CALCIUM MOBILIZATION AND FIBROBLAST PROLIFERATION: A PLATFORM TO SCREEN ANTI-FIBROTIC COMPOUNDS IN PRIMARY HUMAN FIBROBLASTS

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Background: Tissue fibrosis, including pulmonary, hepatic and cardiac fibrosis, is an important stage in the development of many diseases. It can lead to structural damage and dysfunction and even severe carcinogenesis or death. At present, the molecular mechanism of tissue fibrosis has not yet been fully elucidated, but many studies have pointed to a dysregulation of fibroblasts' proliferation, migration and differentiation as a central event in the establishment of fibrosis. Given the lack of effective treatment for many fibrotic conditions, the establishment of in vitro cellular models for testing pharmacological tools and dissect pathways would be of paramount importance in drug discovery programs. In this study, we established in primary human fibroblasts high-throughput compatible read-outs. i.e. measurement of $[Ca^{2+}]i$ and proliferation, whose impairment induces fibrosis development (1).

Methods: Using FLIPR Tetra (Fluorescent Imaging Plate Reader), $[Ca^{2+}]_i$ in human lung fibroblasts was measured in 384well plates following the stimulation with selected agonists/activators at receptors/Ca^{2+} ion channels, with or without pre-treatment with selective antagonists/blockers. The proliferation of lung fibroblasts was measured by incorporation of EdU in newly synthesized DNA, and fluorescence imaging-based detection of Edu with the Click-iT® detection reagent.

Results: A panel of GPCR agonists (including bradykinin, sphingosine-1-phosphate, lysophosphatidic acid, carbachol and substance P) and ion channels activators (such as capsaicin and AITC, acting at transient receptor potential channels) were tested for their ability to elicit increase in $[Ca^{2+}]_i$ in human lung fibroblasts. For some of these pharmacological agents, the effect on proliferation of lung fibroblasts was analyzed. A relationship between $[Ca^{2+}]_i$ mobilization and induction of cell proliferation was observed for the GPCRs studied. To further characterize the response, selective pharmacological inhibitors were used. Lysophosphatidic acid, a known mediator of fibrosis, has been used to validate the technological platform. Lysophosphatidic acid induced calcium mobilization and proliferation with a pEC_{50} value of 8.27 ± 0.16(N=3) and 4.99 ± 0.08(N=3), respectively. Using selective antagonists we demonstrated that both effects are mediated by lysophosphatidic acid receptor subtypes 1/3.

Conclusions: Our study identifies a link between $[Ca^{2+}]_i$ increase and induction of proliferation in a primary culture of lung fibroblasts. This platform can be adapted to fibroblasts from different tissues to model other fibrotic diseases. Furthermore, the availability of patient-derived fibroblasts will allow the development of even more disease-relevant in vitro models.

References

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