Mechanisms and markers of lung ageing in health and disease

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Shareable abstract (@ERSpublications)
Ageing impairs lung function, increasing the risk of diseases like IPF and COPD. Ageing hallmarks and markers aid diagnosis but need optimisation. The AgEnOmics approach uses ageing, omics and environmental exposure to improve understanding of disease. https://bit.ly/3ZFFBVt

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Abstract

Ageing significantly impacts lung function and increases susceptibility to chronic lung diseases. The lung is a complex organ with multiple cell types that undergo cellular age-related perturbations or hallmarks. As knowledge of ageing mechanisms has progressed, we have a better understanding how intracellular adaptations impact cellular crosstalk and integrate to increase the susceptibility to age-related diseases in the lung. Herein, we discuss the prospects of exhaustion of lung progenitor cells, disrupted lung cell plasticity, perturbation in fibroblasts, impaired adaptive immune responses and alterations in lung microenvironment in the promotion of ageing and age-related lung diseases. Additionally, the ageing process trajectory of the lung depends on a combination of biological, genetic, metabolic, biomechanical and sociobehavioural factors that range from protective phenotypes to accelerated ageing phenotypes. We propose the concept of AgEnOmics, which expands the temporal dimension of lung ageing by distinguishing between chronological ageing and accelerated lung ageing phenotypes. Based on this concept, we define biomarkers of biological ageing that will help to define accelerated ageing and early interventions in biological ageing-related lung diseases.

Introduction

Medical advancements have extended human lifespans, creating a growing aged population, the “silver tsunami”. The global population aged 65 years and older is projected to rise from 10% in 2022 to 16% by 2050, representing a 47% increase [1]. This shift in chronological ageing is expected to burden economies and healthcare systems with rising chronic diseases and disabilities. While chronological ageing is the count of years lived, biological ageing is influenced by factors such as genetics and environmental exposures, and results in molecular, cellular and systemic changes that contribute to physiological decline and increased disease and mortality risks. The hallmarks of ageing [2], particularly impact the lungs, which are constantly exposed to environmental factors and regulate systemic homeostasis. The human lungs are highly heterogeneous, hosting a variety of resident and immune cells [3]. Single-cell transcriptomic analyses demonstrate that ageing differentially affects these cells. Immune cells and progenitor alveolar type (AT) 2 (AT2) cells exhibit the highest lung ageing gene signatures, senescence scores and phenotypes, suggesting a potential role in age-related lung dysfunction [4]. However, while these cells show increased expression of senescence-associated genes, further research is needed to determine how they are directly contributing to lung function decline. The accumulation of senescent cells is considered a hallmark of ageing and it is known to be associated with impaired tissue repair, reduced regeneration capacity and chronic inflammation, all of which may contribute age-related chronic lung diseases (CLDs) such as idiopathic pulmonary fibrosis (IPF) and COPD [5].

Hallmarks of ageing and diseased lungs

Age significantly affects lung physiology, with function increasing until 20–25 years, stabilising in adulthood and declining at around 50 years of age [5]. In resident and immune cells of the ageing lung, multiple cellular and subcellular changes occur that diminish lung repair capacity. Following previous definitions, we have grouped the hallmarks of the ageing lung into three different categories based on their hierarchy, their interconnections and the high complexity and regulated dynamics of different cell populations of the lung (figure 1). Primary hallmarks are defined as the causes of cellular damage (genomic instability/telomere attrition, deregulated proteostasis/autophagy and mitochondrial dysfunction) affecting the physiological function of most cell types in the lung including immune, endothelial, epithelial and mesenchymal cells. Secondary hallmarks, established as consequences of the initial damage, encompass senescence, stem cell exhaustion and disrupted cell plasticity. Finally, the integrative hallmarks include inflammageing, dysbiosis, cell competition leading to imbalance of cell populations and the altered cell crosstalk associated with changes in the extracellular matrix stiffness. The integrative hallmarks of the ageing lung summarise drivers of loss of homeostasis and function in the ageing lung promoting inadequate stress responses and with potential pathological consequences (figure 1). It is possible that the integrative hallmarks engage in feedforward loops that accentuate primary and secondary hallmarks, driving the ageing process and accelerating its pace. For example, epithelial cell senescence and the senescence-associated secretory phenotype (SASP) of mesenchymal cells may be both the cause and the consequence of progenitor cell exhaustion and plasticity loss contributing to cell competition and imbalance. Simultaneously, senescence of immune cells promotes dysbiosis that enhances inflammageing. Immunodeficiency leads to accumulation of

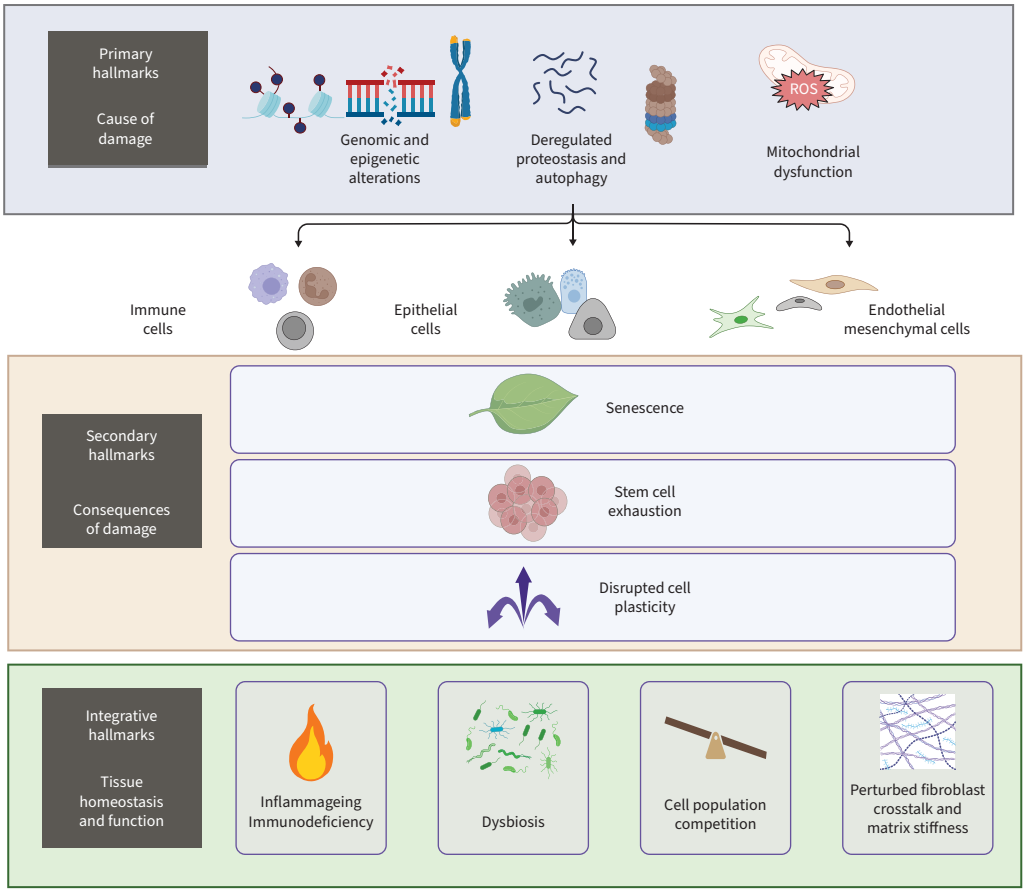

FIGURE 1 Different levels of hallmarks of the ageing lung. A schematic framework categorises lung ageing into primary hallmarks, causes of cellular damage (genomic instability/telomere attrition, deregulated proteostasis/autophagy and mitochondrial dysfunction) affecting immune, epithelial and mesenchymal cells; secondary hallmarks, consequences including senescence, stem cell exhaustion and disrupted cell plasticity; and integrative hallmarks related to tissue homeostasis and function, that encompass inflammageing, dysbiosis, cell population competition leading to cellular imbalance and perturbed fibroblast crosstalk with increased matrix stiffness. ROS: reactive oxygen species. Figure created using BioRender.

