

UVM ScholarWorks

Fgfr1b is Required for Proper Retinal Development in Zebrafish

Item Type	undergraduate thesis
Authors	Davin, Ryan Thomas
Download date	2026-06-05 23:03:21
Item License	http://creativecommons.org/licenses/by-nc-nd/3.0/
Link to Item	https://hdl.handle.net/20.500.14849/5573

Fgfr1b is Required for Proper Retinal Development in Zebrafish

Ryan Thomas Davin

Undergraduate Honors Thesis

UVM College of Arts and Sciences, Department of Biology

May 1st, 2024

Thesis Committee:

Alicia M. Ebert, Ph.D., Advisor

Paula Deming, Ph.D., Chairperson

Bryan A. Ballif, Ph.D.

Table of contents

Abstract	3
Chapter 1: Introduction.	4
Chapter 2: Methods.	12
Chapter 3: Results.	15
Chapter 4: Discussion.	18
Acknowledgements	23
References	24

Abstract

Cellular communication, largely accomplished through the binding of ligands to cell receptors, is essential to proper embryonic development. Fibroblast growth factors (FGF) are one class of these signaling molecules. Previous work in the Ebert Lab investigated Fgf8a in zebrafish at 48 hours post fertilization (hpf), finding that loss of its function leads to smaller eyes with fewer retinal cells and mispatterned retinal vasculature. The receptor being utilized was unknown, with there being five possibilities (Fgfr1a, 1b, 2, 3, and 4). Co-localization of expression using *in situ* hybridization suggested the most likely candidate is Fgfr1b. This study sought to further elucidate the role of Fgfr1b in retinal development through Fgf8a signaling. We hypothesized that Fgf8a from retinal ganglion cells, is acting through Fgfr1b on vasculature to promote blood vessel branching and proper retinal development, predicting that a loss of Fgfr1b would phenocopy loss of Fgf8a. We found *fgfr1b* mutant phenotypes resemble those of *ace* mutants lacking Fgf8a, suggesting Fgf8a is likely signaling through Fgfr1b at this point in development.

Chapter 1: Introduction

Cellular differentiation is a complex process facilitated by a vast network of signaling pathways. Cellular communication is the basis for migration and differentiation of cells, making it essential to proper development of tissues. This communication is largely done through the release and reception of ligands, either from neighboring cells or adjacent tissues. Typically, ligands released from a cell's surface are bound by their complementary receptor on another cell. This binding triggers signaling cascades beneath the membrane of the target cell, often promoting cell division, migration, changes to gene expression, and consequently cell function.

Fibroblast growth factor signaling

The Fibroblast growth factors (FGFs) are a large family of heparin-binding polypeptide growth factors that signal through four different FGF receptor tyrosine kinases (FGFR1-4). Each FGF binds preferentially to some receptors over others.¹⁴ The binding of FGFs to FGFRs can induce many pathways, such RAS, MAPK, PI3K, and PLC γ 1 which regulate proliferation, survival and cell motility (Figure 1.1).⁶ FGFs have been observed to play crucial roles in many aspects of embryonic development such as gastrulation, limb patterning, and nervous system development.² For example, mutant mice lacking FGF2 have disrupted cerebral cortex development. FGF8 and FGF17 proteins were found to be key molecules in cerebellum development. Mutations in FGF receptors similarly have a wide range of consequences. A mutation of FGFR2, for example, is lethal even prior to mature CNS formation.⁴

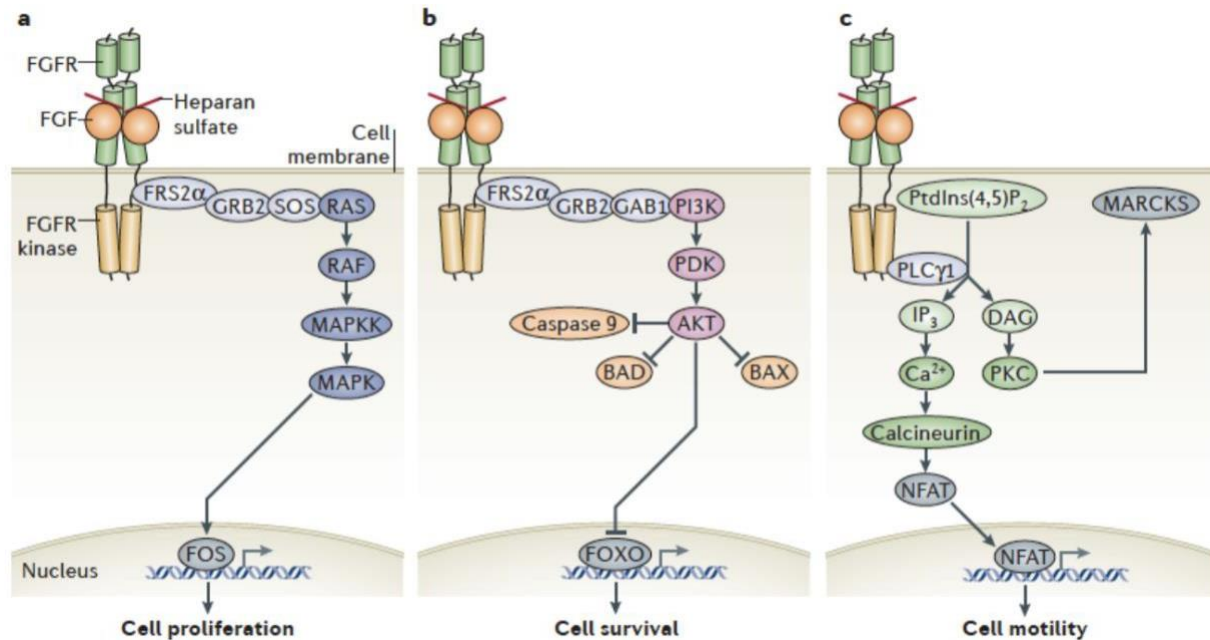


Figure 1.1. FGF signaling pathway. Binding of FGF to the FGF tyrosine kinase receptor induces (a) cell proliferation through RAS/MAPK, (b) cell survival through PI3K, and (c) cell motility through PLCγ1.⁶

The zebrafish model for eye development

This project investigated FGF signaling related to retinal development in zebrafish (*Danio rerio*), as zebrafish are an ideal model organism for studying the eye. External fertilization, large clutch sizes, and rapid development allow for high experimental numbers to be obtained in relatively short amounts of time. All major organ systems are present by 72 hours post fertilization (hpf), and sexual maturity is reached in just three months, making it possible to observe a mutant line over many generations.⁸ Embryos are transparent and with the use of transgenic lines, specific cell/tissue types can be visualized in a live animal throughout development. Because of the strong conservation of the vertebrate genome, and the genetic similarity of zebrafish to humans, much of what is learned using this model can be applied to humans. Of particular importance to our study is that the zebrafish retina and related vasculature pattern nearly identically to human eyes.

