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Review

Extracellular vesicles in atherosclerosis

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ABSTRACT

Extracellular vesicles (EVs), which exist in human blood, are increased in some inflammation-related cardiovascular diseases. EVs are involved in inflammation, immunity, signal transduction, cell survival and apoptosis, angiogenesis, thrombosis, and autophagy, all of which are highly significant for maintaining homeostasis and disease progression. Therefore, EVs are also associated with key steps in atherosclerosis, including cellular lipid metabolism, endothelial dysfunction and vascular wall inflammation, ultimately resulting in vascular remodelling. In this review, we summarize recent studies on EV contents and biological function, focusing on their potential effect in atherosclerosis, including cholesterol metabolism, vascular inflammation, angiogenesis, coagulation and the development of atherosclerotic lesions. EVs may represent potential biomarkers and pharmacological targets for atherosclerotic diseases.

1. Introduction

Cardiovascular diseases (CVDs) comprise multiple maladies, such as coronary artery disease (CAD), hypertension, stroke, and peripheral vascular disease. Furthermore, atherosclerosis (AS) is the main cause of morbidity and mortality in CVDs. AS is a chronic disease characterized by endothelial injury, inflammatory cell infiltration, cell proliferation and fat deposition. It is initiated by the activation and apoptosis of endothelial cells, and endothelial dysfunction plays an important role in the formation of atherosclerotic lesions [1]. The primary initiating event in AS is low-density lipoprotein (LDL) accumulation in the subendothelial matrix. Clinical and experimental studies have provided unequivocal evidence for an etiologic role between cholesterol accumulation and inflammation [2]. Unstable AS results in coronary artery thrombosis, leading to myocardial infarction, ischemic gangrene and stroke [3].

Extracellular vesicles (EVs) are a type of subcellular component produced by paracrine secretion that contain exosomes, apoptotic bodies and microvesicles (MVs) (formerly known as microparticles). EVs carry cholesterol, sphingomyelin, phosphatidylserine, gangliosides and other lipids, as well as a rich variety of proteins, RNAs, and other bioactive substances involved in intercellular signalling, affecting cell survival and apoptosis, angiogenesis, thrombosis, inflammatory immune response, etc. [4].

2. Definition and characteristics of EVs

Characterization and classification of membrane vesicles from different populations has always been a challenge and remains a matter of debate. At present, three different forms of EVs have been classified according to differential mechanisms of formation and physiological characteristics: exosomes, MVs and apoptotic bodies (Table 1). Apoptotic bodies are particles that are cryopreserved or released during the late phase of apoptosis that have high affinity for Annexin V and contain lysed organelles and broken nuclear components that are transported into the nucleus of macrophages [5,6]. This type of EV is identified by the presence of DNA and histone markers. Currently, more popular EVs subtypes, primarily exosomes and MVs, are being studied, and information on the potential role of apoptotic bodies in CVDs is very scarce. Exosomes and MVs are together often called EVs.

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3. EV content and biological function

Communication between cells is necessary for proper development and function of tissue. Classical communication involves adhesion contacts, cell junctions and soluble factors that act on the same, or adjacent, cells that produce them, potentially even acting in an endocrine manner over long distances [7]. Over the years, EVs have been identified to play a role in cellular communication [8]. Therefore, EVs are no longer regarded as “cell dust” but as functional messengers affecting signalling between adjacent and distant cells [7,9]. EVs serve as vehicles for the inter-cellular exchange of biological material and information, for which three principle mechanisms have been proposed: (1) EVs, as modulators of complex signals, activate receptors on target cells by presenting organized clusters of membrane-associated bioactive molecules (e.g., receptors, ligands, antigens). (2) Direct fusion with the target cell plasma membrane promotes phenotypic modification and functional transferring of EV content, including proteins, bioactive lipids or RNA, to receptor cells. (3) Through invagination, a mechanism similar to endocytosis delivers information directly to target cells [10].

