Effects of Lung Fibroblasts on Alveolar Epithelial Cells Under Normal and Smoke Conditions

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Rationale Cigarette smoke is a main cause of chronic obstructive pulmonary disease (COPD), contributing to alveolar epithelial cells (AECs) damage, abnormal injury repair and emphysema. Lung fibroblasts (LFs) could secrete cytokines and produce extracellular matrix (ECM), which plays an important role in injury repair and constitution of stem cell nests. Therefore, we aim to investigate the effects of soluble and insoluble products secreted by LFs on AECs under normal and smoke conditions in vitro.

Methods C57BL/6 mouse primary LFs were isolated by the explant culture method. AECs had the following two sources: MLE12 cell line and C57BL/6 mouse primary AECs isolated by the enzyme digestion method. Cigarette smoke condensate (CSC) was purchased from Murty Pharmaceuticals. Co-culture using a 0.4 µm Transwell chamber and the decellularized ECM scaffold (de-ECM) were used to assess the effects of soluble and insoluble products of LFs on AECs, respectively. Alamar Blue was used to examine cell proliferation, TUNEL stain for apoptosis, and ELISA for IL-1α and IL-1β.

Results MLE12 was stimulated with gradient concentration of CSC, and its proliferation rate reduced at 50 and 100 ug/ml by 17% and 33% at 48 h, respectively. Therefore, 50 and 100 ug/ml were subsequently used as the optimal concentration. When MLE12 was co-cultured with LFs, the proliferation rate increased by about 15% under normal conditions. Co-culture also promoted MLE12 proliferation in CSC environment (48h), indicating that primary LFs produced protective soluble factors for AECs. When HBE16 and MLE12 were respectively cultured on the de-ECM produced by normal mouse primary LFs, HBE16 did not proliferate, but MLE12 could proliferate with reduced rate by 20% compared to that of culture on a plastic flask. In the CSC environment (48 h), de-ECM also reduced proliferation rate of MLE12. When primary AECs were cultured on the de-ECM produced by LFs chronically stimulated with CSC (25, 50 or 100 ug/ml for 10 days), the alveolar cavity-like structure only appeared in the gelatin group after 24 h, but not in the de-ECM group. The apoptosis rate of AECs in the 25 ug/ml de-ECM group was lower than that in the other groups. Additionally, AECs on the CSC-associated de-ECM did not produce IL-1α and IL-1β.

Conclusions Primary LFs could produce soluble factors to promote proliferation of AECs, but the secreted insoluble ECM inhibits their proliferation and the formation of alveolar cavity-like structure. The same phenomenon could also be observed under the smoke condition.

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