Senescent alveolar type 2 cells exhibit a unique metabolomics profile.

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Rationale: Metabolic dysregulation is a characteristic feature of senescent alveolar epithelial cells; however, a detailed characterization of metabolic changes in these cells has yet to be described.

Objectives: To perform a detailed characterization of metabolic changes associated with cellular senescence, using an *in vitro* model of senescent murine alveolar epithelial cells.

Methods: Studies were performed on mouse MLE12 lung epithelial cells. To induced cellular senescence, MLE12 cells were exposed to 10 µg/ml bleomycin for 24 h on two separate occasions (day 0 and 4) and maintained in culture for an additional 3 days until harvest (day 7). Induction of cellular senescence was confirmed by beta-galactosidase activity and measurement of senescence markers. Metabolomics was performed on lysates extracted on day 7 of our protocol and results were compared to extracts from control cells. Raw data were aligned using Compound Discover and imported to SIMCA-P for multivariate analysis. Principle component analysis was used for data visualization and outlier identification. PLS-DA was performed to identify potential biomarkers. Results were then filtered and confirmed by combining VIP values (VIP>15) and t-test ($p<0.05$).

Results: We identified a total of 754 metabolites by positive ion mode and 441 metabolites by negative ion mode that were significantly different between control and senescent cells. When compared to controls, 182 metabolites were significantly upregulated and 122 were significantly downregulated in senescent cells vs. controls. Using the KEEG Database, we identified a significant upregulation in phospholipid biosynthesis, mitochondrial beta-oxidation, fatty acid metabolism, arginine/proline metabolism, phenylalanine/tyrosine metabolism, glutamate metabolism and the Warburg effect in senescent cells. These changes were also associated with an upregulation in multiple metabolites, including proline, uracil, threonine, and phenylalanine. Importantly, we identified similar directional changes of these metabolites in lung tissues from a cohort of IPF patients.

Conclusion: Our data provide a comprehensive evaluation of the metabolome in a model of senescent type 2-like alveolar epithelial cells. Our findings may increase understanding of metabolic changes associated with senescence in the alveolar epithelium of the IPF lung, and guide future translational studies and therapeutic development.

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