ERS International Congress 2022: highlights from the Basic and Translational Science Assembly

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Highlights of basic and translational science presented at #ERSCongress summarising the latest on the dangers in the air we breathe, single-cell RNA sequencing data of the lung and new approaches for lung regeneration and repair @3Assembly https://bit.ly/3Xk5zKT

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Abstract

In this review, the Basic and Translational Science Assembly of the European Respiratory Society provides an overview of the 2022 International Congress highlights. We discuss the consequences of respiratory events from birth until old age regarding climate change related alterations in air quality due to pollution caused by increased ozone, pollen, wildfires and fuel combustion as well as the increasing presence of microplastic and microfibres. Early life events such as the effect of hyperoxia in the context of bronchopulmonary dysplasia and crucial effects of the intrauterine environment in the context of pre-eclampsia were discussed. The Human Lung Cell Atlas (HLCA) was put forward as a new point of reference for healthy human lungs. The combination of single-cell RNA sequencing and spatial data in the HLCA has enabled the discovery of new cell types/states and niches, and served as a platform that facilitates further investigation of mechanistic perturbations. The role of cell death modalities in regulating the onset and progression of chronic lung diseases and its potential as a therapeutic target was also discussed. Translational studies identified novel therapeutic targets and immunoregulatory mechanisms in asthma. Lastly, it was highlighted that the choice of regenerative therapy depends on disease severity, ranging from transplantation to cell therapies and regenerative pharmacology.

Introduction

Over 19 000 delegates attended the first hybrid European Respiratory Society (ERS) International Congress in person in Barcelona, Spain, or virtually. Three symposia, two mini symposia, one year-in-review and one hot topic were organised by Assembly 3 as part of the 2022 ERS Congress programme. In total, 304 abstracts were accepted (~90% acceptance rate), illustrating the high quality of the research submitted and presented by Assembly 3 members at the ERS Congress 2022. This review article highlights some of the most relevant work presented.

Dangers in the air we breathe

This symposium was composed of four talks, each focusing on environmental hazards to respiratory health, from their nature in the environment to their effects at the cellular and molecular level. Since the 1960s, over 350 000 new chemicals have entered use with little-to-no regulatory control [1]. Cezmi Akdis (Davos, Switzerland) provided an overview of environmental factors affecting allergic airway disease. C. Akdis described the concomitant increase in chronic noncommunicable diseases affecting 2 billion individuals, through the "epithelial barrier hypothesis" [2]. This hypothesis proposes that the development of allergic, autoimmune and neurodegenerative diseases is linked to epithelial barrier defects and gut or lung microbial dysbiosis arising from exposure to substances including diesel exhaust, microplastics, detergents or infections such as coronavirus disease 2019 (COVID-19) [3]. Diseases that arise from such exposures can be classified according to the location of tissue injury: 1) at the airway epithelial barrier, for example, asthma and allergic rhinitis; 2) autoimmune/metabolic diseases linked to gut or respiratory barrier defects and microbial dysbiosis, for example, obesity and diabetes; or 3) chronic neuropsychiatric conditions associated with gut barrier defects, for example, Alzheimer's disease [2]. Even trace amounts of toxic substances can damage epithelial barriers, leading to increased bacterial translocation into the bloodstream, thereby inducing an immune response [2, 4]. It is thus imperative to screen potentially harmful substances encountered in our daily life and understand the mechanism with which they damage the epithelium to reduce their potential adverse health effects and develop safe products.

Mary Rice (Boston, MA, USA) discussed the influence of smoke, smog and aeroallergens on children and adults. Climate change directly affects air quality through increased ozone, smog formation, pollen release and wildfires. Increased ozone levels lead to increased frequency of asthma attacks [5, 6], and can induce eosinophilic lung inflammation and airway remodelling [7]. By 2050, ozone-related mortality and hospitalisations are expected to increase by 10–14% [8]. During the past 20 years, the pollen season has lengthened by 20 days and pollen release increased by 21% due to increased temperatures and carbon dioxide emissions [9]. Allergic rhinitis is a common respiratory effect that can lead to asthma and increase respiratory-related mortality [10]. Combined pollen and air pollution exposure shows a clear association with reduced lung function in children [11]. Over a third of ambient fine particulate matter ($PM_{2.5}$) in the USA originates from wildfires [12], driving peaks in particulate matter concentrations that may be many times greater than the average levels. Wildfire smoke significantly increases respiratory admissions and is around five times more toxic for people living with asthma compared to $PM_{2.5}$ from other sources [13]. Besides respiratory morbidity and mortality, $PM_{2.5}$ exposure is also a risk factor for cardiovascular disease, diabetes, neurodegenerative disorders and poor fetal growth [14]. Crucially, the World Health Organization has concluded that there is no safe limit for $PM_{2.5}$ in the air [15].

