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EFFECT OF NOTUM ON ZEBRAFISH SPINAL CORD INJURY RECOVERY

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Background: Zebrafish are of particular interest to researchers due to their retention of neural stem cells into adulthood. The fish can utilize these cells to recover from spinal cord injury (SCI), a lifelong debilitation for humans. One important molecular pathway for this recovery is Wnt signaling (source here). RNA sequencing (RNAseq) of injured spinal cord tissue performed by members of this lab in the presence and absence of a Wnt pathway inhibitor identified the *notum1a* and *notum1b* genes as injury-induced Wnt-dependent targets. The precise role of *notum1a/b* in spinal cord regeneration is still unknown.

Purpose/Research Question(s): The primary goal of the project is to validate that *notum1b* is being expressed in a Wnt dependent manner inside the injured spinal cord. The secondary goal is determining whether *notum1a/b* are required for SCI recovery. Specifically does loss of *notum1a/b* function affect the site of spinal cord injury?

Methodology: *Insitu* hybridizations are performed on injured zebrafish larvae to detect gene expression. A *notum1b* specific probe is made via *in vitro* transcription and is used to stain cells expressing *notum1b* mRNA. The expression pattern can then be examined inside the fish under a microscope to determine where *notum1b* is present in recovery. SCI is done on anesthetized fish with a blade at 5 days post fertilization (dpf), and they are stained using *in situ* hybridization at 3 days post injury (dpi).

Results: *notum1a* mRNA has been detected at the injury site. *notum1b* is still in the preliminary phases and still requires a validated probe to properly examine. Early results are promising in verifying its presence at the injury site. Once those results are repeatable, the *notum1b* probe will be tested on fish treated with IWR1, a Wnt inhibitor, after injury. Preliminary data indicate that *notum1a/b* double mutants do not appear to recover from SCI at 9 dpi.

Discussion: *notum1b* is expected to show Wnt-dependent expression at the injury site in the fish with a proper *in situ*. However, we have no reference for what *notum1b* expression normally looks like, so we must create multiple different probes from different parts of the *notum1b* sequence to find a consistent expression pattern. Positive results using multiple probes will confirm *notum1b* expression at the injury site. Further results on *notum1a/b* function in recovery will follow as a next step.