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This Review is part of a thematic series on **Exosomes in Cardiovascular Disease**, which includes the following articles:

The Role of Exosomes in Myocardial Remodeling [*Circ Res.* 2014;114:315–324]

Exosomes: Nanoparticles Involved in Cardioprotection? [*Circ Res.* 2014;114:325–332]

Exosomes and Cardiac Repair after Myocardial Infarction [*Circ Res.* 2014;114:333–344]

Microvesicles as Cell–Cell Messengers in Cardiovascular Diseases

Ali J. Marian, Editor

Microvesicles as Cell–Cell Messengers in Cardiovascular Diseases

Xavier Loyer, Anne-Clémence Vion, Alain Tedgui, Chantal M. Boulanger

Abstract: Cell–cell communication has proven to be even more complex than previously thought since the discovery that extracellular vesicles serve as containers of biological information on various pathophysiological settings. Extracellular vesicles are classified into exosomes, microvesicles/microparticles, or apoptotic bodies, originating from different subcellular compartments. The cellular machinery controlling their formation and composition, as well as the mechanisms regulating their extracellular release, remain unfortunately much unknown. Extracellular vesicles have been found in plasma, urine, saliva, and inflammatory tissues. Their biomarker potential has raised significant interest in the cardiovascular field because the vesicle composition and microRNA content are specific signatures of cellular activation and injury. More than simply cell dust, extracellular vesicles are capable of transferring biological information to neighboring cells and play an active role in inflammatory diseases, including atherosclerosis and angiogenesis. The molecular interactions regulating these effects involve specific receptor activation, proteolytic enzymes, reactive oxygen species, or delivery of genetic information to target cells. Unraveling their mechanisms of action will likely open new therapeutic avenues. (*Circ Res.* 2014;114:345–353.)

Key Words: angiogenesis effect ■ atherosclerosis ■ cell-derived microparticles ■ exosomes ■ microRNA

Intercellular communication, a key process in multicellular organisms, is generally achieved through direct cell–cell contact or transfer of secreted paracrine molecules. A third mechanism has recently emerged and involves exchange of extracellular vesicles (EVs) that are released under basal or stress conditions. Most cells are capable of releasing EVs of different sizes, composition, and subcellular origin (Table 1). EVs include different types of membrane vesicles (apoptotic bodies [ABs], microparticles/microvesicles, and exosomes), which can be found in conditioned media from cell culture, as well as in body fluids. The classification of these heterogeneous populations of membrane vesicles has been a challenge and a matter of debate, but a working basis for a general consensus has been recently reached.^{1–3} ABs, formed during the late steps of apoptosis, are the largest vesicles with a size of 1 to 5 μm . They contain cellular material,

including cellular organelles and cytosolic content (protein, DNA, and RNAs). Microvesicles, also referred to as microparticles, especially in the cardiovascular field, are distinguished from ABs by their smaller size (100–1000 nm), and different formation mechanisms. They can be released from most cell types, including circulating cells, vascular cells, and cardiomyocytes. Microparticle release increases under stress conditions, but recent findings indicate that apoptosis is not always a prerequisite.^{4,5} During their formation, microparticles retain surface molecules from parent cells, as well as part of their cytosolic content (proteins, RNA, microRNA). The smallest membrane vesicles are exosomes, with a diameter of ≈ 40 to 100 nm. These are formed by an active process involving the fusion of multivesicular bodies with the plasma membrane that allows the release of exosomes into the extracellular medium.

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Nonstandard Abbreviations and Acronyms

ABs	apoptotic bodies
ApoE	apolipoprotein E
EMP	endothelial microparticles
EV	extracellular vesicles
MMP	matrix metalloproteinase

EVs are raising interest as potential circulating biomarkers of cell injury. They also convey part of the biological activity of conditioned media and their presence in body fluids supports their role in cell-to-cell information transfer. This brief review focuses on microvesicles as an important class of cell-cell messengers in cardiovascular diseases.

Extracellular Microvesicles as Biomarkers

All kinds of EVs are easily detectable in cell culture supernatants. Yet analysis of body fluids, in particular human plasma, has mostly focused on microparticles because reliable quantitative techniques (annexin V capture assay and flow cytometry) are only available for this type of EVs. Nevertheless, these methods have several limitations: the capture assay only quantifies microparticles externalizing phosphatidylserine, irrespective of their size, and flow cytometry only detects microparticles of large size (>300 nm). Both these methods have shown increased microparticle plasma levels in cardiovascular disease.⁶ Unfortunately, on the basis of this current available knowledge, it is still unknown whether these changes result from increased rate of vesicle formation and release or decreased clearance.