Barbro Melgert (Groningen, the Netherlands) provided insights into the presence of microplastics and microfibres in the air we breathe. Microplastics, typically 100 nm to 5 mm in size and of varying shape, originate from degradation of bulk materials, or occur as small-by-design particles or microfibres in synthetic clothing. Sedimenting concentrations of 2–2000 particles·m^{-2}·day^{-1} can be measured outdoors while indoor levels can be over five times higher, mostly originating from synthetic fibre-based fabrics, including polyester, nylon and polypropylene [16–18]. Indeed, ~30% of the 20 kg dust produced by an average household per year is comprised of synthetic fibres [19]. A recent study found blood microplastic concentrations of up to 2 µg·mL^{-1} [20], while polyester, nylon and polypropylene fibres up to 200 µm long have been found in the distal lung [21]. The effects of pulmonary microplastic exposure, and resulting respiratory symptoms including bronchiolitis and interstitial lung disease, can be observed in occupational settings and *in vivo* studies, but the underlying mechanisms remain unknown [22]. *In vitro*, synthetic fibres, particularly nylon, inhibit the development of murine airway organoids *via* the leaching of toxic compounds of unknown identity from the fibres, suggesting that microplastics are harmful to developing lungs or to people with pre-existing lung conditions [23].

Air pollution exposure induces airway inflammation and susceptibility to allergens, infections and respiratory diseases, but our understanding of the mechanisms and pathways used to mount and then resolve an immune response is lacking. Neeloffer Mookherjee (Winnipeg, MB, Canada) shared insights

into the effects of inhaled diesel exhaust particles on airway inflammation. Controlled human exposure studies have shown that diesel exhaust not only increased concentrations of inflammation- and oxidative stress-associated proteins in the lung (bronchoalveolar lavage), but also decreased concentrations of antimicrobial/host defence peptides, such as α-defensin (human neutrophil peptide-1), S100A7 (psoriasin) and cystatin [24, 25]. Similarly, cytokines (interleukin (IL)-17, tumour necrosis factor-α and interferon-γ) with high levels in the lungs following allergen or air pollution exposure were shown to alter the abundance of specific host defence peptides in cultured human bronchial epithelial (HBE) cells [26]. These host defence peptides are multifunctional immunomodulators critical for response to infection, able to modulate and resolve inflammation [27].

Diesel exhaust exposure dose-dependently increased plasma concentrations of 115 proteins, including fractalkine, apolipoprotein-B and matrix metalloprotease-12, alongside macrophage inflammatory protein-3 and apolipoprotein-M, all associated with cardiovascular disease [28]. Current work focuses on examining these responses in a sex-disaggregated manner, to understand how responses to diesel exhaust exposure differ between males and females.

The talks in this session highlighted that there is a vast range of agents that can exert mechanistically different effects on the airways and lungs that remain to be fully elucidated. With such a wide variety of sources for these agents and their constant presence in our environment, it is clear that continued research in this area is critical to understand and mitigate their effects.

Deconstructing the developing lung at the single-cell level to determine phenotypes and cell-specific targets of bronchopulmonary dysplasia

The completion of lung development after birth makes the lung highly susceptible to injuries that can potentially lead to impaired alveolarisation and the development of chronic lung injuries such as bronchopulmonary dysplasia (BPD) [29, 30]. Although significant advances have been made to understand the molecular control of alveolarisation, further advances are required for a more in-depth mechanistic understanding.

