WS2.1 Investigation of MiRNA regulation of ER stress in cystic fibrosis airway epithelium

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Objectives: Inflammation, infection and the accumulation of misfolded CFTR have all been reported to instigate an ER stress response in cystic fibrosis. Emerging evidence now implicates microRNA (miRNA) in providing an additional layer of control over the Unfolded Protein Response (UPR). Here the role of miRNA in regulating basal and stimulus-induced ER stress in bronchial epithelial cells *in vitro* and *in vivo* was investigated.
Methods and Results: The miRNA expression profile of bronchial brushings taken from CF and non-CF individuals (n = 5 each) was quantified via *in situ* qRT-PCR. Using *in silico* analysis groups of miRNA predicted to target components of the UPR were identified that were collectively up regulated in CF. Expression of a selection of UPR genes that can be induced via ER stress in non-CF cells was observed to be decreased in CF *in vivo* (ATF6, Grp78, PERK, Erp57, ATF3, Derlin-1, XBP-1) and *in vitro* (ATF6, Grp78, Erp57, XBP-1) under basal conditions. ATF6 was experimentally validated as a direct molecular target of miR-145, -221 and -494 via pre-miR over expression and anti-miR inhibition experiments, and through the use of a luciferase reporter vector containing the full length 3'UTR of ATF6. Reduction of these miRNA and reciprocal ATF6 expression was observed in the presence of LPS.
Conclusions: These results indicate a lack of an active UPR in CF bronchial epithelial cells *in vivo* or *in vitro* under basal conditions which may be in part due to suppression of genes involved by increased miRNA expression in CF.

WS2.2 Partially degraded N-terminal half delF508 CFTR molecule produces aggregation rearrangement of intermediate filaments in fully differentiated human bronchial epithelium

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DelF508 mutation of CFTR results in misfolding of CFTR and failure in processing of the functional apical membrane form. Misfolded delF508 CFTR forms aggregates when overexpressed in the presence of proteosome inhibitors. A recent report showed that defective CFTR induced aggresome formation in a human epithelial cell line. There is no convincing evidence that aggregates of misfolded CFTR to contribute to the pathogenesis of CF. Using a series of domain-specific anti-CFTR antibodies we now show accumulation of specific domains of delF508 CFTR characterized by immunofluorescence and Western blotting. Aggregates accumulated in both well-differentiated ciliated cells and pulmonary neuroendocrine cells. Aggregation occurred in differentiated primary epithelial cells from delF508 CF lung but not in normal and a proliferating CFBE (DelF508) epithelial cell line. Aggregates were found to consist of a mixture of half CFTR peptides, inclusive of the N-terminus sequence and terminating in the middle of the NBD1 domain in association with cytokeratin 18. No C-terminal half peptides were found in the aggregates. The half CFTR is covalently modified, and most likely by ubiquitination. Our findings are consistent with previous studies that showed the N-half with a mutation in the NBD1 domain is much less soluble in vitro and is resistant to limited protease digestion while the C-half, i.e. NBD2 domain, remains unfolded, as the consequence of the misfolding of the NBD1 domain, and is then highly sensitive to protease digestion.
The finding that delF508 peptides disrupt the normal intermediate filament architecture in critical pulmonary cell types could exacerbate CF lung pathogenesis.

WS2.3 *Pseudomonas aeruginosa* enhanced gap junctional communication by a TLR5 and MAPK-dependent mechanism in airway epithelial cells

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Objectives: Gap junction channels, formed by connexin (Cx) proteins, provide low resistance pathways for intercellular propagation of signals that regulate CFTR activity and airway surface liquid volume. To evaluate their role in innate airway defense, we studied the functional expression of Cx43 in airway epithelial cell exposed to *Pseudomonas aeruginosa* (*Pa*).
Methods: Calu-3 airway epithelial cells grown on Transwell inserts were infected with the *Pa* laboratory strain PAO1 or with heat-killed PAO1. Both increased Cx43 mRNA levels and Cx43 protein expression. However, this increased was not observed in cells infected with a PAO1 strain lacking flagella (*fliC*), suggesting that Cx43 up-regulation was mediated by the activation of pathogen recognition receptors. Using a variety of inhibitors, we identified p38, ERK and JNK in the signaling pathways regulating Cx43 expression. PAO1-induced Cx43 expression was reduced by inhibitors of p38 and ERK and strongly enhanced by JNK inhibition. In all conditions, Cx43 expression was associated with concomitant changes in gap junctional intercellular communication. Interestingly, gap junctional communication was associated with increased detection of active caspase-3.
Conclusions: These results indicate that Cx43 is a target of *Pa*-dependent signaling triggered by flagellin binding to TLR5 in Calu-3 cells. During *Pa* infection, Cx43 expression is finely tuned by MAPKs: p38 and ERK increase Cx43 while JNK prevents an excessive up-regulation of gap junctional communication. Further work is in progress to test whether gap junctions propagate apoptotic signals in response to *Pa* infection.

WS2.4 Cx26 regulates Klf gene expression and proliferation of human airway epithelial cells undergoing repair

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Objectives: Aberrant remodeling of the airway epithelium represents a common feature of many types of pulmonary diseases. We aimed to investigate the early mechanisms involved in CF airway epithelial cell (HAEC) repair.
Methods: In a human model of circular wound injury of well-differentiated HAEC cultures, we identified Klf2, Klf4 and the gap junction protein Cx26 as important regulators of cell proliferation. This early step of epithelial repair was associated with the transient (12–96 hours) induction of the nuclear marker Ki67. Induction of cell proliferation was associated with a similar transient expression of Cx26, which like Ki67 was only detected in the repairing area of HAEC cultures. To further examine the relationship between proliferation and Cx26 expression, we used Bmi-1/hTERT immortalized HAECs, which endogenously express Cx26. Treatment of HAECs with mitomycine C decreased Cx26 expression, indicating that proliferation precedes Cx26 expression. However, Cx26 silencing with specific siRNAs was associated with a 2.4-fold increase of Ki67-positive cells. We further show that Klfs are negative regulator of HAEC proliferation and that Cx26 silencing markedly decreased the transcription of Klf2/4. Using primary cultures of CF HAECs, we found that the temporal expression of Klfs during repair was deregulated, leading to a prolonged proliferation phase and accelerated wound closure with global detection of Ki67- and Cx26-positive cells within the whole culture area.
Conclusions: The Klfs/Cx26 balance regulates cell proliferation in repairing HAECs and defective control of Klf gene expression may be responsible of the hyperproliferative state observed in CF HAECs.