Interferon Regulatory Factor 6 Regulates Adherens Junction Proteins and is Associated with Inflammatory Cytokines in Sjögren's Syndrome

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Objectives: This study investigates the role of Interferon Regulatory Factor 6 (*IRF6*) in regulating adherens junction protein expression, endocytic vesicles, and inflammatory cytokines in salivary glands (SG). IRF6 is a transcription factor in ectodermal and craniofacial tissues and has been shown to affect the recycling of E-cadherin, an adherens junction protein pivotal to epithelial integrity. Yet, the function of *IRF6* in SGs remains unknown. Previously, we determined that *Irf6* deficiency in mice causes disorganized branching morphogenesis, compromised acinar differentiation, and immune cell infiltration. We posit that *IRF6* has crucial developmental and immunoregulatory roles that, when disrupted, can lead to altered intercellular adhesion, increased inflammation, and immune dysregulation, contributing to the development of Sjögren's syndrome (SS), which notably affects the salivary and lacrimal glands.

Experimental Methods: Immunofluorescence staining and transfection with siRNA and expression plasmid were used to determine differential expression of target genes associated with adherens junctions, differentiation, and inflammation in SS.

Results: Major SGs of *Irf6* null mice exhibited myoepithelial cell detachment from acinar and ductal cells, reduced E-cadherin and MIST1 differentiation factor expression, and significantly increased IL-6, IL-17A and IL-22 expression. *IRF6* knockdown in human acinar cells led to disrupted cell-cell adhesion, decreased β-catenin, TGFβ3, and RAB11B expression, and dysregulated inflammatory cytokine expression**.** *IRF6* overexpression in human SG adenocarcinoma cells resulted in cell-cell compaction and increased expression of E-cadherin, β-catenin, TGFβ3, and all tested inflammatory cytokines. IRF6 expression was remarkably elevated in acini affected with SS, alongside increased expression of E-cadherin and IL-6 compared to control biopsies. Quantitative analysis revealed significantly higher adherens junction protein intensity and smaller interface areas in acini affected by SS.

Conclusion: Our findings suggest that *IRF6* plays a significant role in regulating adherens junction protein expression in SGs. Increased IRF6 levels in SGs affected by Sjögren's syndrome may explain the compromised intercellular space and elevated inflammatory cytokine presence.

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