Cristina Alvira (Stanford, CA, USA) presented an overview of rodent and human lung development, with a specific focus on distinct phases of alveolar and vascular growth. In addition, alterations and heterogeneity of endothelial cells during late lung development were discussed. By using single-cell RNA sequencing (scRNAseq), C. Alvira's group demonstrated that endothelial cell heterogeneity increased after birth and that perinatal pulmonary endothelial cells were transcriptionally distinct from their adult counterparts. Interestingly, a subtype of pulmonary arterial endothelial cells expressing genes important for vascular development was identified in early postnatal lungs. Moreover, three separate clusters of general capillary cells (gCAP) were identified at different stages during alveolarisation, with early gCAP cluster cells dominating in the beginning of the alveolarisation and late gCAP cluster cells dominating at later stage of alveolarisation. These data suggest that the developing lung contains a transient phase with endothelial cells that exhibit a marked transcriptomic shift between early and late alveolarisation.

Lung function can already be affected at the fetal stage *via* exposures to several factors including prolonged hyperoxia, malnutrition or obesity, and respiratory support such as mechanical ventilation. These factors early in life are associated with an altered lung function, with reduced capacity that persists throughout life. Miguel Alcázar (Cologne, Germany) showed that prolonged oxygen exposure in newborn lungs led to activation of type 1 macrophages and IL-6 activity [31]. Interestingly, blockade of IL-6 signalling led to an increased alveolar type II (AT2) epithelial cell survival and enhanced lung growth following hyperoxia exposure in experimental BPD. Finally, it was demonstrated that the filament protein nestin-1 was reduced during hyperoxia, and could potentially be used to activate AT2 cells and initiate alveolar regeneration.

Clinical antenatal conditions such as pre-eclampsia and chorioamnionitis are associated with an increased risk of neonatal BPD, and it has further been suggested that these conditions can potentially lead to the development of different BPD phenotypes [32–34]. Pre-eclampsia is a placental dysfunction that leads to the release of antiangiogenic and pro-inflammatory mediators, which can affect both the mother and the fetus. To investigate this further, Elizabeth Taglauer (Boston, MA, USA) used the haemoxygenase-1-null mouse model, which has an immunological driven pre-eclampsia phenotype and has previously been demonstrated to be useful, to study the effect on mothers, with focus on postnatal fetal lung effects [35]. Stimulation of lung explants with amniotic fluid from pre-eclampsia samples collected from pregnant female mice was shown to reduce branching and alter expression of genes involved in lung development compared to lung explants exposed to amniotic fluid from healthy controls [35]. Data-independent

proteomics were used to characterise differences in composition between normal and pre-eclampsia amniotic fluid, and suggest that differences in the intrauterine environment in pre-eclampsia can have a significant impact on fetal lung development.

Bernard Thébaud (Ottawa, ON, Canada) ended this session by providing an overview of pre-clinical studies performed using mesenchymal stromal cell (MSC)-based therapies to treat experimental BPD [36–39]. He summarised the results of the clinical studies that have been performed so far [40–42], and ended by discussing advantages and disadvantages of MSC-based therapies for treatment of BPD. The clinical studies performed on MSC-based treatments for BPD have been safe but so far, no significant improved outcomes have been observed. Incomplete reporting both in clinical studies and pre-clinical studies was highlighted as an area with need for improvement; in particular, characterisation of cells according to the International Society for Cell and Gene Therapy guidelines was only reported in 18% of studies and only 43% reported whether fresh or cryopreserved cells were used [43]. While MSC-based therapies are promising based on pre-clinical data, their successful translation into clinical settings will need additional well-designed early phase clinical trials to increase our understanding of MSC biology.

The Human Lung Cell Atlas: a universal reference for all respiratory scientists

Martijn Nawijn (Groningen, the Netherlands) introduced this session focused on the European Union-sponsored project "discovAIR consortium" (https://discovair.org/), which aimed to draft the first Human Lung Cell Atlas (HLCA). He provided examples of the use of the HLCA and illustrated its value in basic and translational research into lung diseases. Malte Lücken (Munich, Germany) explained how to use this universal reference of the cellular landscape of the healthy lung to answer your own research questions. The first challenge tackled by the HLCA for data integration was to harmonise cell annotations from 14 studies, harmonise the metadata and upon integration, reannotate all 58 different cell types according to original annotations and consensus of six experts [44]. This integrated reference is a powerful tool that has been able to recover rare cell identities (⩽0.01% of the sample), identify variant-cell type associations previously detected by genome-wide association studies (GWAS), identify differential pathway activities across the proximodistal axis, and rapidly analyse and contextualise new data [45]. Additionally, HLCA automated the data pre-processing steps to set up label propagation achieving >60% correct annotations compared with manual annotation. This label propagation works well for single-nucleus, single-cell and various sampling methods, and it is applicable to both healthy and diseased states in most instances. Label propagation was able to extend the HLCA to 2.2 million cells and 444 individuals from 46 studies, which can function as a universal transcriptomic reference for healthy lung, and can identify disease-affected cell states and gene signatures.