In asymptomatic patients with subclinical atherosclerosis, levels of microparticles originating from leukocytes are augmented.⁷ In patients with stable coronary artery disease, coronary calcification or acute coronary syndromes, platelet microparticles, and endothelial microparticles (EMPs) are also elevated.^{8–14} Significant increases in plasma leukocyte-derived microparticles levels are associated with unstable plaques.¹⁵ Moreover, several reports demonstrate that plasma EMP level is a novel biomarker of endothelial dysfunction, which is associated with clinical outcomes in several cardiometabolic diseases, including coronary artery

disease, chronic renal failure, diabetes mellitus, and obesity.^{16–20} In heart failure and valvular disease EMP, platelet microparticle and leukocyte microparticle levels are increased. Microparticle levels are also modulated in other vascular diseases, including preeclampsia,²¹ inflammatory vasculitis,²² and antiphospholipid syndrome.²³

Exosomes also can be detected in plasma and body fluids,^{24,25} but limitations of quantitative methods to determine their levels in vivo have precluded researchers from fully appreciating their role under pathophysiological conditions.

Extracellular Microvesicles as Intercellular Messengers

The protein composition of EVs is a complex molecular signature of the type of cell activation that triggers their release. EVs may also carry specific lipids and mRNA, and recent studies demonstrated that they also transport microRNA cargos. More than simply shed membrane, these vesicles are capable of transferring biological information to target cells. As such, they should be considered as intercellular messengers and not only as biomarkers of cell injury or activation. Several in vitro studies have provided strong evidence that EV can transfer information to recipient cells, but less is known about these mechanisms in vivo (Table 2). EVs, especially microparticles, have been shown to mediate several (patho-) physiological processes. They represent a major signal transduction pathway in pathological conditions, including inflammation, atherosclerosis, and angiogenesis.

Inflammation plays a central role in most cardiovascular diseases. Inflammatory conditions per se activate EV release that could then initiate a positive feedback loop via their stimulating effects on cell-cell interaction, adhesion molecule expression, or cytokine release.^{26–30} Microparticle-derived arachidonic acid, oxidized phospholipids, or chemokines can contribute to increased leukocyte adhesiveness.^{31,32} Platelet microparticles have been shown to promote in vitro resident macrophage differentiation into professional phagocytes,³³ but the in vivo relevance of these findings is not clear and requires further study. In apolipoprotein E (ApoE)^{-/-} mice, injection of supernatants from activated platelets (containing platelet microparticles) had no effect on leukocyte adhesion or atherosclerotic plaque size.³⁴ Recently, the contribution of

Table 1. Characteristics of the Different Types of Extracellular Vesicles

	Apoptotic Bodies	Microvesicles or Microparticles	Exosomes
Size	>1 μm	100–1000 nm	<100 nm
Formation mechanisms	Cell shrinkage and cell death	Membrane blebbing	Multivesicular bodies fusion with plasmatic membrane
Characteristics			
Contents	Cell organelles, proteins, DNA, RNA, and miRNA	Proteins, RNA, miRNA, and lipids	Proteins, RNA, and miRNA
Membrane properties	Membrane permeable (PI positive)	Membrane impermeable (PI negative)	Membrane impermeable (PI negative)
Markers	Annexin V positivity	Annexin V positivity and origin cell-specific markers	LAMP1, CD63, and TSG101, MFG8/lactadherin
Detection methods	Flow cytometry and electron microscopy	Flow cytometry for MPs >300 nm and electron microscopy	Flow cytometry with CD68 capture, electron microscopy, Western blot for exosomes enriched markers
Size determination		NTA and DLS	

DLS indicates dynamic light scattering; MPs, microparticles; NTA, nanoparticle tracking analysis; and PI, propidium iodide.

