From the pathophysiology of the human lung alveolus to epigenetic editing: Congress 2018 highlights from ERS Assembly 3 “Basic and Translational Science”

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ABSTRACT The European Respiratory Society (ERS) International Congress is the largest respiratory congress and brings together leading experts in all fields of respiratory medicine and research. ERS Assembly 3 shapes the basic and translational science aspects of this congress, aiming to combine cutting-edge novel developments in basic research with novel clinical findings. In this article, we summarise a selection of the scientific highlights from the perspective of the three groups within Assembly 3. In particular, we discuss new insights into the pathophysiology of the human alveolus, novel tools in organoid development and (epi)genome editing, as well as insights from the presented abstracts on novel therapeutic targets being identified for idiopathic pulmonary fibrosis.

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**Group 3.1. molecular pathology and functional genomics**

*The pathophysiology of the human lung alveolus*

One of the basic science highlights of the European Respiratory Society (ERS) International Congress 2018 in Paris was the hot topic symposium “The pathophysiology of the human lung alveolus”. In this session, four expert speakers presented their recent scientific advances in lung development, repair and regeneration, published as high-impact papers in 2018, to the general respiratory community.

In the first talk, Nikolić et al. [1, 2] presented their work on human lung development. Until recently, most of our knowledge of lung development was based almost exclusively on mouse studies. Molecular studies using human embryonic and fetal lungs were surprisingly rare. Studying normal human lung development on a molecular and cellular level is an essential part of regenerative medicine and meaningful translational studies, with recent evidence suggesting that events in embryonic development contribute to some adult lung diseases later in life [3]. As lung transplantation is a high-risk procedure for a minority of patients with a 5-year survival rate of only 54% [4], there is an urgent need to develop new treatment strategies for patients with end-stage respiratory failure. The most important epithelial stem population in the developing lung is found within the branching tip, and gives rise to all the various epithelial cell types in the adult lung, including alveolar and bronchiolar cells [5]. Expression of specific classical markers identified from mouse lung development were shown to be slightly different in human embryonic tip cells [2, 6, 7], and a wider comparison between mouse and human lung bud tip stem cells found that 96% of orthologous genes were shared, but that there were various differences which are likely to have functional consequences [2]. Self-renewal and differentiation of organoid three-dimensional (3D) culture of these lung bud tip stem cells from human fetal lung tissue was achieved by using a human-specific combination of growth factors and signalling inhibitors [2, 6], with a different combination of growth factors required for mouse tip stem cell renewal [8]. This supports the finding that the transcriptional differences between mouse and human are functionally relevant. The organoid culture system provides a novel tool for studying human lung development in vitro. Other culture systems include the use of alginate beads [9, 10], culture of lung explants [11–16], culture in the mouse kidney capsule [2, 17–19] and co-culture with matrix-embedded fibroblasts [20]. Such methods allow study of the virtually unexplored period of human lung development after 20 weeks of development, by using less than 20-week-old lung tissue and maturing it to a developmental stage at which conditions such as bronchopulmonary dysplasia can be studied.

Next, Melanie Königshoff discussed the role of WNT pathway in initiating alveolar repair in chronic obstructive pulmonary disease (COPD). This was studied by using fresh human lung tissue from surgical samples and then cutting it into precision-cut lung slices [21]. Subsequent 3D culture showed that WNT activation led to increased expression of both type 1 and type 2 alveolar cell markers; with more severe emphysematous disease, this occurred to a lesser degree. Lung repair in the adult COPD lung was associated with a reduction in WNT/β-catenin signalling in the alveolus, and activation of the WNT/β-catenin pathway was found to initiate alveolar repair in both mouse and patient-derived lung tissue ex vivo. Tight regulation of WNT/β-catenin signalling was found to be required for alveolosphere formation. While WNT signalling can activate alveolar repair in vitro, in vivo studies failed to show similar results. WNT signalling comprises a canonical β-catenin dependent and a noncanonical β-catenin independent signalling pathway. The Königshoff laboratory showed that noncanonical WNT5A is secreted by fibroblasts and its expression is higher in human COPD [22], and also increases with age. This switch from canonical to noncanonical WNT signalling also occurs in haematopoietic stem cell ageing [23]. Furthermore, WNT5A inhibits canonical WNT signalling in alveolar epithelial cells and compromises alveolar epithelial cell function [22]. Hence, it was concluded that canonical signalling is required for development and normal adult lung homeostasis, whereas in lung diseases such as emphysema, canonical WNT signalling is disrupted. Therapeutic intervention would require the release of WNT5A-driven WNT/β-catenin inhibition followed by activation of the canonical WNT/β-catenin pathway.