The intricate formation of the retina seen in both zebrafish and humans requires precise differentiation and migration of cells. Eye development begins with the evagination of two optic vesicles from the neural plate of the forebrain. Contact with the overlying ectoderm induces the formation of the lens placode, which gives rise to the lens and later the cornea. Invagination of the optic vesicles results in a bilayered optic cup, which forms the inner neural retina and outer retinal pigmented epithelium (RPE).¹¹ The retina consists of six types of neurons, these being retinal ganglion cells (RGCs), amacrine cells, bipolar cells, horizontal cells, cones and rods, as well as one type of glia, the Müller glia which arises later in development.¹¹ Multipotent progenitor cells simultaneously give rise to these various cell types while migrating to their final destination, resulting in a tri-layered retina (Figure 1.2).¹¹

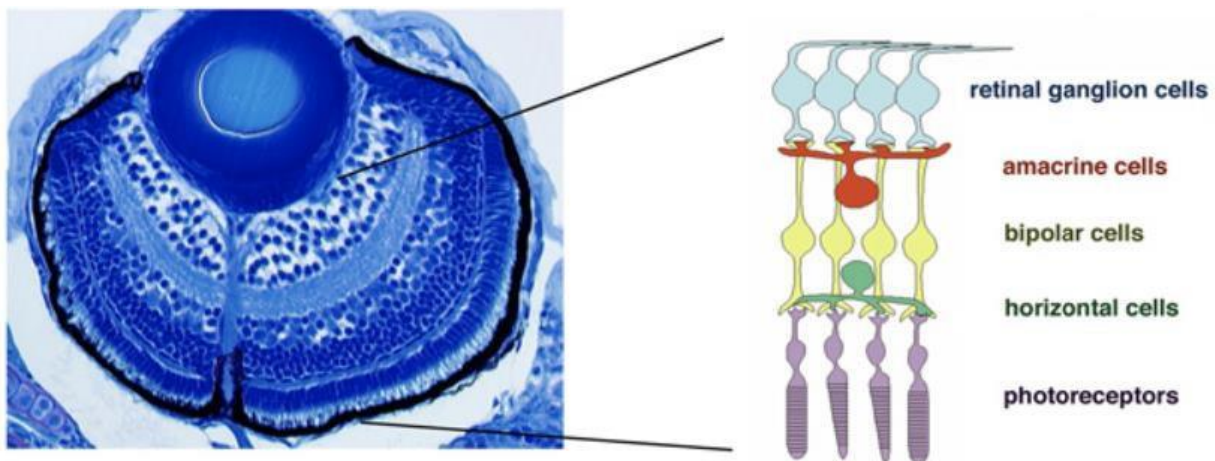


Figure 1.2. Retinal layering in zebrafish. Multipotent progenitor cells give rise to six types of retinal neurons which migrate to form three cellular layers within the retina. The first layer, closest to the lens, houses the retinal ganglion cells (RGC). A layer of interneurons known as amacrine cells sits between the RGC and the preceding layer of bipolar cells. Next there is a layer of horizontal cells, which rests adjacent to the photoreceptor cells.¹⁸

The timing of retinal development corresponds with the invasion of early ocular vasculature at key points in development.³ The choroidal vasculature makes a ring around the optic cup posterior to the retina while the hyaloid vasculature is positioned anteriorly between the lens and the retina.⁷ The hyaloid artery is the earliest component of the retinal vasculature, entering the eye at 18-20 hpf concomitantly with retinal neurogenesis (Figure 1.3).⁷ Vasculature development continues to overlap with key phases of retinal development suggesting the two processes may be influenced by one another.³

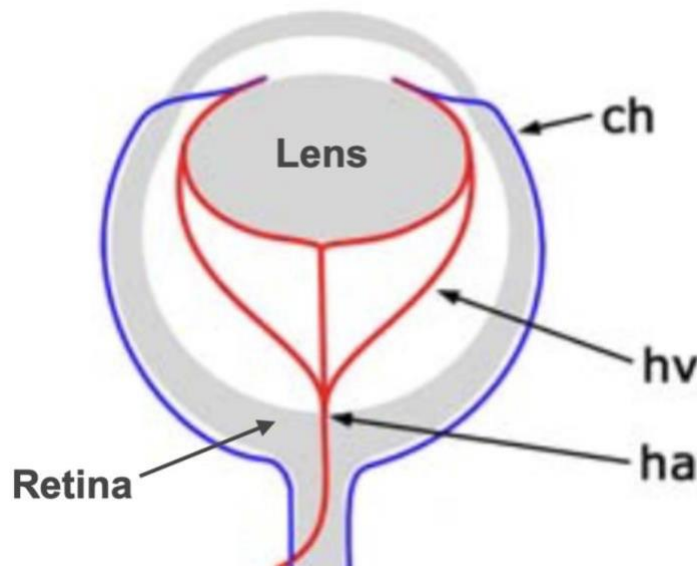


Figure 1.3. Vasculature of the developing eye. Dorsal View. The hyaloid artery (ha) supplies the hyaloid vasculature (hv) posterior to the lens, draining anterior to the lens through the choroidal ring (ch), which flows posterior to the retina. Adapted from (Fruttiger, 2007) and created with BioRender.com.

Vasculature and the developing eye

Further understanding of this vasculature is essential to elucidating its complex relationship with the adjacent, developing eye tissue. Zebrafish are a widely used model for vascular studies due to their ability to survive up to seven days without active blood flow.¹⁸ The silent heart (*sih*) mutant lacks cardiac troponin-T and thus a heartbeat, allowing it to be used in observation of the effects of blood flow, or lack of, on the developing eye. It was found that blood flow was essential for retinal proliferation and cell differentiation³. Through the use of transgenic lines, *nsfB* of *Escherichia coli* can be expressed within different cell types, encoding a nitroreductase (NTR)

enzyme. Metronidazole (Met) targets cells expressing NTR, allowing for specific cell types to be ablated. Through ablation of the endothelial vasculature itself with Met, more severe defects were observed compared to *sih* embryos, suggesting that endothelial cell-derived factors are required for proper laminar organization and development of retinal cells.³ The exact factors mediating these developmental processes remain uncertain.

Fgf8 signaling

Interestingly, the acerebellar (*ace*) mutant, lacking Fgf8, shows a remarkably similar phenotype to the *sih* mutant and the endothelial cell-depleted embryos. Previous research showed Fgf8, working in conjunction with Fgf3, is essential for proper patterning of the lens of the eye and initiation of differentiation in the retina at 24 hpf.⁹ This aligns with mRNA expression of *fgf8a* observed in the zebrafish retina at 24 hpf (Figure 1.4 A-B). Important to note, at this stage in retinal development, loss of Fgf8 alone did not cause significant phenotypes. When both Fgf8 and Fgf3 are lost, retinal ganglion cells (RGCs), which form the optic nerve and relay the light signals from the retina to the optic tectum, do not form.⁹ Further investigating the role of Fgf8 in retinal development, the Ebert Lab found through *in situ* hybridization that *fgf8a* is re-activated at 48 hpf. This expression is localized on retinal ganglion cells, which were tagged with green fluorescent protein for visualization. This suggests further involvement in development than what was previously observed at 24 hpf (Figure 1.4 C-D).

