The Effects of Retinoic Acid on Two Pathways Involved in Zebrafish Tooth Development

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Tooth morphogenesis occurs in zebrafish as a result of cell signaling between epithelial and mesenchymal tissues in the pharyngeal region. Zebrafish are excellent model organisms to study tooth development because the genes and signaling molecules involved are likely conserved across vertebrates. However, all of the cell signaling molecules required for the initiation of tooth development are still unknown. In an effort to identify additional pathways involved and signals necessary for tooth morphogenesis, we are exploring the connection between retinoic acid (RA) and Fibroblast growth factor (Fgf). RA and Fgf are two signaling molecules involved in tooth development that both result in supernumerary teeth when applied exogenously in zebrafish. Because treating embryos with exogenous RA and overexpressing \textit{fgf10a} separately give similar phenotypes, we were interested in seeing whether exposing embryos to both exogenous RA and overexpressing \textit{fgf10a} would result in additive, epistatic or synergistic phenotypes. The results showed a mix of tooth phenotypes with some resembling a RA tooth pattern, some a \textit{fgf10a} pattern and some a combination of the two phenotypes. Having only a \textit{fgf10a} phenotype suggests that \textit{fgf10a} is somehow inhibiting RA activity.

Because RA has the ability to induce tooth development, we are interested in how it regulates tooth related gene expression, including that of \textit{dlx2b}, a transcription factor expressed early in the organogenesis of teeth. Since there is a retinoic acid response element (RARE) located upstream of the \textit{dlx2b} enhancer region in zebrafish, we are testing whether or not RA could regulate \textit{dlx2b} gene expression in tooth development. In an effort to elucidate the relationship between RA and \textit{dlx2b}, I mutated the conserved regions of the RARE site upstream of the \textit{dlx2b} enhancer driving expression of the green fluorescent protein (GFP) in a reporter construct. I injected DNA containing this mutated reporter construct to visualize where the \textit{dlx2b} gene was being expressed in the zebrafish and I am currently analyzing these data. Knowledge of the interactions between RA, \textit{fgf10a} and \textit{dlx2b} could provide information regarding how cell-signaling pathways influence tooth morphogenesis and potentially lead to tooth regeneration therapies in dentistry.

Faculty Mentor: William Jackman

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