INTRODUCTION

In the 1950s, the innovative studies of Palade and Gowan described the endothelium as a dynamic organ with diverse capabilities for the first time.1 Over the last decades, besides as a vast, selectively permeable interface separating the vascular and interstitial compartments of the body,2 the endothelium has been widely investigated as an active organ secreting numerous mediators, which are necessary for normal vascular function.3,4 Endothelial dysfunction is characterized by shifting of the physiological balance of the vessel towards a vasoconstrictive, pro-thrombotic and pro-inflammatory state,5 often preceding atheroma development and being linked to vasculo-pathic diseases such as acute coronary syndromes (ACSs), coronary artery disease (CAD), hypertension, diabetes mellitus (DM), stroke and peripheral arterial disease.6,10 The endothelium is one of the primary targets of circulating microvesicles. Besides, microRNAs emerge as important regulators of endothelial cell function. As a delivery system of microRNAs, microvesicles play an active and important role in regulating vascular endothelial function. In recent years, some studies have shown that microvesicles containing microRNAs regulate the pathophysiological changes in vascular endothelium, such as cell apoptosis, proliferation, migration and inflammation. These studies have provided some clues for the possible roles of microvesicles and microRNAs in vascular endothelial dysfunction-associated diseases, and opened the door towards discovering potential novel therapeutic targets. In this review, we provide an overview of the main characteristics of microvesicles and microRNAs, summarizing their potential role and mechanism in endothelial dysfunction, and discussing the clinical application and existing problems of microvesicles for better translational applications.
OVERVIEW OF MVS AND MiRNAs

2.1 MVs

Extracellular vesicles (EVs) are a heterogeneous population of particles, which are released from various cell types into the extracellular space. According to their size, biogenesis and secretion mechanisms, EVs can be categorized as exosomes, MVs (also known as microparticles) and apoptotic bodies. MVs are irregularly shaped submicron vesicles (100-1000 nm) released from many types of cells, including platelets, ECs, erythrocytes and leucocytes. Moreover, MVs have been found in blood, urine, synovial fluid, extracellular spaces of solid organs, atherosclerotic plaques, tumours and elsewhere. Long considered as inert debris, MVs are now appreciated as an important transcellular delivery system in the exchange of biological signals, and their release is the result of a highly regulated process.

The formation and release of MVs are the result of a complex process with cytoskeleton reorganization and loss of the physiological asymmetry of the membrane bilayer. The formation of MVs appears to occur mostly in lipid-rich microdomains (lipid rafts/caveolae) within the plasma membrane. The underlying mechanism of the formation and release of MVs remains to be fully elucidated, but a consensus has been reached that intracellular Ca2+ plays a crucial role in the formation and release of MVs (Figure 1). Firstly, physiological asymmetry of the membrane bilayer is maintained by several phospholipid transporters: flippase, floppase and scramblase. Under physiological conditions, phosphatidylserine (PS) and phosphatidylethanolamine (PE) are continuously internalized due to the role of flippase, while floppase translocates them to the outside. Scramblase helps promote non-specific bidirectional redistribution across the bilayer. In the presence of external stimuli, increasing intracellular Ca2+ inhibits the flippase and activates floppase and scramblase with PS movement from the inner face to the outer face of cell membrane. Besides, the increased intracellular Ca2+ leads to calpain activation and thus generates the cytoskeleton reorganization and/or damage. Lastly, intracellular Ca2+ activates certain kinases and inhibits phosphatases to ensure the cleavage of the cytoskeleton and facilitate MV release.

The formation and release of MVs are related to a wide range of stimuli, such as pro-inflammatory cytokines (eg tumour necrosis factor-α and interleukin-1β), thrombin, complement proteins (C5b-9), uremic toxins and reactive oxygen species. This makes MVs well suited as surrogate marker of endothelial dysfunction.

2.2 miRNAs

miRNAs are a class of single-stranded, small (about 22 nucleotides long) and generally non-coding RNAs, which have emerged as critical regulators of gene expression via post-transcriptional degradation or translational repression. Each mRNA molecule may be regulated by multiple miRNAs, and a single miRNA may influence the expression of a wide range of genes in a cell. Some miRNAs are expressed ubiquitously, and some miRNAs are expressed in a tissue-specific and/or stage-specific manner. It has been estimated that miRNAs regulate the activity of 30%-50% of protein-coding genes and modulate about 10%-30% of human genome expression. miRNAs play a role in regulating various biological processes including metabolism, cell proliferation and differentiation, and apoptosis. The function of miRNA is directly associated with structure; thus, analysing the structure of the miRNA can help predict its function.
The process of miRNA biogenesis may provide new ideas for the study of miRNA. The generation of mature miRNA is a complicated process (Figure 2). Within the nucleus, a miRNA gene is transcribed by RNA Pol II to generate pri-miRNA, which is subsequently cleaved by RNase III Drosha and DGCR8 into pre-miRNA. Pre-miRNAs generated by both pathways are transported to the cytoplasm by exportin5-RAN•GTP complex, further cleaved by the RNase III Dicer and TRBP to double-stranded miRNA. The guide strand is preferentially incorporated in the RISC. In the simtron pathway, Drosha and possibly an unknown conjugate are involved in simtron biogenesis. After further processed by unknown factors, simtron enters the RISC complex. Functional miRNAs are produced by all three pathways. Most miRNAs are localized intracellularly, but some of them are released into the blood by being packaged in EVs. Both miRNA (including guide strand and passenger strand) and pre-miRNA can be packaged into MVs to deliver information. Pol II: RNA polymerase II; Drosha: RNase III endonuclease; RAN•GTP: GTPase Ran; DGCR8: Di George syndrome critical region 8; TRBP: TAR RNA-binding protein; RISC: RNA-induced silencing complex.

