P407

A-kinase anchoring proteins (AKAPs) as potential novel therapeutic targets to improve cigarette smoke-induced loss of epithelial barrier function

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Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease of the airways. The inflammation in COPD affects the airway epithelial barrier which may play a role in COPD progression. Under healthy conditions, the epithelium forms a barrier against deleterious environmental agents present in cigarette smoke, the latter representing the main etiologic risk factor for COPD. However, the current COPD therapies hardly involve strategies to repair airway epithelial damage. Therapeutic strategies that target cyclic AMP (cAMP), known to restore the barrier in endothelial cells under diseased conditions, might be of interest. Signalling by cAMP is maintained by two cAMP driven effector proteins, PKA (protein kinase A) and Epac (exchange protein directly activated by cAMP). Recent studies from our group demonstrated that these cAMP effectors regulate cellular responses such as inflammation and smooth muscle tone in the airways. Signalling specificity of cAMP is achieved by A-kinase anchoring proteins (AKAPs) that coordinate cAMP signaling by binding to both cAMP effectors and thereby to localize their activity to certain subcellular compartments. Interestingly, AKAP9 is known to associate with E-cadherin, which is a central component of adherens junctions, which maintain a proper epithelial barrier function. We hypothesized here that AKAPs play a critical role in maintaining epithelial barrier function and integrity upon damage by cigarette smoke. For all experiments human bronchial epithelial (16HBE14o-) cells were used. Cells were stimulated with 1% cigarette smoke extract (CSE) with or without the AKAP inhibitor Ht31 (30 μM). E-cadherin and AKAP protein expression was analysed after 24 h. Electrical cell-substrate impedance sensing (ECIS), a technique which measures cell-cell contacts, was used to study the effect of CSE and Ht31 on barrier function. In addition, AKAP9 mRNA expression was analyzed in primary epithelial cells from smokers versus non-smokers and in lung biopsies from non-COPD subjects and COPD patients. Statistical analysis was performed using an ANOVA followed by a post-hoc test or a paired t-test, when appropriate. CSE markedly reduced the epithelial barrier function by 42% and reduced the expression of E-cadherin at cell-cell contacts by 47%. Both processes were completely prevented by disruption of AKAP complexes using Ht31. Interestingly, CSE reduced AKAP9 expression by 53%, while leaving the expression of other AKAPs that are not associated with E-cadherin (AKAP5 and AKAP12) nearly unaffected. Importantly, AKAP9 mRNA expression is also down-regulated in primary epithelial cells from current smokers *vs* non-smokers (0.89 fold of control). AKAP9 mRNA expression is reduced in lung biopsies of COPD patients stage II and IV (0,27 fold of control). Next to AKAP9-dependent targeting of E-cadherin, cigarette smoke may, both directly and via activation of PKA, phosphorylate VASP which in turn activates Rac. When stabilized by IQGAP1, Rac decreases the binding of E-cadherin to β-catenin and thereby reduces cell-cell contacts. Such processes may be prevented by Ht31, and may thereby contribute to the observed restoration of the epithelial barrier. Our data show that AKAPs contribute to CSE-induced disruption of the human bronchial epithelial barrier by translocation of E-cadherin from the cell membrane, via a currently unknown molecular mechanism (Financial support: NAF, grant 3.2.09.034; Rosalind Franklin Fellowship)