Localization of Sonic Hedgehog (Shh) Protein in Zebrafish

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Abstract

Zebrafish possess a unique ability to regenerate their retina when damaged, an ability that mammals lack. Zebrafish do this through a highly regulated cell signaling pathway, called the Sonic Hedgehog (Shh) pathway. This pathway utilizes the Shh protein that plays the role of directing cells to enter the cell cycle and differentiate into brand-new retinal cells. Previous research has shown that a wave of Shh plays this key role in signaling the progenitor cells to differentiate. Localizing the specific sites of the Shh protein during the signaling event brings us insight into the highly intricate signaling pathway of zebrafish. This new knowledge will help us understand the key differences between the human and zebrafish regenerative pathways, in hopes of one day being able to promote retinal cell regrowth in humans as a way to combat blindness.

Introduction

It's understood that in the process of retinal regeneration in Zebrafish, Müller glia cells are the source of new neurons in damaged retinal cells (Gemberling, et al., 2013). Müller glial cells start the process by dedifferentiation and turn into neuronal progenitor cells (NPCs). These cells proliferate and migrate into the area of damage and then differentiate into the appropriate type of cell to replace the previous damaged ones. In this way, zebrafish retinal regeneration is extremely specific. NPCs also have been found to express many of the genes that have been proven to be linked to retinal development (Gemberling, et al., 2013). These cells continue to proliferate until the Sonic Hedgehog protein (Shh) signals these cells to begin differentiating (Bailey & Hyde, 2010). Shh works in a wave-like fashion in which it signals cells to proliferate even more and it signals cells to exit the cell cycle and to begin differentiation into different neuronal cells.

Mammals' Müller glia cells do not have the ability to start a mass regenerative proliferation event. Even when transplanted, they differentiate into other cells and not the damaged ones. In learning the differences in signaling pathways between mammalian and zebrafish Müller glial cells, it could be understood why zebrafish signaling stimulates the Müller glial cells more than mammals as well as how they signal (Bailey & Hyde, 2010). With all the research and findings done, the location of Shh has still not been found during this wave-like event. The location of Shh can provide insight into what kind of cells have the Shh receptors, and potentially, these cells can be the key to understanding the signaling pathway of zebrafish.

The purpose of the following experiments is to attempt to get the Shh primary antibody to bind onto cryosections of damaged zebrafish eyes that have begun the process of regeneration. A secondary antibody will then be bound to the primary, however the secondary will be one that fluoresces when a certain wavelength laser has been shined on it. Then looking at the pictures taken under the laser, the location of the secondary antibody will be the one shining in a certain color, which will indicate the location of Shh.

Materials & Methods

- Sections of fisheyes on a microscope slide were first gone through an antigen retrieval protocol (Raymond Lab) where they were heated in a solution mix to allow for more binding of the antibody
- Sections of fisheyes on a microscope slide were take through an immunohistochemistry protocol with a blocking solution incubation, as well as a Primary antibody (Shh for all) and secondary antibody (varied) incubations.

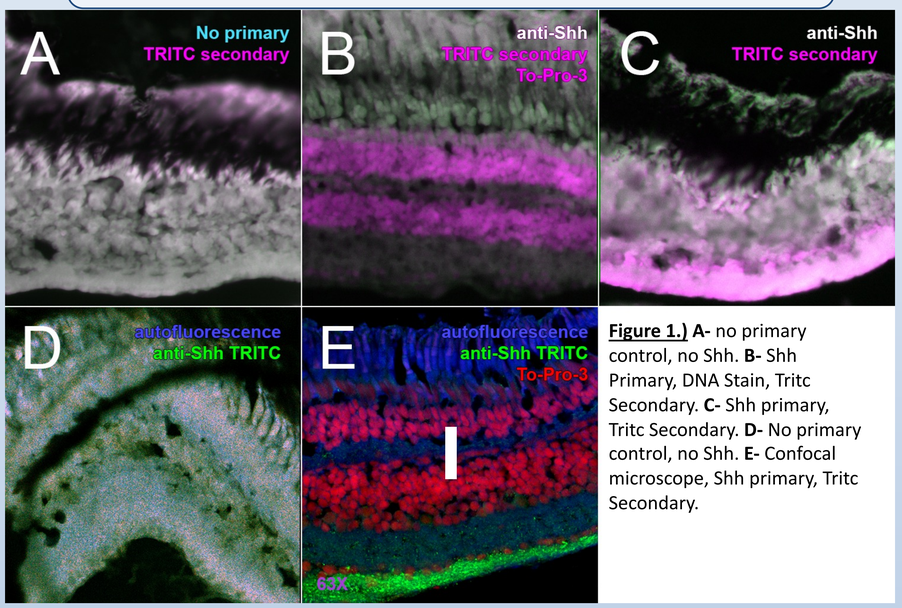

Figure 1.) **A-** no primary control, no Shh. **B-** Shh Primary, DNA Stain, Tritc Secondary. **C-** Shh primary, Tritc Secondary. **D-** No primary control, no Shh. **E-** Confocal microscope, Shh primary, Tritc Secondary.

