Immune/vascular endothelial cell crosstalk drives pathogenic remodelling

Thomas Lins[1]***, Elisabeth Fliesser***[1], ***Anna Birnhuber***[1,2]***, Katharina Jandl***[1,3] ***and Grazyna Kwapiszewska***[1,2,4]

[1]*Ludwig Boltzmann Institute for Lung Vascular Research Graz, Graz, Austria;Graz, Austria;*
[2]*Otto Loewi Research Center, Division of Physiology, Medical University of Graz, Graz, Austria;*
[3]*Otto Loewi Research Center, Division of Pharmacology, Medical University of Graz, Graz, Austria;*
[4]*Institute for Lung Health (ILH), Justus Liebig University, Giessen, Germany*

Background and research objective

Systemic Sclerosis (SSc) is a severe chronic disease with a 50% chance of the lung being directly involved; this can manifest as either pulmonary fibrosis, pulmonary arterial hypertension, or both. It is also accompanied by aberrant immune activation with autoimmunity and vascular abnormalities including the loss of microvasculature.

The Fos-related antigen 2 transgenic (Fra2 TG) mouse line has been established as a functional model for SSc associated pulmonary fibrosis, since it shows the same disease hallmarks. Furthermore, this model suggests a direct involvement of the pulmonary endothelium and the immune cell compartment.

Our aim is to gain a deeper understanding of the exact mechanisms that are involved in this endothelial cell (EC) – immune cell crosstalk.

Methods

We have investigated changes in the lung of Fra2 TG mice at three different time-points (8, 16 and 19 weeks) representing early-onset/mid-stage/end-stage disease. Following methodologies have been applied: lung function measurements, hemodynamic measurements, immunofluorescence staining with tissue clearing, electron microscopy and RNA bulk sequencing of CD31+ /CD45- sorted ECs.

Results

Using electron microscopy, we investigated whether ultrastructural changes were present during early and mid-stage disease development (8 and 16 weeks, respectively) in Fra2 TG mice. In both time points, we observed an increased thickness of the single-layered endothelial cell layer in pulmonary arteries associated with the presence of immune cells; changes were even more pronounced in the later time point (16 weeks).

To investigate the mechanistical changes in EC at early disease onset (8 weeks), we utilized bulk RNA-sequencing of CD31+ and CD45- sorted endothelial cells from eight-week old Fra2 TG mice. The genes that are most dysregulated in the pulmonary endothelium are predominantly involved in extracellular matrix organization and processes directly linked with fibrosis, including mitosis, cell migration, cell motility and proliferation. We also observed changes in genes that are associated with very early mechanisms like developmental processes and morphogenesis. Futhermore, pathways that govern the creation of biologically active compounds like biosynthesis and chemokine ligand production were impacted.

Conclusion

Our findings support the conclusion that ECs have a prominent contribution in the early onset and development of the disease. In future, we will identify key genes that are involved in EC dysfunction in the Fra-2 mediated SSc model and will further investigate their role in disease pathogenesis.

The functional relevance of our findings will be assessed *in vitro* based on proliferation and apoptosis assays, barrier function analysis and spheroid sprouting on human microvascular endothelial cells.