Supplemental Methods

Sampling and preparation of fresh human lungs.

Since 2013, the lung research group of the Institute of Pathology at Hannover Medical School (MHH) provides with a 24h/7d pick-up and work-up service a well-established and unique infrastructure for fast processing of explanted human lungs, long-time storage, and biobanking. With about 130 lung transplantations annually, we provide the basis for comprehensive (histo) pathological and molecular investigations in human lung diseases. Here, the prompt and standardized processing of lung tissue within less than 30 minutes following explantation guarantees incorporation of daily routine work and systematic lung research. The lungs for this study were freshly sampled, followed by routine formalin-fixation and paraffin-embedding (FFPE), conventional light microscopy, µ-CT analysis, microvascular corrosion casting, SEM, and gene expression analysis.

Conventional light microscopy.

For (immuno) histochemical staining FFPE lung tissue samples directly adjacent to the samples used for Electron microscopy were cut to 2 µm thick slices. Histological staining, including Hematoxylin-Eosin (HE) and Elastica van Gieson (EvG), were performed using routine protocols. Immunohistochemical stainings were performed as follows: CD31 (DACO (No. M0823) at a dilution of 1:75), CK7 (DACO (No. M7018) at a dilution of 1:800) and podoplanin (Zytomed (No. MSK057) at a dilution of 1:25) and detected using DAB detection kit (Ventana, No.760-500) or Phosphatase Red detection kit (Ventana 760-501). Conventional microscopy was then performed at a routine diagnostic light microscope.

Vascular Corrosion Casting and scanning electron microscopy.

Briefly, at the time of tissue collection, the afferent vessels were cannulated with an olive-tipped cannula. The vasculature was flushed with saline (at body temperature) followed by glutaraldehyde fixation solution (2.5%, pH 7.4, Sigma Aldrich, Munich, Germany). Fixation was followed by injection of prepolymerized PU4ii resin (VasQtec, Zurich, Switzerland) mixed with a hardener (40% solvent) and blue dye as

casting medium. After curing of the resin, the lung tissue was macerated in 10% KOH (Fluka, Neu-Ulm, Germany) at 40°C for 2 to 3 days. Specimens were then rinsed with water and frozen in distilled water. The casts were freeze-dried and sputtered with gold in an argon atmosphere and examined using a Philips ESEM XL-30 scanning electron microscope at 15 keV and 21 µA (Philips, Eindhoven, Netherlands).

3D morphometry.

For quantitative analyses, stereo-pairs with a tilt angle of 6° were collected from each lung explant using a eucentric specimen holder. The stereo-pairs were used for morphometry of parameters defining the architecture of the microvascular unit. The stereo pairs were color-coded and reconstructed as anaglyphic images. With the known tilt angle, calculations were carried out using macros defined by the KS 300 software (Kontron Electronics, Eching, Germany). Intussusceptive angiogenesis was identified via the occurrence of tiny holes with a diameter of 2-5 µm. The quantitation of these features was expressed as numerical density per vessel area (21,22).

Micro-computed tomography.

High-resolution µ-CT analyses were performed using a Bruker Skyscan 1176 µ-CT scanner. The paraffin-embedded specimen was scanned using the following settings: 50 kV tube potential, 0.5 mm aluminum filtration, 500 µA tube current, 0.18° rotation step over-rotation of 180° and a detector pixel size of 8.6 µm (spatial resolution), applying a frame averaging of 5. Volume image data (2667 slices of 3396x3396 pixels^2 each) was reconstructed with NRecon (v1.7.0.4: Bruker Biospin, Rheinstetten, Germany) and analyzed with CTVox: (v. 3.1.1: Bruker, Biospin). Volumetric analyses were conducted using CTAn (v.1.15.4: Bruker, Biospin).

Gene analysis.

Further analysis of the resulting log2 mRNA counts was performed using custom R code. A Shapiro-Wilks test was performed on all intra-group gene expressions which showed that the vast majority of expression data is normally distributed (>85%, α=0.05). In order to identify significant changes in mRNA expression, we performed t-

tests for pairwise comparisons as well as ANOVA for multigroup comparisons followed by correction for multiple testing via the Bonferroni method. All corrected p-values <0.05 were considered significant.

Immunohistochemical analysis

Selected target proteins were analyzed with regard to their compartment-specific expression assessed via immunohistochemistry. Collagens (Col1, Col3, Col4) and matrix metallo-proteinases (MMP2, MMP9 and MMP14) were analyzed using standard immunohistochemistry as described in [1] with some modifications. Briefly, antibodies were used as follows: Col1 (1:200 from Abcam (ab88147), Col3 (1:400 from Antibodies-online (ABIN214580)), Col4 (1:50 from Cell Marque (CIV22)), MMP2 (1:200 from Abcam, ab86607), MMP9 (1:100 from Abcam, ab76003) and MMP14 (1:100 from Abcam, ab51074). All antibodies were visualized using the (DAB detection kit, Roche)

Proliferation analysis

The roliferation rate of endothelial cells was determined via Immunohistochemistry using the OPAL 7 System as previously described [2] with some modifications. Briefly, CD34/31 positive cells were detected using the anti CD31 monoclonal antibody (1:100, Dako Agilent, Clone JC70A) and the CD34 monoclonal antibody (1:200 Leica, CL-L-End/10). Proliferation was analyzed using the anti-Ki-67 antibody (1:200 Thermo, Clone SP6).

Functional analysis.

The log2 gene expression data of each sample relative to the median gene expression in the controls was committed to the software as an individual observation. The subsequent molecule function activity prediction of IPA was executed with the default settings, thus using information from both in vivo and in vitro experiments. Any z-score obtained as a result gives a quantitative estimate of how a biological function is presumably regulated based on the observed differences

in gene expression. https://www.qiagenbioinformatics.com/products-/ingenuity-pathway-analysis

References

1 Izykowski N, Kuehnel M, Hussein K, *et al.* Organizing pneumonia in mice and men. *J Transl Med* 2016; **14**: 169

2 Mascaux C, Angelova M, Vasaturo A, *et al.* Immune evasion before tumour invasion in early lung squamous carcinogenesis. *Nature* 2019; **571**: 570-575