Next, Alexandra Firsova (Stockholm, Sweden) introduced the power of spatial transcriptomics in healthy and diseased tissue to extend the HLCA with details relating to lung region. Serial sections obtained from five different regions of the respiratory tract (trachea, proximal large airway and three locations of the parenchyma) from five healthy, 10 asthmatic and 20 COPD donor lungs were studied. Upon extensive quality control, SCRINSHOT (64-gene panel with high sensitivity), ISS (160-gene panel with high–medium sensitivity) and ST/VISIUM (untargeted method) were used as integrating spatial transcriptomic methods. Spapros probe selection was used for targeted methods [46]. Expression patterns were compared using VISIUM-based spatial transcriptomic methods and ST-Cell type deconvolution using stereoscopic imaging was used to predict which cell type can appear at specific locations [47]. A machine learning-based segmentation using BIAS software for single-cell level analysis detected 6535 nuclei segmented in 20 min with 70–95% efficiency compared with 9574 nuclei segmented manually in 24 h. Probabilistic cell typing using pciSeq, identification of cell types by SCRINSHOT and niche analysis were utilised to identify cell types, cell communication networks and gene expression [48]. The identification of the cellular niches present in healthy lung serves as a reference for the identification of abnormal disease-associated cell niches.

Amanda Oliver (Cambridge, UK) then discussed how to combine scRNAseq and spatial data to generate a spatial lung atlas. VISIUM analysis was performed to investigate where the cells identified by scRNAseq suspension data [49] aligned in the context of the tissue. 80 cell types/states and their spatial organisation were identified, including 11 novel cell types/states not detected in the consensus dataset. The VISIUM analyses were combined with manual structural annotations and with the cell2location algorithm as unbiased analysis to predict which cell types are present in each location, to colocalise cell types/states [50]. This approach identified localisation of new cell types, such as peribronchial fibroblasts, which have been associated with lower lung function and COPD by functional GWAS, and are enriched in idiopathic pulmonary fibrosis (IPF) samples compared to controls [51]. Additionally, A. Oliver described how spatially informed single-cell transcriptomic analyses can reveal new tissue microenvironments dependent

on specific cell–cell interactions [49], such as the unique IgA-producing plasma cell niche in submucosal glands important for viral infections and vaccination responses, termed the gland-associated immune niche. The combination of spatial and full transcriptome data enabled the identification of novel cell types/states, their localisation, cell–cell interactions and novel tissue niches.

Finally, Herbert Schiller (Munich, Germany) presented an overview of how to integrate perturbations and diseased tissue data in a multimodal analysis of subtissular niches, cell circuits and chronic lung disease including proteome data from the extracellular matrix (ECM). Cell signature deconvolution of bulk RNA-sequencing data showed that aberrant basaloid and myofibroblast states appear early in IPF and persist [52], with $KRT17^{+}$ $KRT5^{-}$ basaloid cells more abundant in early-stage IPF. Laser capture microdissection coupled with mass spectrometry was used for analysis of their interaction with mesenchymal cells. A pilot experiment identified specific niche proteomes with distinct ECM composition, indicating that the matrisome is a good disease predictor. An organotypic *ex vivo* model of human lung fibrogenesis (precision-cut lung slices) was used to functionally validate potential targets. This model showed signs of disease progression upon treatment with a pro-fibrotic cytokine cocktail [53], especially early stages, correlating with *in vivo* models and patient data. Myofibroblast induction observed early in IPF was replicated, thus offering the possibility of being utilised to study the mode of action of drugs such as nintedanib. H. Schiller highlighted the need to integrate multicellular gene programmes in health and disease from human cohorts, mechanistic studies from human organotypic models and *in vivo* models for longitudinal studies. Overall, this session highlighted the potential of the HLCA as an integrated reference of the human lung including scRNAseq, single-cell transcriptomics and spatial maps, as well as perturbations representing transitions to disease and disease data.

