Analysis of *her4.1* in *gef* mutants

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Abstract

Zebrafish make an ideal model organism for studying the development of the retina and can help us understand and eventually create solutions for human eye diseases. The *good effort* (*gef*) mutant zebrafish have smaller eyes compared to the wild-type zebrafish embryos at 3 days post-fertilization. This is due to retinal degradation because of the lack of a functioning Chaf1b protein. The Chaf1b protein-coding region is disrupted due to the deletion of its coding intronic DNA which causes an exon to be lost. Chaf1b is important to the cell because it makes up one of the three parts of the chromosome assembly factor 1 (CAF-1). CAF-1 functions to regulate chromatin and load histones onto DNA, but cannot function without Chaf1b. While it has been hypothesized that Tp53-mediated apoptosis is responsible for the *gef* mutants' small eyes, *tp53*-morphants and homozygous *gef* double mutants have been studied and the cell death seen in *gef* mutants is not correlated with Tp53 activity. Signaling pathway members Her4.1 and Ascl1a, promote retinal cell fate development. Histone deacetylases selectively regulate *her4.1* and *ascl1a* mRNAs during retinal development, making these genes possible affected genes of the *gef* phenotype. *Her4.1* was visualized in *gef* and wild-type zebrafish embryos via the *in situ* hybridization process.

Introduction

The *good effort* (*gef*) mutant zebrafish are characterized by a small-eye and a lack of proper retinal development because of cell death. Adult zebrafish on the other hand, are capable of regeneration of established retinas due to the activity of Müller glial cells. Our lab found that this cell death is not caused by Tp53-mediated apoptosis as proposed, suggesting other factors. We tested whether dysregulation of developmental genes are correlated with cell death. Genes involved in cell-fate specification and proliferation of zebrafish eyes include *her4.1* and *ascl1a*. Expression of Histone deacetylase 1 (hdac-1) is first inhibited in development so that cells can proliferate, inhibiting *her4.1* in order to do this. Later, Hdac-1 is turned off by *her4.1* so that differentiation of cells can occur. Along with this, Hdac-1 also inhibits *ascl1a*. *Ascl1a* stimulates cell proliferation via increased protein Lin28a production (figure 2). These genes can be visualized and interpreted using in situ hybridization. In a *gef* mutant, differing levels of *ascl1a* and *her4.1* would be expected from the wild type. At 52 hours post fertilization (hpf), a wild type embryo has decreased *ascl1a* expression and decreased *her4.1* expression. In situ hybridization technique was performed to visualize gene expression. *Ascl1a in situ* hybridization results failed to stain for expression.

Results

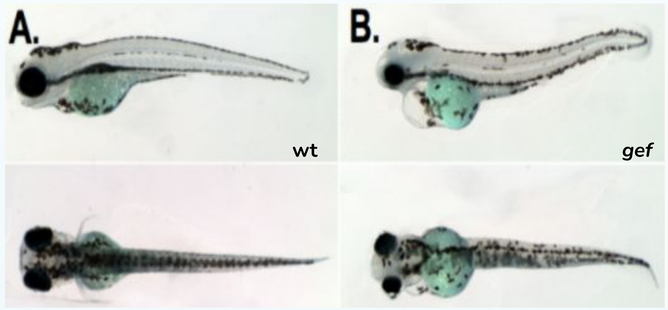

Figure 1. A) dorsal (above) and lateral (below) views of the wild type zebrafish at 3-days old characterized by large eyes. B) dorsal (above) and lateral (below) views of *gef*-mutant zebrafish, characterized by the small-eye phenotype compared to wild-type embryos.

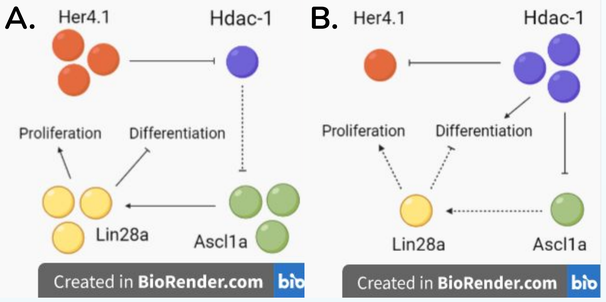

Figure 2. Schematic of the proposed protein activity changes during retinal development. A) Increased Her4.1 expression leads to decreased Hdac-1 expression and increased Ascl1a expression which in turn increases Lin28a expression. This inhibits differentiation while stimulating proliferation. B) Her4.1, Ascl1a, and Lin28a activities are repressed when Hdac-1 is expressed, causing cells to differentiate.

