PHENOVISTA

PHENOTYPIC STUDIES TO DE-RISK FIBROSIS R&D

INTRODUCTION

Thickened, stiff, damaged tissue is the hallmark of fibrosis – a potentially deadly disease that can result from a multitude of causes and affect numerous organs. Although fibrosis itself is a disease, it is also affiliated with other conditions, such as cancer and inflammatory disorders, rendering it a fulcrum point of diverse R&D studies and efforts.

Here we present a brief selection of assays that we have developed to measure various indicators of a fibrotic state using a range of relevant cell types.

Characterize the Effect of a Drug Candidate on the Formation of Stress Fibers

In this experiment, we measured the ability of a small-molecule inhibitor to inhibit the formation of F-actin and α-SMA-containing stress fibers in primary, normal, human lung fibroblasts (NHLFs).

Cells: NHLFs
Fibrotic Inducer: TGF-β
Fibrotic Inhibitor: Alk5i
Markers: Hoechst (nuclei), anti-α-SMA, phalloidin (F-actin)

Study Results:

- TGF-β induced a fibrotic phenotype in a dose-dependent manner, as measured by increases in F-actin and α-SMA area, and alignment of stress fibers.
- Alk5i significantly inhibited all metrics.

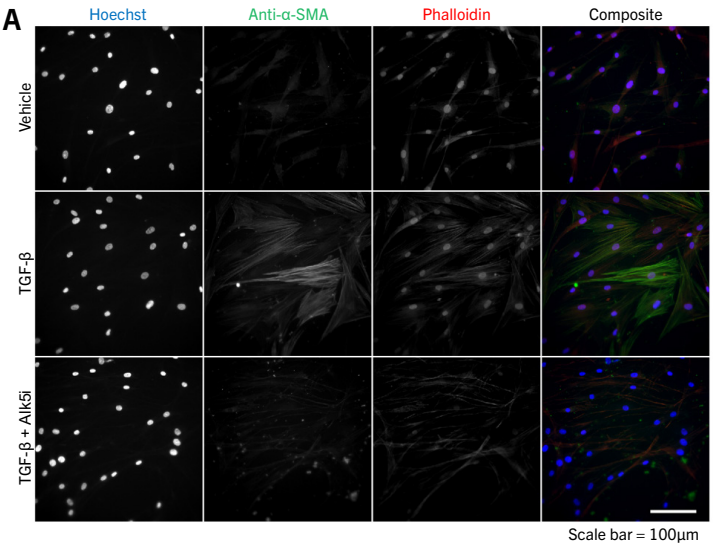

Figure 1A. *Representative images of primary NHLFs stained for nuclei (Hoechst), α-SMA, and F-actin (phalloidin) 72hrs after treatment.*

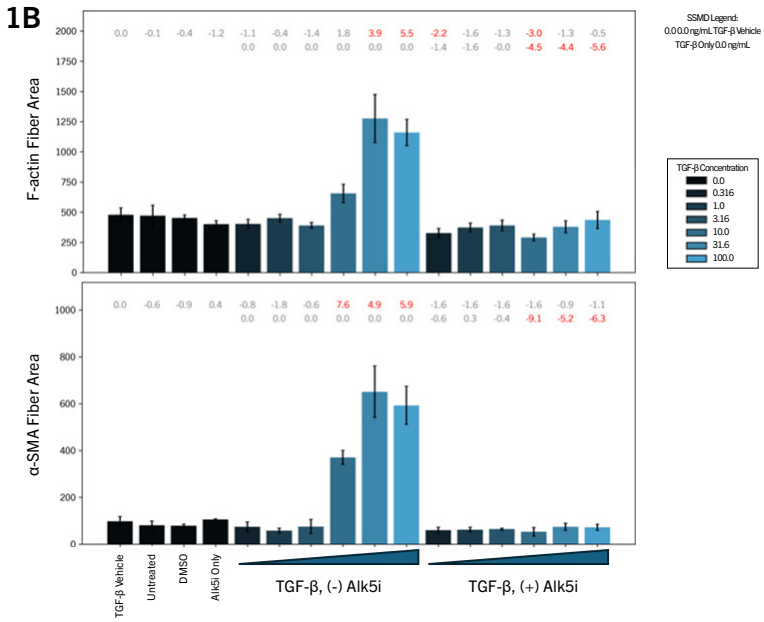

Figure 1B. *Treatment of NHLFs with TGF-β increased fiber area in a dose-dependent manner. Addition of inhibitor Alk5i prevented these increases.*