3.1. Proteins in EVs and their biological function

EVs encapsulate lipids, proteins, nucleic acids, and a variety of other biomolecules before they are released from parent cells, contributing to their remote functional effects [11]. EVs contain a large number of proteins, including proteins from the plasma membrane, cytoskeleton, cytosol and vesicle transport [12]. Some proteins are usually present on the surface of exosomes, including CD9, CD81, CD63, TSG101, and Alix, which have become markers of exosomes that distinguish them from other vesicle subtypes [13]. Several enzymes, including insulin-degrading enzyme (IDE), membrane-type 1 matrix metalloproteinase (MT1-MMP), ADAM-17, heparanase and sialidase, among others, are localized on the surface of exosomes from distinct cell types. The biological activity of these exosomal surface enzymes is still present and degrades natural substrates in the extracellular space [14]. MV surface antigens include tissue factor (TF), vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), platelet cell adhesion molecule-1 (PECAM-1), *E*-selectin, endoglin, phospholipid (PS), vascular endothelial-cadherin (VE-cadherin), and von Willebrand factor (vWf). Another group of proteins commonly found in circulating EVs is cytokines (e.g., IL-6, TGF-β, IL-1β, and TNF) [15]. In addition, EVs contain many proteins, and protein profiling revealed that these proteins include vascular endothelial growth factor, platelet derived growth factor, basic fibroblast growth factor, transforming growth factor beta, MAPK pathway signalling molecules, RHO pathway signalling molecules, Tie-2/TEK and cell adhesion molecules. These molecules are enzymes that catalyse the high efficiency of enzymes, amplifying their effects. Compared to RNA, proteins entering the endothelium act immediately and rapidly, maintaining blood vessels and repairing damaged tissues [16].

3.2. Lipids in EVs and their biological function

In addition to proteins, EVs are also rich in lipids. Exosomes are rich in cholesterol, glycolipids, sphingolipids, glycerophospholipids, and ceramide, except for lysobisphosphatidic acid [17,18]. Sphingolipids and glycolipids are important physiological factors in AS, regulating apoptosis, autophagy, and cell migration [19]. EVs exhibit are more structurally stable than their mother cells because these lipids are usually higher in EV membranes than in the cells from which they originate, so the formation of EVs can lead to cholesterol efflux from the parent cells [20]. EVs also contain bioactive functional lipids, such as prostaglandins, leukotrienes, fatty acids and eicosanoids [21,22].

3.3. Nucleic acids in EVs and their biological function

Finally, EVs contain nucleic acids (both RNA and DNA). EVs carry a variety of functional RNAs, including mRNA, miRNA, circRNA and long noncoding RNAs (lncRNA) [23,24]. Among them, miRNA is currently the most well studied. In the pathogenesis of AS, each stage of plaque development is affected by miRNAs. At present, microRNAs are considered to be related to endothelial cell inflammation, macrophage recruitment, cholesterol inflow and outflow, and ox-LDL accumulation. Quantification of miRNA in EVs is expected to be an important basis for diagnosis of cardiovascular disease [25]. Noncoding RNAs, such as lncRNAs and circRNAs, were more recently identified as EV cargo. In addition, many types of DNA are encapsulated in EVs, including cellular, oncogenic, mitochondrial and viral DNA molecules, but few studies have focused on DNA in EVs [26,27].

4. Extracellular vesicles and AS

Elevated EV levels have been observed in patients with cardiovascular risk factors and after cardiovascular events [28]. EVs may promote development and progression of AS by promoting formation of initial the lesions, intravascular calcification, unstable plaque progression and thrombosis after plaque rupture. Of course, some EVs also protect blood vessels by inhibiting the formation and development of AS. AS is an inflammatory disease elicited by cholesterol accumulation in the arteries. This section reviews the central role of EVs as fine-tuning regulators of cholesterol metabolism in monocytes and macrophages, activation and dysfunction of endothelial cells and platelets, smooth muscle proliferation and vascular inflammation (Fig. 1). In addition, we also discuss thrombus formation, angiogenesis, and plaque stability with respect to EVs during AS and identifying biomarkers for AS, as well as novel therapeutic targets.

4.1. EVs and cholesterol metabolism

Cholesterol is an indispensable molecule in animals, and its levels are carefully regulated through feedback pathways that control the synthesis, esterification and uptake of exogenous cholesterol through lipoprotein uptake and efflux [29]. Removal of cholesterol is essential for cholesterol homeostasis and helps prevent excessive accumulation of esterified cholesterol in cells. The primary method for removing excess cholesterol from cells is the efflux of apolipoprotein A1 (ApoA1), which is mediated by the ATP binding cassette A1 (ABCA1) located in the plasma membrane and belonging to the ABC protein family [30,31].

