Gene expression signature of the ageing lung: breathing new life into COPD

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Due to consistently low birth rates and a higher life expectancy, countries within the European Union are transitioning to a much older population structure.[1] The average number of older persons in the total population will increase significantly in the coming decade, as a large proportion of the post-war, baby-boom generation will reach retirement. Compared with generations prior, the 'baby boomers' were the most physically fit generation; however, they also grew up when smoking rates and exposure to secondhand smoke were at their peak.[2]

Tobacco use is a risk factor for six of the eight leading causes of death in the world including respiratory and cardiovascular diseases, stroke and cancer.[2] Of these diseases, chronic obstructive pulmonary disease (COPD) is a major growing cause of morbidity and mortality[3] with smoking and secondhand smoke exposure being the most important causative factors of the disease.[3] In the European Union, the total direct costs of COPD account for 56% (€38.6 billion) of the total healthcare budget. As there is growing evidence that up to 50% of smokers will eventually develop COPD,[4–6] the ageing 'baby-boomer' population will therefore have a significant effect on future healthcare demands.[7]

COPD is characterised by irreversible chronic airflow limitation that is caused by emphysematous destruction of lung elastic tissue and obstruction in the small airways due to occlusion of their lumen by inflammatory mucus exudates, narrowing and obliteration. In COPD, the airflow limitation measured by reduced forced expiratory volume in 1 s, progresses very slowly over time—potentially due to the high redundancy of small airway generations.[8 9] Therefore, despite symptoms such as cough and phlegm being associated with susceptible smokers aged 20–44 years who will develop COPD, loss of lung function is only observed later in life—early 60s.[10 11] Certainly the incidence of COPD dramatically rises with age as newly diagnosed COPD increases from 2% in the population aged ≤45 years to 12% in patients aged ≥65 years.[12]

Ageing has always been a subject of human fascination. Ageing, is defined as a time-dependent progressive loss of physiological integrity, resulting in impaired function and increased vulnerability to death.[13] The human lung grows progressively until we are ~25 years of age. During 'normal' physiological ageing, healthy subjects will lose on average 25% of their lung function by the age of 75 years. This loss of lung function in the ageing lung is due to loss of elasticity, alveolar enlargement, low-grade inflammation but, in contrast to emphysema, no destruction of alveolar walls.[14]

Rather than ageing being programmed by selected genes, it is now thought that the stochastic interaction between genes, environment and intrinsic defects of the organism, plays an important role in the energy devoted to organ/cell maintenance and repair.[15] Current hypotheses on the molecular mechanisms of ageing encompass telomere attrition, epigenetic alterations, oxidative stress, DNA damage, stem cell exhaustion, cellular senescence, mitochondrial dysfunction, defective protein turnover and many others that have been reviewed elsewhere.[12 13 16] The concept of accelerated ageing contributing to disease is not new or indeed unique to the lung as abnormal ageing of tissues has been implicated in the pathogenesis of many complex diseases reviewed elsewhere.[13 15] The role of accelerated lung ageing is a relatively unexplored avenue that could help to understand how tobacco smoke may accelerate lung ageing in COPD and provide insights into potential new COPD therapeutic targets.

Moving towards the goal of a better understanding the molecular alterations that occur within the lung throughout the ageing process, the study by de Vries[17] investigates genes underlying lung ageing in general and abnormal lung ageing in COPD. de Vries presents a strong signature for lung ageing across three cohorts of well-phenotyped lung tissue samples, identifying 3509 genes, which are differentially expressed with age. Specifically, by employing a linear mixed-effects model with a stringent false discovery rate (FDR) cut-off to <0.0001, they identify 1980 genes that increase expression with age, and 1529 genes that decrease expression with increasing age. Pathway analysis revealed that genes upregulated with age are enriched for biological processes related to calcium signalling and immune responses, whereas genes downregulated with increasing age were enriched for processes related to lung development and cell–cell contacts. Importantly, the age-related lung tissue gene expression signature identified by the authors demonstrates a significant overlap (33.5%) with that from the Genotype-Tissue Expression Consortium (GTEx) study by *Yang et al*, which provided an extensive study of genes and pathways associated with ageing in multiple organ tissues.[18] de Vries further proposes that ectodysplasin A2 receptor (EDA2R) could be a strong candidate gene and biomarker for lung ageing and, interestingly, the authors themselves note that EDA2R was the second highest ranked gene associated with lung ageing in the GTEx project.[18] The EDA2R gene encodes a transmembrane receptor that belongs to the tumour necrosis factor receptor superfamily[19] and has been shown to be transcriptionally regulated by p53.[20] While the exact function of EDA2R and its role in lung ageing needs to be explored, the use of EDA2R as a biomarker specific to lung ageing may be limited as it was also shown in the aforementioned GTEx study by *Yang et al* to be highly associated with ageing of adipose, artery, heart, muscle and skin tissues.[18]

Moving on from the general concept of lung ageing, de Vries applied an interaction term in the linear mixed-effects model to identify genes that were differentially expressed with age between COPD and control tissue samples. This analysis yielded a set of 69 genes that were differentially expressed with age between COPD and control samples, using a more lenient FDR cut-off of <0.25. Gene set enrichment analysis of the ranked gene lists for COPD*age showed genes that were upregulated more with age in patients with COPD were enriched for the mechanistic target of rapamycin (mTOR) signalling pathway, splicing of introns and exons, and genes that belong to the ribosome complex, including the core-enriched genes *FAU* and *RPL38*. Conversely, genes that were downregulated with age more in patients with COPD were enriched in extracellular

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matrix (ECM)–receptor interaction pathways (including collagen genes *COL6A3*, *COL3A1* and *COL4A1*). The implications of these results are as patients with COPD age they have a decrease in ECM-associated gene expression, which follows with the well-established pathophysiology of emphysema known to involve the degradation of ECM and progressive destruction of the alveolar walls. On the other side of this pathogenic seesaw, there is an increased expression of mTOR signalling pathways that could contribute to the abnormal cellular senescence, metabolism and growth seen in the COPD lung.[16]

The findings from de Vries are novel and indeed exciting as they provide evidence of a gene signature associated with accelerated ageing in COPD. However, the authors themselves note some important limitations of the study including differences in demographics of the three cohorts used with respect to their age, smoking status, lung function and severity of disease. Indeed the current study includes paediatric subjects (youngest 4 years of age) and thus it is not surprising that the authors show a number of lung development processes, which are downregulated with age. It would be of interest to see if these same genes and pathways are still identified after removal of the paediatric subjects. In the future, it would also be exciting to see if the age-related lung tissue gene-expression reported can actually predict accelerated ageing in COPD patients. In fact, such an approach was applied by *Yang et al*, whereby the authors used their derived gene signature associated with age to estimate/predict the tissue-specific apparent ages or relative ages in the population using both unsupervised (Principle Component Analysis) and supervised learning methods (Elastic Net Regression). However, this also highlights an important problem in the field of how we define accelerated ageing in lung diseases such as COPD verses chronological age. Indeed the recent approaches for the accurate correlation of gene expression signatures with careful assessment of tissue pathology in idiopathic pulmonary fibrosis[21] and COPD[22] have enabled molecular characterisation of dominant pathogenic processes at different disease stages. Overall, current knowledge in the molecular mechanisms of lung ageing is very limited. Studies such as the one by de *Vries* are important if we are to understand how molecular ageing mechanisms could be minimised to improve the quality of life of patients with obstructive lung diseases such as COPD.

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