Comparison of TGFβ-induced changes in *in vitro* assays of different complexities

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Introduction

- Idiopathic pulmonary fibrosis (IPF) is a complex disease characterized by excessive production of extracellular matrix (ECM) components, resulting in tissue scarring and eventually loss of organ function
- Treatment options are limited, with two approved therapies for IPF, nintedanib and pirfenidone, which only slow down disease progression
- TGFβ (transforming growth factor β) is recognized as one of the key drivers of fibrotic remodeling, and as such has been frequently used in different *in vitro* assays for lung fibrosis

Aim

- The aim of the study was to compare TGFβ-driven fibrotic response in *in vitro* assays of different complexity, from 2D primary cell cultures to 3D lung tissue slices.
- Nintedanib was used as a reference compound since it is one of the approved therapies for pulmonary fibrosis, and SB525334 was used since it is an inhibitor of TGFβ receptor type I (ALK5)

Methods

- Normal human lung fibroblasts (NHLF) were plated in collagen-coated wells
- For αSMA protein detection with immunostaining, NHLF cells were incubated in medium without FBS and stimulated with TGFβ at 1 ng/mL for 48 h
- For collagen 1 protein detection with immunostaining, NHLF cells were incubated in medium without FBS, with Ficoll 70, Ficoll 400 and L-ascorbic acid, and stimulated with TGFβ at 1 ng/mL for 72 h
- Human lung tissue was obtained from donors who underwent medically indicated lung resection
- Lungs were filled with agarose and 500 µm thick PCLS were prepared (Alabama R&D Tissue Slicer)
- PCLS were stimulated with rhTGFβ at 10 ng/mL in Advanced DMEM-F12 medium for 72 h and 120 h
- SB525334 and nintedanib were tested in concentration response by adding them to NHLF or PCLS incubation medium 1 h prior to stimulation with rhTGFβ

Results

Expression of profibrotic markers αSMA and collagen 1 was reduced by SB525334 and Nintedanib in TGFβ-stimulated NHLF cells

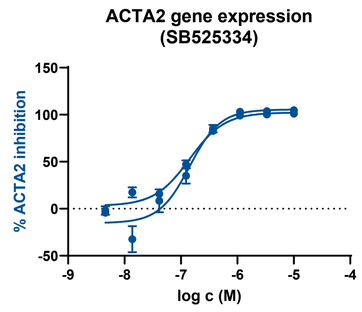

Figure 1. The effect of ALK5 inhibitor SB525334 on ACTA2 and COL1A1 gene expression in TGFβ-stimulated NHLF cells incubated for 48 h (data from 2 independent experiments)

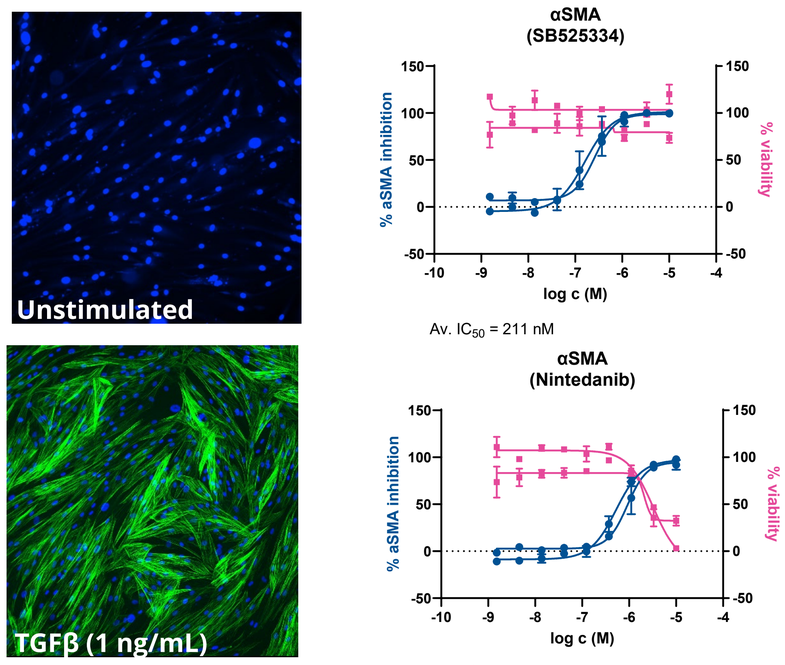

Figure 2. αSMA immunostaining in TGFβ stimulated NHLF cells and the effect of SB525334 and Nintedanib (48 h incubation).
αSMA Hoechst (data from 2 independent experiments)