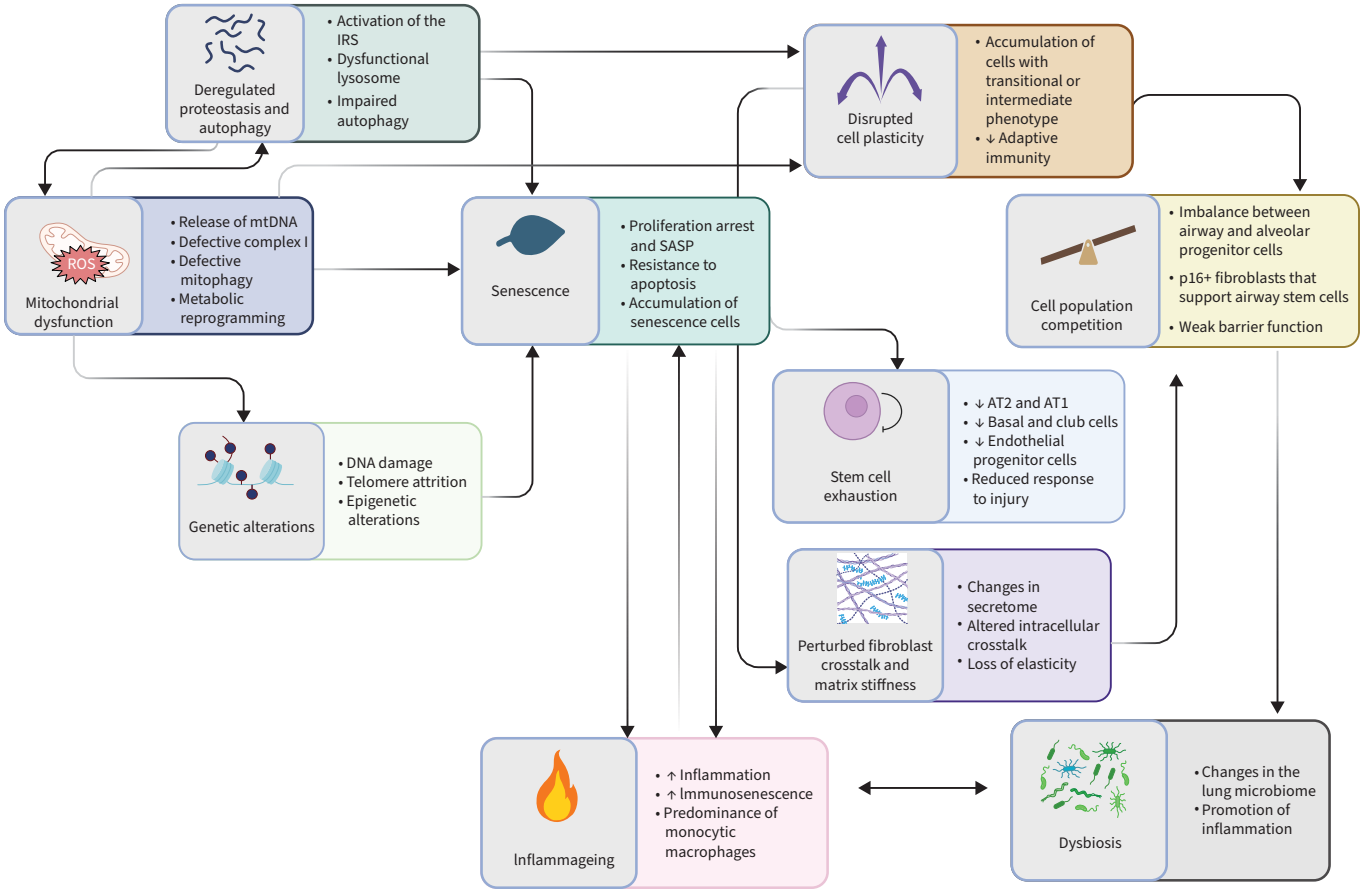

FIGURE 2 Interconnection of hallmarks of the ageing lung. A schematic framework highlighting the interconnection between primary hallmarks, secondary hallmarks and integrative hallmarks related to tissue homeostasis and function. These interconnected features underlie the progressive decline in lung function and the development of age-related pulmonary diseases. Figure created using BioRender. AT1/2: alveolar type 1/2; IRS: insulin receptor substrate; mtDNA: mitochondrial DNA; SASP: senescence-associated secretory phenotype.

senescent cells including fibroblasts that accentuate changes in the matrix composition leading to higher stiffness that might impact cell plasticity. Hence, a major challenge is to understand the relative importance of each of the hallmarks of lung ageing and to clarify their interconnections, with the final goal of defining optimal molecular targets for attenuating or interrupting the ageing process (figure 2).

Primary hallmarks: causes of damage

Genetic alterations

Cellular and DNA replication shorten telomeres, triggering DNA damage signals with telomere-associated DNA damage response foci formation, genomic instability and cellular senescence or apoptosis [2]. Furthermore, epigenetic regulation in lung cells including DNA methylation changes with age and responds dynamically to several environmental exposures. In relation to disease, mutations in telomere maintenance genes have been found in families with inherited IPF [6–8] and most IPF patients have short telomeres regardless of age [9–11]. Some patients with familiar telomerase mutations and a history of cigarette smoke exposure develop emphysema independently or concurrently with IPF [12–14], suggesting critical roles for environmental exposure and genetic predisposition in these diseases. Additional mutations in the gene BICD1 (bicaudal D homolog 1), which regulates microtubule-based cargo loading on the dynein motor complex, have also been identified as playing a role in telomere attrition, leading to increased emphysema risk [15]. In addition, DNA methylation patterns associated with accelerated ageing have been found in diseased lungs with environmental exposures.

Loss of proteostasis and reduced autophagy

Proteostasis and autophagy prevent misfolded protein accumulation. Defects activate the integrated stress response (ISR) and the unfolded protein response, triggering activating transcription factor (ATF) 4, ATF3

and CHOP (CCAAT/enhancer-binding protein homologous protein transcription factor) to restore homeostasis or induce apoptosis. Lysosome function, critical for quality control of macromolecules, is significantly impaired in senescent cells, including a dramatic expansion in lysosomal size and accumulation of lipofuscin and upregulation of lysosomal enzymes such as β-galactosidase. Dysfunctional lysosomes or autophagy have been shown to cause senescence potentially associated with the accumulation of misfolded proteins and dysfunctional mitochondria, which in turn may impair DNA repair mechanisms leading to increased DNA damage. High levels of ISR inhibit AT2–AT1 differentiation and are associated with mitochondrial dysfunction [16–19].

In IPF, alveolar epithelial cells exhibit endoplasmic reticulum (ER) stress markers [20, 21]. Accumulation of defected surfactant protein due to genetic alterations promotes AT2 cell dysfunction, causing intracellular aggregate formation, ER-associated degradation disruption, unfolded protein response activation, autophagy inhibition and mitochondrial homeostasis alterations [22]. ER stress also influences IPF progression by promoting macrophage pro-fibrotic phenotype transitions [23]. Activation of the 26S proteasome has been found in myofibroblast and basal cells of the bronchiolar epithelium in lungs of patients with IPF, which is accompanied by enhanced protein polyubiquitination [24]. Similarly, epithelial cells and fibroblasts of IPF lungs have defective autophagy markers that are associated with senescence, altered cell differentiation and apoptosis resistance [25, 26]. In COPD, ER stress can be induced by cigarette smoke exposure and α1-antitrypsin mutations [27–30]. Cigarette smoke impairs the immunoproteasome function impacting adaptive immune responses and adding to prolonged infections and exacerbations in COPD and IPF [31].

Mitochondrial dysfunction

Mitochondria, the main organelle responsible for cellular metabolism, becomes increasingly dysfunctional with age, leading to mitochondrial DNA (mtDNA) mutations, deficient proteostasis and reduced mitophagy. Mitochondria are major regulators of cell fate, including apoptosis and senescence, and they contribute to the SASP by activating DNA sensors with mtDNA release. Defective autophagy causes dysfunctional mitochondria accumulation that occurs in most lung cell types during ageing leading to systemic release of mtDNA and intracellular DNA sensor signalling pathway activation that promotes proinflammatory immune cell phenotypes and contributes to cellular dysfunction, senescence and cell death [32, 33]. AT2 cells from older lungs show increased frequency of enlarged mitochondria. In IPF lungs, damaged mitochondria accumulate in AT2 cells and have predominant defects in complex I activity and mitochondrial autophagy, known as mitophagy [34]. In addition, AT2 cells from ageing and IPF lungs downregulate β-oxidation enzymes, which was associated with promotion of transforming growth factor-β (TGF-β) pathway activation [35, 36].

Furthermore, reduced expression of the mitochondrial ATP-ADP transporter adenine nucleotide translocase type (ANT) 1 in IPF alveolar epithelial cells drives cellular senescence [37]. In contrast, alveolar macrophages exhibit increased fatty acid oxidation promoting apoptosis resistance [37, 38]. Notably, acetyl-coenzyme A and α-ketoglutarate, metabolites generated through the mitochondrial tricarboxylic cycle, have been recognised as critical for epigenetics, contributing to histone marks and nuclear DNA methylation patterns, suggesting that epigenetic changes found in ageing and IPF lung cells can be connected with mitochondria dysfunction.