Table 2. Summary of Studies Reporting In Vitro or In Vivo Transfer of MV-Content (Protein, Lipid, or Nucleic Acids) to Target Cells

Apoptotic Bodies			Microparticles			Exosomes		
Donor Cells	Target Cells	Factor Transferred	Donor Cells	Target Cells/Organ	Factor Transferred	Donor Cells	Target Cells	Factor Transferred
Transfer evidence in vitro								
EC	EC, VSMC	miRNA-126 ⁴³	Pttt	EC	RANTES, ³² VEGF, ¹¹⁰ bFGF, ^{110,111} PDGF, ¹¹¹ lipid ^{111,112}	CC	EC	miRNA-92a, ⁶² miRNA-9, ⁹⁹ protein (LMP1) ¹¹³
EC	CM	GFP ¹¹⁴	Pttt	Imm.C	GPIIb/IIIa (to neutrophils), ¹¹⁵ PPARG (to monocytes), ¹¹⁶ lipid (to monocytes) ¹¹¹	Pttt	Lung cancer cell	CD41 ⁵¹
			Imm.C	Imm.C	MHC ^{117,118}	EC	VSMC	miRNAs 143/145 ⁹⁸
			Imm.C	Pttt	TF, ¹¹⁹ PAF ^{120,121}	DC	T Cell	MHCI ⁴⁴
			ND	EC	ICAM-1 ⁸⁵	CM	Fb	DNA/RNA ⁹³
Transfer evidence in vivo								
EC	EC	miRNA-126 ⁴³	RBC	Kupffer cells	Radioactive marker ¹⁰⁶	EC	VSMC	miRNAs 143/145 ⁹⁸
EC	CM	GFP ¹¹⁴	HUVECs derived-EMP	Aortic murine EC	Staining and FACS proofs ⁴⁹	DC	T Cell	MHCI ⁴⁴
			HUVECs derived-EMP	Spleen, liver, lung	Nanoparticle quantification by MRI and FACS ¹⁰⁷

bFGF indicates fibroblast growth factor, basic; CC, cancer cell; CM, cardiomyocytes; EC, endothelial cells; FACS, fluorescence activated cell sorting; Fb, fibroblast; GFP, green fluorescent protein; GP, glycoprotein; HUVECs, human umbilical vein endothelial cells; ICAM, intercellular adhesion molecule; Imm.C, Immune cells; LMP1, latent membrane protein 1; MHC, major histocompatibility complex; ND, not determined; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; PPARG, peroxisome proliferator-activated receptor g; Pttt, platelet; RANTES, regulated on activation, normal T cell expressed and secreted; T cell, CD4+ cell; TF, tissue factor; and VSMC, vascular smooth muscle cells.

microparticles to interleukin-1 α release by endothelial cells has been re-evaluated and it seemed that interleukin-1 α release is in fact driven by ABs and not by microparticles.³⁵ Interestingly, microparticles also display anti-inflammatory activities. Leukocyte-derived microparticles hamper the inflammatory response of leukocytes to lipopolysaccharide and stimulate the secretion of the anti-inflammatory cytokine transforming growth factor- β 1.³⁶ This effect is mediated by annexin1 (an anti-inflammatory protein) expressed at the surface of these microparticles.³⁷ In vitro, microparticles are taken up by B cells and monocytes, which modulate their activation toward an anti-inflammatory phenotype.³⁸

Several recent studies indicate that EVs might contribute to the development and progression of atherosclerosis. Atherosclerotic plaques contain large amounts of microparticles, mostly of leukocyte origin.³⁹ Plaque microparticles express major histocompatibility complex class II and costimulation molecules, such as CD40L. They are able to stimulate T lymphocytes that in turn might activate B lymphocytes to produce specific immunoglobulins against atherosclerotic plaque antigens (eg, phosphatidylcholine on oxidized low-density lipoprotein or apoptotic cell debris).^{40,41} Moreover, microparticles generated within the plaque stimulate inflammatory responses and contribute to growth of the necrotic core. EMPs are also found in atherosclerotic lesions; they most likely originate from microvessels within the plaque rather than from luminal endothelial cells. In vitro-generated EMPs can induce plasmacytoid dendritic cell maturation associated with the production of interleukin-6 and interleukin-8. Naïve CD4+T cells primed with these dendritic cells produced Th1 cytokines.⁴² More recently, endothelial ABs have been shown to exert antiatherosclerotic effects in ApoE^{-/-} mice⁴³ through the delivery of miR-126 whose

function is to repress the regulator of G protein RGS16. This triggers an autoregulatory feedback loop that increases the production of CXCL12 that stimulates the incorporation of Sca1+ progenitor cells and promotes plaque stability. Exosomes could also take part in the process of antigen presentation because of the ability of dendritic cell-derived exosomes to stimulate antigen-specific T-cell activation.⁴⁴ In addition, exosomes derived from T cell favor cholesterol accumulation in monocytes⁴⁵ and, therefore, could participate in the development of atherosclerosis.