FIGURE 2 The formation of miRNA. In the canonical pathway, a miRNA gene is transcribed by RNA Pol II to generate pri-miRNA, which is subsequently cleaved by Drosha along with DGCR8 into pre-miRNA. In the mirtron pathway, mirtrons are excised from the host gene by spliceosome and trimming of short introns without Drosha processing. Pre-miRNAs generated by both pathways are transported to the cytoplasm by exportin5-RAN•GTP complex, further cleaved by the RNase III Dicer and TRBP to double-stranded miRNA. The guide strand is preferentially incorporated in the RISC. In the simtron pathway, Drosha and possibly an unknown conjugate are involved in simtron biogenesis. After further processed by unknown factors, simtron enters the RISC complex. Functional miRNAs are produced by all three pathways. Most miRNAs are localized intracellularly, but some of them are released into the blood by being packaged in EVs. Both miRNA (including guide strand and passenger strand) and pre-miRNA can be packaged into MVs to deliver information. Pol II: RNA polymerase II; Drosha: RNase III endonuclease; RAN•GTP: GTPase Ran; DGCR8: Di George syndrome critical region 8; TRBP: TAR RNA-binding protein; RISC: RNA-induced silencing complex. The places labelled with question marks are proposed but not clear.
transport of miRNAs by MVs is the key step of extracellular miRNA-mediated intercellular communication.\textsuperscript{64,67} Multiple miRNAs can be incorporated into one MV, but the exact mechanism of packaging is unclear.\textsuperscript{68} Changes in miRNA content between MVs and their parental cells indicate a selective packaging of miRNAs into MVs.\textsuperscript{69}

In some cases, precursors of mature miRNAs are also released.\textsuperscript{70} Depending on Rho A/ROCK signalling, both precursor and mature forms of miRNAs are exported into MVs in response to TNF-α.\textsuperscript{71} (Figure 1). Besides, not all of the miRNAs in the MVs are associated with the Ago2, the major component of the RISC.\textsuperscript{72} For example, miR-126-5p passenger strand, which does not bind to the Ago2, can exist and promote endothelial repair.\textsuperscript{72}

### 3.2 | miRNAs are protected from circulating RNases and transferred to recipient cells

Secondly, miRNAs are protected from circulating RNases and transferred to recipient cells. Both MVs and proteins contribute to protecting miRNAs in the MVs from RNases even in the unfavourable physiological condition. MV's lipid bilayers contribute to maintaining the stability of the circulating miRNAs to ensure the miRNA transfer to the recipient cells.\textsuperscript{73,74} The protection by MVs is non-specific, whereas by proteins is specific.\textsuperscript{72} Arroyo et al and Turchinovich et al demonstrated that the MV-free miRNAs were protected from RNase A by the association with Ago2 complexes after the disruption of the MVs.\textsuperscript{75,76} Interestingly, not all of the miRNAs in the MVs are associated with Ago2 complexes, and different miRNAs are associated with Ago2 complexes to different degrees. Therefore, the protective effect of Ago2 complex on various miRNAs in MVs is different.\textsuperscript{72}

The protective effect of Ago2 complexes or other proteins on the extracellular miRNAs in the MVs has not been clear.

Interaction between MVs and recipient cells operates through two main mechanisms: (a) receptor-ligand interaction—interaction between specific ligands on MVs surface and receptors on target cells leads to subsequent cascade responses; and (b) direct transferring part of their content or component to target cells.\textsuperscript{31,77} When MVs interact with target cells, perhaps the two mechanisms can influence each other. For instance, surface molecules not only act as adhesion molecules to promote receptor-ligand interaction, but also may act as signalling molecules to regulate the release of the MV content.\textsuperscript{78} Furthermore, a study showed that miRNA-rich MVs and miRNA-poor MVs had different ability to be taken up by recipient cells, but this study did not discuss the mechanisms underlying this increase in MV uptake.\textsuperscript{71}

### 3.3 | miRNAs recognize and repress mRNA targets within recipient cells

Thirdly, miRNAs must retain the ability of recognition and repression of mRNA targets within recipient cells. In cells, miRNAs recognize the specific binding sites usually located in the 3’ untranslated region (3’ UTR) of target mRNA sequences, leading to the reduction of protein expression by inhibiting mRNA translation and/or promoting target mRNA degradation.\textsuperscript{79} Interestingly, recent studies have found that miRNAs may also modulate gene expression in a positive manner.\textsuperscript{80} For instance, Orom UA et al demonstrated that mir-10a could bind to the 5’ UTR of ribosomal protein mRNAs and enhanced their translation.\textsuperscript{81}

Ago2 is also a key effector of miRNA function. Some results may imply that only the secreted miRNAs associated with Ago2 complexes in the MVs are stable and have biological function, compared with the non-Ago2 complex-bound miRNAs, which may be simply degraded in the recipient cells.\textsuperscript{72} However, we cannot exclude the possibility that non-Ago2 complex-bound miRNAs in the MVs may integrate the miRNA machinery of ECs to mediate their mRNA regulatory effects.\textsuperscript{82} Besides, Alexy et al demonstrated that among three precursor miRNAs assessed, pre-miR-155 was released into MVs most consistently in response to TNF-α, and miR-155 appeared to have the greatest capacity to be stably transferred to recipient cells. Perhaps extracellular miRNA-mediated target gene suppression requires transfer of pre-miRNA, which has a greater capacity to be incorporated into the RISC of recipient cells than mature miRNA.\textsuperscript{71}

### 4 | THE ROLE OF MVS CONTAINING SPECIFIC MiRNAs IN VASCULAR ENDOTHELIAL DYSFUNCTION

#### 4.1 | Platelet-derived microvesicles and their miRNA cargo

Platelets have been shown to contain an abundant and diverse array of miRNAs,\textsuperscript{83,84} and two-thirds of peripheral blood MVs are likely derived from platelets.\textsuperscript{85} Platelets do not have nucleus and cannot undergo miRNA generation within the nucleus, so Figure 2 may not be appropriate for platelets. Some members of the cytoplasmic miRNA processing complex such as Dicer, Ago2 and TRBP2 have been reported in platelets, but platelets inherit most of their mature miRNAs directly from the megakaryocytes.\textsuperscript{86} In addition, because prevalence of the platelet-derived microvesicles (PMVs) elevates in cardiovascular diseases,\textsuperscript{22} the interaction between miRNAs in PMVs and the endothelial function has always been a focus of attention.

