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Extracellular matrix proteins: A positive feedback loop in lung fibrosis?

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ABSTRACT

Lung fibrosis is characterized by excessive deposition of extracellular matrix. This not only affects tissue architecture and function, but it also influences fibroblast behavior and thus disease progression. Here we describe the expression of elastin, type V collagen and tenascin C during the development of bleomycin-induced lung fibrosis. We further report *in vitro* experiments clarifying both the effect of myofibroblast differentiation on this expression and the effect of extracellular elastin on myofibroblast differentiation.

Lung fibrosis was induced in female C57Bl/6 mice by bleomycin instillation. Animals were sacrificed at zero to five weeks after fibrosis induction. Collagen synthesized during the week prior to sacrifice was labeled with deuterium. After sacrifice, lung tissue was collected for determination of new collagen formation, microarray analysis, and histology. Human lung fibroblasts were grown on tissue culture plastic or BioFlex culture plates coated with type I collagen or elastin, and stimulated to undergo myofibroblast differentiation by 0–10 ng/ml transforming growth factor (TGF)β_1. mRNA expression was analyzed by quantitative real-time PCR.

New collagen formation during bleomycin-induced fibrosis was highly correlated to gene expression of elastin, type V collagen and tenascin C. At the protein level, elastin, type V collagen and tenascin C were highly expressed in fibrotic areas as seen in histological sections of the lung. Type V collagen and tenascin C were transiently increased. Human lung fibroblasts stimulated with TGFβ_1 strongly increased gene expression of elastin, type V collagen and tenascin C. The extracellular presence of elastin increased gene expression of the myofibroblastic markers α smooth muscle actin and type I collagen.

The extracellular matrix composition changes dramatically during the development of lung fibrosis. The increased levels of elastin, type V collagen and tenascin C are probably the result of increased expression by fibroblastic cells; reversely, elastin influences myofibroblast differentiation. This suggests a reciprocal interaction between fibroblasts and the extracellular matrix composition that could enhance the development of lung fibrosis.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a severely destructive lung disease, resulting in impaired architecture and function of lung tissue (Selman et al., 2004). The incidence of IPF is estimated to be 5 to 10 per 100,000 (Fernandez Perez et al., 2010) and appears to increase in recent years (Nalysnyk et al., 2012). At the core of the fibrotic process are changes in both the structure and the composition of the extracellular matrix. The deposition of excessive amounts of type I collagen is classically seen as the main problem in fibrosing tissues (Meltzer and Noble, 2008).

Fibroblasts are responsible for the maintenance of the extracellular matrix. During fibrosis development, they differentiate toward the myofibroblastic phenotype, characterized by an increased contractile capacity due to the expression of α smooth muscle actin (αSMA) and by increased release of different types of extracellular matrix proteins (Tomasek et al., 2002; Hinz, 2007; Hinz et al., 2012). Extensive literature exists on how this process is regulated by growth factors, such as transforming growth factor (TGF)β_1 (Todd et al., 2012). However, myofibroblast differentiation is also affected by the changes in the composition and architecture of the fibrotic matrix by a) determining which specific attachment sites are available to the cells, b) influencing the mechanical properties of the matrix and c) determining the mechanical loading experienced by the cells during, for example, breathing (Suki and Bates, 2008). The effect of the fibrotic matrix was confirmed recently by seeding lung fibroblasts into decellularized matrix from IPF patients and healthy controls, resulting in an increased expression of myofibroblast markers in IPF matrix (Booth et al., 2012), thus

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indicating that changes in the extracellular matrix composition determine disease progression.

Possible candidates for specific proteins within the extracellular matrix regulating fibrotic cellular processes can be derived from our earlier study (Blaauboer et al., 2013). In that study we measured new collagen formation by analysis of deuterated water incorporation into hydroxyproline in mice with bleomycin-induced lung fibrosis. We combined this with microarray analysis and correlating these results allowed us to identify fibrosis-relevant changes in gene expression within this model. Interestingly, three extracellular matrix proteins were strongly correlated to new collagen formation: elastin, type V collagen and tenascin C. Patients with IPF and its histopathological equivalent usual interstitial pneumonia, also have increased levels of elastin (Cha et al., 2010), type V collagen (Parra et al., 2006) and tenascin C (Kuhn and Mason, 1995; Fitch et al., 2011). Therefore, these proteins are attractive candidates in the search for regulatory roles of extracellular matrix proteins in fibrosis.

