

Comparison of the FMT assay with the Cell Painting approach in healthy patient derived fibroblasts.

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IPF, a fatal chronic disease of the lung, is caused by an inflammatory response that results in aberrant fibroblast activation, the deposition of excessive extracellular matrix, and progressive fibrotic remodelling of the lungs¹. Though the exact pathophysiological mechanisms of IPF remain unknown, myofibroblasts are considered to play a major role in the pathology of IPF. Transforming growth factor β (TGF- β 1), a well-established fibrogenic mediator, induces FMT. In cells undergoing FMT, increased expression of α -smooth muscle actin (α SMA) is observed. In vitro, increased α SMA expression positively correlates with contraction of myofibroblast populated collagen gels, indicating that α SMA is a strong marker of myofibroblast differentiation, and thus a relevant readout for lung fibrosis.

A validated, robust TGF- β 1-induced FMT cell-based imaging assay in normal human lung fibroblasts (NHLF) with α SMA expression as a readout exists in-house to support multiple R&I IPF projects. As an inhibitor control, the highly specific Alk5 inhibitor SB-525334 is used to block TGF- β 1 signalling².

Cell Painting is a non-target high content imaging assay to identify morphological profiles of different cell types with diverse compound treatments³. In this study the compounds used for the FMT assay have been simultaneously analysed via cell painting and the data has been compared. The Cell Painting results correlate well with the FMT data and could potentially be used as an alternative to the FMT assay. Especially while the assay is one day shorter.

Additionally, three patient-derived IPF cell lines have been tested with 27 compounds to see if it is possible to reverse the diseased phenotype to the healthy one, with or without TGF- β 1 treatment. All three donors showed a different response profile when compared to each other, which made it difficult to distinguish if there are compounds being especially responsive to TGF- β 1 treatment or to diseased cells, but not healthy ones. This needs further investigation.

Keywords:

alphaSMA, FMT, Cell Painting, HTS

Reference:

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