A high-fat diet leads to significantly increased plasma endothelial microparticle (EMP) levels in healthy subjects with normal blood lipids and is associated with increases in serum triglycerides after meals [32]. Remnant-like lipoprotein particle-cholesterol (RLP-cholesterol) and platelet microparticles are both elevated in type-2 diabetes mellitus patients. In multivariate analysis, RLP-cholesterol is the only predictor of platelet microparticles, one of several standard AS risk factors [33]. In multiple cell types, particularly monocytes/macrophages, lipid accumulation and cholesterol overload contribute to foam cell formation, a key early event in AS [34,35]. Studies have confirmed that ABCA1 is a key molecule in cholesterol efflux, and several hypotheses surrounding ABCA1 have been proposed [36–38]. ABCA1 mediates cholesterol efflux to lipid-poor apoA-I, generating HDL. There is evidence [39] that the production of apoA-I free microparticles (MPs) is also directly mediated by ABCA1, and research has confirmed that these microparticles are an important part of the classical cholesterol efflux pathway when apoA-I is present, contributing to approximately 30% of ABCA1-and apoA-I mediated cholesterol efflux [20]. Moreover, microparticle release requires ABCA1 activity similar to that required for HDL production, and when ABCA1 is overexpressed, release of cholesterol-rich EVs from different cell types is increased (including from BHK, HepG2, and human THP-1 macrophages) [40]. Exosomes are rich in

cholesterol, and their release provides a compensatory mechanism to reduce excess lipids levels in cells [41].

Platelets play an important role in the progression of atherosclerosis due to their involvement in the formation of thrombosis in vascular occlusion [42]. Platelet-derived exosomes inhibit athero-thrombosis by inhibiting oxidized-LDL binding and cholesterol loading into macrophages, affecting the class B scavenger receptor CD36 and inhibiting platelet thrombosis [43]. Platelet exosomes reduce the expression of CD36 and cholesterol accumulation in cultured murine macrophages [43]. Therefore, these platelet exosomes resist lipid overload and prevent the occurrence of AS. In addition, $CD4^+$ activated T lymphocytes infiltrate atherosclerotic plaques, activate T lymphocyte releasing exosomes, and induce cholesterol accumulation by enriching cholesterol and exposure to phosphatidylserine (PS) at their outer membrane leaflet in human monocytes, thereby promoting AS formation [19]. In addition, lipid-containing MVs derived from various cell types also activate the toll like receptor pathway, which promotes foam cell formation by inducing lipid and cholesterol accumulation in macrophages in vitro.

4.2. EVs and vascular cells

4.2.1. EVs and vascular endothelial cells (VECs)

The normal arterial wall of the intima is composed of a monolayer of endothelial cells. The endodermis is an important barrier between blood and tissue that regulates the exchange of matter between tissues and blood through altering permeability.

In vitro, depending on their cellular origin and the upstream signalling, MVs also exert differential effects on the function of vascular endothelial cells. VECs have been shown to produce large EVs, stimulating a wide spectrum of chemical, physical and biological factors. Inflammatory cytokines, such as IL-1α and TNF-α, stimulate endothelial cells to release MVs [44,45]. EVs are derived from activated endothelial cells that are involved in communication between VECs, VSMCs, and immune cells. MVs mediate endothelial cell inflammation induced by ox-LDL. EVs secreted by ECs also undergo alterations in response to different stressors. They can be further used as markers for the diagnosis of AS and to assess endothelial cell function [46,47].

In addition, EVs released from various tissues (e.g., liver, adipose), platelets, and immune cells also have an important regulatory role on the physiological function of VECs. These MVs promote the adhesion of leucocytes to endothelial cells by increasing expression of VCAM-1, *E*-selectin and ICAM-1, promoting the early development of AS [48–50]. Monocyte-derived MVs increase endothelial thrombogenicity and apoptosis, which may partly explain the role of MVs and exosomes in EC dysfunction associated with hypercoagulability and inflammatory diseases [51]. Additionally, EVs derived from monocytes containing caspase induce apoptosis of ECs and SMCs [52]. Increasing evidence indicates that the regulation of MVs on protein expression depends on their miRNA-cargo. MV-related inflammatory cascades promote binding of monocytes and endothelial cells and their infiltration into atherosclerotic plaques [53–56]. Endothelial MVs and platelet MVs also increase endothelial permeability [57–60]. Increases in endothelial permeability could be mediated by a local increase of apoptosis through delivery of caspase 3 and Rhokinase enzymes from MVs to target cells [59]. MPs from T lymphocytes reduce the activity of endothelial NO synthase (eNOS), thus reducing NO production in ECs and increasing oxidative stress. These effects are dependent on phosphatidylinositol-3-kinase (PI3K), extracellular signal-regulated kinase 1/2 (ERK1/2) and the nuclear factor κ-light chain enhancer activating B cell (NF-κB) pathway. Similarly, MVs derived from circulating T lymphocytes induce endothelial dysfunction. EVs derived from platelets attach to the subendothelium and activate ECs, subsequently recruiting activated platelets to damaged areas endothelial cells. EVs derived from platelets also activate pro-inflammatory cytokines, such as IL-1, IL-6, and IL-8, and induce production of ICAM-1, which may be related to the miRNAs in EVs [61]. In summary, circulating EVs that originate from platelets, lymphocytes, monocytes, and adipose tissue may interact with EC surface adhesion molecules, such as VCAM-1, ICAM-1, and *E*-selectin, promoting endothelial dysfunction by increasing oxidative stress, reducing NO synthesis/bioavailability and producing pro-inflammatory cytokines.

