Editorial

New players in chronic lung disease identified at the European Respiratory Society International Congress in Paris 2018: from microRNAs to extracellular vesicles

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Introduction

Since the first description of microRNAs (miRNAs) in 1993 (1) a large and growing number of studies has explored their roles across a variety of biomedical research disciplines, including lung biology. According to GENCODE (version 27) (2), 1881 of the >7,500 human small non-coding RNAs are miRNAs. These 20–25 nucleotide-long, regulatory RNAs are involved in the translational regulation of gene expression principally via binding to miRNA recognition elements largely in the 3' untranslated regions of target mRNAs. Upon binding they can induce mRNA degradation, deadenylation or inhibition of their translation, leading to decreased target gene expression (3). Originally described to play important roles in developmental biology, miRNAs have since been found to have significant roles in a multitude of biological processes. Expression levels of miRNAs vary greatly between cells and tissues, and aberrant levels of miRNA are associated with many diseases in humans. In fact, these non-coding RNA molecules are now recognized as major regulators in the development and progression of various chronic lung diseases, including cystic fibrosis (CF), idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD) and asthma (4-9).

The role of non-coding RNAs in lung disease

IPF is a progressive lung disease most commonly diagnosed in older adults and characterized by interstitial lung tissue remodeling and rapid respiratory decline. This aging-associated disease has an unknown aetiology, however genetic polymorphisms and several occupational risk factors such as cigarette smoke, farming and pollutants have been strongly associated with IPF (10). Although, the pathogenesis of IPF is not fully understood, epithelial cell injury, fibroblast activation and extracellular matrix remodeling have been identified as key hallmarks of this disease for which limited therapeutic options exist (11).

While the in-depth mechanisms linking IPF to aging have not been fully elucidated, the disease is characterized by various aging-associated abnormalities; for example, mitochondrial dysfunction and telomere shortening. One abstract presented at the Congress in Paris investigated miRNA expression, focusing in particular on those with an anti-fibrotic role, in a mouse model of accelerated aging (12). The human gene Zmpste24 encodes a metallopeptidase that normally processes nuclear lamin A; and its absence has been shown to accelerate aging. Thus, Zmpste24-deficient mice represent a useful model to delineate aging-associated mechanisms contributing to the development of lung

fibrosis. Following bleomycin-induced lung injury aged Zmpste24-deficient mice showed less lung inflammation and attenuated lung fibrosis at 3 weeks compared with wild type (WT) counterparts of the same age. These characteristics occurred in parallel to increased lung expression of several anti-fibrotic miRNAs including miR-23a, miR-27a, miR-29a and miR-29b-1. Several targets of these miRNAs, including collagens, were reciprocally decreased. These results suggest that in the absence of Zmpste24, aged mice are protected from the development of bleomycin-induced lung fibrosis by the upregulation of anti-fibrotic miRNAs.

Another disease widely discussed at the Congress is COPD, which is currently the fourth leading cause of death in the world. The disease is characterized by a persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response of the airways and lungs to noxious particles or gases, e.g., cigarette smoke (CS) (13). The main cause of COPD in Europe and the USA is cigarette smoking or exposure to second-hand smoke/passive smoke exposure. Several studies have focused on determining how miRNAs play a role in cigarette smoke-induced inflammation (7,14). miR-155, for example, is a miRNA increased in the lungs of CS-exposed mice, which is also elevated in the lungs of smokers with and without COPD. miR-155 has known roles in inflammation (15). Now, the authors of an abstract at the European Respiratory Society (ERS) Congress examined the role of miR-155 in inflammation in miR-155 knock out (KO) mice exposed to CS (16). Compared to WT C57BL/6J mice exposed to CS for a 4-week period, miR-155 KO mice had lower neutrophil, dendritic cell, innate lymphoid cell and CD4+ and CD8+ T lymphocyte counts in broncho-alveolar lavage (BAL) fluid. Furthermore, intrapulmonary Th1, Th2 and Th17 subsets, which were increased by CS exposure in WT mice, were reduced in the miR-155 KO mice. CS-induced increase in CXCL1, CXCL2, CCL2, IL-6, IL-1β and TNF was also attenuated in miR-155 KO versus WT mice. Taken together these results demonstrate an important role for miR-155 in CS-induced inflammation.