In 2013, Gidlof et al used RNA-seq to confirm that 9 miRNAs were differentially expressed in PMVs in patients with myocardial infarction. Transfer of miR-320b by PMVs resulted in a down-regulation of intercellular adhesion molecule-1 (ICAM-1) expression in the HMEC-1 cells (human microvascular endothelial cell line) and was attenuated in the presence of brefeldin A, an inhibitor of vesicle formation.\textsuperscript{87} This finding is one of the first reports of vesicle-mediated platelet miRNA transfer, suggesting that transfer of functional platelet miRNAs into vascular ECs via PMVs can play a key role in regulating the inflammatory response of ECs.

In hypertensive conditions, PMVs delivered miR-142-3p into ECs and then enhanced abnormal proliferation of ECs by acting on Bcl-2-associated transcription factor 1 (BCLAF1). These results indicate that PMVs transmit miR-142-3p from activated platelets into
ECs and that miR-142-3p may play a crucial role in EC dysfunction. Anti-β2-glycoprotein I (anti-β2GPI) antibodies are the most common antiphospholipid antibodies in antiphospholipid syndrome (APS). Compared with the control group, anti-β2GPI/β2GPI complex induces the release of PMVs containing higher amounts of miR-96 and miR-26a. These two kinds of miRNAs can inhibit migration and tube formation of HUVECs by targeting selectin-P (SELP) and platelet-derived growth factor receptor alpha (PDGFRα) to enhance vascular endothelial cell damage. Recently, a data showed for the first time that miR-let-7a delivered by PMV targeted the 3′ UTR of antiangiogenic thrombospondin-1 (THBS-1) mRNA and potently inhibited THBS-1 protein synthesis to drive endothelial tubule formation in vitro. PMVs containing miR-223 have been shown to regulate genes in HUVEC, including two endogenous endothelial genes: FBXW7 and EFNA1. In another experiment, platelet-secreted miR-223 via PMVs targeted endothelial cell insulin-like growth factor 1 receptor (IGF-1R) and thus promoted cell apoptosis induced by advanced glycation end products. In both experiments, platelet activation and MV release were induced upon incubation with thrombin, but the effects of MV containing miR-223 on endothelium were different. The difference between the two experiments is that only the first experiment emphasized the combination of miR-223 and AGO2. Perhaps this just shows the characteristic of miRNA regulating multiple target genes, but we cannot exclude the influence of AGO2 on the effect of MVs and their associated miRNA on endothelial function.

FIGURE 3 The role of PMVs containing specific miRNAs in vascular endothelial dysfunction. PMVs produced by platelets under different stimuli contain different contents. Different miRNAs in PMVs act on corresponding target genes to affect gene expression and therefore affect vascular endothelial function, including inflammation, cell proliferation and apoptosis. PMVs: platelet-derived microvesicles; ICAM-1: intercellular adhesion molecule-1; SELP: selectin-P; PDGFRα: platelet-derived growth factor receptor alpha; THBS-1: thrombospondin-1; BCLAF1:Bcl-2-associated transcription factor 1; IGF-1R: insulin-like growth factor 1 receptor; FBXW7 and EFNA1: two endogenous endothelial genes; ↑: up-regulation; ↓: down-regulation.
 Altogether, these findings suggest a specific role of PMVs containing specific miRNAs in vascular endothelial dysfunction (Figure 3).

### 4.2 Endothelial cell-derived microvesicles and their miRNA cargo

Circulating endothelial cell-derived microvesicles (EMVs) act as an important marker of vascular function and major adverse cardiovascular events in patients with endothelial dysfunction.\(^{91-93}\) Interestingly, EMVs have additional biological carrier function, which may provide different explanation for their role in pathology.\(^ {94,95}\) For instance, Hergenreider et al showed that endothelial-derived miR-143/145 could be transferred to SMCs via EMVs and then prevented SMC dedifferentiation to prevent atherogenesis.\(^ {96}\)

To illustrate molecular mechanism of EMVs containing miR-19b in atherosclerosis, two studies demonstrated that EMVs with a high level of miR-19b could inhibit endothelial cell migration and angiogenesis by targeting Rho GTPase-activating protein 5 (ARHGAP5) and transforming growth factor β2 (TGFβ2), which modulate the function of HUVECs.\(^ {97,98}\) During atherosclerosis, miR-126, which is selectively enriched in EMVs from apoptotic endothelial cells, suppresses the inhibitory function of regulator of G-protein signalling (RGS16) and thus unleashes CXCR4 (CXC chemokine receptor type 4) to trigger a self-amplifying feedback loop, which leads to increased production and release of atheroprotective chemokine CXCL12.\(^ {99}\) In 2014, Schober et al first described that miR-126-5p preserved EC proliferation after hyperlipidaemic stress and protected from atherosclerosis by...
suppressing Notch1 inhibitor delta-like 1 homolog (Dlk1), but endogenous miR-126-3p had no such effect. However, the same group later found that application of exogenous miR-126-3p contained by EMVs or apoptotic bodies promoted endothelial repair and inhibited atherosclerosis. Therefore, we can speculate that the function of miRNAs depends on its endogenous expression or exogenous application, and even the same type of miRNA may have different effects.

In 2013, Jansen et al revealed that miR-126 in EMVs released from apoptotic ECs inhibited the target protein sprouty-related, EVH1 domain-containing protein 1 (SPRED1) expression, and then promoted endothelial target cell migration and proliferation in vitro and reendothelialization in vivo to promote endothelial repair. Mechanisms by which SPRED1 inhibits Ras/MAPK (mitogen-activated protein kinase) signalling to reduce migration and proliferation of ECs may be involved in this process. Interestingly, glucose-damaged EMVs contained significantly lower amounts of miR-126 and showed reduced endothelial repair capacity.

