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TGF beta inhibits expression of SP-A, SP-B, SP-C, but not SP-D in human alveolar type II cells

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ABSTRACT

TGF beta is a multifunctional cytokine that regulates alveolar epithelial cells as well as immune cells and fibroblasts. TGF beta inhibits surfactant protein A, B and C expression in fetal human lung and can inhibit type II cell proliferation induced by FGF7 (KGF). However, little is known about direct effects of TGF beta on adult human type II cells. We cultured alveolar type II cells under air/liquid interface conditions to maintain their state of differentiation with or without TGF beta. TGF beta markedly decreased expression of SP-A, SP-B, SP-C, fatty acid synthase, and the phospholipid transporter ABCA3. However, TGF beta increased protein levels of SP-D with little change in mRNA levels, indicating that it is regulated independently from other components of surfactant. TGF beta is a negative regulator of both the protein and the phospholipid components of surfactant. TGF beta did not induce EMT changes in highly differentiated human type II cells. SP-D is an important host defense molecule and regulated independently from the other surfactant proteins.

Taken together these data are the first report of the effect of TGF beta on highly differentiated adult human type II cells. The effects on the surfactant system are likely important in the development of fibrotic lung diseases.

1. Introduction

TGF beta is a critical cytokine for the development of pulmonary fibrosis [1,2]. TGF beta increases expression of smooth muscle actin and extracellular matrix proteins in lung fibroblasts, and these effects have been studied extensively. However, TGF beta also has effects on the alveolar epithelium. Most studies of the effect of TGF beta on alveolar type II cells have focused on the surfactant system in the developing lung. In the fetal lung, TGF beta decreases the expression of SP-A, SP-B and SP-C [3,4]. Using antibodies to endogenous TGFB1, McDevitt and colleagues showed that TGF beta alters a variety of other genes in epithelial cells from fetal human lung, but the largest effects were the decreased expression of SP-A, SP-B, and SP-C [5]. In mice, TGF beta delivered by an adenoviral vector produces fibrosis, increases surface tension, and decreases expression of SP-B and SP-C [6]. Less is known about the effects of TGF beta on regulation of SP-D and the lipid components of pulmonary surfactant.

The inhibitory effect of TGF beta on the surfactant system may contribute to the pathophysiology of pulmonary fibrosis [7]. TGF beta is expressed in many cell types and there are several routes for release and activation [1,2]. Alveolar type II cells in the IPF lung appear to be a rich source of this cytokine [8,9]. There is also significant impairment of pulmonary surfactant in IPF [10]. Surfactant recovered from patients with IPF fails to produce low surface tension and has altered protein and phospholipid composition [10]. The increased surface tension is thought to cause atelectasis, hypoxia, and ultimately appositional atelectasis or collapse induration with loss of alveolar units [11,12]. In addition, mutations in surfactant proteins and ABCA3 cause familiar forms of pulmonary fibrosis and interstitial disease [13–17]. Similarly, in bleomycin induced pulmonary fibrosis, the fibrotic lung has reduced expression of SP-A, SP-B and SP-C, and the isolated surfactant has altered phospholipid composition and impaired ability to lower surface tension [18,19]. Importantly from a therapeutic perspective, surfactant replacement prevents collapse induration and loss of

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alveolar units in rats instilled with bleomycin [20]. In addition, TGF beta inhibits keratinocyte growth factor (KGF, FGF7) induced type II cell proliferation [21], and inhibition of type II cell proliferation would be expected to contribute to the fibrotic response based on the classic studies of Witschi and Adamson [22–25].

Another property of TGF beta suggested to contribute to pulmonary fibrosis is the induction of epithelial to mesenchymal cell transition (EMT) [26–29]. However, the role of EMT in the pathogenesis remains controversial [30–32]. TGF beta induces EMT of alveolar epithelial cells *in vitro* when they are cultured on tissue culture plastic, dedifferentiate, spread on the surface and cease to express the surfactant proteins [27]. However, there is little evidence that TGF beta will induce EMT in highly differentiated cuboidal type II cells [28].

Although there are multiple potential effects of TGF beta on alveolar type II cells that have important implications for human disease, direct effects of TGF beta on adult human alveolar type II cells have not been reported. In this report, we demonstrate that TGF beta inhibits the expression of surfactant proteins SP-A, SP-B, and SP-C, fatty acid synthase, and the phospholipid transporter ABCA3 but not SP-D. These results are important for our understanding of how TGF beta may impair surfactant function in pulmonary fibrosis and lead to collapse induration and loss of alveolar units.