In vitro evidence for a role of ABs, microparticles, and exosomes in angiogenesis has been previously reviewed and indicates that vesicles might have pro- or antiangiogenic activities,⁴⁶ depending on their cellular origin and concentration. In vitro angiogenesis is augmented by microvesicles^{47–49} or exosomes^{50,51} released by different cell types from the vascular compartment, including endothelial cells and platelets. The proangiogenic effects can be attributed to the direct influence on endothelial cell proliferation, tube formation, or endothelial progenitor function/phenotype. The pathophysiological relevance of these observations is supported by the fact that plasma EVs or vesicles isolated from inflammatory tissues affect in vivo angiogenesis and likely contribute to repair mechanisms in ischemic muscles or to complications of the disease in atherosclerotic lesions or in diabetic retinopathy.^{40,48,52,53} In particular, exosomes derived from CD34+ hematopoietic stem cells contribute to hypoxia-induced angiogenesis and improved cardiac function after myocardial infarction.^{54,55} Mesenchymal stem cell exosomes also contribute to placental vascular adaptation to low oxygen tension under physiological and pathological conditions by stimulating endothelial migration and tube formation.⁵⁶ Furthermore, tumor-derived microvesicles and exosomes favor angiogenesis in patients with cancer by

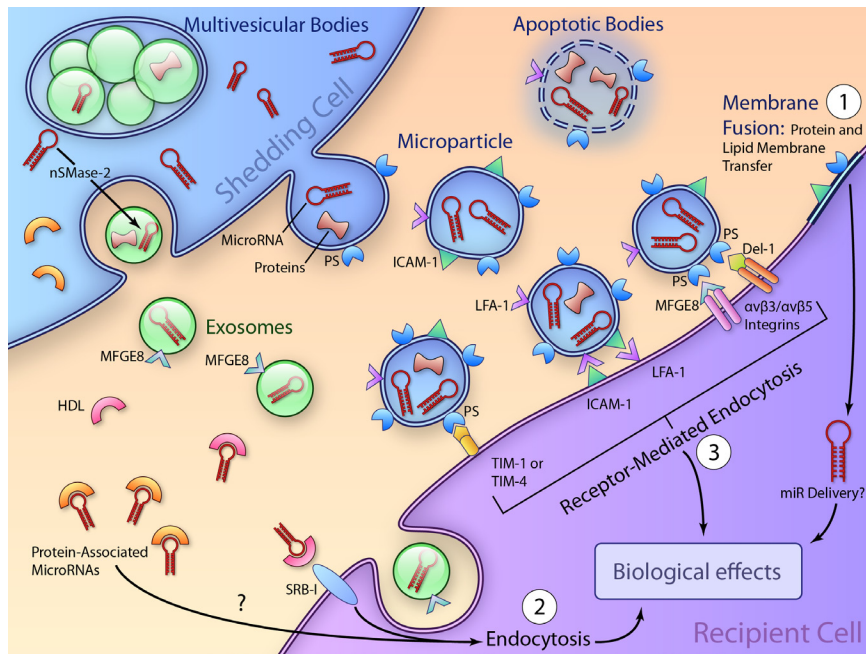


Figure. Mechanisms involving extracellular vesicles in cell-to-cell communication. Once released, exosomes, microparticles, and apoptotic bodies target recipient cells and are able to transfer information (microRNAs, proteins, etc) by membrane fusion (1), endocytosis (2), or receptor-mediated binding (3). (Illustration credit: Ben Smith).

augmenting endothelial proliferation, migration, tube formation, vascular leakiness, or reprogramming bone marrow-derived progenitors toward a provasculogenic phenotype.^{54,57–62} Specific hypoxia-regulated packaging of miRs in tumoral exosomes could affect endothelial angiogenic activity.⁶³ EVs can also promote an antiangiogenic program. Exosomes derived from astrocytes exhibit endostatin-dependent antiangiogenic properties that inhibit laser-induced choroidal neovascularization.⁶⁴ Increased oxidative stress and negative regulation of vascular endothelial growth factor pathways contribute to the antiangiogenic effect of lymphocytic microparticles.⁶⁵ Interestingly, identification of newly discovered microRNA cargos in circulating microvesicles and exosomes provides promising biomarkers of angiogenic potential in patients with critical limb ischemia or diabetes mellitus.^{66,67}

Mechanisms Involved in EV Biological Effects

Molecular Interaction With Target Cell Receptors

Proteins and lipids present on EVs determine their fate and biological activity through specific stimulation of target cells in a receptor/ligand manner (Figure).

