TSP-1 in lung fibrosis

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Abstract Lung fibrosis is often treated with corticosteroids to reduce the inflammatory response, however, no effective treatment options exist for the underlying disease. An important player in the fibrotic cascade is the cytokine, transforming growth factor beta (TGF β). TGF β is converted from an inactive procytokine complex to active TGF β by enzymes such as thrombospondin-1 (TSP-1). It is therefore presumed that TSP-1 deficient mice would fare better to bleomycin-induced pulmonary fibrosis because TGF β would not be efficiently converted to the active form. Interestingly, a recent article by Ezzie and colleagues shows that TSP-1 deficiency does not protect mice from systemic bleomycin challenge. Indeed, they find the opposite, as TSP-1-null mice appear to exhibit greater lung fibrosis than wild type mice, although similar TGF β signaling was observed in the lungs of both mouse strains.

Keywords Lung fibrosis · Bleomycin · TGFbeta

TGF β is secreted by most cell types as a latent, inactive procytokine that consists of the mature, active TGF β protein and latency associated protein (LAP) (Massague 1998). The extracellular release of mature TGF β from this procytokine complex is required for its interaction with TGF β receptors. The proteins and/or mechanisms responsible for this release include changes in extracellular pH, reactive oxygen species, plasmin, calpain or cathepsin and TSP-1 (Massague 1998; Ribeiro et al. 1999). TSP-1 associates with the TGF β procytokine complex by interacting with the

mature TGF β protein as well as latency associated protein (LAP). This allows the molecular interaction of TSP-1 with the N-terminus of LAP, which displaces mature TGF β and disrupts interactions between LAP and mature TGF β (Ribeiro et al. 1999; Schultz-Cherry and Murphy-Ullrich 1993). Mature TGF β then associates with the serine/threonine kinase TGF β receptors to initiate the phosphorylation of Smad2 proteins, their association with Smad4 and translocation into the nucleus to modulate gene transcription (Attisano and Wrana 2002).

Studies have been carried out to evaluate if TSP-1 is an endogenous activator of TGF β in vivo. In the kidney this was assessed by renal arterial transfer of antisense oligos against TSP-1 as well as by continuous intravenous infusion of specific peptides interfering with the activation of TGF β by TSP-1 (Daniel et al. 2003; Daniel et al. 2004). In these studies, treated rats showed a decrease in TGF β activity and/or TGF β receptor signaling as well as glomerular extracellular matrix accumulation as assessed by collagen I, IV, and fibronectin staining, but no change regarding glomerular cell proliferation, cell death or macrophage accumulation compared to control rats. These studies concluded that TSP-1 is an endogenous activator of TGF β in experimental renal disease.

Studies using a similar approach demonstrated that a TSP-1 synthetic peptide also inhibited bleomycin-induced lung fibrosis in mice (Chen et al. 2009). The authors found that inflammation, fibrotic degree and distribution of collagen fibers in the interstitial and alveolar spaces in the TSP-1-treated groups were less than those of the other experimental groups. It is therefore interesting that the recent publication by Ezzie and colleagues show that there is greater subpleural fibrosis in TSP-1 null mice versus their wild type counterparts in response to bleomycin challenge (Ezzie et al. 2010). They observed greater CTGF and

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collagen I mRNA levels as well as a trend towards greater inflammation in the null animals. Intriguingly, they also observed similar Smad2 phosphorylation in the lungs of bleomycin treated wild-type and TSP-1-null animals, strongly suggesting that similar active TGF β was present in the lungs of both mouse strains. It has been established that alveolar macrophages stimulated by bleomycin-induced injury secrete large quantities of biologically active TGF β 1, which plays a critical role in the development of lung fibrosis (Chen et al. 2009). However, although there were greater numbers of lymphocytes in TSP-1-null mice, the number of macrophage and neutrophils in the bleomycin-induced fibrotic lungs were similar in wild-type and TSP-1-null mice (Ezzie et al. 2010).

The differences in the results obtained from genetically blocking TSP-1 production versus mice overexpressing latent TGF β or treated with TSP-1 blocking peptides, highlight the importance of TSP-1 in lung development and homeostasis. Particularly, the different mechanisms in these two model systems of studying lung fibrosis may yield important insight. Is the acute inhibition of TSP-1 in cases of systemic fibrosis a viable treatment option? Lung cancer studies have suggested that TSP-1 levels are a good prognostic tool to assess metastasis and patient outcome (Papadaki et al. 2009; Yamaguchi et al. 2002), so it follows that this will be an important target in fibrosis as well. The study from Ezzie et al. (2010) also raises interesting questions with respect to compensatory gene regulation of the TGF β pathway in TSP-1-null mice. Since TSP-1 is not the only regulatory factor that activates TGF β , could other proteins/mechanisms (for e.g. plasmin) be affected? The authors discuss that other reports demonstrate that integrins are important in regulating lung fibrosis after bleomycin challenge. Specifically, the integrin α v β 6 is critically important in the pathogenesis of pulmonary fibrosis and TGF β activation after intratracheal bleomycin challenge (Munger et al. 1999). These results and the authors' previous observations that the LAP RGD domains are not required for interacting with TSP-1 (Ali et al. 2008), suggests that TSP-1 may not be necessary for the binding of LAP to facilitate the activation of latent TGF β .

These fascinating differences will ensure that this area of research will yield fruitful insight not only in deciphering

TSP-1 biology but also mechanisms of TGF β regulation, and will benefit the treatment of multiple diseases including fibrosis and cancer.

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