Analysis of *her4.1* and *ascl1a* in *gef* Mutants

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Abstract

Zebrafish are a model organism for studying developmental abnormalities, especially in the eye. The *good effort* (*gef*) mutant zebrafish have smaller eyes than wild-type embryos due to rapid retinal degeneration that becomes apparent only after two days post fertilization. The genetic problem arises from a deletion of intronic DNA sequence which leads to the loss of an exon and disrupts the coding region of the Cha1b protein. Chaf1b is a subunit of the Chromosome assembly factor 1 (CAF-1), a complex of three proteins which has a role in histone loading and chromatin regulation. This small eye phenotype has been hypothesized to be due to Tp53-mediated apoptosis, however, phenotypic analysis from complex *tp53*-morphants or *tp53*^{zdf1} mutants and *gef* mutants suggests that the cause of cell death is not Tp53 dependent. Instead, we hypothesize that these issues are due to faulty signaling pathways, such as her4.1 and ascl1a. These specific signaling molecules are involved in retinal cell fate specification. Both of these molecules are under direct control of histone deacetylases which selectively regulate both genes during retinal development. Differences in *her4.1* and *ascl1a* expression levels between *gef* and wild-type zebrafish embryos were analyzed by *in situ* hybridization.

Introduction

These genes were chosen based on their roles in cell fate specification and proliferation. Her4.1 is a protein that inhibits expression of hdac1, a protein that controls a zone of regeneration, causing cells in proximity to differentiate (Mitra et al. 2018). Interestingly, hdac1 behavior changes based on whether cells need to proliferate or if they are in a stage known as post-proliferation. Analysis of the genes was done via in situ hybridization. We hypothesize that her4.1 will be downregulated in *gef* mutants, and ascl1a will be upregulated. Her4.1 inhibits cells from undergoing proliferation. In this scenario, a decrease in *her4.1* levels will induce differentiation in areas where it shouldn't occur. This would then lead to the upregulation of *ascl1a*, a gene that would stimulate proliferation. During development cells would be induced to differentiate, possibly when they shouldn't be, thus leading to this cell death.

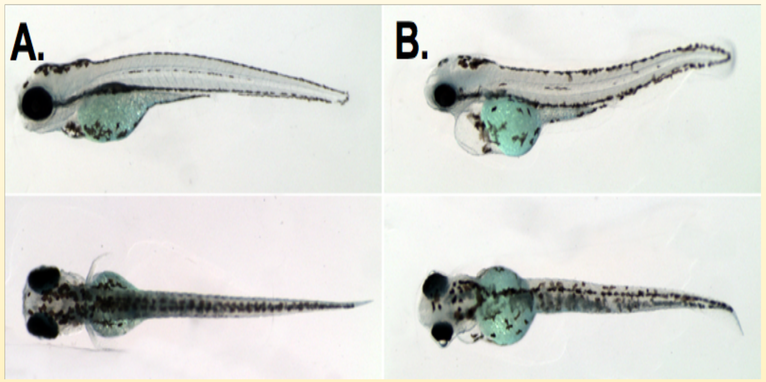

Figure 1. Lateral (top row) and dorsal (bottom row) images of embryos at three days post fertilization (3 dpf). **A)** This embryo exhibits the wild type phenotype, demonstrated by its large eyes. **B)** This embryo exhibits the *gef* phenotype, including abnormally small eyes. Due to the stunted retinal development, the lens can also be seen protruding in the *gef* mutants.

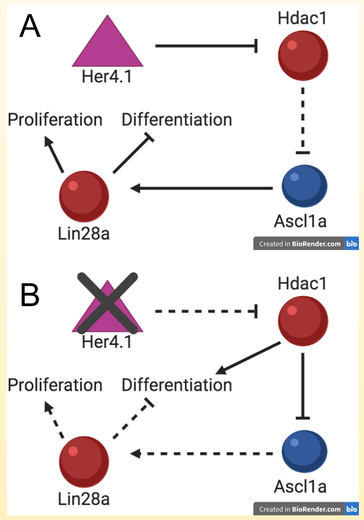

Figure 2. This schematic summarizes the genetic pathway of Lin28a protein expression. Production of Lin28a protein prevents cell differentiation and induces proliferation. A is the proliferation pathway (early development) and B is the differentiation pathway (late development). Early in development Her4.1 inhibits Hdac1 so that Ascl1 can stimulate Lin28a to stimulate proliferation and inhibit differentiation. Late in development Her4.1 expression is decreased, this leads to Hdac1 inhibiting Ascl1a and stimulating differentiation.

