Effects of Particulate Matter on an Animal Model of Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease characterized by excessive extracellular matrix deposition, alveolar epithelial cell (AEC) hyperplasia, and honeycomb cyst formation, leading to irreversible respiratory decline. Exposure to particulate matter (PM) is associated with the development and worsening of idiopathic pulmonary fibrosis (IPF). Prolonged exposure to particulate matter has been epidemiologically associated with the development and progression of idiopathic pulmonary fibrosis (IPF). However, direct evidence establishing a causal relationship remains unclear, necessitating further research in this area. Investigating this relationship is crucial not only to elucidate the underlying mechanisms but also to identify biomarkers associated with disease progression and prognosis.

The bleomycin (BLM)-induced lung fibrosis model is the most commonly used preclinical model for studying pulmonary fibrosis. Typically, a single intratracheal dose of BLM is administered to young mice, leading to an acute inflammatory response followed by transient fibrosis that resolves over time. However, this model has significant limitations. First, the fibrosis observed in the single-dose BLM model lacks the progressive and irreversible nature of UIP seen in human IPF. Second, young mice, which are frequently used in preclinical studies, do not accurately represent the aging-related susceptibility and cellular senescence seen in IPF patients, who are typically older adults. The aged lung microenvironment is associated with increased oxidative stress, mitochondrial dysfunction, and impaired AEC repair mechanisms, which are not adequately captured in young-mouse models. Third, while fibroblast activation and extracellular matrix accumulation are present in the single-dose BLM model, key UIP features such as honeycombing, bronchiolar metaplasia, and severe AEC apoptosis with persistent senescence are either absent or not well-developed. Finally, due to its self-limiting nature, the conventional BLM model does not provide an adequate platform for studying chronic environmental exposures, such as particulate matter (PM), which may exacerbate fibrosis progression in susceptible individuals.

To overcome these limitations, we developed a repetitive BLM-induced model (RBM) in aged mice that better mimics UIP-like lung fibrosis. Repeated low doses of bleomycin were administered to achieve sustained fibrotic remodeling, and its similarity to IPF was validated by comparison with publicly available transcriptomic datasets of the single-dose BLM model and IPF, further evaluating its merits through transcriptome analysis. Additionally, this chronic fibrosis model was utilized to investigate the interplay between fibrosis and PM2.5 exposure in IPF progression using transcriptomic analysis.

Aged mice (over 22 weeks) received intratracheal BLM (0.001 unit/g) every two weeks for 16 weeks. Then PM 2.5 (100μg/m^3, 4h/day, 5day/week) was administered using a nose -only exposure system in BLM treated mice for over 3 months. Lung tissues were analyzed for fibrosis severity, collagen deposition, AEC senescence, apoptosis, and extracellular matrix remodeling. RNA sequencing was performed to identify differentially expressed genes (DEGs) by comparing the RBM model with the single-dose BLM model (Gene Expression Omnibus Series; GSE218997) and IPF patient datasets (GSE47460), and only Genes present in the human genome were analyzed. To assess the similarity of genes to IPF, core fibrosis-related biological processes, including fibrosis, ECM remodeling, inflammatory signaling, hypoxia response, and cell senescence, along with their key genes, were extracted using Gene Ontology (GO) analysis (DAVID), the KEGG pathway database, and literature-based curation, and their key gene distribution patterns were compared among the RBM model, the single-dose BLM model, and IPF. In the PM2.5 nose-only inhalation RBM model, mice were exposed to PM2.5 from Week 8 to Week 20 using a nose-only inhalation exposure system to evaluate its effects on transcriptome levels using RNA sequencing.

RBM led to severe fibrosis, extensive collagen deposition, and UIP-like structural changes, including AEC hyperplasia, bronchiolar metaplasia, and cystic dilatation. Compared to single-dose BLM models, RBM showed more persistent fibrosis with reduced inflammatory infiltration and increased apoptotic AECs. Senescence markers such as P-16 and p-Rb were significantly upregulated, consistent with aging-associated fibrotic mechanisms. RNA sequencing revealed 1,329 downregulated genes and 1,116 upregulated genes, with 165 genes uniquely overlapping between the RBM model and IPF patients. Gene Ontology (GO) enrichment analysis of these 165 genes identified 85 ontologies, among which the highlighted GO network was associated with fibrosis, including the extracellular matrix and collagen-containing extracellular matrix. Comparisons of the core genes involved in five fibrosis-related

biological processes revealed that the RBM model exhibited a gene distribution pattern more similar to IPF than the single-dose BLM model. This result means that the RBM model serves as a more suitable disease model for IPF, providing a more accurate representation of its molecular characteristics. When the RBM were chronically exposed to PM2.5, the mice showed aggravated lung fibrosis and inflammation compared to those exposed to RBM alone.

In RNA sequencing analysis of PM2.5 inhalation-only nose RBM models compared with RBM, 380 genes were differentially expressed, including 203 downregulated and 177 upregulated genes. Ontology analysis of these genes revealed enrichment in processes such as the mitotic cell cycle and DNA metabolic processes. Text mining analysis based on literature revealed that among the 380 DEGs, genes related to apoptosis were the most prevalent, accounting for 52.7% of upregulated genes and 69.5% of downregulated genes. The protein–protein interaction network analysis together with text-mining identified 12 key hub genes including BRCA1, CDK1, AURKB, CDC20, PLK1, CCNB1, AURKA, RAD51, FOXM1, FOS, CRY2, PER2 associated with includes cell cycle regulation and DNA repair.

Our findings suggest that repetitive BLM administration in aged mice better replicates the pathological features of UIP, overcoming the limitations of single-dose models in young mice. This model provides a more physiologically relevant platform for studying IPF pathogenesis and assessing environmental factors such as particulate matter (PM) in fibrotic lung diseases.

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