Zebrafish: Advancing Our Understanding of the Genetic Basis of Craniosynostosis

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Abstract

Purpose: Understanding the genetic basis of congenital disorders is prerequisite to efforts to normalize development. Craniosynostosis is particularly problematic as affected children can have lifelong recurrence of craniofacial abnormalities despite repeat surgical procedures for its correction. Zebrafish add a novel model for investigation without the constraints inherent in mammalian models. The present study investigates novel applications of zebrafish for investigation of craniosynostosis.

Methods: 1) Baseline cranial development: Wild-type zebrafish were studied using sequential live-staining of cranial bones to determine rate and direction of calvarial bone growth.

2) Localized gene activation: Recombination-based cloning was used to create transgenic zebrafish (*Tg(dnFGFR1:EGFP))* carrying a mutant *fgfr1* gene downstream of a heat-shock promoter and upstream of a green fluorescent protein reporter. A novel heat-shock protocol was used to induce expression of this mutated protein within selected sutures.

3) Identification and alteration of *twist* genes: RNA *in-situ* hybridization revealed expression patterns of *twist* genes in the developing cranial sutures. The Cas-9/CRISPR method was applied to create *twist3* mutant lines.

Results: 1) The zebrafish cranial vault has a pattern of suture development analogous to mammalian models. While true fusion is not observed, an analogous interdigitation of bones occurs at the suture interface (Figure 1).

2) The localized heat-shock approach successfully induced selective transgene expression in the sutures of *Tg(dnFGFR1:EGFP)* fish (Figure 2).

3) Whole mount RNA in-situ hybridization revealed differential expression of *twist* genes in sutures of developing calvaria (Figure 3), suggesting distinct regulatory functions. *Twist3* mutant strains of fish have been created using the CRISPR method of genome editing.

Conclusions: 1) The zebrafish cranial vault and suture development is analogous to that of prior mammalian models, making this model a useful tool for the study of normal and pathologic cranial development. 2) The present study is the first report of localized gene induction within sutures, opening a new line of investigation in which gene regulation can be altered specifically within sutures without altering function of those genes in the remaining organism. 3) *Twist* genes, important in human suture biology, are also expressed in zebrafish cranial sutures. Moreover, we have successfully applied the newest techniques in genome editing to this model. These studies highlight the potential impact of zebrafish to advance future research in plastic surgery.

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