Localization of the Sonic the Hedgehog (Shh) protein in Zebrafish retinas

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Abstract:

Unlike most vertebrates, zebrafish possess the unique ability to regenerate organ tissues and retinas when damaged. Understanding this regenerative process could be the key to assisting in preventing and healing damaged retinal cells in humans. Zebrafish can regenerate damaged retinal cells when proliferating progenitor cells re-entering the cell cycle and then differentiate into new retinal cells. The differentiation of these cells is highly regulated by many signaling pathways. One such pathway is the Hedgehog (Hh) signaling pathway. It has been shown that Sonic Hedgehog protein (shh) of the Hh pathway plays a significant role in the differentiation of progenitor cells during embryotic development and retinal healing in zebrafish. Localization of the shh protein can help us better understand where the shh protein is expressed in healthy retinas and then in damaged retinas. Analyzing the differences in location among samples can help identify which regions of the retina are predominantly regulated by shh.

Background/Introduction:

Hedgehog (Hh) signaling pathway, Hh, is one of the few signaling pathways that is used during development for intracellular communication (1). Moreover, Sonic Hedgehog (Shh), which is one of three mammalian Hh proteins (1), plays a role in the differentiation of progenitor cells during eye development in Zebrafish (2). Differentiation, which is “the process during which young, immature (unspecialized) cells take on individual characteristics and reach their mature (specialized) form and function.” (3), plays a key role in regeneration.

Studies have shown that Shh drives a wave of neurogenesis across the retina (2). Their results showed that “that Shh is both necessary and sufficient to control a wave of its own expression that sweeps through the GCL.” (2) This wave of neurogenesis allows for the differentiation of cells. While, another study analyzed the relationship between the membrane protein, Growth Arrest-Specific Gene (Gas1), and Hh concentration gradient. This study was conducted in hopes of determining if there is a correlation between the concentration gradient and activity gradient. Although this study's focus was different, focusing more on Gas1 relationship with Hh signaling, rather than the localization of shh protein itself; it provided key information. Showing that shh concentration is pivotal in terms of development. They demonstrated inhibitory effects occurred with lower shh concentrations (3); our goal is to expand upon these studies, and determine the location and relative concentration of Shh proteins.

Our research group utilized the Kassen Immunoblot from microarray paper Western blotting technique in order to obtain relative concentration values of shh. Using eight wild-type, non-damaged, adult zebra fish retinas; as well as eight UV damaged retinas, during the validation procedure. In addition, we used a Polyclonal Antibody (PA5-116416) from ThermoFisher Scientific as the primary antibody, and HRP-conjugated anti-rabbit IgG antibody as the secondary to detect the shh protein.

Methods:

8 UV damaged retinas and 8 healthy retinas were collected at the beginning of the experiment. We then prepared the retinas into a lyse buffer solution. Following preparations of the retina solution we treated them with the following protocols:

-Ran the protein solutions through a Kassen Western Blot
-A wet transfer of the gel was complete and transferred onto a PVDF membrane
-The PVDF membrane was blocked with Milk Blocking Buffer
-Then treated with the primary (Polyclonal) and secondary (anti-rabbit IgG) antibodies
-Put the ECL, a western blotting substrate from Pierce and was viewed in Vision works detection system

