Changes in the Sonic Hedgehog *(shha)* signalling pathway during Dopaminergic Neuron Regeneration in *Danio rerio*

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Introduction

- Dopaminergic neurons (DA neurons) are the main source of dopamine within the mammalian central nervous system. These neurons, when lost in the zebrafish (*Danio rerio*), has the ability to be regenerated through stem cells previously present in the fish brain. The Sonic Hedgehog (*shha*) signaling pathway, in particular, has been of great interest due to its prominent role in both the development and regeneration of the central nervous system (CNS).

- Using a specific line of transgenic zebrafish Tg(*dat:*CFP-NTR), we seek to determine whether the *shha* signalling pathway is upregulated, and if it is, when and where is the signalling pathway upregulated during dopaminergic neuron regeneration. Determination of the changes in the *shha* signalling pathway will help us further the understanding and the allow for the elucidation of the molecular mechanisms underlying the dopaminergic neuron regeneration in the adult zebrafish brain.

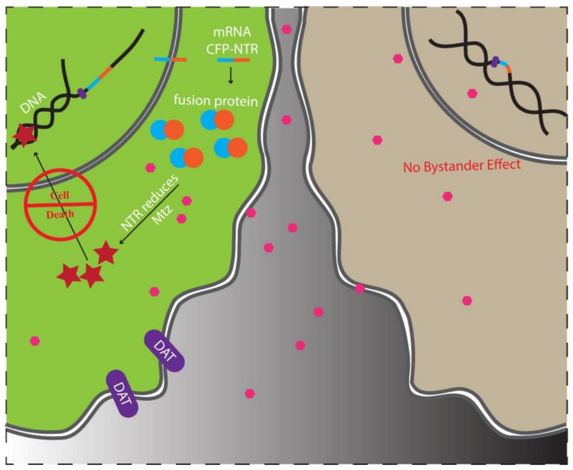

Figure 1: Nitroreductase mediated cell specific ablation. Mechanism of action of Cyan-Fluroscent Protein-Nitroreductase fusion protein (CFP-NTR) and pro-drug Metronidazole (MTZ). MTZ alone is harmless to a normal neuron, as seen in the neuron on the right. However, upon exposure to endogenously produced CFP-NTR, MTZ is reduced into a cytotoxic DNA crosslinking compound that leads to selective DA neuron loss, As seen in the neuron on the left. Figure taken from Godoy[1].

Methods

Zebrafish Model

To specifically ablate the dopaminergic neurons within the adult zebrafish brain, a chemogenetic approach is used by treating the transgenic zebrafish line Tg(*dat:*CFP-NTR) with the pro-drug metronidazole (MTZ). While MTZ alone is harmless to a normal zebrafish brain, the transgenic Tg(*dat:*CFP-NTR) line expresses the Cyan-Fluorescent Protein-Nitroreductase fusion protein specifically in dopaminergic neurons, which, together with MTZ, will result in the selective ablation of DA neurons.

Methods

RNA probe preparation

The *shha* genome is first isolated through the use of phenol chloroform extraction, where the non-polar contaminants are removed through the nonpolar phenol chloroform phase and the polar DNA (along with other polar contaminants, such as salt) are left within the polar aqueous phase. This process is done multiple times to increase purity of extracted product and the final DNA extract is run on an 1% agarose gel to verify presence. The concentration of DNA along with the 260/280 (range >1.8) and the 260/230 (range 2.0 - 2.2) values are obtained through nanodropping to verify purity. A RNA probe is then transcribed from the isolated DNA product and its concentration and presence are again evaluated using 1% agarose gel electrophoresis and nanodropping.

Experiment

The brains of both the DMSO control group (no MTZ treatment) and the experimental group (MTZ treatment) will be collected at time points of 1, 3, 5, 7, and 10 days post treatment (dpt), fixed in paraformaldehyde and cross sectioned. *In situ* hybridization will then be performed on brain cross-sections using *patched-1* and *shha* RNA probes in order to detect their expression and answer the question of when and where is the *shha* signalling pathway upregulated during dopaminergic neuron regeneration.

