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C/EBP transcription factors in lung cellular differentiation and development

av

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Abstract

During embryonic development the lung is lined with a primitive epithelium. As development proceeds, the epithelium matures and differentiates into several cell types that together with cells of mesenchymal origin constitute the adult lung. From studies investigating the regulation of differentiation-dependent genes in the lung, such as the Clara Cell Secretory Protein (*Ccsp/Scgb1a1*), a role for CCAAT/enhancer binder protein (C/EBP) transcription factors in lung cellular differentiation has been suggested. As C/EBPs have been shown to be important regulators of development, differentiation and proliferation in other organs, their expression was investigated during mouse lung development. C/EBPα, C/EBPβ and C/EBPδ were detected from late pseudoglandular stage and expression increased prior to birth, correlating with the extensive cellular differentiation, and onset of *Ccsp/Scgb1a1* expression, that occurs during this period. When investigating combinatorial actions of C/EBPs and other transcription factors in the regulation of *Ccsp/Scgb1a1*, C/EBPα was found to synergistically transactivate the promoter together with the epithelial-specific transcription factor NKX2.1. Together with the expression-pattern of C/EBPα, this indicates that the synergy could be a major determinant for the high-level, epithelial-specific expression of *Ccsp/Scgb1a1* in adult lung, and for the onset of *Ccsp/Scgb1a1* during development. To further examine the role of C/EBPα during lung development we generated transgenic mice ectopically expressing C/EBPα in the lung epithelium using the human Surfactant protein-C promoter, giving a premature, more widespread C/EBPα expression. Lungs from these mice were characterized by fewer and larger developing epithelial tubules. However no defects in overall proliferation or cellular differentiation were observed. A similar phenotype was observed in *Cebpa*$^{-/-}$ mice and we suggest that these similar phenotypes could possibly stem from dysregulation of airway branching. From our results we conclude that C/EBPα has a role in the later stages of lung development.

Glucocorticoids stimulate cellular differentiation during late lung development. In other organs, a role for C/EBPs in glucocorticoid signaling has been suggested. We investigated the role of C/EBPs in the action of glucocorticoids in the lung epithelium by studying the regulation of the *Ccsp/Scgb1a1* and *CYP2B1* genes. Both these genes are positively regulated by glucocorticoids and C/EBPs. However functional binding sites for the glucorticoid receptor have not been found in their promoters. In transient transfection studies using a lung epithelial cell line, we found that glucocorticoids induced expression from the *Ccsp/Scgb1a1* and *CYP2B1* promoters. In both promoters, induction was lost when the C/EBP-binding sites were mutated. Electro-phoretic mobility shift assays revealed that glucocorticoids increased the DNA-binding activity of C/EBPβ and C/EBPδ within 10 minutes. The effect was mediated through the glucocorticoid receptor, independent of protein synthesis and involved phosphorylation of C/EBPβ at residue Thr^{235}. We further demonstrated that C/EBPβ is the predominant C/EBP-factor in both human and mouse lung epithelium and that glucocorticoids increase DNA-binding of C/EBPβ in primary cells from mouse lung. These results indicate a previously unknown role for C/EBP-transcription factors in glucocorticoid signaling in the lung epithelium.

Keywords: C/EBP transcription factors, lung, development, differentiation, glucocorticoid regulation

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