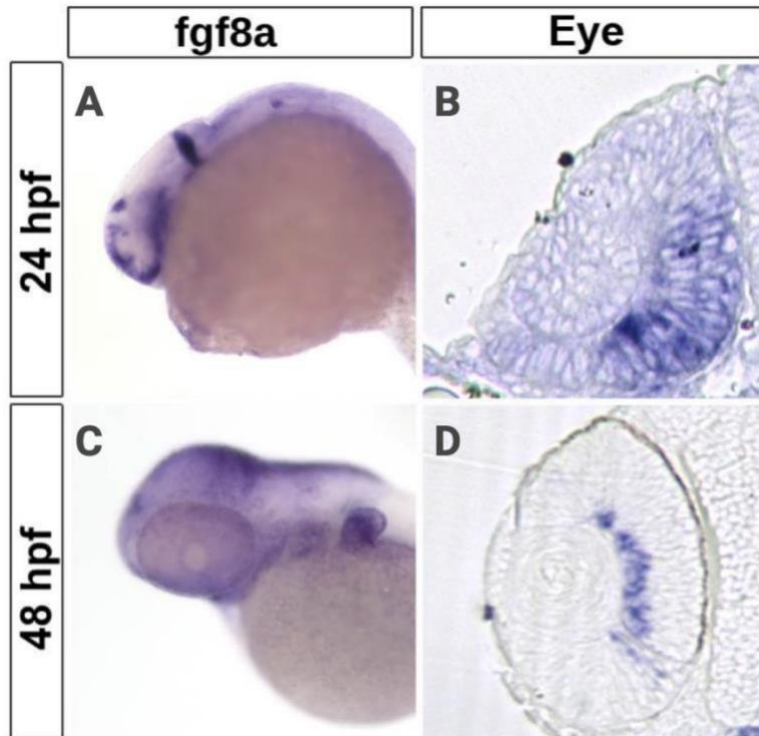


Figure 1.4. Retinal expression of *fgf8a*. *In situ* hybridization of *fgf8a* is shown at 24 (A, B) and 48 hpf (C, D). Lateral view (A, C) and transverse cross section through the eye (B, D) (Ebert Lab, unpublished).

At 48 hpf *ace* mutants had smaller retinas and mispatterned retinal vasculature leading to no blood flow through the eye (Figure 1.5). The smaller eyes were due to significant decreases in cell division.¹⁸ This preliminary data suggests Fgf8 signaling is essential for retina development and retinal vasculature patterning, however, the receptor remains unknown.

Zebrafish possess two copies of *fgfr1* (*fgfr1a* and *fgfr1b*) due to an ancestral duplication, meaning there are a total of five possible FGF receptors with which Fgf8a could be binding (Fgfr1a, 1b, 2, 3, and 4).¹³ The Ebert Lab observed expression patterns of these receptors through *in situ* hybridization to determine likely candidates. The results demonstrated that only *fgfr1b* is expressed highly in the retina at the time *fgf8a* is expressed in RGCs. This is also the preferred binding partner of Fgf8.^{2, 10} Other receptor genes (*fgfr1a*, *fgfr2*, *fgfr3*, and *fgfr4*) do not show as strong expression, if any, in the eye at 48 hpf, making it less likely that they are involved in eye development at this stage.

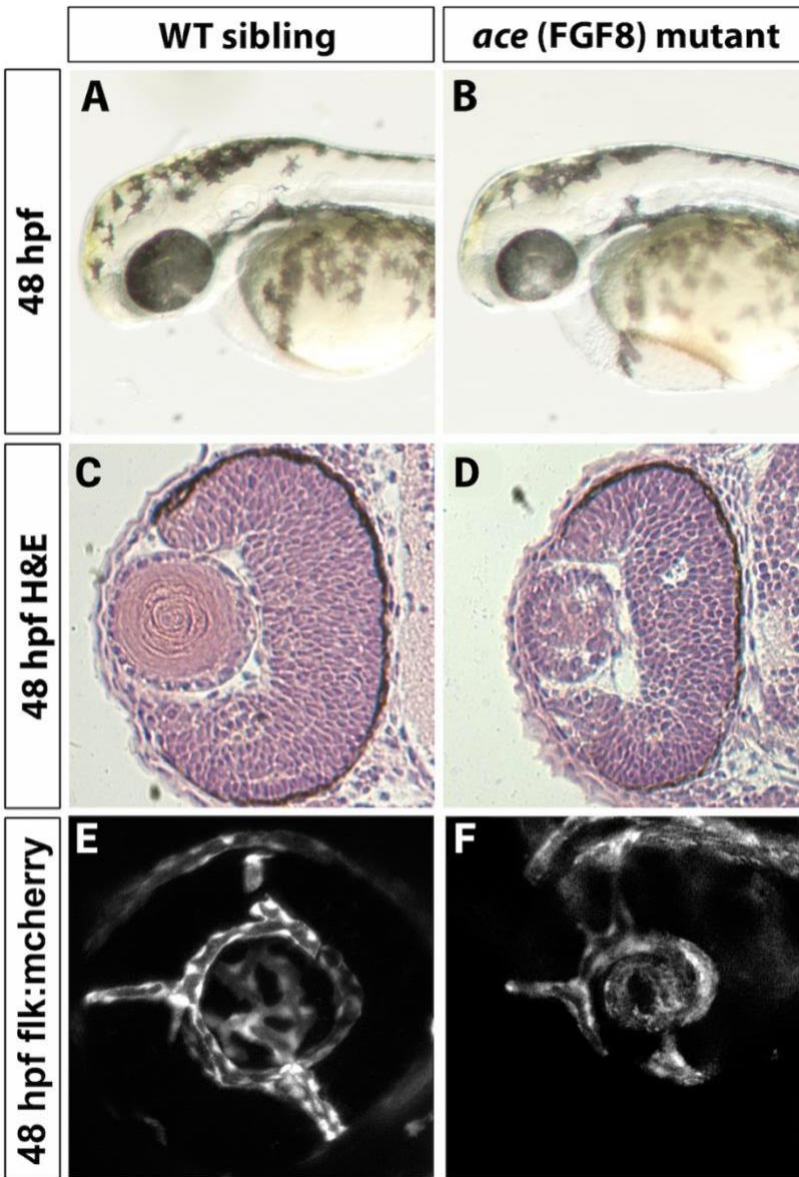


Figure 1.5. Acerebellar (*fgf8*) mutant phenotype against wild-type sibling. Wild-type (A, C, E) and mutant (B, D, F) embryos at 48 hpf. Lateral views (A, B), H&E-stained transverse section through the eye (C, D), and confocal lateral view of retinal blood vessels (E, F). Adapted from (Wysolmerski, 2015) and created with BioRender.com

This suggests *Fgfr1b* could be the receptor binding to *Fgf8a*, leaving us to explore the phenotypes associated with its loss in the developing retina. Gene expression patterns of *fgf8a* and *fgfr1b* were compared with the positions of RGCs and retinal vasculature, which were labeled with fluorescence through transgenic lines *isl2b:GFP* and *kdrl:mCherry* respectively. Interestingly, *fgfr1b* expression appears to be on blood vessels in the retina at 48 hpf (Figure 1.6).