Furthermore, MVs also maintain normal endothelial cell function and protect these cells from damage [62]. EMPs derived from TNF-α-stimulated HUVECs prevent lipid-induced endothelial cell injury, and the mechanism whereby this occurs is related to the Akt/eNOS signalling pathway [63]. Endothelial MVs carrying endothelial activated protein C (APC) and protein C receptor (CD201) also prevent cell death via induction of PAR-1 mediated anti-inflammatory and cytoprotective effects. MVs may also influence endothelial survival and regeneration through either direct interaction of MVs with vascular endothelial cells or endothelial progenitor cells [64]. Further investigation is needed to clarify the mechanism of the various effects of MVs on target cells.

4.2.2. EVs and vascular smooth muscle cells (VSMCs)

Maintenance of vascular tone and proper function of ECs (e.g., blood vessels, blood pressure and vascular function) depends on the function of intercellular signals between VECs and VSMCs. EVs also play an important role in regulating the physiological function of VSMCs. VSMCs themselves release EVs as a means of regulating the exchange of information between ECs and SMCs. Endoplasmic reticulum (ER) stress induces MV formation in VSMCs, leading to endothelial dysfunction and inflammation in the aorta [65]. The X-box binding protein 1 (XBP1) mediates functions of ECs and SMCs, and XBP1 splicing in SMCs controls migration of ECs via EV-mediated miR-150 transfer, maintaining vessel wall homeostasis [66]. In a hyperphosphatemic environment or inflammation-driven AS, EVs released by VSMCs promote extracellular mineralization [67,68]. Endothelial autophagy disorder is an important pathogenic factor in cardiovascular dysfunction and AS, and human aortic smooth muscle cell (HAoSMC)-derived miR-221/222 inhibits autophagy of HUVECs via the PTEN/Akt signalling pathway [69].

Moreover, VSMCs are regulated by EVs secreted from other cell types. MVs can also influence SMC proliferation, phenotype, migration and adhesion. Aggregation-modified atherogenic LDL induces SMCs to release tissue factor-rich MVs [70]. The presence of TF in MVs affects the migration of SMCs through the interaction of protease-activated receptors [71]. EVs from various sources with different microRNA content may also control SMC proliferation and migration [72]. Compared to healthy people, blood from atherosclerotic patients contains increased leucocyte-derived EVs that promote VSMC adhesion and migration [73]. When exposed to high protective shear stress, the transferred EVs from ECs reduce expression of central regulators of SMC phenotypes, ELK1 and CAMK2D, in SMCs [62]. Platelet-derived MVs increase SMC proliferation and migration depending on interactions with CD40 and P-selectin. In particular, platelet-derived MVs induce the transition of pro-inflammatory SMCs and stimulate vascular remodelling [74,75]. EVs from macrophages enriched in CD36 and TNF-α induce matrix accumulation and calcification in SMCs. In addition, EVs from foam cells promote VSMC adhesion and migration via activation of ERK and Akt [76].

4.2.3. EVs and fibroblasts

The vascular adventitia plays an important regulatory role in maintaining the structure and function of blood vessels through direct and indirect effects. The adventitia contains a variety of components that mediate vascular function, such as fibroblasts, stem/progenitor cells, inflammatory cells, vasa vasorum and sympathetic nerves [62]. Fibroblasts, the most important cell type in the adventitia, exhibit histological, biochemical and functional changes in response to vascular lesions due to injury and stress. Fibroblasts secrete a large number of soluble factors, extracellular matrix components and EVs, which

exhibit special phenotypic plasticity [77]. At present, studies on EVs secreted by fibroblasts have primarily focused on wound healing, cancer metastasis and myocardial injury, and less so on AS.