In addition to small non-coding RNAs such as miRNAs, there are various classes of human long non-coding RNAs (lncRNA) numbering in excess of 15,500 different transcripts (17). Circular RNAs (circRNAs) are an abundant subset of lncRNAs that have not been studied in detail however, their roles can include miRNA sponging [by acting as competing endogenous RNA (ceRNA)], transcriptional regulation and sequestration of ribonucleoproteins. Regarding the lung, very few studies focusing on circRNA have been performed so far. One abstract in Paris reported the effect of cigarette smoke extract (CSE) on the expression of circRNA in the distal lung (18). Primary human alveolar epithelial cells were exposed to CSE for 24 hours and the expression profiles of 10,738 circRNA were analysed by microarray. Whilst negatively affecting the overall length and quality of the circRNAs, CSE stimulation also upregulated 65 circRNAs and downregulated 100 by more than two-fold. Bioinformatics analysis suggested that the circRNAs could influence expression of many genes including those involved in response to histidine metabolism, DNA replication, and glutathione metabolism pathways, by interacting with the corresponding miRNAs.

Extracellular vesicles mediate cellular communication in chronic lung diseases

While miRNAs and other non-coding RNAs are produced locally in certain cell types, it is now well established that they can be secreted from parent cells in extracellular vesicles (EV) enabling a functional transfer to other cells and tissues as means of short- and long range inter-cellular communication (19). EVs are a heterogeneous group of lipid vesicles with a typical diameter ranging 30 nm up to 1 µm including exosomes (60–120 nm vesicles with an endosomal origin) as well as larger micro-vesicles (150 nm–1 µm). These small vesicles are actively secreted by cells and their cargo includes lipids, proteins as well as nucleic acids, such as DNA, RNA and miRNA (20). Initially described in cancer, a growing body of literature demonstrated alterations of EVs and their cargo in lung disease (21). Although roles of EVs have just begun to unravel, miRNA have emerged as a key cargo mediating functional effects of EVs on recipient cells in diseases, such as asthma or lung cancer (21-23). Along this line, the authors of an abstract presented at the ERS Congress 2018, investigated the potential of EVs secreted into the airway lumen to act as miRNA transporter (24). The authors focused on alveolar macrophages, the most abundant immune cell in the lung with phagocytic properties, as the EV target cell. Using a small RNA-sequencing technique, they profiled the miRNA content of EVs from asthmatic or cancerous patients and compared miRNA expression of patient-derived macrophages exposed or not to EVs isolated from patients with chronic lung diseases. Macrophages took up miRNA containing EVs leading to cellular transcriptome modifications. Of note, exosomal miRNA further affected macrophage functions such as chemoattraction. This study

provides a proof of concept that EVs isolated within the airway lumen can target macrophages and impact their cellular function. This study represents a first step towards our understanding of the complexity of EV origin and destination in the lung. Future investigations examining the cargo of EVs derived from different cell types, as well as their potentially distinct recipient cells will further advance our knowledge on these emerging extracellular mediators.

One possible application of EV-enclosed miRNA profiles is the development of biomarker signatures for pulmonary diseases, such as IPF. Currently, classical methods to investigate IPF rely on the use of patient-derived samples collected using invasive methods (biopsies or BAL fluid), thus the discovery of non-invasive biomarkers is very appealing. Along this line, induced sputum of patients with IPF has already been reported as a complementary method to BAL fluid (25). In one of the ERS abstracts, the authors profiled miRNAs from EVs isolated from sputum of patients with IPF (26). Interestingly, using miRNA arrays and qPCR, the authors observed that 21 miRNAs are specifically dysregulated in EVs isolated from sputum of IPF patients compared with healthy donor EVs. While these data are encouraging, it has to be noted that they have been obtained from a limited number of patients (10 IPF and 10 control) and need to be confirmed using larger cohorts in future studies.