In 2015, Jansen et al demonstrated that miR-222 in EMVs functionally reduced expression of its target protein ICAM-1 and then promoted anti-inflammatory effects in ECs, while miR-222 in EMVs derived from glucose-treated ECs facilitated ICAM-1 and VCAM-1 expression in resting ECs. Following experiment found that under pro-inflammatory condition, miR-222 in EMVs derived from glucose-treated ECs rather reduced ICAM-1 expression in target ECs. The contradictory results show that the effects of MVs and their miRNA cargo may depend not only on the state of the parent cells but also on the state of recipient cells. In summary, EMVs containing miRNAs are involved in the regulation of endothelial function through different mechanisms (Figure 4).

4.3 | Leucocyte-derived microvesicles and their miRNA cargo

Lymphocyte-derived microvesicles (LMVs) have been shown to have a strong inhibitory effect on proliferation of ECs. Inhibition of miR-181a, which is one of the most abundant miRNAs in LMVs, significantly attenuates the effect of LMVs on EC proliferation. Because overexpression of miR-181a strongly inhibited MAPK1 expression in ECs, Yang C et al speculated the anti-angiogenic effect of miR-181a because of interference with the MAPK1/VEGF signalling. However, the contradictory role of miR-181a in modulating angiogenesis has been reported. These controversial findings may suggest that the role of miR-181a depends on specific cell or tissue. It can also be speculated that the effect of miRNAs in MVs may also be cell- or tissue-specific.

Transcription factor cMyb is responsible for the increased migratory capability of ECs. miR-150 is contained in monocyte-derived microvesicles (MMVs), which can be transferred by MMVs into ECs to take part in down-regulating cMyb. In patients with severe atherosclerosis, MMVs are rich in miR-150; thus, the MMVs containing miR-150 might be part of the reason for the vascularization of atherosclerotic plaques (Figure 4).

4.4 | Tumour cell-derived MVs and their miRNA cargo

Tumour cells have been shown to produce large numbers of MVs. Some studies demonstrated that tumour cell-derived MVs could regulate EC function. Angiogenesis plays a crucial role during tumorigenesis. Zhuang et al described that five tumour cell lines induced up-regulation of miRNAs in EC and modified EC function through MV-derived miRNAs. Furthermore, they described that miR-9 was transferred into ECs by MVs and then activated the JAK/STAT pathway by down-regulating suppressor of cytokine signalling 5 (SOCS5) to induce EC migration.

4.5 | Stem cell-derived MVs and their miRNA cargo

Human bone marrow-derived mesenchymal stem cells (MSCs) and liver resident stem cells (HLSCs) have been shown to release MVs to transfer miRNAs to target cells, while the biological effect of stem cells may depend in part on MV-shuttled miRNAs.

RNA analysis revealed that miRNAs were enriched in adipose-derived stem cells (ASCs) released MVs, and an underlying mechanism of the pro-angiogenesis may be the delivery of miR-31 via MVs from ASCs to ECs. miR-31 in MVs from ASCs contributed to the migration and tube formation of ECs by targeting and suppressing factor-inhibiting HIF-1.

5 | Regulation of miRNAs sorting into MVs

In 2013, Villarroya-Beltri et al demonstrated the RNA-binding protein heterogeneous nuclear ribonucleoprotein A2B1 (hnRNP A2B1) as a key player in miRNA sorting and loading into exosome through combining with GGAG motifs on miRNA. Recently, some studies found that RNA-binding proteins hnRNPQ and hnRNPU were also involved in exosomal miRNA sorting. Besides, Ago2, the Y-box protein 1, ceramide signalling and mRNA-miRNA interaction also play a role in regulating miRNA packing into exosome. However, little is known about the mechanism by which miRNAs are selectively packaged into MVs. The process of sorting and export of miRNAs is ATP-dependent and is affected by extracellular condition. We speculate that the content and mode of miRNAs packaging into MVs may depend on the nature of the agonist, stimulatory or shear conditions.

As far as we know now, firstly, inflammatory factors may be involved in regulating the level and transfer of miRNAs in MVs. Pan Y et al demonstrated that platelets contained abundant miRNAs, particularly miR-223, and the level of the miRNAs in PMVs was up-regulated by inflammatory factors such as thromboxopetin (TPO) and thrombin. Besides, Alexy T et al showed that pro-inflammatory cytokine TNF-α altered the release and transfer of miRNAs in EMVs. Codagnone M et al indicated that anti-inflammatory pro-resolution
lipid mediator lipoxin A4 (LXA4) regulated the miRNAs in EMVs released by TNF-α-stimulated HUVECs. Kuhn S et al provided first evidence that anti-inflammatory adenosine significantly increased miR-142-3p levels in MMVs. Furthermore, a study found miR-221, miR-320a, miR-92a and miR-17 were significantly higher (greater than twofold) in hydrochloric acid-induced MV release from lung epithelium. Secondly, in addition to inflammatory factors, other factors can also regulate the transfer of miRNA in MVs. Collino F et al demonstrated that ribonucleoproteins (T-cell internal antigen-1 (TIA), TIA-1-related (TIAR) and AU-rich element-binding protein (HuR)) in MVs were involved in the selected pattern of miRNAs in MVs. In addition, packing of miRNAs into specific MVs may also be affected by diseases. For instance, miR-222 was demonstrated to be transported into recipient ECs by EMVs and functionally regulated expression of ICAM-1. However, after simulating diabetic conditions, the data showed reduction in the number of miR-222 in EMVs and reduced capacity of anti-inflammatory in vitro and in vivo. It is speculated that the ‘packaging’ also depends on nucleases presenting in the recipient cell, proteins found in MVs and sex-related differences (Table 1).

