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RNA-seq transcriptome analysis of chronic lung injured epithelial cells reveals similar features of IPF lung epithelium.

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Background: Chronic injury to alveolar epithelial cells and aberrant activation of multiple signaling pathways have been recognized as a major hallmark in the IPF pathogenesis. Identification of genes whose expression is most significant in IPF can allow us to study the multiple protein networks that are disrupted in these patients. The major shortcoming of conventional *in vitro* models is that do not resemble the insults induced by chronic repetitive exposures seen in elderly IPF patients. In this study, we sought to develop an *in vitro* chronic injury model to mimic the phenotypic and functional characteristics of the IPF lung epithelium.

Methods: We created an innovative *in vitro* model of chronic bleomycin exposure using murine alveolar type-II epithelial cells (MLE-12). On day 0, MLE-12 cells were treated with 10 ug/ml bleomycin for 24h. Day 2, bleomycin was removed and fresh media was added. Day 4, cells were treated again with 10 ug/ml bleomycin. Day 5, bleomycin was subsequently removed and cells were collected at day 7. RNA sequencing will performed on total RNA isolated from control and chronic injured MLE12 cells on the Illumina Nova Seq and Hiseq platform according to the Illumina Tru-seq protocol (Novogene, USA).

Results: We identified that a total of 8,484 genes with different expression variations between the exposed group and the control group. GO enrichment analysis showed top Go terms for epithelial cell migration, regulation of epithelial proliferation, response to interferon beta and Wnt pathway. KEGG pathway analysis demonstrated up-regulation of cell adhesion molecules, p53 pathway, MAPK signaling pathway, TNF-signaling and PI3K-AKT pathway. Furthermore, Reactome revealed that overrepresented pathways were degradation of the extracellular matrix, fatty acid metabolism and glycosphingolipid metabolism. Moreover, using our 7-day injury protocol, we found that cells exhibited nearly all the features of senescence cells, including increase in β-galactosidase staining, an upregulation in the cycle arrest proteins p-p53, p21, mitochondrial dysfunction, excessive ROS production, impaired autophagy and proteostasis alteration.

Conclusion: Hallmarks of idiopathic pulmonary fibrosis and aging were evidence in our chronic injury model. We believe this model will be ideally suited for use in uncovering novel insights into the gene expression and cellular pathways activation of the IPF lung epithelium and performing high-throughput screening of pharmaceutical compounds.

This abstract is funded by NIH, Grant No: R01 HL136833-01A1

Conflict of interest: No conflict of interest.