg1)-erbB signaling in promoting remyelination on adult regenerating axons. Specifically, our study focused on investigating the role of Nrg1 on Schwann cell myelination as Schwann cell transplantation strategy has been shown to provide therapeutic benefit in promoting remyelination of injured axons in the CNS. For the study, we had the following three specific aims: i) determine the Nrg1-erbB signaling mechanism that promotes Schwann cell myelination, 2) determine the effects of ectopic Nrg1-erbB signaling on Schwann cell myelination and 3) determine whether ectopic Nrg1-erbB signaling could improve myelination on adult regenerating axons.

2. Project successes

During the project period, we published four papers supported by the NJCSCR grant and presented our results at scientific meetings such as the annual meetings for the Society of Neuroscience, the American Society for Neurochemistry and the Gordon Research Conference. Below, I have summarized some of the significant findings from our studies.

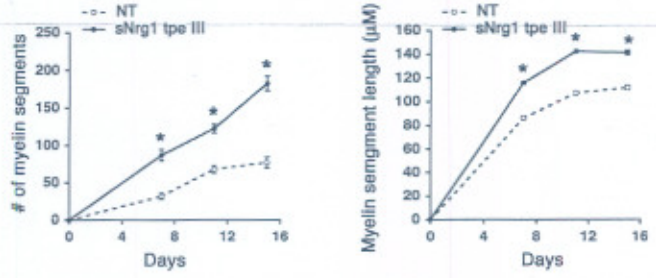

Figure 1 Right: Upon initiating myelination (Day 0), co-cultures were treated with or without soluble Nrg1 type III (1 nM) and the number of total myelin segments was counted on Day 7, 11 and 15. There was a significant increase (*: $p<0.001$) in the number of myelin segments in sNrg1 type III treated cultures compared to the non-treated (NT) control. Left: The lengths of individual myelin segments were measured from myelinating co-cultures, maintained with or without (NT) sNrg1 type III (1 nM) for 11 days.

a. *Ectopic stimulation of Schwann cells with soluble Nrg1 promote myelination and myelin maturation:* To determine whether ectopic addition of Nrg1 improves Schwann cell myelination, we used an in vitro myelinating culture system in which isolated Schwann cells were co-cultured with dorsal root ganglion neurons. Cultures were treated with different doses of Nrg1 and myelination was assessed. As shown in Figure 1, the results show that stimulation with soluble Nrg1 not only increases the number of myelin segments formed on axons but also promote myelin maturation by increasing the myelin segmental lengths.

b. *Soluble Nrg1 functions during the early stages of myelination following establishment of axon-Schwann cell association:* Previous studies have shown that neuronal Nrg1 expressed on axonal membrane is required for Schwann cell-axon association and the subsequent myelination. To determine whether soluble Nrg1 could replace the role of axonal Nrg1, we co-cultured Schwann cells with neurons prepared from Nrg1+/- and Nrg1-/- mice and treated with soluble Nrg1. The data show that while soluble Nrg1 rescues myelination defects on Nrg1+/- neurons (few myelin segments and short