It is clear that some of microparticle biological effects are mediated through direct interaction with receptors expressed at the surface of target cells. Platelet microparticles expressing glycoprotein-Ib interact with neutrophil integrin (Mac-1), resulting in neutrophil activation.⁶⁸ Mechanisms involving integrin interaction have also been reported for microparticles derived from other cell types (endothelial cells, smooth muscle cells, and leukocytes). In atherosclerotic lesions, interaction of CD40L+microparticles with endothelial CD40 promotes *in vivo* angiogenesis, likely contributing to increased plaque vulnerability.⁶⁹ In addition, exposure of Sonic Hedgehog by T-cell-derived microparticles stimulates target Patched/Smoothed receptors to promote angiogenesis.^{53,70} Recently, Burger et al⁷¹ showed that direct interaction of

heparin-binding EGF-like growth factor–positive microparticles with the endothelial EGFR receptor causes pro-oxidative and proinflammatory responses in endothelial cells *in vitro*.

Lipids, in particular externalized phosphatidylserine, are a key determinant of the interaction of membrane vesicles with target cells although oxidized lipids might also play a role. Phosphatidylserine+microparticles of different cellular origin bind to platelet CD36 scavenger receptor, leading to increase ADP-dependent platelet activation and augmented thrombosis in mice.⁷² The phosphatidylserine-moiety of endothelial microparticles can interact with endothelial phosphatidylserine receptor in an annexin I–dependent manner to prevent endothelial apoptosis.⁷³ In addition, proteins, such as milk-fat globule-EGF factor 8 (MFG8)/lactadherin or developmental locus-1, might serve as molecular bridges between phosphatidylserine+EVs and integrins expressed by phagocytic target cells.^{74,75} MFG8 and developmental locus-1 mediate the clearance of circulating phosphatidylserine+microparticles by macrophages (spleen and liver) and endothelial cells, respectively. To date, the functional consequences of such events remain unknown for the phagocytes. Of note, a decreased capacity of plasma microparticles to bind developmental locus-1 could contribute to inefficient intercellular communication during disease initiation and progression in patients with coronary artery diseases.⁷⁶

Indirect Effects

Microparticles contain proteolytic enzymes, such as matrix metalloproteinase-2, -3, -7, and -13, that could contribute to the reported *in vitro* degradation of fibronectin by endothelial microparticles.⁷⁷ Other proteolytic activities have been measured on microparticles and contribute to endothelial tube formation and angiogenesis by stimulating the generation of plasmin.^{78,79} It has also been reported that microparticle derived from human atherosclerotic plaque⁸⁰ contains a disintegrin and metalloproteinase, ADAM17, which can stimulate the

shedding of ADAM17 substrates, including tumor necrosis factor- α , contributing to local changes in the inflammatory/anti-inflammatory balance.

In addition, microparticles augment reactive oxygen species (ROS) production. Particularly, endothelial microparticles stimulate ROS production in vitro and impair endothelial function.⁸¹ The latter study did not demonstrate microparticle transfer and concluded that microparticles per se are responsible for the increase in ROS. Similar conclusion was raised for EVs isolated from ischemic tissues that, unlike other EVs, harbor the necessary nicotinamide adenine dinucleotide phosphate oxidase subunits to release ROS and promote endothelial progenitor cell differentiation.⁴⁸ Other studies also revealed that microparticles derived either from lymphocytes⁸² or from erythrocyte^{83,84} modulate ROS generation.

