MOLECULAR BIOLOGY OF THE LUNG

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Understanding of lung disease on the cellular and molecular level is crucial to develop new approaches for the diagnosis, treatment and prevention of lung disease. Although our knowledge on the molecular level is steadily increasing, we still have a limited understanding of the molecular events underlying the diseases, which is reflected by the fact that very few therapies target specific defects.

The field of molecular biology focuses on the interactions between various systems of a cell and between cells, and particularly includes:

- gene structure, expression, replication, and recombination
- structure, function, chemistry, and *in vivo* modification and processing of proteins and nucleic acids
- cellular and developmental biology
- genetics, structure and growth cycles of viruses, bacteria, and bacteriophages.

This article focuses on selective (signal) molecules and structures, all of which are altered in various lung diseases and are important topics in the field of molecular biological research.

The extracellular matrix

Components of the extracellular matrix (ECM) surround and support the cell and cell–cell interaction. In the lung, the ECM around the conducting airways, alveolar cells and the vascular system has a major impact on lung architecture and function, in particular gas exchange. All lung cell types interact and signal through the ECM *via* adhesive molecules, surface receptors or growth factors.

The lung fibroblast is the main producer of pulmonary ECM, which consists of collagens, elastins and proteoglycans. The interstitium of the lung parenchyma contains mostly collagen type I and III, which are mainly responsible for tensile strength.

The pulmonary ECM is subjected to a continuous turnover of >10% of the total ECM per day. Thus, a dynamic equilibrium between synthesis and degradation of the pulmonary ECM maintains the physiological balance. This balance is tightly controlled by three regulatory mechanisms: 1) *de novo* synthesis and deposition of ECM components such as collagens, mainly by interstitial fibroblasts; 2) proteolytic degradation of existing ECM by matrix metalloproteinases (MMPs), a family of zinc enzymes; and 3) inhibition of MMP activity by specific endogenous antiproteases, the tissue inhibitors of metalloproteinases (TIMPs).

Key points

Major features of lung diseases are:

- altered deposition of extracellular matrix
- impaired surfactant metabolism
- distorted endogenous defence mechanisms

Excessive or inappropriate expression of MMPs is related to the pathogenesis of tissue destructive processes in many of lung diseases, such as MMP-12 in emphysema, or MMP-7 in lung fibrosis.

The surfactant system

The maintenance of normal lung function throughout the life of an organism is ensured largely by alveolar epithelial cells, which form a tight functional barrier essential for gas exchange. The alveolar epithelium is composed of alveolar type I (ATI) and type II (ATII) cells. ATII and ATI cells produce and secrete components of the extracellular matrix and growth factors thereof, which facilitates restoration of the interstitium and, subsequently, functional alveolar structure. ATII cells are cuboidal secretory cells mainly responsible for surfactant secretion. Pulmonary surfactant is a complex mixture of phospholipids and proteins, with surfactant proteins (SP)-A, -B and -C constituting 10% of surfactant. Its main role is to reduce surface tension in the alveoli following the onset of breathing, thereby leading to lung expansion. Mechanical stretching of the lung forces the secretion of lamellar bodies, the intracellular storage granules of surfactant, which form tubular myelin. The surfactant film stabilises the alveolar–air interface with a low surface tension and prevents lung collapse. Following secretion, both surfactant proteins and lipids are recycled by the respiratory epithelium.

Surfactant abnormalities have been described in many infant and adult lung diseases, such as respiratory distress syndrome, bronchiolitis, chronic obstructive pulmonary disease (COPD) or interstitial lung disease.

Defence and clearance mechanisms

From the above-mentioned proteins, surfactant proteins SP-A and SP-D are primarily involved in the innate host defence of the lung. In addition, antimicrobial peptides (AMPs), such as defensins, cathelicidins or lactoferrin, are present in the airways to prevent infection. Moreover, cellular defence mechanisms include macrophage- and neutrophil-mediated release of cytokines, such as interleukins 1 and 8, tumor necrosis factor (TNF)-α, or granulocyte macrophage colony-stimulating factor (GM-CSF).

Pulmonary alveolar proteinosis is caused by disruption of GM-CSF signalling. Loss of GM-CSF signalling in macrophages results in an impaired ability to catabolise surfactant proteins. Abnormal surfactant accumulation leads to respiratory insufficiency.

Mucociliary clearance represents the primary physiological defense mechanism. The ciliated airway cells clear the mucus, which is produced by secretory cells, by forcing the mucus toward the larynx for elemination. An impaired mucociliary clearance is the main feature of cystic fibrosis.