## TABLE 1 Factors that regulate miRNAs in MVs

| miRNA  | Experiment | Factor | Effect | MVs’ origin | Sample | Related diseases                  | Ref. |
|--------|-------------|--------|--------|-------------|--------|-----------------------------------|------|
| miR-223| In vitro    | 1 ng/mL TPO or 0.1 U/mL thrombin | Up-regulate | Human platelet | Venous blood | Enteritis, hepatitis, nephritis, atherosclerosis | 90   |
| miR-221, 320a, 92a, -17| In vivo | Hydrochloric acid (0.1 N, pH 1.5) | Up-regulate | Lung epithelial cell of mouse | Bronchoalveolar lavage fluid | Acute lung injury | 66   |
| miR-126, -21, -155| In vitro | TNF-α (100 ng/mL) | 70%-80% decrease in miR-126 and -21; a significant increase in pre-miR-155 and miR-155; 50% reduction in uptake by recipient cells | Human aortic endothelial cells | - | - | 71   |
| miR-181a,-660, -20b,-29b,-217, -29a,-100,-92a,-214,-139, -494,-19a,-19b, -216,-143,-362, -20a,-126-5p| In vitro | LXA4 (0.1-100 nmol/L) | Up-regulate miR-126-5p and down-regulate the rest of 18 miR | Human umbilical vein endothelial cell | Umbilical cord of human | - | 68   |
| miR-125a, -34a| In vitro | Sex | miR-125a was lower in activation-derived EMVs, whereas expression of miR-34a was higher in apoptosis-derived EMVs from men compared with women. | Human endothelial cell | Venous blood of human | - | 120  |
| miR-142-3p| In vitro | Adenosine (100 μmol/L) | Twofold increase in the miR-142-3p level in MMVs | Bone marrow mononuclear cell | Bone marrow from hind legs of mice | - | 67   |

Note: We listed the factors affecting miRNAs and some basic information in the experiments, as well as the diseases involved in these miRNAs changes. qRT-PCR: quantitative real-time polymerase chain reaction. TPO: thrombopoietin; TNF-α: tumor necrosis factor-α; LXA4: lipoxin A4.

Although the presence of MVs has been known for many years, many basic questions of MVs remain. Firstly, current isolation procedures do not clearly purify specific population of MVs, and this may partly be explained by the lack of standardization of both isolation techniques and protocols, such as specific markers of different MV populations. Furthermore, MV counts, types and contents tend
to vary per collection methods, storage media and the assay itself, which may also affect the type and quantity of miRNAs in MVs.\(^{125}\) Full exploitation of the information encompassed within blood MVs will require complicated proteomic, lipidomic, transcriptomic and metabolomic approaches.\(^{126}\) Secondly, it is not clear how specific miRNAs are packaged into MVs and transferred into target cells in response to different stimuli and pathological conditions. In the same way, the process of how MV-delivered miRNAs are selectively internalized by the specific cells or tissues needs further research.\(^{127}\) Thirdly, although there is a specific effect of miRNAs on MVs, we cannot rule out the influence of other miRNAs or bioactive molecules in the MVs.\(^{26}\) Fourthly, both MVs and exosomes are surrounded by a phospholipid bilayer and carry RNAs and proteins,\(^{128}\) but we do not know whether there are differences in the transmission and function between MV-mediated miRNAs and exosome-mediated miRNAs.

Finally, in terms of treatment, because of the complexity and diversity of miRNA-mRNA interaction, we need to take this into account that the impact of MV-delivered miRNAs on the target cells may be greatly extensive to have bad effects. Besides, before using detections of the MV-delivered miRNAs to better assess diseases, it is necessary to clarify the aspects of the clinical utility of MV as a biomarker, such as evidence of their predictive value, discrimination and reclassification power.\(^{26}\) If all these outstanding issues can be resolved, the use of MV-delivered miRNAs will be an effective and site-specific treatment.

### 6.2 | The clinical prospect of MVs

On the one hand, extracellular miRNAs hold great potential to act as disease biomarkers due to their non-invasive accessibility and remarkable stability.\(^{60,77,129}\) A great number of studies have shown that miRNAs are differently enriched in MVs, and their expression patterns also change with respect to different diseases.\(^{130}\) Therefore, MVs and associated miRNAs are considered to be potential diagnostic biomarkers of diseases.\(^{131-133}\) For instance, in a prospective study, in 176 patients with stable coronary artery disease (CAD), Jansen F at al. found that the level of miR-126 or miR-199a expression in circulating MVs but not in plasma could predict the occurrence of cardiovascular events in stable CAD patients.\(^{134}\) Future studies must identify altered miRNAs in specific subclasses and demonstrate representative ranges of health and disease.\(^{60}\)

On the other hand, MVs and the miRNAs they contain have inspired many studies on pathology and disease resistance.\(^{58,135}\) For a long time, it has been thought that the therapeutic effect of MV-delivered miRNAs is obviously superior to that of traditional treatment. Firstly, acting as therapeutic delivery agents, MVs originate from the host; thus, they have reduced toxicity and may be tolerated by the immune system.\(^{136}\) Secondly, because of the complexity and diversity of miRNA-mRNA interaction, the impact of MV-delivered miRNAs on the target cells may be quite extensive, potentially avoiding the undesired effects caused by switching a single target gene on or off.\(^{137}\) Many studies have shown that MVs delivering miRNAs are involved in the occurrence and development of many diseases.\(^{131,138,139}\) As a strategy to alter the type and quantity of extracellular miRNAs to gain therapeutic advantage, specific miRNAs or their inhibitors can be added to specific MVs.\(^{140}\) Future studies may focus on tailored recombinant MVs with unique cassettes of miRNAs for therapeutic benefits.\(^{141}\)

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### AUTHOR CONTRIBUTIONS

Qiang Zhang, Zeyu Shu and Jin Tan conceived, designed and drafted the manuscript. Yuyang Miao and Zeyu Shu contributed to the revised version of the manuscript. All authors confirmed the final version of the manuscript for submission.

### DATA AVAILABILITY STATEMENT

I confirm that I have included a citation for available data in my references section.

