494. New insights into airway epithelial cell physiology

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***Pseudomonas aeruginosa* causes endoplasmic reticulum stress and loss of tight junctions (TJ) in primary bronchial epithelial cells (PBEC)**

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P.aeruginosa has been shown to decrease epithelial barrier function by disrupting TJ, a process mediated by secreted virulence factors. In *C.elegans*, *P.aeruginosa* was shown to induce XBP-1 splicing (XBP1spl), a key event in endoplasmic reticulum (ER) stress. Moreover, patients with ER stress-associated lung diseases, like CF and COPD, show clinical deterioration after *P.aeruginosa* infection. We hypothesize that *P.aeruginosa* induces ER stress accompanied by loss of TJ, which is exaggerated in ER stress sensitized epithelial cells.

PBEC were exposed to conditioned medium of *P.aeruginosa* strain PAO1 (PAO1-CM) and/or thapsigargin (Tg; chemical ER stress inducer). PAO1-CM caused a time- and dose-dependent ER stress response with a maximal 11.9-fold increase ($\pm$7.8;$p<0.001$) of XBP1spl after 12 hours and 13.0-fold increase ($\pm$6.8;$p=0.02$) of CHOP mRNA after 8 hours, measured by qPCR. This increase was preceded by a 95.1% ($\pm$4.9%) reduction in zona occludens-1 (ZO-1) at 6 hours as assessed by Western blot. After 6 hours, cells showed a gradual decline in resistance until the monolayer was disrupted as assessed by ECIS. In the presence of Tg, PAO1-CM showed an accelerated decline in resistance and loss of TJ, together with a synergistic effect on IL-8 production, but no additional effect on ER-stress markers.

PAO1-CM induces ER stress in PBEC which is associated with altered epithelial barrier function. Tg-induced ER stress increases the response to PAO1-CM. These mechanisms may contribute to the disease progression observed after *P.aeruginosa* infection.

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Mechanism of epithelial Na^+ channel (ENaC) inhibition by hypoxia in alveolar epithelial cells

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Introduction: Transepithelial sodium transport via alveolar epithelial Na^+ channels (ENaC) and Na,K-ATPase constitutes the driving force for removal of alveolar oedema fluid. However, alveolar hypoxia associated with pulmonary edema may impair ENaC activity in alveolar epithelial cells (AEC).

Methods: We studied the mechanism of hypoxia-induced decrease in ENaC activity and alveolar Na^+ absorption in vitro in rat AEC and in vivo in β-Liddle mouse strain harbouring a mutation within the β-ENaC gene abolishing the interaction between ENaC and the ubiquitin protein-ligase Nedd4-2 that targets the channel for endocytosis and degradation in the proteasome.

Results: In vitro, acute exposure of AEC to hypoxia (0.5% O2 for 1-6h) rapidly decreased transepithelial Na^+ transport as assessed by equivalent short-circuit current Ieq and the amiloride-sensitive component of Na^+ current across the apical membrane, reflecting ENaC activity. Hypoxia reduced the expression of α-, β- and γ-ENaC proteins in the plasma membrane, with no change in intracellular expression. Hypoxia-induced inhibition of amiloride-sensitive Ieq was rapidly reversed by the β2-agonist terbutaline, and was fully prevented by preincubation with proteasome inhibitors. In vivo, hypoxic exposure (8% O2 for 24h) reduced amiloride-sensitive alveolar fluid clearance by 69% in wild-type mice without changing the expression level of ENaC proteins in the distal lung, but had no significant effect in homozygous mutated Liddle mice.
Conclusion: These data strongly suggest that decreased cell surface expression of ENaC subunits under hypoxic condition is related to Nedd4-2-mediated endocytosis of ENaC and subsequent degradation in the proteasome.

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IL-17C is expressed in respiratory epithelial cells under inflammatory conditions
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Background: IL-17 is a cytokine that comprises a group of five IL-17 subtypes (IL-17 A to F). IL-17 directly activates epithelial cells leading to the expression of inflammatory mediators and antimicrobial factors. Recent studies showed that IL-17A/F is released by professional immune cells such as CD4+ T cells and macrophages whereas IL-17C is expressed by epithelial cells. It was the purpose of this study to examine the expression of IL-17 in respiratory epithelial cells infected with bacterial pathogens.
Methods: Bronchial epithelial cells were exposed to smoke and infected with bacterial pathogens. Mice were exposed to smoke and colonized with *H. influenza*. Expression and release of IL-17 (A to F) was measured by ELISA and qRT-PCR. IL-17C was detected in human bronchial tissue by immunohistochemistry.
Results: Bacterial pathogens such as *P. aeruginosa* and *H. influenzae* induced the expression and release of IL-17C in human bronchial epithelial cells (HBECs). The same was true for ligands of Toll-like receptors 3 and 5 (flagellin, polyI:C). The expression of IL-17A/B/D/E was not induced by bacterial stimuli in HBECs. Cigarette smoke suppressed the expression of IL-17C in HBECs in response to bacterial infection in vitro and in the upper airways of mice colonized with *H. influenza* in vivo. IL-17C could be detected in bronchial tissue of subjects with infection-related lung diseases.
Conclusion: These data show that IL-17C is involved in the innate immune response of respiratory epithelial cells. Smoke suppresses IL-17C expression in case of infection.

