New avenues for Epac in inflammation and tissue remodeling in COPD

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Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease of the airways and the lung parenchyma, further characterized by airway obstruction and remodeling (1). Symptoms are treated with glucocorticosteroids, anticholinergics, β_2-agonists or phosphodiesterase (PDE)-4 inhibitors (2,3). Both β_2-agonists and PDE4 inhibitors elevate the second messenger cyclic AMP (cAMP), although by distinct mechanisms (4,5). The two main effectors of cAMP are protein kinase A (PKA) and the exchange protein directly activated by cAMP (Epac), which consists of the two isoforms Epac1 and Epac2.

Epac1 and Epac2: Inflammation

An important cytokine that is increased in COPD is interleukin-8 (IL-8). Our data indicate that Epac and PKA decrease cigarette smoke extract (CSE)-induced IL-8 release by airway smooth muscle (ASM) cells, via inhibition of NF-κB and ERK signalling, respectively. Importantly, we also observed reduced Epac1 expression in lung tissue from COPD patients (6,7).

To investigate the potential cause for the downregulation of Epac1, we studied microRNAs (miRNAs). MiRNAs are epigenetic regulators involved in fine-tuning of cellular activities by posttranscriptional repression of mRNA. In COPD patients, miRNA-7 is increased in serum (8). We investigated the potential interaction of Epac1 and miRNA-7. CSE induced miRNA-7 specifically in human ASM cells. In line, miRNA-7 was increased in bronchial smooth muscle of COPD stage II patients isolated by laser dissection. Importantly, Epac1 expression is reduced in miRNA-7 overexpressing human ASM cells (Figure 1). Our data implicate that upregulation of miRNA-7 by cigarette smoke correlates with the downregulation of Epac1 in COPD (8).

Both Epac1 and Epac2 seem to be implicated in the reduction of CSE-induced IL-8 release from human ASM cells. However, phospholipase Cε (PLCε), a direct effector of Epac, acts pro-inflammatory. To translate our findings on inflammation *in vitro* to the *in vivo* situation, Epac1-/-, Epac2-/- and PLCε-/- mice were exposed to cigarette smoke for 5 days. We demonstrated that compared to wild-type (WT) mice exposed to cigarette smoke, the number of total inflammatory cells, macrophages, and neutrophils as well as IL-6 release were lower in Epac2-/- mice, which was also the case for neutrophils and IL-6 in PLCε-/- mice (Figure 2, ref 9). Compared to WT mice exposed to cigarette smoke, the number of macrophages was reduced in Epac1-/- mice. Whereas, the numbers of lymphocytes, only present in low numbers in BALF of air-exposed WT mice, were increased in Epac1-/- mice. Together our data indicated that particularly Epac2 acts pro-inflammatory *in vivo* (9).

Aberrant epithelial repair is also regarded as a pathophysiological feature of COPD. It is of interest that the A-kinase anchoring protein (AKAP) family member AKAP9 enhanced the endothelial barrier function in concert with Epac1. AKAPs compartmentalize cellular cAMP upon generation of multiprotein complexes. We found that CSE reduced the barrier function in human bronchial epithelial cells, a process accompanied by a reduced membrane expression of E-cadherin and AKAP9 (Figure 1). Silencing of AKAP9 reduced the functional epithelial barrier and prevented the ability of st-Ht31, an inhibitor of AKAP-PKA interactions, to restore membrane localization of E-cadherin. Our data indicate that AKAP9 maintains the bronchial epithelial barrier function and may be important in the pathophysiology of COPD (10).

Altogether, Epac1 and Epac2 seem to be involved in cigarette smoke induced inflammation, although with a clear distinction regarding their relative contribution. Compartmentalization of cAMP driven by AKAP family members, may be responsible for the distinct biological effects of cAMP.