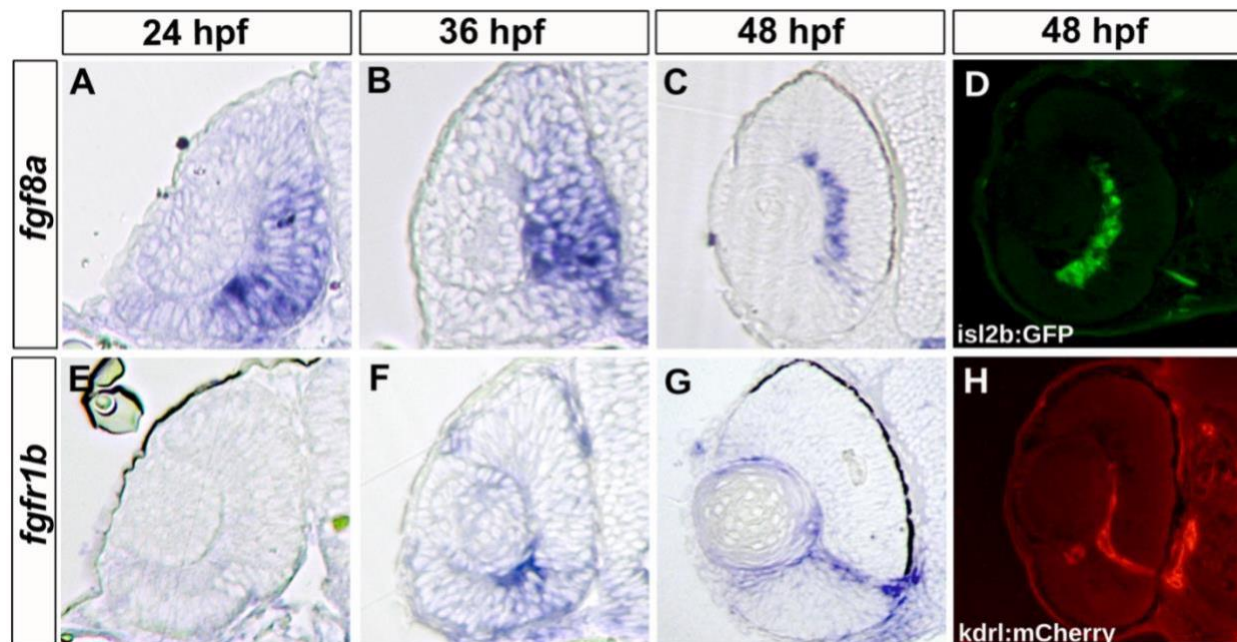


Figure 1.6. Expression of *fgf8a* and *fgfr1b* through in situ hybridization. Transverse sections of *in situ* hybridization highlighting mRNA expression of *fgf8a* (A, B, C) and *fgfr1b* (E, F, G) at 24, 36, and 48 hpf. *isl2b:GFP* (D) labeling retinal ganglion cells, and *kdrl:mCherry* (H) labeling retinal blood vessels (Ebert Lab, unpublished).

This project aimed to confirm the role of Fgfr1b in zebrafish retinal development at 48 hpf by exploring phenotypes of *fgfr1b* mutants lacking that receptor. I hypothesized that Fgf8a from retinal ganglion cells, acts through Fgfr1b on retinal vasculature to promote blood vessel branching and allow for proper retinal development. I predicted that a loss of Fgfr1b would phenocopy loss of Fgf8a, and explored this prediction by comparing eye size and cell counts of mutant and control zebrafish embryos.

Chapter 2: Methods

Zebrafish husbandry

All experiments were approved by both the University of Vermont Institutional Animal Care and Use Committee (IACUC) Protocol number: PROTO201900024 and the University of Vermont Institutional Biosafety Committee (IBC): REG201900054. We utilized the *fgfr1b* mutant line from the Sanger zebrafish mutation project: SA13288.¹⁵ This line was crossed with strains *Tg:(isl2b:GFP)* and *Tg:(kdrl:mCherry)*, which allowed for the labeling of retinal ganglion cells and blood vessels, respectively. Adult zebrafish were cared for and mated using standard protocols. Embryos were raised at 28.5 °C, staged as per Kimmel et al., 2008, and fixed for experiments in 4% paraformaldehyde. Tricaine was used as an anesthetic for all procedures and euthanization.

Genotyping embryos and adult fish

Adult fish were anesthetized with 0.4% tricaine prior to fin clipping. A small portion of the tail fin was amputated using a razor blade. The fin tissue was digested in 50 mM NaOH at 95 °C and neutralized with 1.5 M TrisHCl pH 8.8, spun down to pellet cellular debris and the supernatant was moved into a clean tube. PCR amplification was performed using primers shown in Table 2.1, and DNA was cleaned using overnight sodium acetate/ethanol precipitation. The *fgfr1b* mutation results in the substitution of an adenine for a cytosine on chromosome 10: 42636167, introducing a *bfaI* restriction cut site (3'-5' GATC).¹⁵ Digested PCR products were separated by gel electrophoresis and imaged. Wild-type sequences do not cut, resulting in one band at 375 base pairs, homozygous *fgfr1b* mutants cut and show as one band at 187/188 base pairs, while heterozygotes show both bands. Genotyping embryos followed a similar protocol to adult fish. Individuals were euthanized with tricaine prior to the removal of tails

at 48 hpf. Tails were similarly digested in NaOH at 95 °C and neutralized with TrisHCl pH 8.8. All subsequent steps were identical to those described above. To ensure gel electrophoresis was effective in genotyping for this project, samples underwent Sanger sequencing. This confirmed the genotypes determined through gel electrophoresis.

Table 2.1. Primers used in genotyping.

Gene	Forward Primer 5'-3'	Reverse Primer 5'-3'
<i>fgfr1b</i>	ACGTGGCAATAGGGCCTAAG	CTCTACATCACTGCCGACCA

Eye measurement and analysis

Whole mount lateral images were taken at 48 hpf on a Nikon SMZ800 dissecting scope with a SPOT Insight 4 camera at 3x magnification. Eye diameters (μm) were measured using Spot Imaging 5.2 software (Diagnostic Instruments Inc.). Total n-values were WT = 37 and *fgfr1b* = 26. Data were analyzed using an unpaired Student's t-test with a 95% confidence interval and graphed using GraphPad Prism.¹⁶ Images were cropped and adjusted for brightness and contrast using Adobe Lightroom (Adobe systems Inc.)

Embedding and sectioning

Following imaging, embryos were fixed in 4% paraformaldehyde (PFA) in phosphate buffered saline and stored at 20 °C until use. Embryos were dehydrated in 100% ethanol and embedded in JB4 resin following manufacturer's protocol (Polysciences Inc.; Warrington, PA, USA). A SMZ800 Nikon dissecting scope was used in orienting embryos for transverse sections. Embryos were sectioned at 7 μm on a Leica RM2265 microtome, mounted on glass slides, stained with hematoxylin and eosin (H&E), and imaged on an Olympus iX71 inverted microscope at 20X.

