Extracellular vesicles in lung health, disease, and therapy

Mark J. McVey,1,2,3,4 Mazharul Maishan,1 Kaj E. C. Blokland,5,6,7 Nathan Bartlett,5 and Wolfgang M. Kuebler1,2,8,9

1Keenan Research Centre for Biomedical Science, St. Michael’s Hospital, Toronto, Ontario, Canada; 2Department of Physiology, University of Toronto, Toronto, Ontario, Canada; 3Department of Anesthesia, University of Toronto, Toronto, Ontario, Canada; 4SickKids Department of Anesthesia and Pain Medicine, Toronto, Ontario, Canada; 5School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, New South Wales, Australia; 6National Health and Medical Research Council Centre of Research Excellence in Pulmonary Fibrosis, Sydney, New South Wales, Australia; 7Department of Pathology and Medical Biology, Groningen Research Institute for Asthma and COPD, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands; 8Department of Surgery, University of Toronto, Toronto, Ontario, Canada; and 9Institute of Physiology, Charité-Universitätsmedizin Berlin, Berlin, Germany

Submitted 13 December 2018; accepted in final form 12 March 2019

McVey MJ, Maishan M, Blokland KE, Bartlett N, Kuebler WM. Extracellular vesicles in lung health, disease, and therapy. Am J Physiol Lung Cell Mol Physiol 316: L977–L989, 2019. First published March 20, 2019; doi:10.1152/ajplung.00546.2018.—Both physiological homeostasis and pathological disease processes in the lung typically result from complex, yet coordinated multicellular responses that are synchronized via paracrine and endocrine intercellular communication pathways. Of late, extracellular vesicles have emerged as important information shuttles that can coordinate and disseminate homeostatic and disease signals. In parallel, extracellular vesicles in biological fluids such as sputum, mucus, epithelial lining fluid, edema fluid, the pulmonary circulation, pleural fluid, and lymphatics have emerged as promising candidate biomarkers for diagnosis and prognosis in lung disease. Extracellular vesicles are small, subcellular, membrane-bound vesicles containing cargos from parent cells such as lipids, proteins, genetic information, or entire organelles. These cargos endow extracellular vesicles with biologically active information or functions by which they can reprogram their respective target cells. Recent studies show that extracellular vesicles found in lung-associated biological fluids play key roles as biomarkers and effectors of disease. Conversely, administration of naïve or engineered extracellular vesicles with homeostatic or reparative effects may provide a promising novel protective and regenerative strategy to treat lung disease. To highlight this rapidly developing field, the American Journal of Physiology-Lung Cellular and Molecular Physiology is now launching a special Call for Papers on extracellular vesicles in lung health, disease, and therapy. This review aims to set the stage for this call by introducing extracellular vesicles and their emerging roles in lung physiology and pathobiology.

extracellular vesicles; health; lung disease

INTRODUCTION

Despite their known existence for over 50 years (39), extracellular vesicles (EVs) have only recently gained significant traction in lung research over the past 5 years. As such, EVs have been recognized as important novel biomarkers, effectors of pathomechanisms, and therapeutic targets in a range of lung diseases. In the lung, EVs can be released from numerous parent cells both spontaneously and in response to specific stimuli such as inflammation or mechanical stress. EVs mediate micrometer- to nanometer-level intercellular communication within biological fluids, a feature that is of particular relevance to the respiratory tract, which contains numerous biological fluid niches such as sputum, mucus, epithelial lining fluid, edema fluid, the pulmonary circulation, pleural fluid, and lymphatics (Table 1).

In response to this rapidly emerging field, the American Journal of Physiology-Lung Cellular and Molecular Physiology is now issuing a special Call for Papers on extracellular vesicles in lung health, disease, and therapy. To start this special call off, this review highlights the evolving physiological, pathophysiological, and clinical relevance of EVs in the lung. Following a brief introduction of EVs, we will focus on three major, rapidly developing facets of pulmonary EV research, namely, EVs’ capacity as biomarkers, as effectors of disease, and as potential therapeutics (79).
Disease | Biological Fluid Compartment of EV Sampling and Key Findings | Reference
--- | --- | ---
Asthma | ↑ PMVs (blood), 11 different EXO miRNAs between asthma and healthy controls (exhaled breath), ↑ eosinophil | (25, 32, 76)
COPD | ↑ EMVs/epMVs (sputum/blood), ↑ EXO miRNA from epithelial cells leads to myofibroblast differentiation (BALF) | (40, 51)
ARDS | ↑ LMVs correlates with survival (blood/BALF), ↓ MVs (blood) | (36, 106)
IPF | ↑ EXO miRNA correlates with mortality (blood) | (69)
Lung cancer | ↑ EXOs correlates with cancer (blood/sputum/pleural fluid/saliva) | (65, 112)
Sarcoidosis | ↑ EXO vitamin D-binding protein correlates with sarcoidosis (BALF) | (72)
Scleroderma | ↓ LMVs + ↑ EMVs correlates with poor outcomes in scleroderma (blood) | (46)
Tuberculosis | ↑ EV miRNA correlates with tuberculosis (pleural fluid) | (65)
Pneumonia | ↑ EV miRNA correlates with pneumonia (pleural fluid) | (65)
PH | ↑ EMVs correlates with poor PH outcomes, poor right heart function (blood/urine) | (3, 102)
PE | ↑ PMVs correlates with PE (blood) | (44)

ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease; EMVs, endothelial microvesicles; epMVs, epithelial cell microvesicles; EVs, extracellular vesicles; EXOs, exosomes; IPF, idiopathic pulmonary fibrosis; LMVs, leukocyte microvesicles; miRNA, micro-RNA; MVs, microvesicles; PE, pulmonary embolism; PH, pulmonary hypertension; PMVs, platelet microvesicles; ↑ , increase in; ↓ , decrease in.

**EXTRACELLULAR VESICLES**

EVs represent an inclusive overarching hierarchical term describing a range of small (less than or equal to ~1–2 μm) subcellular membrane-enclosed vesicles of differing composition, origin, and endowment with lipids, sugars, proteins (both surface markers and internal cargos), genetic material (DNAs and RNAs), or even intact organelles (mitochondria; Fig. 1; 45, 79). Within the spectrum of EV subgroups, vesicles are stratified on the basis of size, the mechanism by which they are formed, and their cargos or surface markers. In terms of EV size, apoptotic bodies (ABs; greater than or equal to ~1 μm) are typically larger than microvesicles (MVs; ~100–1,000 nm; also known as microparticles but referred to as MVs for the remainder of this review), which are both larger than exosomes (EXOs; less than or equal to ~100 nm). ABs are formed as cellular products of apoptosis, MVs result from membrane blebbing from the cell surface, and EXOs are released from multivesicular bodies (MVBs) within parent cells. EV-specific enrichment of certain markers or cargos helps stratify different EVs: ABs contain histones and genomic DNA; MVs contain surface markers characteristic for their specific parent cells, such as cluster of differentiation 41 (CD41, or integrin-αIIb) on platelet MVs; and EXOs contain tetraspanins [CD9, CD63, CD81, and heat shock 70-kDa protein (HSP70)] as well as elevated acetylcholinesterase activity (10, 14, 58, 79).