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### REFERENCES

1. Fishman A. Endothelium: a distributed organ of diverse capabilities. Ann N Y Acad Sci. 1982;401:1-8.
2. Krogh A. The Anatomy and Physiology of Capillaries. New Haven, CT: Yale University Press and London, UK: Oxford University Press; 1922.
3. Tousoulis D, Simopoulou C, Papageorgiou N, et al. Endothelial dysfunction in conduit arteries and in microcirculation. Novel therapeutic approaches. Pharmacol Ther. 2014;144:253-267.
4. Ray S, Miglio C, Eden T, Del Rio D. Assessment of vascular and endothelial dysfunction in nutritional studies. Nutr Metab Cardiovasc Dis. 2014;24:940-946.
5. Chistiakov DA, Revin VV, Sobenin IA, Orekhov AN, Bobryshev YV. Vascular endothelium: functioning in norm, changes in atherosclerosis and current dietary approaches to improve endothelial function. Mini Rev Med Chem. 2015;15:338-350.
6. Gkaliagkousi E, Gavrilakli E, Triantafylou A, Douma S. Clinical significance of endothelial dysfunction in essential hypertension. Curr Hypertens Rep. 2015;17:85.
7. Giraldo-Gruese M, From ED. Endothelial dysfunction to arterial stiffness in diabetes mellitus. Curr Diabetes Rev. 2018.
8. Matsuza Y, Guddeti R, Kwon T, Lerman L, Lerman A. Treating coronary disease and the impact of endothelial dysfunction. Prog Cardiovasc Dis. 2015;57:431-442.

9. Abdel Hamid M, Bakhoun SW, Sharaf Y, Sabry D, El-Gengehe AM, Latif A. Circulating endothelial cells and endothelial function predict major adverse cardiac events and early adverse left ventricular remodeling in patients with ST-segment elevation myocardial infarction. J Interv Cardiol. 2016;29:89-98.

10. Shin HK, Oka F, Kim JH, Atochin D, Huang PL, Ayata C. Endothelial dysfunction abrogates the efficacy of normobaric hyperoxia in stroke. The Journal of Neuroscience. 2014;34:15200-15207.

11. Lovren F, Verma S. Evolving role of microparticles in the pathophysiology of endothelial dysfunction. Clin Chem. 2013;59:1166-1174.

12. Sun X, Belkin N, Feinberg MW. Endothelial microRNAs and atherosclerosis. Curr Atheroscler Rep. 2013:15:372.

13. György B, Szabo TG, Pasztoi M, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cell Mol Life Sci. 2011;68:2667-2688.

14. Yáñez-Mó M, Siljander P, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracellular Vesicles. 2015;4:27066.

15. Fujita Y, Kosaka N, Araya J, Kuwano K, Ochiya T. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013;200:373-383.

16. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles and friends. Nat Rev Mol Cell Biol. 2004;5:277-286.

17. Melkì I, Tessandier N, Zufferey A, Boilard E. Platelet microvesicles and vascular dysfunction in obstructive sleep apnoea. Eur Respir J. 2014;44:207-216.

18. Renaud-Picard B, Toussaint J, Leclercq A, et al. Membrane microvesicles and respiratory disease. Rev Mol Respir. 2017:34:1058-1071.

19. Győrgy B, Módos K, Pállinger E, et al. Detection and isolation of cell-derived microvesicles are compromised by protein complexes resulting from shared biophysical parameters. Blood. 2011;117:e39-48.

20. Wolf P. The nature and significance of platelet products in human plasma. Br J Haematol. 1967;13:269-288.

21. Edelstein LC. The role of platelet microvesicles in intercellular communication. Platelets. 2017;28:222-227.

22. Arora V, Shukla SK, Garg A, et al. Phosphatidylserine exposure on circulating microparticles is increased in inflammatory diseases. J Cell Physiol. 2016;231:1638-1644.

23. Goyal P, Tiwari A, Aggarwal SK, et al. Microparticle generation: identification of a novel pathway involving ROCK-II activation by caspase-2. Blood. 2006;108:1868-1876.

24. Yamazawa R, Jiko C, Choi S, et al. Structural basis for selective binding of export cargoes by exportin-5. Proc Natl Acad Sci USA. 2015;112:E1106-E1115.

25. Wienholds E, Kloosterman WP, Miska E, et al. MicroRNA expression evidence for the expression of numerous novel primate- and tissue-specific microRNAs. Proc Natl Acad Sci. 2011;108:105-112.

26. Anglicheau D, Muthukumar T, Suthanthiran M. MicroRNAs: small RNAs with big effects. Transplantation. 2010;90:105-112.

27. Coaching MJ, Jao KH, Zeng L. The salient role of microRNAs in atherogenesis. J Mol Cell Cardiol. 2018;122:99-113.

28. Lekakis J, Abraham P, Balbarini A, et al. Methods for evaluating atherosclerotic properties. J Cell Physiol. 2016;231:1638-1644.

29. Donaldson CJ, Lao KH, Zeng L. The salient role of microRNAs in atherogenesis. J Mol Cell Cardiol. 2018;122:99-113.

30. Li Y, Jiang J, Liu W, et al. microRNA-378 promotes autophagy and inhibits apoptosis in skeletal muscle. Proc Natl Acad Sci. 2019;116:281-297.