In COPD, mitochondrial dysfunction is observed in cells exposed to cigarette smoke [39, 40]. Low ANT2 expression impairs ciliary motility [41], while reduced levels of the E3 ubiquitin ligase PARK2 protein leads to the accumulation of damaged mitochondria [42] and increased reactive oxygen species (ROS) [43]. COPD patients exhibit reduced mitochondrial membrane potential and electron chain transport complex proteins I and II [40]. Increased short isoforms of OPA1 (optic atrophy protein 1), which maintains mitochondrial structure and function, alters interactions with proteins such as prohibitins 1 and 2, disrupting mitochondrial quality and mitophagy [44].

Secondary hallmarks: consequences of damage

Cellular senescence

Senescent cells exhibit irreversible proliferation arrest, apoptosis resistance and SASP expression. While senescence aids development, wound healing and tumour suppression, senescent cell accumulation causes persistent SASP factor secretion of highly heterogeneous profiles or “genotypes”, regulated by factors such as cell type, stimuli and age, ultimately influencing age-related disease pathology [2]. Although physiological senescence is associated with development, wound healing and tumour suppression, persistence and accumulation of senescent cells has been associated with organ dysfunction and disease. Calculation of the transcriptomic senescence score in human lungs have confirmed a significantly higher

senescent cell accumulation in aged rather than young lungs. Resident and monocytic macrophages exhibit the highest transcriptomic senescence scores, followed by endothelial cells, epithelial AT2, AT1 and club epithelial cells [4, 45].

In IPF lungs, senescent cell markers are detected in AT2 cells, airway epithelial cells, immune cells and fibroblasts [46, 47]. Senescent fibroblasts, which are resistant to apoptosis, contribute to lung fibrosis by secreting collagens and other extracellular matrix (ECM) proteins as part of their SASP. In ex vivo organoid models, these fibrotic fibroblasts express collagen triple helix repeat containing 1 (CTHRC1) [48]. Transcriptomic features of senescent cells have been found in AT2 cells, AT2–AT1 transitional cells, and aberrant basaloid alveolar epithelial cells expressing keratin (KRT) 17. Within the IPF lung, these cells are associated with the activation of p53 signalling pathway and high levels of CD38 [49, 50].

In COPD, cigarette smoke induces cellular senescence [51], particularly in lung fibroblasts, which express proinflammatory SASP factors and exhibit impaired responsiveness to repair signals such as TGF-β [52, 53]. Senescence markers are also detected in AT2, airway basal, endothelial and smooth muscle cells, where they are linked to NF-κB signalling pathway activation and an enhanced proinflammatory SASP repertoire [54]. This contributes to impaired lung repair and increased emphysema risk [55].

Stem cell exhaustion

Local stem cells play a critical role in tissue maintenance and repair by differentiating and replacing damaged cells. Lineage tracing studies in young mice indicate that AT2 cells serve as the primary progenitors of alveolar epithelial cells, supporting self-renewal and AT1 differentiation. Approximately 7% of AT2 cells undergo turnover per year during homeostatic renewal [56, 57]. However, with ageing, stem cell niches decline, reducing the capacity of tissue regeneration and repair after injury. Consistently, AT2 cells from ageing mice exhibit impaired sphere-forming ability compared to those from young mice [58, 59]. Basal and club airway progenitor cells also decline progressively during ageing.

Progenitor AT2 cells are described as Wnt-activated with Axin2 expression, positioning them near Wnt-secreting mesenchymal cells. A decline in canonical Wnt signal has been observed in the ageing lung microenvironment [60], while an increase in active WNT signalling in AT2 cells has been described, leading to oncogene-induced senescence [59]. Additionally, fibroblasts have been reported to secrete Wnt5A, an inhibitor of canonical Wnt signalling and a promoter of ageing-like phenotypes in hematopoietic stem cells, which disrupts alveolar epithelial cell function and contributes to emphysema development [60–62]. Similarly, ageing endothelial progenitor cells exhibit both numerical and functional declines, including reduced proliferative capacity and impaired migration to injury sites, leading to defects in vascular repair [63]. Additionally, after injury, lung neuroendocrine stem cells in the lung function as airway sensors and shape immune responses and tissue remodelling [64]. Although functional perturbations and declining production of hormones, peptides and enzymes have been described in the brain, the impact of ageing on lung neuroendocrine cells is currently unknown.

In COPD, the differentiation capacity of both AT2 and endothelial progenitor cells is impaired [65–68]. Additionally, COPD lungs exhibit defects in basal cells, the resident airway epithelial stem cells responsible for differentiating into ciliated and secretory cells [68].

Disrupted cell plasticity

Ageing is characterised by cumulative injuries over a lifetime and it is hypothesised that ageing-associated shifts in cell identity promote dedifferentiation, biased differentiation and the acquisition of features from alternative lineages, contributing to the pathogenesis of age-related diseases [69]. For instance, loss of plasticity has been observed on immune cells. Lymphocytes become less flexible to adapt to immune challenges with limited capacity to switch between different effector functions [70]. Using single-cell RNA sequencing (scRNA-seq) in the lung, several groups have identified an alveolar epithelial transitional cell state, characterised by downregulation of AT2 markers, mild upregulation of AT1 markers and expression of high levels of unique markers including KRT8, *Cldn4*, *Sfn* and TGF-β pathway genes including integrin β6 [71]. This is in contrast to the physiological AT2 proliferation and differentiation into AT1 in physiological conditions after injury. It is not well understood why differentiation is stalled in the transitional cell state and if it remains unclear whether ageing works to sustain an expansion of transitional cells or impairs their differentiation to AT1 cells. Studies in animal models suggest that primary hallmarks of ageing, like loss of proteostasis with ISR activation or mitochondrial dysfunction, stall AT2–AT1 differentiation, leading to transitional cell accumulation that impairs alveolar regeneration and promotes fibrosis [19, 34, 72]. Consistently, an increase in transitional cells has been observed in fibrotic lungs and

severe lung injuries such as COVID-19. Loss of plasticity is also observed on immune cells. For instance, lymphocytes become less flexible to adapt to immune challenges with limited capacity to switch between different effector functions. The transitional cell state in human IPF has been termed basaloid due to the expression of markers of basal airway cells including KRT17. Basaloid cells have high expression of senescence markers. Pseudotime analysis in mouse models and human IPF lungs predicted that basaloid cells represent an alternative terminal cell fate for transitional cells other than AT1 cells [71, 73]. In human adult bronchioles, new progenitor cell populations have been identified that express airway secretory (secretoglobin family 3A member 2 (SCGB3A2) and secretoglobin family 1A member 1 (SCGB1A1)) and AT2 cell markers (surfactant protein C (SFPC) and surfactant protein B (SFPTB)), suggesting their potential to generate both airway and alveolar epithelial lineages [74, 75]. Similar respiratory alveolar secretory cells have been described in nonhuman primates and human organoids. In these models, AT2 cells can transiently acquire a progenitor state referred to as AT0 ($SFPTB^+$/SCGB3A2/$SFPTC^+$), with the ability to differentiate into AT1 or terminal bronchiolar secretory cells (TRB-SCs) or terminal alveolar stem cells ($SFTPB^+$/$SCGB3A2^+$) [76]. AT0 cells have been observed in lungs affected by acute lung injury, COPD and IPF, but not in healthy lungs. In contrast, TRB-SCs were exclusively found in mildly affected or bronchiolised areas of IPF lungs and other fibrotic interstitial lung diseases (ILDs), suggesting a role for these progenitor cells in lung fibrosis [76]. Notably, the number, proliferative capacity and differentiation potential of TRB-SCs decline with ageing in human lungs, possibly contributing to the age-related decline in COPD pathobiology. Interestingly, AT2 cells with inhibited epidermal growth factor signalling or a deficiency in the mitochondrial metabolic pathway of fatty acid oxidation exhibit a bias towards the A0 differentiation state. Metabolomic studies suggest that active fatty acid oxidation is crucial for healthy ageing and influences biological ageing [75–84].

Similarly to AT2 cells, ageing and IPF endothelial cells have an activated cell state phenotype enriched for hypoxia, glycolysis and YAP/TAZ activity in $ACKR1^+$ (atypical chemokine receptor 1) venous and $TrkB^+$ (tropomyosin receptor kinase B) capillary endothelial cells. These findings offer insights into ageing-related lung epithelial and endothelial cell dysfunction that may contribute to defective lung injury repair and persistent fibrosis [85].

Integrative hallmarks: tissue homeostasis and function

Immunodeficiency and inflammageing

Ageing individuals experience a decline in immune function along with other physiological impairments, such as reduced mucociliary clearance, diminished elastic recoil of the lung, decreased respiratory muscle capacity, increased gut and lung lymphatic permeability, loss of the lung barrier, and heightened aspiration that increases chronic infection risk. The ageing immune system experiences a decline in its ability to mount specific and rapid responses to challenges, along with a reduced capacity for memory responses, a phenomenon known as immunosenescence. This decline is accompanied by an increase in nonspecific proinflammatory and autoimmune reactions, a process known as inflammageing [86]. In concordance, ageing lungs exhibit a higher number of neutrophils, eosinophils and T-cells, along with increased levels of proinflammatory mediators [87]. Exposure to cigarette smoke exacerbates this inflammatory environment and disrupts normal lung immune function. Transcriptomic analyses of the ageing human lung found that monocyte-like macrophages were among the cell populations with the highest transcriptional changes, followed by FABP4 (fatty acid-binding protein 4) resident macrophages.