![Fig. 1. Extracellular vesicles vary in size (apoptotic bodies > microvesicles > exosomes). Different subtypes of extracellular vesicles are formed and released from distinct mechanisms such as during cell apoptosis (apoptotic bodies), cell membrane blebbing (microvesicles), or from multivesicular bodies (exosomes) fusing with the plasma membrane. Apoptotic bodies can contain histones, genetic material (DNA), and intact or fragments of organelles, in part because of their large size. Microvesicles can contain cell-of-origin surface markers and may be formed by elevated intracellular calcium ([Ca²⁺]). Exosomes are formed by invaginating endosomes and, because of their intracellular origin, contain tetraspanins and heat shock proteins. PS, phosphatidylserine. [Components of this figure have been obtained and modified from Servier Medical Art, https://smart.servier.com, with permission under the Creative Commons Attribution 3.0 Unported License.]
Within the lung all three subtypes of EVs are relevant, though this review focuses primarily on MVs and EXOs. Though present expert consensus statements encourage use of the global term EV, regardless of size and subtype variability, we will specify MVs or EXOs where relevant and possible based on the descriptions from primary studies cited throughout this article.

**EV Formation and Clearance**

In general, EVs in specific biological fluids represent a sample of accessible EV-forming parent cells. This is why, for example, in healthy human blood, where platelets are the most numerous circulating cell type, platelet MVs ($10^5$–$10^{12}$/ml) are the most abundant type of EVs (56). EVs are estimated to circulate in the order of minutes in blood and organs such as the lung before being taken up by the reticuloendothelial system (20), in particular by hepatic Kupffer cells (122), albeit circulating half-life and uptake may vary, e.g., under inflammatory conditions (113a). The lungs are of particular interest as both a source of EVs and a target for EVs’ functional effects. Unlike blood, which typically contains a mixture of EVs from lungs, extrapulmonary organs, and circulating blood cells, biological fluids specific to the lung such as pleural fluid and bronchoalveolar lavage fluid (BALF) predominantly contain lung-specific sources of EVs. Triggers for EV formation vary and may differ in effect between parent cells. Some EVs are produced constitutively, although stress, infection, or normal physiological processes can at least transiently increase circulating levels (31, 79). Just as diseases and injuries are associated with elevations in EVs, physiological activity such as exercise can increase EV levels (31, 56). Generally speaking, ABs are formed as the dying cell breaks apart during the late stages of apoptosis, can contain organelar and nuclear fragments, and eventually become phagocytosed and their contents recycled (26). In contrast, MVs are released by healthy cells through different regulated processes where the plasma membrane blebs outward and is pinched to release the MV (84). The best-studied mechanism of MV formation is triggered by calcium influx, which causes the loss of bilayer phospholipid asymmetry and the externalization of phosphatidylserine (PS), which ultimately induces plasma membrane blebbing (79). EXOs are formed inside MVBs from intracellular contents as well as endocytosed materials and are released by MVB budding to and inward clefting of the plasma membrane (38, 107). Having said this, specific subsets of EVs can undergo distinct changes in numbers and composition in specific biological fluids in both health and disease conditions due to acute or chronic perturbations above or below a tonic/basal rate of production, generating a dizzying complexity when one attempts to decipher a complete “EVome.”

**EV Analysis**

EV science is rapidly progressing, in part aided by improvements in techniques for accurately isolating, characterizing, and sizing EVs. EVs are typically collected from biological fluids and then undergo isolation and/or enrichment. Collection and processing (differential centrifugation/filtration) of EVs require care to minimize contamination with cells, crystals, or debris as well as erroneous artificial (postcollection) generation of EVs (98). Though there is an abundance of tools available to analyze EVs, each has relative strengths and weaknesses; for example, flow cytometry is high throughput but lacks sensitivity to detect small EVs, whereas techniques such as electron microscopy offer exquisite resolution but can only assess small numbers of EVs (117). For analysis of EV cargo, genomic arrays have been widely used to detect mRNAs, micro-RNAs (miRNAs), and more recently, long noncoding RNAs (54), whereas comprehensive proteomic and lipodemic analyses by mass spectrometry have only recently become implemented (101). Advances in EV science pertaining to the preclinical collection and processing of EVs as well as their characterization have been recently summarized by the International Society for Extracellular Vesicles as “Minimal Information for Studies of Extracellular Vesicles 2018” with the goal of establishing minimal criteria for investigation and publication of EV studies (115). It is strongly encouraged that authors submitting papers in answer to this call for publications should adhere to these guidelines when reporting the details of their studies.

**BIOMARKERS OF LUNG DISEASE**

Ideal biomarkers are easily measurable, accessible, reproducible, and accurate signals that have strong predictive association with a specific physiology or disease. With increasing interest in precision medicine, the search for specific biomarkers of lung disease is intensifying. Traditional diagnostic lung biomarkers such as pulmonary function tests (PFTs) and imaging (X-rays/computed tomography scans and ultrasound) satisfy only a few “ideal biomarker” criteria such as accessibility and ease of collection and have drawbacks such as exposure to ionizing radiation (X-rays) or being subjective because of their reliance on patients’ efforts (PFTs). On this background, EVs are presently emerging as promising biomarker candidates for a number of airway, alveolar, interstitial, and vascular lung diseases that currently have little to no reliable means of predicting prognoses, response to therapy, or outcomes.

**Biomarkers of Lung Airway Disease**

Asthma’s classic biomarker is PFTs in conjunction with methacholine challenge testing. Unlike other non-EV inflammatory markers such as TNF-α or C-reactive protein, platelet MVs are selectively elevated in blood of patients with asthma compared with nonasthmatic matched controls (25). Smaller than MVs, EXO formation from eosinophils isolated from patients’ blood has also been shown to be elevated in patients with asthma compared with healthy subjects (76). EXOs’ cargos, specifically miRNAs, make them especially attractive as biomarkers because the uniqueness of miRNA types and abundance increases EXO specificity for diseases such as asthma (32). Smoking, the major risk factor for chronic obstructive pulmonary disease (COPD), is a prime example of how the cargo or characteristics of EVs may improve their utility as a biomarker: Although absolute concentrations of EXOs are similar between smokers and nonsmokers, EXOs’ miRNA content in BALF from smokers shows key differences with functional relevance for EXO-mediated bronchial epithelial cell responses (40). Beyond the cargo, the parent cell of origin is an important distinction that may increase the value of EVs as useful biomarkers, e.g., in COPD (51).
Biomarkers of Alveolar Disease

Acute respiratory distress syndrome (ARDS), characterized by alveolar flooding with proteinaceous fluid, is typically associated with increased levels of leukocyte-derived MVs in the BALF, with higher levels in both blood or BALF positively predicting survival (36). Alternatively, though studies in mice show elevations in alveolar MVs associated with acute lung injury (80), a study of 280 critically ill patients showed that patients who went on to develop ARDS actually had lower “total” numbers of plasma MVs; hence quantification of both MV subsets as well as entire MV populations may act as a biomarker (106).

Biomarkers of Interstitial-Parenchymal Disease

In both patients with idiopathic pulmonary fibrosis (IPF) and mouse models of lung fibrosis, blood EXO miRNA (miR)-21-5p levels correlate with disease severity and mortality and, as such, present a promising candidate as a biomarker (69). Lung-related proteins from sputum MVs and EXOs may be useful biomarkers for lung cancer (112). Lower concentrations of endothelial and leukocyte MVs in plasma appear to correlate with lower pulmonary diffusing capacity in scleroderma, and BALF EXO-derived vitamin D-binding protein is emerging as a relatively specific marker for lung sarcoidosis (46, 72).

Biomarkers of Pleural Disease

Developing a means of conducting a liquid biopsy capable of aiding in diagnosing pleural effusions would help initiate timely focused therapy. Early evidence of this possibility includes specific patterns of miRNA in EVs from pleural fluid that show unique signatures for patients with pneumonia distinct from patients with tuberculosis or lung cancer (65).

Biomarkers of Pulmonary Vascular Disease

Circulating blood endothelial MVs have been reported as being higher in patients with pulmonary hypertension (PH) who had worse complication rates, which could become a useful sentinel to guide caregivers to customize care (3). Endothelial MVs have also been detected in urine and are again elevated in PH and correlate with ultrasound findings such as changes in the tricuspid annular plane systolic excursion (102). For patients with pulmonary embolism, plasma platelet MVs have been shown to be elevated (44).

