

Investigating the Role of *IRF6* in Salivary Gland Development and Inflammation Modulation leading to Sjögren's syndrome

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Objectives: Interferon Regulatory Factor 6 (*IRF6*) is a crucial regulator of cell differentiation in ectodermal and oral epithelial tissues. Haploinsufficiency of *IRF6* in humans causes syndromic cleft lip and palate, namely Van der Woude syndrome and Popliteal pterygium syndrome. We have previously determined that *Irf6* deficiency causes disorganized branching morphogenesis, lack of differentiated mucous acinar cells, disrupted cell-cell adhesion, and immune cell infiltration in salivary glands of *Irf6*-null mice. Immune infiltration into acini suggests a potential association with Sjögren's syndrome, an autoimmune disorder marked by mononuclear cell infiltration into the salivary and lacrimal glands. We posit that IRF6 has an immunoregulatory role in early development that, when disrupted, can lead to immune dysregulation, increased inflammation, and potential autoimmunity. The aim of this investigation is to further visualize inflammatory presence in salivary glands as well as morphological and cellular proliferation-differentiation differences in human acinar cells.

Experimental Methods: Immunofluorescence staining was performed using interleukin 6 (IL-6), interleukin 10 (IL-10), alpha-smooth muscle actin (α SMA), E-cadherin, aquaporin 5 (AQP5), Transforming Growth Factor Beta 3 (TGF β 3), Epiregulin (Ereg), and latent transforming growth factor beta binding protein 4 (LTBP4). Salivary acinar cells were transfected with either *Irf6*-containing plasmid or siRNA to overexpress IRF6 protein or knock down *IRF6* endogenous expression, respectively. Western blot analysis was performed to detect baseline and altered IRF6 expression.

Results: Salivary glands of *Irf6*-null mice revealed differential expression and localization patterns of inflammatory, cell adhesion, and proliferation-differentiation biomarkers. Human acinar cells showed decreased cell-cell adherence and loss of morphological definition following knockdown of IRF6 by siRNA transfection.

Conclusion: Our findings suggest that IRF6 contributes to immunoregulation in the salivary glands and is necessary for cell-cell adhesion and differentiation in human acinar cells. We will further investigate the expression of IRF6 and its target genes in biopsies of human salivary gland tissues affected by Sjögren's syndrome.

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