Extracellular Vesicles

Chantal Boulanger, Guest Editor

Extracellular Vesicles in Cardiovascular Disease

Potential Applications in Diagnosis, Prognosis, and Epidemiology

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Abstract: **Extracellular vesicles originate from diverse subcellular compartments and are released in the extracellular space. By transferring their cargoes into target cells and tissues, they now emerge as novel regulators of intercellular communication between adjacent and remote cells. Because vesicle composition and biological content are specific signatures of cellular activation and injury, their potential as diagnostic and prognostic biomarkers has raised significant interest in cardiovascular diseases. Characterization of circulating vesicles- or nonvesicles-bound nucleic acids represents a valuable tool for diagnosing and monitoring cardiovascular diseases, recently referred to as a liquid biopsy. Circulating extracellular vesicles offer a noninvasive and almost continuous access to circulating information on the disease state in epidemiological investigations. Finally, genetic engineering and cell-specific application of extracellular vesicles could display a novel therapeutic option for the treatment of cardiovascular diseases. In this review, we summarize the current knowledge about extracellular vesicles as diagnostic and prognostic biomarkers, as well as their potential applications for longitudinal epidemiological studies in cardiovascular diseases. (*Circ Res*. 2017;120:1649-1657. DOI: 10.1161/CIRCRESAHA.117.310752.)**

Key Words: biomarkers ■ cardiovascular diseases ■ extracellular vesicles ■ liquid biopsy

Intercellular communication is essential for the maintenance of tissue homeostasis and disease development. Classical pathways include short distance cellular crosstalk via direct cell–cell contact or long-range communication through cytokines or hormones. In addition, a third mechanism for intercellular communication has emerged, which involves the intercellular transfer of extracellular vesicles (EVs).[1] This rather nonclassical route of interaction has the potential to deliver diverse biological messages to EV-recipient cells at a level beyond that of soluble factor signaling because EVs can carry and transfer a multitude of bioactive molecules, surface receptors, and genetic information.[2–4] These functional contents depend on the cellular origin and the particular (patho) physiological condition at the time of EV packaging and secretion.[5]

EVs consist of a lipid bilayer membrane, enclosing the biological contents derived from the cell of origin.[6] The characterization and the classification of these different populations of membrane vesicles have been challenging and a matter of debate, but a working basis for a general consensus has been recently reached.[7,8] EVs can be classified according to their size and their biogenesis into 3 main classes. Exosomes represent the smallest subgroup of EVs (ranging from 30 to 100 nm) and are formed within the endosomal network and are released on fusion of multivesicular bodies with the plasma membrane. Microvesicles/microparticles range from 200 nm to 1 µm and are directly shed from the plasma membrane in response to activation. Apoptotic bodies (1–4 µm) are released as blebs of cells undergoing apoptosis.[9–11] Most cell types, including endothelial cells, vascular smooth muscle cells, thrombocytes, monocytes/macrophages, and cardiomyocytes, are capable to release EVs of different sizes, composition, and subcellular origin. EVs can be found in plasma,[12] saliva,[13] urine,[14] and inflammatory tissues.[15] Because of their presence in most body fluids, which makes them easily accessible, EVs have been investigated as potential biomarkers in cardiovascular diseases (CVDs) and other diseases.[16–19]

CVDs, including coronary artery disease (CAD), stroke, hypertension, and peripheral arterial disease, are the major cause of cardiovascular morbidity and mortality worldwide. The underlying disease, atherosclerosis, is initiated and propagated by a continuous damage of the vascular

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Nonstandard Abbreviations and Acronyms	
ACS	acute coronary syndrome
CAD	coronary artery disease
CVD	cardiovascular diseases
EV	extracellular vesicle
miRNA	microRNA

endothelium leading to endothelial activation and apoptosis, the development of endothelial dysfunction, and subsequent atherosclerotic lesion formation.[20] Endothelial injury triggers the release of EVs.[21,22] Accordingly, patients with vascular diseases associated with a systemic endothelial damage, such as atherosclerosis, show significantly increased levels of circulating endothelial cell–derived EVs.[23] Because vesicle composition and content represent specific signatures of cellular activation and injury, EVs represent useful tools for diagnosing and monitoring CVD and other diseases.[24–27]