Overall, EVs show potential promise as biomarkers over a range of different lung diseases. Caution is, however, warranted when interpreting present clinical studies as the majority of available studies show association of EV (subtype) numbers or cargo with specific diseases, yet very few studies have so far addressed the diagnostic and prognostic value of EVs, their ability to discriminate between different lung diseases (rather than healthy control patients), or their usefulness to guide individual, personalized medicine. Further complexity is added by the present limitations of EV analysis and data interpretation in the clinical setting. Number and size distributions of EVs do not convey information on their cellular origin and may vary depending on factors such as magnitude/chronicity of the stressor(s) on parent cells, preclinical handling steps of collected EVs, and the methods used for enumeration/sizing EVs, which presently limits the utility of these EV parameters as biomarkers. On the other hand, using EV cargos as a means of establishing specificity for EVs as biomarkers is challenging as the cargos may change over time, making a single assessment potentially unrepresentative of a specific pulmonary disease state. Finally, even the use of phenotypic (parent cell) surface markers of EVs to enhance their specificity as biomarkers may prove inexpedient as 1) MV-sized EVs are small and have a low antigenic density, making detection of surface markers challenging, whereas 2) EXO-sized EVs are even smaller and (as they are formed from intracellular compartments and then extruded) lack typical cell surface markers, requiring characterization of intravesicular cargos such as genetic materials to show inter-EXO specificity, for which, again, limitations apply in terms of temporal variability as discussed above.

MECHANISMS OF LUNG DISEASE

The present interest in EVs relates as much to their potential as biomarkers as it does to their putative mechanistic role in the onset and propagation of disease. Via their membrane composition in terms of lipids or antigens, as well as via their cargo including nucleic acids or mitochondria, EVs act as information shuttles that convey signals from parent to target cells with the ability to modulate or “reprogram” the latter (130). As such, EVs provide a versatile and efficient means to communicate information between cells over short (paracrine) or long (endocrine via the blood or exocrine into the urine, mucus, etc.) distances. Yet analogously, EVs may also disseminate disease processes to juxtaposed cells as well as remote organs. With no intention for completeness, we briefly highlight this emerging concept for a few major lung diseases.

Lung Airway Disease

In different forms and models of lung airway disease, EVs have been found to promote airway remodeling and disseminate inflammation. Specifically, eosinophil-derived EXOs from subjects with asthma, yet not healthy controls, induce apoptosis and prevent wound closure in airway epithelial cells while enhancing smooth muscle cell proliferation, thus recapitulating in vitro key features of asthmatic airway remodeling with epithelial injury and increased muscularization (15). In patients with COPD, circulating EXOs contain increased levels of inflammatory cytokines, in particular IL-8 and IL-1β, as well as the proinflammatory Wnt ligand Wnt5a, which, in mice, disseminate inflammation to extrapulmonary organs such as the thymus, liver, and spleen (28). Finally, MVs isolated from sputum of patients with cystic fibrosis (CF) elicit peri-bronchial and perivascular inflammation when instilled intratracheally in mice (92). On the basis of the observation that MVs from CF sputum yet not from CF blood showed LPS activity, the authors of the latter study concluded that the proinflammatory effect of MVs in CF airways may be related to the binding of shed LPS, yet this direct mechanistic link remains to be proven.

Alveolar Disease

Endothelial-derived MVs are elevated in animal models of acute lung injury (63, 113), and in patients with ARDS and are in turn capable of inducing significant lung injury as demonstrated by alveolar-capillary barrier failure, lung edema, and neutrophil infiltration at pathophysiologically relevant concen-
trations in mice (24). These effects are linked to and presumably at least in part mediated by the detrimental effects of endothelium-derived MVs on endothelial function, causing a loss of endothelial NO formation from endothelial NO synthase with concomitant overproduction of reactive oxygen species (24), and a concomitant release of inflammatory cytokines including TNF-α and IL-1β (13). The latter may again be packaged into MVs, facilitating local and systemic dissemination of inflammatory responses as shown, e.g., in mechanical overventilation (132), a setting that is again associated with increased levels of circulating endothelial MVs (90).

In addition to endothelial MVs in the circulation, MVs derived from alveolar macrophages are abundant in BALF in animal models of acute lung injury (110). These MVs again contain high levels of TNF-α and are able to trigger inflammatory responses both in vitro and in vivo (110).

Epithelial cell-derived EVs are important mediators of inflammatory alveolar lung injuries such as those caused by hyperoxia. In a mouse model, hyperoxia led to upregulation of epithelial EVs (≤120 nm, i.e., EXO sized) in the BALF and blood. These EVs were able to upregulate and activate proinflammatory responses in systemic and pulmonary macrophages and to recruit macrophages and neutrophils to the inflated lung (82). A separate study showed that depending on its etiology [acid aspiration (sterile) vs. bacterial (infectious) exposures], acute lung injury led to increases in epithelial cell-derived EVs or macrophage-derived EVs, respectively, in the BALF, which both recruited macrophages and worsened disease presentation in mice (61).

Finally, MVs generated from stored red blood cells have been shown to activate neutrophils and, as such, may trigger or promote the development of transfusion-related acute lung injury (9, 125). Hence, MVs from various parent cells (endothelium, macrophages, and red blood cells) have been implicated in the pathogenesis of acute lung injury and ARDS; yet, surprisingly, a recent clinical study in 280 critically ill patients, of which 90 developed ARDS, revealed that elevated total MV numbers are associated with a reduced risk of ARDS (106). This finding emphasizes the value of parent cell and/or cargo characterization for both biomarker and mechanistic studies and concomitantly suggests that subpopulations of MVs may have protective or homeostatic effects, a notion we discuss below in potential therapeutics for lung disease.

Various EVs from different subpopulations of parent cells, including monocytes, epithelial cells, endothelial cells, and platelets, have prothrombotic properties. Coagulation and fibrinolysis can be altered in inflammatory lung diseases such as asthma and ARDS, where procoagulant perturbations in tissue factor (TF) and thrombin generation or increased levels of plasminogen activator inhibitor type 1 can promote thrombosis and worsen lung injury (7, 8, 30, 70). As such, EVs can in part mediate changes in pulmonary coagulation that promote lung injury. Specifically, many EVs contain externalized surface PS, which can catalyze enzymes within the coagulation cascade (42). Platelet MVs often are adorned with parent cell surface glycoproteins that retain their adhesive functions and can promote coagulation by enhancing platelet aggregation or fibrinogen binding (42). Direct evidence for the significance of platelet MVs in coagulation is derived from patients with Scott syndrome, who develop hemorrhagic complications due to a genetic inability to produce platelet MVs and a deficiency in the expression of PS (42). Monocyte MVs disrupt endothelial barriers by promoting apoptosis, increasing their expression of procoagulant TF and reducing expression of TF pathway inhibitor and thrombomodulin (both anticoagulant factors), and as such create a prothrombotic milieu (66). Furthermore, patients with ARDS have been found to have increased expression of receptor for advanced glycation end products (RAGE) and TF on MVs (likely epithelial MVs), which can promote coagulation (8). Finally, endothelial MVs can contain von Willebrand factor and E-selectin, which in turn promote clot formation (42). Although the various procoagulant effects of EVs are most prominent and best studied in ARDS, they may also contribute to other lung diseases associated with inflammatory or procoagulatory states such as, e.g., chronic thromboembolic PH.

