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Screening Anti-Fibrotic Drugs Using Primary Human Pulmonary Fibroblasts: A Cell Migration and Gene Expression Assay



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BACKGROUND

Pulmonary fibrosis is a progressive, restrictive lung disease characterized by scarring and stiffening of lung tissue, ultimately leading to respiratory failure. This condition is driven by the excessive proliferation and migration of fibroblasts, specialized cells residing within the lung. These activated fibroblasts deposit excessive amounts of extracellular matrix (ECM) proteins, fundamentally disrupting the lung's architecture and hindering its ability to function properly. Therefore, targeting fibroblast proliferation and associated ECM deposition represents a logical therapeutic strategy for both delaying disease onset and, potentially, reversal of disease pathology. In this study, we utilized primary human pulmonary fibroblasts to develop a functional screening assay for identifying novel anti-fibrotic drugs. Pirfenidone and nintedanib, the only Food and Drug Administration (FDA)-approved antifibrotic drugs for the treatment of idiopathic pulmonary fibrosis were used to validate the assay.

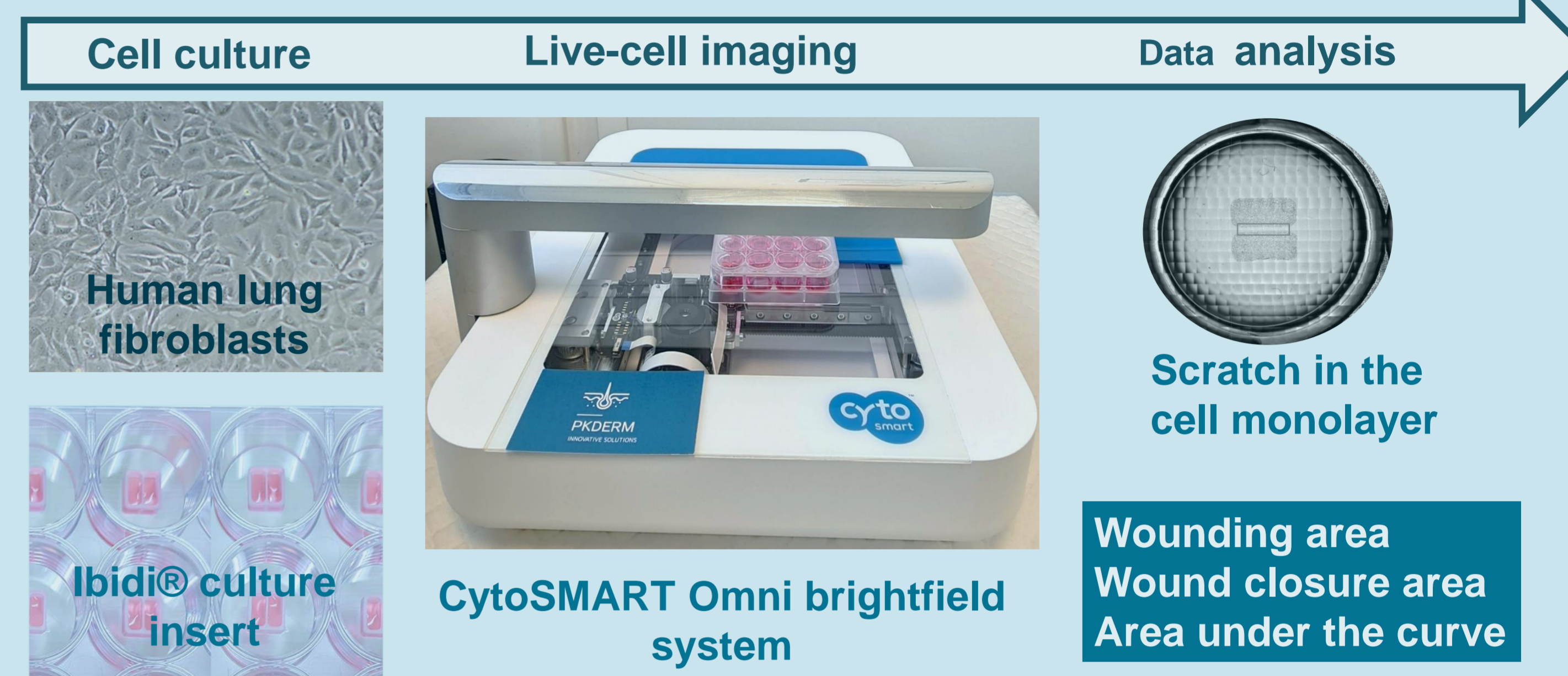
METHODS

Morphological response: Primary human pulmonary fibroblasts (HPFs) were treated with TGF- β alone or in combination with nintedanib. Untreated cells served as control. After 24 hours, HPFs were fixed and permeabilized for fluorescence microscopy. F-actin was stained with phalloidin to assess the cell morphology, while DAPI was used to stain the cell nuclei.

Fibrotic Response: HPFs were treated with either TGF- β alone or in combination with nintedanib. Untreated cells served as control. After 24 hours, mRNA expression of fibrosis markers, LRRC15, COL1A1, COL3A1, FN1, ACTA2, and MMP12, was measured using RT-qPCR with TaqMan[®] technologies.

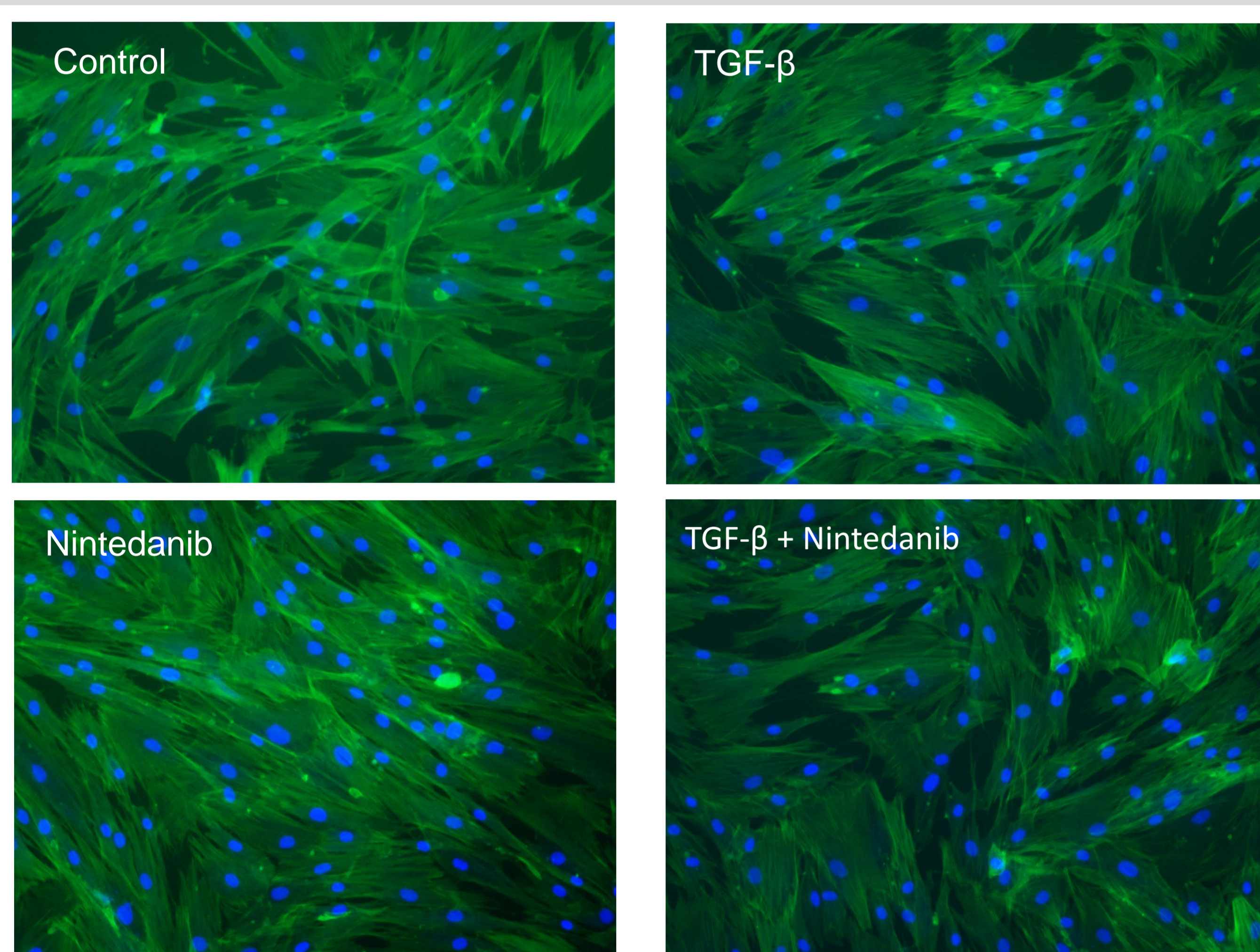
Wound healing assay: HPFs were seeded on Ibidi[®] 2-well culture inserts to create standardized 500 μ m wounds. The inserts were removed at confluency, and cells were exposed to 1 μ M Nintedanib, 10 μ M Pirfenidone, or 0.1 μ M Taxol. Untreated cells served as control. Live-cell imaging using the CytoSMART Omni brightfield system within a CO₂ incubator allowed for quantification of cell migration dynamics into the wounded area.