Next to their use as potential biomarkers, EVs might also be involved in molecular mechanisms underpinning IPF pathogenesis. As discussed above EVs can carry proteins that can initiate receptor-mediated cellular functions on the recipient cell. Recent studies indicate that these proteins can include hydrophobic and normally short-range Wingless/integrase-1 (Wnt) signaling proteins. The Wnt signaling pathway consists of secreted glycoproteins that interact with several transmembrane receptors and co-receptors on their target cells to initiate an intracellular response (27). In addition to extracellular transporters and intracellular transmission, EVs may play an important role allowing transport of Wnt signaling proteins resulting in a long-distance movement of these lipid-modified proteins with strong membrane affinity signaling molecules (28-30).

The type of EVs that are released and the content of their cargo may depend on the type of stimuli and the severity of the disease (31). The presence of EVs in BAL fluid was first discovered by Admyre *et al.* in 2003 (32). At this year's ERS Congress, one abstract presented a pre-clinical study investigating the protein cargo in EVs derived from BAL fluid of bleomycin-treated mice using an unbiased proteomic based approach (33). The authors reported an increase in EV number and protein in BAL from fibrotic mouse lungs compared to controls. Several proteins were identified in BAL-derived EVs such as chitinase-like protein 3 precursor (Chil3) or collagen proteins. The function of the BAL-derived EVs was further evaluated *in vitro* by using a murine 3D tissue culture system and alveolar epithelial cells. *In vitro*, EVs induced metabolic activity in lung tissue together with transcriptomic changes in epithelial cells and altered Wnt signaling. Of note, impaired Wnt signaling was initially identified in IPF and has been demonstrated to be aberrantly active both in human and experimental pulmonary fibrosis (34,35).

Another EV-related Congress highlight abstract investigated acute lung injury (ALI), which is a severe lung disorder characterized by an acute inflammation following beyond others for example a bacterial infection by i.e., *Streptococcus pneumoniae* (36). *Streptococcus pneumoniae* is one of the major causes of bacterial pneumonia and pneumolysin (PLY) has been demonstrated to be its major virulence factor. PLY is thought to disrupt the lung epithelial and endothelial barriers, leading to permeability edema and activation of the immune system. It activates the immune system by activating epithelial cells which leads to an induction of danger-associated molecular patterns (DAMP) release (37). One of the presented abstracts aimed to investigate if PLY can induce epithelial cells to release mitochondria enclosed in microvesicles (MVs) (38). In order to do this, human alveolar epithelial cells (A549) and human lung tissue were treated with live *Streptococcus pneumonia* or PLY. MVs were isolated from the conditioned medium through differential centrifugation. Exposure of live *Streptococcus pneumonia* to A549 or human lung tissue resulted in an increase of extracellular release of Annexin V/EPCAM positive MVs compared to the control. Furthermore, Annexin V/EPCAM positive MVs released from *Streptococcus pneumonia* exposed A549 cells also stained positively for Mito-tracker. The presence of mitochondrial content was further confirmed by Tom20 (mitochondrial protein) expression of the released MVs. This study concludes that PLY triggers the epithelial cells to release MVs containing mitochondria, which in turn may act as mitochondrial DAMPs (mtDAMPs). Accumulating evidence indicates that mtDAMPs can activate the innate immune response, inducing a pro-inflammatory immune response, and has been associated with dysregulated processes in human diseases (39). This study gives important insight in the potential of EVs to transport mitochondrial cargo,

which might contribute to activation of the immune system and should be further investigated.