**Interstitial-Parenchymal Disease**

In recent years, EVs have also been recognized as an important mechanism promoting the migration and metastasis of cancer cells. Transcriptional analyses revealed that these effects are often linked to exosomal miRNAs and long non-coding RNAs (27), which, e.g., regulate cancer cell invasion by promoting the expression of matrix metalloproteinases (124) or bone metastases by facilitating osteoclastogenesis (126). In nonneoplastic interstitial lung disease, elevated levels of EXOs have been detected in BALF of patients with idiopathic pulmonary fibrosis (71). Similar to what we discussed above for cigarette smoke-induced pulmonary inflammation (28), these EXOs contained high levels of the Wnt ligand Wnt5A and induced proliferation of primary human lung fibroblasts in a Wnt5A-dependent manner, suggesting partially parallel exosomal cell-cell communication pathways in the progression of different lung diseases.

**Pulmonary Vascular Disease**

Last but not least, miRNA delivery via EXOs has been implicated in PH, in that EXOs from patients with idiopathic pulmonary arterial hypertension or animal models of PHs were found to contain a unique set of miRNAs regulating cell proliferation, apoptosis, and inflammation and were able to induce right ventricular hypertrophy in healthy mice (1).

Overall, cell-cell communication via EVs plays an important role in the onset and/or progression of numerous lung diseases. In part for methodological reasons, mechanistic analyses of EV cargo and effects have so far focused predominantly on inflammatory cytokines in MVs and RNAs in EXOs, while other constituents such as carbohydrates or lipids have been addressed less.

**Respiratory Viruses Hijack EVs for Viral Transmission and Play a Key Role in the Pathogenesis of Respiratory Diseases**

Understanding how viruses interfere with host cell processes such as EV formation, cargo, and release is critical to understanding the pathogenesis of respiratory diseases. It is now recognized that viruses drive the pathogenesis of various chronic lung diseases. The vast majority of asthma exacerbations are triggered by a respiratory virus infection, which is usually a rhinovirus (49, 50). Viruses are also a common trigger of COPD exacerbations (41). The role of viruses in interstitial lung diseases such as IPF is less clear, but the
evidence is increasing. Chronic infection with hepatitis C, adenovirus, and herpesviruses has been suggested to be associated with IPF, although the data from different studies are conflicting (83). In terms of acute exacerbations of IPF, respiratory viruses (parainfluenza, rhinovirus, and coronavirus) have been reported in a small number of cases (83). Novel concepts propose that viruses may drive chronic lung disease via interaction with EVs, but how?

There is now abundant evidence that respiratory viruses have evolved to exploit EVs as transporters of viral particles or viral genomes for transmission of infection (2). The ability of these viruses to hijack EVs is due in part to similarities between the exosomal pathway and aspects of the virus replication cycle (35). During viral infection the EV machinery can be exploited to generate EXOs containing virus-expressed molecules such as proteins, mRNAs, and miRNAs that can be carried to uninfected recipient cells and modify susceptibility to infection (100). Direct transmission of virions via EXOs has also been observed resulting in a more efficient spread of infection to target cells (85).

There are a number of mechanisms by which virus-modified EVs can promote infection. This can be via direct interaction with host cell proteins involved in the biogenesis of EVs. A group of well-studied cellular proteins called endosomal sorting complexes required for transport (ESCRT), which are essential for EXO biogenesis, can be exploited by viruses to hijack the exosomal pathway for viral transmission to unaffected cells (96, 118). Several enveloped respiratory viruses including hantavirus, respiratory syncytial virus, and influenza A virus have been reported to utilize the Ras-related protein Rab (Rab) pathway for virion transfer to the plasma membrane to exit the host cell. Rab proteins are highly conserved proteins that are essential for vesicular formation, trafficking, and fusion in eukaryotic cells. Furthermore, these proteins have been implicated in the release of EXOs, and alteration of these proteins might lead to interference with exosomal cargo resulting in packaging of viral factors and infectious virus particles. However, the regulatory functions of Rab proteins in the packaging of viral factors into EVs are not yet fully understood (129).

Viruses can exploit EVs to evade host immunity to facilitate persistent infection. Although this does not apply to respiratory viruses, there are several lines of evidence that some persistent viruses such as hepatitis A virus and hepatitis C virus employ EVs as a strategy to evade neutralizing antibodies or other immune responses that promote viral clearance (97). Encapsulation of viral particles in EVs may also enhance virus spread over greater distances (62). This highlights two concepts to increase/prolong the infectivity of viruses: 1) modifying cell tropism by utilizing EV surface proteins rather than viral-expressed receptor-binding proteins (29, 95) and 2) evading clearance by the immune system by shielding immunogenic viral proteins.

Nonenveloped respiratory viruses such as members of the picornavirus family (rhinovirus and coxsackievirus) have also evolved strategies to exploit EVs. These viruses rely on lysis to egress an infected cell. Once a cell has undergone apoptosis it can no longer support viral replication. Loading infectious virus particles into EVs provides an alternative strategy for these viruses to exit that does not result in the death of the cell (19). It was noted that the source of these EVs were not MVBs derived from the exocytic pathway, but rather autophagosomes identifying an alternative mechanism involving autophagy (47). In support of this, it was recently reported that rhinovirus, poliovirus, and coxsackievirus were found in large clusters of autophagosome-derived EVs (19). Autophagosomes are present in all eukaryotic cells and part of the bulk degradative pathway. The size of an autophagosome can be roughly 400 nm, whereas the size of a rhinovirus virion is 30 nm. Therefore, a single autophagosome has the capacity to contain hundreds of viral particles, massively increasing the potential to cause infection.

Despite ever-increasing evidence the present understanding of EV biology and function in respiratory viral transmission, host immunity, and disease is still in the early stages. Improved understanding of the mechanisms of viral transmission to unaffected cells might open the way for new therapeutic interventions and diagnostics.

**POTENTIAL THERAPEUTICS FOR LUNG DISEASE**

The need for more effective treatments for various lung diseases continues to spur the search for innovative therapies and their clinical application. One emerging intervention is cell therapy using mesenchymal stromal/stem cells (MSCs) owing to their multiple functions including homing (52, 89), niche-dependent anti-inflammatory (34, 59, 67, 87, 94, 131) or proinflammatory (16, 64, 119) effects, enhancing bacterial clearance (37, 55, 88), and secretion of therapeutic factors (53, 68, 128). Although administration of MSCs has demonstrated therapeutic benefit in various models of lung diseases as reviewed elsewhere (21, 73, 75, 78), it has recently become evident that their mechanism of action is not via engraftment, but predominantly paracrine and attributable to transfer of EVs to target tissues (99).

**Therapeutic Potential of MSC-Derived EVs**

MSC-derived EVs are a growing research area for clinical applications in a number of diseases. Compared with full, intact MSCs, cell-free cell therapy by MSC EVs may offer a series of advantages in terms of greater safety, production, and off-the-shelf availability (5) that would avoid the challenges and costs of producing vast quantities of cells by in vitro expansion (103). Therefore, capturing the therapeutic functions of MSCs in a more stable and easily administered package is the basic tenet of cell-free therapy using EVs that has been explored in different respiratory illnesses (Fig. 2).