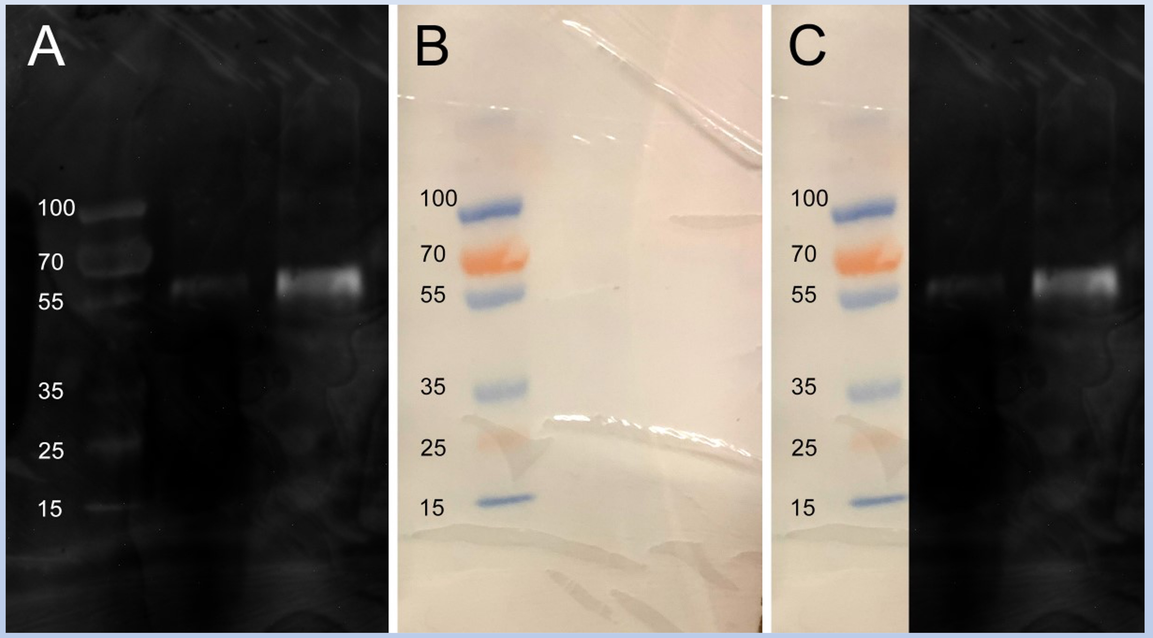
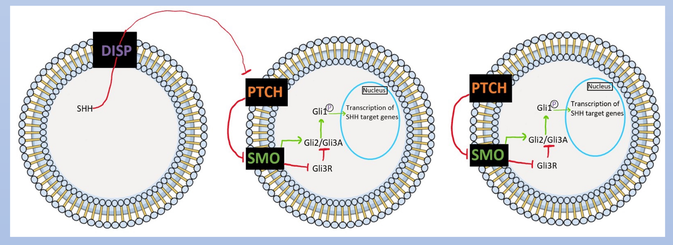
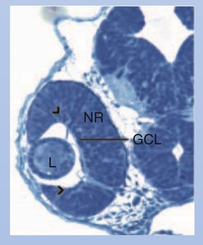
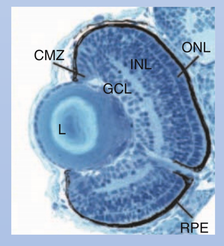


Figure 2: Western blot PVDF membrane shows the presence of tubulin proteins present in the samples. The length of the segments were measured in kDa.







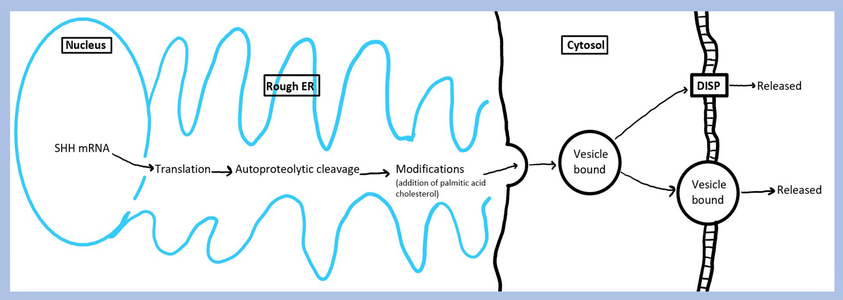


Figure 1: The Shh pathway from RNA to protein expression within Zebrafish retinas.

Results/Conclusion:

We performed the western blot, membrane transfer, and blocking procedure. After completion of the blocking, the membrane was treated with a shh polyclonal antibody. Then the membrane was treated with ECL blotting substrate for one minute then immediately placed in the Vision works viewing system. After 80µ sec of exposure, there was no visible lines detected. The membrane was then exposed for ten minutes and still no bands were detected. A follow up experiment was performed using an actyletublin antibody to determine if protein was present on the membrane. A similar 80µ sec exposure was performed with no bands being detected. The membrane was then exposed for 10 minutes and bands were detected (Fig 2). The tubulin protein, which is ~50 kDa, was detected on the membrane.

Follow up experiments will be conducted using a more concentrated retinal solution. Increasing the concentration should allow for a better chance of binding for the shh polyclonal antibody. If a positive binding result of the shh antibody is confirmed, further experiments will be carried out on cross sections of health, damaged, and embryotic zebra fish retinas.

Citations/ Acknowledgements:

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[1] TJ Bailey and DR Hyde. Zebrafish: Retinal development and regeneration.
2010.
[2] Adler Keith and Miller Morgan. sonic hedgehog protein(shh).
[3] Alena Shkumatava, Sabine Fischer, Ferenc Muller, Uwe Strahle, and Carl J Neumann. Sonic hedgehog, secreted by amacrine cells, acts as a short-range signal to direct differentiation and lamination in the zebrafish retina. 2004.