However, EVs are not merely inert debris that reflect vascular dysfunction after release. Once released, EVs target neighboring or remote recipient cells and can be incorporated via ligand/receptor signaling or fusion of vesicle and target cell plasma membranes. During this process, EV cargoes can enter cell cytoplasm or nucleus, influencing function and phenotype of the EV-recipient cells.[9,28,29] EVs can transfer proteins, cytokines, mRNA, and noncoding RNAs to target cells and influence their biological behavior.[28–30] By transferring biological messages to target vascular cells, EVs have emerged as crucial regulators of vascular homeostasis and CVD progression.[22] Accordingly, the role of EVs has changed from being only a marker of vascular integrity toward a relevant effector in intercellular signaling.[31–33] These properties and the progress made in our understanding of EV crosstalk propose unmodified or genetically engineered EVs as novel tools for various therapeutic approaches combatting CVDs.

In this review, we summarize the current knowledge about EVs as diagnostic and prognostic biomarkers, as well as their potential applications for longitudinal epidemiological studies in CVDs.

EVs in CVD: Diagnostic Implications

EV subgroups are subdivided according to their size and releasing stimulus. Although exosomes are permanently exported from cells, microvesicles are released in response to cell activation or injury.[17,18] Accordingly, levels of circulating EVs are detectable in plasma of healthy subjects and elevated in patients with cardiovascular risk factors or already present CVDs or myocardial disases.[34,35] During their formation, EVs are equipped with surface molecules from their parent cells, as well as selected cytosolic content. Particularly, characterization of circulating vesicles- or nonvesicles-bound nucleic acids represents a valuable tool for diagnosing and monitoring oncological diseases and CVDs, recently referred to as a liquid biopsy.[36] Therefore, both EV counts and EV content are of potential interest for the use as disease-specific biomarkers for CVDs.[37–39]

EVs in CAD

EVs in Patients at Risk and Established Stable CAD

CAD and its sequelae are the main cause of death worldwide. Incidence and progression of CAD are highly influenced by cardiovascular risk factors. Patients with cardiovascular risk factors, such as diabetes mellitus, hypertension, metabolic syndrome, or hypercholesterolemia, show increased levels of circulating endothelial- and platelet-derived microvesicles.[40–43]

Cardiovascular risk factors or CAD affect not only microvesicle levels but also their biological content. In this context, a study cohort of diabetic patients revealed a reduced expression of vascular endothelial microRNA (miRNA)-126-3p and miRNA-26 in circulating endothelial microvesicles. Furthermore, patients with low miRNA-26a and miRNA-126 levels were at higher risk for a concomitant CAD, highlighting potential implications of microvesicle-incorporated miRNA expression on vascular integrity. miRNA profiling of isolated EVs from 6 smoker/control pairs revealed a decrease of platelet-derived miRNA-223 and an increase of miRNA-29b and RNU6-2, contributing potentially to the development of smoking-related cardiovascular pathologies. Of note, an increase of circulating EVs after cigarette smoke could be prevented with the preceding consumption of wine in young, healthy nonsmokers.[44] In patients with stable CAD, vascular miRNAs were shown to be reduced in circulating microvesicles, whereas their expression in isolated exosomes was not altered.[45]

Patients with familial hypercholesterolemia are at high cardiovascular risk and develop premature CAD because of life-long vascular exposure to high low-density lipoprotein cholesterol levels. Familial hypercholesterolemia is associated with higher numbers of platelet-derived microvesicles, as well as of tissue factor–rich microvesicles compared with age-, sex-, and treatment-matched control patients. Furthermore, tissue factor–rich microvesicles showed procoagulant activity and were associated with atherosclerotic plaque burden, indicating that tissue factor in the microvesicles is functional and might modulate CAD progression.[46]

