Regulation of matrix turnover: fibroblasts, forces, factors and fibrosis

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Abstract
Fibroblasts are multifunctional cells that are responsible for matrix homoeostasis, continuously synthesizing and degrading a diverse group of extracellular molecules and their receptors. Rates of turnover of matrix molecules and the proteases that degrade them are normally under the control of diverse chemical and mechanical cues, with cytokines, growth factors, proteases, lipid mediators and mechanical forces playing roles. The maintenance of this homoeostasis is vital to the preservation of normal tissue function and is clearly lost in chronic diseases of the joints, skin and internal organs where destruction and excessive deposition is seen. Current research is focusing on defining the key pathways of activation either in resident fibroblasts, matrix-producing cells derived from circulating fibrocytes, or from transdifferentiation of resident cells. The common downstream signalling pathways are also being defined, as well as the gene interactions leading to altered cell phenotype. The present article reviews these findings and our current concepts of the key molecular events leading to tissue damage and excessive matrix deposition in tissue fibrosis. These studies are leading to an appreciation of the complexity of events with multiple pathways involved, but, as the facts emerge, we are finding promising new ways to treat fibrosis and halt the inexorable progression that is a feature of so many fibrotic and remodelling disorders.

Introduction
Chronic disorders of the joints and internal organs are a major cause of morbidity and mortality and an enormous burden on world healthcare systems. A feature of these diseases is the destruction and remodelling of extracellular matrix with deleterious effects on tissue function. This is seen in many disease settings, including disorders of the joints (e.g. rheumatoid arthritis and osteoarthritis), liver (hepatic cirrhosis) and kidney (renal sclerosis). It is also a feature of many lung diseases, including asthma and COPD (chronic obstructive pulmonary disease), where excessive matrix deposition may occur in large or small airways, as well as pulmonary fibrosis, where there is excessive deposition in alveolar structures and severely compromised gas exchange. This article explores the current concepts of the processes that regulate matrix turnover leading to fibrosis, with the lung explored as a paradigm of this group of disorders. We will develop the theme that abnormalities in multiple pathways involved in wound healing and inflammation can lead to the development of fibrotic disorders and suggest that this rationale for pathogenesis may be more productive in driving the search for new therapies.

Matrix turnover
It is now well recognized that most of the body’s structural cells are actively synthesizing and degrading a diverse group of matrix components, and the rates of these processes are rapid in normal tissues [1]. In recent years, we have characterized the key processes in remodelling and have identified the diverse structural components of lung airway and parenchymal structures (see [2] for a review). Collagen types I and III are the most abundant proteins found in airways and blood vessels as well as alveolar septae. These collagens are synthesized by many cell types, but predominantly by mesenchymal cells: fibroblasts, myofibroblasts and smooth muscle cells (reviewed in [3]). In various disease settings, fibroblasts differentiate into myofibroblasts, and recent data suggest plasticity between epithelial cells and mesenchymal cells and that cytokines and chemokines can promote these differentiation processes [4].

Fibroblasts and the fibrotic phenotype
Fibroblasts are widely distributed in all lung structures and have long been recognized as extremely dynamic cells which play key roles in matrix homoeostasis. For example, in response to growth factors or mechanical stimuli, they are capable of generating more that 5000 molecules of pro-collagen per cell per min [5,6]. They are also in communication with a large number of cell types and respond to a host of cytokines and growth factors (Figure 1). More recent studies using microarray technologies have reiterated further the gene diversity of this response. We have previously profiled human foetal lung fibroblast global gene expression in response to TGF-β1 (transforming growth factor β1) using oligonucleotide microarrays [7]. Almost 150 genes were up-regulated at least 2-fold, representing several major

Key words: cytokine, fibroblast, fibrosis, integrin, lung, matrix turnover.

Abbreviations used: COX, cyclo-oxygenase; PAR, proteolytically activated receptor; PGE₂, prostaglandin E₂; TGF-β, transforming growth factor β.

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functional categories, including genes involved in cytoskeletal reorganization, matrix formation, metabolism and protein biosynthesis, cell signalling, proliferation, survival and gene transcription. This included 80 that were not previously known to be TGF-β1-responsive [7]. Such diversity is reflected in vivo with studies of pulmonary fibrosis in human and animal models, showing that almost 500 genes are expressed more than 2-fold, including many of the above, as well as large cluster of diverse matrix genes [8,9].