Retinal cell counts for mutants and wild-type siblings were quantified, graphed, and statistical analysis was performed using an unpaired Student's t-test.¹⁶

Chapter 3: Results

We mated heterozygous adults and determined genotypes for each embryo by separating *bfaI* restriction digested PCR products with gel electrophoresis. Bands of wild-type *Fgfr1b* were 375 bp in length, mutant bands appeared at 187/188 bp, and heterozygotes showed both bands. We then aimed to confirm the results of gel electrophoresis by sequencing select samples. Through Sanger sequencing we confirmed that this mutation resulted in the substitution of adenine with cytosine, and that the *bfaI* restriction digest with gel electrophoresis was a suitable method of genotyping *fgfr1b* embryos. This genotyping process is outlined in Figure 3.1.

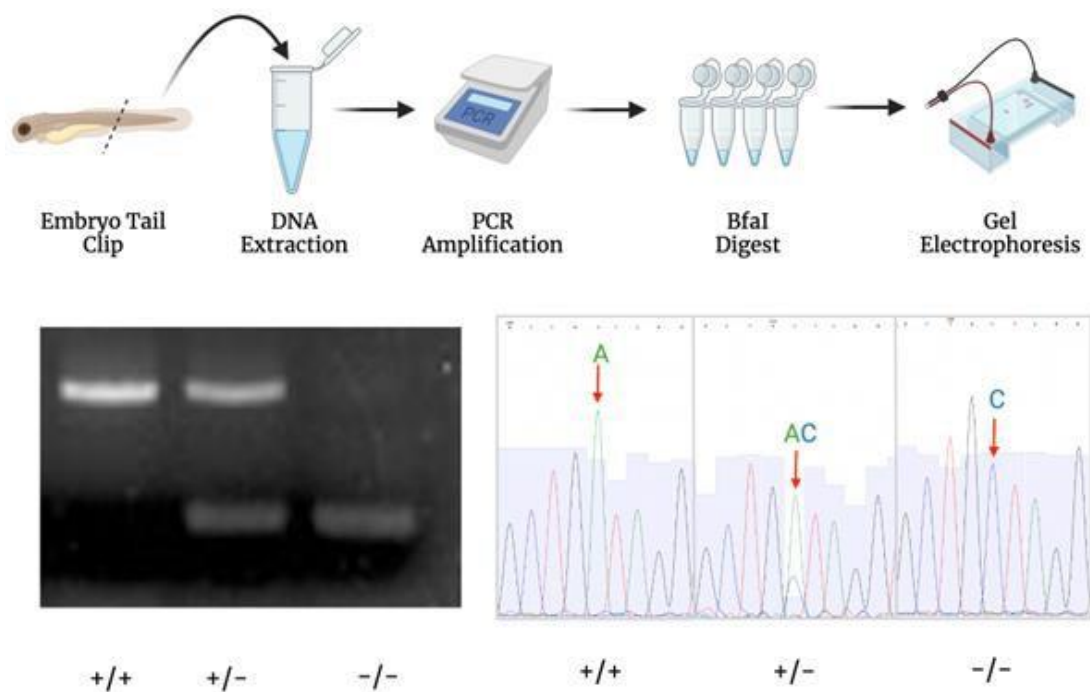


Figure 3.1. Embryo genotyping workflow. DNA extracted from embryo tails was amplified through PCR, digested with *bfaI*, and run on an agarose gel. The *fgfr1b* mutation introduces a *bfaI* cut site. Wild-type (+/+) bands are 375 bp (uncut), heterozygote (+/-) bands are 375 bp and 187 bp, and mutant (-/-) bands are 187/188 bp. Genotyping was confirmed with Sanger sequencing. Created with BioRender.com.

We next aimed to explore phenotypic consequences of the *fgfr1b* mutation, particularly with regard to its effect on the eye. Eye diameters (μm) were measured using lateral whole mount images (Figure 3.2) of each group. When compared to wild-type siblings ($n = 37$), *fgfr1b* mutants ($n = 26$) had significantly smaller eyes (Student's t-test; $p \leq 0.0218$) (Figure 3.3). The mean eye diameter for wild-type embryos was $219.2 \mu\text{m}$ while that of mutants was $205.9 \mu\text{m}$.



Figure 3.2. Whole mount images at 48 hpf. Lateral images were taken of wild-type (WT) siblings (top) and *fgfr1b* mutants (bottom). Created with BioRender.com.

We then performed hematoxylin and eosin (H&E) staining on transverse retinal sections at 48 hpf to assess phenotypes further. Looking closer at retinal morphology, we found that *fgfr1b* mutants appeared to have impairment of cellular lamination when compared to the control group. In counting the total number of cells per central retinal section, we found that mutant embryos had significantly fewer cells than wild-type siblings (Student's unpaired t-test; $p < 0.0001$). Wild-type

fish (n = 8) had an average cell count of 442 while mutants (n = 12) averaged only 276 cells (Figure 3.4).

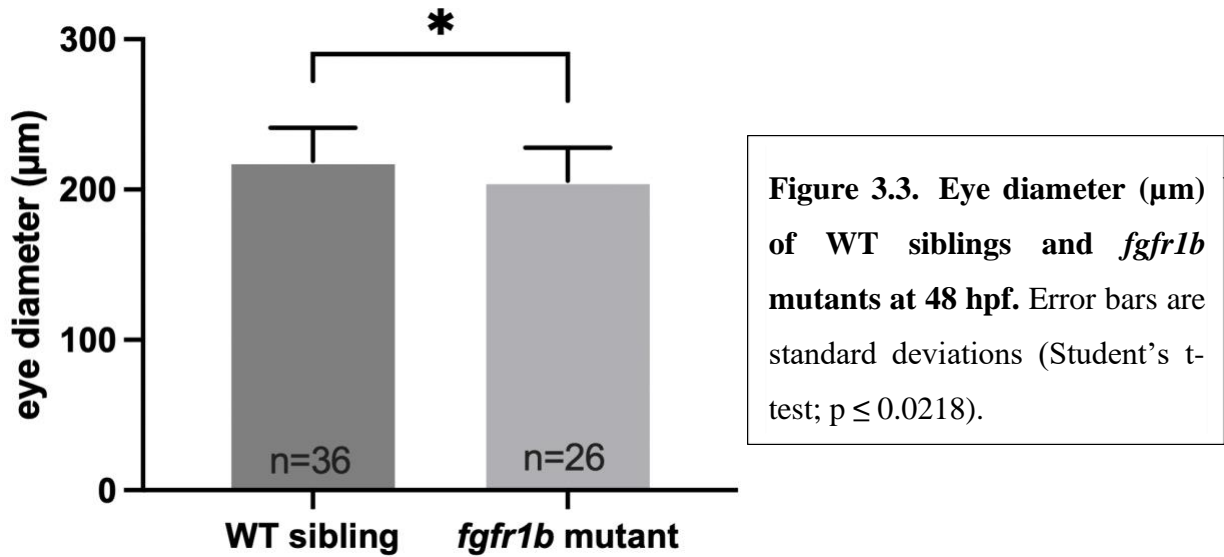


Figure 3.3. Eye diameter (µm) of WT siblings and *fgfr1b* mutants at 48 hpf. Error bars are standard deviations (Student's t-test; $p \leq 0.0218$).