31. Conner J, Pak CH, Zwaal RF, Schroit AJ. Bidirectional transbilayer movement of phospholipid analogs in human red blood cells. Evidence for an ATP-dependent and protein-mediated process. J Biol Chem. 1992;267:19412-19417.
54. Bronze-da-Rocha E. MicroRNAs expression profiles in cardiovascular diseases. Biomed Res Int. 2014;2014:985408.
55. Jansen F, Yang X, Hoelscher M, et al. Endothelial microparticle-mediated transfer of MicroRNA-126 promotes vascular endothelial cell repair via SPRED1 and is abrogated in glucose-damaged endothelial microparticles. Circulation. 2013;128:2026-2038.
56. Anene C, Graham AM, Boyne J, Roberts W. Platelet microparticle-delivered microRNA-Let-7a promotes the angiogenic switch. Biochim Biophys Acta. 2018;1864:2633-2643.
57. Valadi H, Ekstrom K, Bossios A, Sjostrom M, Lee JI, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9:654-659.
58. Alexandru N, Andrei E, Niculescu L, Dragan E, Ristoiu V, Georgescu M. MicroRNAs expression profiles in cardiovascular diseases. Mol Cancer. 2010;9:56:580.
59. Chen X, Liang H, Zhang J, Zen K, Zhang CY. Horizontal transfer of extracellular vesicles and their microRNA signature from liquid biopsy as early biomarkers of diabetic micro/macrovacular complications. Int J Mol Sci. 2017;18:1974.
60. Mauze S, Weber M, Gray WD, Searles CD. TNF-α mediates the release and transfer of microparticle-encapsulated miRNAs to endothelial cells via microparticle-mediated transfer of miRNAs that suppress tumor growth. Blood. 2017;130:567-580.
61. Kuhn S, Splith K, Ballschuh C, et al. Mononuclear-cell-derived microparticles attenuate endothelial inflammation by transfer of mRNA in an atherosclerotic hamster model. Acta Physiol (Oxf). 2017;212:230-249.
62. Leri A, Collino F, Deregibus MC, et al. Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. PLoS ONE. 2010;5:e11803.
63. Laffont B, Corduan A, Ple H, et al. Activated platelets can deliver mRNAs, microRNAs and protein mRNAs and enhances their translation. Circulation. 2016;134:734-751.
64. Groves R, Hyde S, Keddie K, et al. The clinical significance of platelet microparticle-associated microRNAs. Clin Chim Lab Med. 2017;55:64-666.
65. Li H, Zhang X, Wang F, et al. MicroRNA-21 lowers blood pressure in spontaneous hypertensive rats by upregulating mitochondrial translation. Circulation. 2016;134:734-751.
66. Yang X, Zhang D, Wu J. Lung epithelial cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. Leukemia. 2006;20:847-856.
67. Lenti A, Collino F, Deregibus MC, et al. Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. PLoS ONE. 2010;5:e11803.
68. Lee H, Zhang D, Wu J. Lung epithelial cell-derived microvesicles regulate macrophage migration via microRNA-17/221-induced integrin beta1. Recycling. 2017;199:1453-1464.
69. Kuhn S, Splith K, Ballschuh C, et al. Mononuclear-cell-derived microvesicles attenuate endothelial inflammation by transfer of mir-142-3p in a CD39 dependent manner. Purinergic Signalling. 2018;14(4):423-432.
70. Codagnone M, Recchiuti A, Lanuti P, et al. Lipoxin A4 stimulates endothelial miR-126-5p expression and its transfer via microvesicles. FASEB J. 2017;31:1856-1866.
71. Diehl P, Fricke A, Sander L, et al. Microvesicles: major transport vehicles for distinct microRNAs in circulation. Cardiovasc Res. 2012;93:633-644.
72. Nemez M, Alexandru N, Tanko G, Georgescu A. Role of microRNA in endothelial dysfunction and hypertension. Curr Hypertens Rep. 2016;18:87.
73. Alexy T, Rooney K, Weber M, Gray WD, Searles CD. TNF-α alters the release and transfer of microparticle-encapsulated miRNAs from endothelial cells. Physiol Genomics. 2014;833-40.
74. Li L, Zhu D, Huang L, et al. Argonaute 2 complexes selectively protect the circulating microRNAs in cell-secreted microvesicles. PLoS ONE. 2012;7:e46957.
75. Chen X, Liang H, Zhang J, Zen K, Zhang CY. Horizontal transfer of microRNAs: molecular mechanisms and clinical applications. Protein & Cell. 2012;3:28-37.
76. Duttagupta R, Jiang R, Gollub J, Getts RC, Jones KW. Impact of cellular microRNAs on circulating miRNA biomarker signatures. PLoS ONE. 2011;6:e20769.
77. Arroyo JD, Chevillet JR, Kroh EM, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci USA. 2011;108:5003-5008.
78. TurcinoVíč, Weiz L, Langheinze A, Burwinkel B. Characterization of extracellular circulating microRNA. Nucleic Acids Res. 2011;39:7223-7233.
79. Mause S, Weber M, Gray WD, Searles CD. TNF-α mediates the release and transfer of microparticle-encapsulated miRNAs to endothelial cells via microparticles. Blood. 2013;122:253-261.
80. Laffont B, Corduan A, Ple H, et al. Activated platelets can deliver mRNAs, microRNAs and protein mRNAs and enhances their translation. Mol Cell. 2008;30:460-471.
81. Orom UA, Nielsen FC, Lund AH. MicroRNA-21a binds the 3′UTR of the tumor suppressor EPB41L3. J Hum Hypertens. 2015;29(3):149-156.
82. Turchinovich A, Weiz L, Langheinze A, Burwinkel B. Characterization of extracellular circulating microRNA. Nucleic Acids Res. 2011;39:7223-7233.
83. Arroyo JD, Chevillet JR, Kroh EM, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci USA. 2011;108:5003-5008.
93. Burger D, Turner M, Xiao F, Munkonda MN, Akbari S, Burns KD. High glucose increases the formation and pro-oxidative activity of endothelial microparticles. Diabetologia. 2017;60:1791-1800.

94. Jansen F, Yang X, Baumann K, et al. Endothelial microparticles reduce ICAM-1 expression in a microRNA-222-dependent mechanism. J Cell Mol Med. 2015;19:2202-2214.

95. Bodega G, Alique M, Bohorquez L, et al. Young and especially senescent endothelial microvesicles produce NADPH: the fuel for their antioxidant machinery. Oxid Med Cell Longev. 2018;2018:3183794.

96. Hergenreider E, Heydt S, Treguer K, et al. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat Cell Biol. 2012;14:249-256.

