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ΜΕΛΕΤΗ ΤΟΥ ΡΟΛΟΥ ΤΗΣ ΙΝΤΕΓΚΡΙΝΟΣΥΝΔΕΟΜΕΝΗΣ ΚΙΝΑΣΗΣ ΣΤΗΝ ΠΝΕΥΜΟΝΙΚΗ ΙΝΩΣΗ

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ABSTRACT

Fibrosis is a pathologic condition characterized by increased production and decreased degradation of extracellular matrix components, especially collagen. The cell that produces collagen and therefore is of significant importance for fibrogenesis, is the myofibroblasts. As far as molecular

mechanisms are concerned, the fibrotic molecular mechanism resembles the normal wound healing response. The wound healing response involves an initial inflammatory phase that is followed by myofibroblasts proliferation and collagen accumulation. Finally, during tissue regeneration, myofibroblasts undergo apoptosis and collagen in excess is degraded. During fibrosis, myofibroblasts escape apoptosis and collagen production continues till tissue's normal architecture and function is compromised.

Fibrosis can occur in every human tissue. Therefore, although there are basic fibrotic mechanisms that are common for every tissue, there are also important fibrotic features that exhibit tissue specificity. Recently, myofibroblast origin is regarded as one of the features exhibiting tissue specificity since it has been proven that myofibroblasts can originate from several sources including the epithelium, endothelium, resident fibroblasts, bone marrow derived cells, hepatic stellate cells etc.

The present study focuses on pulmonary fibrosis which is a common feature of a large group of lung diseases, called interstitial lung diseases, ILDs. The most common ILD is idiopathic pulmonary fibrosis (IPF) for which there is no effective treatment available since the molecular mechanisms underlying the disease are unknown. One of the unknown elements in the pathogenesis of IPF is the origin of myofibroblasts since the existing studies are inconclusive, implicating epithelia, endothelial, bone marrow derived and resident sources, to a bigger or smaller extent, in myofibroblasts accumulation. Identification of the primary myofibroblast source, or of the extent that each source contributes to the overall accumulation is considered to be crucial for the generation of an effective treatment.

Integrin-linked kinase (ILK) is a serine/threonine kinase involved in the regulation of several cell processes. Due to its interaction with the $\beta 1$ and $\beta 3$ cytoplasmic tails of integrins, ILK is involved in the regulation of structural functions such as maintenance of tissue integrity and cell shape, resistance to mechanical forces and cell movement. Moreover, it has been proposed that ILK phosphorylates substrates such as protein kinase B (Akt) and glucogen synthase

kinase-3 β , meaning that ILK is indirectly involved in the regulation of several other functions involving resistance to apoptosis, angiogenesis etc.

As far as fibrosis is concerned, ILK has been extensively studied in the kidney and to a lesser extent in the liver. In both cases, it is involved in mechanisms regarding myofibroblasts production. In renal fibrosis, ILK mediates, in response to TGF- β , the initiation of epithelial to mesenchymal transition (EMT), a process through which epithelial cells transform into myofibroblasts. In liver fibrosis, ILK mediates a similar process through which hepatic stellate cells transform into myofibroblasts. However, ILK's role in pulmonary fibrosis has not been studied so far.

In order to examine ILK's role in pulmonary fibrosis, we performed both an *in vitro* and an *in vivo* approach. For the *in vitro* study, human alveolar epithelial cells of the A549 cell line were used. Cells were incubated with human recombinant TGF- β in order to induce acquisition of fibrotic features and EMT. After we showed that ILK is upregulated in response to TGF- β through a smad-dependent mechanism, we used the siRNA technique in order to unravel ILK's role in alveolar EMT. Our results showed that, in contrast to renal fibrosis, ILK is not involved in the initiation of EMT but in later stages such as the expression of mesenchymal markers and possibly cell migration. Using the same technique, we also evaluated ILK's role in several cells processes and our results showed that it is involved in maintenance of cell shape, cell adhesion and cell migration. However, in contrast to its role in many other cell types, ILK is not involved in protection from apoptosis in alveolar epithelial cells.

For the *in vivo* study we initially used the adenovirus TGF- β overexpression model but our results were inconclusive. Then we used the bleomycin model where we showed that ILK is initially increased in response to bleomycin. During the intermediate time intervals, it decreases significantly while at the later time points it tends to increase till it reaches control levels. To better understand the reasons of the variations in ILK levels, we performed immunohistochemistry and double immunofluorescent experiments so as to define the cell populations that express ILK in every time interval. Our results

showed that ILK's downregulation during intermediate intervals is due to the apoptosis and/or necrosis of epithelial populations which express ILK. The re-increase observed during late time intervals is due to the influx of cell populations expressing ILK to a bigger or a lesser extent such as cells of the immune system and a subpopulation of myofibroblasts.

Conclusively, our results show that ILK is a multifunctional protein that can have distinct roles among different fibrotic tissues. We believe that our findings support the idea that key molecules and mechanisms involved in fibrosis should be examined in a tissue specific manner.