4.3. EVs and vascular inflammation

4.3.1. Immune cell-derived EVs exert direct pro-inflammatory effects

EVs derived from immune cells and ECs can directly promote inflammation by producing pro-inflammatory mediators. EVs regulate the immune system by trafficking receptors and inflammatory mediators [52]. The mechanisms of inflammation induced by EVs are as follows: stimulation of the production and release of inflammatory cytokines, such as ICAM-1, VCAM-1, P-selectin, and *E*-selectin, stimulation of the expression of adhesion molecules on ECs and leucocytes, and activation of coagulation and hemostasis [62]. EVs promote inflammation through their lipid fraction, which activates toll-like receptor (TLR)-4 on macrophages [78]. Neutrophil-derived EVs induce ECs to secrete IL-6 and tissue factors (TFs). In AS, chemical attraction of neutrophils to endothelial cells may aggravate endothelial dysfunction and promote monocyte migration into the fragile damaged vascular wall [79]. The primary source of leukotriene B4 (LTB4) is from macrophages. LTBs are associated with AS, whereas LTB4 exerts a potent pro-inflammatory effect by activating G-protein coupled receptors [80,81]. In turn, LTB4 may act as a chemical attractant to stimulate monocyte aggregation to arterial walls, inducing monocyte differentiation into macrophages. In addition, EVs from dendritic cells, macrophages and plasma contain enzymes involved in leukotriene synthesis [82].

4.3.2. EVs exert pro-inflammatory effects by inducing immune cell activation

B-, T- and NK/T-lymphocytes are derived from lymphoid progenitor cells. Activated T-lymphocytes induce monocytes to produce cytokines [83]. EVs derived from monocytes significantly promote inflammation by interacting with other cells, including monocytes themselves, endothelial cells, fibroblasts and SMCs, primarily through endothelial cells [84]. Many studies have shown that EVs from infected macrophages promote inflammation both in vivo and in vitro. MiRNAs derived from EVs of atherosclerotic macrophages, particularly miR-146a, may accelerate the development of AS in a variety of ways, such as reducing cell migration and promoting macrophage retention in the vascular wall [85]. Elevated environmentally related CO_2 levels stimulate neutrophils to produce MVs containing high levels of IL-1β, leading to diffuse inflammatory vascular damage [86]. MVs derived from activated or apoptotic leucocytes are the primary subgroup of MVs in human carotid plaques. During inflammatory states, MVs induce the release of pro-inflammatory cytokines from platelets and leucocytes, promoting adhesion of monocytes to endothelial cells [13].

Neutrophils, leucocytes and platelet-derived EVs also play anti-inflammatory roles [52]. Furthermore, EMP is released from ECs and can be absorbed by adjacent ECs. EMP transfers to receptor cells through functional miR-222, promoting anti-inflammatory effects in vitro and in vivo by reducing ICAM-1 expression.

4.4. EVs, angiogenesis and vasculogenesis

EVs derived from the cell membranes carry pro- and anti-angiogenic mediators [62]. Effector molecules in the EVs include metalloproteinases, growth factors, tissue factors, CD40L and other molecules are capable of inducing production of angiogenic factors, such as VEGF and TGF-β. Furthermore, localization of membrane type 1 matrix metalloproteinases (MT1-MMPs) on the surface of exosomes secreted by different cell types is thought to play an important role in promoting cell migration, especially during invasion and metastasis of tumours [14]. MT1-MMP also cleaves many soluble molecules with diverse effects. Cleavage of MT1-MMP is associated with angiogenesis, epithelial morphogenesis, inflammation and wound healing [87].

EVs derived from ECs, platelets and leucocytes play an important role in the regulation of neovascularization in AS plaques. The normal interstitial microenvironment does not induce neovascularization and only creates a favourable “soil environment” for angiogenesis. EVs secreted from ECs contain β1 integrin, MMP-2 and MMP-9, which promote capillary-like structure formation in vitro [88]. In addition, MVs secreted by activated ECs and platelet induce angiogenesis depend on VEGF and FGF-2 [62]. The transfer of microRNAs from EVs to recipient ECs also regulates angiogenesis. For example, EVs secreted by ECs under stimulation of IL-3 induce angiogenesis by translocating miR-126-3p and pSTAT5 into EC recipients [89]. SMC-derived EVs transfer to activate the VEGF pathway, maintaining homeostasis of the vascular wall through miR-150 [66].

In addition to playing a crucial role in hemostasis and thrombus formation, platelet-derived MPs promote angiogenesis by transferring cytokines, including VEGF, FGF-2, and PDGF, and activating PI3K, SRCK, and ERK [90]. Platelet stimulation greatly affects the level of MVs and proteomes produced by platelets [91].

In addition to pro-angiogenic features, EV from various cell types and plasma, are able to inhibit angiogenesis through reducing NO and increasing ROS production or through interaction with CD36 on ECs [92,93].