Resident alveolar macrophages play a crucial role in maintaining lung homeostasis and preventing excessive inflammation. However, with ageing, their numbers decline in the lung, accompanied by reduced proliferative capacity due to high concentrations of hyaluronan in lung lining fluid, inhibiting proliferative responses to growth factors such as granulocyte-macrophage colony-stimulating factor. Additionally, ageing and repeated lung injury reshapes macrophage populations, leading to the replacement of resident alveolar macrophages with monocytic-derived alveolar macrophages, which adopt a profibrotic phenotype over time. In line with these changes, IPF lungs predominantly contain profibrotic monocytic macrophages characterised by high expression of osteopontin (also known as secreted phosphoprotein 1 (SPP1)) and C-C motif chemokine ligand 16 [88–92].

Notably, IPF lungs also exhibit a reduction in natural killer (NK) cells [87], which play a vital role in clearing senescent cells. Mouse models of bleomycin-induced lung fibrosis with NK cell depletion demonstrate increased senescent cell accumulation and persistent collagen deposition [88]. Additionally, B-cell activation, along with memory IgA^+ B-cells and plasma cells, have been observed in the IPF lung, leading to fibroblast activation [93]. Overall, these disruptions in intracellular communication contribute to increased senescence, matrix stiffness and promotion of fibrotic responses.

Dysbiosis

The microbiome plays a key role in lung ageing, including bacterial overgrowth and loss of distinct anaerobic bacteria. Fungal infections are more prevalent in ageing and immunosuppressed individuals and impaired epithelial barrier integrity in the ageing lung promotes translocation of fungal organisms and/or cell wall components promoting innate immune response activation [94]. Dysbiosis triggers inflammation and immune dysfunction, promoting age-related diseases. In IPF, lung microbiome disruption leads to immune cell recruitment and subsequent inflammation making lung dysbiosis a potential disease progression and mortality predictor [78]. The microbiome of IPF and healthy lung tissue is diverse and the composition can be linked to diagnosis, mortality and disease severity [95]. Despite these findings, other studies have not found evidence of microbiome alterations in IPF lungs, making this topic controversial within the field of age-related lung diseases [96]. Similarly, in COPD, both lung and gut dysbiosis are associated with disease development, suggesting that local and systemic dysbiosis can exacerbate disease [79]. In COPD lung tissue from Global Initiative for Chronic Obstructive Lung Disease stage 4 patients, differences in the abundance of major lung microbiome phyla have been detected, suggesting a host immune response to microorganisms within the lung microbiome that might contribute to the disease pathogenesis [97].

Cell competition

In response to challenges such ageing, specific lung cell populations must dynamically adjust their numbers by expanding or contracting based on their fitness. Cellular fitness is influenced by a variety of factors, including epigenetic patterns, metabolism, microenvironmental exposures and both primary and secondary hallmarks of ageing. Ultimately, changes in cellular fitness and competition underlie the cell population imbalances observed in the ageing lung [98].

Lineage tracing experiments in mice suggest that AT2 cells play an essential role in maintaining alveolar homeostasis during steady state. Following severe injury, bronchial alveolar stem cells (BASCs), which are stem cells that only exist in the mouse, are found at the junction between bronchioles and alveoli and club cells in terminal bronchioles and contribute to alveolar repair [80]. With ageing, AT2 cells decline, while bronchiolar club cells and BASCs predominate [76]. This imbalance between alveolar and bronchiolar progenitor cells has been associated with reduced histone H3 lysine 9 di-methylation (H3K9me2) mediated by the methyltransferase G9a, which increases chromatin accessibility of bronchiolar genes, enhances the frequency of BASCs and promotes their repair responses after injury [76]. Progenitor cells exhibit varying capacities for alveolar differentiation and repair. While club cells exhibit lower conversion efficiency, they retain remarkable cell plasticity and can regenerate most damaged alveoli when AT2 cells are depleted or senescent, as observed in ageing lungs. Epithelial stem cell fitness levels are determined by Myc, which is tuned by the Hippo and Wnt pathway, both known to be differentially regulated during ageing and lung injury [99, 100]. Cells expressing high Myc levels become supercompetitors and lowering the fitness of AT2 stem cell fitness drives expansion of airway stem cells and might cause bronchiolisation of distal regions of the lung in case of severe lung injury [99]. Notably, niche signals including interacting mesenchymal cells can dictate cellular fitness [101, 102]. Airway subepithelial resident lung fibroblasts expressing p16 senescence marker, develop a secretory phenotype after injury promoting regeneration of $SCGB1A1^{+}$ epithelial airway stem cells [102]. These $p16^{+}$ fibroblasts have been shown to be reduced during alveologenesis but accumulate in the ageing lung [102].

Perturbed fibroblast crosstalk and matrix stiffness

Lung fibroblasts play an essential role in maintaining lung homeostasis and modulating repair responses. Advances in scRNA-seq have enabled the identification of diverse fibroblast subpopulations, including $LGR5^{+}$ (leucine-rich repeat-containing G-protein coupled receptor 5) resident alveolar fibroblasts, which support epithelial progenitor cells [102, 103]. In organoid systems, ageing fibroblasts co-cultured with AT2 cells exhibit an impaired capacity to induce AT2 differentiation, which can be at least partially rescued by treatment with retinoic acid [104–107].

Proteomic analysis of lung aged mouse fibroblasts secretome reveals high expression of secreted frizzled-related protein 1 (sFRP1), a regulator of cell growth and differentiation. In the injured lung, sFRP1 is secreted on extracellular vesicles and contributes to the accumulation of KRT8-positive transitional ATII cells [108]. sFRP1/RUNX1 (runt-related transcription factor 1) fibroblasts are alveolar fibroblasts that respond to the accumulation of transitional state epithelial cells [109]. Notably, $sFRP1^{+}$ aged fibroblasts promote the reactivation of dormant cancer cells, leading to metastatic tumour formation [110]. Changes in the secretion of components of the ECM have been also shown in the human and mouse ageing lungs with predominance of collagen type I, VI, IX, fibulin (FBLN2) and the latent transforming growth factor beta binding protein 4 (LTBP4), resulting in loss of elasticity.

In IPF, lung fibroblasts acquire a senescent phenotype, resisting apoptosis while secreting ECM components that contribute to fibrosis [33, 107]. Various lung cell types contribute to the ECM by secreting multiple proteins. Proteomic studies in the ageing lung indicate significant alterations in ECM components, including a predominance of collagen IV and reduced solubility of fibrillar collagens [86]. These changes increase lung parenchymal and blood vessels stiffness, activating mechanoreceptor signalling that promotes epithelial cell differentiation into transitional cells and fibroblasts into myofibroblasts and fibrotic phenotypes, further exacerbating lung fibrosis.

Body fluid markers of ageing in CLDS

Biomarkers offer insight into health, aiding in diagnosis, therapy and prognosis. Effective biomarkers should be noninvasive, cost-effective, reproducible, sensitive and disease- and disease severity-specific. While the previous section reviewed potential biomarkers of ageing in lung tissue and biopsies, this section will focus on putative biomarkers found in various bodily fluids of patients with IPF and COPD. The selection of an appropriate sample source is critical, as different fluids may capture distinct aspects of the ageing process. Table 1 provides an overview of key ageing biomarkers identified in bodily fluids and lung tissue of patients with IPF and COPD, highlighting their relevance to disease pathology. Additionally, figure 3 illustrates how different sample sources enable the detection of specific ageing hallmarks. The transparency within the figure indicates the strength of detection for each biomarker type, emphasising the varying utility of different specimens in capturing the biological signatures of lung ageing. Together, table 1 and figure 3 provide a comprehensive view of how ageing biomarkers in bodily fluids and tissue can aid in understanding and monitoring IPF and COPD.

Blood

Blood is an easily accessible and widely utilised source of biomarkers, providing valuable insights into systemic physiological changes associated with disease progression, treatment response and early disease detection. Technological advances have enhanced their precision and sensitivity.

Lung senescence is primarily assessed in blood through analysing systemic SASP factors. In IPF and COPD, elevated levels of SASP factors, including growth differentiation factor 15 (GDF15) and interleukin (IL)-6, have been detected in whole blood, plasma, and serum [111–118]. Similarly, COPD blood outgrowth endothelial cells (BOECs) exhibit nuclear foci of γH2AX and 53BP1, markers of DNA double-strand breaks, along with increased expression of senescence proteins p16 and p21 [67]. Additionally, COPD peripheral blood mononuclear cells (PBMCs) show increased levels of sirtuin (SIRT) 1 inhibitor miR-570-3p [119]. Further, elevated talin-1 and high mobility group box 1 levels have been found in extracellular vesicles from IPF plasma [120]. Long noncoding RNAs, known regulators of stem cell function, are differentially expressed in both IPF and COPD blood, plasma, serum and PBMCs [121–124]. Furthermore, higher levels of myeloid-derived suppressor cells and enrichment of stemness-associated pathways, such as Wnt and Notch signalling, have been observed in IPF blood and serum [125, 126].