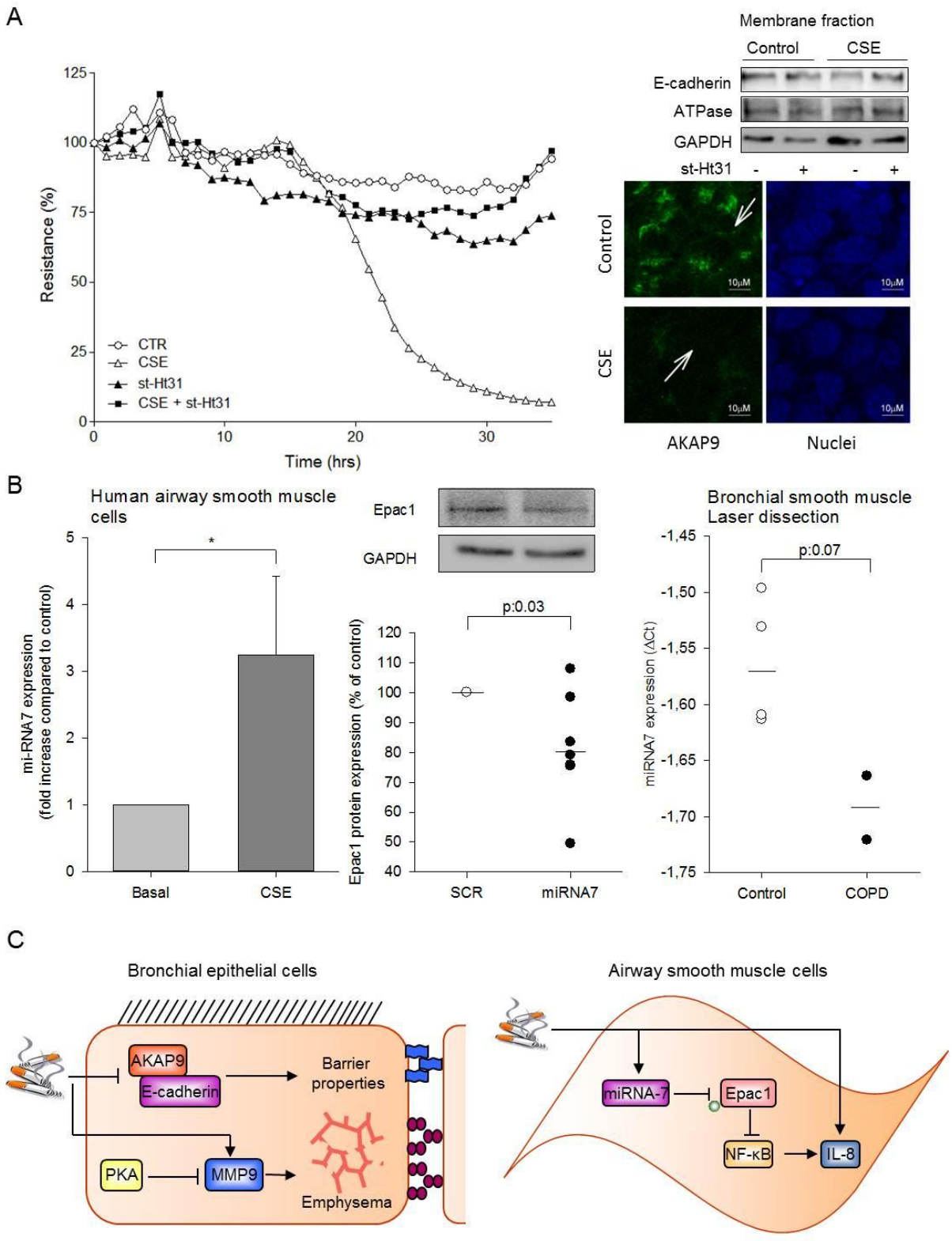

Figure 1: (A) In human bronchial epithelial cells, cigarette smoke extract reduces the barrier function (resistance)which is prevented by St-Ht31, a inhibitory peptide of AKAP-PKA interactions. In addition, the expression of both AKAP9 and E-cadherin is reduced by cigarette smoke extract (further details see ref 9). (B) In airway smooth muscle cells, cigarette smoke extract enhances miRNA-7 levels. Overexpression of miRNA-7 inhibits the protein expression of Epac1. In bronchial smooth muscle of COPD patients a upregulation of miRNA-7 is observed. (C) Epac: implications for structural lung cell functioning(further details see ref 6).