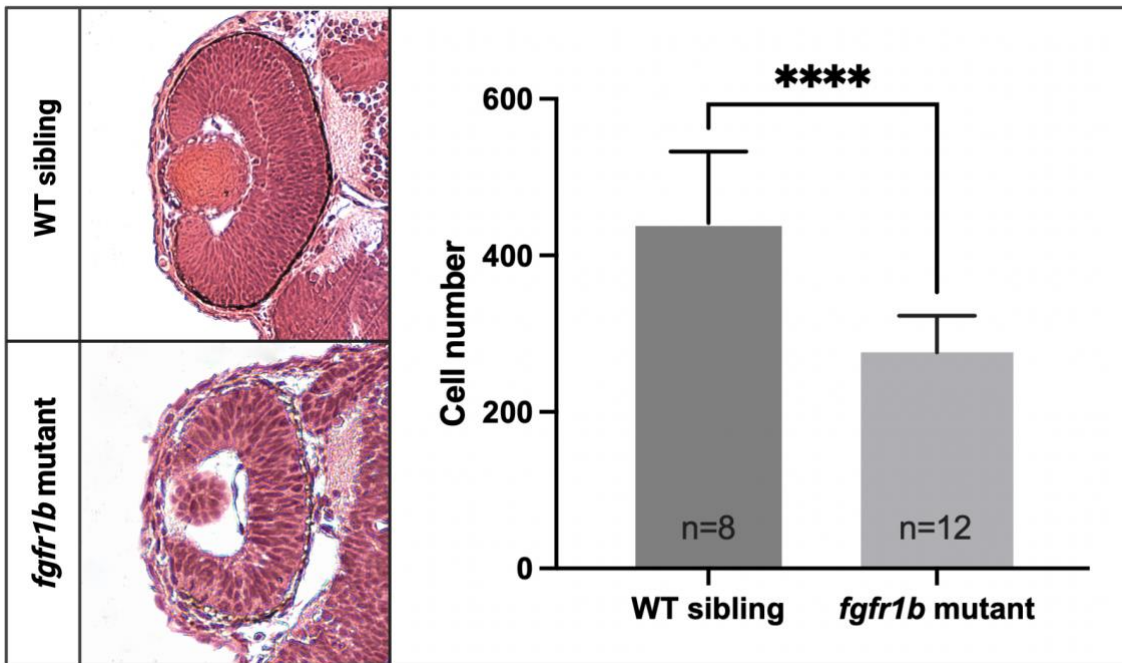


Figure 3.4. Total retinal cell counts of transverse retinal sections of wild-type siblings (top) and *fgfr1b* mutants (bottom). Error bars are standard deviations (Student's t-test; $p < 0.0001$). Created with BioRender.com.

Chapter 4: Discussion

This project sought to explore the role of *Fgfr1b* in retinal development of zebrafish. We found that *fgfr1b* mutant embryos show a similar phenotype to *ace* mutants lacking *Fgf8a*, having overall smaller eyes and fewer retinal cells than wild-type siblings. This provides evidence that at 48 hpf *Fgfr1b*, expressed on the vasculature, is a likely receptor of *Fgf8a* from retinal ganglion cells. We found this signaling pathway to facilitate proper retinal development through support of neuronal growth. We predict *Fgfr1b* to play a large role in the organization of retinal vasculature due to the concurrence of vessel growth and RGC differentiation.¹¹

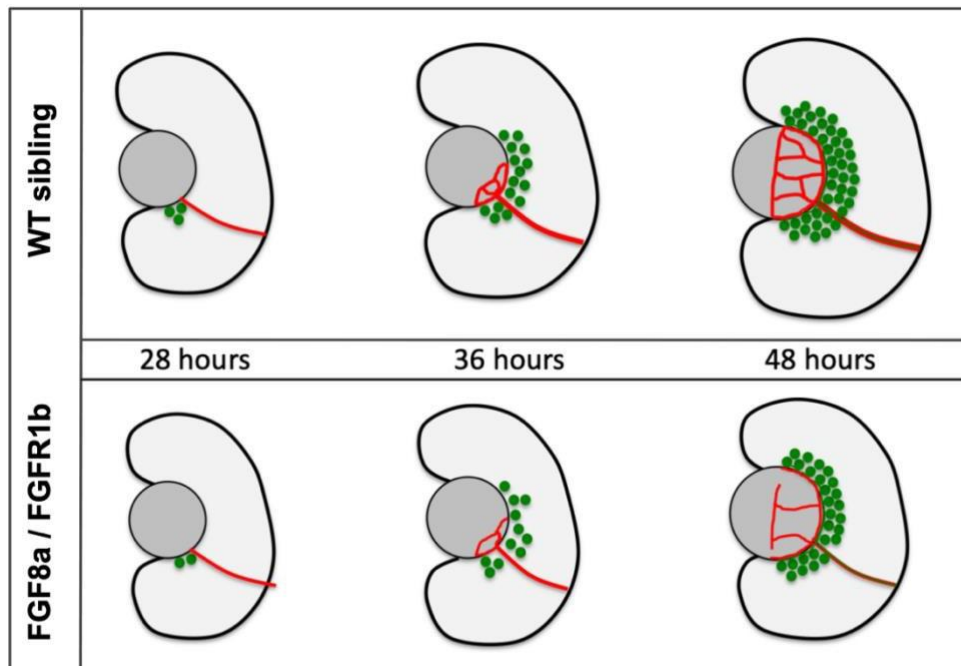


Figure 4.1. Model of retinal and vascular development. Retinal ganglion cells (green) are born at the same time as vessels (red) enter the eye. As more neurons are born, they release additional *Fgf8a*. This signals to the *Fgfr1b*-expressing vessels to grow and branch to further support these neurons. Without FGF signaling, this process is disrupted leading to fewer neurons and mispatterned retinal vessels.

With Fgfr1b identified as a likely receptor of Fgf8a, potential roles for the four other FGF receptors (Fgfr1a, Fgfr2, Fgfr3, & Fgfr4) should be explored to determine if Fgfr1b is acting alone in this developmental process. At 48 hpf, *in situ* hybridization showed *fgfr1b* expression to have the highest expression of the receptors, however, *fgfr2* and *fgfr4* are also expressed in the retina at this stage (Figure 4.2). Mutations of these genes should be investigated to determine if phenotypes match those of the *fgfr1b* and *ace* mutants. This would suggest that Fgf8a may signal through more than just Fgfr1b. Further evidence for this could be drawn from double knockout experiments using CRISPR/Cas9 to target both *fgfr1b* and another suspected receptor. If Fgf8a is in fact signaling through both receptors, we would expect to see a combined effect leading to more severe phenotypic consequences on the eye.