For type V collagen and tenascin, pro-fibrotic mechanisms have been described. Exposure to type V collagen results in inflammatory responses (Braun et al., 2010), that could affect the development of fibrosis, for example *via* fibrosis-relevant cytokine release by immune cells (Todd et al., 2012). Also tenascin C could play a regulatory role in the development of fibrosis since it increases cell migration (Trebaul et al., 2007) and migration of cells is important for the recruitment of myofibroblasts. These observations emphasize the reciprocal relationship between changes in matrix composition and cellular contributions to fibrosis development. In this study, we aimed to further unravel this reciprocal relationship between cells and matrix during fibrosis development. For this, we first analyzed the expression of elastin, type V collagen and tenascin C at different time points during the development of bleomycin-induced lung fibrosis. Then we addressed the role of lung fibroblasts and myofibroblasts in the changed expression of these extracellular matrix proteins. Since it is not known if elastin has similar fibrosis-inducing effects as type V collagen and tenascin C, we investigated the effect of elastin on lung fibroblasts and the differentiation to myofibroblasts.

2. Results

2.1. Elastin, type V collagen, and tenascin C strongly correlate with new collagen deposition in bleomycin-induced lung fibrosis

In vivo new collagen formation, as measured by incorporation of deuterated water into hydroxyproline, correlated strongly with gene expression of elastin ($r = 0.93$, Fig. 1A), the α-1 chain of type V collagen ($r = 0.84$, Fig. 1B) and tenascin C ($r = 0.88$, Fig. 1C) during the development of bleomycin-induced lung fibrosis.

2.2. Increased protein deposition of elastin, type V collagen and tenascin C during bleomycin-induced lung fibrosis

Resorcin-fuchsin staining in lung sections of healthy control mice indicates the presence of elastin around blood vessels and at the tips of alveolar septae (Fig. 2A). In fibrotic lung sections, elastin was increasingly found in fibrotic areas at all time points (Fig. 2B–D).

Type V collagen was present in healthy lung tissue in blood vessel walls and in a thin layer around bronchioles (Fig. 2E). In fibrotic lungs, during the first two weeks after fibrosis-induction by bleomycin instillation type V collagen was increased in fibrotic areas (Fig. 2F and G). At 4 weeks, type V collagen immunostaining was decreased compared to the high levels in the first two weeks (Fig. 2H).

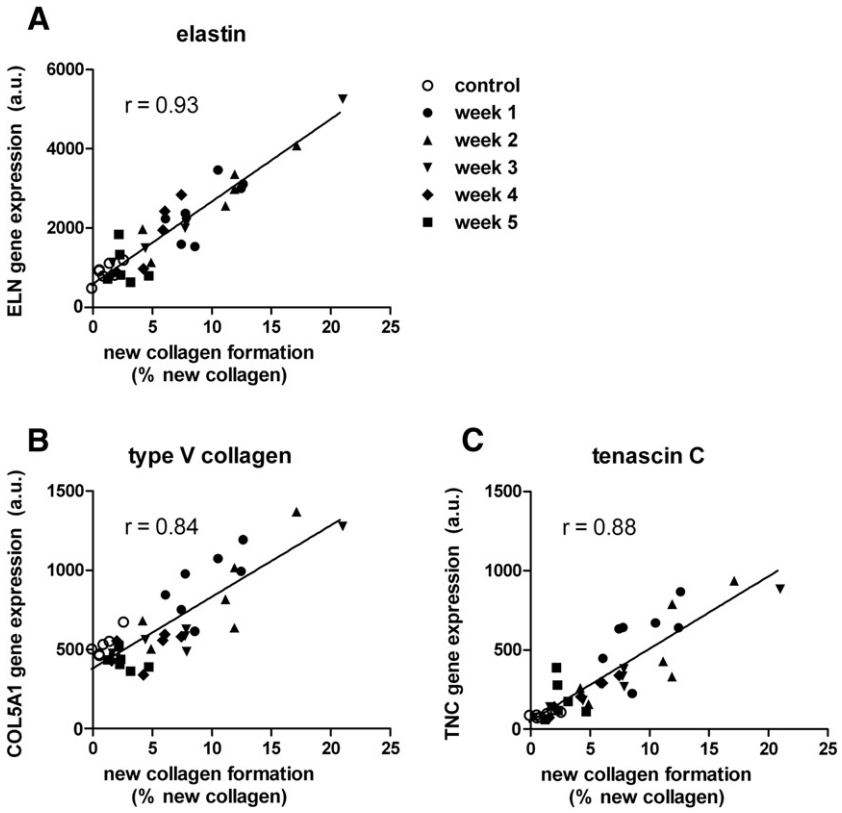

Fig. 1. During bleomycin-induced lung fibrosis, gene expression of elastin, type V collagen, and tenascin C is highly correlated to new collagen formation; the core process of fibrosis. Correlation between new collagen formation as measured by deuterated water incorporation in hydroxyproline and A) elastin gene (ELN) expression, B) gene expression of the α-1 chain of type V collagen (COL5A1), and C) tenascin C gene (TNC) expression. Each point represents data of one experimental animal, lines represent trend-lines from linear regression.

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