4.5. EVs, coagulation and thrombosis

Many studies have shown that MVs, especially those from endothelial cells and platelets, have potential roles in AS and coagulation. Coagulation-promoting MVs are located in human advance vulnerable plaques [94]. When atherosclerotic plaques rupture, their vascular are exposed to the blood stream, activating the coagulation cascade and accompanying platelet recruitment. Thrombosis reduces the arterial blood supply, leading to hypoxia, ischemic heart disease, stroke, hypertension and diabetes.

4.5.1. Endothelial EVs and coagulation

Lipids are an important component of EV membranes, and specific lipid content (sphingomyelin, phospholipids, and cholesterol) [95] in EVs is higher than that in EV-derived cells. Phosphatidylserine interacts with the cationic domain of coagulation factors represented by gamma carboxyglutamic acid [96]. In addition to phosphatidylserine, endothelial MVs exhibit TF on the surface, a potent initiator of the extrinsic coagulation pathway. Pro-coagulation and thrombogenic activities of endothelial MVs were demonstrated using cultured ECs in vitro and were illustrated in vivo as well [97,98]. Endothelial MVs also participate in the dissemination and amplification of procoagulant and pro-inflammatory responses via upregulation of procoagulant activity in other cells [99]. MVs from activated endothelial cells can bind to adhesion molecules (ICAM1, VCAM-1, *E*-Selectin, L-Selectin) on other cell types and transfer bioactive tissue factor in vitro. Exposure of activated endothelial cells causes MVs on the monocytes surface to acquire procoagulation activity [100]. Endothelial and leucocyte MVs generate plasmin on their surface through the active surface tissue plasminogen activator [101]. Plasmin generation capacity in endothelial MVs causes them to be partly responsible for maintaining vascular patency.

4.5.2. Platelet EVs and coagulation

Platelet MVs (PMVs) are an indispensable part of thrombosis, and the procoagulant activity of platelet MVs in blood circulation is 50–100 times higher than that of activated platelets [62]. There are tissue factor (TF), integrin glycoprotein (GP) IIb/IIIa (CD41/61), GPIX (CD42a), GPIB (CD42b), P-selectin (CD62p), factor VIII and Va, P-selectin glycoprotein ligand 1 (PSGL-1, CD162), CD40 ligand (CD154) and protein disulfide isomerase (PDI) on the surface of MVs, which may further promote thrombosis [62,102]. TF can transfer functionally to monocytes and other cells through MVs. Activation of protease-activated

receptor 2 releases TF-rich MVs via filamin A signalling [103]. In fact, hyperinsulinemia increases MVs, which are rich in procoagulant TF [104] and are also found in type 2 diabetes (T2D) patients [105]. Recent studies have shown that IL-33 induces differential TF expression and activity in intermediate monocyte subsets and releases TF-rich MVs [106]. Together, these results indicate that MVs are closely related to the coagulation process. PMVs promote coagulation by binding to the subcellular matrix after endothelial injury [107]. Researchers have demonstrated that elevated PMV levels enhance platelet and fibrin adhesion in damaged atherosclerotic vessel walls under high shear stress [108,109].

In addition, leucocyte-derived MVs participate in platelet activation and coagulation, and P-selectin plays a key role in the influence of leucocyte-derived MVs on thrombosis. Neutrophil-derived MV contains αMβ2 mac-1, which can regulate their interaction with stationary platelets to activate platelets, thereby increasing expression of P-selectin and sustaining thrombosis. Circulating platelet-derived MVs promote platelet and fibrin deposition on the arterial wall, which directly affects AS [108].

5. Potential role of EVs in AS therapy

EVs have therapeutic potential due to their bioactive cargoes, their small size, the ease with which they penetrate biofilms, their low immunogenicity, and their special lipid bilayer membrane structure that prevents degradation of their contents and resists RNAse damage to nucleic acids [110]. Due to the excellent membrane stability of EVs, circulating RNA (miRNA, lncRNA) is stable and widespread in body fluids and blood levels in patients.

