The Role of *Sema3E* in Optic Fissure Closure of the Eye in Zebrafish

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Introduction

Microphthalmia, anophthalmia, and coloboma are significant birth defects causing up to ten percent of childhood blindness that can be identified in two per ten thousand newborns. Up to seventy percent of microphthalmia, anophthalmia, and coloboma are associated with optic fissure (OF) closure defects and fall into a category of developmental eye defects.

The gene, *Sema3E*, has been associated with colobomas in two unrelated children. *Sema3E* is a member of the class three semaphorins (Sema3s). Sema3s are a family of molecules known to direct cell movement (Callander et al. 2007). **The objective of this project is to investigate the role of *Sema3E* in eye development, specifically in eye size and OF closure.**

Zebrafish eyes are genetically and morphologically similar to human eyes, and zebrafish develop rapidly and externally. These characteristics make zebrafish prime candidates for studying the genetics and cell biology of eye development *in vivo.* Figure 1 displays the developmental time line of zebrafish vision.

Figure 1:

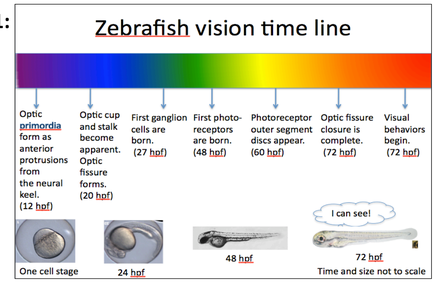
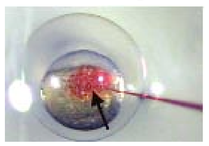


Figure 2: Microinjection at one cell stage.



Bill et al. 2009

Methods

To test the function of *Sema3E*, zebrafish embryos were microinjected at the one cell stage with a morpholino oligonucleotide (MO). MOs bind messenger RNA and inhibit translation through a steric blocking mechanism.

Figure 2 shows a microinjection being performed at the one cell stage of development. Two *Sema3E* MO doses, 4ng and 6ng, were injected into embryos and compared to un-injected wild-type (WT) embryos.

The embryos were observed at 24 hours post fertilization (hpf) to assess the viability after MO treatment. Observations were then made at 48 hpf and the computer programs AxioVision and ImageJ (NIH) were used to image, collect eye size measurements, and measure the green fluorescent protein (GFP) signal.

Results

Sample Type Representatives for Eye Size Reduction

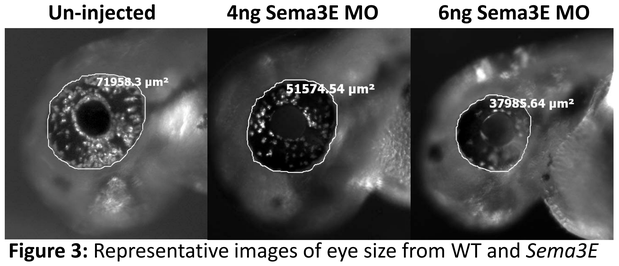


Figure 3: Representative images of eye size from WT and *Sema3E* knockdown embryos. Embryos are 48 hpf.

Sema3E MO Induces Eye Size Reduction

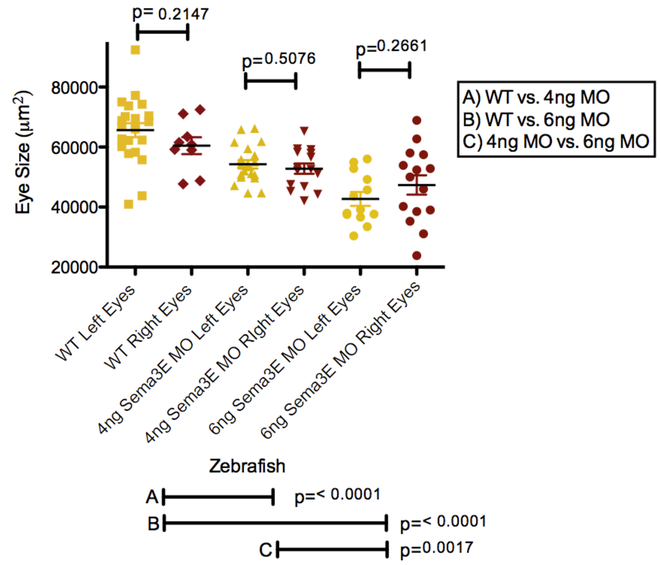


Figure 4: Each eye measurement represents an individual zebrafish taken at 48 hpf. Left and right eyes were measured from each sample type of zebrafish (WT, 4ng Sema3E MO, and 6ng Sema3E MO) to demonstrate that there is no significant size difference between left and right eyes within each sample type. Importantly, eye size was significantly reduced in *Sema3E* knockdown embryos compared to WT controls. Additionally, the significant eye size reduction was MO dose dependent.

Sample Type Representatives for GFP Signal

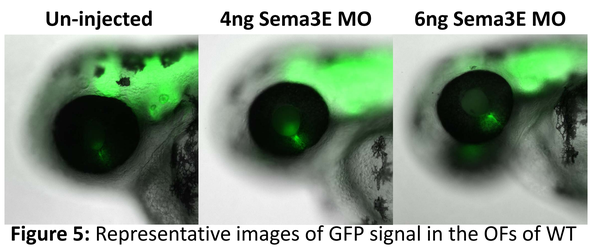


Figure 5: Representative images of GFP signal in the OFs of WT and *Sema3E* knockdown embryos. Embryos are 48 hpf.

Sema3E MO Increases High GFP Signal Frequency

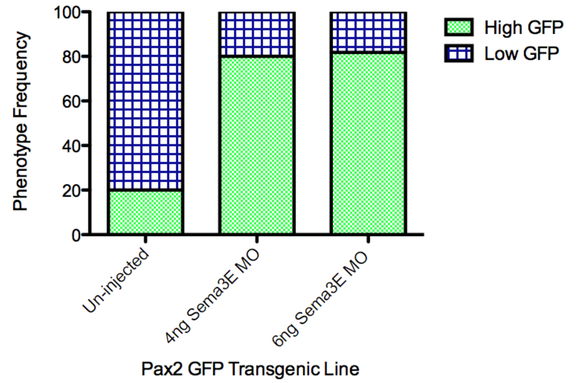


Figure 6: The percentage of embryos in each sample with a high or low GFP signal in the OF was determined. Embryos were classified as high or low GFP signals by comparing them to the appropriate representative image (see Figure 5). A greater proportion of *Sema3E* knockdown embryos have higher GFP signals than un-injected WT embryos.

Conclusion/Discussion

✦Loss of *Sema3E* results in microphthalmia.

✦*Sema3E* is required for correct developmental optic fissure closure.

Current/Future Research

Retinal layers of 72 hpf zebrafish eye are being examined by immunohistochemistry and hematoxylin and eosin (H&E) staining to understand the mechanism(s) by which *Sema3E* is involved in eye development.

Acknowledgements

•Jessica Gergen at the University of Minnesota Zebrafish Core Facility for zebrafish care and embryo collections.
•Shou Lin at UCLA for providing the Pax2 GFP transgenic line.

References

Callander, Davon C., Ryan E. Lamont, Sarah J. Childs, and Sarah McFarlane. 2007. Expression of multiple class three semaphorins in the retina and along the path of zebrafish retinal axons. *Developmental Dynamics* 236, no. 10 (10): 2918-2924. doi:10.1002/dvdy.21315.

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Funded by Developmental Eye Defects, NIH NEI/5R01EY01926702

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