Pro-fibrogenic cytokines and pulmonary fibrosis

A large number of mediators, produced by many different cell types, are known to promote fibroblast proliferation, collagen synthesis, migration and differentiation [3,10,11] (Figure 1). TGFβ is one of the most potent pro-fibrotic mediators and a strong candidate as a central player in remodelling diseases, including asthma [12] and pulmonary fibrosis [10]. Blocking TGFβ by a number of strategies inhibits experimental pulmonary fibrosis, and several groups are exploring inhibitors of TGFβ activation, TGFβ-blocking antibodies and receptor kinase inhibitors as strategies to prevent fibrosis. A serious reservation, however, is the role that TGFβ plays as an inhibitor of immune responses, since mice deficient in this cytokine exhibit a severe wasting syndrome with evidence of mononuclear cell infiltration into the heart and lungs [13]. However, more recent evidence from TGFβ-inhibition studies suggests that these reservations may be unfounded (reviewed in [14]).

Endothelin and angiotensin: vasoconstrictors that regulate remodelling and fibrosis

There are parallels between the actions of agents that regulate tone in the vasculature and their effects on fibroblasts: vasoconstrictors often induce remodelling, whereas vasodilators are inhibitors. Two examples are endothelins and angiotensin II, both agents that exhibit pro-fibrotic features in vitro [15,16], and receptor antagonists for these agents have shown some success in blocking fibrosis in animal models. Relevance to humans remains uncertain and will await the results of trials with drugs currently used in humans in other settings, but it is of interest that a polymorphism in ACE (angiotensin-converting enzyme) has been shown to influence outcome in patients with ARDS (acute respiratory distress syndrome) [17].

Cytokines and lipid mediators as inhibitors of fibroblast function and fibrosis

Fibrosis is thought to result if, in response to injury, the normal homoeostasis is lost, and, in the microenvironment around fibroblasts, there is an excess of mediators driving cells into a pro-fibrotic phenotype (Figure 2). This concept has led us to consider ways of changing this balance in patients with fibrotic disorders. The focus has mostly been on inhibition of pro-fibrotic cytokines, but there is also considerable interest in the use of anti-fibrotic molecules to inhibit lung fibrosis. This was given impetus by the reports in a small group of patients that IFNγ (interferon-γ) was an effective treatment for idiopathic pulmonary fibrosis [18], although...
this early promise was not borne out in recent multi-centre
trials with large numbers of patients. Another molecule of
interest is PGE₂ (prostaglandin E₂), a product of arachidonic
acid metabolism. PGE₂ is a paracrine mediator and autocrine
inhibitor of fibroblast proliferation and collagen deposition
[19,20]. Its production is reduced in fibroblasts from patients
with fibrosis following stimulation with mediators such as
IL-1 (interleukin 1) [21] or TGFβ [22] due to a decreased
capacity to up-regulate COX (cyclo-oxygenase)-2. Furthermore,
COX-2-deficient mice are more susceptible to bleo-
mycin-induced pulmonary fibrosis [23]. Taken together,
these observations support the hypothesis that there may
be a defect in the COX2–PGE₂ axis in patients developing
fibrosis. This defect may, in part, involve the presence of gene
polymorphisms. In support of this hypothesis, a functional
promoter polymorphism in the COX-2 gene (PTGS2) which
reduces gene expression [24] has recently been associated with
susceptibility to sarcoidosis [25]. Moreover, the association
appears to be driven by those patients with persistent
progressive disease who are most likely to develop pulmonary
fibrosis [25]. These data highlight the importance of gene
interactions and also suggest that therapeutic strategies
to overexpress anti-fibrotic molecules might be fruitful. To
begin to explore this, we have developed an integrin-targeting
gene-delivery system which shows high delivery efficiency,
while avoiding the immune and inflammatory side effects
that are associated with the use of adenoviral vectors [26].

