Role of extracellular vesicles in lung diseases

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Abstract

Extracellular vesicles (EVs) are anuclear particles composed of lipid bilayers that contain nucleic acids, proteins, lipids, and organelles. EVs act as an important mediator of cell-to-cell communication by transmitting biological signals or components, including lipids, proteins, messenger RNAs, DNA, microRNAs, organelles, etc, to nearby or distant target cells to activate and regulate the function and phenotype of target cells. Under physiological conditions, EVs play an essential role in maintaining the homeostasis of the pulmonary milieu but they can also be involved in promoting the pathogenesis and progression of various respiratory diseases including chronic obstructive pulmonary disease, asthma, acute lung injury/acute respiratory distress syndrome, idiopathic pulmonary fibrosis (IPF), and pulmonary artery hypertension. In addition, in multiple preclinical studies, EVs derived from mesenchymal stem cells (EVs) have shown promising therapeutic effects on reducing and repairing lung injuries. Furthermore, in recent years, researchers have explored different methods for modifying EVs or enhancing EVs-mediated drug delivery to produce more targeted and beneficial effects. This article will review the characteristics and biogenesis of EVs and their role in lung homeostasis and various acute and chronic lung diseases and the potential therapeutic application of EVs in the field of clinical medicine.

Keywords: Lung diseases; Biomarker; Lung disease pathogenesis; Extracellular vesicles; Clinical application

Extracellular vesicles (EVs) are a heterogeneous group of anuclear vesicles encased by a phospholipid bilayer. EVs can be derived from various cell types such as immune cells, epithelial cells, endothelial cells, stem cells, tumor cells, etc. Under both physiological or various pathological states, EVs can be released into the extracellular space and body fluids such as blood, urine, saliva, cerebrospinal fluid, semen, bile, breast milk, amniotic fluid, ascites, bronchial lavage fluid, etc.[1] In 1946, Chargaff and West[2] isolated minute pellets containing thromboplastic protein from oxalated plasma, which was the earliest report of microvesicles (MVs) in the literature. In 1969, Anderson[3] found vesicles with various shapes with ranges in size from 300 angstrom (A) to 1 μm in the cartilage matrix from each layer of normal mouse tibial epiphyseal plates. Crawford[4] also isolated microparticles containing lipids and proteins with adenosine triphosphatase (ATPase) activity from human and mammalian platelet-free plasma by ultracentrifugation; the investigators demonstrated that the microparticles were originated from the surface membrane of platelets or the inner membrane structure of cells. Initially, researchers believed that vesicles were a population of particulate matters that packaged cell metabolic wastes. Until the late 1990s,

scholars were surprised to discover that EVs also played an important role in mediating intercellular transfer of information through multiple pathways including direct contact and signal molecule transmission. The biological information exchange of EVs from parent cells into target cells is a crucial mechanism of local and long-distance communication between cells. For example, Raposo et al[5] found that exosomes derived from human and murine B lymphocytes can induce antigen-specific major histocompatibility complex (MHC) class II restricted T cell responses and participate in antigen presentation in vivo. Later, Wolfers et al[6] also found that tumor-derived exosomes contained tumor antigens and could transfer tumor antigens to dendritic cells (DCs) which initiated T cell-mediated anti-tumor immune responses.

EVs, as a carrier of information transmission, contain a diverse array of biologic cargoes including protein, lipids, metabolites, nucleic acids (such as messenger RNA [mRNA], DNA, non-coding RNA, small interfering RNA, micro-RNA), transcription factors, signal transduction molecules, and even organelles such as the mitochondria.[11] They exchange these biological infor-

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mation through horizontal transfer of genetic information between cells which can activate phenotypic and functional changes in target cells[7] and result in various pathophysiological responses such as regulating immunity and participating in cell proliferation, inflammatory response, angiogenesis, or vascular endothelial permeability, etc. Research in the past decades has demonstrated that EVs were involved in various pathological and physiological events in various tissues and organs, especially in the immune and inflammatory regulation.[8] Numerous studies have demonstrated that EVs play a crucial role in all kinds of pulmonary physiological and pathological conditions.[9] EVs also have been studied as potential disease biomarkers to improve diagnosis or predict disease prognosis in lung diseases.[10] Recently, EVs have been applied to enhance target drug delivery for human gene therapy in various pulmonary disorders, which showed a potential application in translational medicine.[11,12] In this review, we highlighted the recent literature to deepen our understanding of the biogenesis of EVs and their role in maintaining the pulmonary homeostasis or further aggravating various lung diseases such as during acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD), asthma, pulmonary arterial hypertension (PAH), and idiopathic pulmonary fibrosis (IPF). Moreover, we discuss the potential diagnostic and therapeutic applications of EVs in lung diseases for clinical use.

Classification of EVs

It has been widely known that EVs are composed of heterogeneous membrane vesicles from different sources. The size of EVs generally varies from 50 to 1000 nm, even reaching up to 10 μm which are termed oncosomes and are specifically derived from malignant cells.[13] Based on the biological origin, EVs are usually divided into three subtypes: exosomes, MVs, and apoptotic bodies (ABs) [Figure 1]. The diameter of exosomes is between 50 and 150 nm and the density is ranged from 1.13 to 1.19 g/mL. Exosomes can be obtained by differential centrifugation or sucrose gradient ultracentrifugation. The markers of exosomes mainly include CD9, CD63, CD81, lysosome-associated membrane protein 1, heat shock proteins (Hsp60, Hsp70, Hsp90), apoptosis-linked gene 2 interacting protein X (Alix), tumor susceptibility gene 101, etc.[8,14] MVs, also known as microparticles, are formed by direct sprouting, blistering, and fission from the plasma membrane. The diameter of MVs ranges from 100 to 1000 nm and can be collected by differential centrifugation and ultracentrifugation. MVs contain phosphatidylserine, lipid-related molecules (tissue factor, lipid structural proteins), annexin, integrins, etc, and are rich in cholesterol, sphingomyelin, and ceramides.[13,14]

ABs originate from the blebbing of the plasma membrane of apoptotic cells with a diameter of >1 μm (usually 1–5 μm). ABs are usually considered to be a separate subgroup of EVs. The externalization of phosphatidylserine is an important feature of ABs. Its markers mainly include phosphatidyl-serine, thrombospondin and complement protein C3b. ABs also contain fragmented DNA, noncoding RNA, and organelles.[15] ABs are involved in the horizontal transfer of oncogenes, the horizontal transfer of DNA, and the presentation of T cell epitopes and immunosuppression when they were taken up by phagocytes.[16] In summary, exosomes and MVs are produced and released by living cells in a resting or activated state, whereas ABs are released by apoptotic cells. However, currently, there is no clear classification standard for various specific subtypes of EVs. The International
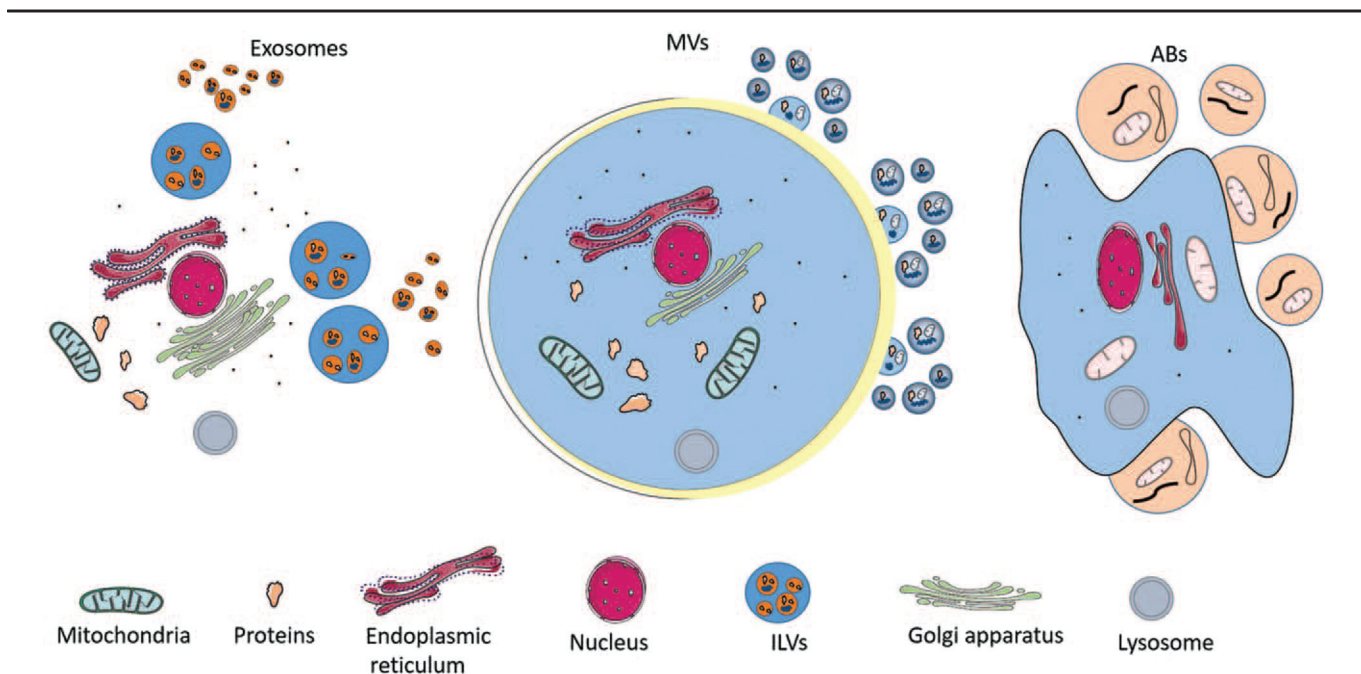
Figure 1: Classification and biogenesis of EVs. (A) Exosomes with a size range of 50 to 150 nm are released by inward budding of MVBs from the plasma membrane. (B) MVs with a size range of 100 to 1000 nm are produced by outward budding of the plasma membrane. (C) ABs with a size range of 1 to 5 μm are produced by blebbing of the plasma membrane of apoptotic cells. ABs: Apoptotic bodies; EVs: Extracellular vesicles; ILVs: Intraluminal vesicles; MVBs: Multivesicular bodies; MVs: Microvesicles.

Association of Extracellular Vesicles recommends that it is more reasonable to distinguish EVs based on their physical characteristics including its size, density, biochemical composition, or cell origin, unless EVs have reliable specific markers.[17]

Biogenesis of EVs

The production and release of exosomes and MVs include assembly and sorting, and subsequent budding and vesicles release. For exosomes, investigators believe that it originates from early endosomes which result from the internalization of the plasma membrane. Various molecules in the endosomes are recruited into small membrane domains, and then these small membrane domains are divided into small membrane vesicles via inward budding, which are termed intraluminal vesicles (ILVs) and further formed multivesicular bodies (MVBs).[18] When MVBs fuse with the plasma membrane, the components of ILVs are released into extracellular space to produce exosomes and this process is regulated by P53-mediated exocytosis.[11] The secretion of exosomes would significantly increase under the stimulation of multiple soluble agonists (such as cytokines or chemotactic or growth factors), physical forces, chemical factors (such as oxidative stress or hypoxic conditions), and shear stress.[19,20] The release of exosomes from MVBs into the extracellular fluid mainly includes the following four steps: (1) transportation of MVBs; (2) fusion of MVBs with plasma membrane; (3) shedding of MVBs from plasma membrane; and (4) release of exosomes.[16]

The biogenesis of exosomes includes the endosomal sorting complex required for transport (ESCRT)-dependent mechanism and non-ESCRT-dependent mechanism, in which the recruited substance of MVBs and the cell type determine the manner of exosomes release.[11] ESCRT is composed of four complexes (ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III) assembled by 20 proteins and involved in the budding and the release of ILVs from the plasma membrane. The ESCRT-0 subunit is responsible for recruiting ubiquitinated substances to accumulate on the plasma membrane. The ESCRT-I and ESCRT-II subunits cooperate to mediate the budding of ILVs. The ESCRT-III subunit participates in the membrane bending, inward budding, and release of ILVs into extracellular spaces to produce exosomes.[21,22] A non-ESCRT-dependent mechanism is involved with the tetra-spanin family (such as CD63, CD9, CD81, and CD82) and ceramide, which also play an important role in the production and secretion of exosomes.[11,23] Tetraspanin contributes to the formation of the microstructure surrounded by the plasma membrane and the sorting of the substances in the ILVs.[11] Multiple ILVs are involved in advanced endosomes (called multivesicles) and fuse with the plasma membrane, and further mediate the release of exosomes. The process for transporting multivesicles to the plasma membrane relies on the cytoskeleton, related molecular motors, and molecular converters,[18] in which small guanosine triphosphatases (GTPases) act as a molecular converter in regulating transporting multi-vesicles within intracellular compartments.[24]

MVs are formed via outward budding of the plasma membrane, which relies on the interaction between actin and myosin and subsequent adenosine triphosphate-dependent contraction.[11] First, lipids, membrane-associated proteins, cytosolic proteins, and RNA are aggregated and sorted into individual membrane microdomains, in which lipids such as cholesterol, sphingomyelin, and ceramide are also recruited and play an important role in membrane bending.[16] Cytoplasmic proteins are also collected into cell membranes via plasma membrane anchors and the formation of high-order complex.[11] Then, the polymerization state of actin on the cell membrane altered and the myosin backbone contracted which was mediated by GTPase RhoA and Rho-related protein kinase.[1] Thus, the budding and shedding of MVs generated. The ESCRT-III/vacuolar protein sorting 4 (VPS4) complex plays a crucial role in mediating the shedding and release of MVs off the cell membrane.[25] In addition, the budding of MVs largely depends on calpain, cytoskeleton reorganization, and intracellular calcium concentration. Lastly, the activation of flippase and calpain changes the distribution of asymmetric phospholipid on the cell membrane and the shape of the cytoskeleton, then induces an increase in calcium concentration and thus leads to the secretion of MVs.[26]

EVs as an Important Mediator in Intercellular Communication

After being released from cells, EVs can directly act on adjacent or target cells through extracellular fluids such as peripheral blood or lymphatic fluid.[18] EVs are covered with a large number of glycans and sugar-binding proteins, which participate in cellular physiological activities and cell-to-cell communication in pathological conditions by transferring various substances (including proteins, lipids, nucleic acid, organelles, etc) to regulate cell adhesion, the intercellular transmission of information, and interaction between cells and the extracellular matrix. EVs have significant effects in immune regulation, damage and repair of epithelial–endothelial cells barrier, the genesis and development of inflammation, tumor progression and metastasis, etc.[14] Nevertheless, it is still unclear whether subtypes of EVs and the specific combination between target cells and parent cells influence the target specificity in the trafficking of EVs to target cells. The scholars considered that the size and the difference in surface ligands ofEVs may affect their recognition and capture by target cells.[7] Furthermore, surface membrane proteins on EVs and receptor cells (including integrins, proteoglycans, T cell immunoglobulins, and tetraspanins) are involved in the specific localization and uptake of EVs by target cells.[27,28] For example, heparan sulfate proteoglycans on cancer cell-derived EVs are responsible for the internalization and subsequent functional activity by target tumor cells.[28] Exosomes attach to DCs mediated by phosphatidylserine, CD9 and CD81 on exosomes and alpha (v)/beta(3) integrin, CD11a and CD54 on DCs.[27] Different surface ligands and receptors on EVs and target cells determine the attachment specificity and capture.

When transported to target cells, EVs can be taken up via the following three mechanisms: (1) direct interaction between surface receptors of EVs and ligands on target cells; (2) endocytosis or pinocytosis by target cells; and(3)

fusion between EVs and the membrane of target cells.[29] First, EVs can directly transmit information to target cells by acting on the surface proteins of target cells without transferring its content.[7] For example, the MHC complex on EVs can directly activate the homologous T cell receptors on T lymphocytes and thus mediate the corresponding immune response.[30] EVs exchange chemokine receptor 5, a receptor required for the transmission and reproduction of human immunodeficiency virus-1, between monocytes and endothelial cells and result in viral infections in the tissue using atypical mechanisms for viral infection.[31] Second, as mentioned earlier, EVs also transfer their contents into target cells through direct fusion with the plasma membrane or endocytosis by target cells.[7,18] When the EVs are taken up and internalized by the target cells, the contents of EVs are released into the cytoplasm of recipient cells through re-fusion with the plasma membrane.[8] In general, the fusion between EVs and target cells is triggered by receptor binding to some specific surface proteins. For example, an endogenous fusion protein syncytin 1 on the surface of exosomes can bind to type 2 amino acid transporters to induce the fusion of EVs into cells and then transfer the gene products in EVs into recipient cells.[32,33] EVs derived from tumor cells can promote the degradation of the extracellular matrix via transmembrane proteases in EVs and the horizontal migration of mRNA and microRNA (miRNA), and then promote tumor invasion and metastasis.[34] In addition, the fusion of EVs with receptors on the cell membrane also can transport various lipids, including eicosanoids, fatty acids, cholesterol, and lipid translocases, to the recipient cells, which helps regulate the biological activity of lipids.[35]

Role of EVs in Pulmonary Homeostasis

Emerging evidences suggest that EVs are involved in maintaining pulmonary microenvironment homeostasis and regulating physiological functions.[36-39] Many types of respiratory cells can release EVs, including alveolar epithelial cells, alveolar macrophages, pulmonary vascular endothelial cells, airway and vascular smooth muscle cells, fibroblasts, stromal cells, and immune cells.[40] Epithelial cells, endothelial cells, and alveolar macrophages are considered as three important sources of EVs in lung tissues.[41] EVs derived from the bronchial epithelium play a critical part in different physiological processes of lung immune regulation and inflammation to maintain lung homeostasis.[42] In physiological conditions, EVs derived from epithelial cells contain mucin such as MUC1, MUC4, or MUC16, and can neutralize respiratory pathogens such as human influenza virus which usually bind to alpha-2,6-linked sialic acid, and further weaken the ability of virus to infect target epithelial cells, and participate in innate mucosal defense in the respiratory system.[43]

EVs derived from alveolar macrophages can be taken up by various types of respiratory cells, including lung epithelial cells, endothelial cells, fibroblasts, and monocytes, and are involved in regulating alveolar and bronchial inflammation and pulmonary homeostasis via intercellular communication.[40] Investigators have demonstrated that EVs derived from alveolar macrophages contained suppressor of

cytokine signaling 1 and suppressor of cytokine signaling 3. When alveolar epithelial cells take up these EVs, the activation of Janus kinase/signal transducer and activator of transcription signaling pathway and secretion of inflammatory cytokines can be inhibited.[44] The study also found that EVs derived from alveolar macrophages could alleviate airway inflammation by transferring microRNA (miR)-223 into lung epithelial cells and monocytes to produce an anti-inflammatory effect.[45] In addition, EVs derived from endothelial cells in the blood circulation may also inhibit the activation of monocytes by transferring anti-inflammatory miR-10a into pulmonary cells to play a beneficial role in lung homeostasis.[46] In summary, in the physiological state, EVs from pulmonary cells can exchange proteins, lipids, miRNAs, or other anti-inflammatory molecules into specific pulmonary recipient cells to maintain airway biology and pulmonary homeostasis [Figure 2].

Role of EVs in Pathogenesis and Development of Various Lung Diseases

As mentioned above, multiple respiratory cells and immune cells could release EVs. However, depending on the type of stimuli and donor cells, the components in the EVs would be largely different and thus have protective or harmful effects on the airway microenvironment.[37] In the physiological condition, respiratory cells and immune cells would secret protective EVs to defense endogenous stress and maintain respiratory homeostasis. Nevertheless, under chronic and long-term damages or acute and severe injuries, mitochondria metabolism and the homeostasis and balance of the respiratory microenvironment is disrupted. Overloaded oxidative stress products, inflammatory cytokines, chemokines, or other detrimental products are secreted and released into the extracellular fluid and peripheral circulation.[47] And abundant inflammatory cells, immune cells, or T cells are activated. In the pathological condition, respiratory cells would release EVs containing various cytokines, chemokines, miRNA, or proteins to transfer inflammatory signals, oxidative stress signals, immune regulation signals to initiate a detrimental pathophysiological process in the pulmonary microenvironment.[37] EVs derived from alveolar epithelial cells and macrophages contain abundant tumor necrosis factor (TNF) after lipopolysaccharide (LPS) stimulation and further initiate ALI.[48] Under hyperoxia-induced oxidative stress condition, EVs derived from alveolar epithelial cells carried caspase-3 and further aggravated oxidative stress and inflammatory responses.[49]

Furthermore, environmental stimulations and pathological conditions could induce the production and regulate the level of EVs in the lungs, and simultaneously modify the contents of EVs, such as cigarette and toxic exposure, bacterial or viral infections, hyperoxia, and DNA damages.[37] Studies showed that the concentration of EVs was significantly higher in the lungs of smokers[50] or under chronic hypoxia condition.[51] In addition, Rab GTPases are important regulators involved in the formation and release of EVs.[52] Some miRNAs such as miR-124a could downregulate the secretion of EVs via inhibiting the function of Rab32.[53] Interleukin (IL) 25 derived from lung epithelial cells reduced the release of EVs from
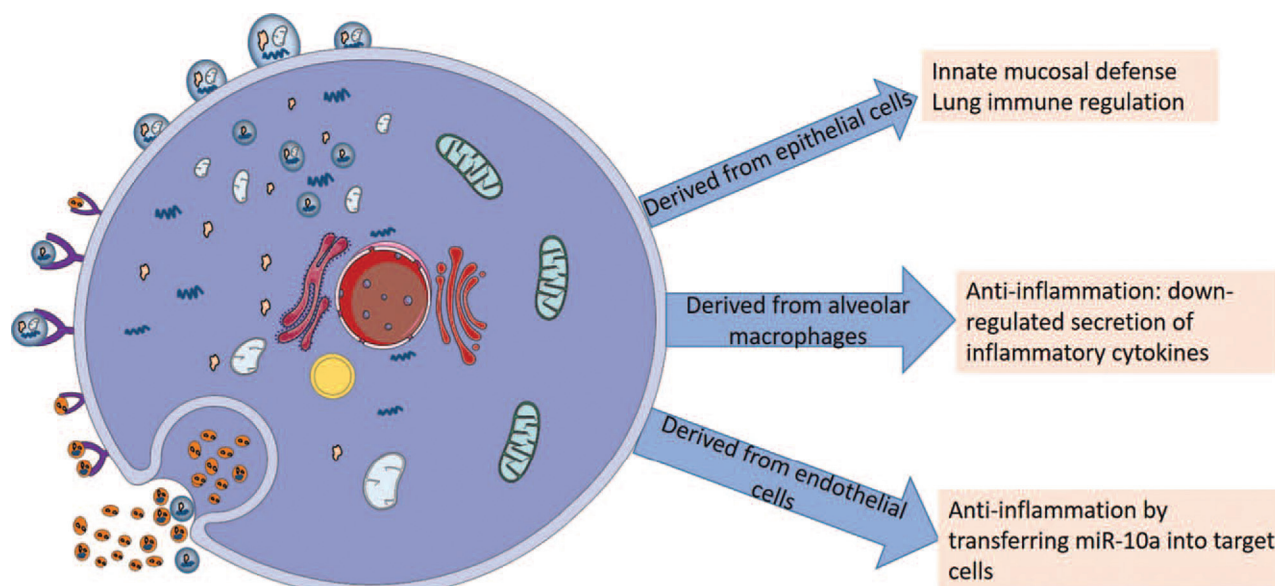
Figure 2: The update and roles of EVs in maintaining pulmonary homeostasis. EVs can be taken up via receptors, endocytosis or pinocytosis, or through membrane fusion. The transfer of the components of EVs into the target pulmonary cells can play an important role in maintaining lung homeostasis by regulating lung immune state, innate mucosal defense, and suppressing inflammation. EVs: Extracellular vesicles.

macrophages via downregulating the expression of Rab27a and Rab27b.[54] Moreover, reduced protein kinase D1 level in pancreatic cancer cells led to higher production of EVs along with changed components, which further increased metastasis of pancreatic tumors to the lung *in vivo*.[55]

ALI/ARDS

Numerous studies have shown that EVs play important roles in the pathogenesis and progression of ALI/ARDS.[56] EVs are released by various cell types, including alveolar epithelial cells, alveolar macrophages, endothelial cells, neutrophils, platelets, and monocytes.[57] Alveolar macrophage-derived EVs carrying TNF played a significant role in initiating the inflammatory response in the early stages of ALI.[48] Alveolar macrophage-derived exosomes collected from LPS-induced ALI mice significantly increased the expression of TNF-α via the internalization by neighboring macrophages.[54] Soni *et al*[48] found that alveolar macrophage-derived EVs also mediated the intercellular communication with alveolar epithelial cells which induced the increased secretion of keratinocyte-derived cytokine and intercellular adhesion molecule-1 (ICAM-1) and increased the infiltration of inflammatory cells into the alveolar space, accelerating lung injury. And alveolar macrophages are the main sources of EVs in bronchoalveolar lavage fluid (BALF) from infectious stimuli (such as bacteria, viruses infections)-induced ALI and EVs derived from BALF of infectious stimuli-induced ALI elevated the accumulation of cytokines such as toll-like receptor 6 (TLR6), myeloid differentiation factor 88, IL-1β, and IL-10 in the alveolar macrophages.[58] Moreover, type I alveolar epithelial cells are the main sources of EVs in the BALF collected from sterile stimulation (such as acid aspiration or oxidative stress)-induced ALI. EVs derived from BALF of sterile stimuli-induced ALI increased the expression of TLR2,

myeloid differentiation factor 88, TNF-α, and IL-6.[58] A subpopulation of miRNA-rich EVs accounted foronly 6% of the total number of BALF EVs derived from type I alveolar epithelial cells.

And alveolar EVs transmitted miRNAs to alveolar macrophages which activated inflammation, recruited granulo-cytes, and transformed macrophages into M1 type which accelerated pulmonary inflammation.[59] Furthermore, monocyte-derived EVs can also adhere to lung epithelial cells and further promote the secretion of inflammatory cytokines such as IL-8 and monocyte chemoattractant protein-1 (MCP-1) and aggravate ALI.[60] EVs derived from LPS-stimulated monocytes resulted in the death of pulmonary vascular endothelial (VE) cells and endothelium injury.[61] Besides, endothelial cell-derived EVs could increase the pulmonary and systemic release of IL-1β and TNF, thus promoting the recruitment of neutrophils and enhancing inflammatory cascade in lung tissues.[62] All in all, EVs derived from alveolar macrophages and epithelial play main roles in the pathogenesis and development of ALI/ARDS.

COPD

The pathological alterations of COPD mainly include the following aspects: emphysema, airway inflammation, remodeling of vasculature, airway fibrosis, and abnormal cellular morphology.[63] The level of miR-21 in serum-derived EVs was significantly higher in patients with COPD.[64] The previous study showed that CD31(+) endothelial EVs indicated the apoptosis of endothelial cells while CD62(+) endothelial EVs suggest the activation of endothelial cells and the destruction of the pulmonary capillary beds.[65] The results from Thomashow *et al*'s[65] study showed that compared with control subjects, CD31 (+) endothelial EVs were significantly elevated in patients with COPD, especially among mild COPD patients. Furthermore, CD31(+) endothelial EVs were positively correlated with the percent of emphysematous changes on

chest CT imaging but inversely correlated with blood flow of pulmonary microvessels and diffusion capacity of the lungs. This indicated pulmonary vasculature damages occurred in patients with mild COPD and emphysema.[65]

Moreover, CD62(+) endothelial EVs were notably increased in patients with severe COPD and hyperinflation.[65] Levels of platelet endothelial cell adhesion molecule endothelial microparticles (EMPs), VE-cadherin EMPs, and E-selectin EMPs in patients with acute exacerbation of COPD were significantly higher than those in patients with stable COPD and the non-COPD healthy control.[66] And the levels of EMPs were significantly higher at baseline of COPD patients with frequent exacerbations than COPD patients without frequent exacerbation, which may suggest sustained inflammation and damage to pulmonary capillaries even in the stable stage for COPD patients with frequent exacerbation.[66] Furthermore, the elevated level of E-selectin EMP also revealed a significantly negative relationship with the annual changes of forced expiratory volume in the first second (FEV_1) among stable COPD patients.[67]

The mechanisms of EVs in aggravating the pathogenesis of COPD are multiple.[68] As we described above, epithelial, macrophage, and endothelial cells are the three main producers of EVs in lung tissues. Moon et al[69] found that cigarette smoke extract (CSE) exposure induced lung epithelial cells to release full-length cellular communication network factor 1 (flCCN1)-enriched EVs, which led to the recruitment of neutrophils and upregulated pulmonary inflammation. And long-term (6 months) CSE exposure cleaved flCCN1 into cleaved flCCN1 which interacted with integrin-α7, enhanced the release of matrix metalloproteinase protein 1 and led to pulmonary emphysema. α-1-antitrypsin plays a significant role in preventing the lung from inflammation and elastase disruption. The study of Lockett et al[70] indicated that endothelial cells could release α-1-antitrypsin-riched EVs and these EVs adhered to and transferred α-1-antitrypsin into epithelial cells, preventing the development of emphysema. CSE exposure significantly inhibited the secretion of α-1-antitrypsin-riched EVs and thus contributed to the pathogenesis of COPD. Moreover, research demonstrated that the up-regulation of miR-21 in the lungs triggered the modification of exosomal components. Exosomes transferred miR-21 from bronchial epithelial cells to bronchial fibroblasts, upregulated the expression of hypoxia-inducible factor-1α, and led to increased secretion of a-smooth muscle actin and collagen I, which induced differentiation of myofibroblast and resulted in emphysema.[71] In addition, under the stimulation of CSE, macrophages secreted matrix metalloproteinase protein 14-positive EVs which enhanced the proteolytic activity in the lungs, leading to the development of emphysema.[72] The study from Fujita et al[73] demonstrated that CSE exposure upregulated the expression of miR-210 both in the human bronchial epithelial cells (HBEC) and HBEC-derived EVs. And miR-210 in the HBEC-derived EVs downregulated autophagy activity of lung fibroblast via inhibiting autophagy related 7 activity and promoted the differentiation of pulmonary fibroblast into myofibroblast.[73] Preclinical studies have found that bacteria-derived EV also contributed to the pathogenesis of COPD. Repeated inhalation of Escherichia coli (E. Coli) derived EVs induced neutrophilic inflammation and emphysema and upregulated the activity of elastase in the lung in an IL17α-dependent mechanism via TLR4 signaling pathway.[74] The above studies indicated that the components in the EVs potentially mediated and regulated pathological alterations in the development of emphysema and COPD [Figure 3].
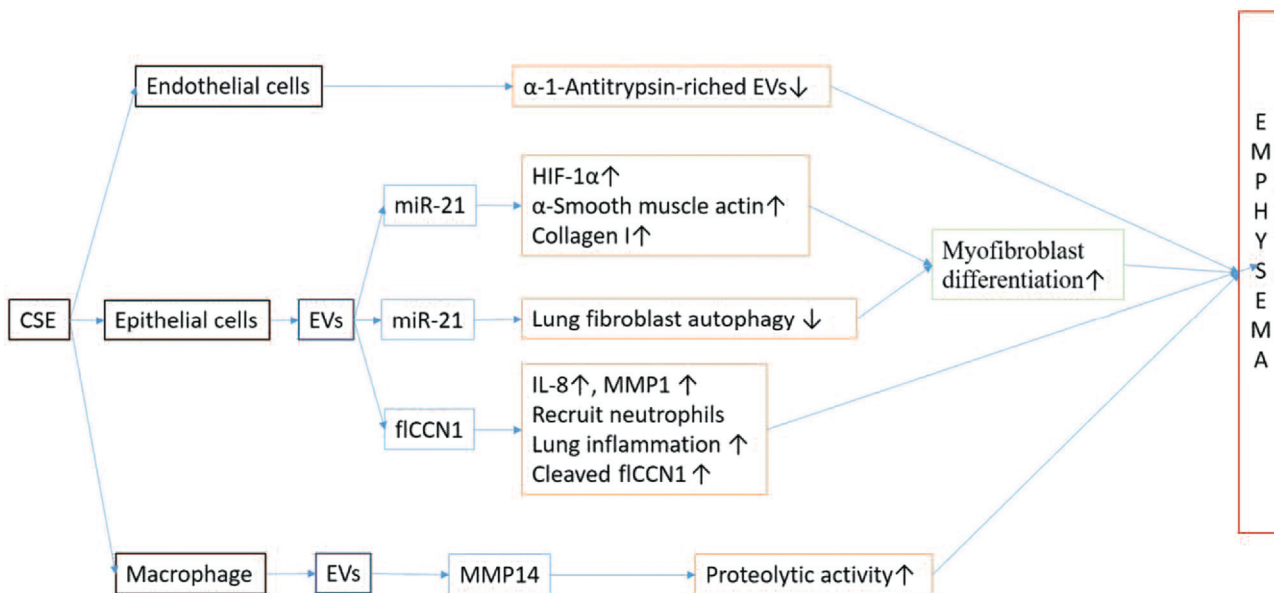

Figure 3: Diagram of potential roles of EVs in the pathogenesis and development of COPD. CCN1: Cellular communication network factor 1; COPD: Chronic obstructive pulmonary disease; CSE: Cigarette smoking exposure; EVs: Extracellular vesicles; flCCN1: Full-length CCN1; HIF-1α: Hypoxia-inducible factor-1α; IL-8: Interleukin 8; miR: MicroRNA; MMP: Matrix metalloproteinase protein.

PAH

A clinical study found that the levels of circulating endothelium-derived CD105+ EVs and EVs bearing active tissue factor were significantly increased in patients with PAH compared with control subjects. And compared with jugular vein blood, higher quantities of pro-coagulant EVs were notably detected in the occluded pulmonary artery blood.[75] EVs derived from different types of PAH contain different components. A study showed that the level of CD3+ EVs in the circulation of patients with idiopathic PAH or chronic thromboembolic PAH was much higher than that in patients with connective tissue diseases related to PAH, congenital heart disease-related PAH, and pulmonary fibrosis-induced PAH.[76] Similarly, the expression of E-selectin-positive EVs in peripheral blood in patients with thromboembolic PAH was significantly higher than that in patients with other types of PAH.[76] Chronic hypoxia is a common pathological feature in the pathogenesis of PAH. Zhang et al[51] found that the level of circulating exosomes in hypoxia rats was significantly higher than that in normoxia rats and the concentration of exosomes showed a positive relationship with mean pulmonary arterial pressure, pulmonary vascular resistance, right ventricular hypertrophy index, and the percentage of medial wall thickness, etc. In vitro treatment of circulating EVs isolated from hypoxic rat declined activity of nitric oxide (NO) synthase, decreased the production of NO in pulmonary arteries and aortas, and increased the production of reactive oxygen species (ROS) in endothelial cells.[77] And in vivo, intravascular administration of circulating hypoxic EVs inhibited the relaxation of pulmonary arteries. It was considered that EVs played a crucial part in inducing endothelial dysfunction of pulmonary arteries.[77] Intravenous administration of EVs derived from the lung and plasma of monocrotaline (MCT)-induced PAH mice could induce right ventricular hypertrophy and remodeling of the pulmonary vasculature in healthy mice, which indicated that EVs mediate the pathological process of pulmonary vascular remodeling in the development of PAH.[78] Excessive proliferation of pulmonary artery smooth muscle cells is another important feature in the pathogenesis of PAH. Research has indicated that exosomes derived from pulmonary artery endothelial cells injured with hypoxia caused an excessive proliferation of pulmonary artery smooth muscle cells.[51] Khandagale et al[79] found that EVs isolated from plasma of patients with PAH triggered activation of human pulmonary endothelial cells and angiogenesis in vitro and significantly increased the release of pro-angiogenic proteins, namely vascular endothelial growth factor A and fibroblast growth factor, which indicated that EVs were involved in regulating the function of pulmonary endothelial cells and angiogenesis in the pathogenesis of PAH. The level of endothelial cell-derived MVs was significantly increased in the plasma of patients with PAH,[80] suggesting that endothelial cell-derived MVs may play a pivotal role in the development of PAH.

In the pathogenesis of PAH, the transforming growth factor-β (TGF-β) signaling pathway and bone morphogenetic protein receptor 2 play an important role in the proliferation of pulmonary arteries through Smad signaling pathway.[81] Endothelial cell-derived caveolin-1 positive EVs in hypoxia-induced PAH mice promoted the secretion of TGF-β by macrophages and resulted in dysfunctional nitric oxide synthase (NOS) production and excessive production of peroxynitrate, which led to reduction of bone morphogenetic protein receptor 2 and induced vascular remodeling.[82] In addition, the activities of proliferator-activated receptor gamma coactivator 1-alpha and sirtuin 1, two classic biomarkers associated with mitochondria biogenesis, were significantly decreased in the circulating EVs isolated from PAH rats.[83] The higher levels of lipid peroxidation, nicotinamide adenine dinucleotide phosphate oxidase activity, and lower activity of superoxide dismutase and catalase were detected in the circulating EVs derived from MCT-induced PAH rats, which indicated that EVs may mediate oxidative stress and mitochondrial dysfunction in accelerating the development of PAH.[83] In addition, EVs-mediated mRNA transfer has a significant role in the pathogenesis of PAH. Pulmonary artery smooth muscle cell-derived exosomal miR-143-3p enhanced the migration of endothelial cells, accelerated pulmonary angiogenesis and remodeling, and thus contributed to the development of PAH.[84] The expression of miR-211 in circulating exosomes derived from hypoxia-induced PAH rats was notably increased. And the administration of miR-211 overexpressed exosomes further exacerbated PAH in vivo while downregulating the expression of miR-211 relieved PAH.[51] In all, circulating EVs play a role in the pathogenesis and development of PAH by mediating excessive oxidative stress, endothelial dysfunction of pulmonary arteries, accelerating pulmonary angiogenesis, and remodeling of pulmonary vasculature [Figure 4].

Asthma

Asthma is a chronic inflammatory airway disease characterized by airway hyper-responsiveness and reversible airway obstruction. Multiple studies during the past 20 years have shown that EVs derived from a wide range of cells including mast cell, eosinophil, neutrophil, B cell, T cell, epithelial cell, DC, fibroblast, myeloid-derived regulatory cell, etc, had a profound impact on the pathogenesis and progression of asthma.[85,86] The review from Sastre et al[86] described the role of exosomes in asthma and allergic sensitization. And it showed that exosomes had parts in presenting antigen, increasing ROS production and T helper 2 (Th2) cytokines release, recruiting granulocytes and eosinophil, stimulating the activity and proliferation of lymphocytes, inducing T cell activation and response, and modulating airway inflammation.[86] The effects of mast cell-derived EVs on the pathology of asthma remain controversial. The work from Xie et al[87] found that mast cell-derived EVs enriched with high-affinity immunoglobulin E (IgE) receptors were involved with binding to free IgE, decreasing the activation of mast cells, and inhibiting inflammatory responses in the development of asthma. However, the study of Skokos et al[88] showed that mast cell-derived EVs containing immune factors such as MHC II, CD86, and ICAM-1 could promote the recruitment of lymphocytes such as T cells and B cells into the lungs. It has been widely acknowledged that the eosinophil is the most important
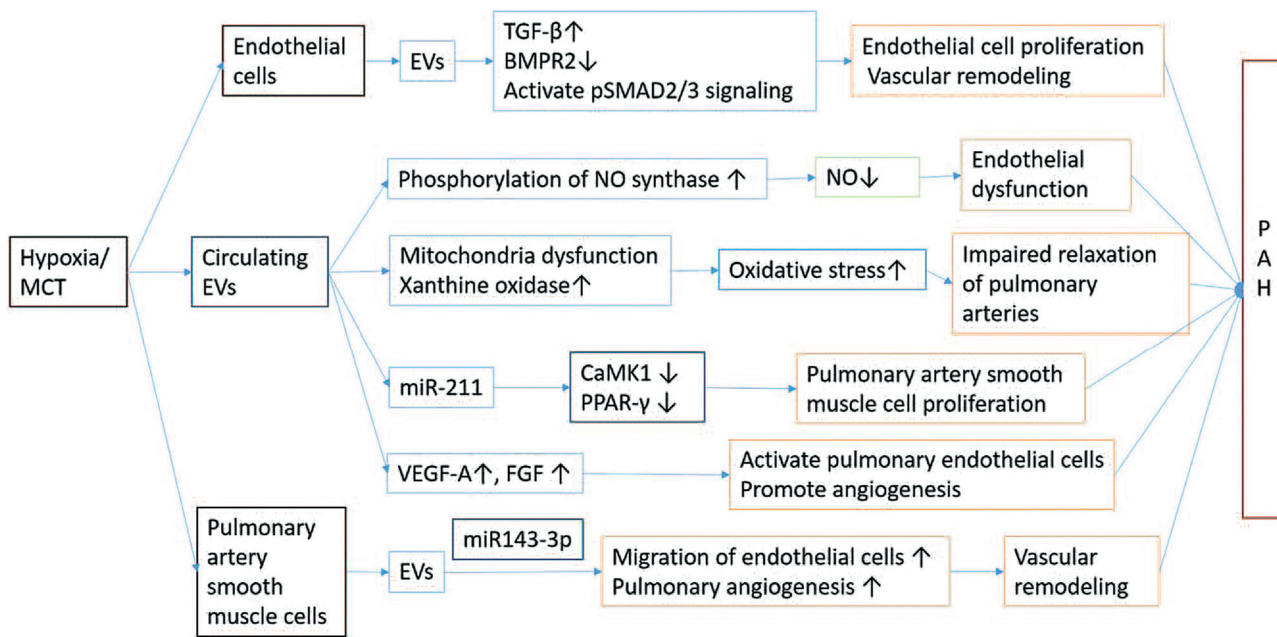
Figure 4: Diagram of potential roles of EVs in the pathogenesis and development of PAH. BMPR2: Bone morphogenetic protein receptor 2; CaMK1: Calcium/ calmodulin dependent protein kinase I; EVs: Extracellular vesicles; FGF: Fibroblast growth factor; MCT: Monocrotaline; miR: MicroRNA; NO: Nitric oxide; PAH: Pulmonary arterial hypertension; PPAR: Peroxisome proliferator-activated receptor; pSMAD2/3: Phosphorylated SMAD2/3; TGF-β: Transforming growth factor-β; VEGF-A: Vascular endothelial growth factor A.

effector cell in the pathophysiology of asthma. Among patients with asthma, the level of eosinophil-derived EVs was significantly increased,[89] and eosinophil-derived EVs were involved with increased release of ROS and NO, and these substances acted as chemotaxis factors to increase the adhesion of eosinophils via ICAM-1 and integrin α2.[90] Eosinophil-derived exosomes from asthmatic patients may also contribute to the proliferation of bronchial smooth muscle cells and induce airway remodeling in the progression of asthma. Furthermore, the study of Kulshreshtha et al[91] indicated that lung epithelial cell-derived exosomes could induce the production of IL-13 and proliferation and chemotaxis of macrophages in the development of asthma.[91] B cell-derived EVs could act as an immune-stimulatory factor to present allergen-associated peptides such as CD86, CD81, CD19, MHC, and Bet v 1-derived peptides which can result in the proliferation of T-cell and production of Th2-like cytokine (IL-5 and IL-13).[92] Therefore, EVs have a pivotal role in the pathogenesis of asthma, and EVs derived from different cell types might accelerate or prevent the development and progression of asthma. Further research is required to determine specific effects of EVs on asthma [Figure 5].

Moreover, the studies using EVs derived from BALF of asthma patients also indicated the potential role of EVs in the pathogenesis of allergic airway inflammation conditions. A study revealed that the levels of lipid, protein, and miRNA in BALF-derived exosomes in patients with asthma were significantly higher than that in the healthy population, and the levels were also related to increased levels of blood eosinophils and IgE.[93] BALF-derived EVs from asthma and healthy individuals exhibit different phenotypes and functions, in which EVs isolated from BALF of asthmatic patients showed increased release of leukotriene C4 and IL-8 from bronchial epithelial cells and further accelerated airway inflammation.[94] In addition, the spectrum of miRNA in BALF-derived exosomes in patients with asthma is quite different from healthy people. Twenty-four miRNAs in the EVs derived from BALF of asthma patients showed large differences with healthy controls and were highly correlated with FEV$_1$ and enhanced the release of asthma-related inflammatory cytokines, including IL-13, IL-10, IL-6, and IL-8.[95] A work from Zhao et al[96] also showed that the level of miRNA-126 in serum exosomes of patients with allergic asthma was significantly higher than that of healthy controls which may indicate a potential role of miRNA-126-enriched exosomes in the pathogenesis of asthma. The level of miR-223 and miR-142a in BALF-derived EVs was significantly increased in the allergen-treated mice model.[97] In summary, EVs contents, such as miRNA, derived from BALF of asthma patients aggravate the pathological changes of asthma via enhancing the release of asthma-related inflammatory cytokines and airway inflammation.

IPF

A couple of reviews have demonstrated that EVs also play an important role in driving pulmonary fibro-proliferation.[98-101] The review from Ibrahim et al[98] demonstrated that EVs drove and accelerated the development and progression of interstitial lung disease via the following three main signaling pathways: Wingless-N-type (Wnt)/ β-catenin pathway, TGF-β pathway, and cellular senescence.[32] Martin-Medina et al[102] reported that BALF-derived EVs from patients with IPF were enriched with Wnt5A, a significant signaling mediator that activated fibroblast proliferation and collagen production in the pathogenesis of IPF. Moreover, Chanda et al[103] demonstrated that EVs derived from IPF patients were enriched
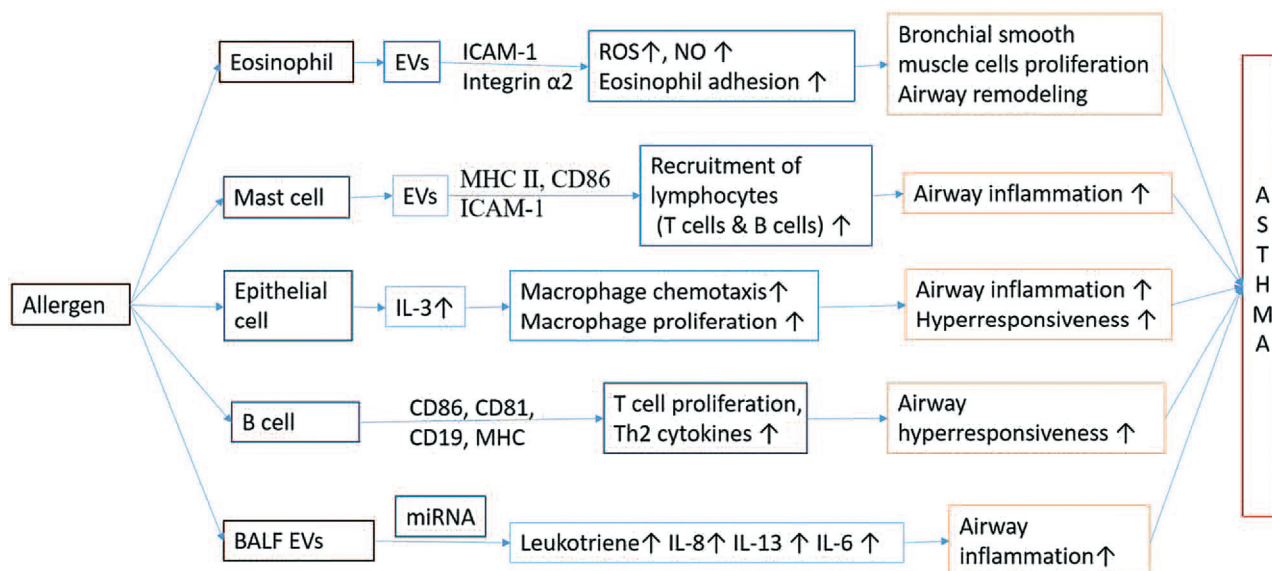
Figure 5: Diagram of potential roles of EVs in the pathogenesis of asthma. BALF: Bronchoalveolar lavage fluid; CD: Cluster of Differentiation; EVs: Extracellular vesicles; ICAM-1: Intercellular adhesion molecule-1; IL: Interleukin; MHC: Major histocompatibility complex; miR: MicroRNA; NO: Nitric oxide; ROS: Reactive oxygen species; Th2: T helper 2.

in fibronectin and could activate Src kinase and focal adhesion kinase and further enhance fibroblast proliferation and invasion in the progression of IPF. In addition, the study by Kang *et al*[104] showed that TGF-β induced human and mouse fibroblasts to release program death ligand-1-enriched EVs to activate fibroblast proliferation, promote collagen deposition, and further contribute to pulmonary fibrosis. Furthermore, the work of Parimon *et al*[105] showed that syndecan-1 downregulated the cargoes of multiple anti-fibrotic miRNAs (miR-34b-5p, miR-144–3p, and miR 503–5p) in the EVs of IPF to alter epithelial preprogramming and plasticity in pulmonary fibrosis by regulating fibrogenic signaling networks such as TGF-β signaling pathway, Wnt signaling pathway and cellular senescence. In addition, the miRNA in EV also plays a part in promoting the pathogenesis of IPF. The study showed that upregulation of miR-142–3p could aggravate the progress of IPF by altering the balance of proliferation and differentiation of mesenchymal cells.[106] Njock *et al*[107] also found that the miRNA spectrum in salivary exosomes of patients with IPF was significantly different from that in the healthy population; the level of miR-142–3p was significantly elevated and the level of let-7d–5p was significantly reduced in exosomes of IPF patients compared with healthy people. Moreover, the level of miR-142–3p in exosomes was negatively correlated with pulmonary diffusion area, while the level of let-7d-5p in exosomes was positively related to pulmonary diffusion area in patients with IPF. Both *in vivo* and *in vitro* experiments have proved that miRNA let-7d had anti-fibrosis effects; downregulation of let-7d-5p can increase the development of IPF by increasing epithelial–mesenchy-mal transition and collagen deposition.[108] miR-451a in EVs may also play an essential part in contributing to alleviating pulmonary fibrosis. Downregulated miR-451a levels in EVs increased the level of odd-skipped related 1, the putative target for miR-451a, to further promote epithelial–mesenchymal transition and

lung fibrosis.[109] The above studies highlighted the role of the content of EVs in enhancing the epithelial-mesenchymal transition and collagen deposition to further lead to the development of IPF [Figure 6].

The Application of EVs as Disease Biomarker

Multiple researches have demonstrated the potential of EVs as a promising biomarker for lung diseases. A clinical study of PAH found that the levels of platelet/endothelial cell adhesion factor and endothelial-cadherin-derived MVs in the circulation of patients with PAH were much higher than those of healthy controls. And the level of MVs was positively correlated with mean arterial pressure, right atrial pressure, and pulmonary vascular resistance, and negatively correlated with cardiac index, indicating the potential of endothelial cells-derived MVs as a promising biomarker to predict the severity of hemodynamic changes in patients with PAH.[110] The concentration of circulating leukocyte EVs derived from BALF of ARDS patients was significantly associated with the better outcome which suggested BALF-derived leukocyte EVs as a prognostic biomarker at the onset of ARDS.[111] And a higher level of plasma-derived EVs was correlated with a lower risk in critically ill patients with ARDS.[112] In addition, sphin gosine-1-phosphate receptor-3-enriched EVs also have been identified as a promising biomarker for disease prognosis and severity of ALI.[113] The profiling of miRNA contained in the lung-derived EVs has been used as a new disease biomarker in multiple lung diseases.[100] A clinical study of COPD showed that the level of exosomal miR-21 in the serum of smokers was significantly higher than that in non-smokers and was negatively correlated with FEV_1/FVC, which indicated the potential value of exosomal miR-21 as a disease biomarker in the diagnosis and treatment of COPD.[71] In addition, the level of E-selectin-positive MVs was significantly increased in COPD patients with
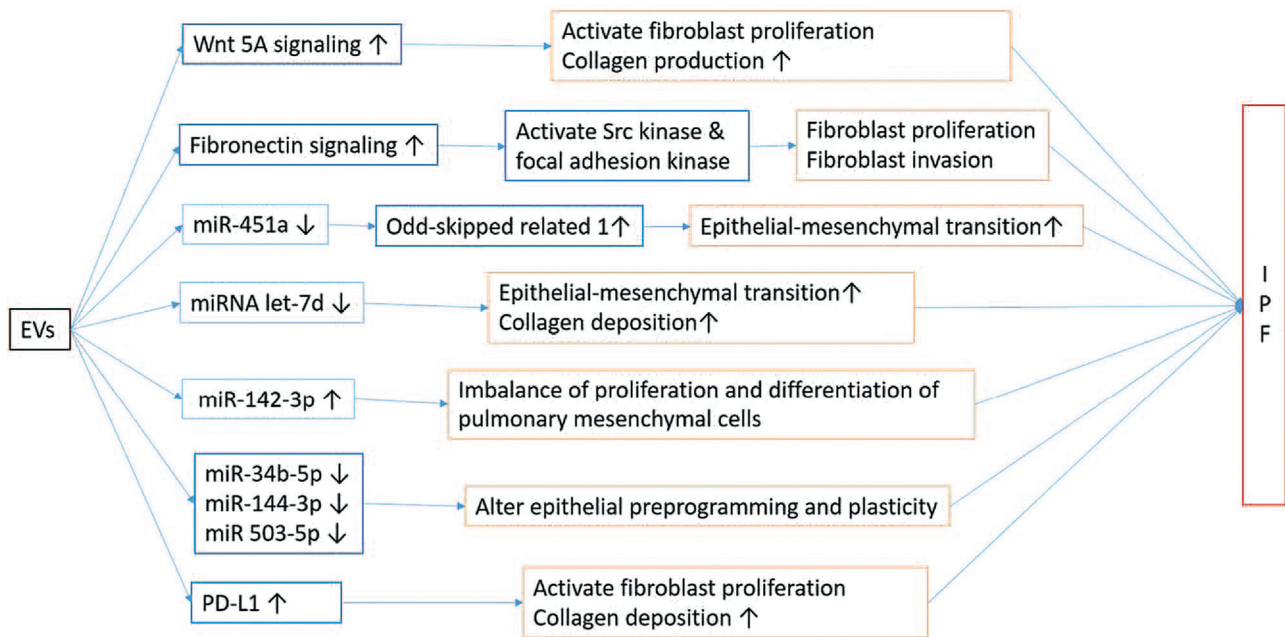
Figure 6: Diagram of possible roles of EVs in the pathogenesis and development of IPF. EVs: Extracellular vesicles; IPF: Idiopathic lung fibrosis; miR: MicroRNA; PD-L1: Programmed death ligand-1; Wnt: Wingless-N-type.

recurrent acute exacerbations and remained at a high level even when the symptoms of COPD disappeared. This work indicated that the high expression of E-selectin-positive MVs may be a potential predictor for acute exacerbations in patients with COPD.[66] In patients with IPF, the level of miR-21-5p in serum EVs was significantly higher than that in a healthy population. The baseline level of miR-21-5p in serum EVs was closely related to the decline rate of forced vital capacity within half a year and the mortality rate in 30 days, which indicated that the level of miR-21-5p in serum EVs may serve as an effective prognostic factor for IPF.[114]

Moreover, the levels of circulating exosomal RNA, such as miR-140-3p, miR-128, miR-196b-5p, and miR-486-5p, were significantly upregulated in patients with severe asthma compared with patients with mild-to-moderate asthma or healthy controls.[115] However, further research is needed to explore the value of EVs as biomarkers in asthma.

The Therapeutic Application of EVs in Lung Diseases

EVs can promote or inhibit the development of diseases or the pathogenesis of various lung disorders. Strategies to produce EVs with beneficial effects to treat lung injuries have been developed in the field of pulmonary diseases. In this section, we will discuss the latest advances of EVs as therapeutic strategies in various lung diseases.

Mesenchymal stem cells (MSC) derived EVs (MSC EVs) are the most frequently studied EVs as a therapeutic strategy to improve and repair lung damages such as in multiple lung diseases. MSC is an important subgroup of stem cells that can self-renew and differentiate under specific conditions. In ALI/ARDS, MSC can interact with

the respiratory microenvironment to regulate the immune system, increase bacterial removal, decrease inflammation, and repair the capillary barrier. Multiple investigators have shown MSC EVs can also have similar therapeutic effects.[12] A large number of preclinical studies have explored the therapeutic effects of MSC EVs in ALI/ARDS. Zhu et al[116] found that in the LPS-induced ALI mouse model, intra-tracheal instillation of MSC EVs can significantly decrease the infiltration of inflammatory cells and suppress inflammatory cytokines, repair the lung endothelial–epithelial cell barrier, and reduce pulmonary edema within 48 h. Monsel et al[117] demonstrated that intravenous injection of MSC EVs can improve mortality, reduce lung inflammation, reduce protein permeability, and increase bacterial clearance in a preclinical ALI mouse model induced by severe E. coli pneumonia. The study of Park et al[118] showed that MSC EVs have therapeutic effects similar to the above-mentioned ALI mouse models in ex vivo perfusion human lungs injured by severe E. coli pneumonia; intravenous infusion of MSC EVs restored alveoli fluid clearance, improved lung protein permeability, reduced pulmonary edema, and more importantly, also reduced the bacterial load in the damaged alveoli. The therapeutic effects of MSC EVs have also been described in preclinical models of viral pneumonia.[119] Recently, the administration of nebulized MSC EVs has also shown a promising therapeutic effect on enhancing survival rate, decreasing lung inflammation, and improving histological damages in a preclinical ALI mouse model and clinical safety in healthy volunteers.[120] MSC EVs also showed therapeutic potential in the treatment of COPD. Harrell et al[121] demonstrated that administration of MSC EVs could not only significantly alleviate chronic airway inflammation, improve pulmonary function, decrease the production of pro-inflammatory cytokines in various pulmonary cells

including lung-infiltrated macrophages, neutrophils, and natural killer T cells, in cigarette smoking-induced COPD mice model, but also improve lung function, exercise capacity, and life quality in patients with COPD.

In a preclinical study of allergic asthma, the treatment of MSC EVs also showed promising therapeutic effects in attenuating airway hyper-responsiveness, ameliorating airway remodeling, and downregulating production of Th2 cytokines and infiltration of eosinophils in the lungs.[22-24] In the ovalbumin-induced allergic asthma mice model, both human adipose tissue derived MSC and MSC EVs significantly reduced total leukocyte and eosinophils in BALF, levels of IL-5, eotaxin, and TGF-β in the lungs, collagen fiber deposition in the airways and lung parenchyma, and CD3+CD4+ T cells in the thymus.[122] Moreover, compared with MSC treatment, MSC EV treatment notably decreased the eosinophil count in the lung parenchyma, IL-4 level in lung tissue and CD3+CD4+ T cells in BALF, and improved lung mechanics, which showed MSC EVs treatment may have better effects on improving lung mechanics and eosinophils inflammation in allergic airway disorder.[122] In the *Aspergillus* hyphal extract induced allergic airway inflammation mice model, intravenous injection of EVs derived from either human MSC or mice MSC both significantly improved overall tissue resistance, lung elasticity, and airway resistance, and largely decreased histologic inflammation, total cell count, differential cell counts and the levels of IL-4, IL-5, IL-6, IL-17, and IL-12 in the BALF.[123] MSC treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydro-chloride, a cross-linker inhibiting the release of soluble proteins and MSC EVs, further blocked above multiple therapeutic effects from MSCs or MSC EVs treatment.[123] This study indicated MSC EVs played an essential role in ameliorating allergic airway inflammation. Besides, in the group 2 innate lymphoid cell-dominant asthma mice model, systemic treatment with MSC EVs significantly declined the levels of group 2 innate lymphoid cell and Th2 cytokines, mitigated pulmonary inflammatory cell infiltration, the secretion of mucus, and airway hyper-responsiveness.[124] The above studies demonstrated promising therapeutic effects of MSC EVs on alleviating allergic airway inflammation and pulmonary mechanics in different patterns of asthma induced by different incentives.

For PAH, a series of studies showed positive therapeutic effects of MSC EVs in improving hypertrophy and thickness of right ventricle (RV), decreasing mean pulmonary artery pressure and mean RV pressure, and promoting the mitochondrial activity to prevent the progression of PAH.[125-127] In the MCT-induced rat PAH model, intravenous administration of MSC EVs significantly improved the RV hypertrophy and reduced pulmonary arteriole area index, thickness index, the mean pulmonary artery pressure, and mean RV pressure.[125] In the hypoxia-induced mice PAH model and semaxanib/hypoxia rat PAH model, Hogan *et al*[126] demonstrated that MSC EVs treatment substantially increased metabolic flux into the tricarboxylic acid cycle, improved mitochondria function, and regulated metabolic dysfunction. And their study also indicated that MSC EVs treatment

significantly reduced the proliferation of pulmonary artery smooth muscle cells, lowered increased right ventricular systolic pressure, RV/left ventricle and septum ratio, and systolic pulmonary artery pressure, and further inhibited vascular remodeling in PAH.[126] Similarly, Aliotta *et al*[127] also demonstrated that MSC EVs reversed the increase in the RV/left ventricle and septum ratio and pulmonary arterial wall thickness-to-diameter ratio in the MCT-induced PAH mice model. The above research confirmed the therapeutic effects of MSC EVs on preventing vascular and ventricular remodeling and thus alleviating PAH pathogenesis. In interstitial lung disease, studies found that MSC EVs suppressed lung fibroblast differentiation, and proliferation, or inhibited pulmonary inflammation and remodeling in *in vitro* and *in vivo* experimental IPF models.[128,129] *In vitro*, the study from Wan *et al*[128] identified that MSC EVs blocked activation, proliferation, migration, invasion, and differentiation of pulmonary fibroblasts. In the bleomycin-induced pulmonary fibrosis mice model, intravenous injection of MSC EVs significantly reduced collagen deposition, percentage of apoptotic cells in the lung parenchyma, fibrosis, and alveolar distortion.[129] In addition, MSC EVs treatment also reprogrammed monocytes population into the immune-regulatory, anti-inflammatory monocytes phenotype, decreased pulmonary inflammation level, and remodulated alveolar macrophage phenotypes toward that of control mice.[129] These studies exhibited MSC EVs could revert fibroblast proliferation, decreased collagen content, and lung inflammation in pulmonary fibrosis.

Other strategies for therapeutic application of EVs include inhibiting the production, release, and uptake of EVs, as well as targeted inhibition of the components of EVs which could accelerate the progression of lung diseases.[130] In the alveolar macrophages, the fusion of MVBs and plasma membranes is a pivotal step for the release of exosomes, which is mainly mediated by Rab27a and Rab27b.[131] Under the stimulation of LPS, exosomes were released from alveolar macrophages and internalized by neighboring macrophages, which promoted the secretion of TNF-α from macrophages. Therefore, inhibition of Rab27a and Rab27b may reduce the inflammatory responses induced by TNF-α. And lung epithelial cell-derived IL-25 down-regulated expression of Rab27a and Rab27b in alveolar macrophages and further inhibited the release of exosomes and alleviated the expression and secretion of TNF-α.[54] The distribution of negatively charged phospholipid bilayers such as phosphatidylserine on the surface of EVs is an important feature of EVs, which plays a significant role in the uptake of EVs by recipient cells.[132] The uptake of EVs derived from hypoxia-induced stem cells by human umbilical cord endothelial cells can be inhibited by annexin, which can bind to phosphatidylserine with high affinity. Target interference for phosphatidylserine such as using the antibody for phosphatidylserine could significantly prevent the uptake of EVs by umbilical cord endothelial cells.[133] In addition to inhibiting the uptake of EVs, double annexin, an annexin dimer, can significantly block the budding of EVs from the plasma membrane and thus further prevent the release of MVs from endothelial cells.[134] Moreover, the recent study found that

pre-incubation with a small dose of high molecular weight hyaluronic acid significantly increased the trafficking of MSC EVs to injured lung tissues and improved the therapeutic efficacy of MSC EVs in severe bacterial pneumonia.[135] In addition, EVs present a potentially promising application in drug delivery as an effective cargo carrier. Novel technologies, such as miRNA-loaded EVs, are attracting much interest as a therapeutic strategy to treat pulmonary diseases.[100] A large number of studies and reviews have shown that blocking the harmful effects of EVs and increasing EVs-mediated drug delivery to improve their therapeutic efficacy in multiple lung diseases, including PAH, COPD, ALI/ARDS and asthma, have important clinical significance.[14,100,135,136] Our study also demonstrated that MSC EVs pre-treated with high molecular weight hyaluronic acid significantly increased the trafficking and targeting of MSC EVs into injured lung tissues and further promoted the therapeutic potency of MSC EVs in bacterial pneumonia–induced ALI.[135] Besides, in a prospective non–randomized open–label clinical cohort study among severe COVID–19 patients with moderate–to–severe ARDS, intravenous infusion with allogeneic bone marrow MSCs derived EVs declined increased neutrophil count, C–reactive protein, ferritin, and D–dimer, and improved patient's oxygenation status with increased arterial oxygen to fraction of inspired oxygen ratio and lymphopenia with increased CD3+, CD4+, and CD8+ lymphocyte counts.[137] However, the therapeutic application of MSC EVs or functional EVs remains in the preclinical experimental models of inflammatory lung diseases because of high production cost, instability of therapeutic effects, uncertain safety issues, usage and dosage, etc. Further research in decreasing the production cost and increasing the therapeutic efficacy of EVs is needed to explore the optimal application of EVs from bench to bedside.

Conclusion

In the past few decades, the field of EVs has undergone tremendous development. EVs have become important messengers of intercellular communication via transferring their contents (including proteins, lipids, nucleic acids, organelles, etc) into target cells to regulate respiratory immunity, repair or enhance the damage of the epithelial–endothelial barrier, and influence the inflammatory response. EVs play essential biological roles in both maintaining lung homeostasis and exacerbating the pathogenesis and progression of various lung diseases including ALI/ARDS, COPD, asthma, PAH, and IPF depending on the respiratory microenvironment condition. In addition, EVs and the components such as miRNA in EVs are being recognized as disease biomarkers to guide the diagnosis, treatment, and prognosis of multiple lung diseases. However, many biological characteristics and function of EVs in regulating pulmonary pathophysiology remain to be further explored and production cost and therapeutic effects of EVs also remain to be solved, which is of great significance for the successful translation of preclinical research into clinical diagnosis and treatment.

Conflicts of interest

None.

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