**Lung Airway Disease**

Administration of MSCs has shown therapeutic benefit in various animal models of asthma by reducing inflammation and airway hyperresponsiveness (111). These beneficial effects can at least in part be successfully replicated by MSC EVs, which also improved respiratory mechanics while reducing lung inflammation and airway collagen fiber deposition in a murine ovalbumin-induced allergic model of severe asthma (23) and mitigated Aspergillus-induced T helper 2 (Th2)/Th17-mediated airway inflammation and hyperreactivity in mice (22).
Alveolar Disease

A growing body of evidence suggests that cell-based therapy with MSCs holds therapeutic promise for the treatment of ARDS (43), and phase 1 clinical trials assessing safety did not detect any adverse effects of MSC therapy in patients with ARDS (123). Notably, many if not all of the previously documented beneficial effects of MSCs can again be replicated by respective EVs. Specifically, the loss of alveoli, increase in alveolar volume, and increase in medial thickness of small pulmonary vessels in a rat model of hyperoxia-induced bronchopulmonary dysplasia were prevented by MSC EVs, improving lung vascularization and alveolarization (93). Specifically, MSC MVs have been shown to restore alveolar fluid clearance and reduce edema in ex vivo perfused human lungs rejected for transplantation (33) and those injured by bacterial pneumonia (91). Pretreating MVs with an antibody against the hyaluronic receptor CD44 eliminated this restorative effect and prevented uptake of MSC MVs by alveolar epithelial cells, potentially indicating that binding and uptake of MVs are mediated via the epithelial glycocalyx (121). In Escherichia coli endotoxin-induced lung injury in mice, administering MSC MVs reduced pulmonary edema, alveolar protein leak, neutrophil influx, and accumulation of inflammatory cytokines (133). Knockdown of keratinocyte growth factor (KGF) in MSCs by siRNA partially eliminated these therapeutic effects of MVs. Similarly, E. coli-induced pneumonia in mice was ameliorated by MSC EVs in a KGF-dependent manner (81). These findings have fueled the notion that MSCs or MSC MVs, respectively, exert their beneficial effects primarily via the well-documented barrier-stabilizing and anti-inflammatory effects of KGF (57, 120). However, a recent randomized placebo-controlled phase 2 trial on treatment of patients with ARDS with pure KGF did not detect any benefit of KGF on clinical outcome, but rather suggested potential harmfulness (77). The discordance between these and previous preclinical findings discussed above suggests that MSC MVs (and intact MSCs) facilitate temporally or spatially controlled delivery of KGF and/or delivery of protective mediators other than KGF that are crucial for their beneficial effects. One of these mediators could, e.g., be angiopoietin-1, another barrier-stabilizing mediator, since angiopoietin-1 knockdown in MSCs eliminated the ability of MVs to reduce LPS-induced lung edema, protein permeability, and inflammation (114). In addition, MSCs have been shown to deliver mitochondria via MVs to alveolar epithelial cells, thus restituting alveolar bioenergetics and function (45).

Pulmonary Vascular Disease

In a murine model of monocrotaline-induced PH, MSC MVs were able to prevent pulmonary arterial wall thickening and...
right ventricular hypertrophy (17). This protective effect was also demonstrated by EXOs, owing to their contents of anti-inflammatory and antiproliferative miRNAs (1). MSC EXOs also inhibited vascular remodeling in hypoxic PH by upregulation of miR-204 and suppression of STAT3 (60). Intriguingly, it has been demonstrated that following isolation from cell culture, EXOs may be efficiently loaded with specific miRNAs using a combination of calcium chloride and heat shock (130), creating the possibility for designer EVs with desired contents.

On the basis of the promising results from the few exemplary studies highlighted above and the rapid expansion of this research field it seems fair to say that we have thus far merely scratched the surface of EVs’ therapeutic potential, focusing on only a handful of the plethora of biomolecules contained in EVs. Although MSC EVs bear significant promise as cell-free cell therapy, better insight into the cargo of EVs, its selective packaging by parent cells in response to specific stimuli, and its mode of action is required and may allow for bioengineering of optimized EVs with potentiated therapeutic function.

**SUMMARY**

Emerging insight into EV biology and function in the context of lung disease has fueled considerable interest in EVs’ roles as biomarkers, effectors of health and disease, and therapeutics. EVs, especially when further stratified by vesicle type, parent cells, and cargo, hold significant promise as biomarkers of diverse lung diseases (Table 1). In addition, EVs actively mediate onset and progression of lung diseases through distinct mechanisms involving, e.g., the delivery of proinflammatory cytokines or regulatory RNAs. Finally, EVs offer a unique opportunity for therapeutics, as either druggable targets or possible vehicles for therapeutics or therapeutic use of specific EVs themselves to treat lung diseases and injuries (Fig. 2).

Yet, caution is warranted not to treat EVs as a new panacea for maintenance of lung health or treatment of disease, respectively, as fundamental challenges and questions remain that must be navigated and resolved. First, many studies use varied strategies for collection, processing, and analysis of EVs, which makes comparisons across space and time a major limitation at present (105). Second, in most studies, analyses of EV cargo and surface markers have focused on a few candidate proteins or RNA arrays, whereas complete analysis of the EVome in different disease conditions is still outstanding. Third, key aspects of basic EV biology still remain poorly understood. For example, although the basic principles by which MVs and EXOs are formed and released have been described (11, 104), the specific mechanisms that fine-tune these processes remain unresolved. Just as different cell types treated with the same stimulus may form different EVs, EVs induced by different stimuli from the same cell could carry different components with different effects on target cells (18, 116). Although heterogeneity is thus emerging as an essential feature of EVs, the regulatory pathways underlying this diversity remain ill defined. At least part of this puzzle could be resolved if we would better understand the mechanisms by which cargo is loaded into EVs during their formation. This is by no means a random process, but highly selective (48). For example, packaging of miRNAs and long noncoding RNAs by chondrocytes into EXOs changes dramatically in osteoarthritis, which cannot be explained by corresponding changes in expression levels in the parent cells (108). Another example for selective loading by a yet unexplained mechanism is TNF-α release from LPS-treated macrophages: while ATP switches off the release of soluble TNF-α, it concomitantly packages membrane TNF-α into MVs (109).

Similarly, important characteristics of EV (pharmacokinetics and (pharmacokinetics) remain to be clarified both in general and for specific EV populations in specific lung diseases. EVs have been shown to interact with target cells by either receptor binding, fusion, endocytosis, or release of soluble mediators. Notably, MSC EVs have been shown to rely on CD44, a widely expressed glycoprotein, for uptake and transfer of biomolecular contents to epithelial cells (12, 33). As the primary ligand for CD44 is the glycosaminoglycan hyaluronic acid, a primary constituent of the glycocalyx (121), one may speculate whether initial anchoring of EVs to the intact surface layer of the cell is necessary for their subsequent uptake and, thus, how disease-related changes in this surface layer will affect EV-target cell interaction. Subsequent uptake may occur by a variety of endocytic pathways, including clathrin-dependent endocytosis, caveolin-mediated uptake, macroinocytosis, phagocytosis, and lipid raft-mediated internalization, presumably depending on the abundance of proteins and glycoproteins on the surface of both the vesicle and the target cell (86). Likewise, mechanisms of EV clearance remain incompletely characterized and likely differ between health and disease. Clearance from the circulation or air spaces commonly occurs via uptake by epithelial or endothelial cells, resident macrophages, or blood monocytes. Clearance rate is commonly considered to occur within minutes but changes as a function of both EV and host characteristics such as, e.g., PS expression or physical state (with, e.g., trained individuals being able to clear exercise-induced EVs much faster than untrained subjects; 4). Taken together, there remains a sizeable body of unknowns that presently limit the potential clinical use of EVs as therapeutics or vehicles for therapeutics (74), yet at the same time provide fertile and promising ground for future basic and translational research.

Finally, though the field is progressing in terms of identifying particular aspects of EVs, reproducible association of specific EV populations to particular diseases has so far not been validated in large multicenter clinical trials. This is important as routine stresses in healthy individuals such as exercise or heat stress can lead to at least transient changes in the types and numbers of EVs, which may lead to false interpretation of risk in the context of EVs as biomarkers (6, 31). This latter challenge segues to other exciting possibilities, such as assessing not only host eukaryotic EVs but also prokaryotic EVs such as bacterial EVs, often referred to as outer membrane vesicles, as biomarkers for infection and effectors of lung health and disease (51).