Cell death modalities regulating the onset and progression of chronic lung diseases

Simon Pouwels (Groningen, the Netherlands) provided a general overview on cell death modalities. Classically, cell death has been categorised as accidental/nonprogrammed cell death (necrosis) or regulated cell death (apoptosis). In 2009, the Nomenclature Committee on Cell Death introduced a molecular classification based on the signalling pathways leading to cell death, including nonprogrammed necrosis, apoptosis, autophagy, entosis, methuosis, paraptosis, mitoptosis, parthanatos, ferroptosis, pyroptosis, neutrophil extracellular trap (NET)osis and necroptosis [54–59].

Among the major consequences of cell death is the release of danger-associated molecular patterns (DAMPs), which can activate pattern recognition receptors and induce inflammatory responses [60, 61]. Pouwels *et al.* [62] demonstrated that the exposure of HBE cell lines and primary cells to toxic cigarette smoke extract (CSE) concentrations triggers necrosis and DAMP release (*e.g.* mitochondrial (mt)DNA, double-stranded DNA and HMGB1), with airway epithelium from COPD patients being more prone to release (*e.g.* mtDNA). In addition, they found that conditioned medium from CSE-exposed cells triggered pro-inflammatory cytokine release (*e.g.* IL-6 and IL-8) in which involvement of DAMPs was implicated [63]. Pre-treatment with receptor-interacting serine/threonine protein kinase 1 (RIPK1) inhibitor necrostatin-1 (Nec-1) prevented CSE-induced decrease in cellular viability and DAMP release [64]. Similarly, in a mouse model of cigarette smoke-induced airway inflammation, Nec-1 treatment significantly reduced neutrophilic infiltration DAMP release [64]. This identifies RIPK1 as potential therapeutic strategy in obstructive lung diseases associated with cigarette smoke-induced inflammation, such as COPD.

Jin-Ah Park (Boston, MA, USA) described the role of mechanical stress in airway remodelling and cell migration in asthma. J-A. Park indicated that the consequences of this type of cellular stress for cell death modalities are currently under investigation. During asthma attacks or exacerbations, the airway undergoes constriction where the airway epithelium buckles, triggering mechanical compression of epithelial cells [65–67]. Using a model of mechanical compression (30 cmH_2O for 3 h) on mucociliary-differentiated air–liquid interface (ALI)-cultured primary HBE cells, J-A. Park and co-workers found that this leads to features of partial epithelial-to-mesenchymal transition (pEMT) as seen in the airways of asthmatic patients [68–71]. Mechanically compressed cells undergo a transition referred to as unjamming transition (UJT) in which cells become migratory, fluid-like and elongated in comparison to stationary and cobblestone-like jammed cells [72]. This phenotypic switch from a stationary to a migratory phenotype following UJT differs from cells undergoing a classic transforming growth factor-β1-induced pEMT [73]. J-A. Park's laboratory is now exploring the molecular mechanisms underlying cellular level changes seen in UJT, including basal stress fibre formation and subsequent increased traction forces involved in migration.

Ken Bracke (Ghent, Belgium) discussed the involvement of regulated cell death modalities, such as necroptosis and ferroptosis, in the pathogenesis of COPD. The machinery of the necrotic cell death

complex (necrosome) in airway epithelial cells consists of phosphorylation of RIPK1 and RIPK3, which can subsequently phosphorylate MLKL [60]. K. Bracke and co-workers observed increases of total and phosphorylated MLKL in the airway epithelium as well as phosphorylated RIPK3 in the lung tissue of patients with severe COPD [74]. In addition, RIPK1 was increased in AT2 cells and bronchial epithelial cells from COPD patients. When mechanistically exploring the role of different components of the necrosome, using transgenic mice with deficient (Ripk3) or inactivated ($Ripk1^{S25D}$) necroptosis pathway components, they found reduced features of airway inflammation and remodelling upon cigarette smoke exposure [63, 74]. In addition, $Ripk1^{S25D}$ mice were almost completely protected from the development of emphysema following elastase exposure. Pharmacological inhibition of RIPK1 with the RIPK1 kinase inhibitor GSK547 prevented emphysema and airway remodelling in a chronic smoke model. Finally, evidence is emerging for involvement of other modes of cell death in COPD, such as ferroptosis (iron- and lipid peroxidation-dependent cell death) [60, 75, 76] and pyroptosis (inflammasome-driven cell death) [77–80], and regulation of cell death pathways could thus be a promising new therapeutic target for COPD.