Anjali Jacob discussed the use of induced pluripotent stem cells (iPSCs) to model the human lung alveolar epithelium [24]. The discovery of iPSCs [25] allowed the possibility of producing isogenic, patient-specific mature lung epithelial cells that could be used for disease modelling, drug discovery and cell-based therapy. Cell cultures can be guided from the pluripotent stem cell stage to alveolar epithelium by recapitulating normal development in a stepwise process termed directed differentiation. Specifically, surfactant producing type 2 alveolar cells (AEC2s) have been implicated in various lung diseases, such as interstitial lung diseases (ILDs) and COPD, making iPSC-derived AEC2s a useful tool to study alveolar diseases. The directed differentiation protocol into functional lamellar body-like containing, surfactant-secreting and fetal-like SFTPC⁺ AEC2 takes at least 28 days. WNT activation was required for efficient production of SFTPC⁺ distal lung progenitors, but later on a withdrawal of WNT activation increased the expression of SFTPC⁺ AEC2 progenitors, illustrating that WNT signalling has different effects on various stages of the differentiation process.
Surfactant protein B deficiency, which causes lethal neonatal respiratory distress, was shown as an example of how iPSC-derived AEC2 cells could be used for disease modelling. Dermal fibroblasts from a patient with SFTPB-deficiency were reprogrammed into iPSCs and then the 121ins2 mutation was corrected by gene editing; subsequently, the gene-corrected iPSCs were differentiated into SFTPB-expressing and lamellar-body containing alveolospheres.

In addition to their use in disease models, human iPSCs can also be used to study human lung development, and thus complement studies with primary cells from human embryonic and fetal lungs. Furthermore, this provides an opportunity for improved maturation and validation of iPSC-derived alveolar cells.

Finally, William Zacharias completed the session by presenting an important study regarding the regeneration of the lung alveolus after diffuse lung injury, which is a complex and not uniform regionalised process in which the exquisitely organised alveolar architecture must be regenerated [26] and probably involves different models of regeneration processes. There are three cell types which are thought to be involved in alveolar regeneration: 1) in a mouse model of influenza injury, Sox2− negative epithelial cells delaminate from the airway, and migrate distally to create Krt5+ pods in the distal lung as a result of hypoxia, but are unable to recover a normal alveolar structure [26, 27]; 2) the bronchoalveolar stem cell that is present in the bronchoalveolar duct junction which gets activated after influenza injury and in vitro can be differentiated to both proximal and distal lineages [28, 29]; and 3) the AEC2 cell is the major stem cell in the alveolus, as it can both self-renew and differentiate into type 1 cells [30]. A WNT-responsive subpopulation within the AEC2 population, termed alveolar epithelial progenitor (AEP) [31, 32], acts as a major facultative progenitor cell in the distal lung which can differentiate and regenerate injured alveolar epithelium after influenza infection [32]. In addition, AEPs can be isolated by a specific cell surface marker and grown as 3D organoids in both human and mouse for further functional analysis [32].

### Group 3.2. airway cell biology and immunopathology

**Organoids, pluripotent stem cells and epigenetic editing**

This scientific symposium summarised the latest research on the use of organoids for drug screening and research, but also on novel tools to modify the genome and epigenome for therapeutic purposes. It was composed of five expert speakers showing their latest research in this field.