97. Liang H, Li S, Chen H. Endothelial microparticles-mediated transfer of miR-126-3p by endothelial microparticles reduces vascular smooth muscle cell proliferation and limits neointima formation by inhibiting LRP6. J Mol Cell Cardiol. 2017;104:43-52.

98. Yang C, Mwaikambo B, Zhu T, et al. Lymphocytic microparticles inhibit angiogenesis by stimulating oxidative stress and negatively regulating VEGF-induced pathways. Am J Physiol Regul Integr Comp Physiol. 2008;294:R467-R476.

99. Yang C, Xiong W, Qiu Q, et al. Role of receptor-mediated endocytosis in the antiangiogenic effects of human T lymphoblastic cell-derived microparticles. Am J Physiol Regul Integr Comp Physiol. 2012;302:R941-R949.

100. Tahiri H, Omri S, Yang C, et al. Lymphocytic microparticles modulate angiogenic properties of macrophages in laser-induced choroidal neovascularization. Sci Rep. 2016;6:37391.

101. Yang C, Mwaikambo B, Zhu T, et al. Lymphocytic microparticles inhibit angiogenesis by stimulating oxidative stress and negatively regulating VEGF-induced pathways. Am J Physiol Regul Integr Comp Physiol. 2008;294:R467-R476.

102. Yang C, Xiong W, Qiu Q, et al. Role of receptor-mediated endocytosis in the antiangiogenic effects of human T lymphoblastic cell-derived microparticles. Am J Physiol Regul Integr Comp Physiol. 2012;302:R941-R949.

103. Tahiri H, Omri S, Yang C, et al. Lymphocytic microparticles modulate angiogenic properties of macrophages in laser-induced choroidal neovascularization. Sci Rep. 2016;6:37391.

104. Yang C, Mwaikambo B, Zhu T, et al. Lymphocytic microparticles inhibit angiogenesis by stimulating oxidative stress and negatively regulating VEGF-induced pathways. Am J Physiol Regul Integr Comp Physiol. 2008;294:R467-R476.

105. Zhang Y, Liu D, Chen X, et al. Secreted monocytic miR-150 enhances late angiogenic properties of macrophages in laser-induced choroidal neovascularization. Mol Cell Proteomics. 2015;14:259-272.

106. Yuan A, Farber EL, Rapoport AL, et al. Transfer of microRNAs by embryonic stem cell microvesicles. PLoS ONE. 2009;4:e4722.

107. Maase SF. Microparticles as intercellular carriers of the microRNA signal: insights for novel diagnostic and therapeutic approaches. Thromb Haemost. 2016;115:236.

108. Boulanger C, Loyer X, Rautou P, Amabile N. Extracellular vesicles in coronary artery disease. Nat Rev Cardiol. 2017;14:259-272.

109. Aikawa E. Extracellular vesicles in cardiovascular disease: focus on vascular calcification. J Physiol (Lond). 2016;594:2877-2880.

110. Shao H, Im H, Castro CM, Bäckefeld X, Weissleder R, Lee H. New technologies for analysis of extracellular vesicles. Chem Rev. 2018;118:1917-1950.

111. Milioli M, Ibáñez-Vea M, Sidoli S, Palmisano G, Careri M, Larsen MR. Quantitative proteomics analysis of platelet-derived microRNAs reveals distinct protein signatures when stimulated by different physiological agonists. J Proteomics. 2015;121:56-66.

112. Xia L, Zeng Z, Tang WH. The role of platelet microparticle-associated microRNAs in cellular crosstalk. Front Cardiovasc Med. 2018;5:29.

113. da Silveira JC, de Avila A, Garrett HL, Bruemmer JE, Winger QA, Bouma GJ. Cell-secreted vesicles containing microRNAs as regulators of matute development. J Endocrinol. 2018;236:R15-R27.

114. Etheridge A, Lee I, Hood L, Galas D, Wang K. Extracellular microRNAs: a new source of biomarkers. Mutat Res. 2011;717:85-90.

115. Di Masi S, Karkeni E, Laurant P, Tabka Z, Landrier JF, Riva C. Microparticle miRNAs as biomarkers of vascular function and inflammation response to aerobic exercise in obesity. Obesity. 2018;26:1584-1593.
a biomarker of heart failure in univentricular heart disease. PLoS ONE. 2017;12:e0183624.
134. Jansen F, Yang X, Proebsting S, et al. MicroRNA expression in circulating microvesicles predicts cardiovascular events in patients with coronary artery disease. J Am Heart Assoc. 2014;3:e001249.
135. Wang X, Tian L, Jiang X, et al. The expression and function of miRNA-451 in non-small cell lung cancer. Cancer Lett. 2011;311:203-209.
136. Zhang L, Valencia C, Dong B, Chen M, Guan P-J, Pan L. Transfer of microRNAs by extracellular membrane microvesicles: a nascent crosstalk model in tumor pathogenesis, especially tumor cell-microenvironment interactions. J Hematol Oncol. 2015;8:14.
137. Li J, Zhang Y, Liu Y, et al. Microvesicle-mediated transfer of microRNA-150 from monocytes to endothelial cells promotes angiogenesis. J Biol Chem. 2013;288:23586-23596.
138. Huber V, Vallacchi V, Fleming V, et al. Tumor-derived microRNAs induce myeloid suppressor cells and predict immunotherapy resistance in melanoma. J Clin Investig. 2018;128:5505-5516.
139. Zhang W, Chen S, Liu ML. Pathogenic roles of microvesicles in diabetic retinopathy. Acta Pharmacol Sin. 2018;39:1-11.
140. Leavitt RJ, Limoli CL, Baulch JE. miRNA-based therapeutic potential of stem cell-derived extracellular vesicles: a safe cell-free treatment to ameliorate radiation-induced brain injury. Int J Radiat Biol. 2019;95:427-435.
141. Simonson B, Das S. MicroRNA therapeutics: the next magic bullet? Mini Rev Med Chem. 2015;15:467-474.

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