Both IPF and COPD are characterised by chronic inflammation, partly SASP-mediated. In IPF serum, elevated C-reactive protein levels are associated with reduced 5-year survival rates [127–129]. Additionally, blood neutrophil levels are associated with IPF fibrosis progression and COPD disease severity and mortality [130, 131]. COPD patients with elevated blood eosinophil levels have an increased risk of exacerbations [132].

In IPF, higher levels of Krebs von den Lungen-6 (KL-6) in blood and serum amyloid A in serum have been detected [114, 133].

Telomere attrition is a driver of cellular senescence and a central hallmark of ageing [134–136]. Importantly, telomerase reverse transcriptase (TERT) mutations are a main cause of familial pulmonary fibrosis and have been linked to telomere attrition [8]. Shorter telomeres have been observed in IPF and COPD blood samples [9, 14]. In COPD, shorter telomeres are associated with higher risks of exacerbation, increased mortality and worsened lung function, including impaired alveolar gas exchange, hyperinflation and more severe extrapulmonary complications [12, 13].

Epigenetic clocks, which measure DNA methylation levels and other factors in blood, provide a way to assess biological *versus* chronological age and indicate whether the body maintains proper homeostatic epigenetic regulation. Initially developed to integrate chronological age with epigenetic changes, these clocks now include lifestyle factors that influence disease risk and mortality [137]. Both COPD and IPF are associated with epigenetic changes and accelerated ageing clocks, further linking these diseases with biological ageing processes. Several 5′-cytosine-phosphate-guanine-3′ (CpG) sites are strongly associated

TABLE 1 Comprehensive list of putative ageing fluid and tissue biomarkers in as idiopathic pulmonary fibrosis (IPF) and COPD

Ageing hallmark	IPF	COPD
Cellular senescence	Lung: ↑ SA-β-gal [86, 219], ↑ SASP [86, 111–113, 219–223], ↓ proliferation and apoptosis [67, 224], ↑ lamin A/C isoform [225] Blood: ↑ SASP [111–116], ↑ mosaic loss of Y chromosome [226, 227] BALF: ↑ SASP [111] Sputum: ↑ SASP [193]	Lung: ↑ SASP [119, 228–231], ↑ cellular damage/stress [159, 228, 232], ↓ lamin B1 [228] Blood: ↑ SASP [117, 118], ↑ cellular damage/stress [66, 117], ↑ mosaic loss of Y chromosome [227] BALF: ↑ SASP [173, 174] Sputum: ↑ SASP [193], ↑ cellular damage/stress [119, 194]
Telomere attrition	Lung: ↓ telomere length [233], ↓ hTERT and hTERC [177] Blood: ↓ telomere length [8, 9], mutant TERT [8] BALF: ↑ hTERT and hTERC [177]	Lung: ↓ telomere length [230], ↓ telomere activity [230] Blood: ↓ telomere length [14] BALF: ↑ ultrashort telomeres [176]
Epigenetic alterations	Lung: Δ DNA methylation [33, 234–236], Δ CpG island methylation [237], ↓/↑ miRNA [238–242] Blood: ↓/↑ miRNA [141, 142]	Lung: Δ DNA methylation [243], ↓/↑ miRNA [140, 244] Blood: ↑ miRNA [140], ↓ CpG island methylation [139] BALF: ↓/↑ miRNA [187] Sputum: ↓ miRNA [203]
Chronic inflammation	Blood: ↑ Inflammation and fibrosis [114, 127, 133, 245] BALF: ↑ alveolar macrophages [167], ↑ immune response/ inflammation [166, 167] Sputum: ↑ inflammatory/defence immune cells [193], ↑ KL-6 [193]	Lung: ↑ inflammatory/defence immune cells [92, 246], ↑ inflammation and fibrosis [92, 238], ↑ Ki-67 [92] BALF: ↑ inflammatory/defence immune cells [92] Sputum: ↑ inflammatory/defence immune cells [190]
Mitochondrial dysfunction	Lung: ↑ mitochondrial stress/damage [16, 247, 248], ↓ ANT1 [37] Blood: ↑ mtDNA [32, 179], ↑ mtDNA/nDNA [249], ↑ oxidative stress [147] BALF: ↑ mtDNA [32, 108, 179], ↓ mitochondrial quality control [181], ↑ ROS [181]	Lung: ↑ mitochondrial stress/damage [44, 250], ↓/↑ mitochondrial function [16, 247] Blood: ↓ mtDNA-CNs [144, 145], ↑ cell-free mtDNA [146] BALF: ↑ ATP [180] Sputum: ↑ MDA [196], ↑ oxidative stress [197]
Loss of proteostasis	Lung: ↓/↑ HSPs [239, 251], ↑ autophagy and protein degradation [252, 253], ↑ UPR [239, 252], ↑ PGK1 [239], ↓ LC3II [252], differentially expressed proteins [239], dysregulated oxidative phosphorylation [254] Blood: ↑ HSP47 [255], ↑ anti-HSP70 IgG autoantibody [256] BALF: ↑ S100A9 [168], ↑ oxidatively modified proteins [187]	Lung: ↑ HSP70 [257], ↑ autophagy and protein degradation [229, 258, 259], ↑ proteasome and protein degradation [260], ↑ inflammation [198, 241], ↑ caspase-3 activity [260], ↓ CHOP [261], ↓ LAMP2A [262] Blood: ↑ HSPs [260, 261], ↑ protein damage [262, 263], ↑ protein quality control [264], ↑ autophagy [265], ↓ sCD163 [264], ↓ p62 [265] BALF: ↑ innate immune system [166], ↓ immune processing/ presentation [30] Sputum: ↑ cellular stress response [195, 197]
Deregulated nutrient-sensing	Lung: ↓ nutrient-sensing pathways [242, 266], ↓/↑ insulin/IGF signalling [267, 268], ↓/↑ energy-sensing and metabolism [242, 253, 269], ↑ protein synthesis regulation [242], ↑ leptin [158] Blood: ↓/↑ nutrient-sensing pathways [150], ↑ insulin/IGF signalling [152], ↑ adipokines [158, 159] BALF: ↓/↑ insulin/IGF signalling [181, 183], ↑ IGF-1^{+} alveolar lymphocytes [184], ↑ BCL-2 [184] Sputum: ↑ IGFBP-2 [193]	Lung: ↓ nutrient-sensing pathways [229, 269–271], ↑ leptin [272, 273] Blood: ↓/↑ nutrient-sensing pathways [66, 151, 162], ↓/↑ insulin/ IGF signalling [152–154, 156], ↑ metabolism [153], ↑ adipokines (19344528, 23525184, 29552529) BALF: ↑ adiponectin [186] Sputum: ↑ leptin [160], ↑ glucose [200], ↓ FOXO3 [201]
Stem cell exhaustion	Lung: ↑ lncRNAs [274, 275], ↑ stem cell maintenance/self-renewal [276], ↑ Wnt signalling pathways [173, 174, 277, 278], ↑ Notch1 [279], ↓/↑ bone morphogenetic protein pathways [280–282] Blood: ↑ MDSC [125], ↑ Wnt [126], ↑ Notch [126], ↓/↑ lncRNAs [121, 283] BALF: ↑ Wnt5A [173]	Lung: ↓/↑ lncRNAs [284, 285], ↓/↑ signalling pathways [190, 286, 287], ↓/↑ stem cell maintenance/renewal [276, 288] Blood: ↑ lncRNAs [122–124], ↑ exosomes [289] Sputum: ↑ Wnt5A [61], ↓/↑ bone morphogenetic protein pathways [195]
Dysbiosis	Lung: diverse microbiome composition [78] Blood: ↑ calprotectin [163] BALF: ↑ bacterial burden [188], ↑ *Streptococcus*, *Pseudobutyrivibrio* and *Anaerorhabdus* [189], ↑ ABC transporter systems [189], ↑ biofilm formation [189], ↑ two-component regulatory system [189]	Lung: ↑ *Proteobacteria* and *Actinobacteria* [97], ↓ *Firmicutes* and *Bacteroidetes* [189] Blood: ↑ TMAO [164], ↑ zonulin [164, 165] BALF: ↑ LBP [190] Sputum: ↑ *Moraxella* and *Haemophilus* [204]