Conclusions

Collectively, the studies presented at the Annual Congress of the ERS in Paris 2018 provide new insights into the expression and function of non-coding RNAs in IPF and COPD, and shed new light on their inter-cellular transport via EVs. Novel data presented at the Congress also suggest that EVs might be functionally implicated in other pulmonary pathologies; i.e., by influencing Wnt signaling in IPF or by transferring mtDAMPs and virulence factors in ALI. Finally, these newly identified regulators and processes might play an essential role in the development of respiratory diseases and/or represent clinical biomarkers, both highlighting the need for further studies in this field.

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Footnote

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References

1. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993;75:843-54.
2. Harrow J, Frankish A, Gonzalez JM, et al. GENCODE: the reference human genome annotation for The ENCODE Project. Genome Res 2012;22:1760-74.
3. Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nat Rev Genet 2011;12:99-110.
4. Oglesby IK, Chotirmall SH, McElvaney NG, et al. Regulation of Cystic Fibrosis Transmembrane Conductance Regulator by MicroRNA-145, -223, and -494 Is Altered in F508 Cystic Fibrosis Airway Epithelium. J Immunol 2013;190:3354-62.
5. Oglesby IK, Bray IM, Chotirmall SH, et al. miR-126 is downregulated in cystic fibrosis airway epithelial cells and regulates TOM1 expression. J Immunol 2010;184:1702-9.
6. Lino Cardenas CL, Henaoui IS, Courcot E, et al. miR-199a-5p Is upregulated during fibrogenic response to tissue injury and mediates TGFbeta-induced lung fibroblast activation by targeting caveolin-1. Scott HS, editor. PLoS Genet 2013;9:e1003291.
7. Conickx G, Mestdagh P, Avila Cobos F, et al. MicroRNA Profiling Reveals a Role for MicroRNA-218-5p in the Pathogenesis of Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med 2017;195:43-56.
8. Hassan T, Carroll TP, Buckley PG, et al. miR-199a-5p silencing regulates the unfolded protein response in chronic obstructive pulmonary disease and α1-antitrypsin deficiency. Am J Respir Crit Care Med 2014;189:263-73.
9. Maes T, Cobos FA, Schleich F, et al. Asthma inflammatory phenotypes show differential microRNA expression in sputum. J Allergy Clin Immunol 2016;137:1433-46.
10. Wolters PJ, Blackwell TS, Eickelberg O, et al. Time for a change: is idiopathic pulmonary fibrosis still idiopathic and only fibrotic? Lancet Respir Med 2018;6:154-60.
11. Lederer DJ, Martinez FJ. Idiopathic Pulmonary Fibrosis. Longo DL, editor. N Engl J Med 2018;378:1811-23.
12. Selman Lama M, Pardo A, Calyeca K, et al. Premature aging in Zmpste24 deficient mice protects them from bleomycin induced lung fibrosis through upregulation of antifibrotic microRNAs. Eur Respir J 2018;52. [Epub ahead of print].
13. De Rose V, Molloy K, Gohy S, et al. Airway Epithelium Dysfunction in Cystic Fibrosis and COPD. Mediators Inflamm 2018;2018:1309746.
14. Conickx G, Avila Cobos F, van den Berge M, et al. microRNA profiling in lung tissue and bronchoalveolar lavage of cigarette smoke-exposed mice and in COPD patients: a translational approach. Sci Rep 2017;7:12871.
15. Mashima R. Physiological roles of miR-155. Immunology 2015;145:323-33.
16. de Smet E, Mestdagh P, Avila Cobos F, et al. The role of microRNA-155 in cigarette smoke-induced inflammation. Eur Respir J 2018;52. [Epub ahead of print].
17. Glasgow AMA, De Santi C, Greene CM. Non-

