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Abstract Title

Wnt Signaling and Tooth Development in Mexican tetra (*Astyanax mexicanus*): A Comparative Model for Developmental Dental Abnormalities

Authors

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Background

Developmental defects in odontogenesis can alter tooth number and impair children's oral health and quality of life. Hypodontia affects about 6.5% of children and adolescents, while hyperdontia occurs in about 2%. Surface and cave morphs of *Astyanax mexicanus* have diverged genetically through adaptation to contrasting environments

Objective

We hypothesize that this divergence alters Wnt-dependent signaling and gene expression during odontogenesis, leading to differences in tooth number. Understanding these mechanisms may provide insight into pathways involved in developmental dental abnormalities.

Methods

Surface and cave morphs of *Astyanax mexicanus* were exposed at 10 hpf to the Wnt activator LiCl or inhibitor Wnt-C59. Mandibular tooth numbers were quantified at 6, 10, 15, 20, 30, and 60 dpf (n=15/group/time point) following whole-mount Alizarin Red staining. Developmental stages showing informative tooth-number differences will be selected for RNA-seq of treated and untreated fish, with head regions collected for RNA isolation. Differential expression and GSEA will identify Wnt-responsive genes and enriched developmental pathways. Selected genes will be validated by qPCR and in situ hybridization.

Results

Wnt modulation produced morph-specific effects on mandibular tooth number. In cavefish, treatment did not significantly alter mandibular tooth number, whereas Wnt-C59 significantly reduced mandibular tooth number in surface fish at 15 and 20 dpf ($p < 0.05$). At 20 dpf, Wnt-C59-treated surface and cavefish differed

significantly in mandibular tooth number ($p < 0.001$). RNA-seq of untreated fish at 72 hpf identified 22 differentially expressed tooth-development-related genes ($FDR < 0.05$), with 12 upregulated and 10 downregulated in cavefish. Notable changes included higher *bglap1* (~19.2-fold), *bmp6* (~4.6-fold), and *spp1* (~3.8-fold), and lower *wnt11f2* (~5.8-fold) and *spp2* (~8.7-fold) expression in cavefish.

Conclusion

The differential responses to Wnt-C59, together with early transcriptomic differences, support our hypothesis that genetic divergence modifies Wnt responsiveness and odontogenic gene regulation. These findings link genetic background to tooth-number variation and may clarify mechanisms underlying developmental dental abnormalities.

Table/Figure File

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