Understanding the cellular mechanism by which the Hedgehog pathway signals for dental papilla formation in *Danio rerio*

James Crimp, 2013

The hedgehog-signaling pathway (Hh) is widely acknowledged as one of the most important signaling systems involved in early embryonic development. As an evolutionarily ancient system, it has evolved a wide variety of functions across a diverse array of species. One of the most important roles of the pathway is in organogenesis, where it has been shown to be necessary for the growth of primary organs such as the heart and the lungs, in addition to a variety of appendages such as hair, feathers, and teeth (Brownell et al., 2011). While in teeth, most past research has focused on the role of Hh signaling in murine dentition, our lab has made several new discoveries by studying the pathway in the tooth development of the *Danio rerio*, better known as the zebrafish (Jackman et al., 2010).

To study this pathway, we have employed the use of the inhibitor cyclopamine, a chemical that binds to a signaling factor downstream of Hh, preventing it from taking effect. Using this inhibitor in a line of embryos that express green fluorescent protein in the developing tooth germ, we are able to visualize the effect of disrupted Hh signaling. Preliminary results have shown that when hedgehog is inhibited, the dental papilla, the part of the tooth germ that eventually forms the inner layer of dentin in mature teeth, does not form or is reduced in size.

My research this summer has focused on explaining the cellular mechanism behind this papilla loss. To do this, I am exploring two main lines of reasoning. The first is that Hh signaling leads to papilla formation by preventing cell death over the course of its growth. The second is that Hh signals for cellular proliferation in the developing papilla, without which it does not form. To investigate these options, I am employing the use of two antibodies, caspase 3 and phospho histone H3 that mark cells that are actively undergoing apoptosis or dividing, respectively. Using a staining amplification protocol, I am able to amplify the signal of these two antibodies, allowing me to compare cell death and proliferation between Hh inhibited and wild-type embryos. So far, our lab has found upregulated cell death in cyclopamine treated embryos but this cell death is not localized to the tooth germ, showing that likely Hh acts through some other mechanism to signal for papilla formation. Additionally, I have found evidence of dividing cells in the untreated dental papilla and am in the process of comparing this division to papilla cells in Hh-inhibited embryos. Overall, I hope to continue my research to eventually form a complete explanation for Hh's role in signaling for papilla formation.

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Funded by the Maine INBRE Fellowship

References:

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