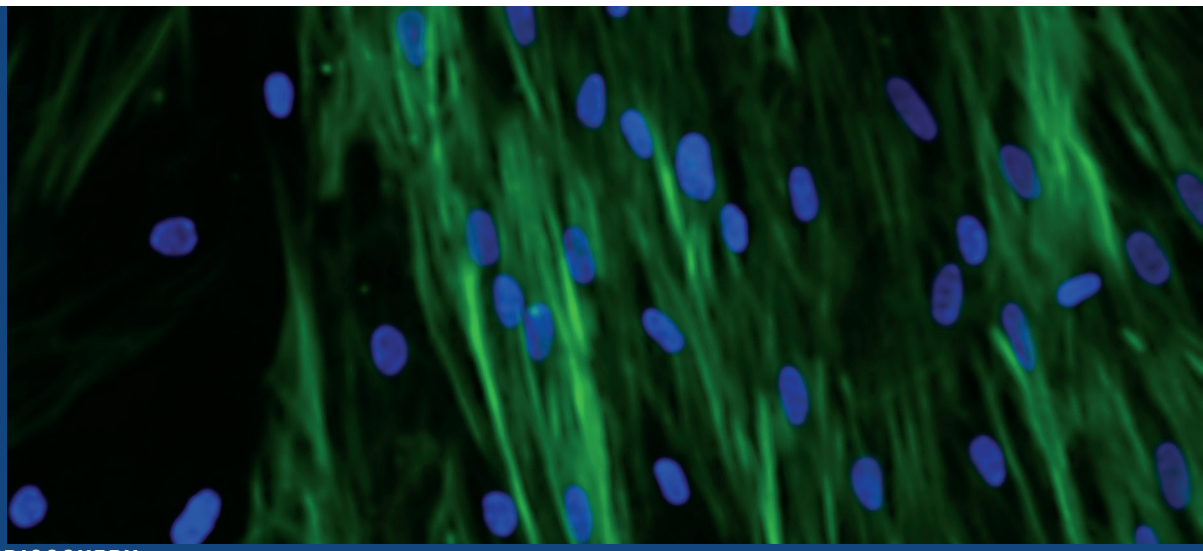
charles river

Overview

Fibrosis results from misregulated complex pathways involving multiple cell types such as epithelial cells and fibroblasts. We've developed an optimized, off-the-shelf panel of *in vitro* fibrosis assays using our own patient-derived donor cells to assess the translational potential of small molecules as novel therapies.



DISCOVERY

Off-the-Shelf Assay:

Complex Biology *In Vitro* Assays: Fibrosis

Epithelial-to-Mesenchymal Transition (EMT) Assay

Click to learn more

Epithelial-to-Mesenchymal Transition (EMT) Assay in Human Lung Cells Derived from Idiopathic Pulmonary Fibrosis (IPF) Patients and Healthy Donors

Epithelial mesenchymal transition (EMT) was proposed as a mechanism for collagen overproduction and increased number of fibroblast-like cells leading to fibrosis. Several studies demonstrated that EMT occurs in human lung epithelial cell lines and primary human bronchial epithelial cells (HBECs) upon exposure to TGF-β1. In cells that have undergone EMT, increased synthesis of fibronectin (FN1, an important component of the extracellular matrix) is a relevant readout for lung fibrosis. A validated, robust TGF-β1 induced EMT cell-based assay has been developed using primary human bronchial epithelial cells that are derived from IPF patients and healthy donors, to evaluate therapeutic candidates with various modes of action in this disease area. Trans-differentiation of EMT is measured with biomarker fibronectin (FN1) via high content analysis (HCA).

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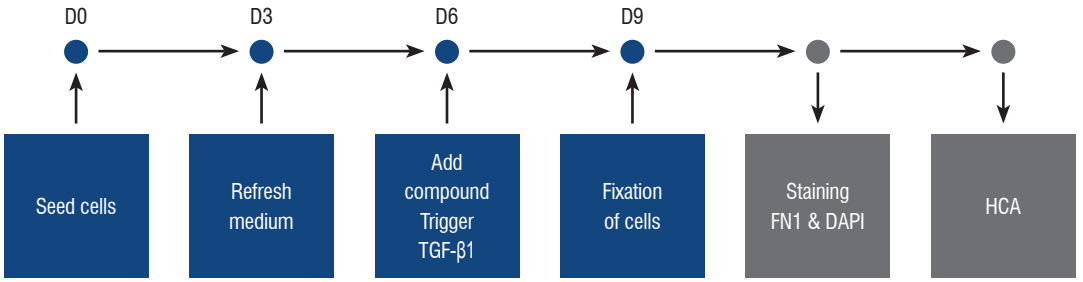
EMT Assay Principle

Lung-derived primary human bronchial epithelial cells are seeded, then refreshed in preparation for addition of small molecule compounds and the TGF-β1 trigger. After 3 days, the cells are fixed, then stained using DAPI and FN1 and imaged via HCA.