Transforming growth factor-β

The transforming growth factor (TGF)-β superfamily is critically involved in embryonic development, organogenesis and tissue homeostasis. TGF-β superfamily members act as multifunctional regulators of cell growth and differentiation. The TGF-β superfamily comprises more than 40 members, including TGF-βs themselves. Three TGF-β isoforms have been characterised so far: TGF-β1, TGF-β2 and TGF-β3. TGF-β1 is the most important isoform in the cardiopulmonary system, as it is ubiquitously expressed and secreted by several cell types, such as endothelial, epithelial and smooth muscle cells, as well as fibroblasts and most cells of the immune system. TGF-β is secreted in covalent association with the latent TGF-β binding protein (LTBP), thus providing a reservoir in the ECM. For active signalling, TGF-β needs to dissociate from the complex by a mechanism that involves proteases, such as plasmin or matrix metalloproteinases, as well as interaction with integrins. Active TGF-β ligands bind to the type II TGF-β receptor, which binds to the type I TGF-β receptors. Subsequent transphosphorylation of the type I receptor results in recruitment of specific intracellular signals mediators, called Smad proteins. Smad2 and Smad3 have been shown to be phosphorylated by the type I receptor,

followed by complex formation with Smad4 and, finally, nuclear translocation and regulation of gene transcription (fig. 1). The receptor-regulated Smad2 or Smad3, in combination with the co-Smad Smad4, positively regulate TGF-β-induced effects, while the inhibitory Smads (Smad6 and Smad7) negatively regulate TGF-β signalling.

Increased TGF-β signalling is the key pathophysiological mechanism that leads to fibrotic lung disease, which is characterised by an increase in activated (myo)fibroblasts and excessive deposition of ECM.

There is emerging interest in the role of TGF-β in the pathogenesis of COPD, particularly

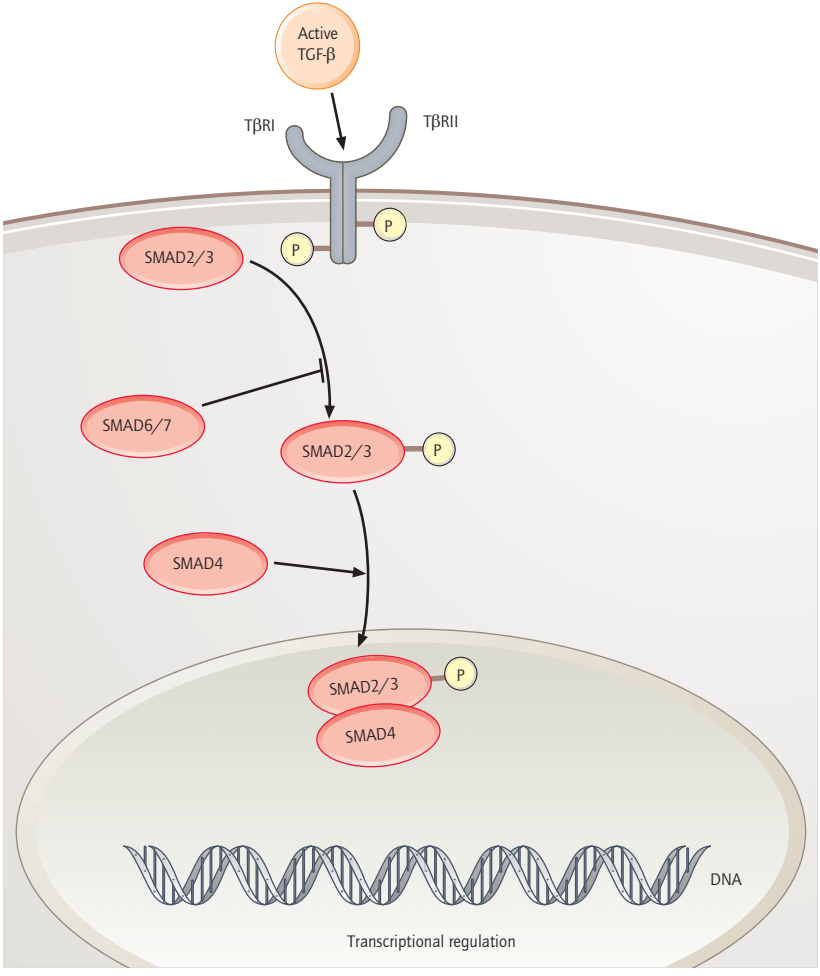

Figure 1. Transforming growth factor-β signalling.

since genetic studies have demonstrated an association of gene polymorphisms of the TGF-β superfamily with COPD. In addition, increased expression of TGF-β1 in COPD has been reported, suggesting an impact of TGF-β signalling in the development and progression of COPD.

Nuclear factor-κB

Nuclear factor (NF)-κB is a ubiquitous transcription factor present in all cell types. In its resting stage, this factor resides in the cytoplasm as a heterotrimer consisting of p50, p65 and the inhibitory protein IκBα. Upon activation, the IκBα protein undergoes phosphorylation, ubiquitination and degradation. p50 and p65 are then released to be translocated to the nucleus, where they bind specific DNA sequences present in the promoters of various genes and initiate transcription. IκBα kinase or IKK is responsible for the initial phosporylation. Several kinases have been shown to activate IKK, such as AKT, mitogen-activated protein/extracellular signal-regulated kinase kinase kinase 1 (MEKK1), and protein kinase C. In the nucleus, NF-κB induces a range of gene expression, in particular of mediators of inflammation, cell proliferation, metastasis and angiogenesis.

Many noxious substances related to lung disease, such as cigarette smoke, radiation, chemotherapeutic agents, or cytokines and growth factors, activate NF-κB, and increased NF-κB signalling has been associated with COPD or asthma.

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