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Regulation of Gene Expression in Pulmonary Inflammation and Differentiation: A Role for C/EBP Transcription Factors

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ABSTRACT

CCAAT/enhancer-binding protein (C/EBP) transcription factors play essential roles in gene regulation. The lung-enriched isoform C/EBPα is known to inhibit proliferation, promote differentiation and stimulate gene expression characteristic of the mature differentiated pulmonary epithelium. C/EBPβ, also enriched in the lung, plays a role in cell differentiation and the regulation of inflammatory and host defense genes in several organs. The activity of C/EBPβ is decreased in smokers with chronic obstructive pulmonary disease (COPD), indicating a role in COPD pathogenesis. The objective of this thesis was to investigate the unique or overlapping roles of C/EBPα and C/EBPβ in lung epithelial differentiation, and to assess the contribution of C/EBPβ in regulating pulmonary inflammation.

To investigate unique *vs.* overlapping roles of C/EBPα and C/EBPβ in the lung, the pulmonary phenotype of mice lacking C/EBPα (*Cebpa*$^{\Delta LE}$ mice), C/EBPβ (*Cebpb*$^{\Delta LE}$ mice) or both C/EBPα and C/EBPβ (*Cebpa*$^{\Delta LE}$; *Cebpb*$^{\Delta LE}$ mice) specifically in the lung epithelium, all generated by *SFTPC*-Cre mediated excision, was investigated. Cell culture experiments suggested that C/EBPα and C/EBPβ bind the same elements within a lung-specific promoter, and that their functions are partially overlapping. Pre-natal *Cebpa*$^{\Delta LE}$ mice and *Cebpa*$^{\Delta LE}$*; Cebpb*$^{\Delta LE}$ mice displayed immature lungs similar to the lungs of premature infants, and *Cebpa*$^{\Delta LE}$*; Cebpb*$^{\Delta LE}$ mice exhibited even more impaired airway epithelial cell differentiation than the *Cebpa*$^{\Delta LE}$ mice. The proportion of *Cebpa*$^{\Delta LE}$ mice that survived and reached adulthood spontaneously developed a majority of the histopathological hallmarks of COPD, possibly caused by infiltrating inflammatory cells – similar to what is observed in COPD and what is mechanistically proposed to drive COPD pathogenesis. These findings are indicative of a relationship between immature lungs at birth, C/EBPs and the development of inflammatory lung disease.

Considering the previous documentation of decreased airway epithelial C/EBPβ activity in smokers with COPD, C/EBPβ could have a role in COPD pathogenesis. The role of C/EBPβ in regulating inflammatory and innate immune responses in the lung was on this account investigated by employing a translational approach encompassing clinical samples as well as *in vitro* and *in vivo* experiments. *CEBPB* was significantly down-regulated in the airway epithelium of both current and former smokers compared to never-smokers, and in cigarette smoke extract-treated primary human airway epithelial cells *in vitro*, suggesting that C/EBPβ plays a role in smoking-induced disease. Supporting this, inhibition of *CEBPB* in human airway cells *in vitro* resulted in a compromised inflammatory response to smoke. Moreover, cigarette smoke-exposed *Cebpb*$^{\Delta LE}$ mice displayed reduced respiratory neutrophilia and induction of inflammatory mediators, including the neutrophil chemoattractant *Groa*, compared to smoke-exposed controls. LPS-challenged *Cebpb*$^{\Delta LE}$ mice also exhibited blunted respiratory neutrophilia and lower pulmonary expression of *Groa*, compared to LPS-challenged control littermates. In addition, suppression of LPS-induced neutrophilia and inflammatory gene expression by formoterol, a long acting β_2-adrenoceptor agonist used in treatment of COPD, was impaired in *Cebpb*$^{\Delta LE}$ mice. C/EBP transactivation was increased by treatment with formoterol *in vitro*, possibly through a β_2-adrenoceptor and cAMP-dependent mechanism. This demonstrates that both inflammatory as well as anti-inflammatory stimuli involve regulation of gene transcription by C/EBPβ.

Taken together, these findings demonstrate that C/EBPα and C/EBPβ play pivotal and partly overlapping roles in airway epithelial differentiation, and that C/EBPβ and the lung epithelium orchestrates inflammatory responses as well as anti-inflammatory signaling by β_2-adrenoceptor agonists in the lung. Thus, C/EBPs may influence tissue regeneration in lung homeostasis and disease as well as inflammatory and anti-inflammatory signaling, and are potential contributors to COPD pathogenesis.

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