EVs, though small in size, appear to play large roles in intercellular communication. Recognizing this, the American Journal of Physiology-Lung Cellular and Molecular Physiology now issues a special Call for Papers related to lung EV research.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.
REFERENCES

1. Aliotta JM, Pereira M, Wen S, Dooner MS, Del Tato M, Papa E, Goldberg LR, Baird GL, Ventetuolo CE, Queinnec PY, Klinger JR. Exosomes induce and reverse monocrotaline-induced pulmonary hypertension in mice. Circ Cardiovasc Res 110: 319–330, 2016. doi: 10.1093/cvr/cvw054.

2. Altan-Bonnet N. Extracellular vesicles are the Trojan horses of viral infection. Curr Opin Microbiol 22: 77–81, 2016. doi: 10.1016/j.mib.2016.05.004.

3. Amabile N, Heiss C, Chang V, Angelis F, Damon L, Rame EJ, McEnally D, Grossman W, De Marco T, Yeghiazarians Y. Increased CD62e on endothelial microplatelet levels predict poor outcome in pulmonary hypertension patients. J Heart Lung Transplant 28: 108–108, 2009. doi: 10.1016/j.healun.2009.06.005.

4. Ayers L, Nieuwland R, Kohler M, Kraenkel N, Ferry B, Leeson P. Microparticles from stored red blood cells activate neutrophils and cause lung injury after hemorrhage. J Immunol 214: 648–655, 2012. doi: 10.3389/fphys.2012.00359.

5. Bainton CM, Amabile N, Tedgui A. Apoptosis: a review of programmed cell death. Am J Physiol Lung Cell Mol Physiol 306: L657–L669, 2013. doi: 10.1152/ajplung.00036.2012.

6. Bastańca JA, Fremont RD, Kropski JA, Bossert FR, Ware LB. Dynamic microvesicle release and clearance within the cardiovascular system: tricks and mechanisms. Clin Sci (Lond) 129: 915–931, 2015. doi: 10.1042/CS20140623.

7. Baglio SR, Pedgelt DM, Baldini N. Mesenchymal stem cell secreted vesicles provide novel opportunities in stem cell-free therapy. Front Physiol 3: 359, 2012. doi: 10.3389/fphys.2012.00359.

8. Berré-Haddad Y, Robert S, Salers P, Zekraoui L, Farnarier C, Dinarello CA, Dignat-George F, Kaplanakis Y. Sterile inflammation of endothelial cell-derived apoptotic bodies is mediated by interleukin-1α. Proc Natl Acad Sci USA 108: 20684–20689, 2011. doi: 10.1073/pnas.1116848108.

9. Boulanger CM, Amahile N, Tedgui A. Circulating microvesicles: a potential prognostic marker for atherosclerotic vascular disease. Hypertension 48: 180–186, 2006. doi: 10.1161/HYP.0000000000001772.

10. Bruno S, Grange C, Deregibus MC, Calogerou RA, Saviozzi S, Collino F, Morando L, Busca A, Falda M, Bussolati B, Tetta C, Camussi G. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. J Am Soc Nephrol 20: 1053–1067, 2009. doi: 10.1681/ASN.2008070798.

11. Buesing KL, Denisco JR, Kaul S, Pritchard KA Jr, Juve KS, Oldham KT. Endothelial-derived microvesicles induce endothelial dysfunction and acute lung injury. Shock 26: 464–471, 2006. doi: 10.1097/01.shk.0000228791.10550.36.

12. Duarte D, Taveira-Gomes T, Sokhatka O, Palmares C, Costa R, Negráio R, Guimarães JT, Delgado L, Soares R, Moreira A. Increased circulating platelet microvesicles as a potential biomarker in asthma. Allergy 68: 1073–1075, 2013. doi: 10.1111/all.12190.

13. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol 35: 495–516, 2007. doi: 10.1177/0192623307312037.

14. Falcone G, Felsani A, D’Agnano L. Signaling by exosomal microRNAs in cancer. J Exp Clin Cancer Res 34: 32, 2015. doi: 10.1186/s13046-015-0148-3.

15. Feller D, Kun J, Ruzsics I, Rapp J, Sarosi V, Kvell K, Helyes Z, Pongracz JE. Cigarette smoke-induced pulmonary inflammation becomes systemic by circulating extracellular vesicles containing Wnt5a and inflammatory cytokines. Front Immunol 9: 1724, 2018. doi: 10.3389/fimmu.2018.01724.

16. Fong Z, Hensley L, McNichol KL, Hu F, Madden V, Ping L, Jeong SH, Walker C, Lanford RE, Lemon SM. A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. Nature 496: 367–371, 2013. doi: 10.1038/nature12029.

17. Frantzakaki F, Armaganidis A, Orfanos SE. Immunothrombosis in acute respiratory distress syndrome: cross talks between inflammation and coagulation. Respir Res 93: 212–225, 2017. doi: 10.1186/s13287-016-0350-6.

18. Frühbeis C, Helmig S, Altan-Bonnet N. Surrogate fluorescent microvesicles on pulmonary arterial hypertension in rats. Exp Lung Res 36: 221–236, 2010. doi: 10.1080/01903660903273863.

19. Fujita Y, Yoshikawa Y, Ito S, Araya J, Kuwano K, Ochiya T. Microvesicles derived from human mesenchymal stem cells restore alveolar fluid clearance in human lungs rejected for transplantation. Am J Transplant 16: 2404–2412, 2015. doi: 10.1111/ajt.13271.

20. Ghanam S, Pene J, Torrey-Moquet G, Jorgensen C, Yssel H. Mesenchymal stem cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell phenotype. J Immunol 185: 302–312, 2010. doi: 10.4049/jimmunol.0902007.

21. Gruenewald J. Viruses and endosome membrane dynamics. Curr Opin Cell Biol 21: 582–588, 2009. doi: 10.1016/jceb.2009.03.008.
EVS IN THE LUNG

36. Guervilly C, Lacroix R, Ford JM, Roch A, Camoin-Jau L, Papazian L, Dignat-George F. High levels of circulating leukocyte microparticles are associated with better outcome in acute respiratory distress syndrome. Crit Care 15: R31, 2011. doi:10.1186/cc9978.

37. Gupta N, Krasnodembskaya A, Kapetanaki M, Mouded M, Tan X, Serikov V, Matthay MA. Mesenchymal stem cells enhance survival and bacterial clearance in murine *Escherichia coli* pneumonia. Thorax 67: 533–539, 2012. doi:10.1136/thoraxjnl-2011-201176.

38. Harding C, Heuser J, Stahl P. Endocytosis and intracellular processing of transferrin and colloidal gold-transferrin in rat reticuloocytes: demonstration of a pathway for receptor shedding. Eur J Cell Biol 35: 256–263, 1984.

39. Hargett LA, Bauer NN. On the origin of microparticles: from “platelet dust” to mediators of intercellular communication. *Palm Circ* 3: 329–340, 2013. doi:10.4102/apv.v14i0.114760.

40. Héliot A, Landkocz Y, Roy Saint-Georges F, Gosset P, Billet S, Hervieu S, Vignaux A, Bauduin S, Landkocz Y, Roy Saint-Georges F, Gosset P, Billet S. Endocytosis and intracellular processing of viral RNA in extracellular vesicles mediate WNT-5A signaling in idiopathic pulmonary fibrosis. Respir Res 16: 191, 2015. doi:10.1186/s12931-015-0653-8.

41. Hugel B, Martinez MC, Kunzelmann C, Freysinet JM. Membrane microparticles: two sides of the coin. *Physiology (Bethesda)* 20: 22–27, 2012. doi:10.1152/physiologyonline.00029.2004.