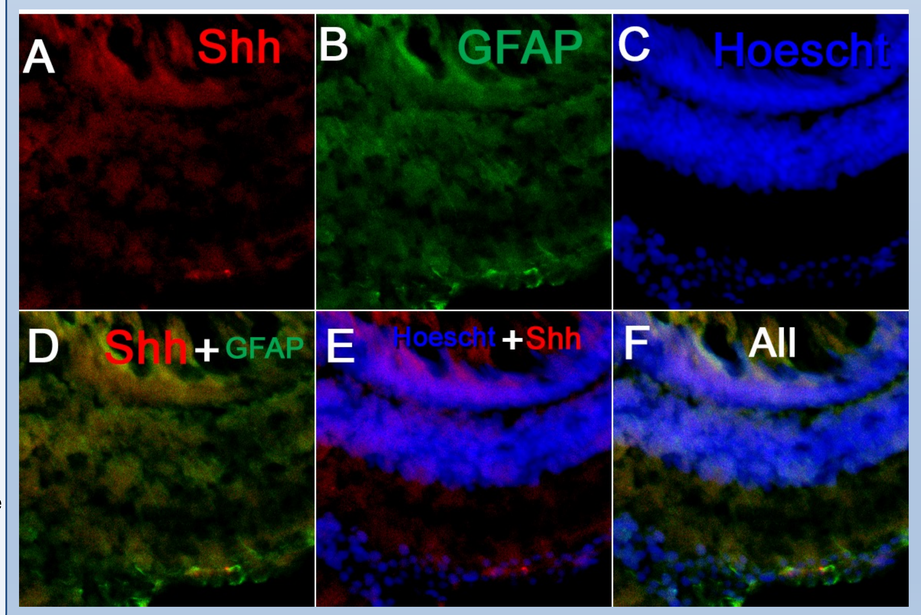

Figure 2.) **A-** Shh primary, Tritc. **B-** GFAP Primary **C-** Hoescht DNA stain **D-** Shh and GFAP primary **E-** Hoescht DNA stain and Shh primary **D-** Shh primary, GFAP stain, and Hoescht DNA Stain all together

Results

As seen in Figure 1 B,C and E, Shh was found in the outer nuclear layer. In figure 1 A and D served as the no primary control in order to assure that the primary antibody is binding specifically. The next figure shows our future goals, which is to identify which cells the Shh is binding to. In figure 2, is some over lap between the location of Shh and Muler Glial cells. However, further assure direct interaction between signal molecule and the cell.

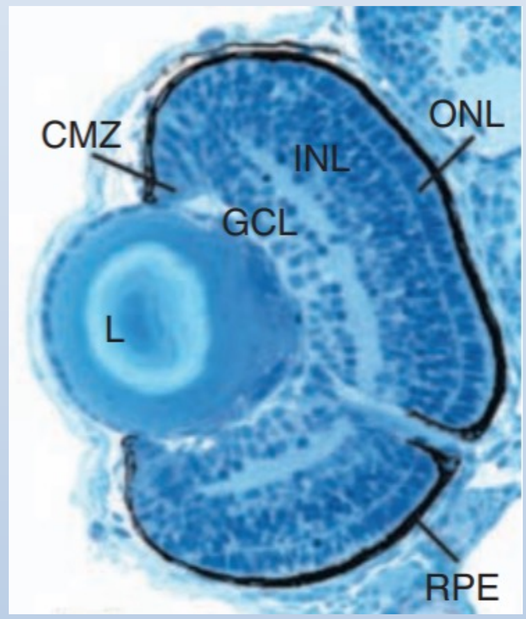

Figure 3 – Retinal Anatomy: CMZ, Circumferential marginal zone; ONL, Outer nuclear layer; INL, Inner nuclear layer; GCL, Ganglion cell layer; RPE, Retinal pigmented epithelium.

References

TJ Bailey and DR Hyde. Zebrafish: Retinal development and regeneration. 2010

Gemberling et al. The zebrafish as a model for complex tissuue regeneration. 2013

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