5.1. Circulating EVs as biomarkers of AS

EVs are considered as potential biomarkers for the occurrence and development of diseases. Elevated levels of MVs, especially platelet, leucocyte and endothelial cell-derived MVs, have been shown to increase vascular damage, promote thrombosis and promote inflammation. In the case of stress and injury, EVs production increases, resulting in endothelial damage and thrombocyte activation and aggregation, most of the studies exploring circulating MVs in patients with ACS observed elevated endothelial cell or platelet–derived MVs in patients with ACS compared with healthy or sex- and age-matched controls [111,112]. In acute stroke, the level of endothelial MVs is correlated with lesion size and clinical prognosis [113]. EVs surface markers from clinical studies showed $CD144^+$ endothelial MVs independently predicted future cardiovascular events. Furthermore, Clinical long-term follow-up studies found that high levels of $CD31^+$/Annexin V^+ microbubbles represent a higher risk for coronary revascularization and cardiovascular death. $CD31^+$/Annexin V^+ MVs increases in patients with impaired coronary artery function and cardiovascular risk factors, so it can be used as an independent factor to predict the occurrence of cardiovascular events in patients with stable CAD [114]. Moreover, $CD3^+$/$CD45^+$ and SMA-α^+ circulating MPs increased in people with high cardiovascular risk [115]. In patients with severe carotid stenosis, a subset of leucocyte-derived MVs were shown to be a biomarker associated with plaque vulnerability [116].

In addition to protein content in EVs, the expression profile of nucleic acids (mRNAs, miRNAs, lncRNA) in vesicles also represents a potential biomarker for coronary AS. Patients with low levels of miRNA-126-3p and miRNA-199-5p were at increased risk for future cardiovascular events [100]. Exposure to atherosclerotic lipoproteins alters the miRNA profile of human coronary artery smooth muscle cell (HCASMC)-derived MVs, which is reflected in patients with hypercholesterolemia (FH). Exposure of HCASMCs to pathophysiological conditions (e.g., hypercholesterolemia) results in decreased expression of miR-143-3p and miR-222-3p in MPs rather than in cells. Compared with normocholesterolemic controls, expression of miR-222-3P in circulating MPs in familial FH patients was reduced. Compared to non-atherosclerotic areas, MPs from atherosclerotic plaques exhibited decreased levels of miR-143-3P and miR-222-3P [117]. Exosomes derived from activated platelets containing miR-223, miR-339 and miR-21 were transferred to SMCs, subsequently inhibiting the expression of PDGFRβ, and the miRNA in these exosomes may represent biomarkers to predict atherosclerotic thrombosis [118]. Genomic and proteomic analysis of EVs may provide an overall pattern for clinicians, potentially containing new diagnostic and prognostic information during the initial stages of atherosclerosis.

5.2. Therapeutic potential of circulating EVs

In recent years, studies have demonstrated that stem cell-derived EVs exert biological functions similar to stem cells themselves, exhibiting advantages such as small size, effortless penetration of biofilms, low immunogenicity, special lipid bilayer membranous structures that prevent degradation of contents, and resistance to RNase damage to nucleic acids to preserve biological activity. These vesicles play a therapeutic role in many diseases through regulating immune-inflammatory reactions and VEC/VSMCs function, resisting fibrosis, promoting blood vessel and cell regeneration, attenuating apoptosis and improving proliferation of damaged cells [119]. Exosomes derived from mesenchymal stem cells (MSCs) play an important role in the treatment of CVDs, such as myocardial infarction (MI), reperfusion injury and pulmonary hypertension (pH). In addition, inflammation stimulates the release of EVs with enhanced anti-inflammatory properties in MSCs, partly due to the alteration of the COX2/PGE2 pathway [120]. Compared to traditional MSC therapy, MSC-exosomes therapy reduces injury caused by MSC transplantation, avoids the risk of accidental differentiation into osteoblasts, adipocytes, chondrocytes or other cell types, and decreases the possibility of malignant transformation of MSCs into tumour cells [121]. Endothelial progenitor cell (EPC)-derived MPs (MPEs) reduce the accumulation of lipids and macrophages in the liver with consequent alleviation of dyslipidaemia, hypertension and cytokine/chemokine profiles (VEGF, IL-6, IL-8), suppressing the development of AS [122,123]. In addition, EVs are ideal drug carriers, and compounds can be directly transferred into EVs by electroporation or liposome transfection.

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Competing interests

The authors declare that they have no competing interests.

Consent for publication

All the authors agree to publication of this review.

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Appendix

Table 1
Classification of different types of extracellular vesicles.

