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Hypoxia-inducible miR-155 dysregulates epithelial barrier by attenuating claudin-7 in eosinophilic esophagitis

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Attenuated HIF-1α contributes to barrier dysfunction in the chronic allergic disease eosinophilic esophagitis (EoE). We sought to identify the regulatory mechanisms behind this and hypothesised that miR-155-5p regulates HIF-1α and more globally epithelial barrier function in EoE.

MiR-155-5p is significantly increased in EoE patients (in-situ hybridization, *P≤0.01*), with miR-155-5p most abundant in relatively undifferentiated basal cells. MiR-155-3p is not expressed in these cells in culture. Esophageal epithelial cells (EPC2-hTERT) cultured in extended hypoxia mimicking inflammatory hypoxia in EoE showed elevated miR-155-5p (2 fold; *P≤0.05*), which was inversely correlated with HIF-1α suppression (0.5 fold; *P≤0.001*). A miR-155-5p stably overexpressing cell line also confirmed this relationship, displaying decreased HIF-1α mRNA (0.6 fold; *P≤0.01*).

Stable miR-155-5p overexpressing (miR-155OE) esophageal epithelial cells were used to examine the potential consequences of elevated epithelial miR-155-5p. Physiologically relevant 3D-Air-Liquid-Interface (ALI) cultures demonstrated functionally impaired barrier via transepithelial electrical resistance (TEER) (0.6 fold, *P≤0.01*), as well as perturbed 3D-ALI histology in miR-155OE cultures. In elucidating the potential molecular mediators of this, a screen of desmosomes, adherens junctions and tight junctions was performed. qRT-PCR and western blotting identified a significant decrease in the key esophageal tight junction molecule, claudin-7 (0.56 fold, *P≤0.05*). RNA sequencing on miR-155OE 3D-ALIs showed decreased enrichment for genes involved in cell cytoskeleton (GO:0005856), and focal adhesion (GO:0005925). Cross-comparative analysis on patient-derived RNA sequencing identified 167 overlapping gene signatures with important functions in EoE pathology, gene ontology pathway analysis demonstrated genes involved in cell cytoskeleton (GO:0005882), and Keratin Filament (GO:0045095). Immunofluorescence staining and qRT-PCR of physiologically relevant oesophageal epithelial organoids showed no change in basal-cell associated KRT14 in miR-155OE cultures, however there was a significant decrease in suprabasal-cell associated *KRT4* (0.2 fold, P≤*0.001*) suggesting an attenuation of epithelial differentiation processes.

Immunofluorescence characterisation of dysfunctional epithelial barrier in miR-155OE organoids identified claudin-7 mislocalization. Further molecular analysis confirmed a significant decrease in claudin-7 mRNA (0.55 fold, *P≤0.*001) and protein (0.3 fold, *P≤0.*05) in miR-155OE cultures relative to control. Haematoxylin and Eosin staining of miR-155OE organoids also showed dilated interepithelial spaces, consistent with the histopathological hallmark in patients with EoE. These dilations were also observed in our claudin-7 knock down cells cultured as organoids.

Together, this data supports the relationship between elevated miR-155 and decreased epithelial barrier in the context of EoE, proposing miR-155-5p as a likely therapeutic target.