coding RNA in cystic fibrosis. Biochem Soc Trans 2018;46:619-30.
18. Ni Z, Shen YC, Wen FQ. Cigarette smoke extract alters genome-wide profiles of circular RNAs in primary human alveolar epithelial cells . Eur Respir J 2018;52. [Epub ahead of print].
19. Valadi H, Ekström K, Bossios A, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007;9:654-9.
20. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol 2018;19:213-28.
21. Nana-Sinkam SP, Acunzo M, Croce CM, et al. Extracellular Vesicle Biology in the Pathogenesis of Lung Disease. Am J Respir Crit Care Med 2017;196:1510-8.
22. Levänen B, Bhakta NR, Paredes PT, et al. Altered microRNA profiles in bronchoalveolar lavage fluid exosomes in asthmatic patients. J Allergy Clin Immunol 2013;131:894-903.
23. Parimon T, Brauer R, Schlesinger SY, et al. Syndecan-1 Controls Lung Tumorigenesis by Regulating miRNAs Packaged in Exosomes. Am J Pathol 2018;188:1094-103.
24. Sanchez-Elsner T, Lekkas A, Mellows T, et al. Exosomal microRNAs in the lung: eliciting long-term intercellular communication. Eur Respir J 2018;52. [Epub ahead of print].
25. Antoniou KM, Alexandrakis M, Tzanakis N, et al. Induced sputum versus bronchoalveolar lavage fluid in the evaluation of patients with idiopathic pulmonary fibrosis. Respiration 2005;72:32-8.
26. Guiot J, Njock MS, Henket M, et al. Sputum exosomal microRNAs in IPF. Eur Respir J 2018;52. [Epub ahead of print].
27. Skronska-Wasek W, Gosens R, Königshoff M, et al. WNT receptor signalling in lung physiology and pathology. Pharmacol Ther 2018;187:150-66.
28. Takada S, Fujimori S, Shinozuka T, et al. Differences in the secretion and transport of Wnt proteins. J Biochem 2017;161:1-7.
29. Parchure A, Vyas N, Mayor S. Wnt and Hedgehog: Secretion of Lipid-Modified Morphogens. Trends Cell Biol 2018;28:157-70.
30. Gradilla AC, Simon E, Aguilar G, et al. From intra- to extracellular vesicles: extracellular vesicles in developmental signalling. Essays Biochem 2018;62:215-23.
31. Lee H, Abston E, Zhang D, et al. Extracellular Vesicle: An Emerging Mediator of Intercellular Crosstalk in Lung Inflammation and Injury. Front Immunol 2018;9:924.
32. Admyre C, Grunewald J, Thyberg J, et al. Exosomes with major histocompatibility complex class II and co-stimulatory molecules are present in human BAL fluid. Eur Respir J 2003;22:578-83.
33. Burgy O, Martin-Medina A, Mayr CH, et al. Extracellular vesicles from broncho-alveolar lavage act on cellular behavior during lung fibrosis. Eur Respir J 2018;52. [Epub ahead of print].
34. Chilosi M, Poletti V, Zamò A, et al. Aberrant Wnt/beta-catenin pathway activation in idiopathic pulmonary fibrosis. Am J Pathol 2003;162:1495-502.
35. Königshoff M, Balsara N, Pfaff EM, et al. Functional Wnt Signaling Is Increased in Idiopathic Pulmonary Fibrosis. Schmidt HHHW, editor. PLoS One 2008;3:e2142.
36. Johnson ER, Matthay MA. Acute lung injury: epidemiology, pathogenesis, and treatment. J Aerosol Med Pulm Drug Deliv 2010;23:243-52.
37. Czikora I, Alli AA, Sridhar S, et al. Epithelial Sodium Channel-α Mediates the Protective Effect of the TNF-Derived TIP Peptide in Pneumolysin-Induced Endothelial Barrier Dysfunction. Front Immunol 2017;8:842.
38. Letsiou E, Teixeira-Alves LG, FatykhovaD, et al. Lung epithelial microvesicles induced by pneumolysin contain mitochondrial cargo. Eur Respir J 2018;52. [Epub ahead of print].
39. West AP. Mitochondrial dysfunction as a trigger of innate immune responses and inflammation. Toxicology 2017;391:54-63.

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