Characteristic	Apoptotic bodies	Microvesicles	Exosomes
Size (nm)	500–2000[a]	200–2000[a]	40–200[a]
Density (g/ml)	1.16–1.28[a]	1.16–1.19[a]	1.13–1.18[a]
Origin and formation mechanisms	Endoplasmic reticulum, cell surface, outward blebbing of apoptotic and death cell membrane	Cell surface, outward blebbing (budding) of the plasma membrane	Origin the inward budding of endosomal membrane, secreted by fusion of the MVB with plasma membrane
Contents	Nuclear fraction, DNA RNA, microRNA(miRNA) protein, cell organelles	RNA, miRNA, other non-coding RNA, cytoplasmic protein and membrane protein, cell organelles, lipid	RNA, miRNA other noncoding RNA, cytoplasmic protein and membrane protein and membrane protein, major histocompatibility complex(MHC)
Markers	Phosphatidylserine, genomic DNA, caspase3	Integrins, selectins, CD40	Tetraspanins (CD9, CD63, CD81), Alix, TSG101

MVB, multivesicular body; PDCD6IP, programmed cell death 6 interacting protein (also known as Alix); TSG101, tumour susceptibility gene 101 protein.
[a] Data from Huilin Shao, Hyungsoon Im, Cesar M. Castro et al. New technologies for analysis of extracellular vesicles. *Chem. Rev.* 2018, 118, 1917–1950.

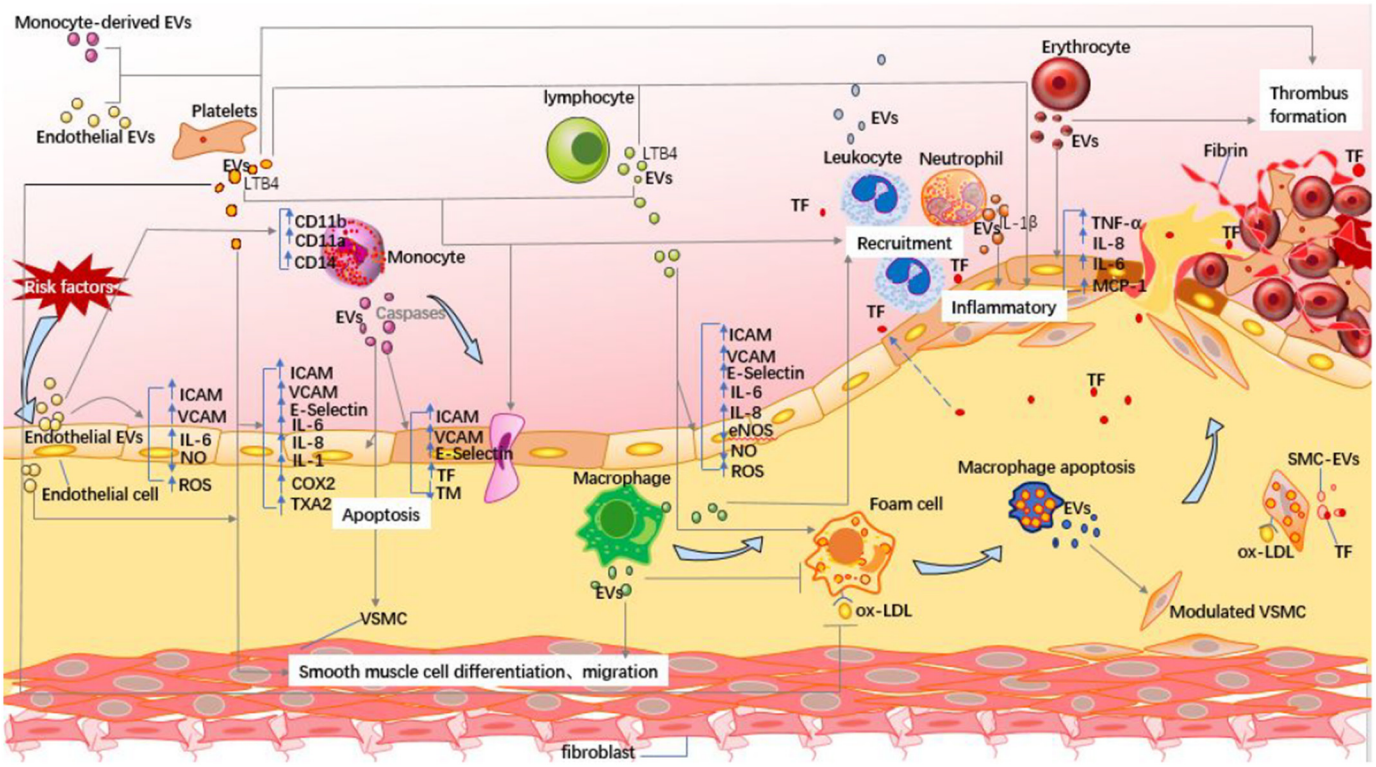

Fig. 1. Role of extracellular vesicles in atherosclerosis.

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