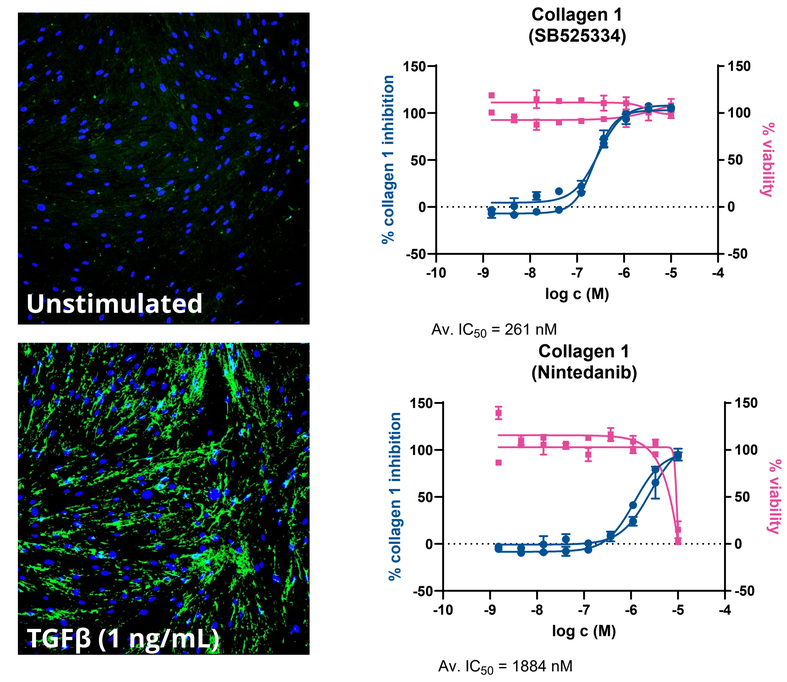

Figure 3. Collagen 1 immunostaining in TGFβ stimulated NHLF cells in presence of Ficoll and L-ascorbic acid and the effect of SB525334 and Nintedanib (72 h incubation).
Collagen 1 Hoechst (data from 2 independent experiments)

TGFβ increased expression of fibrotic markers in human PCLS from multiple donors, while treatment with SB525334 and Nintedanib inhibited their expression

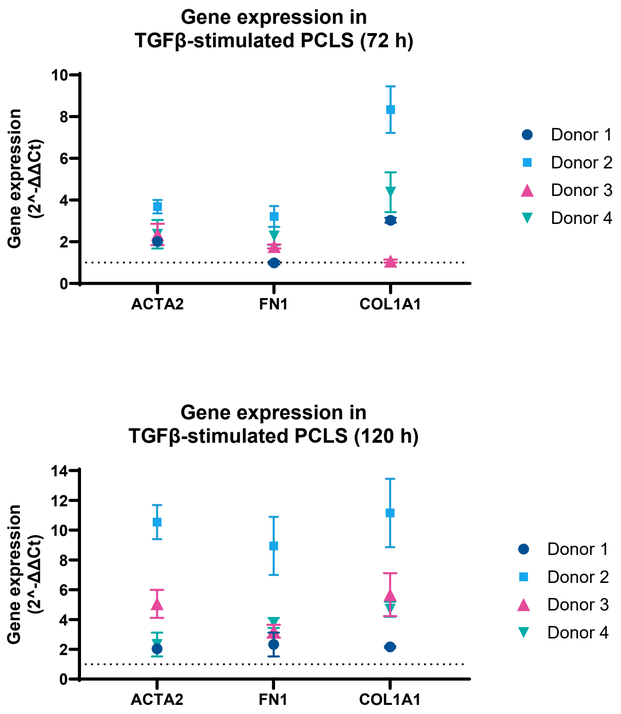

Figure 4. ACTA2, FN1 and COL1A1 gene expression in TGFβ (10 ng/mL) stimulated PCLS prepared from 4 donors (72 h and 120 h incubation). Data normalized to untread PCLS for each donor. Mean ± SEM.