Federica Meloni (Pavia, Italy) discussed the contribution of NETosis to lung fibrosis in COVID-19. NETosis is a reactive oxygen species (ROS)-dependent regulated form of cell death characterised by extrusion of DNA from the cell [81, 82]. Neutrophils use this mechanism to kill invading microbes, producing NETs [83]. Increased presence of NETs has been observed in lung tissue and plasma of deceased patients with COVID-19 [84] and bronchoalveolar lavage of severely ill COVID-19 patients [85]. *In vitro* work has demonstrated that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) induces NETosis in human neutrophils and is associated with upregulation of intracellular neutrophil ROS [86]. Furthermore, histopathological analysis of lung tissue from deceased patients with COVID-19 showed a subset of reactive pneumocytes co-expressing epithelial and mesenchymal markers, suggesting EMT/cellular reprogramming, which thus may contribute to the fibrotic responses seen in COVID-19 [85]. F. Meloni and co-workers established a co-culture model for SARS-CoV-2 infection using human AT2 epithelial A549 cells co-cultured at the ALI with alveolar macrophages at the apical side and neutrophils at the basal side. SARS-CoV-2 infection induced NET production by neutrophils, which was accompanied by up-regulated smooth muscle α-actin expression and induction of EMT in A549 cells [85]. Activation of NADPH oxidase, ROS production, peptidyl arginine deiminase 4 and gasdermin D were implicated in SARS-CoV-2-induced NET formation. Hence, future therapies for fibrosis in COVID-19 could be based on the modulation of these specific pathways of NETosis, as well as modulation of NETosis by MSC-derived exosomes.

Novel targets and approaches in pulmonary translational research

This session showcased exciting avenues in translational lung research, covering lung development, homeostasis and pathologies. The use of organoid models generated from induced pluripotent stem cell (iPSC)-derived alveolar progenitor cells [87] has revolutionised our understanding of epithelial cell plasticity. Alejandro Rodriguez Ruiz (Leiden, the Netherlands) presented advances in increasing the differentiation efficiency of iPSC into high *FOXA2*, low *SOX17* expressing anterior foregut endoderm cells. One of the described conditions resulted furthermore in a three-fold increase in efficiency of alveolar lung progenitor differentiation. Laurent Renesme (Bordeaux, France) presented a single-cell atlas of the fetal lung containing lungs from pseudoglandular as well as the early canicular stage. The authors identified nine cell populations, including rare populations like neuroendocrine cells. During development, the mesenchymal compartment is decreased while the epithelial cell compartment is increased. This project will help understand crosstalk of cellular compartments during lung development and will be the groundwork for many future mechanistic studies including organoid development [88]. The session also featured a direct application of scRNAseq in lungs of trisomy 21 patients, who are susceptible to lung diseases and present with prenatal histological, cellular and molecular anomalies in the lung [89]. Even though the underlying mechanisms are not yet understood, Soula Danopoulos (Torrance, CA, USA) showed a downregulation of *SOX2* mRNA indicative of differential regulation of epithelial cells. Notably, there was an accumulation of club, ciliated and alveolar epithelial cell markers, revealing an increased epithelial cell differentiation in trisomy 21 lungs.

Multiple interesting approaches were described for COPD diagnosis and treatment. Nuria Olvera Ocana (Barcelona, Spain) presented a multi-omics approach to characterise COPD patients. Using mRNA, microRNA and methylome data, a multilayer network based on the lung tissue similarities uncovered communities that differ in clinical COPD characteristics and shed light on molecular mechanisms. Immune response pathways such as T-cell receptor signalling were among the identified mechanisms characterising specific communities. Ali Önder Yildirim (Munich, Germany) also highlighted the importance of epigenetic reprogramming in macrophages of COPD patients that show an upregulation of the arginine methyl transferase PRMT7 [75]. Notably, $Prmt^{+/-}$ mice were protected from emphysema development.

Mechanistically, PRMT7 is important for transendothelial migration and ultimately, induction of ferroptosis, a regulated cell death modality, driving tissue destruction. This suggests inhibition of arginine monomethylation as a promising intervention in monocyte-driven inflammatory lung diseases.

