Identification of Human Lung Lymphatic Endothelial Cells as Potential Targets of Inflammatory and Neoplastic Diseases

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Background. The lymphatic system is composed of a vascular network with blind-end, regulating tissue fluid homeostasis, immune cell trafficking and dietary fats absorption. The lymphatic system also plays a pivotal role in a variety of diverse pathologic conditions such as inflammation, wound healing and tumour metastasis.
Pulmonary lymphatics are required for efficient respiration, mainly attributable to alveolar clearance in which their contribution is even greater than that exerted by blood vessels. Patients affected by idiopathic pulmonary fibrosis show severe damage of subpleural and interlobular lymphatics, suggesting alveolar clearance as a new putative pathogenetic mechanism.
Cell culture models may be crucial to study the pathophysiologic role of lung microvascular endothelium. Most of our knowledge is derived from studies on cultured human umbilical cord vein endothelial cells (HUVECs) due to their accessibility and reproducible isolation. However, these cells do not represent a model of the adult human microcirculation. Moreover, a high heterogeneity among endothelial cells from various districts has been demonstrated, with greater difference on the lymphatic circulatory system.
Methods. We describe a simple and not-expensive method, requiring minimal equipment and accessories, to harvest, isolate and expand lymphatic and blood endothelial cells from human lung (Lu-LECs and Lu-BECs, respectively). Specifically, samples from lung cancer (T) and spared distal lung (D) of 28 patients undergoing lobectomy or pneumonectomy, were processed. We used a two-step purification tool based on sorting with monoclonal antibody to CD31- and podoplanin coated paramagnetic beads to obtain a pure population of Lu-LEC. Immunohistochemical analysis was performed on harvested pulmonary tissues to assess the presence or absence of neoplastic cells and to identify blood and lymphatic vasculature.
Results. The purity of cultured endothelial cells was ascertained by morphologic criteria, immunocytochemistry, flow cytometry and functional assays. T and D Lu-LECs and Lu-BECs were positive for CD31. Moreover, to define the lymphatic phenotype, we examined the specific markers podoplanin, LYVE-1 and Prox-1. No immunophenotypic differences between D and T endothelial cells were detected by FACS. These cells were characterized *in vitro* for the ability to express several receptor tyrosine kinases (RTKs) implicated in cell survival and proliferation and in the development and progression of cancer. Cultured blood and lymphatic endothelial cells consistently express VEGFR-2, VEGFR-3, PDGFR-beta, c-met, and IGF-1R. Moreover, FGFR-1 and EGFR-1 were present in a large fraction of T and D Lu-LEC.
Conclusion. We have been able to obtain 28 primary lines of LECs from the human lung. These cells may represent an important tool for *in vitro* studies on lymphatic biology, lympho-angiogenesis, wound healing, anti-cancer therapy and interaction with microbial agents. Organ-specific endothelial cells are essential to elucidate signalling pathways involved in the pathogenetic mechanisms of inflammatory and neoplastic lung diseases and to provide novel approaches to reach the goal of a true personalized therapy.