Workflow of standardized wound healing assay

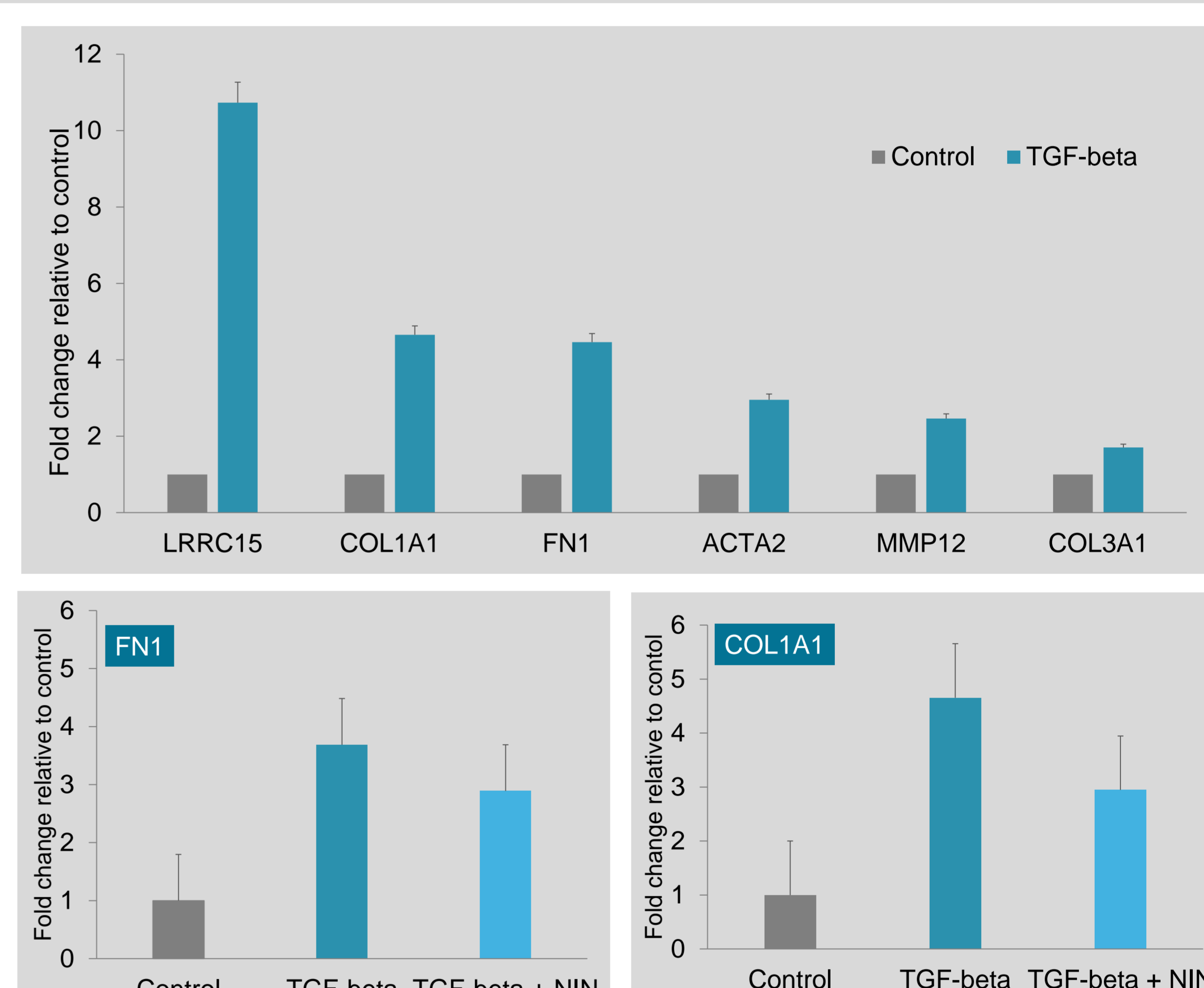


RESULTS

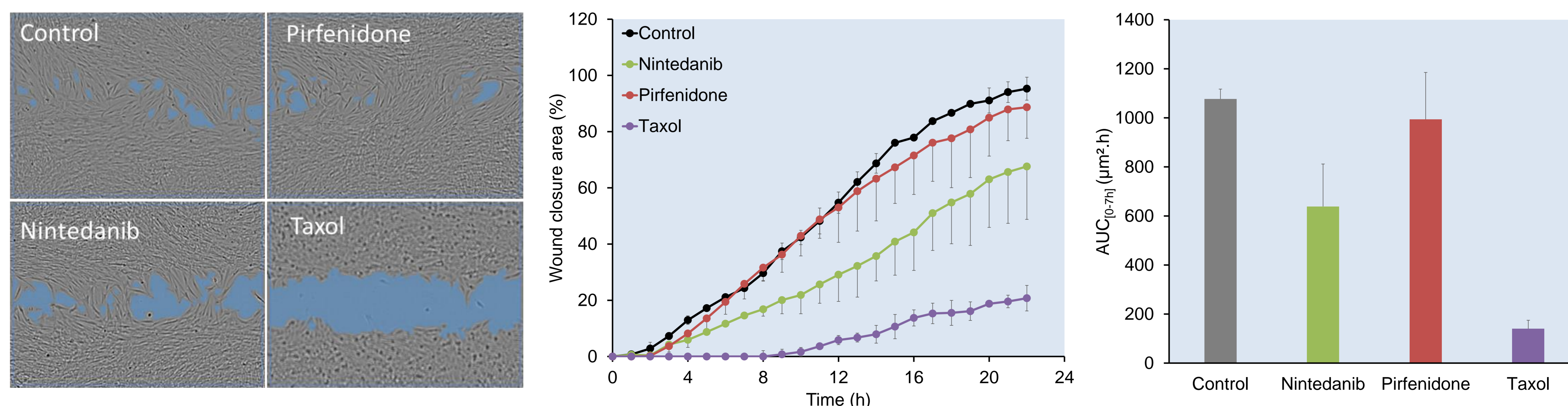
Effect of Nintedanib on Actin fibers



Expression of fibrosis markers in human lung fibroblasts



Effects of Nintedanib and Pirfenidone on Wound Healing and Cell Migration



CONCLUSIONS

- TGF- β treatment markedly increased the mRNA expression of fibrotic markers FN1, COL1A1, and LRRC15, indicating activation of fibrosis at the gene expression level. Importantly, despite these changes, no alterations in cell morphology were observed.
- Nintedanib treatment counteracted the profibrotic effects of TGF- β . It downregulated the expression of FN1 and COL1A1, and also markedly decreased cell migration, a key feature of fibrosis. Pirfenidone, on the other hand, did not affect cell migration.

This study established a new functional assay that combines cell migration and gene expression analysis. This approach offers a simple and versatile tool for screening potential antifibrotic drugs for pulmonary fibrosis.