Supplementary Information

Fibulin-1c regulates transforming growth factor-β activation in pulmonary tissue fibrosis

Gang Liu,1,2,3 Marion A. Cooley,4 Andrew G. Jarnicki,1,5 Theo Borghuis,6 Prema M. Nair,1 Gavin Tjin,7 Alan C. Hsu,1 Tatt Jhong Haw,1 Michael Fricker,1 Celeste L. Harrison,1 Bernadette Jones,1 Nicole G. Hansbro,1,2,3 Peter A. Wark,1 Jay C. Horvat,1 W. Scott Argraves,4 Brian G. Oliver,5,6 Darryl A. Knight,1 Janette K. Burgess,6,7 and Philip M. Hansbro1,2,3

Affiliations

1Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute and The University of Newcastle, Newcastle, New South Wales, Australia
2School of Life Sciences, University of Technology Sydney, Sydney, New South Wales, Australia
3Centenary Institute, Sydney, New South Wales, Australia
4Department of Oral Biology and Diagnostic Sciences, Augusta University, Augusta, Georgia, USA;
5Department of Pharmacology and Therapeutics, University of Melbourne, Parkville, Victoria, Australia
6University of Groningen, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD (GRIAC), Department of Pathology and Medical Biology, Groningen, The Netherlands
Woolcock Institute of Medical Research, Discipline of Pharmacology, The University of Sydney, Sydney, New South Wales, Australia

Corresponding authors

Correspondence to Philip M. Hansbro: Philip.Hansbro@newcastle.edu.au
Supplementary Figures and Figure legends

Supplementary Figure 1. Excess collagen deposition in whole lungs and around the airways in bleomycin-induced experimental pulmonary fibrosis. A single bleomycin challenge induced pulmonary fibrosis in WT mice. Controls received PBS. (A) A time-course of lung sections stained with Verhoeff's-Van Gieson stain. Scale bar=500 μm; inserts show expanded images of indicated regions, scale bar=50 μm. Images are representative of n=24-40 airways from n=4-8 mice per group. (B) A time-course of quantification of collagen area around small airways with normalization to the perimeter of the basement membrane (Pbm) (n=7–8). (C) Total collagen levels were assessed by measuring hydroxyproline in whole lung tissues (n=8). Statistical
differences were determined with two-tailed student t-test. **P<0.01, ****P<0.0001 compared to PBS-challenged mouse controls.
Supplementary Figure 2. Fbln1c is increased around the airways in bleomycin-induced experimental pulmonary fibrosis. A single bleomycin challenge was used to induce pulmonary fibrosis in WT mice. Controls received PBS. A time-course of lung sections were assessed for Fbln1 protein levels around small airways using immunohistochemistry. Scale bar=500 μm; inserts show expanded images of indicated regions, scale bar=50 μm. Images are representative of n=24-40 airways from n=4-8 mice per group.
Supplementary Figure 3. Bleomycin challenge of Fbln1c−/− mice does not increase collagen fibers around the airways. A single bleomycin challenge was used to induce pulmonary fibrosis in WT and Fbln1c−/− mice. Controls received PBS. Collagen fibers were imaged by second harmonic generation (SHG) microscopy. Collagen backward signal (B_{SHG}) is violet, and collagen forward signal (F_{SHG}) is cyan, scale bar=100 μm. Images are representative of n=40 airways from n=4 mice per group.
Supplementary Figure 4. Bleomycin challenge of Fbln1c−/− mice does not increase the mRNA levels of Mmps or Timp1 in whole lung tissues. A single bleomycin challenge was used to induce pulmonary fibrosis in WT and Fbln1c−/− mice. Controls received PBS. (A) Mmp1, (B) Mmp3, (C) Mmp8, (D) Mmp12, (E) Mmp13 and (F) Timp1 mRNA levels in lungs determined using qRT-PCR (n=6-8). Statistical differences were determined with one-way ANOVA followed by Bonferroni post-test. *P<0.05, **P<0.01, ***P<0.001 compared to PBS-challenged WT mice. †P<0.05, †††P<0.001 compared to bleomycin-challenged WT mice.
Supplementary Figure 5. TGF-β challenge of Fbln1c−/− fibroblasts does not affect Smad3 mRNA levels, and bronchoalveolar lavage fluid (BALF) from Fbln1c−/− mice reduces Smad gene levels in fibroblast from WT mice. Primary fibroblasts were isolated from the lungs of WT and Fbln1c−/− mice and stimulated with TGF-β or media control for 24 h. (A) Smad3, (B) Smad2 and (C) Smad4 mRNA levels in fibroblast lysates determined by qRT-PCR (n=6 of each genotype). Primary mouse lung fibroblasts from WT mice were incubated with bronchoalveolar lavage fluid (BALF, 20µl each mouse from WT and Fbln1c−/− mice after 28 days bleomycin challenge and controls for 6 hours. (D) Smad3, (E) Smad2, (F) and Smad4 mRNA levels in fibroblast
lysates determined by qRT-PCR. Statistical differences were determined with one-way ANOVA followed by Bonferroni post-test. *P<0.05 compared to media control.