Proteases in the regulation of fibroblast
function and remodelling

The serine and matrix metalloproteinases have long been
assumed to play key roles in emphysema, where degradation
of matrix and destruction of parenchymal lung structures is
a feature. However, there is also compelling evidence that
these molecules are also central to the pathogenesis of acute
lung injury and pulmonary fibrosis [27]. Thus inhibitors of
neutrophil elastase inhibit lung injury and fibrosis, and we
have recently demonstrated that mice deficient in this protein-
ase are protected from lung fibrosis [28].

Figure 2 | Cytokines in the balance in fibrosis
Data taken from [11].

It is also clear that proteinases of the coagulation cascade,
including Factor VIIa, Factor Xa and thrombin, exert pro-
inflammatory and pro-fibrotic effects and probably play key
roles in acute lung injury and remodelling disorders of the
lung [29–32]. These proteinases exert their cellular effects
via a family of at least four PARs (proteolytically activated
receptors). These receptors have emerged as interesting
targets to prevent fibrosis on the basis of studies showing
that thrombin inhibitors can partially block experimental
fibrosis [33,34] and that mice deficient in the high-affinity
thrombin receptor, PAR1, are protected from both lung
inflammation and fibrosis following bleomycin injury.

Mechanical forces and fibroblast function

The potent actions of mechanical events on altering fibroblast
phenotype has long been recognized in tissues exposed to
changing forces, such as muscle [35], skin [36] and tissues of
the cardiovasculature [37]. More recently, the potential role
for mechanical forces in altering lung cell phenotype has been
highlighted [38,39]. We still know little of the mechanisms
of mechanosensing and the subsequent molecular pathways
leading to altered gene expression. In the context of fibrosis,
it is of interest that mechanical forces promote matrix pro-
duction and that this effect is via multiple cell-surface
receptors and intracellular signalling pathways [40,41] and
involves autocrine actions of TGFβ [5].

Apoptosis and pulmonary fibrosis

Apoptotic pathways are also key to the resolution of inflam-
mation and fibrosis following lung injury [42,43]. For exam-
ple, the removal of inflammatory cells or fibroblasts may be
vital elements of remodelling, and there is evidence for di-
munition of pro-apoptotic pathways in fibroblasts taken
from patients with pulmonary fibrosis compared with con-
trols [44]. Furthermore, fibroblasts derived from injured
lungs can induce apoptosis of epithelial cells. Moreover, it has also been shown that excessive apoptosis of epithelial cells is a feature of experimental fibrosis, and inhibitors of the pro-apoptotic molecule Fas or the caspases, which signal from the death receptors, inhibited fibrosis [45,46].

Cell plasticity: epithelial–mesenchymal transition and blood fibrocytes

The pathways leading to fibrosis are now recognized to involve considerable cell plasticity, with the excessive numbers of pro-fibrotic cells being derived from several sources (Figure 3). It has been proposed that epithelial–fibroblast interactions are central to remodelling both in the airways [47] and in the fibrotic foci within the lung parenchyma of patients with idiopathic pulmonary fibrosis [48,49]. Epithelial cells can transdifferentiate into mesenchymal cells with a pro-fibrotic phenotype [50,51]. They can also release many pro-fibrotic cytokines, including TGFβ, IGF-1 (insulin-like growth factor-1) and endothelin-1, which stimulate fibroblast proliferation and pro-collagen production. Furthermore, these cells may play key roles in the activation of growth factors via cell-surface integrins. One of these integrins, expressed only by epithelial cells is αvβ6. Mice deficient in the β6 subunit are protected from pulmonary fibrosis and lack the ability to activate TGFβ. Furthermore, recent studies suggest that activation of PAR1 generates active TGFβ via this αvβ6-dependent mechanism [52]. These data, taken together with the sparsity of inflammatory cells in some fibrotic conditions, and the ineffectiveness of current anti-inflammatory drugs, have led to the suggestion that remodelling and fibrosis may proceed independently of inflammation and that epithelial cells have a central role possibly involving products of the coagulation cascade.

Previous studies have also suggested that fibrocytes, bone-marrow-derived blood cells that express type I collagen, are targeted to the lung both in experimental disease [53] and in human pulmonary fibrosis [54]. A better understanding of this process and the molecular interactions that lead to this recruitment is needed, and recent studies have implicated several different chemokines in the recruitment process [55].

References

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