The underlying disease of CAD, atherosclerosis, is initiated and propagated by a continuous damage of the vascular endothelium leading to endothelial dysfunction.[47] The latter is not only a prerequisite of atherosclerosis but also largely influences the outcome of patients at cardiovascular risk or with CAD.[48] Several studies have demonstrated that endothelial microvesicles are associated with endothelial dysfunction not only in CAD but also in diabetes mellitus or chronic renal failure.[23,49,50]

Atherosclerosis is a chronic inflammatory disease.[51] In response to leukocyte activation, asymptomatic patients with subclinical atherosclerosis display increased circulating levels of leukocyte-derived microvesicles. These findings suggest leukocyte-derived EVs as possible tool to detect beginning atherosclerotic alterations before they might have a clinical impact to initiate treatment.[52] In a more advanced stage of the disease, such as coronary calcification or stable CAD, rather circulating platelet- and endothelial cell–derived microvesicles are elevated in plasma of patients.[53,54] Cardiac stress in 1 stimulus for EV release into the circulation.[55] In response

to dobutamine stress echocardiography, platelet, erythrocyte, and endothelial microvesicles were released into the circulation and then cleared from during the next hour. Of interest, this dynamic release of microvesicles was abolished in patients with signs of cardiac ischemia, suggesting that stress-induced microvesicle rise seems to be a normal physiological response that is diminished in those with vascular disease.[56] In patients with significant coronary stenosis, circulating endothelial EVs significantly dropped after cardiac stress, possibly because of increased endothelial uptake.[57]

In summary, in patients at risk or established ischemic or nonischemic CAD, levels of circulating EVs of different cellular origin are significantly altered.

EVs in Acute Coronary Syndrome

An acute coronary syndrome (ACS) is usually caused by coronary plaque rupture or erosion with subsequent luminal thrombus formation with or without simultaneous vasospasm resulting in an acute myocardial infarction.[58] Plaque hemorrhage may expand the plaque rapidly, leading to the development of unstable angina.[59]

As a result of sudden endothelial injury and thrombocyte aggregation and activation, most of the studies exploring circulating EVs in patients with ACS observed elevated platelet- or endothelial cell–derived microvesicles in patients with ACS compared with healthy or age- and sex-matched controls.[60–62] In contrast, elevated levels of erythrocyte derived without alterations in platelet- and endothelial cell–derived microvesicles in patients with ST-segment–elevation myocardial infarction have also been described.[63] In patients with unstable carotid plaques, a subset of leukocyte-derived microvesicles was shown to be associated with plaque vulnerability and was therefore suggested as promising biomarker to identify asymptomatic patients at risk for neurological events.[64,65] Comparing intracoronary and systemic circulating microvesicle levels in patients with ST-segment–elevation myocardial infarction , microvesicle quantification from culprit lesions aspirates revealed locally increased levels of endothelial- and platelet-derived microvesicles that significantly decreased after percutaneous coronary intervention.[66] Furthermore, intracoronary levels of microvesicles originating from both endothelial cells and platelets in patients with ST-segment–elevation myocardial infarction undergoing primary percutaneous coronary intervention correlated with microvascular obstruction.[67]

Increasing evidence suggests EVs as active regulators of CVD progression. In this context, isolated plasma microvesicles from patients with ACSs impaired endothelium-dependent NO-mediated vasodilation in vitro, suggesting that systemic endothelial dysfunction in these patients could be effectuated at least to some extend by microvesicles.[68] In line with these findings, isolated microvesicles from patients with ST-segment–elevation myocardial infarction induced secretion of interleukin-8 in both THP-1 and primary human monocytes, implicating a proinflammatory effect on target cells of certain microvesicles in conditions of acute coronary ischemia.[69]

One study investigating exosomes in patients with ACS showed a significantly upregulated miRNA-208a expression in serum exosomes of patients with ACS. Moreover, the survival rate of patients with high miRNA-208a expression was reduced, indicating that exosomal miRNA-208a could be useful for the early diagnosis and prognosis of ACS.[70] Another study revealed an increased number of exosomes and vesicle-bound cardiac miRNAs after coronary bypass operation.[71]