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Synthetic response of stimulated respiratory epithelium: Modulation by prednisolone and iKK2 inhibition
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Background: The airway epithelium plays a central role in wound repair & host defence & is implicated in the immunopathogenesis of asthma. Whether there are intrinsic differences between the synthetic capacity of epithelial cells derived from asthmatics & healthy controls & how this mediator release is modulated by anti-inflammatory therapy remains uncertain.
Aims: We sought to examine the synthetic function of epithelial cells from different locations in the airway tree from subjects with & without asthma & to determine the effects of anti-inflammatory therapies upon this synthetic capacity.
Methods: Primary epithelial cells were derived from 17 asthmatics & 16 controls. The release of 13 mediators from nasal & bronchial basal & air-liquid interface differentiated epithelial cells before & after stimulation with IL-1β, IL-1β & IFNγ or Poly-IC (TLR3 agonist) were measured using MSD or ELISA & the effects of prednisolone, rosiglitazone, & an inhibitor of nuclear factor κ-β2 (IKK2i) were determined.
Results: The pattern of release of cytokines & chemokines was significantly different between nasal & bronchial basal & differentiated epithelial cells, but not between health & disease. Stimulation of the epithelial cells caused marked up-regulation of most mediators which were broadly corticosteroid unresponsive, but attenuated by IKK2i.
Conclusion: Synthetic capacity of primary airway epithelial cells varies between location & degree of differentiation, but is not disease specific. Activation of epithelial cells by pro-inflammatory cytokines & TLR3 agonism is attenuated by IKK2i, but not corticosteroids suggesting that IKK2i may represent an important novel therapy for asthma.

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FOXO transcription factors regulate innate immune mechanisms in respiratory epithelial cells
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Background: Bacterial pathogens are a leading cause of lung infections and contribute to acute exacerbations in patients with chronic respiratory diseases. The innate immune system of the respiratory tract controls and prevents colonization of the lung with bacterial pathogens. FOXO transcription factors are key regulators of cellular metabolism, proliferation and stress resistance. In this study, our aim was to investigate the role of FOXO transcription factors in innate immune functions of respiratory epithelial cells.
Methods: Bronchial epithelial cells were transfected with siRNA specific for FOXO1 and FOXO3 and infected with *Haemophilus influenza* and *Pseudomonas aeruginosa*. Expression of inflammatory cytokines and antimicrobial peptides were determined. Epithelial uptake of bacteria was examined. FOXO3 was detected in human bronchial tissue.
Results: Infection with bacterial pathogens potently activated FOXO transcription factors in respiratory epithelial cells *in vivo* and *in vitro*. Active FOXO was also detectable in bronchial tissue of subjects with different infection-related lung diseases. siRNA mediated knock down of FOXO in epithelial cells resulted in reduced expression of factors of the innate immune system such as antimicrobial peptides and proinflammatory cytokines under basal conditions and upon infection. FOXO deficiency further affected internalization of *Haemophilus influenzae* in bronchial epithelial cells. TLR3 activated innate immune responses in a FOXO-dependent manner.
Conclusion: FOXO transcription factors are involved in the cellular responses to bacterial stimuli and possess a central role in regulating innate immune functions of respiratory epithelial cells.