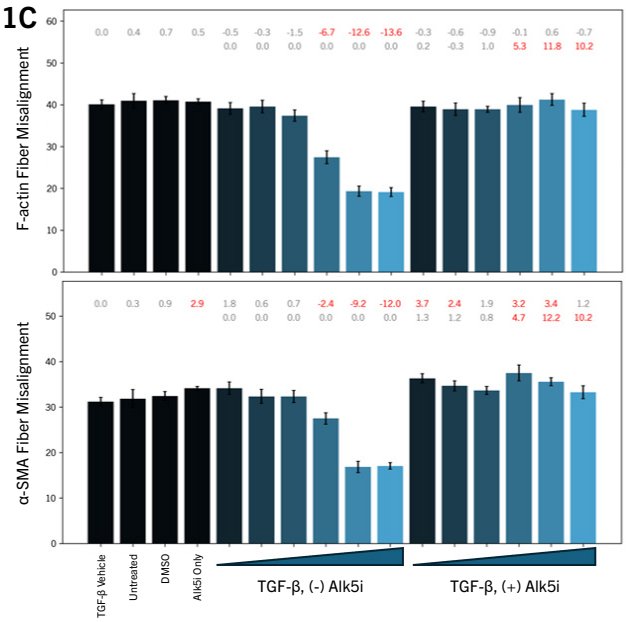

Figure 1C. *Treatment of NHLFs with TGF-β increased fiber alignment (decreased deviations in relative fiber directionality) in a dose-dependent manner. Addition of inhibitor Alk5i prevented these increases.*

Characterize the Effect of a Drug Candidate on Fibrosis Pathway Activation

Here, we measured the ability of a drug candidate (test article, TA) to prevent that activation of the TGF-β/SMAD pathway in fibrosis in primary, renal, proximal tubule, epithelial cells (PTECs) through quantifying the prevalence of nuclear translocation of phospho-SMAD2/3 (pSMAD).

Cells: PTECs
Fibrotic Inducer: TGF-β
Fibrotic Inhibitor: Test article (TA)
Markers: Hoechst (nuclei), anti-pSMAD, phalloidin (F-actin)

Study Results:
- TGF-β increased the occurrence of nuclear translocation of pSMAD.
- All tested doses of the test article prevented this pathway activation.

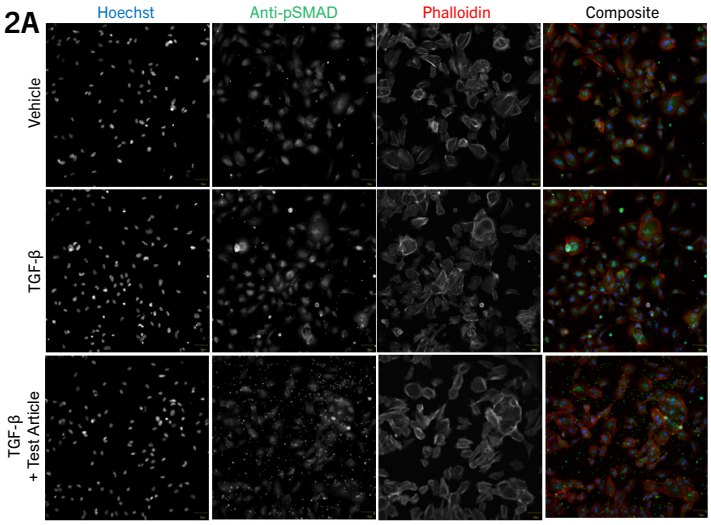

Figure 2A. *Representative images of PTECs stained for nuclei (Hoechst), phospho-SMAD, and F-actin (phalloidin) 30 minutes after treatment.*