42. Huppert LA, Liu KD, Matthay MA. Therapeutic potential of mesenchymal stromal cells in the treatment of ARDS. *Transfusion* 59, Suppl 1: 869–875, 2019. doi:10.1111/trf.14483.

43. Inami N, Nomura S, Kikuchi H, Kajiura T, Yamada K, Nakamori H, Takahashi N, Tsuda N, Hikosaka M, Masaki M, Iwasaka T. P-selectin and platelet-derived microparticles associated with monocyte activation markers in patients with pulmonary embolism. *Clin Appl Thromb Hemost* 9: 309–316, 2003. doi:10.1097/01.tht.0000090000.4006.

44. Ishii M, Das SR, Emini MT, Wei M, Sun J, Westphalen K, Rowlands DJ, Quadri SK, Bhattacharya S, Bhattacharya J. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat Med* 18: 759–765, 2012. doi:10.1038/nm.2736.

45. Iversen LV, Ullman S, Östergaard O, Nielsen CT, Halberg P, Hjort MN, Sarasin L, Gadsby D, Tiro J, Hørsted K, Aichler M, Lindner M, Gesierich W, Guenther A, Walch A, Coughlan D, Chandrasena A, Pan J, Bajaj A, Johnson M, Frank JA. Keratinocyte growth factor enhances barrier function without altering claudin expression in primary alveolar epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 299: L724–L734, 2010. doi:10.1152/ajplung.00233.2010.

46. Lancaster GI, Febralbo MA. Exosome-dependent trafficking of HSPTO: a novel secretory pathway for cellular stress proteins. *J Biol Chem* 280: 23349–23355, 2005. doi:10.1074/jbc.M502172200.

47. Le Blanc K, Mougiakakos D. Multipotent mesenchymal stromal cells and the innate immune system. *Nat Rev Immunol* 12: 383–396, 2012. doi:10.1038/nri3209.

48. Lee C, Mitsialis SA, Aslam M, Vitali SH, Vergadi E, Konstantinou G, Sdrimas K, Fernandez-Gonzalez A, Kourembanas S. Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. *Circulation* 126: 2601–2611, 2012. doi:10.1161/CIRCULATIONAHA.112.114173.

49. Lee H, Zhang D, Laskin DL, Jan YJ. Functional evidence of pulmonary extracellular vesicles in infections and noninfectious lung inflammation. *J Immunol* 201: 1500–1509, 2018. doi:10.4049/jimmunol.1800264.

50. Lemon SM. Type A viral hepatitis. New developments in an old disease. *N Engl J Med* 313: 1059–1067, 1985. doi:10.1056/NEJM198510243131706.

51. Li H, Meng X, Gao Y, Cai S. Isolation and phenotypic characteristics of microparticles in acute respiratory distress syndrome. *Int J Clin Exp Pathol* 8: 1640–1648, 2015.

52. Li W, Ren G, Huang Y, Su J, Han Y, Li J, Chen X, Cao K, Chen Q, Shou P, Zhang L, Yuan ZR, Roberts AI, Shi S, Le AD, Shi Y. Mesenchymal stem cells: a double-edged sword in regulating immune responses. *Cell Death Differ* 19: 1505–1513, 2012. doi:10.1038/cdd.2012.26.

53. Lin J, Wang Y, Zou YQ, Chen X, Huang B, Liu J, Xu YM, Li J, Zhuang J, Yang WM, Min QH, Sun F, Li SQ, Gao QF, Wang XZ. Differential miRNA expression in pleural effusions derived from extra-cellular vesicles of patients with lung cancer, pulmonary tuberculosis, or pneumonia. *Tumour Biol* 37: 15835–15845, 2016. doi:10.1007/s13277-016-5410-6.

54. Lovren F, Verma S. Evolving role of microparticles in the pathophysiology of endothelial dysfunction. *Clin Chem* 59: 1166–1174, 2013. doi:10.1373/clinchem.2012.197911.

55. Maggini M, Mirkin G, Bognanni I, Holmberg J, Piazziom IM, Nenposnachy I, Costa H, Cañones C, Raiden S, Vermeulen M, Geffner JR. Mouse bone marrow-derived mesenchymal stromal cells activate macrophages into a regulatory-like profile. *PLOS One* 5: e00252, 2010. doi:10.1371/journal.pone.000252.

56. Mahmood A, Lu D, Chopp M. Intravenous administration of marrow stromal cells (MSCs) increases the expression of growth factors in rat brain after traumatic brain injury. *J Neurotrauma* 21: 33–39, 2004. doi:10.1089/neu.2008.0159.563363463.

57. Makiguchi T, Yamada M, Yoshioka Y, Sugihara H, Koarai A, Chiba S, Fujino N, Tojo Y, Ota C, Kubo H, Kobayashi S, Yanai M, Shimura S, Ochiya T, Ichinose M. Serum extracellular vesicular mir-21-5p is a predictor of the prognosis in idiopathic pulmonary fibrosis. *Respir Res* 17: 110. 2016. doi:10.1186/s12931-016-0427-3.

58. Manuyakorn W, Mairiang D, Sirachainan N, Kadegasem P, Kamchasitawan W, Benjaponpitak S, Chuanasritian W. Blood coagulation and asthma exacerbation in children. *Int Arch Allergy Immunol* 170: 75–83, 2016. doi:10.1159/000446775.

59. Martin-Medina A, Lehmann M, Burgel O, Hermann S, Baarsma HA, Wiest DE, Stafstrom PM, Ciolek H, Hofer TP, Frankenberger M, Aichler M, Lindner M, Gesierich W, Guenther A, Walch A, Coughlan D, Wolters P, Lee JS, Behr J, Königshoff M. Increased extracellular vesicles mediate WNT-5A signaling in idiopathic pulmo-
nary fibrosis. *Am J Respir Crit Care Med* 198: 1527–1538, 2018. doi:10.1164/rcrm.201708-1580OC.

72. Martínez-Bravo MJ, Wahlund CJ, Qazi KR, Moulder R, Lukic A, Rádmark O, Lahesmaa R, Grunewald J, Eklund A, Gabriellsön S. Pulmonary sarcoidosis is associated with esoxosomal D-binding protein and inflammatory molecules. *J Allergy Clin Immunol* 139: 1186–1194, 2017. doi:10.1016/j.jaci.2016.05.051.

73. Masterson C, Jerkic M, Curley GF, Laffey JG. Mesenchymal stromal cell therapies: potential and pitfalls for ARDS. *Minerva Anestesiol* 81: 179–194, 2015.

74. Mateescu B, Kowal EJ, van Balkom BW, Bartel S, Bhattacharyya SN, Buzás EI, Buck AH, de Candia P, Chow FW, Das S, Driedonks TA, Fernández-Messina L, Haderk F, Hill AF, Jones JC, Van Keuren-Jensen KR, Lai CP, Lässer C, di Liegro I, Lunavat TR, Lorenzowicz MJ, Maas SL, Mager I, Mittelbrunn M, Momma S, Mukherjee K, Nawaz M, Pegtel DM, Pfaff MW, Schifflers RM, Tahara H, Théry C, Tosar JP, Wauben MH, Witwer KW, Nolte–t Hoen EN. Obstacles and opportunities in the functional analysis of extracellular vesicle RNA: an ISEV position paper. *J Extracellular Vesicles* 6: 1286095, 2017. doi:10.1080/20013787.2017.1286095.

75. Matthay MA, Piti S, Lee JW. Concise review: mesenchymal stem (potental) cells: biology and preclinical evidence for therapeutic potential for organ dysfunction following trauma or sepsis. *Stem Cells* 35: 316–324, 2017. doi:10.1002/stem.2551.