Jeffrey M. Beekman and his co-workers presented the advantages of using intestinal organoids as ex vivo models for personalised medicine in cystic fibrosis (CF). CF is a monogenetic disease characterised by mutations in a gene encoding an epithelial chloride channel (the cystic fibrosis transmembrane conductance regulator (CFTR)) causing thick mucus accumulation and resulting in increased susceptibility to infections [33]. The standard clinical end-points are currently spirometry (forced expiratory volume in 1 s (FEV1)) and sweat chloride concentration (SCC). Both of these are influenced by non-CFTR dependent factors, including environmental and genetic factors. These end-points have been useful for differential diagnosis, but it is essential to develop novel models for establishment of drug efficacy in individual patients. To this end, Beekman and co-workers used the intestinal organoid model, which is derived from easily accessible rectal biopsies and can thus be obtained from every individual. Moreover, organoids can be cultured for at least several months, recapitulating essential tissue features in a self-organising system. An in vitro forskolin-induced organoid swelling (FIS) assay, mediated by cAMP-dependent chloride pumping into the lumen, allowed for prospective drug screening with high predictive value, being fully CFTR-dependent [34, 35]. This FIS assay was used for: 1) in vitro typing of CFTR function in a newborn cohort; and 2) in vitro assessment of therapeutic response using multiple modulators in different CFTR genotypes [36]. Their work demonstrated that both the in vivo measurement of SCC and the in vitro FIS assay are able to distinguish CF phenotypes in the same way, but only FIS allows the identification of the most severe cases [36]. In addition, they observed exciting correlations between changes in FEV1 or SCC in vivo and changes in organoid swelling in vitro [36]. However, the two in vivo parameters did not correlate. They propose innovative CF care by generating organoids as living biobanks that allow for personalised scientific studies with better clinical care.

Other lung diseases, such as COPD or ILDs can unfortunately not be diagnosed with such an organoid-based system, due to the current lack of a functional readout. However, work presented by Melanie Königshoff and colleagues showed the suitability of lung organoids for studying signalling pathways involved in lung development, injury and repair. In both diseases epithelial damage is evident, and diminished epithelial progenitor renewal and regeneration is thought to contribute to a disturbed lung repair. Thus, their work aims to use alveolar organoids to gain insight into mechanisms underlying disease, and also to understand molecular events that control and drive normal repair in order to develop novel therapies [37–39]. Königshoff and colleagues used organoids derived from Epcam+ epithelial progenitors of murine lung tissue, supported by the addition of fibroblasts to the culture. Fibroblasts are
essential for organoid formation, although their exact role is still unclear [30, 40]. As explained above, WNT/β-catenin signalling is involved in many aspects of lung development [41] and is also implicated in abnormal repair responses [42–45]. To study β-catenin activity, they used a TCF/LEF-driven green fluorescent protein (GFP) reporter and sorted GFP high and low cells and assessed the role of WNT/β-catenin signalling in organoid formation. The colony forming efficiency of organoid formation was analysed by assessing total nuclei per sphere and the percentage of proliferative, Ki67+ cells per sphere, as well as expression of several differentiation markers. Interestingly, WNT/β-catenin signalling was involved in both alveolar and airway organoid initiation, but WNT was not necessary for their proliferative expansion. This may have important implications for COPD and specifically emphysema, as the activation of WNT/β-catenin signalling may thus be able to initiate or improve disturbed repair responses in the lung tissue of COPD patients. In addition, their recently published work suggests that regulation of retinoic acid signalling is essential for epithelial progenitor growth and differentiation during lung repair processes [46].

