2.08 Human epididymis protein 4 (HE4) in the pulmonary microenvironment: A window into fibrosis

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Background: Human epididymis protein 4 (HE4) is a secretory protease inhibitor produced by epithelial cells and a biomarker in cancer and recently in fibrosis. HE4 activates fibroblasts and high levels are associated with poor lung function. However, epithelial cell supernatant with HE4 knockdown appears pro-fibrotic, suggesting a regulatory role for HE4. **Aim:** To understand the function of HE4 in the pulmonary microenvironment through characterisation of the epithelial cell supernatant with HE4 knockdown injured epithelial cells. **Methods:** 16HBE14o- cells were treated with siHE4 RNA and lack of HE4 confirmed by ELISA. Cells were exposed to hypoxia (1%O_2, 6h) and normoxia and supernatants collected. Supernatants (n=3 independent experiments) were pooled and subjected to the Proteome Profiler (R&D Systems). Relative cytokine concentrations were then assessed using ImageJ and selected candidates confirmed by ELISA and scRNAseq. **Results:** The absence of HE4 in hypoxia injury increased the profibrotic mediators TNF-alpha, GM-CSF, CXCL-12, IL-6 and CCL-3. Using ELISA, we confirmed significantly increased expression of TNF-alpha, IL-6 and CXCL-12. Single cell RNAseq analysis confirmed epithelial and fibroblast (CxCl12) expression.**Conclusion:** The upregulation of profibrotic cytokines in the absence of HE4 in hypoxic conditions suggests a regulatory and wider immunosuppressant role of HE4 in pulmonary fibrosis. **Conflicts of interest:** Authors declare no conflicts of interest