↑: higher concentration/amount or upregulated; ↓: lower concentration/amount or downregulated; ↑/↓: variably up or downregulated; Δ: alterations; ABC: ATP-binding cassette; ANT1: adenine nucleotide translocase 1; BALF: bronchoalveolar lavage fluid; BCL-2: B-cell lymphoma 2; CHOP: CCAAT/ enhancer-binding protein homologous protein transcription factor; CpG: 5′-cytosine-phosphate-guanine-3′; FOXO3: forkhead box O3; HSP: heat shock protein; hTERC: human telomerase RNA component; hTERT: human telomerase reverse transcriptase; IgG: immunoglobulin G; IGF: insulin-like growth factor; IGFBP: insulin-like growth factor-binding protein; KL-6: Krebs von den Lungen-6; LAMP2A: lysosomal-associated membrane protein 2A; LBP: lipopolysaccharide-binding protein; lncRNA: long non-coding RNA; MDA: malondialdehyde; MDSC: myeloid-derived suppressor cell; miRNA: micro RNA; mtDNA: mitochondrial DNA; mtDNA-CN: mtDNA copy number; nDNA: nuclear DNA; PGK1: phosphoglycerate kinase 1; ROS: reactive oxygen species; SA-β-gal: senescence-associated beta-galactosidase; SASP: senescence-associated secretory phenotype; S100A9: S100 calcium-binding protein A9; sCD163: soluble cluster of differentiation 163; TERT: telomerase reverse transcriptase; TMAO: trimethylamine N-oxide; UPR: unfolded protein response; Wnt: wingless-related integration site.

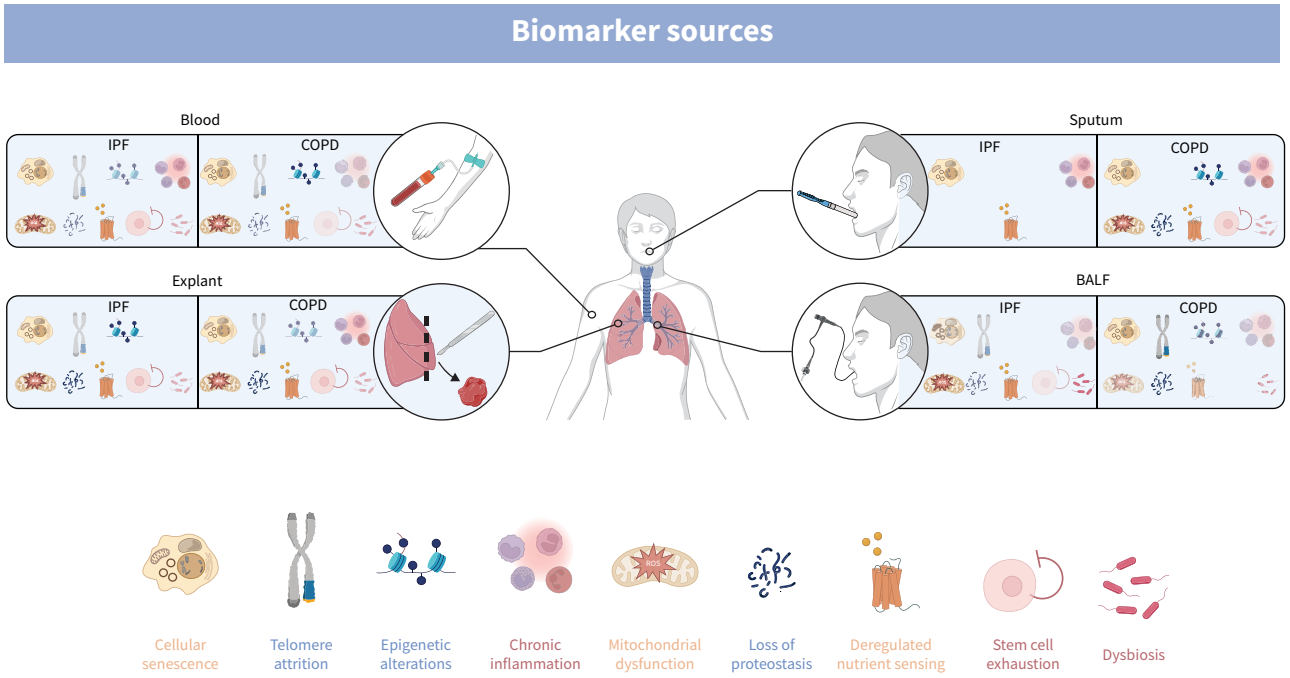

FIGURE 3 Markers of lung ageing in different sample sources. Biomarkers of ageing can be detected in different bodily fluids as well as lung tissue. Different sources allow for the detection of different hallmarks of ageing that are displayed here. Transparency indicates how well the ageing hallmark can be detected in the sample source. Figure created using BioRender. IPF: idiopathic pulmonary fibrosis; BALF: bronchoalveolar lavage fluid.

with smoking and smoke exposure, contributing to COPD pathogenesis [138]. In COPD, CpG sites are hypomethylated and have been linked with disease presence and severity [139]. One study found that two DNA methylation-based epigenetic clocks, DNAmGrimAge and DNAmTL, were predictive of 1-year mortality in COPD patients, while another demonstrated a strong correlation between epigenetic measures of age, such as DNA methylation-based telomere length in blood, and physical indicators, including exercise capacity and activity levels. Additionally, various micro RNAs (miRNAs) are differentially regulated in IPF and COPD blood, further implicating epigenetic regulation in disease progression [140–142]. Another emerging tool in ageing research, the proteomic ageing clock, has identified several plasma proteins that mediate the link between chronological age and survival in IPF and COPD [143].

Mitochondrial dysfunction has recently gained interest in the study of ageing-associated CLDs. In IPF, elevated plasma mtDNA levels and a higher ratio of mtDNA and nuclear DNA in blood have been observed. In COPD, decreased mtDNA copy numbers in peripheral leukocytes and increased levels of plasma cell-free mtDNA have been detected [144–146]. Additionally, the amount of oxidative stress marker oxidised glutathione (GSSG), and the ratio of GSSG to glutathione were significantly increased in IPF blood [147]. Furthermore, production and generation of superoxide anions were significantly higher in IPF. In COPD serum, elevated levels of mitokine fibroblast growth factor 21 (FGF21), produced due to mitochondrial dysfunction, were associated with a higher risk of exacerbation [148]. Interestingly, circulating proteins such as lipopolysaccharide (LPS)-binding protein or Crk-like protein have also been found to be useful in identifying subjects with preclinical pulmonary fibrosis [149].

SIRT1 and SIRT3, key regulators of cellular ageing, are differentially expressed in IPF or COPD BOECs, PBMCs and serum [67, 150, 151]. Additionally, insulin-like growth factor-binding protein (IGFBP)-1 and IGFBP-2 levels are higher in IPF, whereas blood, plasma and serum IGF-1 levels are lower in COPD [152–155]. Higher insulin and glucose levels along with increased homeostasis model assessment of insulin resistance have been detected in COPD blood [156]. Similarly, elevated blood glucose levels have been associated with a higher risk for IPF, suggesting that hyperglycaemia may contribute to IPF progression [157]. In both IPF and COPD, leptin and adiponectin levels are elevated in serum and plasma with increased levels during acute exacerbations [154, 158–161]. Further, mTOR (mechanistic target of rapamycin) levels are higher in COPD blood [162].

In IPF, significantly higher levels of serum calprotectin, which is known to have antimicrobial properties, correlated with disease severity [163]. In COPD, elevated plasma trimethylamine-N-oxide levels were found to be related to long-term all-cause mortality independent of exacerbation type [164]; however, this link may be explained by age and comorbidities. Further, increased plasma zonulin levels, a protein that regulates the intestinal barrier, have been observed in male COPD patients [165].

Fungal colonisation and impaired barrier integrity in the lung have been associated with elevated levels of 1,3-beta-d-glucan (BDG), a key polysaccharide component of the fungal cell wall that acts as a pathogen-associated molecular pattern promoting inflammation. High levels of circulating BDG have been associated with the severity of acute respiratory failure in the absence of fungal infection as well as worse function, morbidity and matrix degradation markers in COPD patients [166, 167].

Bronchoalveolar lavage fluid (BALF)

BALF is a valuable source for lung disease biomarkers as it reflects the local lung environment and contains a variety of cell types. However, obtaining BALF is invasive, requires sedation and specialised equipment, and cannot be performed on patients at every clinical visit, making longitudinal analysis and continuous monitoring of biomarkers challenging.

