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Effect of substrate stiffness on pulmonary fibroblast activation by TGF-β

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ABSTRACT

Peptide crosslinkers containing the sequence C-X-CG (X represents various adhesive peptides) were incorporated into poly(ethylene glycol) (PEG) hydrogel networks with different mechanical properties. Pulmonary fibroblasts (PFs) exhibit increased adhesion to rigid hydrogels modified with X = RGDS, DGEA and IKVAV (0.5 and/or 5 mM) compared with a scrambled control (X = HRPNS). PFs exhibit increased adhesion to softer hydrogels when X = DGEA at low (0.5 mM) peptide concentration. PFs seeded onto hydrogels modified with X = RGDS produce alpha-smooth muscle actin (α-SMA), a myofibroblast marker, and form an extensive cytoskeleton with focal adhesions. Decreasing substrate stiffness (achieved through hydrolytic degradation) results in down-regulation of α-SMA expression by PFs. Substrate stiffness increases the sensitivity of PFs to exogenously applied transforming growth factor beta (TGF-β1); PFs on the most rigid gels (E = 900 kPa) express α-SMA when treated with low concentrations of TGF-β1 (1 ng ml^{-1}), while those on less rigid gels (E = 20–60 kPa) do not. These results demonstrate the importance of both mechanical and chemical cues in studying pulmonary fibroblast activation, and establish PEG hydrogels as a viable material for further study of IPF etiology.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive, fatal disease. Two hundred thousand patients are currently living with IPF in the US, and 50,000 cases are diagnosed annually. It is the most common form of interstitial lung disease, and generally has a poor prognosis [1]. While the cause is not well understood, IPF is thought to be the result of an abnormal wound-healing process in the lungs [2].

Fibroblast differentiation into the myofibroblast phenotype is a critical event in the wound-healing process. In a normal wound-healing response, the disappearance of myofibroblasts is mediated by apoptosis [3,4]. In fibrosis, myofibroblasts remain active, producing larger amounts of collagen relative to the fibroblast phenotype [5,6], which has been correlated to subepithelial fibrosis in the lung [7–9]. Fibrosis prevents the normal expansion and contraction of the lung tissue and therefore impairs its function. Many biochemical cues have been identified in the signaling pathways for these fibrotic diseases, particularly transforming growth factor beta (TGF-β1) [10] and connective tissue growth factor (CTGF) [11–13]. TGF-β1 influences the differentiation of fibroblasts into myofibroblasts; this differentiation is depicted in Fig. 1 (figure redrawn and adapted from Ref. [14]). Current therapeutics under study for treatment of pulmonary fibrosis, such as the drug Gleevac, target TGF-β1. While it is known that TGF-β1 plays a role in IPF, the mechanism by which it is upregulated is still being investigated.

Mechanical injury has been shown to induce myofibroblast formation [7], but many patients with IPF do not present an injury. Because mechanical injury to the lung epithelial cells induces fibroblast differentiation [7,15], the effect of normal mechanical forces in the lungs [16] may affect fibroblast differentiation and fibrosis. Pulmonary fibroblasts (PFs) have been seeded onto elastic membranes and exposed to dynamic stretching; increased cell proliferation occurred [17], and gene expression was directly regulated [17–20]. However, this type of stress is fundamentally different from the compressive forces that are thought to be dominant in the lung. It is therefore imperative to investigate both compressive and tensile stress on PF, with independent control over the chemistry and the mechanics of the cell's microenvironment.

The Hinz group has published several papers describing the transmission of mechanical force to the extracellular matrix (ECM) by (pulmonary) myofibroblasts [21–23]. They have recently shown that the contractile activity of myofibroblasts can release latent TGF-β1 from the ECM, resulting in further activation of the wound-healing phenotype [24]. This highlights the important role of the balance of tension between cell cytoskeleton and the matrix elasticity, but does not show whether or not quiescent PF can be activated to the myofibroblast phenotype through mechanical

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