Compared with microvesicles, there are only limited data available exploring the role of exosomes in CAD. This might be because of some practical advantages microvesicles have over exosomes, including a more simple isolation and quantification procedure. Because of their smaller size and in contrast to microvesicles, exosomes are below the detection limit of the commonly used flow cytometers and require special equipment for their quantification, such as nanoparticles tracking analysis or light scattering.[72] Nevertheless, exosomes represent a totally different population of vesicles from that of microvesicles and may provide unique information on vascular health and disease. Therefore, additional clinical studies characterizing the role of circulating exosomes in CAD are urgently needed. The diagnostic role of EVs in the different stages of CAD from cardiovascular risk factors to ACS is summarized in the Figure.

EVs in CVDs: Prognostic Implications

EVs have been shown to transfer their biological content into recipient endothelial cells, vascular smooth muscle cells, or into atherosclerotic plaques modulating vascular function and CVD progression. In view of these findings, it is comprehensible that circulating levels of EVs have been shown to be associated with the occurrence of major adverse cardiac events.

In a heterogeneous population of patients with various cardiovascular risk factors at high risk for CAD, levels of circulating $CD144^+$ endothelial microvesicles independently predicted future cardiovascular events.[73] In line with these findings, it was demonstrated that patients with stable CAD and high levels of circulating $CD31^+$/annexin V^+ microvesicles were at higher risk for coronary revascularization and cardiovascular deaths in a 6-year follow-up. Furthermore, addition of $CD31^+$/$annexin^+$ microvesicle levels in a classical risk factor model displayed an incremental value.[74] In patients with pulmonary hypertension, circulating level of $CD62e^+$ endothelial microvesicles was associated with the 1-year outcome.[75] In acute stroke, level of endothelial microvesicles correlated with lesion volume and clinical outcome.[76]

Exploring the prognostic value of microvesicle biological contents, it was shown that microvesicle-bound cystatin C, serpin F2, and CD14 were related to an elevated risk for future vascular events and mortality in patients with clinically manifest vascular disease.[77] Furthermore, our group demonstrated that intravesicular miRNA expression profiles are associated with cardiovascular outcome in patients with stable CAD. Patients with low levels of miRNA-126-3p and miRNA-199-5p were at higher risk for future cardiovascular events. Furthermore, endothelial cell–derived microvesicles were shown to be the major source of circulating microvesicle-bound miRNA-126-3p and miRNA-199-5p,[78] whereas others found that microvesicle-associated miRNA-126-3p is also of platelet origin.[79,80]