Besides the development of new disease models, the development of novel molecular biology tools is rapidly emerging. During this session, Patrick Harrison discussed the relatively new technology to modify genomes using the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) system. This complex of bacterial origin consists of the Cas9 protein, an exonuclease producing double strand DNA breaks, and a single guide RNA directing the Cas9 protein to a specific location in the genome [47]. Thus, in theory gene modifications can be induced by simply designing a single guide RNA for the gene of interest, and by adding a modified gene construct that will be incorporated into the DNA at the location of interest after the double strand break by homologous recombination. Depending on the envisaged effect, entire genes can be deleted, super-exon donors can be inserted to activate gene expression or single base pairs can be edited to correct mutations [48]. This very promising technology is currently mainly being investigated for the correction of monogenetic diseases, such as α1-antitrypsin deficiency (AATD). In one recent study, the authors have attempted to correct the AATD-causing amino acid substitution of a glutamine residue to a lysine residue in a mouse model using an adeno-associated vector system. Of note, even if the efficacy of gene editing in the cells was rather low (~3%), it resulted in a functional effect and increased levels of α1-antitrypsin in blood [49]. Although the first studies using CRISPR-Cas9 for genome modification seem encouraging, one has to keep in mind that the safety of this technology for use to treat human disease is not yet clarified and recent reports about CRISPR-Cas9 off-target effects (extensively reviewed in [50]) further indicate the need for caution in pushing this new technology to clinical studies.

Another intriguing application for the CRISPR-Cas9 system that was presented by Marianne Rots and co-workers is epigenetic editing. As compared with genome editing, epigenetic editing does not aim to introduce permanent alterations of the genome, but rather to correct aberrant epigenetic marks, in order to influence the expression or function of a specific gene. Epigenetic marks are defined as transient but mitotically stable changes to the DNA molecule, but not its sequence [51]. Mainly, these include DNA methylation, histone modifications (post-translational modifications such as acetylation of methylation) and non-coding RNAs [52]. Epigenetic marks have been described to be influenced by the environment (e.g. smoking) [53] and their alterations have been associated with many diseases to date, including COPD [52]. This has led to the development of a new therapeutic approach to correct these aberrant marks in order to restore or silence target gene expression, so-called epigenetic editing. In principle, an epigenetic editor consists of an epigenetic enzyme (such as a DNA-methyltransferase) and a DNA-targeting system to bring this editor to a specific location in the genome [54]. Thus, it is not surprising that the discovery of CRISPR-Cas9 has also revolutionised the field of epigenetic editing, due to the ease of targeting specific genomic regions. For this purpose, a deactivated Cas9 protein is used as double strand breaks are not needed for epigenetic editing; indicating a possible advantage in terms of safety and prevention of off-target effects. In one proof of principle study that identified methylation marks in the transcription factor SAM pointed domain containing ETS transcription factor, a major regulator of mucus secretion, in airway epithelial cells of patients with COPD [55], correction of those marks in human lung cell lines by epigenetic editing was able to reduce the mucus production [56]. However, as discussed above there is a need for further studies assessing the safety and in vivo efficacy of CRISPR-Cas9 based systems, and also to optimise delivery to primary cells [52].

Most of the research discussed above has been performed in in vitro models of single cell types or multicellular organoids. Thus, the last talk of this session, given by Martin Stampfl, focused on the optimisation of animal models for respiratory research. The presentation reflected the results of the ERS task force TF-2014-05 chaired by Martin Kolb and Philippe Bonniaud. The objectives of this task force were to provide a better understanding of the complexity of animal models, to improve scientific quality and efficacy, to avoid unethical animal research, to reduce research costs in experimental pulmonology, and to educate young scientists about proper design, with the right approach for the right question as well.
as execution, and interpretation of findings from animal models [57]. The final aim was to move towards the three Rs in animal experimental models: replace, reduce and refine. The results have recently been published as an ERS statement “Optimising experimental research in respiratory diseases” [57]. The purpose of the document is to provide guidelines to improve animal models in the respiratory medicine field. Asthma, pneumonia, COPD and idiopathic pulmonary fibrosis (IPF) are chronic diseases for which animal models are being used to gain insight into their pathogenesis and novel treatment options. However, the use of animal models is not always justified and the approach is often not appropriate. Many models have studied the effect of treatments as prevention, in diseases where that prophylactic treatment is not feasible in clinical practice, and a therapeutic intervention is what should be sought. An example of this is the bleomycin model for IPF that had been used since 1980 mainly for preventive treatment approaches, but was redirected for therapeutic intervention after 2007, which lead to successful applications in clinical practice [58–60]. It is of utmost importance to understand the similarities but also the differences between humans and animal models, as mechanistic pathways observed in vitro might not be replicable in vivo. Thus, the use of multiple models is recommended to facilitate reliable conclusions as there is no model that reflects every condition in the clinic and one has to take the weaknesses and errors of the individual model into account.