Chronic inflammation is a hallmark of many CLDs and is detectable in BALF. In IPF BALF, KL-6 levels are higher compared to other ILDs [168]. Additionally, IPF BALF contains an elevated number of alveolar macrophages. In co-cultures of T-cells with IPF alveolar macrophages, IL-4 and IL-5 levels are increased [169]. In COPD bronchial washing fluid, neutrophils and eosinophils are also increased [170]. Both IPF and COPD BALF show elevated levels of the inflammatory marker S100A9 [171, 172]. Moreover, concentrations of S100A8 and neutrophil defensins 1 and 2 are elevated in COPD BALF [172]. SASP factors, such as matrix metalloproteinases (MMPs), contribute to chronic inflammation and are increased in both IPF and COPD BALF [111, 173, 174]. However, their specific expression patterns differ between the diseases. MMP1, MMP8 and MMP9 are elevated in emphysema patients, whereas MMP1 and MMP7 are higher in IPF. Additionally, extracellular vesicles from IPF BALF show upregulation of Wnt pathway components, Wnt5a and sFRP1, suggesting that age-related effects on stem cell function are detectable in BALF [108, 175].

Telomere attrition is also observed in BALF cells. In COPD, higher levels of ultra-short telomeres have been detected, correlating with disease severity [176]. In IPF, human (h) TERT and h-TERC mRNA levels are elevated [177], though some IPF patients exhibit a transcriptional endotype of telomere and mitochondrial dysfunction [178]. Along these lines, mtDNA levels in IPF are higher, especially during acute exacerbations [32, 179, 180].

Mitochondrial dysfunction is also evident in BALF. In IPF BALF cells, reduced expression of PTEN-induced kinase 1 (PINK1) and E3 ubiquitin ligase (PARK2), along with elevated mitochondrial ROS levels, has been observed [181]. In COPD supernatants, increased ATP concentrations and deregulated nutrient-sensing have been detected signs [182]. In IPF, IGFBP-3 levels and numbers of IGF-1-positive alveolar lymphocytes are increased [183, 184], whereas IGF-1 expression is downregulated in healthy lung cells [35]. Furthermore, BCL-2 (B-cell lymphoma 2) expression has been shown to be higher in IPF BALF cells [184] and increased adiponectin levels have been found in COPD BALF [185].

Signs of impaired proteostasis resulting in immune dysfunction have been found in COPD BALF cells with reduced immunoproteasome and major histocompatibility class I (MHC I) gene expression [31]. In addition, higher concentrations of oxidatively modified proteins in IPF BALF were observed [186]. Several miRNAs were also found to be differentially regulated in COPD BALF [187].

Significant correlation between airway microbiota and alveolar inflammation has been observed in IPF. Lung bacterial burden is twice as high in IPF patients and is predictive of disease progression, while microbiota composition was found to correlate with elevated alveolar profibrotic cytokines [78, 188]. The IPF microbiome is enriched in microbes associated with ABC transporter systems, biofilm formation and two-component regulatory systems [189]. In BALF from patients with diffuse lung diseases, the highest LPS concentrations were found in individuals with eosinophilic disorders, while LPS levels were lower in those with sarcoidosis, lung fibrosis or smoking-related ILDs. Interestingly, significantly elevated levels of LPS-binding protein have been observed in smokers and COPD patients [190]. In addition, *Actinobacteria*, *Firmicutes* and *Proteobacteria* have been found to be the most prevalent species in COPD BALF [191].

Sputum

Sputum is an easily accessible sample source, allowing for frequent, longitudinal sampling and biomarker monitoring during disease progression or treatment. Despite its potential, it is not widely used clinically

and is primarily used in preclinical studies to identify novel biomarker candidates [192]. One limitation is the uncertainty of how well sputum reflects lung-specific processes.

Elevated levels of chronic inflammation markers such s neutrophils, eosinophils, macrophages and KL-6 have been identified in both IPF and COPD sputum [193]. Additionally, elevated concentrations of SASP factors IL-6, IL-8, TNF-α, TGF-β, MMP-7 and MMP-9 have been detected in both COPD and IPF sputum supernatants. Interestingly, sputum levels of MMP-7 were higher in IPF than in COPD, whereas concentrations of IL-6 and TNF-α were higher in COPD. Furthermore, COPD sputum contains higher concentrations of SIRT1 inhibitor miR-570-3p and chemokines GROα (growth-regulated oncogene α) and MCP-1 (monocyte chemoattractant protein-1) [119, 194].

In COPD sputum, dysregulated developmental pathways mediating stem cell function, mitochondrial dysfunction and altered proteostasis can also be detected. Notably, Wnt5A expression in sputum of COPD patients has been found to correlate with disease severity [61]. Additionally, during acute COPD exacerbations, bone morphogenetic protein (BMP)-4 is upregulated, whereas BMP-6 is downregulated in sputum [195].

Oxidative stress is another hallmark of COPD, with elevated markers such as 27-OHC (27-hydroxycholesterol) and HSP70 (heat shock protein 70) found in sputum [196–199]. Nutrient-sensing and metabolic alterations can also be detected in sputum, including elevated IGFBP-2 in IPF and increased levels of leptin and glucose in COPD, both of which tend to rise with disease severity [160, 193, 200]. Additionally, FOXO3 (forkhead box O3) is reduced in COPD sputum cells [201].

Sputum analysis has also revealed epigenetic alterations in smokers with wood smoke exposure and aberrant promoter methylation of p16 or GATA4 genes, which is associated with reduced lung function and other COPD-related phenotypes [202]. Furthermore, several miRNAs are downregulated in COPD sputum supernatants, suggesting a role for epigenetic regulation in disease progression [203].

Finally, dysbiosis of the COPD microbiome has been demonstrated by a significant increase in respiratory tract-associated bacteria of COPD sputum, further highlighting the role of microbial imbalance in disease pathology [204].

Limitations and outlook for ageing biomarkers

Biomarkers of lung ageing offer valuable insights into CLDs, but limitations should be considered when interpreting their clinical relevance [205, 206]. Inflammageing, the chronic low-grade inflammation associated with ageing, can alter biomarker levels independently of lung disease, making it difficult to distinguish between normal ageing and disease-related changes [207]. Additionally, comorbidities such as cardiovascular disease, diabetes and chronic kidney disease are closely related and share overlapping biomarkers, potentially confounding lung-specific ageing signatures [208]. Individual variability in genetics, lifestyle and environmental exposures further complicates biomarker-based assessments, as ageing is highly heterogeneous, leading to differences in disease progression and treatment response [209, 210]. Moreover, many older individuals with CLDs take multiple medications, including corticosteroids, immunosuppressants and statins, which can influence biomarker levels and obscure true age-related changes in lungs. These factors highlight the need for multi-biomarker approaches that account for systemic influences and individualised assessments to improve the accuracy and applicability of lung ageing biomarkers in CLD diagnosis, prognosis and treatment strategies.

Less invasive alternatives for sample collection, such as exhaled breath analysis, nasal swabs, urine tests and “mini-bronchoalveolar lavage” (mini-BAL) offer promising solutions. Exhaled breath analysis captures volatile organic compounds and metabolic byproducts that reflect lung disease. Nasal swabs are an accessible sampling method that can serve as a surrogate for lung-specific biomarkers. Urine tests offer systemic biomarker readouts, particularly useful for monitoring oxidative stress, metabolic shifts and inflammatory mediators. Mini-BAL presents a lower-cost, bedside alternative to standard BAL, reducing risks for critically ill or mechanically ventilated patients [211]. The sampling is adequate for microbiological analysis, biomarkers, or immune profiling. While less invasive sample collection methods allow for more frequent serial sampling that can elucidate disease progression, treatment response or biomarker validation, data on their effectiveness is still sparse.

Conclusions and future directions

Biological as opposed to traditional chronological age years that an individual has lived accounts for physiological changes and environmental exposures that increase susceptibility to age-related diseases such

as IPF and COPD. The hallmarks of ageing drive lung function decline and offer valuable insights for diagnosis and prognosis of CLDs, emphasising the need for reliable biomarkers to assess lung health and disease pathogenesis. The use of easily accessible bodily fluids as biomarkers sources presents a unique opportunity for early disease diagnosis and stratification of at-risk patients. However, challenges remain regarding precision and clinical integration.

Current models of lung ageing do not fully account for the relationships related to genetics, environmental exposures and ageing. Biological age can be assessed by ageing clocks that use various modelling approaches to assess interindividual variations in ageing rate based on different hallmark changes. Most prominently, biological ageing has been determined by the use of epigenetic clocks, including in the lung [137–139]. However, these do not fully capture the complexity of the ageing process [212, 213] and further clocks based on transcriptomic, proteomic and metabolomic signatures have been introduced [214]. To advance our understanding of lung ageing, an integrated approach that combines multiomic analysis of ageing hallmarks and environmental factors is necessary to account for both chronological and biological ageing on multiple levels. This approach would more comprehensively model how genetic predisposition determines disease susceptibility and how the timing of environmental exposures impacts premature lung ageing [215, 216]. Figure 4 illustrates the variability of biological ageing among individuals, showing how certain factors accelerate ageing while others promote a protective phenotype. This schematic highlights the multifactorial determinants of ageing, emphasising the need for a holistic approach in studying lung ageing and disease progression.