EMT Assay Setup

Setup:

- Cells → HBEC from IPF donors or healthy donors
- Seeding density → 2,500 cells/well in 96-well plates
- Trigger → 5 ng/mL TGF-β1
- Assay controls → 0.1% DMSO (negative control) and 1 µM SB525334 (positive control)
- Compounds → 8-point concentration response curves (in biological duplicate)
- Fix → 72 hours post-trigger
- Readout → FN1 and DAPI staining (high-content analysis)

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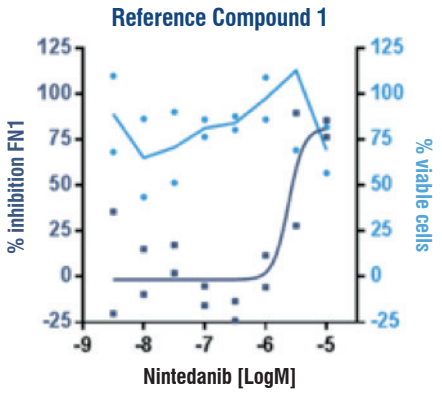
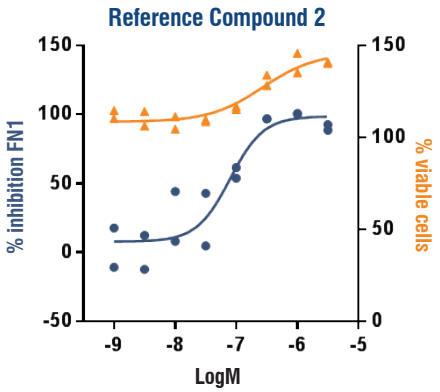
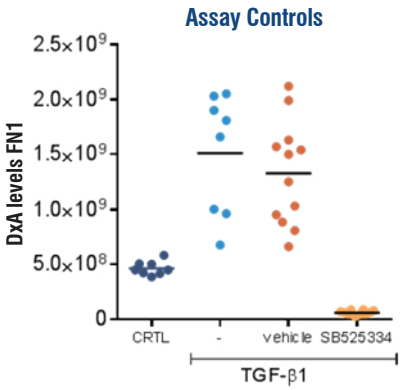
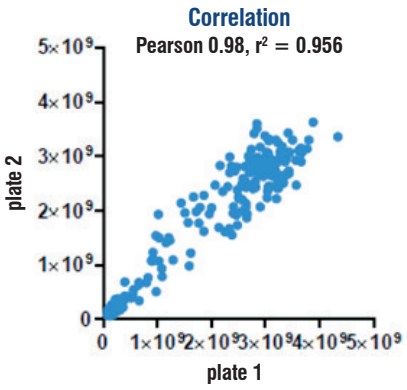
Assay Performance

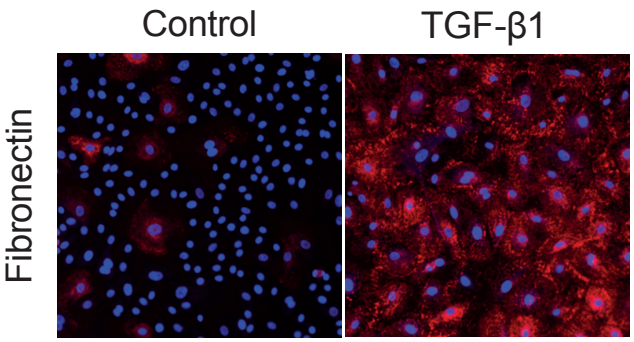
Representative concentration response data shown below from IPF patient-derived HBEC, 72 hours post TGF-β1 trigger.











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Summary

TGF-β1 consistently induced upregulation of the biomarker FN1, indicative for epithelial to mesenchymal transition (EMT). As a side-effect, TGF-β1 stimuli showed a slight effect on the number of nuclei. Among the several effects of TGF-β1 is regulation of the cell cycle in the G1 phase, through increased expression and/or stabilization of cyclin-dependent kinase (CDK) inhibitors (CKI), ultimately resulting in growth arrest of human airway epithelial cells. TGF-β1 trigger could be inhibited by treatment with an ALK-5, showing full inhibition of FN1 regardless the presence of TGF-β1. As a result, the TGF-β1-induced cell cycle blockade was reversed in a concentration-dependent fashion, as was shown by increased number of nuclei. IC50 values were consistent between different donors (not shown), and strong Pearson correlation denotes consistency between biological replicates. Using these fibrosis assays, the trans-differentiation of EMT can be monitored to evaluate therapeutic candidates.

Therapeutic candidates can be evaluated in 8-step CRC, using three different IPF patients/healthy donors in biological duplicate for their effect on FN1 modulation. In addition, potential cytotoxic side effects of the tested therapeutic candidate will be assessed by monitoring the loss of nuclei as a measure for cell death. Results will be provided as percentage inhibition (PIN values) and % viable cells.

For our clients' scheduling convenience, we perform EMT assays on a routine bi-monthly basis. Results are issued within 6–8 weeks of receipt due date.

EMT Assay – Compound Receipt Due Dates

May 2020	August 2020	November 2020
4	14	13

Assay Reference Codes

Epithelial-to-Mesenchymal Transition (EMT) Assay – IPF Human-Derived Donor Cells
Assay reference code: OTS103-EMT-LUNG-IPF

Epithelial-to-Mesenchymal Transition (EMT) Assay – Healthy Human-Derived Donor Cells
Assay reference code: OTS104-EMT-LUNG-HEALTHY

Complementary Fibrosis Assays

Fibroblast-to-Myofibroblast Transition (FMT) Assay

M1 Polarization Assay

M2 Polarization Assay