New avenues for Epac in inflammation and tissue remodeling in COPD

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Interaction between miRNA-7 and Epac in airway smooth muscle cells

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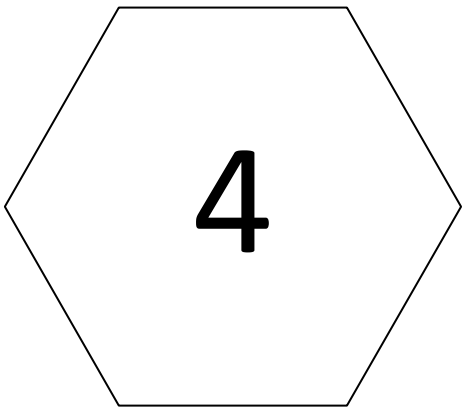

Naunyn-Schmiedeberg's Archives of Pharmacology 2014, 387(8):795-7

Chronic obstructive pulmonary disease (COPD) is an inflammatory disorder of structural airway cells with cigarette smoke as the main etiologic factor. COPD patients are treated with agonists of the β_2-adrenoceptor, which induce their biological effects by elevating cyclic AMP and subsequently activate protein kinase A (PKA) and the "exchange proteins directly activated by cAMP", Epac1 and Epac2. PKA and Epac are involved in key processes that contribute to the pathogenesis of COPD, including inflammation (Schmidt et al, 2013). The expression of specifically Epac1 protein is reduced in cultured human airway smooth muscle (HASM) cells, immortalized by stable ectopic expression of human telomerase reverse transcriptase enzyme, after exposure to cigarette smoke extract (CSE) and in lung tissue from COPD patients (Oldenburger et al, 2012). Since specific activation of Epac reduces CSE-induced cytokine release (Oldenburger et al, 2012), loss of Epac1 may contribute to the inflammatory process in COPD.

MicroRNAs (miRNAs) are epigenetic regulators involved in fine-tuning of cellular activities by posttranscriptional repression of mRNA. In COPD, miRNAs have been implicated in the regulation of inflammatory processes (Oglesby et al, 2010) and miRNA-7 is increased in serum of COPD patients (Akbas et al, 2013). *In silico* analysis indicates that Epac1 is a putative target of miRNA-7 (targetscan.org). Therefore, we hypothesized that increased miRNA-7 levels are involved in the attenuation of Epac1 expression in COPD patients. We investigated the expression of Epac and miRNA-7 in lung tissue of control and COPD patients as well as in CSE exposed structural airway cells.

HASM cells, MRC-5 fibroblasts and human bronchial epithelial (16HBE14o-) cells were exposed to CSE as described previously (Oldenburger et al, 2012). RT-PCR for miRNA-7 was performed by using stemloop primers (Stemloop: GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGCACTGGATACGACACAACAAA; Forward: GCGGTTGGAAGACTAGTGAT; Reverse: CCAGTGCAGGGTCCGAGGTCCG) and quantified results were normalized to RNU6B (Stemloop: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAAATATGG; Forward: TGCGGCTGCGCAAGGATGA; Reverse: CCAGTGCAGGGTCCGAGGTCCG).

CSE increased miRNA-7 expression specifically in HASM cells, leaving miRNA-7 in 16HBE14o- and MRC-5 cells unaffected (Fig. 1A). To correlate the expression of miRNA-7 and Epac1, HASM cells were lentivirally transformed to overexpress miRNA-7. Although Epac2 protein (Fig. 1C) was not altered, ectopic overexpression of miRNA7 significantly decrease Epac1 protein (Fig. 1B; p=0.03). These data suggest that Epac1 is targeted by miRNA-7 and that increased miRNA-7 expression is related to CSE-induced downregulation of Epac1 in HASM.
Since Epac1 expression is also attenuated in lung tissue of COPD patients (Oldenburger et al, 2012), we analyzed miRNA-7 expression in total lung homogenates of controls and of patients with GOLD stage II and IV COPD. Human lung tissue from COPD patients (GOLD stage II) and non-COPD controls were collected according to the Research Code of the University Medical Center Groningen (http://www.rug.nl/umcg/onderzoek/researchcode/index) and national ethical and professional guidelines ("Code of conduct; Dutch federation of biomedical scientific societies"; htttp://www.federa.org). No alterations were found in miRNA-7 expression in total lung homogenates from COPD patients compared to controls (Fig. 1D). In line with the specific induction of miRNA-7 in cultured HASM cells, miRNA-7 was increased in bronchial smooth muscle of COPD stage II patients isolated by laser dissection (Emmert-Buck et al, 1996), compared to controls (Fig. 1E).

