Ltbp4 regulates Pdgfrβ expression *via* TGFβ-dependent modulation of Nrf2 transcription factor function

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Abstract

Latent transforming growth factor beta binding protein 4 (LTBP4) belongs to the fibrillin/LTBP family of proteins and plays an important role as a structural component of extracellular matrix (ECM) and local regulator of TGFβ signaling. We have previously reported that *Ltbp4S* knock out mice (*Ltbp4S*−/−) develop centrilobular emphysema reminiscent of late stage COPD, which could be partially rescued by inactivating the antioxidant protein Sestrin 2 (Sesn2). More recent studies showed that *Sesn2* knock out mice upregulate Pdgfrβ-controlled alveolar maintenance programs that protect against cigarette smoke induced pulmonary emphysema. Based on this, we hypothesized that the emphysema of *Ltbp4S*−/− mice is primarily caused by defective Pdgfrβ signaling. Here we show that LTBP4 induces Pdgfrβ signaling by inhibiting the antioxidant Nrf2/Keap1 pathway in a TGFβ-dependent manner. Overall, our data identified Ltbp4 as a major player in lung remodeling and injury repair.

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Introduction

Latent transforming growth factor beta binding protein 4 (LTBP4) belongs to a family of four (LTBP1–4) secreted extracellular matrix (ECM) proteins that share structural homology with fibrillins. Like the other LTBPs, LTBP4 consists of numerous EGF-like motifs whose functional significance is not yet clear; they are found in the extracellular domain of membrane bound proteins and in some secreted proteins. Interspersed between the EGF-like modules of LTBP4 are three 8-Cys repeat domains of which the third covalently binds latent TGFβ1 (TGFβ1-LAP, where LAP stands for latency associated propeptide) and deposits it into the ECM. There, TGFβ1-LAP complexed to LTBP4 is stored until needed. When needed, active TGFβ is released from LTBP and LAP in a process referred to as "latent TGFβ activation", which is accomplished by a variety of factors including proteases, integrins, reactive oxygen species and many others (reviewed in [1,2]). In addition to its TGFβ related function, LTBP4 is required for the assembly of elastic fibers in the ECM [3].

Mammalian cells express two major isoforms of LTBP4, which by analogy to the long and short isoforms of LTBP1 discovered earlier, are also called long (LTBP4L) and short (LTBP4S). These isoforms are encoded by two N-terminal splice variants expressed independently from their own promoters [4].

We and others previously reported that mice with an inactivating mutation in *Ltbp4S* (*Ltbp4S*−/− mice) are born with alveolar septation defects that deteriorate with age [5,6]. By the age of 5–6 months lungs from *Ltbp4S*−/− mice show symptoms reminiscent of

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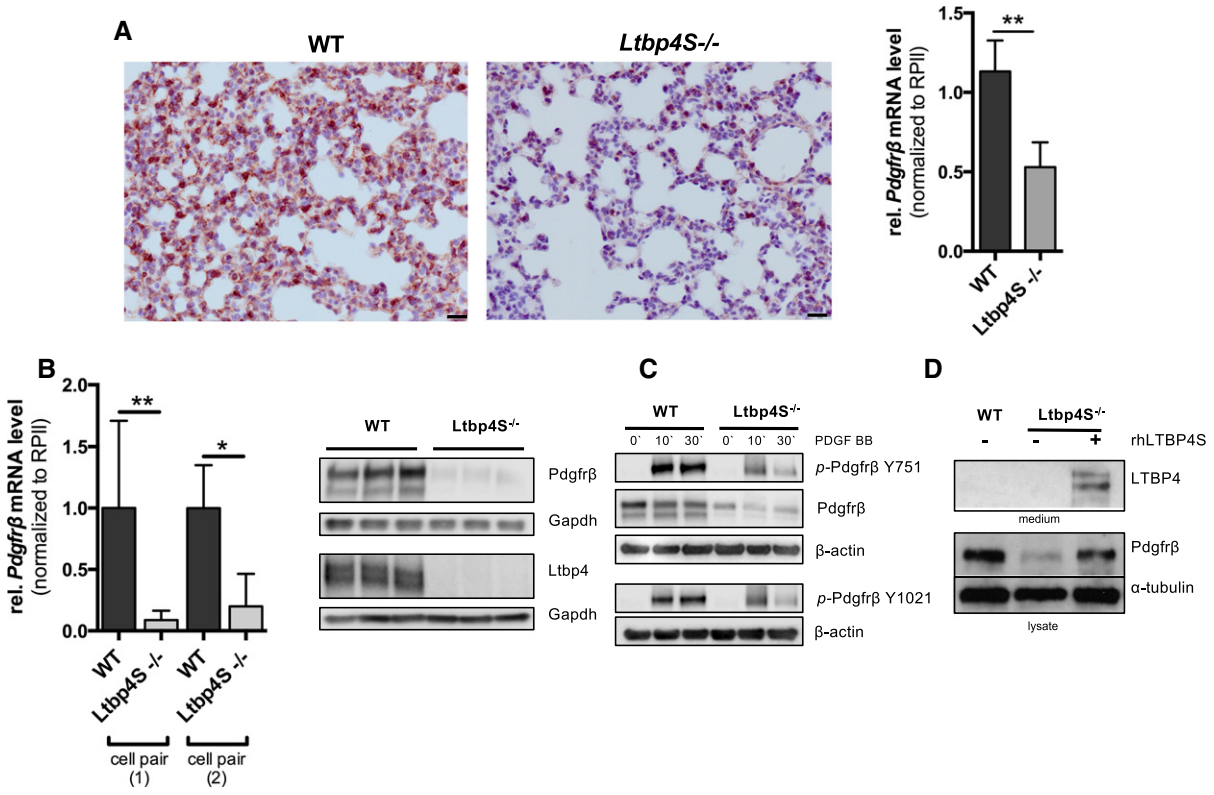