2. Methods

2.1. Type II cell isolation and culture

Primary alveolar type II cells were isolated from human lungs from de-identified organ donors whose lungs were not suitable for transplantation. The Committee for the Protection of Human Subjects at National Jewish Health deemed this research as non-human subject research. The lung was perfused, lavaged, and digested with elastase as described previously [33]. The lung was minced, and the cells were partially purified by centrifugation on a discontinuous density gradient made of Optiprep (Accurate Chemical Scientific Corp., Westbury, NY) with densities of 1.085 and 1.040. The type II cells were then isolated by nonadherence to IgG coated petri dishes. The isolated cells were suspended in Advanced DMEM/F12 medium (Life Technologies, Grand Island, NY) or regular DMEM (Thermo Fisher Scientific, Waltham, MA) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 2.5 μg/ml amphotericin B, 100 μg/ml streptomycin, 100 μg/ml penicillin G (GIBCO BRL, Life Technologies Inc., Rockville, MD), and 10 μg/ml gentamicin (Sigma-Aldrich, St. Louis, MO) or frozen down in 90% FBS and 10% DMSO to be used for culture at a later date.

For air liquid interface (ALI) cultures, the epithelial cells were plated on gels composed of 80% rat-tail collagen and 20% Matrigel

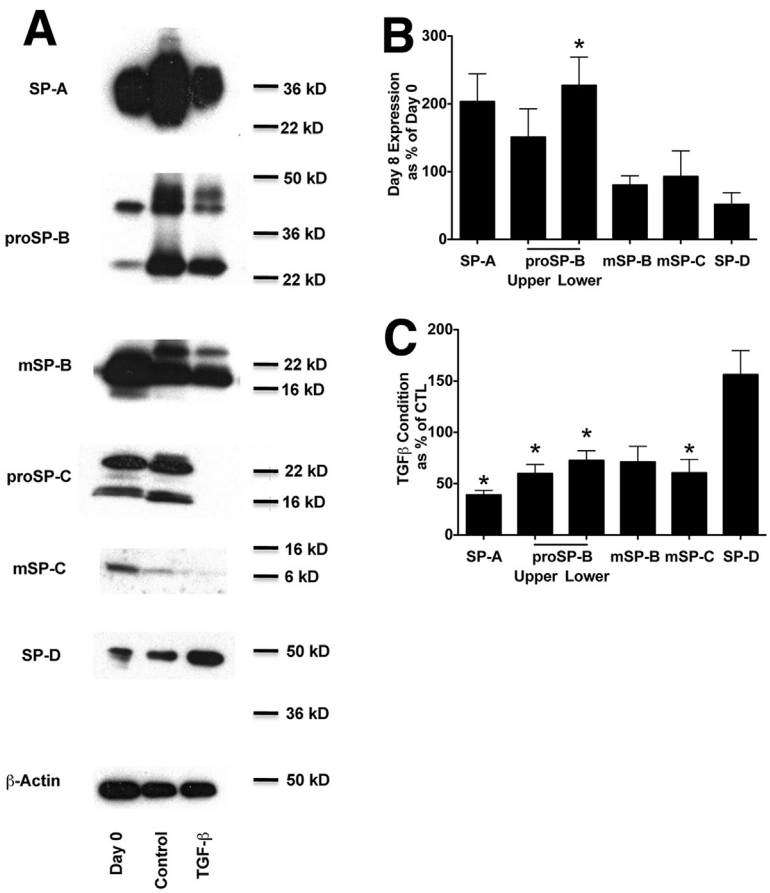

Fig. 1. TGF beta inhibits the expression of SP-A, SP-B, and SP-C but increases the expression of SP-D. Panel A. Protein expression under air/liquid conditions. Western analyses of surfactant protein levels in freshly isolated cells and cultured with or without TGF beta for the final 4 days. The columns for the Western are Day 0 (freshly isolated type II cells), control (cells cultured for eight days and under air/liquid conditions for the last 6 days), and TGF beta (cells cultured for eight days and in the presence of 5 ng/ml TGF-B for the last 4 days. Representative of four independent experiments. **Panel B.** Protein expression for comparing the level of expression on day 8 in culture to the freshly isolated type II cells. Westerns from four separate experiments were quantitated by ImageJ 64 software. * indicates $p < 0.05$ compared to day 0 control. **Panel C.** Data for protein expression in the presence of TGF beta is compared to the control value on day 8. Results are from four separate experiments. * indicates $p < 0.05$.

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