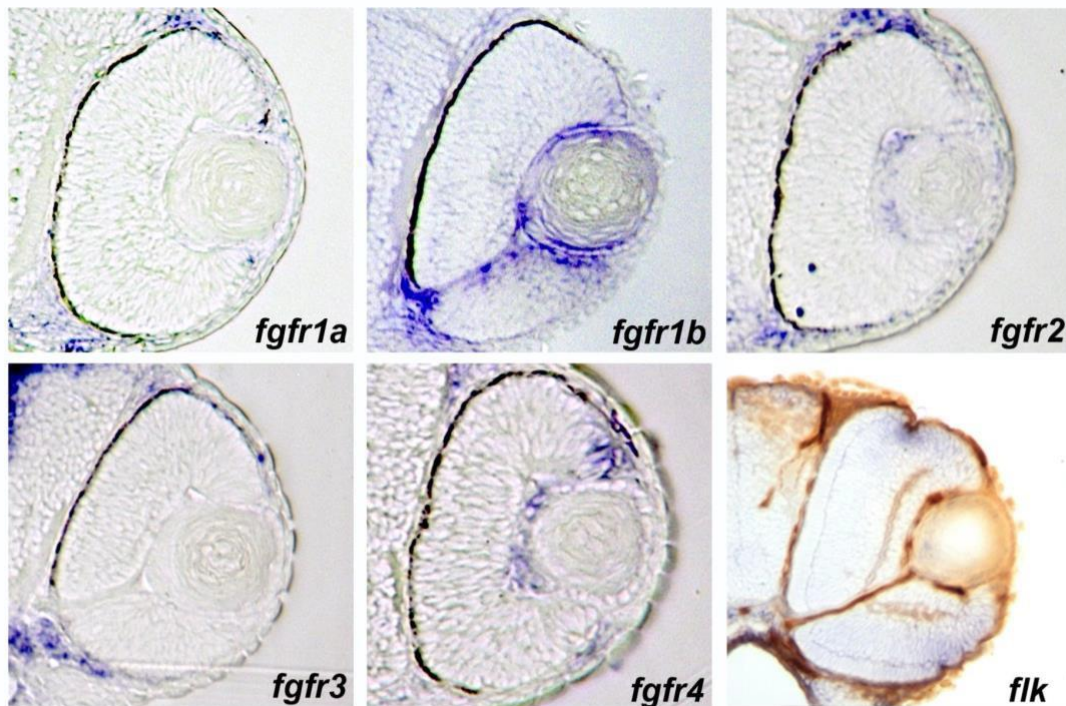


Figure 4.2. *Fgfr* expression patterns. *In situ* hybridization on transverse sections through the retina at 48 hpf of all five *fgfrs* and blood vessel marker *flk*.¹⁴

The exact mechanisms causing these phenotypes remain unknown. A number of factors could be contributing to the deformed retinas observed in mutant embryos, such as hypoxia, malnutrition, or the absence of a signaling molecule. More likely than not, this disrupted retinal formation is due to the combined effects of these factors. As previously described, work with the *sih* mutant assessed the effect of an overall lack of blood flow, depriving the retina of oxygen and key circulating nutrients.¹⁸ Embryos with ablated vessels similarly lacked blood flow, but more importantly lacked any factors that may be secreted from vascular endothelial cells themselves. More severe defects were found in the retinas of endothelial cell-depleted embryos compared to *sih* embryos,³ suggesting the vasculature is in fact supporting retinal development in addition to simply supplying blood. The exact role played by vascular endothelial cells is yet to be confirmed, but we suspect that when bound by Fgf8a, Fgfr1b on the surface of these cells could be triggering reciprocal signaling pathways that further promote neuronal growth and vascular organization in the retina.

Before such pathways can be fully understood, the retinal vasculature of *fgfr1b* mutants must be assessed. Vascular disorganization, similar to what was found in *ace* mutants, would provide further evidence that Fgf8a signals through Fgfr1b. In order to assess retinal vasculature, *fgfr1b* mutants on the *kdrl:mCherry* transgenic line must be identified and the development of pigmentation must be blocked, either with 0.003% phenylthiourea (PTU) at 24 hpf or other means. This would allow for 20x confocal Z-stacks of eye vasculature to be taken so that comparisons can be drawn between mutant and wild-type embryos.

Obtaining such images fell under the initial goal of this project. We were unable to accomplish these experiments due unexpected obstacles in the embryonic genotyping process. The use of embryonic genomic DNA (gDNA) for PCR amplification proved more difficult than

expected, despite genotyping of adult fish posing no challenges. Upon our *bfaI* digest yielding no results, we sought to verify the quality of our gDNA. Through test PCR amplification with *efl α* primers we found the gDNA extraction to be successful. Gel electrophoresis of PCR products with *fgfr1b* primers showed nonspecific binding and no clear amplification of our target gene. We found that PCRs were most often successful when performed immediately following gDNA extraction, but successful amplification still remained infrequent. The PCR was repeated with new primers and with various concentrations of gDNA to no avail. The number of amplification cycles was increased, and numerous annealing temperatures were tested (58, 60.5, 60.9, & 62.0 °C). Despite these efforts, we were unable to identify sufficient mutants to allow for vascular imaging. Future efforts should consider utilizing CRISPR-Cas9 technology to target *fgfr1b* and bypass the genotyping process. This would be worth the greater expense as it would allow for an efficient increase in sample size. With that done, efforts could be shifted to exploring the potential reciprocal signaling of Fgf8a-activated Fgfr1b.

This project contributes to future understanding of the exact mechanisms through which retinal vasculature and neurons in the retina are communicating. The understanding of interactions between adjacent tissue systems in development is necessary to understand individual cell behavior.¹¹ One study investigating these complex tissue interactions found translocation of retinal neurons to be essential to proper lamination and growth.¹² Concomitant development and migration of various cell types was found to be essential for avoiding spatial competition.¹² Mitotic division occurs at the apical surface of the retina as retinal progenitor cells (RPCs) undergo interkinetic nuclear migration.¹ With migratory mechanisms disrupted, the apical site for division would be overcrowded, likely leading to decreased proliferation. As seen in some cases, the binding of FGFs to FGFRs facilitates cellular migration,⁶ meaning the phenotype found

in *fgfr1b* mutants could stem from disruption to a similar pathway responsible for the translocation of retinal neurons. Fgfr1b could potentially be releasing a factor that organizes or temporally regulates interkinetic nuclear migration while the vasculature and adjacent neurons simultaneously develop. Without Fgfr1b, apical mitosis would be inhibited by overcrowding, resulting in decreased eye size and retinal cell counts characteristic of *fgfr1b* mutants. Regulation of interkinetic nuclear migration, however, is just one potential explanation for how downstream effects of Fgfr1b could be involved in the promotion of neuronal growth.