76. Mazzeo C, Canas JA, Zafra MP, Rojas Marco A, Fernández-Nieto M, Sany V, Mittelbrunn M, Izquierdo M, Baixaulli F, Sastre J, Del Pozo V, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de la Cueva T, Fernández-Messina L, Fiserova K, Martinez de the lifetime of their work. *Science* 310: 983–989, 2007. doi:10.1126/science.1118311.

77. McAuley DF, Cross LM, Hamid U, Gardner E, Elborn JS, Cullen Morel O, Jesel L, Freyssinet JM, Toti F. ilicit. *J Cyst Fibros* 12: 721–728, 2013. doi:10.1016/j.jcf.2013.03.002.

78. Porro DA, De Gioia S, Trotta T, Lepore S, Panaro MA, Battaglino A, Ratcliff L, Castellani S, Bufo P, Matthay MA, Cones E. Pro-inflammatory effect of cystic fibrosis sputum microparticles in the murine lung. *J Cyst Fibros* 12: 721–728, 2013. doi:10.1016/j.jcf.2013.03.002.

79. Porzionato A, Zaramella P, Dedja A, Guidolin D, Van Wemmel K, Macchi V, Jurga M, Perliongo G, De Caro R, Baraldi E, Muraca M. Intratracheal administration of clinical-grade mesenchymal stem cell-derived extracellular vesicles reduces lung injury in a rat model of bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol* 316: L6–L19, 2019. doi:10.1152/ajplung.001569.2017.

80. Potian JA, Aiviv H, Ponzi NO, Harrison JS, Ramaswah P. Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens. *J Immunol* 171: 3241–3244, 2003. doi:10.4049/jimmunol.171.7.3241.

81. Raab-Traub N, Dittmer DF. Viral effects on the content and function of extracellular vesicles. *Nat Rev Microbiol* 15: 559–572, 2017. doi:10.1038/nrmicro.2017.60.

82. Raiborg C, Stenmark H. The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. *Nature* 458: 445–452, 2009. doi:10.1038/nature07961.

83. Ramanath M, Kwekkeboom J, Tilanus HW, Haagmans BL, Baumert TF, van der Laan LJ. Exosome-mediated transmission of hepatitis C virus between human hepatoma HuH7.5 cells. *Proc Natl Acad Sci USA* 110: 13109–13113, 2013. doi:10.1073/pnas.1221899110.

84. Ramirez MI, Amorim MG, Gadelha C, Milic I, Welsh JA, Freitas VM, Nayaz M, Akhar N, Couch Y, Makin L, Cooke F, Vettese AL, Batista PX, Freezer R, Pekuz JA, Rosa-Fernandes L, Carreira AC, Devitt A, Jacobs L, Silva IT, Coakley G, Nunes DN, Carter D, Palmisano G, Dias-Neto E. Technical challenges of working with extracellular vesicles. *Nanoscale* 10: 881–906, 2018. doi:10.1039/C7NR08360B.

85. Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. *Mol Ther* 23: 812–823, 2015. doi:10.1038/mt.2015.44.

86. Rodrigues M, Fan J, Lyon C, Wan M, Hu Y. Role of extracellular vesicles in viral and bacterial infections: pathogenesis, diagnostics, and therapeutics. *Theranostics* 8: 2709–2721, 2018. doi:10.7150/thno.20576.

87. Rosa-Fernandes L, Rocha VB, Carrecci V, Urbani A, Palmisano G. A perspective on extracellular vesicles proteomics. *Front Chem* 5: 102, 2017. doi:10.3389/fchem.2017.00102.

88. Rose JA, Wanner N, Cheong HI, Queisser K, Barrett P, Park M, Hite C, Naga Prasad SV, Erzurum S, Assingh K. Flow cytometric quantification of peripheral blood cell β-adrenergic receptor density and urinary endothelial cell-derived microparticles in pulmonary arterial hypertension. *PLoS One* 11: e0156940, 2016. doi:10.1371/journal.pone.0156940.

89. Rubino D, Garcia S, Paz MF, De la Cueva T, Lopez-Fernandez LA, Lloyd AC, Garcia-Castro J, Bernad A. Molecular characterization of spontaneous mesenchymal stem cell transformation. *PLoS One* 3: e1398, 2008. doi:10.1371/journal.pone.001398.
the circulation by scavenger receptors. Blood 105: 2141–2145, 2005. doi:10.1182/blood-2004-04-1578.

123. Wilson JG, Liu KD, Zhuo H, Caballero L, McMillan M, Fang X, Cosgrove K, Vojnik R, Callie CS, Lee JW, Rogers AJ, Levitt J, Wiener-Kronish J, Bajwa EK, Leavitt A, McKenna D, Thompson BT, Matthay MA. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. Lancet Respir Med 3: 24–32, 2015. doi:10.1016/S2213-2600(14)70291-7.

124. Wu DM, Deng SH, Liu T, Han R, Zhang T, Xu Y. TGF-β-mediated exosomal Inc-MMP2-2 regulates migration and invasion of lung cancer cells to the vasculature by promoting MMP2 expression. Cancer Med 7: 5118–5129, 2018. doi:10.1002/cam4.1758.

125. Xie R, Yang Y, Zhu Y, Gao L, Jiang X, Sun J, Bian M, Yang J. Microparticles in red cell concentrates prime polymorphonuclear neutrophils and cause acute lung injury in a two-event mouse model. Int Immunopharmacol 55: 98–104, 2018. doi:10.1016/j.intimp.2017.11.029.

126. Xu Z, Liu X, Wang H, Li J, Dai L, Li J, Dong C. Lung adenocarcinoma cell-derived exosomal miR-21 facilitates osteoclastogenesis. Gene 666: 116–122, 2018. doi:10.1016/j.gene.2018.05.008.

128. Yuan L, Wu MJ, Sun HY, Xiong J, Zhang Y, Liu CY, Fu LL, Liu DM, Liu HQ, Mei CL. VEGF-modified human embryonic mesenchymal stem cell implantation enhances protection against cisplatin-induced acute kidney injury. Am J Physiol Renal Physiol 300: F207–F218, 2011. doi:10.1152/ajprenal.00073.2010.

129. Zerial M, McBride H. Rab proteins as membrane organizers. Nat Rev Mol Cell Biol 2: 107–117, 2001. doi:10.1038/35052055.

130. Zhang D, Lee H, Zhu Z, Minhas JK, Jin Y. Enrichment of selective miRNAs in exosomes and delivery of exosomal miRNAs in vitro and in vivo. Am J Physiol Lung Cell Mol Physiol 312: L110–L121, 2017. doi:10.1152/ajplung.00423.2016.

131. Zhang QZ, Su WR, Shi SH, Wilder-Smith P, Xiang AP, Wong A, Nguyen AL, Kwon CW, Le AD. Human gingiva-derived mesenchymal stem cells elicit polarization of M2 macrophages and enhance cutaneous wound healing. Stem Cells 28: 1856–1868, 2010. doi:10.1002/stem.503.

132. Zhang S, Dai H, Zhu L, Lin F, Hu Z, Jing R, Zhang W, Zhao C, Hong X, Zhong JH, Pan L. Microvesicles packaging IL-1β and TNF-α enhance lung inflammatory response to mechanical ventilation in part by induction of cofilin signaling. Int Immunopharmacol 63: 74–83, 2018. doi:10.1016/j.intimp.2018.07.034.

133. Zhu YG, Peng XM, Abbott J, Fang XH, Hao Q, Monsel A, Qu JM, Matthay MA, Lee JW. Human mesenchymal stem cell microvesicles for treatment of Escherichia coli endotoxin-induced acute lung injury in mice. Stem Cells 32: 116–125, 2014. doi:10.1002/stem.1504.