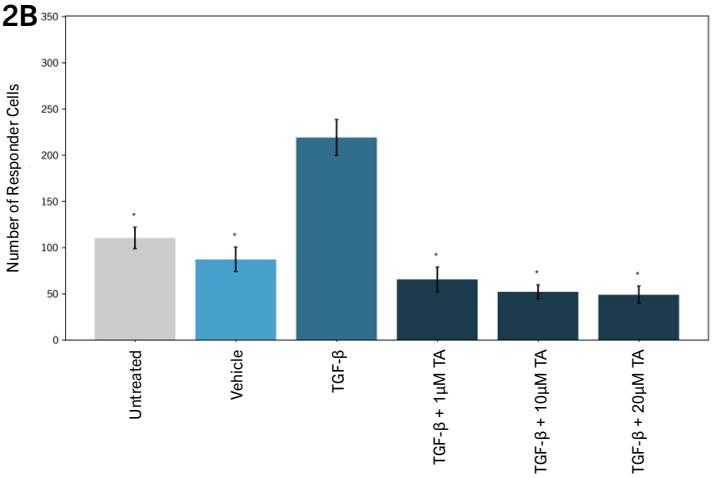

Figure 2B. *Treatment of PTECs with TGF-β increased number of cells with phospho-SMAD nuclear translocation. Addition of the test article prevented this increase.*

Characterize the Effect of a Clinical Candidate on Deposition of Extracellular-Matrix Proteins

Deposition of excess extracellular-matrix (ECM) proteins is implicated as the cause of scarring and stiffening of tissues in fibrosis. In this experiment, we measured the ability of test articles to inhibit this hallmark of disease.

To inhibit the deposition of two different ECM components – collagen type I and fibronectin - in two primary, human cell types – normal hepatic stellate cells (HSCs) and normal dermal fibroblasts (NHDFs).

Cells: HDFs
Fibrotic Inducer: TGF-β
Fibrotic Inhibitors: Five test articles (TA1-5)
Markers: Hoechst (nuclei), anti-collagen type I

Study Results:
- Collagen I deposition increased with TGF-β treatment.
- Client-provided did not significantly inhibit excess collagen I deposition elicited by TGF-β.

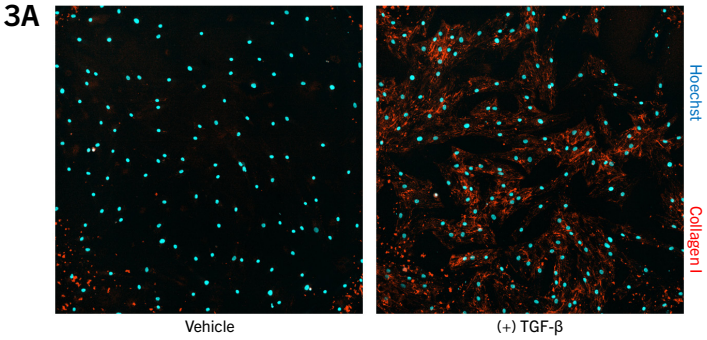

Figure 3A. *Representative images of HDFs stained for nuclei (Hoechst) and collagen I 72hrs after treatment with TGF-β.*

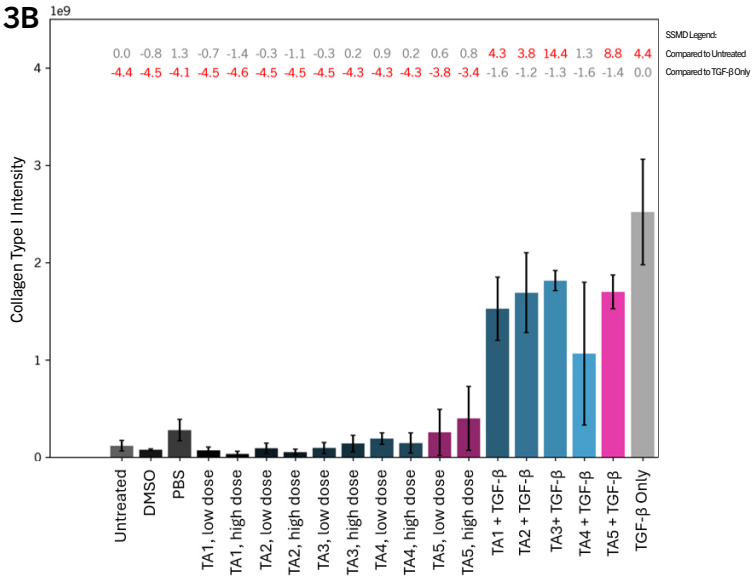

Figure 3B. *Treatment of HDFs with TGF-β increased collagen I deposition. Treatment with client's TAs alone did not induce excess collagen I deposition. Treatment with client TAs did not prevent excess collagen deposition induced by TGF-β.*