Delivery of EV Cargo to Target Cells

One major mechanism proposed for EV signaling is vesicle fusion with target cell membrane and transfer of protein contents or transfer of genetic information, particularly RNA and miRNAs (Figure). This likely involves docking of microparticles on the cell surface possibly through a ligand–receptor interaction and subsequent fusion of their respective membranes. The fusion process is regulated by the lipid composition of EV membrane, and several reports indicate that the presence of phosphatidylserine contributes to membrane fusion.⁸⁵

Transfer of microparticle contents to recipient cells has been described for a large variety of cells, including platelets, endothelial cells, and monocytes, and for a large spectrum of transferred molecules, such as cytokines/chemokines, growth factors, receptors.⁸⁶ As an example, intercellular adhesion molecule-1 is transferred from microparticles isolated from atherosclerotic human plaques and functionally integrated in endothelial cells after membrane fusion.⁸⁵ This transfer is blocked by the phosphatidylserine-binding protein annexin V, indicating that phosphatidylserine is actively involved in the process. P-selectin/PSGL-1 interaction is not required, which differs from what has been found in monocytes, in which P-selectin/PSGL-1 induces phosphatidylserine exposure.⁸⁷ Platelet or megakaryocyte-derived microparticles can deliver and transfer functional CXCR4 to CXCR4-deficient cells.⁸⁸ Although docking and fusion of microparticles with target cells are considered as 2 separate processes, a cooperative interaction between the 2 cannot be ruled out. Phagocytosis and clearance of EVs by the target cells might help transfer the content of EVs into cells.

Transfer of Genetic Information

Transfer of genetic information by EVs is emerging as a new epigenetic mechanism of regulation. EVs contain and transport DNA, mRNA and miRNAs.^{89,90} The vesicle protects RNA from degradation by nucleases and can circulate in the blood or the extracellular space, which has raised interest in detecting particular RNAs as potential biomarkers. ABs and other EVs can deliver DNA to target cells.^{91–93} Microparticles derived from endothelial progenitor and embryonic stem cells⁹⁴ are capable of delivering functional mRNAs to recipient

cells.⁴⁷ Using microparticles derived from endothelial progenitor cells expressing a green fluorescent protein-mRNA, Deregibus et al⁴⁷ demonstrated the microparticle-dependent transfer and translation of mRNA to recipient cells. There is still little evidence for mRNA delivery and transfer by exosomes, mainly because of these vesicles' size that limits them to small RNA and in particular miRNAs.

EVs carry and shuttle a broad range of premature and mature miRNAs.^{95,96} Interestingly, the EV miRNA profile may not mirror the one found in the parent cell, suggesting a specific regulation of miRNA packaging in EVs.⁹⁷ Several recent studies focused on the mechanisms and functions of miRNA transfer from EVs. As described above, injection of ABs enriched in miR-126 conferred supported antiatherosclerotic effects.⁴³ EVs secreted by Krueppel-like factor 2–transduced or shear-stress–stimulated human umbilical vein endothelial cells are enriched in miR-143/145 and can regulate the expression of target genes in cocultured smooth muscle cells.⁹⁸ These EVs also transfer functional miR-126 that promotes accelerated endothelial migration and proliferation leading to reduced atherosclerosis in ApoE^{-/-} mice.⁴⁹ Recently, it has been reported that tumor exosomes contain miR-9 that can be delivered to endothelial cells, where it represses Janus kinase–Signal Transducers and Activators of Transcription pathway and in turn modulates tumor growth and angiogenesis.⁹⁹ Although miRs are also bound to high-density lipoprotein, circulating EVs seem as the major mechanism for the transfer of plasma miRs to target cells, whether they are bound to microvesicle cargo ships or to argonaute complexes that share physical properties with exosomes.^{100,101}

Open Questions

EVs and Extracellular Matrix

Little is known about the interaction between EVs and the extracellular matrix. EVs may need to cross this barrier to reach other cell types or to reach the circulation. Matrix metalloproteinase activities as reported for some microparticles might first degrade the matrix, facilitating the tissue distribution of subsequently released vesicles. Alternatively, the smallest EVs might be able to cross the matrix and reach the circulation through endothelial fenestrae.

Transfer of Biological Information: Local or Remote Effect?

