Ultrastructural characterization of bronchoalveolar lavage (BAL) fluid cells in usual interstitial pneumonia and sarcoidosis

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Characteristic feature for usual interstitial pneumonia (UIP) is occurrence of transformed fibroblasts i.e. myofibroblasts within interstitium of lung at the alveolar level [1]. Myofibroblasts have been observed to be expressed also in sarcoidosis [2]. Myofibroblasts in lung tissue typically exhibit a cell surface structure known as fibronexus junction associated with cell-cell and cell-matrix interactions [3].

The aim of this study was to examine by electron microscopy and immunoelectron microscopy the ultrastructural features of BAL-derived myofibroblasts with special emphasis on the structure of fibronexus in two differently behaving fibrotic lung disorders such as UIP and sarcoidosis.

Cells from BAL fluid from patients with UIP and sarcoidosis were cultured and studied by transmission electron microscopy for detecting the ultrastructural features of myofibroblasts, and by immunoelectron microscopy for analyzing the ultrastructural localization of fibronectin and alpha-smooth muscle actin (α -SMA) which have observed to be the main components of fibronexus. The amount of fibronectin and α -SMA-positive cells were investigated also by immunohistochemistry.

Results from transmission electron and immunoelectron microscopy showed ultrastructural features of myofibroblasts i.e. α -SMA positive bundles in the cytoplasm of cells and extracellular fibronectin-containing structures on the surface of the cell, thus forming fibronexus-structures. Both fibroblasts and myofibroblasts were derived from BAL fluid. The labels for α -SMA and fibronectin were distinctly expressed in myofibroblasts in UIP and sarcoidosis. More myofibroblasts were derived from the BAL fluid from the patients with UIP than the patients with sarcoidois.

We conclude that cells cultured from BAL fluid of patients with UIP and sarcoidosis showed divergent amount of myofibroblasts with variable ultrastructural morphology which phenomenon could be linked with the different clinical behavior of these lung diseases.

References

1. Kuhn C & McDonald JA. The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. Am J Pathol. **138** (1991) p1257–1265.

- Fireman E, Shahar I, Shoval S, Messer G, Dvash S & Grief J. Morphological and biochemical properties of alveolar fibroblasts in interstitial lung diseases. Lung 179 (2001) p105-117
- Singer II, Kawka DW, Kazazis DM & Clark RAF. In vivo co-distribution of fibronectin and actin fibers in granulation tissue: Immunofluorescence and electron microscope studies of the fibronexus at the myofibroblast surface. J Cell Biol 98 (1984) p2091-2106
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Figure 1. TEM images from BAL cell lines derived from UIP patients. Image A shows the fibronexus structure (FN), fibronectin bundle is located at the surface of the cell. Image B shows the double immunolabeling of α -SMA (white arrow) along intracytoplasmic thin filaments. Fibronectin (black arrow) is located on the extracellular site of plasma membrane.