Results

RNA probe synthesis

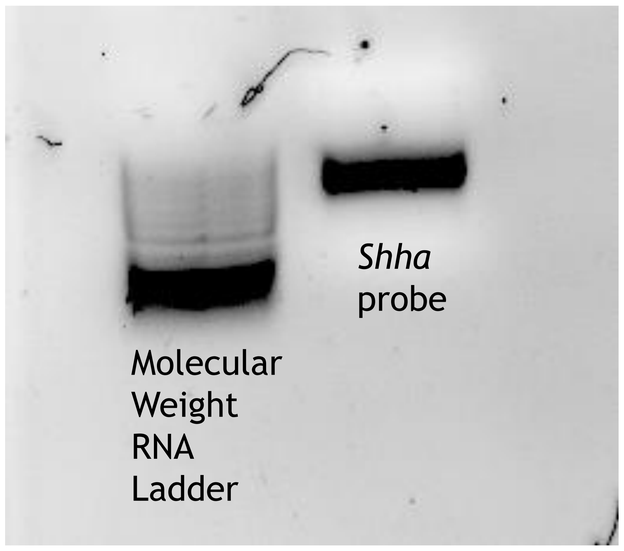

Figure 2: Agarose Gel Electrophoresis of *shha* RNA probe. Following RNA probe synthesis using previously extracted DNA, the probe is run along a molecular RNA ladder on an 1% agarose gel to verify presence and purity. A clear band within the RNA probe lane is desired, as seen in the figure above.

Results

In Situ hybridization

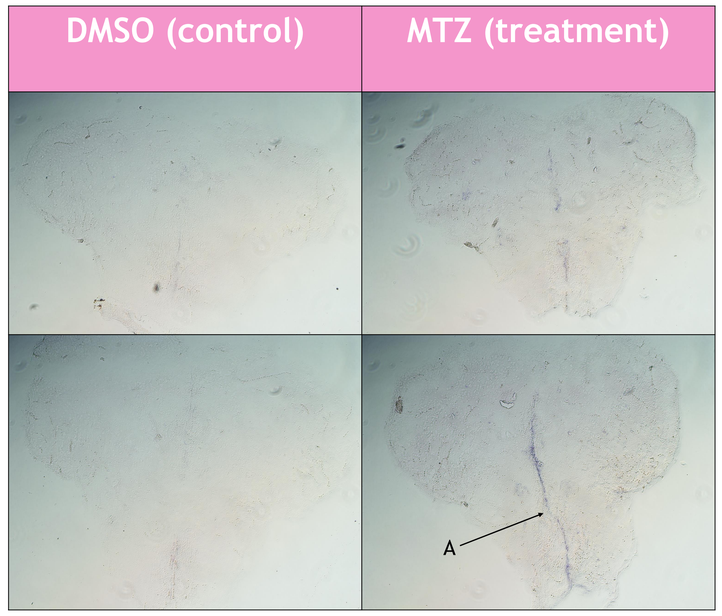

Figure 3: Comparison of cross-section telencephalon of control group and MTZ treated zebrafish, 5 days post treatment. Following cross sectioning of control and MTZ treated zebrafish brains, *in situ* hybridization is done on the cross sections and the staining patterns are obtained and analyzed. Intensity of the light, the filter, and exposure times are pre-set and is not altered during the experiment to ensure uniformity between images. It is seen that there is comparatively darker staining within the cross-sections of the MTZ treated fish as opposed to the DMSO group, showing a higher level of activation of the *shha* genes. There is also observed staining at the region labelled A in the MTZ treated group, which is not present in the DMSO group, suggesting a possible location for mass DA neuron regeneration.

Conclusion

Through this experiment, we were able to successfully determine that the Sonic Hedgehog signalling pathway is upregulated following selective ablation of DA neurons within the zebrafish's brain. We were also able to determine that upregulation mostly happens in areas that are already expressing a low level of *shha*, but there are some regions that are activated in the MTZ group which are not observed within the DMSO controlled group.

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Bibliography

1. Godoy, Rafael Soares (2015). *Chemogenetic Ablation of Dopaminergic Neurons in the Brain of Larval and Adult Zebrafish (Danio Rerio): Phenotypes and Regenerative Ability.* University of Ottawa, Ottawa, Ontario.