Institutionen för medicin

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

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Thoraxaulan N2:U1, Karolinska Universitetssjukhuset, Solna

Torsdagen den 7 juni 2012, kl 09.00

av

Abraham B. Roos
MSc

Huvudhandledare:
Docent Magnus Nord
Karolinska Institutet
Institutionen för medicin

Bihandledare:
Professor Johan Grunewald
Karolinska Institutet
Institutionen för medicin
Enheten för lungmedicin

Med Dr Lukas Didion
Weill Cornell Medical College
Department of Genetic Medicine

Professor Göran Tornling
Karolinska Institutet
Institutionen för medicin
Enheten för lungmedicin

Fakultetsopponent:
Professor Donna Davies
University of Southampton
Faculty of Medicine
Clinical and Experimental Sciences

Betygsnämnd:
Docent Lena Palmberg
Karolinska Institutet
Institutet för miljömedicin
Enheten för lung och allergiforskning

Professor Gunnar Nilsson
Karolinska Institutet
Institutionen för medicin
Enheten för klinisk immunologi och allergi

Docent Ellen Tufvesson
Lunds Universitet
Institutionen för kliniska vetenskaper
Lungmedicin och allergi

Stockholm 2012
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 (CebpaΔLE mice), C/EBPβ (CebpbΔLE mice) or both C/EBPα and C/EBPβ (CebpaΔLE; CebpbΔLE 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 CebpaΔLE mice and CebpaΔLE; CebpbΔLE mice displayed immature lungs similar to the lungs of premature infants, and CebpaΔLE; CebpbΔLE mice exhibited even more impaired airway epithelial cell differentiation than the CebpaΔLE mice. The proportion of CebpaΔLE 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 CebpbΔLE mice displayed reduced respiratory neutrophilia and induction of inflammatory mediators, including the neutrophil chemoattractant Groa, compared to smoke-exposed controls. LPS-challenged CebpbΔLE 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 β2-adrenoceptor agonist used in treatment of COPD, was impaired in CebpbΔLE mice. C/EBP transactivation was increased by treatment with formoterol in vitro, possibly through a β2-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 β2-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.