

LETTER TO THE EDITOR

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# Small airway epithelial-C/EBP $\beta$ is increased in patients with advanced COPD

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## Abstract

The expression of CCAAT/enhancer-binding protein (C/EBP) $\beta$  in the small airway epithelium of COPD is unknown. C/EBP $\beta$  was assessed in peripheral lung tissue of non-smoking/smoking controls and patients with GOLD I-IV COPD by quantitative immunohistochemistry. The expression of C/EBP $\beta$  was decreased in smokers compared to never smokers. Furthermore, C/EBP $\beta$  was significantly elevated in advanced COPD vs. asymptomatic smokers, and the expression correlated to lung function decline. As C/EBP $\beta$  exerts pro-inflammatory effects in the context of cigarette smoke, the elevated C/EBP $\beta$  in advanced COPD may be an indication of a breakdown of regulatory mechanisms and excessive inflammation.

**Keywords:** COPD, C/EBP $\beta$ , Airway epithelium

## Findings

Chronic obstructive pulmonary disease (COPD) is characterized by small airway inflammation. While glucocorticoids (GCs) and  $\beta_2$  agonists are mainstay in the management of COPD, these classes of drugs are, by and large, ineffective in preventing disease progression [1]. The lack of efficient pharmaceutical options is in part due to the incomplete understanding of the intricate molecular mechanisms contributing to the disease.

The transcription factor CCAAT/enhancer binding protein (C/EBP) $\beta$  regulates inflammatory [2] and host defense genes [3] in the airway epithelium. Lung epithelial-C/EBP $\beta$  activates the inflammatory response to cigarette smoke [4], as well as lipopolysaccharide (LPS) [3]. Suppression of LPS-induced airway inflammation by  $\beta_2$  agonists is, however, also mediated by lung epithelial-C/EBP $\beta$  [3]. In addition, glucocorticoids increase the expression and transcriptional activity of C/EBP $\beta$ . Transactivation by glucocorticoids has in contrast to pro-inflammatory stimuli been suggested to up-regulate host defense genes [5, 6]. Hence, cigarette smoke and microbial ligands, as well as GCs and  $\beta_2$  agonists may all activate airway-epithelial C/EBP $\beta$  in COPD, with the possibility of

different outcomes depending on the stimuli. There is currently insufficient knowledge of the expression of C/EBP $\beta$  in the small airways of COPD, in particular in end-stage disease where GC/ $\beta_2$  agonist therapy is mainstay.

We obtained peripheral tissue specimens from patients with stable GOLD I-IV COPD ( $n = 30$ ), as well as controls with or without a smoking history ( $n = 14$ ) (Table 1). The study was approved by the Swedish Research Ethics Committee in Lund, Sweden. All study subjects signed informed consent to participate. Formalin-fixed and paraffin-embedded tissue sections were pre-treated with a pH 6.1 buffer (EnVision™ FLEX Target Retrieval Solution, Dako, Glostrup, Denmark). The expression of C/EBP $\beta$  was visualized by immunohistochemistry using a polyclonal rabbit anti-C/EBP $\beta$  antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and EnVision™ Peroxidase/DAB Detection System kit on an Autostainer Plus (DakoCytomation, Glostrup). Automated immunohistochemistry allowed for minimized operator error between tissue samples.

## C/EBP $\beta$ is decreased in the small airway epithelium of asymptomatic smokers

Strong immunoreactivity to C/EBP $\beta$  was observed in the peripheral airway epithelium, as well as in alveolar macrophages of COPD patients and asymptomatic controls (Fig. 1a-c). C/EBP $\beta$  positive cells were furthermore identified within and in the epithelial interface of lymphoid

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**Table 1** Baseline demographics and clinical characteristics

