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**Title:** Ultrastructural and functional properties of cultured lung cancer-associated myofibroblasts

Dr. Siri 1034 Lehtonen siri.lehtonen@oulu.fi<sup>1,2</sup>, Ms. Henna 1035 Karvonen henna.karvonen@oulu.fi<sup>2,3</sup>, Dr. Raija 1036 Sormunen raija.sormunen@oulu.fi<sup>4,5</sup>, Dr. Elisa 1037 Lappi-Blanco LappiElisa.Lappi-Blanco@ppshp.fi MD<sup>5</sup>, Prof. Magnus 1038 Sköld Magnus.Skold@ki.se MD<sup>6</sup> and Prof. Riitta 1039 Kaarteenaho riitta.kaarteenaho@oulu.fi MD<sup>2,7</sup>. <sup>1</sup> Department of Anatomy and Cell Biology, Institute of Biomedicine, University of Oulu, Oulu, Finland ; <sup>2</sup> Respiratory Research Unit and Clinical Research Center, Oulu University Hospital, Oulu, Finland ; <sup>3</sup> Department of Internal Medicine / Respiratory Research Unit, Institute of Clinical Medicine, University of Oulu, Oulu, Finland ; <sup>4</sup> Biocenter Oulu, University of Oulu, Oulu, Finland ; <sup>5</sup> Department of Pathology, Institute of Diagnostics, University of Oulu and Oulu University Hospital, Oulu, Finland ; <sup>6</sup> Department of Medicine, Division of Respiratory Medicine, Karolinska Institutet, Stockholm, Sweden and <sup>7</sup> Unit of Medicine and Clinical Research, and Center for Medicine and Clinical Research, Pulmonary Division, University of Eastern Finland, and Division of Respiratory Medicine, Kuopio University Hospital, Kuopio, Finland .

**Body:** Cancer-associated stromal cells are important regulators of tumor progression. Our aim was to culture and characterize these cells. Cells were cultured from 13 adeno-, 6 squamous cell and 2 other carcinomas. For comparison, the cells were cultured from tumor-free lung tissue of the same patients (4 non-smokers, 9 smokers and 8 smokers with COPD). The cultured cells were analysed by immunocytochemistry, transmission electron microscopy (TEM), immunoelectron microscopy (IEM), Western and gel contraction assay. Cultured cells from both cancer and tumor-free lung were mixtures of fibroblasts and myofibroblasts. Cancer associated cells contained more alpha smooth muscle actin ( $\alpha$ SMA) ( $p=0.006$ ) and cancer associated myofibroblasts exhibited more  $\alpha$ SMA labeling by IEM ( $p=0.01$ ) than the cells cultured from tumor-free lung (fig 1). Amount of  $\alpha$ SMA was lower in smokers' cells cultured both from tumor and tumor-free lung than those from non-smokers (fig 1). Cells from lung cancers and smokers with COPD were more contractive than those from non-involved lung. Extracellular component of fibronexus by TEM associated with the contraction capacity of the cells.

Our study shows that cultured cancer-associated myofibroblasts differ both structurally and functionally from cells of non-involved lung and this difference is maintained to some extent during prolonged cell culture. Smoking and COPD affected also the properties of the cells.