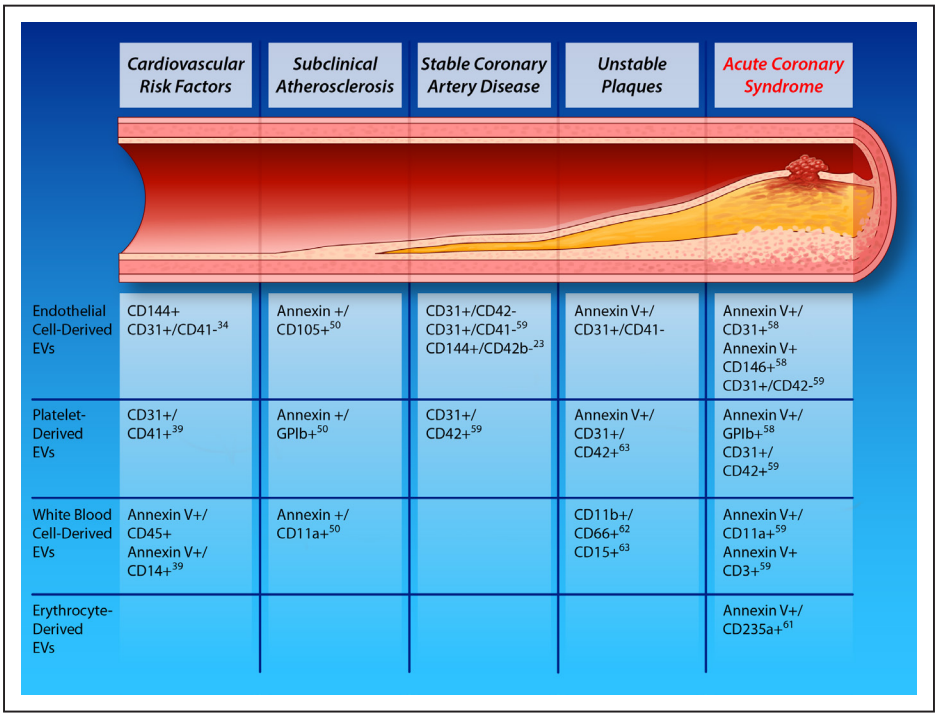

Figure. Extracellular vesicles (EVs) as biomarker in different stages of coronary artery disease. Levels of circulating EVs are detectable in plasma of healthy subjects and are elevated in patients with cardiovascular risk factors or already present cardiovascular diseases. This figure summarizes EV surface markers from clinical studies, which showed increased circulating levels of endothelial-, platelet-, white blood cell–, and red blood cell–derived EVs in different stages of coronary artery disease from patients at risk to acute coronary syndromes (Illustration credit: Ben Smith).

Biodistribution and Targeting of EVs

Although considering circulating EVs as potential biomarkers, it is important to highlight that plasma levels of EVs represent the balance between their production and their clearance. Half-life and target tissue of circulating EVs have been only explored in experimental studies. Diverse studies have shown that the half-life of purified exogenous EVs, artificially introduced into circulation, is short. Labeled rabbit EVs were cleared in rabbit circulation within ≈10 minutes.[81] Splenocyte-, red blood cell–, and melanoma cell–derived EVs all showed a clearance of >90% after 30 minutes.[82–84] However, human platelet–derived EVs displayed a longer half-life of 5.5 hours.[85] Biodistribution of EVs most probably depends on the parent cell source, as well as their target cells and their availability to internalize the circulating EVs. A biodistribution study with red blood cell–derived EVs showed a major uptake by the liver (44.9%), followed by bone (22.5%), skin (9.7%), muscle (5.8%), spleen (3.4%), kidney (2.7%), and lung (1.8%).[84] In contrast, melanoma-derived EVs were mainly incorporated by lungs and spleen,[83] and murine endothelium has been shown to be the main target of intravenously injected endothelial- and platelet-derived EVs.[86,87] More detailed studies comparing different injection sites, donor cells, and healthy and disease conditions are necessary to establish the clearance and the organ uptake of the various EV populations.[88]

Although the rate of EV formation depends on the activation status of the releasing cells, clearance of EVs occurs through different mechanisms. About endothelial microvesicles, 1 major route is the uptake by the endothelium in an annexin V-phosphatidylserinreceptor–dependent mechanism.[89] Platelet-derived microvesicles have been shown to be taken up by endothelial cells via endothelial developmental locus 1 or by macrophages in a lactadherin-dependent pathway.[87,90] Other potential mechanisms of microvesicle clearance include uptake of microvesicles from the circulation by liver Kupffer cells[84] and phagocytosis of microvesicles by splenocytes.[91] About the clearance of circulating exosomes, several studies have demonstrated that intravenously injected exosomes accumulate in macrophages of the mononuclear phagocyte system tissues, such as liver and spleen, or in lung endothelial cells.[83,92,93] Of note, systemic macrophages depletion greatly increased the number of intravenously injected exosomes, proposing macrophages as major contributor to exosomes clearance in mice.[92] Other proteins involved in exosomes uptake include tetraspanins, integrins, and lactadherin. Exosomes are enriched with tetraspanins, which can form specific complexes with different integrin subforms. Of note, the molecular composition of such complexes influenced target cell selection in vitro and in vivo with a major exosome uptake in hematopoietic cells and solid organs.[94] Expression of lactadherin on EVs mediates the cellular uptake by binding phosphatidylserine and cell surface integrin proteins CD51 and CD61 on recipient cells.[95]