Remarkably, processes resembling interkinetic nuclear migration are seen being performed by cells of retinal organoids.¹⁷ Organoids are a recently developed model for studying the development of human organ systems, often employing induced pluripotent stem cells to recreate organ tissues *in-vitro*. Though organoid models have made huge advances in allowing for the study of cell behavior in the human retina, they lack the influences of many surrounding tissue structures, such as the retinal vasculature.¹⁹ Consequently, a major challenge associated with retinal organoid is the significant variability in their development, making it difficult for results to reliably be applied to human health efforts.^{17, 19}

Our project highlights the need for clearer understanding of the communication between these adjacent tissues. Elucidation of the pathway between vascular endothelial cells and retinal neurons, as seen with Fgf8a/Fgfr1b could allow for more accurate recreation of the eye's developmental environment, and more reliable culturing of retinal organoids. We hope this study sparks efforts to identify signaling factors involved in the bi-directional communication of the developing retina and vascular tissues; we also hope that our identification of Fgfr1b as a key player in retinal development supports growing efforts to address human disease through the use of *in-vitro* studies.

Acknowledgments

This project would not have been possible if not for the unrelenting support of my mentors, friends, and family. Firstly, I would like to thank my advisor Dr. Alicia Ebert for the invaluable guidance over the last three years, and for the countless hours spent teaching me new skills, both for in lab and in life. Thank you, Dr. Paula Deming, for being on my committee and supporting this project. I also want to thank Dr. Bryan Ballif, Taylor Bean, Phoebe Cousens, and the rest of Ebert and Ballif labs for their continued willingness to share with me their advice and enthusiasm. Lastly, I wish to give my sincerest gratitude to my family for their endless love and support. I could not have done it without them.

Funding provided by the University of Vermont's College of Arts and Sciences APLE Award, the University of Vermont FOUR Mini Grant, and NSF grant IOS1456846.

References

1. Baye, L. M., & Link, B. A. (2007). Interkinetic nuclear migration and the selection of neurogenic cell divisions during vertebrate retinogenesis. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *27*(38), 10143–10152. <https://doi.org/10.1523/JNEUROSCI.2754-07.2007>
2. Böttcher, R. T., & Niehrs, C. (2005). Fibroblast Growth Factor Signaling during Early Vertebrate Development. *Endocrine Reviews*, *26*(1), 63–77. <https://doi.org/10.1210/er.2003-0040>
3. Dhakal, S., Rotem-Bamberger, S., Sejd, J. R., Sebbagh, M., Ronin, N., Frey, R. A., Beitsch, M., Batty, M., Taler, K., Blackerby, J. F., Inbal, A., & Stenkamp, D. L. (2021). Selective Requirements for Vascular Endothelial Cells and Circulating Factors in the Regulation of Retinal Neurogenesis. *Frontiers in Cell and Developmental Biology*, *9*, 628737–628737. <https://doi.org/10.3389/fcell.2021.628737>
4. Ford-Perriss, M., Abud, H., & Murphy, M. (2001). Fibroblast Growth Factors In The Developing Central Nervous System. *Clinical and Experimental Pharmacology & Physiology*, *28*(7), 493–503. <https://doi.org/10.1046/j.1440-1681.2001.03477.x>
5. Fruttiger, M. (2007). Development of the retinal vasculature. *Angiogenesis (London)*, *10*(2), 77–88. <https://doi.org/10.1007/s10456-007-9065-1>
6. Goetz R, Mohammadi M. (2013) Exploring mechanisms of FGF signalling through the lens of structural biology. *Nature Reviews Molecular Cell Biology*. *14*, 166-180
7. Hartsock, A., Lee, C., Arnold, V., & Gross, J. M. (2014). In vivo analysis of hyaloid vasculature morphogenesis in zebrafish: A role for the lens in maturation and maintenance of the hyaloid. *Developmental biology*, *394*(2), 327–339. <https://doi.org/10.1016/j.ydbio.2014.07.024>
8. Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental dynamics: an official publication of the American Association of Anatomists*, *203*(3), 253–310. <https://doi.org/10.1002/aja.1002030302>
9. Martinez-Morales, J.R., Del Bene, F., Nica, G., Hammerschmidt, M., Bovolenta, P., Wittbrodt, J., 2005b. Differentiation of the vertebrate retina is coordinated by an FGF signaling center. *Developmental cell* *8*, 565-574
10. Moon, A. M., Guris, D. L., Seo, J., Li, L., Hammond, J., Talbot, A., & Imamoto, A. (2006). Crkl Deficiency Disrupts Fgf8 Signaling in a Mouse Model of 22q11 Deletion Syndromes. *Developmental Cell*, *10*(1), 71–80. <https://doi.org/10.1016/j.devcel.2005.12.003>
11. Norden, C. (2023). A Fish Eye View: Retinal Morphogenesis from Optic Cup to Neuronal Lamination. *Annual Review of Cell and Developmental Biology*, *39*(1), 175–196. <https://doi.org/10.1146/annurev-cellbio-012023-01303>
12. Rocha-Martins, M., Nerli, E., Kretzschmar, J., Weigert, M., Icha, J., Myers, E. W., & Norden, C. (2023). Neuronal migration prevents spatial competition in retinal morphogenesis. *Nature*, *620*(7974), 615–624. <https://doi.org/10.1038/s41586-023-06392-y>
13. Rohner, N., Bercsényi, M., Orbán, L., Kolanczyk, M. E., Linke, D., Brand, M., Nüsslein-Volhard, C., & Harris, M. P. (2009). Duplication of fgfr1 Permits Fgf Signaling to Serve as a Target for Selection during Domestication. *Current Biology*, *19*(19), 1642–1647. <https://doi.org/10.1016/j.cub.2009.07.065>
14. Rohs P, Ebert AM, Zuba A, McFarlane S. (2013). Neuronal expression of fibroblast growth factor receptors in zebrafish. *Gene expression Patterns*. *13*, 354-361. <http://dx.doi.org/10.1016/j.gep.2013.06.006>
15. Sprague, J., Doerry, E., Douglas, S. and Westerfield, M. (2001). The Zebrafish Information Network (ZFIN): a resource for genetic, genomic and developmental research. *Nucleic Acids Res.* *29*, 87-90.
16. Student's t-test was performed using GraphPad Prism version 9.4.1 for macOS, GraphPad Software, Boston Massachusetts USA, www.graphpad.com
17. Vielle, A., Park, Y. K., Secora, C., & Vergara, M. N. (2021). Organoids for the Study of Retinal Development and Developmental Abnormalities. *Frontiers in cellular neuroscience*, *15*, 667880. <https://doi.org/10.3389/fncel.2021.667880>
18. Wysolmerski, Erin, "FGF8a is Required for Proper Vascularization of the Zebrafish Retina" (2015). *Graduate College Dissertations and Theses*. 348. <https://scholarworks.uvm.edu/graddis/348>
19. Zhang, Z., Xu, Z., Yuan, F., Jin, K., & Xiang, M. (2021). Retinal Organoid Technology: Where Are We Now?. *International journal of molecular sciences*, *22*(19), 10244. <https://doi.org/10.3390/ijms221910244>