Additionally, cellular senescence of fibroblasts is well described in COPD [90]. Danuskhi Herath (Créteil, France) discussed a specific fibroblast senescence-associated secretory phenotype factor, secreted phospholipase A2 (sPLA2)XIIa, which can be detected in the blood of COPD patients. Mechanistically, sPLA2XIIa induces paracrine senescence, an effect that is exaggerated in COPD patients. This signalling is mediated by proteoglycans, mitogen-activated protein kinase and STAT3, leading to mitochondrial dysfunction-associated senescence. sPLA2XIIa and its signalling pathway could thus be potential targets for dampening senescence and inflammation in COPD.

Asthma remains a global healthcare burden, highlighting the need for more detailed mechanistic understanding of disease pathogenesis [91]. Kamini Rakkar (Nottingham, UK) presented transcriptional and methylation changes of nasal brushes from severe asthma patients before and 3 months after mepolizumab treatment. Notably, mepolizumab treatment induced both methylation and transcriptomic changes associated with cellular movement, neutrophil activation and survival. This analysis will help to understand molecular pathways associated with mepolizumab treatment and therapy resistance. Abhinav Singla (Essen, Germany) studied the effect of WNT5A in asthma and demonstrated with a mouse model that deficiency in WNT5A specifically in smooth muscle cells leads to increased sensitivity to methacholine challenge. WNT5A-deficient mice showed an increased Th2 response resulting in stronger inflammation and tissue remodelling, and highlight an immunoregulatory role of WNT5A in asthma.

IPF is an age-associated disease without any curative pharmacological therapeutic options [92], with median time from diagnosis to death of 2–4 years [93]. Mada Ghanem (Paris, France) identified fibroblast growth factor (FGF)21, an endocrine regulator of lipid and glucose metabolism (mainly secreted by the liver), as increased in plasma of IPF patients. Exposure to FGF21 mechanistically did not change lung fibroblast phenotypes but might exert a protective role in IPF by inhibiting AT2 cell apoptosis. It is of importance to characterise the “long distance” lung interactions with other organs in pathological setups. Excess deposition of ECM is a hallmark of IPF and recent studies shed light on the ECM as a potent instructor of fibrotic responses [94]. Yuexin Chen (Munich, Germany) presented a spatial ECM proteomic approach that combines histopathology and laser microdissection with state-of-the-art proteomics. This allows the characterisation of disease-specific niche proteomes and has led to the discovery of a fibroblast foci-specific gene programme that potentially directs epithelial–mesenchymal crosstalk in IPF.

In conclusion, this session highlighted promising novel approaches to characterise normal and impaired lung development and disease, as well as presenting potential avenues to diagnose and target different chronic lung diseases.

Year in review: preclinical discovery and testing of new approaches to lung repair

The “scientific year in review” session was presented by Reinoud Gosens (Groningen, the Netherlands) who discussed eight papers focused on the repair of distal lung damage, identification of new cell types involved in lung repair, regenerative cell therapy and pharmacological solutions.

The first part focused on newly identified cell types in the distal lung involved in repair. Based on surfactant protein C *(Sftpc)*$^{CreERT2/+}$;*tdTomato*$^{flox/flox}$ mice used to lineage-label AT2 cells in the adult lung, Ahmadvand *et al.* [95], showed that there were two subpopulations of Tomato^{+} cells, an SFTPC-high population and a less abundant SFTPC-low population. Surprisingly, there was a selective expansion of the latter population in the right lung after pneumonectomy of the left lobe, indicating that this SFTPC-low population could be involved in tissue repair. Murthy *et al.* [48] found, through scRNAseq analysis, a specific type of SCGB3A2^{+} cell population that was able to give rise to AT2 and AT1 cells. Additionally, Basil *et al.* [96] found a SCGB3A2^{+} and LAMP3^{+} cell in the terminal bronchus, termed the respiratory airway secretory cell, that could also differentiate into AT2 cells. Interestingly, these SCGB3A2^{+} cells were more abundant in smokers, the elderly, COPD patients and patients with a high number of pack-years, compared to never-smokers. Furthermore, in an experimental setting, respiratory airway secretory cells expanded after cigarette smoke exposure, indicating these cells are likely involved in lung repair. Both papers show that these SCGB3A2^{+} cells in the terminal bronchiole may act as stem cells for AT2 cells and are likely involved in alveolar repair.