Cells: HSCs
Fibrotic Inducer: TGF-β
Fibrotic Inhibitors: Alk5i and one test article (TA)
Markers: Hoechst (nuclei), anti-fibronectin

4A

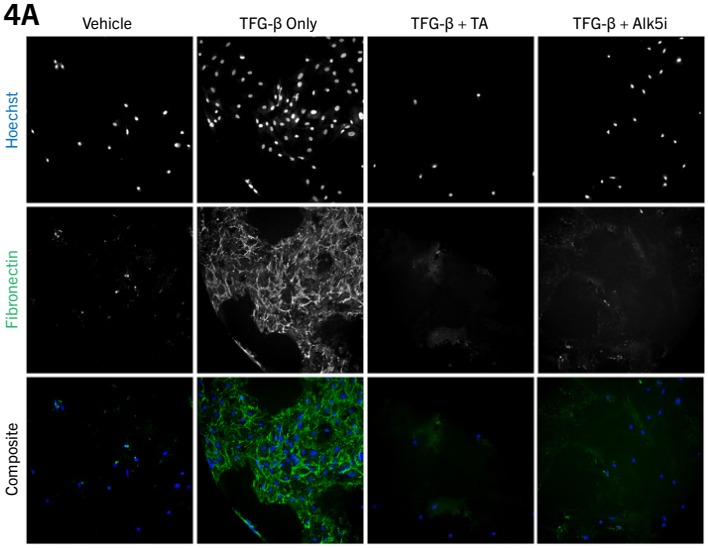

Figure 4A. *Representative images of HSCs stained for nuclei (Hoechst) and fibronectin 72hrs after treatment with TGF-β.*

Study Results:

- Fibronectin deposition increased with TGF-β treatment in a dose-dependent manner.
- Both the test article and Alk5i significantly inhibited excess fibronectin deposition.

4B

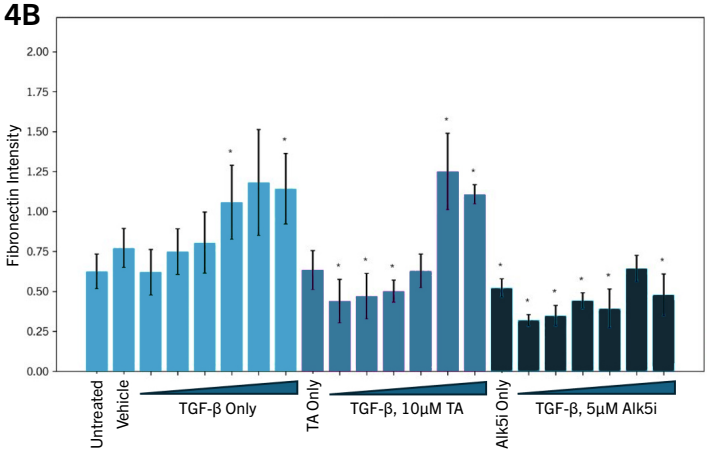

Figure 4B. *Treatment of HSCs with TGF-β increased fibronectin deposition. Treatment with client's TA inhibited this deposition at lower levels of TGF-β. Alk5i significantly inhibited fibronectin deposition at all tested concentration of TGF-β.*

PHENOVISTA SERVICES

As demonstrated by these examples, we design phenotypic studies to measure diverse aspects of fibrosis-relevant biology, from stress fiber formation to pathway activation.

We develop assays in close collaboration with our clients to ensure that your specific questions will be answered. You can choose from a range of services to select the best fit for your needs.

Custom Assay Services
Custom assays to answer your specific, complex biological questions.

Cell Painting
Compare your compounds' effects against those of reference compounds.

Ready-2-Go Assay Services
Defined assay offerings across a range of disease and therapeutic areas.

Imaging & Analysis
Send us plates of fixed & stained cells, and we'll send you data.

Questions? Leave us a message at https://phenovista.com/contact-us