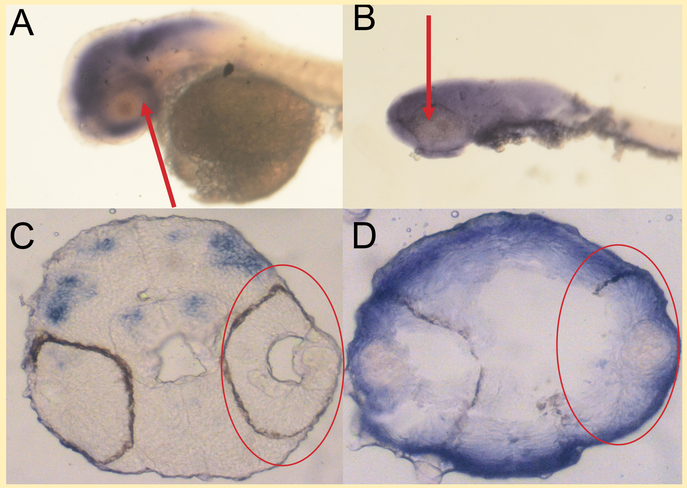

Figure 3. A –B: Photographs of wholemount 2 dpf embryos stained for *ascl1a* via in situ hybridization. C – D: Images of 10 µm cryosections of 2 dpf, embryos, stained via in situ hybridization. **A, C)** Wild type embryo. **B, D)** A *gef* mutant. The arrows and circled areas indicate important retinal differences in staining.

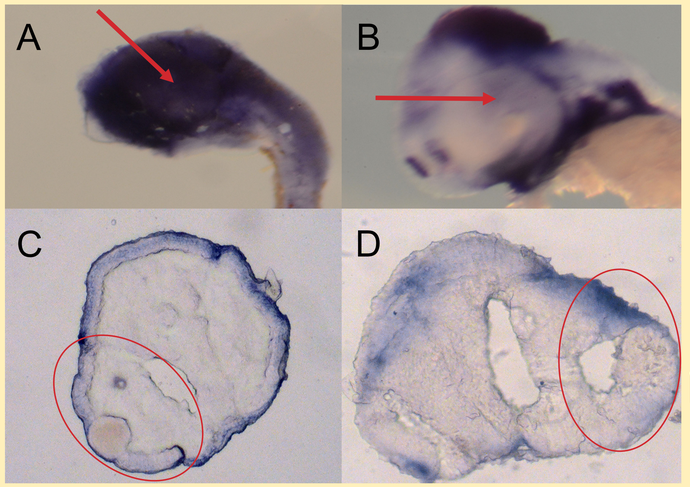

Figure 4: A –B: Photographs of wholemount 2 dpf embryos stained for *her4.1* via in situ hybridization. C – D: Images of 10 µm cryosections of 2 dpf embryos, stained via *in situ* hybridization. **A, C)** Wild type embryo. **B, D)** A *gef* mutant. The arrows and circled areas indicate important retinal differences in staining. The lighter staining in C could be interpreted as the stain not penetrating.

Conclusion

The lack of expression of *ascl1a* in wild-type retinas indicates *lin28a* cannot be transcribed. When *lin28a* is produced, cell fate specification is inhibited. However, expression of *ascl1a* in the *gef* mutant retinas suggest production of *lin28a*. Therefore, this would promote proliferation in the *gef* mutant retinas. These two findings are the opposite of what we originally thought as it suggests that the wild type embryos do not promote proliferation in their eye tissue while the *gef* do promote it.

Little to no expression of *her4.1 in* wild type retinas means that there is no inhibition of *hdac1*. This allows for hdac1 to be produced, so cell fate specification is promoted. At this stage in development, this is the appropriate regulation of the pathway. Conversely, expression of *her4.1* in the *gef* mutant retina suggests her4.1 is blocking the transcription of *hdac1*, and therefore is inhibiting cell fate specification in the eyes. These results demonstrate, both Ascl1a and Her4.1 are upregulated in *gef* mutants leading to unnecessary proliferation and apoptosis. Due to these regulation patterns, *hdac1* is strongly repressed leading to inappropriate signaling in *gef* mutants during this stage of development. This being the reason for the cell death seen in *gef* mutants.

Acknowledgements

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References

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Mitra, S., Sharma, P., Kaur, S.P., Khursheed, M.A., Gupta, S., Ahuja, R., Kurup, A.J., Chaudhary, M., & Ramachandran, R. (2018). Histone Deacetylase-Mediated Müller Glia Reprogramming through Her4.1-Lin28a Axis Is Essential for Retina Regeneration in Zebrafish. *iScience*.