An emergeing framework addressing this complexity is GETomics, which seeks to elucidate the dynamic interactions between genes (G) and environment (E) by integrating data from fundamental omics (*e.g.* genomics, epigenomics and proteomics) and clinical omics (*e.g.* phenomics, physiomics and radiomics) with environmental exposures (exposome) over time (T) [217]. GETomics underscores the dynamic nature of gene-environment interactions, emphasising that their effects are influenced not only by an individual's chronological age but also by prior exposures, epigenetic modifications and immune memory. The cumulative nature of these interactions plays a crucial role in determining lung health outcomes.

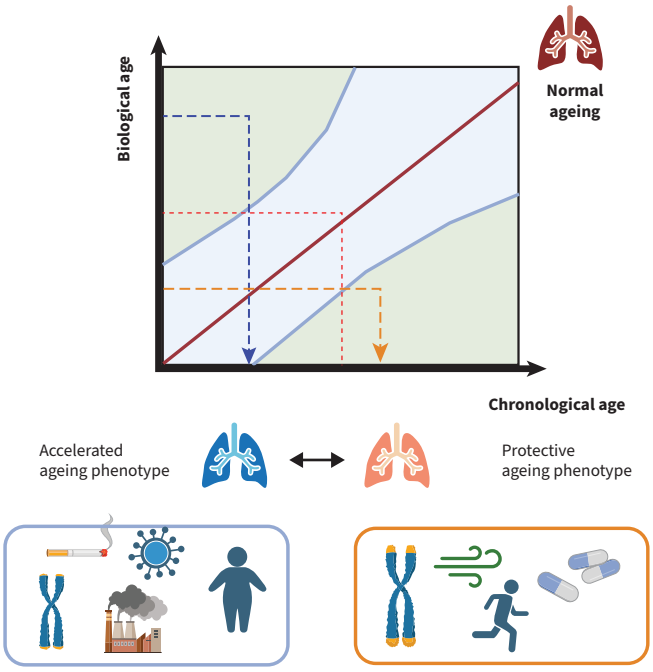

FIGURE 4 Multifactorial determinants of biological ageing. Plot of biological age (*y*-axis) *versus* chronological age (*x*-axis) illustrates the variability of biological ageing among individuals. Schematic representation of the ageing continuum: on the left, factors associated with accelerated ageing, including telomere attrition, sun exposure, obesity, smoking and industrial environmental exposures, shift the phenotype upward, while on the right, protective factors such as exercise, maintenance of healthy chromosomes and beneficial supplements promote a protective ageing phenotype. Figure created using BioRender.

Building on this, we propose the concept of AgEnOmics, an approach that expands the temporal dimension of lung ageing by distinguishing between chronological age and accelerated lung ageing phenotypes based on the analysis of multiple ageing hallmarks. By assessing biological age in a multiomic approach rather than chronological age, AgEnOmics enhances disease risk prediction and enables early interventions by identifying accelerated ageing phenotypes. Building on GETomics, it integrates genetic predisposition, environmental exposures and their timing and frequency to better characterise lung ageing and CLD progression [218], such as by epigenetic reprogramming, immune system alterations and metabolic shifts.

Figure 5 illustrates the AgEnOmics framework, which integrates multiomics with environmental exposure data to refine our understanding of lung ageing. This model incorporates novel technological tools, preclinical models and computational biology to identify biomarkers and develop personalised interventions for lung diseases.

AgEnOmics has the potential to enhance clinical decision-making by enabling more precise patient stratification, allowing for targeted prevention and personalised therapeutic strategies. Identifying individuals with accelerated lung ageing phenotypes may provide opportunities for early intervention, particularly in diseases such as COPD and IPF, where early-stage detection and treatment can significantly improve outcomes. Moreover, incorporating ageing biomarkers into clinical practice could refine disease classifications, providing a more nuanced understanding of lung health across the lifespan.

Despite its promise, implementing AgEnOmics presents several challenges. Determining multiomic biological age requires the identification and validation of multiple ageing biomarkers, demanding

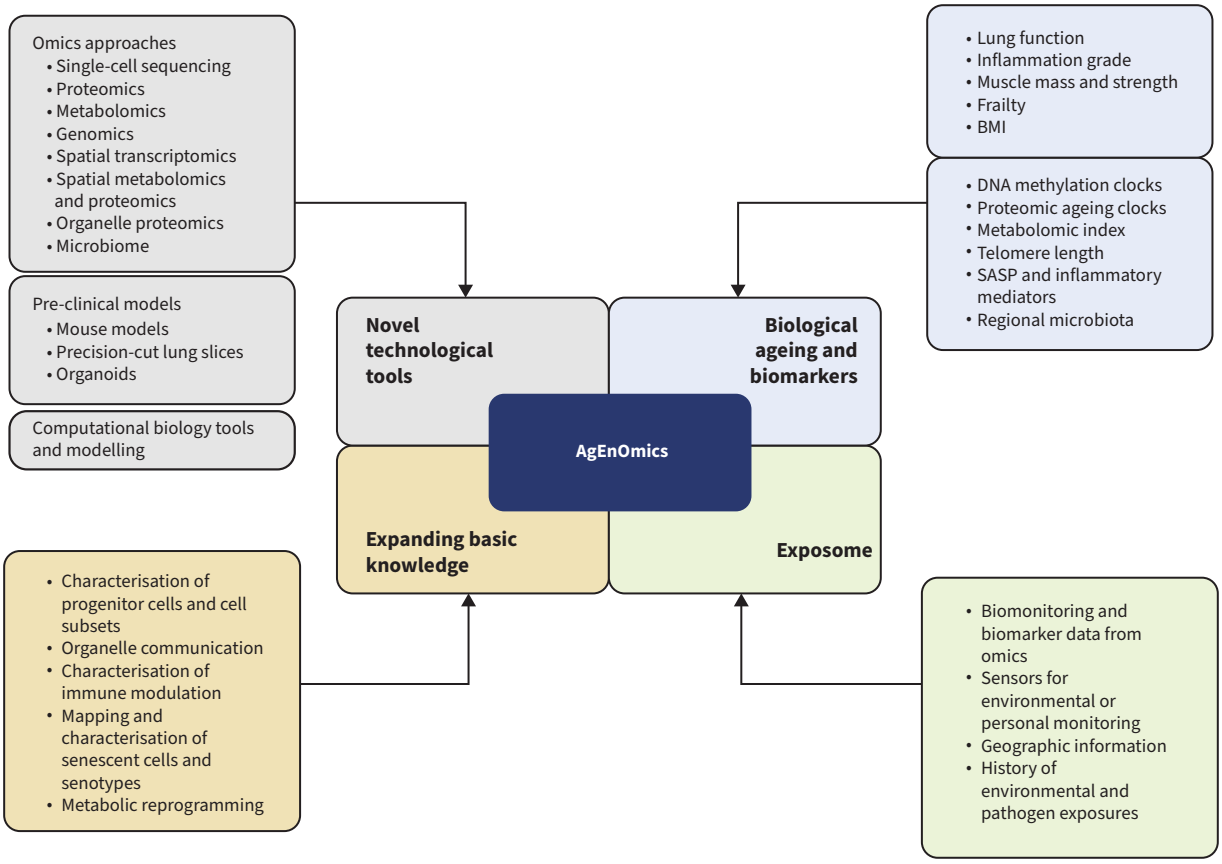

FIGURE 5 Strategies for future advances in biology of lung ageing and diseases. A central "AgEnOmics" framework integrates multiomics (genomics, epigenomics, proteomics, clinical omics) with environmental exposure data to distinguish biological from chronological lung ageing, identify biomarkers and inform therapeutic strategies. Surrounding this core concept are layers of novel technological tools, omics approaches, preclinical models and computational biology methods, all convergeing to expand basic knowledge and drive personalised interventions for lung diseases. Figure created using BioRender. BMI: body mass index; SASP: senescence-associated secretory phenotype.

substantial financial, temporal and biological resources. Additionally, integrating these biomarkers into routine clinical workflows requires significant infrastructure, much like other omics-based approaches. However, through international collaborations and large-scale cohort studies, an initial phenotyping framework could be established to identify the most relevant and clinically feasible biomarkers for widespread use.

Looking ahead, incorporating ageing biomarkers into clinical trials and routine medical practice may advance our understanding of lung disease and ageing mechanisms. By prioritising early detection, the future of age-related lung diseases can be transformed, reducing the healthcare burden associated with the "silver tsunami".

Questions for future research

- How do markers for lung ageing correlate with biomarkers for disease?
- Are accelerated ageing hallmarks cause or consequence of disease?
- How do environmental factors drive premature lung ageing in relation to timing of exposure and the underlying genetics?
- How can we combine multiomics and environmental factors to account for both chronological and biological ageing?
- How does microbial dysbiosis influence disease progression, immune response and treatment outcomes in patients with IPF and COPD?

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