Despite these experimental data, there is still a lack of detailed mechanistic insights in EV release and clearance processes. Nevertheless, an emerging body of evidence suggests that analysis of circulating EV numbers and biological contents could contribute to a better risk stratification in intermediate risk patients and improved identification and treatment of high-risk patients for cardiovascular events in the future.[96]

Impact of Cardiovascular Drugs on EVs Levels

Different studies reported the impact of commonly used cardiovascular therapeutics on circulating EV levels. Blood pressure–lowering medications (β-blockers, calcium channel inhibitors, angiotensin II, and receptor inhibitors) have been shown to be associated with reduced the number of circulating platelet-, endothelial-, and monocyte-derived EVs.[97–100] These findings implicate an inhibitory effect of antihypertensive agents on vascular and inflammatory activation processes.

In contrast, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, such as simvastatin, promoted the release of endothelial cell–derived EVs by inhibiting prenylation, presumably via a caspase 8–dependent mechanism. By facilitating endothelial cell–derived EV release, this pleiotropic effect of statins may improve the overall condition of the remaining vascular endothelium.[101] However, the reports exploring the impact of statins on EV release are still a matter of debate: in vitro data showed that statins exerted anti-inflammatory effects on endothelial cells and decreased vesiculation processes by inhibiting the Rho-kinase pathway.[102,103] In line with these rather contradictory in vitro findings, clinical studies reported conflicting results exploring the effect of statins on endothelial- and platelet-derived EVs.[104–108]

In patients with diabetes mellitus, acarbose was shown to lower the levels of circulating platelet-derived EVs.[109] Besides affecting EV release, drugs can also influence EV composition and their subsequent biological effects. In this context, endothelial EVs displayed an inhibitory effect on monocyte adhesion toward the endothelium after antihypertensive and lipid-lowering therapy, most probably by changing their biological composition.[105] In vitro, insulin lowered tissue factor concentration at the surface of monocytes-derived EVs.[110]

Platelet aggregation inhibitors, such as aspirin or ticlopidine, have an inhibitory effect on platelet activation and platelet EV release.[111–113] Furthermore, abciximab influences EV release from leucocytes in patients with ACS.[114] The influence of novel antiplatelet drugs, such as prasugrel or cancrelor, on EV formation has been investigated only in vitro to date. Preincubation of blood samples from healthy volunteers with an active metabolite of prasugrel or cangrelor reduced procoagulant activity of platelet-derived EVs.[115]

To date, no clinical data on the influence of prasugrel, ticagrelor, or cangrelor on circulating EV levels and contents are available. This issue will be addressed by the ongoing clinical trial TIGER-M (Evaluation of Ticagrelor Anti Platelet and Pleiotropic Effects in Patients Undergoing Percutaneous Coronary Intervention for an Acute Coronary Syndrome) that explores the impact of ticagrelor on the level and miRNA content of circulating EVs compared with clopidogrel (https://clinicaltrials.gov/ct2/show/NCT02071966). The investigators assume that ticagrelor benefits are mediated by a reduction of circulating endothelial cell–derived EV levels, a marker of endothelial dysfunction in patients with CVD, and by a modification of their intravesicular miRNA pattern.

In summary, various cardiovascular medications affect the levels of circulating EVs in patients. Whether this rather is mediated by a direct regulatory effect on EV production/clearance processes or by an improved control of cardiovascular risk factors, such as hypertension or diabetes mellitus, needs to be addressed in future studies.

