Immunohistochemistry for Myosin in Zebrafish Embryos

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Abstract

Myosin is an important component to muscle contractility. Since the heart requires the ability to contract to pump blood, myosin will also be located in the heart. The goal of this research project was to utilize immunohistochemistry and hematoxylin/eosin staining on zebrafish embryos at different stages of development, from unfertilized to moments before hatching, in order to find the when zebrafish hearts are developed far enough to produce myosin.

Background

Zebrafish (*Danio rerio*) are freshwater teleost, often found in home aquariums and kept at 28.5˚ C (Nesan et al. 2012) and between 6.8 and 7.2 pH. Not only can they produce many embryos in a short amount of time, the amount of time between fertilization and hatching is between three and six days, and their translucent bodies during development allow for observations that would be difficult to analyze in other species. Zebrafish contain many conserved genes that are similar to humans, making them an ideal model organism for studying diseases and disorders (Bakkers 2011). Among the observations that can be observed are cardiac development and malfunction in zebrafish. Zebrafish embryos are able to survive cardiac malformation and malfunction until the larval stages because their small size allows diffusion of nutrients and oxygen from the surrounding water to keep them viable, allowing the condition to be studied on a live specimen, with a fully formed heart produced around 30 hours post fertilization (hpf) (Glickman and Yelon 2002).

Immunohistochemistry is an analytical tool used to examine protein expression in tissues to identify particular tissue types and manifestations of changes in gene expression. Utilizing an anti-myosin antibody, up and down regulation of the gene can be measured. Antibodies that are against that gene in zebrafish, cultivated from a separate organism, and then a secondary antibody against the first with tagging allows the gene regulation to be seen. An important IHC result would be a difference in protein presence or expression pattern between the control and experimental groups

Procedure

Zebrafish eggs were collected shortly after initiating the “light” cycle to encourage breeding. In the first set of experiments, the eggs were collected and immediately euthanized and placed in fixative. These eggs represented both unfertilized and undifferentiated eggs. They embryos remained in 4% PFA overnight at 4˚ C, were dehydrated in various concentrations of MeOH, left overnight in 100% MeOH, rehydrated the following day and incubated in Tris-HCl buffer. After the fixative procedure was finished, the eggs were dechorionated and then treated with either IHC, H&E, or served as negative controls for IHC. The same procedure was repeated for embryos that had been reared past the 24 hpf stage except they were dechorionated prior to fixation. After their staining was completed, they were dehydrated again in 99% EtOH and whole mounted.

Preliminary Results

Fig 1 Control 40x. This zebrafish embryo was treated with IHC, but did not receive the primary antibody. There was no blue staining.

Fig 2 IHC 40x. This zebrafish embryo was treated with IHC, receiving the primary antibody against myosin-light chain. There was blue staining evident along the tail and back.

Fig 3 IHC zoom. This is the zebrafish from Fig 2 at 100x magnification. The staining is much clearer

Fig 4 IHC 100x Tail. This is the tail of the zebrafish from Fig 2.

Fig 5 IHC Zebrafish 40x. This is another zebrafish subject to the IHC and primary antibody. Again, there is staining along the tail and back where muscle would form

Fig 6 IHC Tail Only40x. This is another zebrafish subject to the IHC and primary antibody. Only the tail stained blue.

Fig 7 H&E Zebrafish 40x. This is another zebrafish subject to hematoxylin and eyosin staining.

Fig 8 IHC Embryo 40x. This embryo was undifferentiated and was subjected to IHC with the primary antibody. No staining occurred.

Discussion

After several trials, the IHC began to produce the expected results. Originally, there was little to no staining, or everything was stained blue. This could be from using pre-made solutions instead of using freshly made solutions. Though the heart could not be seen in any of the zebrafish, either due to positioning on slide or debris from the water, there IHC did indicate the muscle that would be located in the tail. The primary antibody used was only to detect myosin-light chains and was not specific for cardiac muscle. That being said, either the antibody does not detect cardiac muscle, or the fixation process did not penetrate the tissues enough to allow the IHC to stain the tissue.

Future Direction

Since the reagents did not come in early enough to do everything that I hoped would be done this semester, I would like to continue this research with other antibodies and with an experimental group. I hope to raise embryos in cortisol and aldosterone to see the effects on cardiac development. I would also like to make fresh buffers and fixative solutions to see if that improves the results.

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