Remodeling: Epac1 and Epac2

Tissue remodeling is another feature of COPD and covers different characteristics of the disease such as mucus hypersecretion, airway fibrosis and emphysema. The majority of extracellular matrix (ECM) proteins, including collagens and fibronectin, are produced by fibroblasts. Alterations in the tightly controlled balance of production and degradation of ECM proteins causes structural changes in the lung such as emphysema, characterized by excessive degradation of parenchymal ECM , and (small) airway fibrosis characterized by excessive deposition of ECM proteins (1). Although a role for cAMP in the regulation of remodeling has been shown (4,5), the exact role of the cAMP effectors Epac and PKA on the different aspects of remodeling is not completely known.

Interestingly, it has been reported that cAMP-elevating drugs reduce collagen I synthesis in human lung fibroblasts. A PDE inhibitor and a cAMP analog have been shown to reduce MMP9 and TIMP1 gene expression and activity in different cell types. CSE exposure of the human bronchial epithelial cell line 16HBE14o- leads to increase of MMP9 mRNA and thereby of the MMP9/TIMP1 ratio. Induction of MMP9 mRNA was reduced by specific PKA activation. Pro-MMP9 levels induced by CSE were reduced by the fenoterol, an effect specifically mimicked by pharmacological activation of PKA. PKA inhibition may enhance the MMP9/TIMP1 ratio and thereby reduce emphysema. In contrast, activation of PKA may reduce emphysema by a decrease of the MMP9/TIMP1 ratio (further details see ref 6).

In addition, we tried to identify the role of Epac1 and Epac2 in remodeling processes in the lung by exposure of Epac1-/- and Epac2-/- mice to cigarette smoke. We demonstrated that Epac1-/- mice expressed higher levels of TGF-β1, collagen I and fibronectin. We propose that Epac1, but not Epac2, acts anti-fibrotic. Concerning mucus hypersecretion, Epac1-/- and Epac2-/- were characterized by a constitutively higher expression of MUC5AC mRNA at basal level (Figure 2). We observed that goblet cells tended to be increased in Epac2-/- and PLCε-/- mice, whereas primarily Epac1-/- mice tended to stain positive for the inducer of goblet cell differentiation SPDEF (9).

Overall, we identified distinct roles of Epac1 and Epac2 in remodeling processes. This suggest that Epac1 alone controls remodeling. In contrast, both Epac1 and Epac2 seem to control MUC5AC (9). Similar as for inflammation, compartmentalization of Epac by AKAPs will most likely define a potential role of AKAP-dependent compartmentalization of cAMP in remodeling processes.