### Group 3.3 mechanisms of lung injury and repair

**New insights into the signalling pathways that underlie IPF pathophysiology**

Besides the use and development of novel models for respiratory disease, important findings relating to newly discovered novel therapeutic agents were discussed in an oral presentation session of scientific abstracts in Paris. As discussed above, IPF pathogenesis is a process of excessive lung tissue repair in response to injury that is considered a failure of normal tissue regeneration [61]. IPF is considered the most fatal and common disease among the ILDs [62], with a mean survival rate of 3.8 years [63]. Novel advances in understanding IPF pathophysiology, such as new insights into signalling pathways and mechanisms are the prerequisite for the development of novel therapies. Thus, the most important findings from presented abstracts in Paris are summarised in the following sections and grouped based on whether they relate to collagen deposition, metabolic dysfunction, extracellular vesicles or cellular senescence.

**Collagen deposition**

Fibroblast proliferation, migration and differentiation are described as central players that promote collagen deposition in IPF lungs [63]. The abstracts presented in Paris explored collagen deposition in IPF lungs through the following signalling pathways: 1) paired related homeobox 1 (PRRX1) transcription factor [64], 2) FK506-binding protein 10 (FKBP10) [65], and 3) activator protein 1 (AP-1) transcription factor Fra-2 [66].

In IPF, lung fibroblasts are known to express increased levels of activated transforming growth factor (TGF)-β that enhances matrix metalloproteinase production and promotes collagen deposition [67]. At the congress, fibroblast activation via transcription factor expression and collagen deposition in fibrotic lungs was discussed, which contributes to enhanced lung matrix stiffness. One of the abstracts showed PRRX1 transcription factor as a modulator of fibroblast proliferation, migration and differentiation [64]. Another abstract identified an increase of the transcription factor AP-1/Fra-2 in macrophages by RNA sequencing of lungs of a mouse model for bleomycin-induced lung fibrosis model [66]. Fra-2 expression was also associated with increased expression of collagen VI in the lungs of IPF patients [66]. Another abstract in the session showed that knockdown of FKBP10 attenuated the expression of collagens I and IV, thereby reducing fibroblast migration [65, 68, 69]. In line with this, FKBP10 is upregulated in IPF lungs [69]. Taken together, these three ERS abstracts showed new possible signalling pathways to target lung fibroblast phenotype and function, as well as matrix collagen deposition. However, more comprehensive studies describing the different types of collagen proteins, such as collagen IV, and their functional implication in the extracellular matrix deposition and their applicability to patients are warranted.

**Metabolic dysfunction**

Another important pathway driving IPF pathogenesis is metabolic cell dysfunction, which mediates fibroblast activity and death mechanisms [70]. Besides the epithelial–mesenchymal transition being suggested as a player in IPF pathogenesis, catalytic activity of fibroblasts may also contribute to lung fibrosis [71]. In particular, receptor tyrosine kinases and mitogen-activated protein kinases are suggested to play a role in fibrosis development. At the 2018 ERS congress the following signalling pathways involved in the metabolic changes in IPF were discussed: 1) growth hormone-releasing hormone (GHRH) [72], 2) MAP kinase phosphatase 5 (MKP5) [73], and 3) glutathione peroxidase 4 (GPX4) [74].
Mitochondrial dysfunction has been demonstrated to be increased in the plasma and bronchoalveolar lavage of IPF patients [75], which might regulate lung mesenchymal cell injury [76]. One study presented in Paris showed that GHRH is increased in the bleomycin-induced lung fibrosis model, and its antagonism decreases fibrosis by reduced expression of transcription factor 3 (STAT3) and nuclear factor (NF)-κB [72]. Moreover, another abstract from this session revealed decreased lung fibrosis by attenuation of TGF-β signalling in MAP kinase phosphatase 5 (MKP5) knockout mice [73]. Accordingly, the abstract presented by Tsubouchi et al. [74] from Japan showed TGF-β expression in IPF lungs induces lipid peroxidation and myofibroblast differentiation. In summary, these three abstracts showed the involvement of fibroblast metabolic dysfunction in IPF and suggest future research on this topic to develop novel therapies. Metabolic changes in IPF may suggest an interesting mechanism of cell damage, which can contribute to the matrix stiffness occurring in pulmonary fibrosis and can thus serve as a potential treatable trait.

