

Title: Senescence in Healthy Lung Aging Contributes to Changes in the Extracellular Matrix Proteome

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Rationale: Aging is characterized by several hallmarks, including senescence, implicated in chronic lung diseases. Previous studies have focused on cellular outcomes of senescence, however, effects on the extracellular matrix (ECM) altered upon lung aging remain elusive. We aim to decipher changes in the ECM proteome in healthy aging lung and investigate whether trigger- and cell-specific senescence is sufficient to drive ECM changes. **Methods:** Twenty healthy human donor lung tissues (4mo-95yrs) were obtained for label-free liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis completed in the Schilling Lab. Precision cut lung slices (PCLS), an *ex vivo* model for multicellular interactions and ECM, were prepared from human lung tissue into 300um thick and 1cm diameter slices. Samples were treated with bleomycin (15ug/mL) to induce senescence. **Results:** 5,138 protein groups were identified and quantified with at least two unique peptides in human lung tissue aging cohort. In old (>65years) vs young adult (< 50 years) lung tissues, 740 protein groups reached significance with 508 up-regulated, and 232 down-regulated. Statistical analysis revealed significant changes in abundance and expression of proteins, with the largest group representing ECM proteins, identified through the MatrisomeDB. To further investigate whether these changes are mediated by cellular senescence, we analyzed senescent human PCLS confirmed by assessing p21/p16 mRNA and protein levels, and b-galactosidase staining. 2,899 protein groups were similarly identified in the senescent human PCLS cohort, with 61 key proteins significantly altered with aging and senescence. Nineteen ECM proteins, including secreted factor S100A9 and matrix metalloproteinases (MMP9, MMP2) regulated across age and senescence were termed Senotype A (senescence-type) “ECM-based Healthy Aging Senotype”. Twenty-six ECM proteins, including nidogens (NID1), collagens (COL1A1, COL3A1), and laminins (LAMA3, LAMB2) regulated only with senescence-induction were termed Senotype B “ECM-based Fibrosis Senotype”. **Conclusion:** The lung ECM is significantly altered over the healthy lifespan. Autocrine and paracrine signaling of senescent cells contributing to ECM alterations. Two ECM-based lung senotypes were identified for healthy and diseased senescence-driven dysregulation. **Acknowledgements:** NIA U54 AG075931 (Koenigshoff) and U54 AG075932 (Schilling)