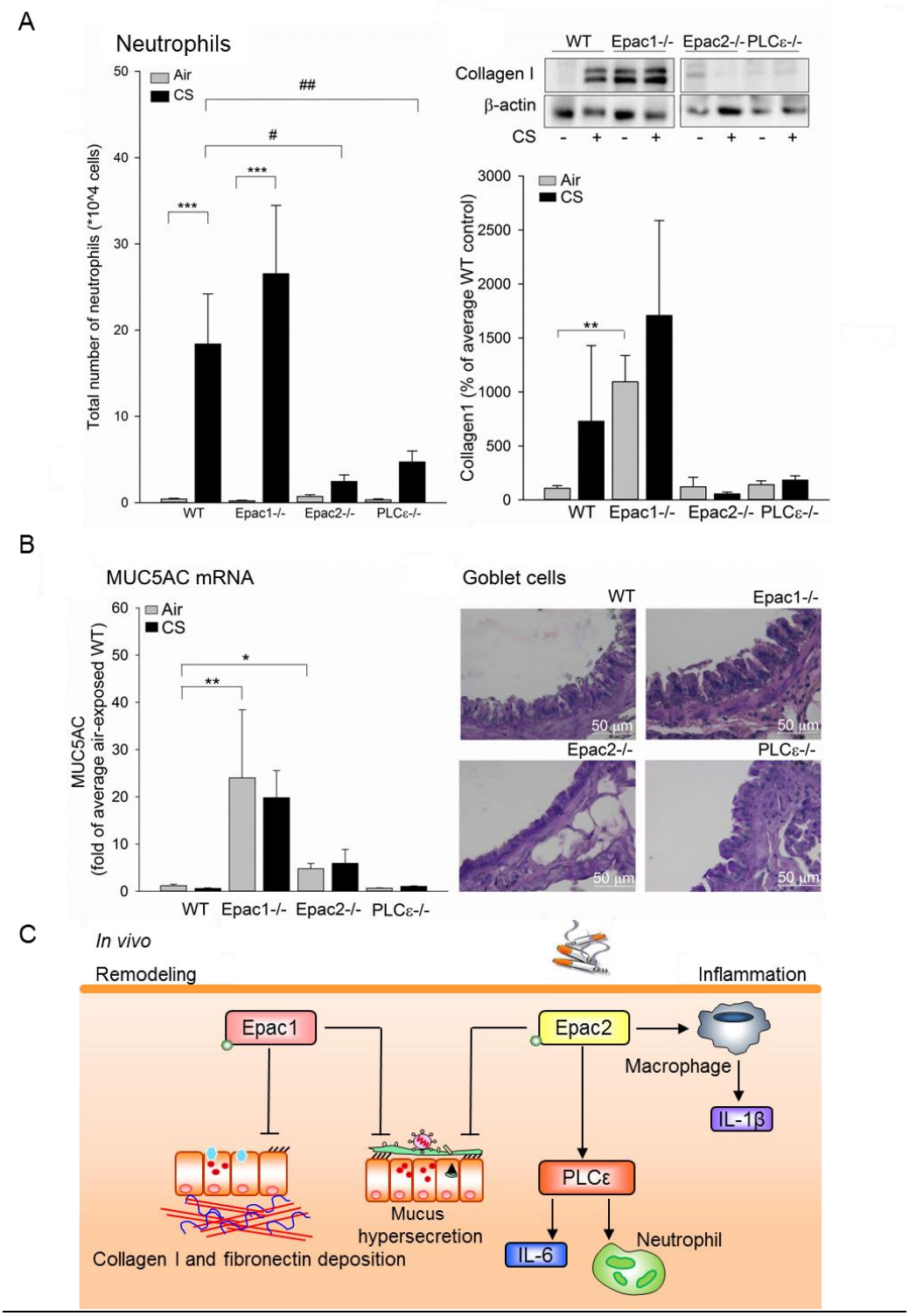

Figure 2: (A) In an acute mouse model of cigarette smoke exposure, Epac2, possibly via PLCε, enhances neutrophils (left panel). Epac1 bears the capacity to reduce the deposition of collagen I in lung tissue (right panel) . (B) Both Epac1 and Epac2 regulate mucus hypersecretion in mice. (C) Epac1 and Epac2: implications in inflammation and remodelling in vivo. Epac2 contributes to cigarette smoke induced-increase in macrophages and IL-1β release. Possibly via PLCε, Epac2 also enhances neutrophils and IL-6 release. Epac1 inhibits collagen I and fibronectin deposition. Both Epac1 and Epac2 play an inhibitory role in mucus hypersecretion. For further details see refs 6, 9.

Conclusions

These studies implicate an important role for cAMP in COPD. We defined distinct roles of Epac1 and Epac2 in inflammation and remodeling. In an acute model of cigarette smoke exposure in mice, we unraveled pro-inflammatory actions of Epac2. We showed that Epac1 alone is able to reduce remodeling, whereas for the reduction of MUC5AC a concerted action with Epac2 seemed to be required. Based on our findings, the development of selective Epac1 and Epac2 activators and/or inhibitors could be of additive value to alleviate symptoms of COPD. Targeting of these selective Epac activators or inhibitors to specific areas in the lung represents a future goal. Research into compartmentalization of cAMP by AKAPs should be another subject of future research.

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