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Title: Transcriptional analysis of the human BPIFA1/SPLUNC1 gene

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Body: Despite being one of the most highly expressed genes in the respiratory tract nothing is known about the molecular basis of the cell type specific expression of the BPIFA1/SPLUNC1 gene. Though the gene is very highly expressed in differentiated tracheobronchial epithelial cells cultured at an air liquid interface these cells are refractory for traditional promoter analysis and only a limited number of cell lines express the gene robustly. We have shown that the adenosquamous carcinoma cell line, NCH H647 is one such cell and in the present study we have used this line to begin to characterize the proximal promoter of the human BPIFA1 gene. We performed a bioinformatic analysis to identify the likely proximal promoter and we generated a deletion series of reporter constructs in pGL3 corresponding to this region located within 1kb upstream of the transcription start site. Transfection of these constructs confirmed that these elements contained positive regulatory regions and we could show that this element resides within the first 300bp of the putative promoter region. Transfection of these constructs into a second expressing cell line (NCI H292) confirmed the localisation of this element. As we have previously shown that BPIFA1 expression is down regulated by treatment with interferon gamma (IFNg) we treated cells transfected with the constructs alongside others that had been transfected with the IFNg responsive promoter from the SCGB1A1/CCSP gene. These results suggest that BPIFA1/SPLUNC1 is repressed by an IFNg responsive element located in the proximal promoter. These proximal promoter elements will be essential for uncovering the molecular basis of the tissue specific expression of the BPIFA1/SPLUNC1 gene.