Parameter	Never smokers	Smokers w/o COPD	GOLD I-II COPD <sup>b</sup>	GOLD III-IV COPD <sup>c</sup>	<i>p</i> ANOVA
Subjects (n) <sup>a</sup>	8	6	18	12	
Gender (female/male)	6/2	3/3	5/13	6/6	
Age (years)	63 ± 4.8	56 ± 3.2	68 ± 1.8	61 ± 1.2	<0.05
Height (m)	1,64 ± 0.033	1.72 ± 0.05	1,74 ± 0.02	1,7 ± 0.031	ns
Weight (kg)	64,6 ± 4.6	69.2 ± 4.4	73,1 ± 3.5	67,7 ± 4.1	ns
Body mass index	23,9 ± 1.3	23.3 ± 1.1	24,4 ± 1.1	23,3 ± 0.94	ns
Pack years	N/A	43 ± 9.7	45 ± 3.5	41 ± 3.2	ns
Smoker/ex-smoker	N/A	3/3	7/11	0/12	
FEV1/FVC	85,9 ± 5.7	77.8 ± 2.4	61,4 ± 1.9	33,4 ± 2.1	<0.001
FEV1 (% of predicted)	109,8 ± 6.2	93.8 ± 4.2	74,1 ± 2.7	26,2 ± 2.7	<0.001
Corticosteroids (yes/no/unknown)	0/8/0	0/6/0	2/16/0	9/2/1	
Bronchodilator (yes/no/unknown)	0/8/0	0/6/0	6/12/0	9/2/1	

<sup>a</sup>All surgeries were performed at Skåne University Hospital, in Lund, Sweden

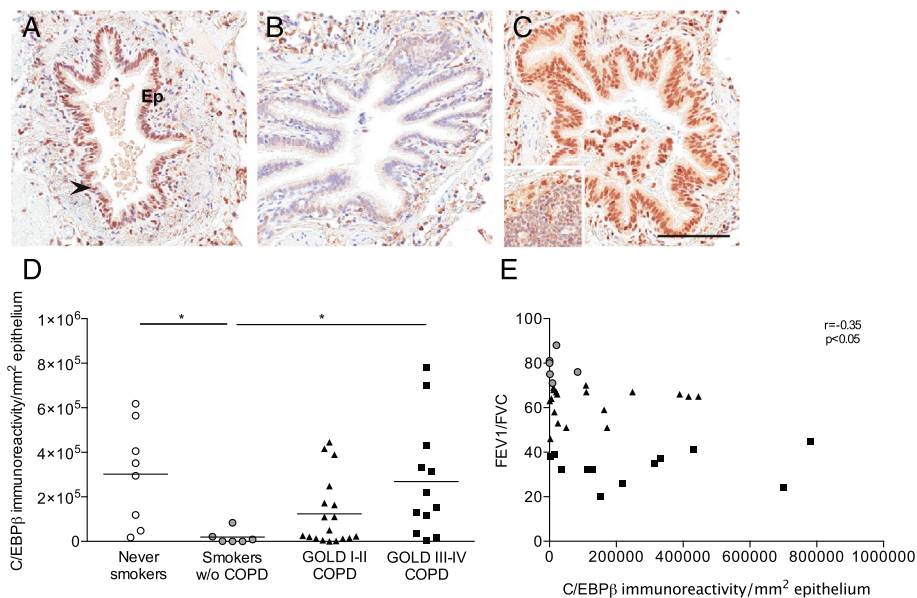
<sup>b</sup>Tissue samples were obtained during lung resection surgery for bronchial tumour

<sup>c</sup>Tissue samples were obtained from GOLD II COPD patients during lung resection surgery for bronchial tumour, and from GOLD IV COPD patients during lung transplantation

follicles [7], in lung tissue collected from patients with COPD (inlet of Fig. 1c).

An Aperio ScanScope Slide Scanner (Aperio Technologies, Vista, CA) was used to generate digital images of the tissue sections, and morphometric analyses were

performed using Aperio ImageScope v.10.0 software (Aperio Technologies) [8]. Computerized image analysis revealed that the expression of C/EBPβ was significantly lower in the airway epithelium among asymptomatic controls with a smoking history compared to never



**Fig. 1** Reduced expression of C/EBPβ in asymptomatic smokers and elevated expression in advanced COPD. Light micrographs of the immunoreactivity to CCAAT/enhancer-binding protein (C/EBP)β in peripheral pulmonary tissue of a (a) never-smoker, (b) asymptomatic smoker, and (c) patient with very severe chronic obstructive pulmonary disease (COPD). Immunoreactivity was detected by DAB (brown). Sections were counterstained with Mayer's Hematoxylin (blue). Scale bar indicates 100 μm. The epithelium (Ep) is denoted and an arrowhead indicates a positive cell in (a). Inlet of (c) shows immunoreactivity to C/EBPβ in a tertiary lymphoid follicle. **d** Immunoreactivity (defined as number of positive pixels/mm<sup>2</sup>) to C/EBPβ in peripheral lung epithelium of never-smokers, asymptomatic smokers and patients with mild-moderate (global initiative of COPD, GOLD I-II) and severe-very severe (GOLD III-IV) COPD. Horizontal lines indicate mean value. **e** Pearson correlation coefficient analysis of the immunoreactivity/mm<sup>2</sup> to C/EBPβ in peripheral lung epithelium and the forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) of smokers without COPD and patients with GOLD I-IV COPD. Clear circles: never smokers without airway obstruction; grey circles: asymptomatic smokers, triangles: GOLD I-II COPD; squares: GOLD III-IV COPD. *n* = 6-18. \**p* < 0.05