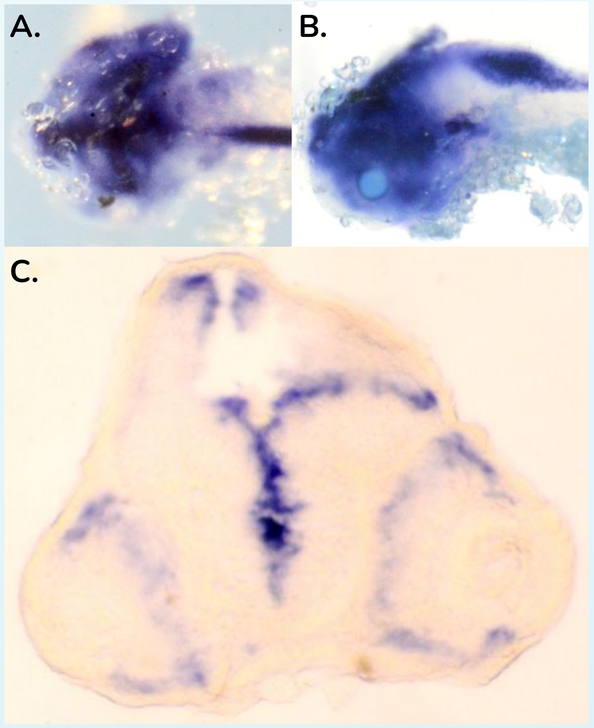

Figure 3. A-C show *in situ* hybridization staining results of *her4.1* expression in wild-type zebrafish embryos at 52 hpf. There is minimal purple staining proximal to the lens, while the rest of the retina shows generalized staining. A) dorsal view of a whole mount. B) left lateral view of a whole mount. C) 15 µm transverse cryosection showing specific staining in the retinas and brain.

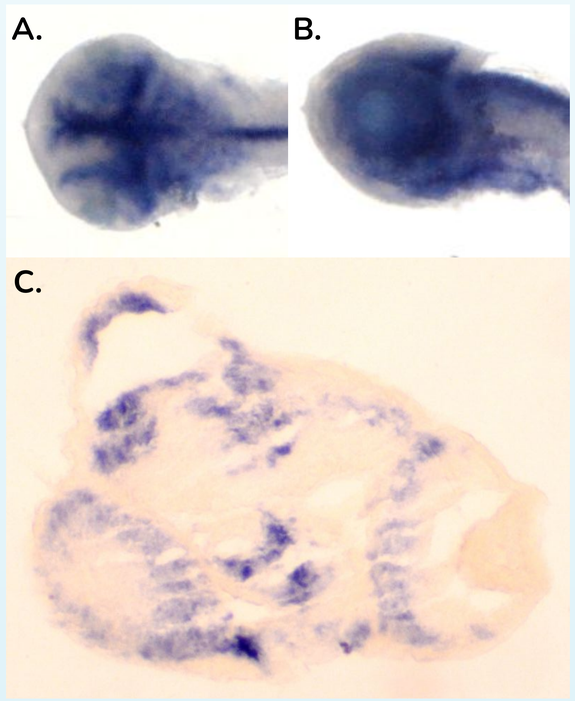

Figure 4. A-C show *in situ* hybridization staining results of *her4.1* expression in *gef*-mutant zebrafish embryos at 52 hpf. There is minimal purple staining proximal to the lens, while the rest of the retina shows generalized staining. A) dorsal view of a whole mount. B) left lateral view of a whole mount. C) 15 µm transverse cryosection showing specific staining in the retinas and brain.

Methods

At 52 hpf, zebrafish embryos were acridine-orange stained to visualize cell death, to distinguish mutant and wild-type. *In situ* hybridization technique was used to visualize the amounts of *her4.1* and *ascl1a* present using complementary RNA probes. After successful *her4.1* staining, whole mount pictures were taken. Zebrafish were then cryosectioned in 15 µm transverse slices and results were photographed.

Conclusion

The results from the *in situ* hybridization and cryosection images visualizing *her4.1* show similar, specific staining in the retina. This suggests that Her4.1 protein levels are not significantly different in the *gef*-mutant retina compared to wild-type embryos. Since the wild-type and *gef* embryos have similar results, we can conclude that the cell death and the small eye phenotype in *gef* mutants is independent of *her4.1*. This suggests that certain notch signaling pathway members are not involved in the *gef*-mutant phenotype, since others have been analyzed previously.

Further experiments could include making probe for sonic hedgehog signaling pathway genes and continuing *in situ* hybridization and cryosection analysis of these genes. Sonic hedgehog genes are involved in differentiation pathways. This could help determine the cause of cell death seen in the *gef* mutant that is unrelated to the p53 pathway.

Acknowledgements

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References

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