**Extracellular vesicles**

In this new era of cellular communication, extracellular vesicles have gained important attention in the context of several lung diseases, including IPF pathophysiology [77]. Extracellular vesicles (EVs) are cell-free membrane structures that facilitate intracellular communication by allowing cells to exchange proteins, genetic material and lipids via vesicle trafficking. They can be roughly divided into three types: exosomes, microvesicles and apoptotic bodies [77], based on their size and cellular origin. The most extensively studied exosomes are ∼50–150 nm in size, are derived from the endosome and have therefore been characterised by enrichment of tetraspanin membrane proteins, such as CD9, CD63, and CD81. EVs also contain DNA, RNA and a variety of non-coding RNAs, which can be functionally transferred to a recipient cell [78]. At the ERS congress, recent findings on EV contribution to pulmonary fibrosis from human studies and mouse models were presented in abstracts [79]. EVs were reported to be increased in human lung pulmonary fibrosis, thereby inducing changes in fibroblasts function in IPF [79, 80]. In this study, exosomes were increased in the bronchoalveolar lavage of mice with bleomycin-induced fibrosis, as well as in IPF patients. In addition, the proliferation of human lung fibrotic fibroblasts was increased upon co-cultivation with exosomes. The observation that antibody-mediated neutralisation of WNT-5A, accompanied by EV destruction, decreased the fibroblast proliferation in this co-cultivation supported a role of the WNT-5A signalling pathway herein [79, 80]. Another abstract from this session showed that collagen IV is increased in EVs in a mouse model of pulmonary fibrosis [66]. EVs are of strong interest in tissue repair and regeneration and exosomes have already been shown to be a targetable pathway in IPF as they are associated with matrix deposition, and disease severity [79–81].

**Cell senescence**

Most patients diagnosed with IPF are usually older than 50 years and cell senescence has been demonstrated to contribute to IPF disease development [82]. Cell senescence markers, such as senescence-associated β-galactosidase, P21, cyclin dependent kinase inhibitor 2A (P16-INK4a) and DNA damage response have been demonstrated to be increased in injured epithelial cells in IPF [83]. Similarly, one of the abstracts in this session discussed p16 as a regulator of ageing-related diseases [84]. In this study, P16-INK4a knock-out mice were shown to protect epithelial alveolar type 2 cell injuries, collagen deposition and myofibroblast differentiation in fibrosis [84]. Thus, this pathway might be a promising target for IPF and other ageing-related diseases which affect the lung, such as COPD. Thus, cell senescence in IPF represents a good example of a disease target for the development of therapeutics.

**Future perspectives in IPF signalling pathways**

Newly identified mechanisms and the underlying signalling pathways associated with the pathophysiology of IPF are emerging from bench-to-bedside studies and vice versa. This new knowledge is being used to improve disease characterisation and patient phenotyping, thereby ultimately leading to personalised treatments. In addition, a comprehensive understanding of molecular, genetic and functional players involved in the pathophysiology of IPF will help to find new therapeutic targets, thereby improving translational medicine and patients’ treatment.

**Summary**

In summary, basic science is becoming increasingly visible at the annual ERS International Congress. Importantly this brings recent developments in basic science to a largely clinical audience and thereby provides a platform to discuss the translational value of recent studies in order for scientists and clinicians to jointly develop novel therapeutic approaches.
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