smokers ( $p < 0.05$ , Fig. 1d). The lower expression of C/EBP $\beta$  was associated with a reduced immunoreactivity to the Marker of Proliferation (M) KI67 (rabbit polyclonal antibody A0047, DakoCytomation) suggestive of an attenuated proliferation of the airway epithelium ( $0.02 \pm 0.004$  vs.  $0.0082 \pm 0.0026$ ; mean  $\pm$  SEM,  $p < 0.01$ ).

The reduced expression of C/EBP $\beta$  corroborates our previous finding of significantly decreased *CEBPB* mRNA in the bronchial epithelium of current and former smokers, compared to never smokers [4], and reduction of *CEBPB* mRNA and C/EBP $\beta$  protein in bronchial epithelial cells stimulated with cigarette smoke extract *in vitro* [4]. Thus, C/EBP $\beta$  is down-regulated by cigarette smoke in both the proximal and distal airway epithelium. This may be part of a compensatory mechanism of feed back inhibition, as an adaptive attempt to control chronic inflammation. C/EBP $\beta$  contributes to the differentiation of the airway epithelium during organogenesis, and promotes club cell differentiation at the expense of goblet cell differentiation [9]. As cigarette smoke stimulates goblet cell differentiation *in vitro* [10], decreased expression of C/EBP $\beta$  in the distal airways may thus provide a mechanistic explanation for goblet cell hyperplasia induced by cigarette smoke. While the smokers included in our study were asymptomatic, decreased C/EBP $\beta$  may over time lead to clinical presentation with mucus hypersecretion. In support of this, the activity of C/EBP $\beta$  in the bronchial epithelium is decreased in smokers with chronic bronchitis [11], compared to asymptomatic smokers.

#### Airway epithelial-C/EBP $\beta$ is elevated in advanced COPD

The expression of airway epithelial-C/EBP $\beta$  was significantly increased in advanced (GOLD III-IV) COPD, compared to asymptomatic smokers ( $p < 0.05$ , Fig. 1d). Furthermore, a negative correlation between lung function and the airway expression of C/EBP $\beta$  was observed ( $r = -0.35$   $p < 0.05$ , Fig. 1e), suggesting a role for C/EBP $\beta$  in disease progression. The expression of the lung-enriched C/EBP $\alpha$ , which cooperates with C/EBP $\beta$  in various cellular functions [2], was not significantly different in any of the groups within the cohort (C/EBP $\alpha$  rabbit polyclonal antibody (14AA) sc-61, Santa Cruz Biotechnology, Dallas, TX, USA; data not shown).

Mechanistically, it is possible that the elevation of C/EBP $\beta$  represents a breakdown of the suggested feed back inhibition observed in cigarette smoke-induced inflammation, leading to escalating inflammatory processes in end-stage COPD. Alternatively, chronic bacterial colonization among COPD patients [12] may activate C/EBP $\beta$ . It is, however, also possible that steroid and  $\beta_2$  agonist treatment effected the expression of C/EBP $\beta$  in our study, as airway epithelial-C/EBP $\beta$  is induced/activated by GCs and  $\beta_2$  agonists [3, 6]. This may represent a novel mechanism by which GCs and  $\beta_2$  agonists modulate

the transcriptional profile of the airway epithelium in advanced COPD. Future studies should address whether the elevation of C/EBP $\beta$  is disease- or treatment-specific, and if GCs and  $\beta_2$  agonists induces C/EBP $\beta$  to promote host-defenses and act anti-inflammatory, or pro-inflammatory.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

MM, LB, JSE, MRS and ABR conceived of and designed the study. MM and ABR performed experiments. MM, JSE, MRS and ABR analyzed and interpreted data. MSR and ABR wrote the manuscript. All authors read and approved the final manuscript.

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