Most of the biological transfer of information by EVs has been reported as occurring in the close vicinity of the site they originate from. For instance, transfer of miR-143/miR-145 from endothelial cells to vascular smooth muscle cells occurs within a short distance from the vessel wall. This raises several questions. If we assume that endothelial EVs loaded with miRNAs are released from both luminal and apical sites, what would be the proportion of EVs containing miR-143/miR-145 released into the circulation when compared with what is shuffled toward vascular smooth muscle cells? Because shear stress occurs on the luminal side of endothelial cells, one could speculate that a large amount of EVs containing these miRNAs might be released into the bloodstream. The fact that circulating miR-145 levels are

detectable in human plasma supports this hypothesis.^{102,103} Furthermore, exosomes enriched in cardiomyocytes miRNAs have been found in urine samples.¹⁰⁴ Taken altogether, these findings open the question for a possible long-range effect of EVs present in plasma. Alternately, EVs may reach the blood compartment as the last step before their clearance by Kupffer cells in the liver and macrophages in the spleen. Whatever the fate of circulating EVs, they need to bind phagocytes or targets cells under flow conditions. Few studies have addressed this issue.

Vesicle Clearance: Compatibility With Cell–Cell Communication?

It is important to point out that plasma levels of EVs reflect the balance between their production and their clearance. In cardiovascular diseases, these respective contributions to the levels of EVs in plasma have not been fully investigated. In healthy animals, microparticles are rapidly cleared from the circulation by the liver and the spleen but also by lung (within 10 minutes after injection).^{105–107} In fact, macrophages are the major phagocyte involved in their clearance because of their ability to recognize phosphatidylserine-bound MFGE8.⁷⁵ Expression of MFGE8 by dendritic exosomes promotes phagocytosis of apoptotic cells, a crucial step in inflammation and sepsis.¹⁰⁸ Macropinocytosis seems to be 1 way for exosomes to interact with cells, but there is no information on their clearance in vivo by specific organs.¹⁰⁹

The possible role of EVs as cell–cell messengers needs to be weighed against their rapid clearance in vivo, which makes the long-range transmission of information unlikely. Although circulating in the bloodstream, EVs can be caught by macrophages and taken up by phagocytic tissue such that they can no longer transfer their information to remote cells. In addition, EVs in urine could represent a way to eliminate biological information for targeted tissues or a protection against abnormal processes.

Evidence for Selective Delivery of Vesicle Content?

Microvesicle composition reflects the type of activation that initiated their release, and different microvesicle phenotypes could be obtained from 1 single cell type. It remains to be determined whether the target of the information contained by EVs is a specific cell type or whether this is a random process. We might speculate that all types of EVs can signal to all cell types in view of the literature. For example, endothelial microparticles can transfer information to a large variety of target cells.⁸⁶ In this regard, the nonselectivity of EV delivery to recipient cells could contribute to amplify or reduce the disease state by delivering specific molecular information from activated cells (proteins, mRNA, microRNA, or phospholipids). In addition, understanding how EVs might selectively recognize a specific cell type could represent a strategy for selective delivery of therapeutic molecules.

Need for Standardized Methods to Isolate and Characterize Membrane Vesicles

The growing interest for EVs has led to an exponential number of publications in the past 50 years. Most studies consider heterogeneous cellular origins and mixtures of different

vesicles types (such as microvesicles and exosomes). The respective functional effects of each class of EV are, therefore, often underappreciated. Obviously the recent position papers on isolation and characterization of membrane vesicles^{1,3} will help resolve these issues.

The current cut-off values (100 nm and 1 μ m) used to distinguish the 3 populations should be used with caution because technological limitations prevent a thorough assessment of the diameter range of these different EV populations, when evaluating their cellular origin. Therefore, one cannot exclude the possibility of some overlap between ABs and microparticles in the micron range or between microparticles and exosomes in the 100-nm range.

Potential contamination of membrane vesicle preparations should be routinely assessed using specific markers of ABs, microvesicles/microparticles, and exosomes to document the purity of each fraction. Many studies do not provide these data that limit their interpretation. In addition, isolation of vesicles from human body fluids or pathological samples requires proper controls to demonstrate their presence in the tissue before isolation, which adds additional complexity because vesicles might have different cell origins. Although this often limits the analysis of their mechanisms of action, the (patho-)physiological relevance of such findings is crucial.

Conclusions

Increasing numbers of studies highlight the contribution of EVs in signal transmission in a cell–cell communication process in different situations, including inflammation, atherosclerosis, and angiogenesis. However, the extent of their contributions to (patho)physiological processes and the mechanisms involved remain uncertain. Deciphering the molecular mechanisms governing EV formation, release, and clearance, as well as those involved in cell–cell communication, will enable us to envision new therapeutic strategies for limiting disease progression or favoring tissue repair.

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Disclosures

None.

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