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Corticosteroids improve airway epithelial regeneration and restore oxidative stress-induced epithelial barrier dysfunction
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Background: The airway epithelium plays an emerging role in the pathogenesis of respiratory diseases. Its barrier function may be defective in asthma and COPD.
Aims: To study the protective effect of inhaled corticosteroid budesonide (BUD) on different aspects of epithelial integrity, including cell-cell contact formation, regeneration upon wounding and barrier function during mucociliary differentiation at the air-liquid interface (ALI).
Methods: We studied the effect of BUD (10^{-7}-10^{-9}M) on epithelial resistance during ALI culture, upon exposure to H_2O_2 and/or cigarette smoke extract (CSE) and upon wounding by electroporation using the ECIS system in 16HBE cells and primary human bronchial epithelial cells (PBECs) from asthma patients, COPD patients and healthy controls.
Results: BUD significantly improved the recovery of cell-cell contacts upon wounding in 16HBE cells. Oxidative stress induced a transient defect in 16HBE barrier function, which was attenuated by BUD and likely mediated by the protection against EGFR-dependent disruption of junctions. In PBECs, CSE exposure resulted in a substantial barrier dysfunction, with no significant differences between the subject groups. BUD significantly improved baseline resistance and counteracted the CSE-induced barrier dysfunction. Finally, BUD slightly but significantly increased epithelial barrier function during ALI-culture, but did not affect MUC5A/C production.
Conclusions: BUD improves the restoration of epithelial cell-cell contacts upon oxidative stress and wounding and may thus provide beneficial effects on epithelial barrier function, repair and remodelling in the airways of asthma and COPD patients.

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ANO1 expression and activity in cystic fibrosis
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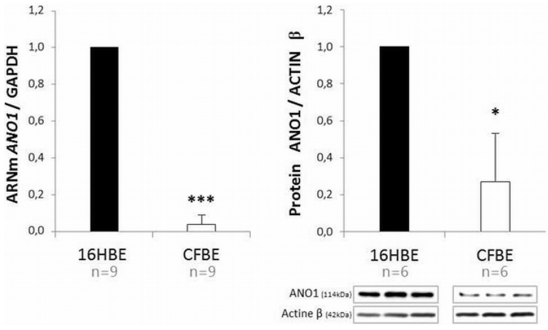
Defective CFTR function in the airway epithelium is responsible for cystic fibrosis (CF) patient lung disease. CFTR represents the most important pathway for apical chloride (Cl-) secretion in human bronchial epithelial cells. Calcium activated Cl-channels (CaCCs) are also an important pathway of Cl- secretion. In 2008, three independent teams suggest that ANO1 (Anoctamine 1) also called TMEM16a could be a CaCC candidate. Mice lacking ANO1 exhibit a defect in epithelial Cl-transport and pathology similar to CF.

The main aim of this study is to characterize ANO1 protein in CF vs non CF context.
For this study, we used different CF and non CF models whose cell lines, mice and lung explants from CF and non CF patients.
Our results show that ANO1 expression and activity are significantly decreased in CF compared to non CF models.



ANO1 is expressed at plasma membrane of bronchial epithelial cells and there is no difference in localization between CF and non CF cells. To understand the differential expression between CF and non-CF cells/tissus we will investigate miRNA expression that could modulate ANO1 protein.
We conclude that decreased ANO1 activity in CF cells could be explained by decreased ANO1 ARNm and protein expression and may contribute to the worsening of ionic imbalance and decrease lung function.
All of these results lead us to think that this Cl- channel could be a potential pharmacological target for the treatment of cystic fibrosis patients.

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Cystic fibrosis epithelial cells are primed for apoptosis as a result of increased Fas

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Apoptosis is a physiological process essential for homeostasis of epithelial organisation and function. Cystic fibrosis (CF) lung disease is characterised by chronic infection and inflammation and previous work suggests that apoptosis is dysfunctional in the CF airways with conflicting results. In addition, controversy exists regarding how CFTR misfolding contributes to apoptosis. In this study, we evaluated the relationship between CFTR mutation and apoptosis in CF airway epithelial cells. Basal activity of the executioner caspase, caspase-3, was significantly increased in CF tracheal and bronchial epithelial cell lines and primary bronchial epithelial cells compared to non-CF controls. In addition, activity of the upstream initiator caspase, caspase-8, was significantly increased in CF epithelial cells compared to controls, suggesting involvement of extrinsic apoptosis signalling, which is mediated by the activation of death receptors, such as Fas (CD95). Increased levels of Fas were observed in CF epithelial cells and bronchial brushings, reciprocal decreases in a selection of microRNAs predicted to target Fas were evident in the brushing samples, and neutralization of Fas significantly inhibited caspase-3 activity in CF epithelial cells compared to untreated cells. Furthermore, activation of Fas significantly increased caspase-3 activity and apoptosis in CF epithelial cells compared to control cells. Overall, these results suggest that CF airway epithelial cells are more sensitive to apoptosis via increased levels of Fas and subsequent activation of the Fas death receptor pathway. Further work will delineate the mechanism underlying increased Fas expression in CF epithelial cells.