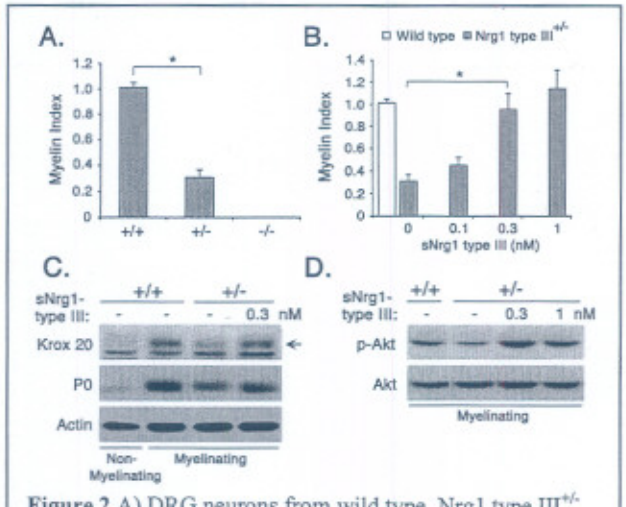

Figure 2 A) DRG neurons from wild type, Nrg1 type III$^{+/-}$ and Nrg1 type III$^{-/-}$ mouse embryos were co-cultured with rat Schwann cells and maintained in myelinating media for 11 days. Quantification of the number of myelin segments showed a significant decrease (*: $p<0.001$) in myelination in Nrg1 type III$^{+/-}$ cultures whereas no myelination was observed on Nrg1 type III$^{-/-}$ neurons. (B) Myelination in Nrg1 type III$^{+/-}$ co-cultures (grey bars) treated with soluble Nrg1 type III. Wild type cultures were used as control (white bar). Soluble Nrg1 type III increases Krox 20 expression (C) and Akt activation (D) in Nrg1 type III$^{+/-}$ co-cultures.

internodal length) (Figure 2 A and B), it fails to rescue myelination in Nrg1-/- neurons (not shown). Since Schwann cells associate normally on Nrg1+/- axons but not on Nrg1-/- axons, the result suggests that soluble Nrg1 promotes stages of myelination subsequent to axon-Schwann cell association.

c. *The pro-myelinating function of soluble Nrg1 is mediated by an increase in the PI3-kinase activation and Krox 20 expression*. To define the signaling function of soluble Nrg1, we assessed various signaling pathways that are activated in Schwann cells upon soluble Nrg1 treatment. We show that the pro-myelinating function of Nrg1 is mediated by an increase in the PI3-kinase activation and expression of myelin proteins and Krox 20, a transcription factor required for myelination (Figure 2 C and D).

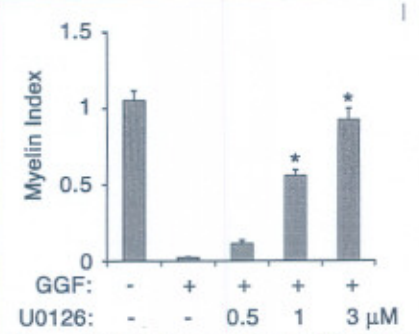

Figure 3 Inhibitory effect of GGF on myelination is blocked by inhibition of the Mek/Erk activation. Quantification of myelination in cultures treated with GGF and increasing doses of U0126 (0.5, 1 and 3 μM). Treatment with U0126 abolished the inhibitory effect of GGF and increased myelination (*: $p < 0.001$).

d. *Soluble Nrg1 elicits a concentration-dependent, biphasic effect on Schwann cell myelination:* A surprising finding of our study was the dose-dependent effect of soluble Nrg1 that promotes or inhibits myelination. While soluble Nrg1 promotes myelination at relatively low doses, it inhibits myelination at high doses in a manner that is dependent on Mek1/2 activation: pharmacologic inhibitor to Mek1/2 blocked the inhibitory effect of Nrg1 (Figure 3). Furthermore, inhibition of the endogenous Mek1/2 activity in co-cultures promoted myelination, indicating that activation of the Ras/Raf/Erk pathway functions as a negative signal for myelination (not shown).

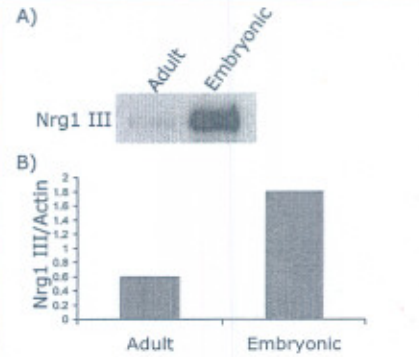

Figure 4 A) Cell lysates were prepared from adult and embryonic neuron cultures and subjected to western blotting. The Nrg1-type III expression was reduced in the adult neurons compared to the embryonic neurons. B) Quantification of the Nrg1-type III expression by normalizing over actin expression.

