



**Karolinska  
Institutet**

Department of Medicine, Respiratory Medicine Unit

## **INTRACELLULAR SIGNALING IN THE LUNG:**

A role for C/EBP transcription factors in chronic obstructive pulmonary disease, glucocorticoid signaling and lung development

### **AKADEMISK AVHANDLING**

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## ABSTRACT

Over the last decade, a previously unknown role has been established for members of the C/EBP transcription factor family in lung gene expression. In other organs, C/EBPs are well known regulators of cell differentiation and linked processes such as proliferation, apoptosis, gene expression as well as central regulators of inflammatory responses and infectious defenses. The scope of this thesis is to investigate the role of C/EBP transcription factors in the human and mouse lung as well as a putative role in the pathogenesis of chronic obstructive pulmonary disease (COPD). As a first approach, DNA-binding activity of C/EBPs was studied in the airway epithelium of humans and in adult mice. We found that C/EBP $\beta$  is the dominant DNA-binding C/EBP transcription factor. Interestingly, we also found that C/EBP $\beta$ -activity is increased in the airways of asymptomatic smokers, whereas smokers that develop COPD lack this increase. We hypothesize that altered activity of C/EBP $\beta$  in airway epithelial cells has a previously unknown role in the pathogenesis of COPD. Here it could affect the production of inflammatory mediators and genes involved in anti-oxidative and infectious defenses in addition to affecting epithelial cell proliferation, thereby making the lungs more susceptible to destruction and inflammation which augment the progression of the disease. Inflammation in COPD typically exhibits partial resistance to the anti-inflammatory action of glucocorticoids. When studying glucocorticoid signaling in the lung epithelium, we found that the glucocorticoid receptor, at least partially, mediates the effects of glucocorticoids in lung epithelium by inducing phosphorylation of C/EBP $\beta$ , thereby augmenting its DNA-binding activity. This raises the possibility that the decrease in C/EBP-binding activity in the airway epithelium of patients with COPD may have a causative role for the relative resistance to glucocorticoids seen in this disease.

As a means to deepen our understanding of the C/EBP family's role in the lung, as well as to critically address whether C/EBPs have a role in COPD pathogenesis and related pathological processes, an animal model was used due to the limitations in sampling the human lung. As a first approach to evaluate whether the mouse is a suitable model to study C/EBP functions in the lung, we investigated the expression of C/EBPs in the human and mouse lung epithelium in addition to the lung epithelium of COPD patients. By using immunohistochemistry we found that the adult expression pattern of C/EBPs in the mouse lung is highly similar to the expression pattern of C/EBP $\alpha$  and C/EBP $\beta$  in the human lung, suggesting the mouse as a suitable model to study the C/EBP family's role in lung. In addition, we found that C/EBP $\alpha$  displays dynamic expression during lung development that together with the respiratory distress of neonatal C/EBP $\alpha$  knockout mice, suggest a crucial role for C/EBP $\alpha$  in the development of the lung. We generated a gain-of-function mouse model ectopically expressing C/EBP $\alpha$  in the lung epithelium (*SFTPC-Cebpa* mice), and a loss-of-function mouse model using the Cre-LoxP technique, with lung epithelial disruption of the C/EBP $\alpha$  gene (*Cebpa*<sup>ΔLE</sup> mice) to address this hypothesis. Both *Cebpa*<sup>ΔLE</sup> mice and *SFTPC-Cebpa* mice display strikingly similar impaired lung phenotypes during development characterized by a decreased number of growing epithelial tubules which are larger in size as well as a thickened interstitium, indicating that the tempo-spatial expression of C/EBP $\alpha$  is important for correct lung development. Further, adult *Cebpa*<sup>ΔLE</sup> mice, that survive the perinatal lethality, demonstrate a severe pathological picture with 1) goblet cell hyperplasia, bronchiolar metaplasia, fibrosis and mucus plugging, together pathologically defined as bronchiolitis, 2) emphysema and 3) extensive macrophage and lymphocyte infiltrations. C/EBP $\alpha$  has a vital role in lung development and lung epithelial differentiation. Repair processes generally descend from mechanisms and signaling pathways used during organ or tissue development. Therefore, C/EBP $\alpha$  could have a potential role also in remodeling processes, which in COPD patients either is impaired or inadequate. The diagnosis of COPD is based on clinical, radiological and functional features but there are well-recognized histopathological correlates including all the histopathological findings in the *Cebpa*<sup>ΔLE</sup> mice. In line with this, it is tempting to speculate that the pathological processes in COPD and *Cebpa*<sup>ΔLE</sup> mice share at least some underlying mechanisms, with a linkage between the epithelial differentiation-repair process inherent in COPD and the epithelial differentiation during lung development. In summary, the findings presented in this thesis suggest that investigations of the role of C/EBPs in the pathogenesis of COPD could provide important knowledge, that may potentially serve as a base for the development of new treatments for this devastating disease.