The Role of fgf10a and fgf4 in *Danio rerio* Embryonic Tooth Development

Lauren Waters, Class of 2022

In order to understand the mechanisms of early embryonic development, including that of humans, we need to better understand the role that genes play in morphogenesis. Zebrafish (*Danio rerio)* are valuable model organisms for studying such vertebrate development, as they carry orthologs for many mammalian genes. One group of genes that are shared between mammalian and zebrafish genomes are fibroblast growth factors (FGFs), which are a family of cell signaling proteins that are crucial for normal vertebrate development.

This summer, I investigated the role of two genes from the FGF family-- fgf10a and fgf4-- in zebrafish embryonic tooth development. In order to understand the role that genes play in embryonic tooth development, the Jackman lab creates genetically altered zebrafish using a CRISPR "knockout" procedure on zebrafish embryos. The CRISPR/Cas9 injection introduces a double stranded break into the target gene, which results in imprecise double stranded break repair, and produces random mutations such as indels and point mutations at the locus. The addition of the GFP plasmid in this CRISPR/Cas9 injection allows for GFP to be inserted at the location of the double stranded break. With the insertion of GFP at the locus of the target gene, we can see the expression pattern of the target gene in the embryonic tooth germs while also observing any phenotypic effects that a mutant gene has on tooth development. Ultimately, the goal of these CRISPR injections is to obtain a stable line of genetically altered organisms with consistent GFP expression so that we are able to continue to study the effects of the target gene without further injections.

At the beginning of this summer, the Jackman lab had not yet collected a stable line of fgf10a mutant fish, although we had a line of fgf4 mutant fish. Therefore, I began my project with a CRISPR/Cas9 injection targeted at the fgf10a locus, with the plan of collecting a few fish with good GFP expression to raise. However, this first round of injections did not produce any embryos with significant GFP expression. As a result, I designed a new crRNA for the fgf10a locus that would potentially produce better results.

Concurrently with my fgf10a injections, I was also working to obtain a genetic sequence of the Jackman lab's fgf4 mutant line through PCR and subsequent genetic sequencing. Prior to my research this summer, a genetic sequence had not been obtained, because the primers for the 3' UTR of the fgf4 locus had always failed with PCR. It was hypothesized that this was because there was a much larger deletion on the 3'UTR end of the fgf4 gene than expected, so the previous 3' primers had not functioned since there was no complementary sequence present for the primers to attach to and start the PCR. Therefore, I designed four different PCR primers for the 3' end, each a greater number of base pairs (up to ~5000 bp) away from the expected 3' end of the gene to account for this potential deletion. However, when the PCR product with each of these 3' primers was run on an electrophoresis gel, only a band for the control was present, leading to further questions about the identity of the fgf4 mutant line, how large the 3'UTR deletion actually was, and if we would ever be able to obtain a genetic sequence of the line. Therefore, I began to do CRISPR injections targeting the fgf4 gene as well as fgf10a, in order to raise a new stable line of fgf4 mutant fish that we would be able to sequence.

Ultimately, after many fgf4 and fgf10a CRISPR injections, I was able to collect six fgf4 mutant embryos and four fgf10a mutant embryos to raise (Fig 1). I am excited to continue to observe these mutants as they become mature enough to be crossed and spawned in the upcoming months. Along with this, I was able to antibody stain and image the teeth of the fgf4 mutant embryos, finding consistent GFP expression localized at the position of the enamel knot in the tooth germs (Fig 2). I look forward to

further investigating this fgf4 enamel knot expression pattern, as well as solidifying the fgf10a expression pattern with my honors project this upcoming semester.

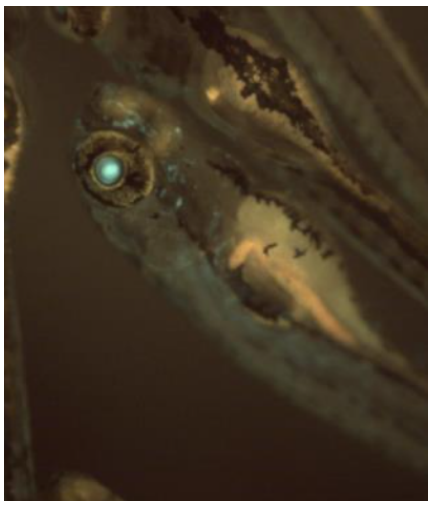

Figure 1. GFP expression pattern of an fgf4 rKO zebrafish 5dpf that was kept to be raised. Embryos injected with CRISPR/Cas9 system at 1 cell stage. Imaged with fluorescent microscopy.

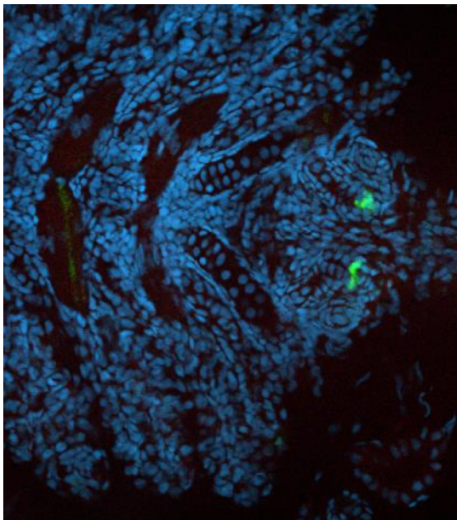

Figure 2. GFP tooth germ expression pattern localized to enamel knot in fgf4 rKO embryos at 102hpf. Embryos fixed at 127 hpf in 4% formaldehyde, stained with an antiGFP antibody (green), alizarin red-S calcium stain (red), and DAPI nuclear stain (blue). Images of fixed embryos were obtained using a Zeiss Axio Imager M2 microscope with an Apotome 2 structured illumination attachment.

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