The Role of the Wnt3a and the Beta-Catenin Signaling Pathway at the Motor Endplate following Traumatic Nerve Injury

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Introduction: Major peripheral nerve injuries lead to significant functional deficits. Although the general belief is that the regenerative capacity of the PNS when compared to the CNS is much more robust after nerve insult, this does not translate in the clinical setting. A reason for this phenomenon may be explained by end-organ atrophy. We recently showed that preservation of the motor endplate after traumatic nerve injury improves functional recovery after surgical repair1. Wnt signaling proteins, particularly the canonical Wnt/beta-catenin pathway, play an important role in the development and the maintenance of motor endplates. Previous studies have shown that multiple signaling pathways and proteins including agrin, MuSK, and the Wnt pathway influence the neuromuscular junction (NMJ). In particular, Wnt3a has been shown to inhibit agrin-induced acetylcholine receptor (AChR) clustering by suppressing rapsyn expression via beta-catenin dependent but TCF/LEF independent signaling. The current study focuses on assessing the NMJ after long-term denervation injury. Levels of Wnt3a and activated beta-catenin are quantified throughout various timepoints to determine if destabilization of the NMJ corresponds to an increase in the concentration of these proteins within the motor endplate. As such, we explored the role of Wnt/beta-catenin at the NMJ after traumatic nerve injury.

Methods: Animal Model. All procedures involving living animals were approved by the Institutional animal care and Use committee of the University of California, Irvine. Homozygous pairs of the 129 SV/EV wildtype mice were a generous gift from Dr. Wee Yong at the University of Calgary. Genotyping was performed by Transnetyx (Cordova, TN).

Surgery. For denervation studies, 6-week old male mice were anesthetized with ketamine/xylazine. A 10 mm segment of the right sciatic nerve was excised.

Immunohistochemistry and Immunoblotting. Whole mounts of plantaris muscles were extracted from wildtype mice ipsilateral and contralateral to the side of denervation at 1 month and 2 months. IHC analysis was performed to define the integrity of the nerve terminal and motor endplates after denervation and for Wnt3a. Gastroc-soleus muscle complexes were harvested for Wnt3a and beta-catenin levels and quantified using standard western blot techniques. A t test was performed with p value < 0.05 constituting significance.

Results: Wnt3a protein levels were elevated at 1 month (0.633±0.0540 vs 0.937±0.128) and 2 months post-injury (0.488±0.0170 vs. 0.970±0.232; p<0.002) relative to control. Moreover, activated beta-catenin showed a similar increase (0.532±0.0250 vs. 1.050±0.204; p<0.026). Additionally, Wnt3a staining in uninjured muscles was not present. However, after denervation injury, Wnt3a was upregulated and recruited into the post-synaptic muscle, specifically to the degrading AChRs and motor endplate band at increasing levels until 2 months.

Discussion: The NMJ undergoes a gradual degradation with loss of its different constituents after prolonged denervation. For the terminal axon and per-synaptic Schwann cells, this process is immediate as the mechanism for Wallerian degeneration is initiated after nerve transection. For the ACh receptor, signs of destabilization are not grossly seen until the later time points; although evidence for this process can be found in loss of receptor area early after injury. Long-term denervation leads to extensive atrophy of the motor endplate, which translates to deficits in functional recovery. Post-synaptic AChRs at the NMJ appear to destabilize after denervation by a process that involves the Wnt/beta-catenin pathway. Our data implicates this pathway as a potential source of this motor endplate instability after injury.

Significance: Recent data has shown that preservation of the motor endplate significantly enhance regeneration and functional recovery following traumatic nerve injury. Therefore, this study aims to develop a novel method of preserving the motor endplate in muscles by inhibition of the canonical Wnt/beta-catenin pathway. Our data presents a potential novel target to optimize functional outcomes following surgical management of traumatic nerve injuries.

Acknowledgments:

Metalloproteinase 3 deletion preserves denervated motor endplates after traumatic nerve injury. Ann Neurol., 73: 210-223. doi: