Extracellular Vesicles, MicroRNAs, and Pulmonary Hypertension

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Abstract

Pulmonary hypertension (PH) is a devastating disease that results in a progressive increase in pulmonary vascular resistance, right ventricular failure, and ultimately death of patients. Recent advances in our understanding of pathogenesis of diseases, including PH, have led to the study of extracellular vesicles (EV) as mediators of disease. Subsets of EV are microvesicles (MV), exosomes (Exo), and apoptotic bodies, and they are released from a variety of cell types and carry cargo such as proteins and microRNAs (miR). MicroRNAs contained within these EV play an important role in disease including in the pathogenesis of PH as well as other lung diseases.

Keywords

Extracellular vesicles · Pulmonary hypertension · Endothelial cells · Smooth muscle cells · microRNAs

7.1 Extracellular Vesicles (EV)

Extracellular vesicles (EV) are nano-sized, membrane-bound vesicles released from cells that can mediate intercellular communication [1]. Different EV types, including exosomes (Exo), microvesicles (MV), and apoptotic bodies, have been

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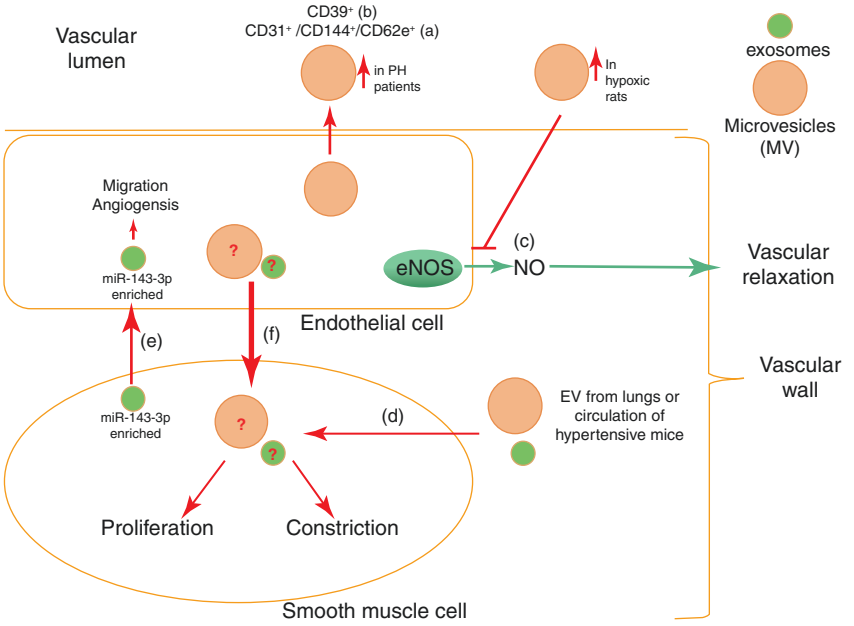

Fig. 7.1 Endothelial (EC) and smooth muscle (SMC) cells release extracellular vesicles (EV) and interact through transfer of EV. Increased circulating levels of endothelium-derived microvesicles (MV) have been documented in PH patients (*a*). Visovatti et al. demonstrated increased CD39 expression and function in circulating MV of idiopathic PAH patients, which may be associated with the increased ATPase/ADPase activity in MV (*b*). Tual-Chalot et al. showed that circulating MV from hypoxic rats can suppress endothelial-dependent vascular relaxation in rat aorta and pulmonary arteries by decreasing NO production (*c*). More recently, Aliotta et al. reported that healthy mice injected with circulating or lung EV isolated from MCT-treated mice show elevated right ventricular-to-body weight ratio and pulmonary arterial wall thickness-to-diameter ratio compared to that of mice injected with control EV (*d*). Deng et al. showed a high abundance of miR-143-3p in PASMC-derived exosomes and a paracrine pro-migratory and pro-angiogenic effect of these miR-143-3p-enriched PASMC-derived exosomes on PAEC (*e*). However, the cross talk between EC and SMC through EV transfer, especially from EC to SMC, and underlying molecular mechanisms remain unclear (*f*)

characterized on the basis of their biogenesis or release pathways: *Exosomes (Exo)* are 50–100 nm membrane vesicles of endocytic origin. They are released into the extracellular space by fusion with the plasma membrane. Exosomes contain endosome-specific proteins such as Alix and TSG101, components of microdomains in the plasma membrane such as cholesterol, ceramide, integrins, and tetraspanins, mRNAs, microRNA (miRNAs), and other non-coding RNAs. ExoCarta, an exosome database, provides a comprehensive list of exosomes identified (http://exocarta.org/) [2, 3]. *Microvesicles (MV)*, also referred to as microparticles (MP), especially in the cardiovascular field, are sized 20–1000 nm. They are formed through the outward budding and separation of the plasma membrane. During their formation, microvesicles retain surface molecules from parent cells and part of their

cytosolic content (proteins, RNAs, microRNAs) [3, 4]. *Apoptotic bodies* are the largest vesicles of the EV with a size of 1–5 μm. They are formed through outward blebbing of the cell membrane during the late steps of apoptosis. Apoptotic bodies contain cellular organelles, proteins, DNAs, RNAs, and microRNAs [2, 3, 5].

7.2 EV in Pulmonary Hypertension (PH)

Pulmonary hypertension (PH) is a devastating disease that results in a progressive increase in pulmonary vascular resistance, right ventricular failure, and ultimately death of patients [6, 7]. Recent studies have shown that abnormal EV secretion is associated with the pathogenesis of PH, and increased circulating levels of endothelium-derived MV have been documented in various cardiovascular diseases including PH [4, 8]. In PH patients the levels of circulating endothelial $CD31^+$ ($PECAM^+$)/$CD41^-$, $CD144^+$($VE\text{-}cadherin^+$), and $CD62e^+$ ($E\text{-}selectin^+$) positive microvesicles are increased compared with control subjects. Moreover, PAH patients exhibit higher values of endothelial $PECAM^+$ and $VE\text{-}cadherin^+$-positive MV versus those with chronic pulmonary disease-related PH [8]. Higher levels of endothelium-derived MV bearing E-selectin are also noted in thromboembolic PH as compared with non-thromboembolic PH subjects [9], suggesting that the etiology of the disease may influence MV levels [10].

MV are not only a biomarker of PH but rather actively contribute to development of PH [11, 12]. Visovatti and colleagues demonstrated increased CD39 expression and function in circulating MV of idiopathic PAH patients, which may be associated with the increased ATPase/ADPase activity in MV [13]. The endothelium-dependent relaxation of rat pulmonary arteries is suppressed after incubation with MV obtained from rats exposed to chronic hypoxia as compared to control arteries exposed to normoxia, accompanied by attenuated eNOS activity and increased ROS production [11]. In another study, Lee and colleagues demonstrated that mesenchymal stromal cell-derived exosomes exert a pleiotropic protective effect on the lung and inhibit vascular remodeling and hypoxic PH with suppression of STAT3/miR-17 levels and induction of miR-204 levels in the lung [14]. Moreover, a recent study reported that healthy mice injected with EV isolated from MCT-treated mice show elevated right ventricular-to-body weight ratio and pulmonary arterial wall thickness-to-diameter ratio compared to that of mice injected with control EV, providing direct in vivo evidence that EV contribute to pulmonary vascular remodeling and PH [12].

7.3 MicroRNA Transfer Through EV in PH

MicroRNAs (miRNAs, *miRs)* are small single-stranded non-coding RNAs that mediate post-transcriptional degradation or translation repression of target messenger RNAs (mRNAs) [15, 16]. Many miRNAs have been identified to play important roles in disease development, including PH [17–19]. In addition to the primary

intracellular locations, miRNAs can be exported extracellularly into the circulation system [20–23] through the transfer of EV. A recent study by Aliotta et al. has reported dysregulated miRNA profiling in the circulating exosomes of monocrotaline (MCT)-induced PH in mice, as well in patients with idiopathic pulmonary artery hypertension (IPAH) [24], suggesting that exosome (and maybe also other EV)-mediated miRNA signaling may play a role in the pathogenesis of PH. This hypothesis is supported by their finding that healthy mice injected with EV isolated from MCT-treated mice show induced right ventricular hypertrophy and pulmonary vessel wall thickening [12].

In the pulmonary vasculature, endothelial and smooth muscle cells (EC and SMC) are the two key cell types that play a major role in the pathobiology of PH [25]. The miRNA cross talk between EC and SMC via EV in pulmonary vasculature is exemplified by a recent study by Deng et al. [26]. This study demonstrates that migration and angiogenesis of pulmonary arterial endothelial cells (PAEC) are induced not only by exosome-derived miR-143 but also by co-culture of PAEC with pulmonary arterial SMC (PASMC) under conditions where direct cell-cell contact is prevented. The miR-143-enriched exosomes derived from PASMC are internalized by PAEC which lead to increased EC migration and angiogenesis. This study also shows that miR-143 is upregulated in the pulmonary vasculature of murine models of PH and in patients with PH. Genetic deletion of miR-143 or pharmacological inhibition of miR-143 in mice prevented the development of hypoxia-induced pulmonary hypertension. Hence, cross talk between EC and SMC via miR-143-enriched exosomes may be involved in the pathogenesis of PH under in vivo conditions.

Our knowledge about the cross talk between EC and SMC through EV transfer, especially the information transfer from EC to SMC, is still very limited and further studies are warranted.

7.4 Future Direction and Clinical Implications

The release of extracellular vesicles (EV) is a phenomenon shared by most cell types, including EC and SMC [1]. EV released into the extracellular space can enter body fluids and/or potentially reach neighboring and/or distal cells. The cargo of EV includes the proteins, lipids, nucleic acids, and membrane receptors of the cells from which they originate. Hence, EV can function as the "mail carrier" and transfer information (microRNAs, proteins, etc.) to their target cells, thus representing an important mechanism for intercellular communications [3, 4, 27–32]. The EV-mediated intercellular communications are evolutionarily conserved [33]. Therefore, EV are rich sources of biomarkers for diagnosis and/or prognosis of human diseases [34–41] and provide us potential therapeutic approaches [42, 43].

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