The second part of the session focused on how to facilitate endogenous tissue repair and discussed regenerative strategies through cell therapy. Louie *et al.* [97] demonstrated the beneficial effects of

organoid transplantation therapy in a bleomycin-injury mouse model. Labelled alveolar organoids were transplanted into bleomycin pre-treated mouse lungs where they engrafted, while maintaining their SFTPC expression. Tissue slides of the bleomycin-only treated mice compared with the organoid-transplanted bleomycin-treated mice showed an improvement of lung structure and less fibrosis. Hisata *et al.* [98] used an elastase injury model of emphysema and administered purified lung vascular endothelial cells transduced with adenoviral E4ORF1, which sustains survival, expansion and pro-regenerative capacity of endothelial cells, intravenously at 1 and 2 weeks after elastase administration. Alveolar destruction was rescued in the endothelial cell-treated mice; there was also an improvement in lung function (inspiratory capacity) and in mean cord length (a measurement for emphysema in mice). The endothelial cell treatment and organoid transplantation could be interesting therapeutic strategies for chronic lung diseases.

The last part of the session focused on key findings in regenerative pharmacology. Wu *et al.* [99] used transcriptomics-guided discovery to identify overlapping up- and downregulated genes in alveolar epithelial progenitors of mice exposed to cigarette smoke and in COPD patients. After subselection, several potential drug targets were identified and, using a murine alveolar organoid assay, drugs targeting these genes were tested alone or in the presence of 5% CSE. Drugs targeting prostacyclin and prostaglandin E2 increased airway and alveolar organoid numbers in presence of CSE, indicating that these compounds could overcome the CSE effect. This finding was further tested *in vivo*, where mice were treated with misoprostol (a prostaglandin E2 analogue) and iloprost (a prostacyclin analogue) and exposed to cigarette smoke. Thereafter, organoid cultures were established from the resected lung tissue and both drugs prevented a reduction in alveolar organoid number compared to untreated mice. Further studies revealed that expression of clock-pathway genes was distorted by cigarette smoke and prostaglandin I2 as well as prostacyclin were able to correct this effect and might therefore contribute to lung regeneration. Another drug-screening approach was used by Costa *et al.* [100] with Food and Drug Administration-approved drugs using a Wnt-pathway reporter cell line, a key pathway in lung regeneration and repair. A luciferase assay in a Wnt-reporter cell line identified amlexanox as a possible Wnt-pathway activator. Indeed, amlexanox was able to stimulate growth in a lung organoid assay compared to controls and was also effective *in vivo* in an elastase injury model, showing a positive effect on lung function and lung structure compared to vehicle elastase-treated mice. These two papers highlighted *ex vivo* organoid assays as a relatively simple screening tool to enable identification of existing drugs with potential benefit for COPD treatment.

Finally, Polverino *et al.* [101] studied the effects of metformin, an AMP kinase activator commonly used for diabetes, on emphysema development in 6-month cigarette smoke-exposed mice. Mice that received 1% metformin in their chow had less emphysema development and reduced apoptosis levels compared to untreated cigarette smoke-exposed mice. Additionally, the group used data from a large COPD cohort (COPDGene) to look retrospectively at emphysema development in metformin users compared to nonusers and found less emphysema progression in the metformin group. Although this was a retrospective study, these data combined with the effects found in mice give an interesting insight in the possibilities of metformin as a regenerative drug to treat emphysema.

In conclusion, this year in review session gave an overview of new cell types that may stimulate or be involved in repair and possible uses of cell therapy in lung regeneration, and gave examples of how to use drug repurposing to facilitate regenerative drug discovery in lung repair.

Conclusion

This article highlighted some of the biggest challenges we have to overcome to improve the air quality and the respiratory landscape overall. It demonstrated the power of new technologies such as scRNAseq as well as how they can be combined with novel spatial transcriptomic methods to identify new targets and new mechanisms of health and disease to identify potentially new therapeutic targets. Novel organoid technology was described in the context of cell therapies and regenerative pharmacology, emphasising new avenues to tackle the current issues faced by respiratory professionals. Overall, the basic and translational science presented at the 2022 ERS Congress demonstrated the value of the increasing number of tools and resources we have in overcoming the current challenges and improve the respiratory landscape.

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