EVs in Epidemiological Studies

Potential Use of EVs for Population-Based Epidemiological Research

Beyond diagnostic and prognostic usefulness, EVs might be beneficial for population-based research in several ways. First, circulating EVs offer a noninvasive approach to receive biological information at any time point about vascular or myocardial homeostasis or a certain disease state for epidemiological investigations. Second, the bioavailability of EVs from body fluids (urine, blood, serum, etc) could be used for longitudinal studies to obtain knowledge about the progression of CVDs without a need for invasive coronary diagnostics or radiological examinations. In an oncological study, the levels of epithelial cell adhesion molecule and CD24 present on circulating EVs correlated with the aggressiveness of ovarian cancer.[116] Supporting the notion that EV compositions represent disease progression speed, exosomes isolated from the urine of patients with high-grade bladder cancer expressed higher levels of epidermal growth factor–like repeats and discoidin domains 3, a molecule that promotes angiogenesis and tumor progression, compared with exosomes from healthy individuals.[117,118] In CVDs, the level and the biological content of circulating endothelial EVs were associated with the outcome of patients with stable CAD, providing a possible tool to predict disease progression by circulating EV characterization.[74,78] Finally, cardiovascular epidemiologists might use circulating EVs to investigate whether EV characteristics may be influenced by exposure to certain proatherogenic factors (eg, smoking, physical inactivity, and obesity[119–121]). However, exploration of EV analyses might be helpful to monitor the efficiency of pharmacological treatment in patients with cardiovascular risk factors (hypertension and hypercholesterolemia) or a manifest cardiovascular pathology.[122]

Current Limitations of EVs Analysis in Epidemiological Studies

Despite recent technical progress, the methods for EV isolation and characterization are still time consuming and technically demanding.[123] These factors, together with the high costs currently associated with the necessary processes, may limit research—especially in epidemiological studies, where thousands of samples need to be analyzed. Therefore, further improvements in EV purification, isolation, and content characterization are needed. In addition, current isolation technologies make it difficult to distinguish different EV subpopulations. This explains the wide use of the term EVs in publications instead of specific types of EVs (eg, exosomes and microvesicles). In addition, contamination from RNA–protein complexes, protein aggregates, and other particles may affect the EV quantification and characterization results. Therefore, further research is needed to develop simple technologies at a reasonable price to isolate

highly pure EVs for downstream analysis (transcriptomics, miRnomics, and proteomics).[124]

Future Directions in EV Research

Presuming a further improvement of EV collection and characterization methods, genomic and proteomic analyses of EVs biological content will give clinicians an overall pattern to diagnose and monitor CVDs.[125,126] The composition and the quantity of EVs could provide additional information on the severity of the disease.[33] In regard to epidemiological studies, EVs may represent a valuable indicator of treatment efficacy.[127] Several drugs commonly used in patients with CVDs or some dietary nutrients target EV levels, contents, and functions and show a cardiovascular protective effect. The potential of EVs to locally and systemically influence recipient cell function has inspired the scientists to develop pharmacology engineered or genetically modified vesicles on the basis of naturally released or artificial vesicles.[128–130] Once underlying mechanistics of EV uptake and systemic clearance will be understood more deeply, EVs may serve as potential clinical delivery tool for novel therapies by modifying underexpressed or overexpressed molecules of interest within EVs. Finally, EV classification, isolation, and purification need to be standardized to ensure that EV analysis is feasible in the clinical setting. Recent position papers of the International Society of Extracellular Vesicles research have made a first step into the right direction to standardize EV analysis internationally between different laboratories.[7,131,132] These consensus papers have recently been complemented by online education and courses developed by International Society of Extracellular Vesicles on the Basics of Extracellular Vesicles (https://www.coursera.org/learn/extracellular-vesicles), and 2 webinars on EVs recorded from the International Society of Thrombosis and Hemostasis Academy. Both courses include up-to-date information on isolation and detection of microvesicle.

Conclusions

Circulating EVs are detectable in plasma of patients with CVDs, and EV expression pattern may serve as diagnostic and prognostic biomarkers in diverse cardiovascular pathologies. EVs have various functional effects on CVD progression depending on the cellular origin, the functional state of the releasing cells, and transfer capacity of intravesicular functional bioactive molecules. Therefore, they may participate in physiological and pathological processes in CVDs. Additional insights into EVs biogenesis and clearance, cellular sorting mechanisms of EVs content, and the functional and biological consequences of their delivery to target cells may offer new diagnostic and prognostic information in patients with CVDs.

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Disclosures

None.

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