Fig. 1. Regulation of Pdgfrβ signaling by Ltbp4. A. Reduced Pdgfrβ expression in lungs of *Ltbp4S–/–* mice. *Left panel:* Representative lung sections stained with anti-Pdgfrβ antibody and counterstained with hematoxilin. Scale bar: 20 μm. *Right panel: Pdgfrβ* mRNA in lung tissue homogenates quantified by qRT-PCR. Results are represented as means ± SD of N = 3 lungs. B. Pdgfrβ mRNA and protein expression in MLFs of WT- and *Ltbp4S–/–* mice. *Left panel:* qRT-PCR. Results are represented as means ± SD of three independent experiments. *Right panel*: Western blot showing Ltbp4S and Pdgfrβ expression in MLFs derived from three separate experiments. C. Western blot showing Pdgrβ phosphorylation after stimulation with 25 ng/ml recombinant PDGFR BB. D. Rescue of Pdgfrβ expression in Ltbp4S–/– MLFs after transfecting with the LTBP4S/pEF-IRES expression plasmid (see Experimental procedures). *$p < 0.05$, **$p < 0.01$.

centrilobular emphysema associated with late stage chronic obstructive pulmonary disease (COPD) [5,7]. We could partially rescue this phenotype by inactivating the antioxidant protein Sestrin 2 (Sesn2) and attributed this to an activation of TGFβ- and mTOR signaling [7]. However, more recent studies showed that Sesn2 also regulates Pdgfrβ signaling. In mice, the mutational inactivation of Sesn2 prevents the development of cigarette smoke induced pulmonary emphysema by upregulating Pdgfrβ-controlled alveolar maintenance programs [8]. We showed that Pdgfrβ upregulation is mediated by second messenger superoxide anions (O_2^-) accumulating in Sesn2 depleted cells as a result of Nrf2 (nuclear factor erythroid 2-related factor 2)/Keap1(Kelch-Like ECH-Associated Protein 1) pathway inhibition [9].

Nrf2 is a well-characterized, global antioxidant gene inducer, whose activity is tightly controlled by cytoplasmatic association with its inhibitor Keap1. Upon oxidative stress, Nrf2 dissociates from Keap1, translocates into the nucleus and transactivates antioxidant genes (reviewed in [10]). Sesn2 stimulates this process by promoting autophagic degradation of Keap1 [11].

We demonstrated that Sesn2 and Nrf2/Keap1 are part of a Sesn2/Pdgfrβ suppressor pathway that is highly upregulated in the lungs of individuals with late stage COPD [9]. As upregulation of Pdgfrβ protected Sesn2 knock out (KO) mice against cigarette smoke induced emphysema [8], we speculated that a similar mechanism might be responsible for the emphysema rescue phenotype of Sesn2 depleted *Ltbp4S* KO mice. Here we show that Ltbp4 is required for latent TGFβ activation in the ECM and TGFβ signaling for the inhibition of the Sesn2/Pdgfrβ suppressor pathway. Overall, our data suggest that Ltbp4 plays a major role in lung remodeling and might be involved in the pathogenesis of COPD.

Results

Repressed Pdgfrβ expression in lungs of *Ltbp4S–/–* mice

To investigate a possible interrelationship between Ltbp4 and Pdgfrβ, we estimated Pdgfrβ expression

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