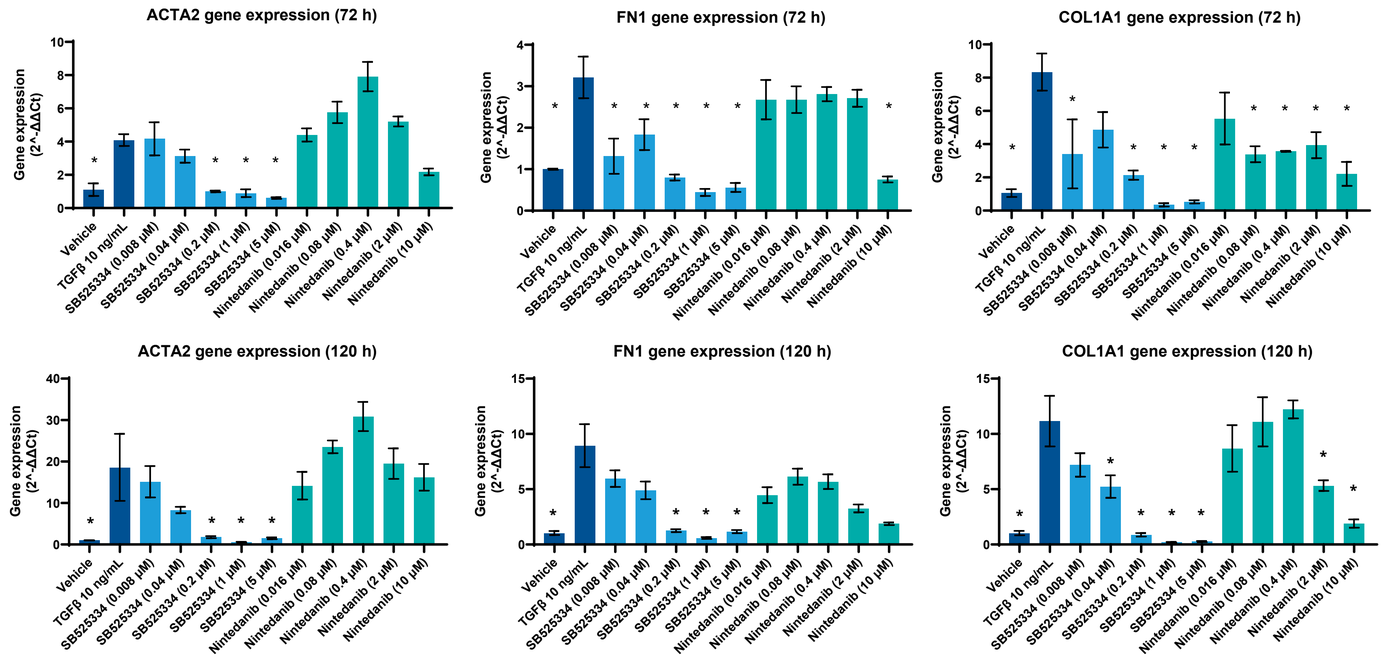

Figure 5. The effect of SB525334 and nintedanib on ACTA2, FN1 and COL1A1 gene expression in TGFβ-stimulated PCLS (72 h and 120 h incubation). Mean ± SEM. *p<0.05 vs. TGFβ, One-way ANOVA with Dunnett's multiple comparisons test.

Conclusions

- TGFβ induced fibrotic response in primary lung fibroblasts, with increased gene and protein expression of αSMA and collagen 1, enabling robust *in vitro* assay for testing anti-fibrotic compounds
- Monitoring the deposition of αSMA and collagen 1 fibers in NHLF assay required two different medium compositions, since addition of Ficoll 70, Ficoll 400 and L-ascorbic acid was essential for formation of collagen 1 fibers
- In a more complex system of human PCLS containing multiple different cell types including fibroblasts and epithelial cells, treatment with TGFβ induced fibrotic changes already after 72 h, with further increase up to 120 h
- The ability of PCLS to respond to TGFβ stimulation was confirmed on tissue from several different donors
- The relevance of both NHLF cell and PCLS models was confirmed by the activity of clinically approved therapy nintedanib