e. *Soluble Nrg1 is sufficient to induce myelination on normally non-myelinated axons:* Small diameter axons, such as the ones of sympathetic neurons do not become myelinated, but ensheathed by the Schwann cells. The neurons express low levels of the membrane-bound Nrg1, insufficient for myelination. We show that ectopic stimulation of Schwann cells with soluble Nrg1 induces myelination on sympathetic neurons (data not shown), demonstrating the pro-myelinating role of the Nrg1.

f. *Adult axons express low levels of the membrane-bound Nrg1*: Since regenerating axons in adult animals are often poorly myelinated, we speculated that adult axons do not express sufficient levels of Nrg1 required for myelination. We prepared neurons from adult and embryonic dorsal root ganglion and compared the levels of the membrane-bound Nrg1. As shown in Figure 4 adult neurons expressed a significantly lower level of Nrg1 compared to embryonic neurons. Based on our results above, we speculate that a therapeutic strategy using soluble Nrg1 may improve remyelination on injured adult axons.

3. Project challenges

In our original aims we had proposed to generate both loss-of-function and gain-of-function phenotypes for erbB2, a receptor for Nrg1, to investigate the role of Nrg1-erbB signaling during

Schwann cell myelination and to determine whether increasing the erbB2 activity would promote remyelination. Modulating the erbB expression in Schwann cells or knocking-down erbB activity by expressing dominant negative mutant was proven to be difficult. As an alternative approach, we investigated the effect of the ligand, Nrg1 and generated a gain-of-function (ectopic stimulation with soluble Nrg1) and a loss-of-function (use of Nrg1-deficent neurons) phenotypes and successfully completed most of the aims of the proposed study.

4. Implications for future research and/or clinical treatment

Rebuilding of myelin in demyelinated lesions in the CNS by transplanting exogenous myelin-forming glial cells is a concept that has been explored and tested for many years. Schwann cells offer the possibility of autologous transplantation as they are easily obtained and expanded in culture, and myelinate when transplanted in demyelinated lesions. However Schwann cell remyelination of adult axons is often incomplete, resulting in the formation of thinner myelin sheathes and shorter internode compared to normal nerves. The pro-myelinating effect of soluble Nrg1 presented in this study is significant as it provides a potential therapeutic strategy for improving myelination by Schwann cells. However, it should be cautioned that concentrations above the threshold level could have a devastating consequence on the pathologic condition. Further understanding of the inhibitory role of Ras/Raf/Erk pathway on myelination might provide insights into developing a combined strategy for improving myelination.

5. Plans to continue this research, including applications submitted to other sources for ongoing support

To investigate the therapeutic potential of soluble Nrg1 to improve remyelination on adult axons, we will further define the Nrg1-signaling property of adult axons to determine whether there is a deficiency in activating crucial pro-myelinating signals in the associated Schwann cells. We will also determine whether soluble Nrg1 improves myelination on adult axons by increasing the pro-myelinating signal. Lastly, we will determine the effect of soluble Nrg1 on remyelination in vivo. A grant proposal is in preparation to be submitted to NIH. We have also begun to investigate molecular mechanisms regulating oligodendrocyte myelination using in vitro myelinating co-culture sytem.

6. List of publications

1. Syed, N. and H. A. Kim. Soluble neuregulin and Schwann cell myelination: A therapeutic potential for improving remyelination of adult axons. *Submitted* to Molecular Cellular Pharmacology
2. Syed, N., K. Reddy, D. P. Yang, C. Taveggia, J. L. Salzer, P. Maurel, and **H. A. Kim.** Soluble neuregulin-1 has bi-functional, concentration-dependent effects on Schwann cell myelination *Journal of Neuroscience* 2010 30: 6122-6131
3. Yang, D. P., D. P. Zhang, K. S. Mak, D. E.Bonder, S. L. Pomeroy and **H. A. Kim**. Schwann cell proliferation during Wallerian degeneration is not necessary for regeneration and remyelination of the peripheral nerves: axon-dependent removal of newly generated Schwann cells by apoptosis. *Molecular and Cellular Neuroscience*, 2008 38(1): p. 80-88