In conclusion, our studies point for the first time to a possible link between miRNA-7 and Epac1. We hypothesize that the induction of miRNA-7 in HASM cells and bronchial smooth muscle of COPD patients is induced by exposure to cigarette smoke. Thus, our data may suggest that the downregulation of pulmonary Epac1 expression in COPD patients is related to the upregulation of miRNA-7.

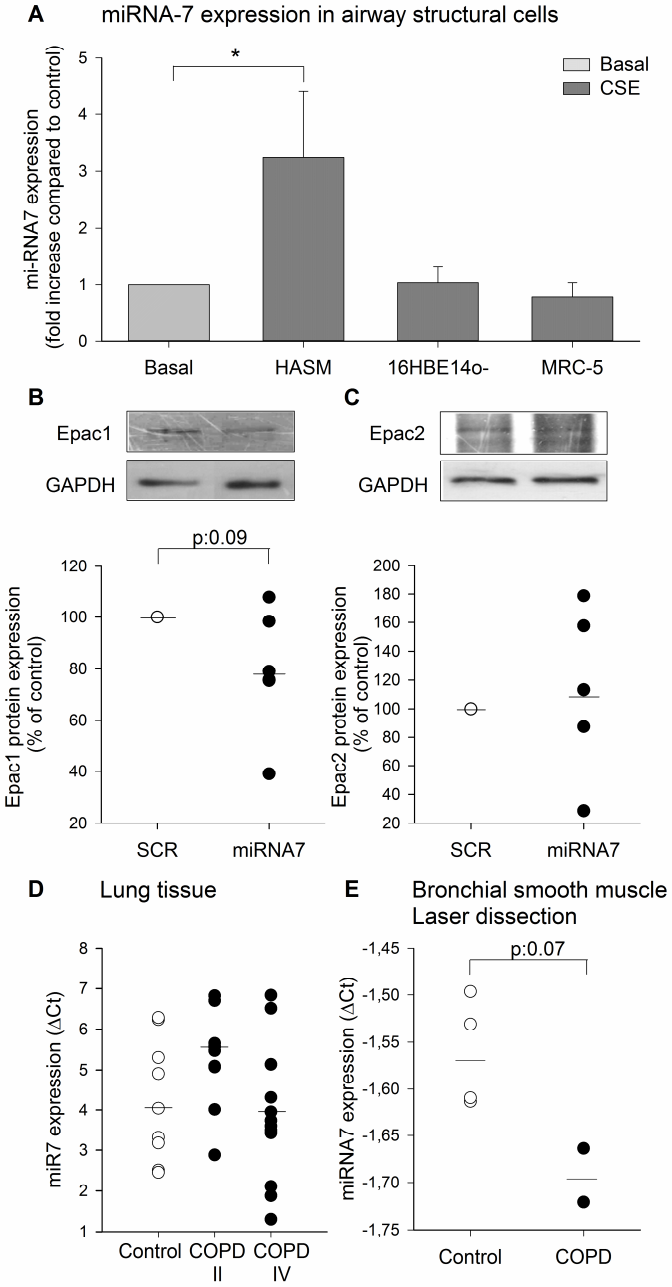

Figure 1: HASM, 16HBE14o- and MRC-5 cells were exposed to 15% CSE for 24 hrs and miRNA-7 levels were analyzed (n:4-5)(A). Epac1 (B) and Epac2 (C) protein expression was analyzed in miRNA-7 overexpressing HASM cells using scrambled mRNA as control (n:7). miRNA-7 expression was analyzed in total lung homogenates of controls and COPD

patients stage II or IV (D; n:9-13) (Oldenburger et al, 2012). Laser dissection was performed to isolate bronchial smooth muscle from lung tissue of control and COPD stage II patients to determine miRNA-7 expression (E; n:2-4) (Emmert-Buck et al, 1996). Data represented as mean ± SEM (A), or as individual data points (B-E). A Mann-Whitney Rank Sum Test (A-C) or a Fisher's Exact Test (E) was performed to determine statistical difference

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