AAO Foundation Award Final Report

Principal Investigator	Joel S. Gaikwad, PhD
Co-Investigator	Mark Reynolds, DDS, MS
Secondary Investigators	
Award Type	Biomedical Research
Project Title	Analysis of Osteoblast Gene Expression by Subtractive Cloning
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Institution	University of Texas Health Science Center at Houston, Dental Branch
Summary/Abstract	Odontogenesis involves epithelial-mesenchymal signaling interactions that lead to cuspal morphogenesis, cell differentiation and the formation of enamel, dentin, cementum and bone matrices. Although previous studies have increased our understanding of molecules that regulate tooth initiation and early morphogenesis, the precise nature of the molecules controlling late morphogenesis and cell differentiation is not known. We reported a unique phenotype involving dentition in mice lacking the transcription factor, Runx2. The markedly hypoplastic tooth organs and defects in the maturation of ameloblasts and odontoblasts point to an important and non-redundant role for Runx2 in tooth development. Importantly, Runx2 mutations are responsible for the defects in human cleidocranial dysplasia (CCD). The objective of our study was to test the hypothesis that the absence of Runx2 affects downstream target genes that are important in tooth morphogenesis and cytodifferentiation. Our methods included generating a cDNA library from E14.5 Runx2(-/-) and (+/+) first molar organs using a sensitive PCR-based method called suppression subtractive hybridization (SSH). **Results:** Sequence analysis of 61 differentially expressed genes revealed that 96.03% of the clones matched known genes in the GenBank/EBML database while 3.96% did not match any entries. Other tooth-specific downstream target genes of Runx2 include: mitochondrial proteins, extracellular matrix proteins, kinases, receptors, growth factors, transcription molecules and genes involved in apoptosis. One of the isolated clones termed Zfp is a gene that encodes for a transcription factor and belongs to the zinc finger family of proteins which reveals an interesting temporal and spatial pattern during odontogenesis. RT-PCR analysis confirmed a downregulation of Zfp expression in Runx2(-/-) mice molar tooth organs. **Conclusion:** We have successfully generated and screened a cDNA library enriched in genes expressed in Runx2(+/+) and Runx2(+/-) molar tooth organs

	and performed preliminary studies to assess the role of Zfp in tooth development. This data will contribute to a better understanding of the role of Runx2 in odontogenesis and the molecular basis for human CCD.