*Am J Physiol Cell Physiol* 306: C621–C633, 2014.
First published January 22, 2014; doi:10.1152/ajpcell.00228.2013.

**Review**

# Extracellular membrane vesicles as a mechanism of cell-to-cell communication: advantages and disadvantages

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Submitted 29 July 2013; accepted in final form 16 January 2014

**Turturici G, Tinnirello R, Sconzo G, Geraci F.** Extracellular membrane vesicles as a mechanism of cell-to-cell communication: advantages and disadvantages. *Am J Physiol Cell Physiol* 306: C621–C633, 2014. First published January 22, 2014; doi:10.1152/ajpcell.00228.2013.—Microvesicles represent a newly identified mechanism of intercellular communication. Two different types of microvesicles have been identified: membrane-derived vesicles (EVs) and exosomes. EVs originate by direct budding from the plasma membrane, while exosomes arise from ectocytosis of multivesicular bodies. Recent attention has focused on the capacity of EVs to alter the phenotype of neighboring cells to make them resemble EV-producing cells. Stem cells are an abundant source of EVs, and the interaction between stem cells and the microenvironment (i.e., stem cell niche) plays a critical role in determining stem cell phenotype. The stem cell niche hypothesis predicts that stem cell number is limited by the availability of niches releasing the necessary signals for self-renewal and survival, and the niche thus provides a mechanism for controlling and limiting stem cell numbers. EVs may play a fundamental role in this context by transferring genetic information between cells. EVs can transfer mRNA and microRNA to target cells, both of which may be involved in the change in target-cell phenotype towards that of EV-producing cells. The exchange of genetic information may be bidirectional, and EV-mediated transfer of genetic information after tissue damage may reprogram stem cells to acquire the phenotypic features of the injured tissue cells. In addition, stem cell-derived EVs may induce the de-differentiation of cells that survive injury by promoting their reentry into the cell cycle and subsequently increasing the possibility of tissue regeneration.

extracellular vesicle; membrane vesicle; stem cell; regenerative medicine

CELL-TO-CELL COMMUNICATION is necessary for proper coordination, both during development and among different cell types within adult tissues. Cells are known to communicate via secreted molecules and by cell surface molecules, which are deciphered by the target cell upon receptor binding, or by direct cell-to-cell contact, mediated by specialized molecules. Gap junctions represent one such contact, comprising clusters of intercellular channels formed by the integral membrane protein connexin, which allow the direct diffusion of ions and small molecules between adjacent cells. Communication via intercellular channels is regulated at multiple levels, such as by changing the unitary conductance of single channels or by altering the number of channels. Numerous molecules can diffuse through these junctions, which are thereby involved in the control of cell migration, proliferation, differentiation, and apoptosis, as well as in carcinogenesis (for reviews, see Refs. 69, 111, 176). In addition to interacting with each other, cells within multicellular organisms also interact with the extracellular matrix (ECM), mediated by several kinds of receptors including syndecans, dystroglycans, and integrins. Integrins in particular are involved in both cell-ECM and in certain cell-cell adhesion interactions (i.e., integrin $\alpha_4\beta_1$ and $\beta_2$-integrins). Integrins participate in cell-cell adhesion by binding receptors such as disintegrins and metalloproteases or immunoglobulins. Vertebrates also express a set of leukocyte-specific integrins, which are able to recognize the Ig-superfamily receptors. In addition to mediating cell adhesion, integrins make transmembrane connections to the cytoskeleton, thus activating intracellular signaling pathways involved in development, immune responses, leukocyte trafficking, cancer, and other human diseases (for a review, see Ref. 88).

It has recently been demonstrated that microvesicles released by cells represent another important mediator of cell-to-cell communication and are also an integral part of the intercellular microenvironment (39, 110, 138). This reveals a new scenario in terms of our understanding of signal and molecule transfers between cells, not only locally, but also over long distances. The presence of microvesicles in the extracellular space was initially reported by Chargaff and West (35), as a precipitable factor in platelet-free plasma (35), and then again in 1960 (20). However, microvesicles were considered for many years to be inert cellular debris until De Broe et al. (42) suggested that microvesicles released from human cells may result from a specific process. It is now accepted that most cell types release microvesicles (e.g., epithelial, fibroblast, hematopoietic, immune, tumor, and stem cells) (129, 169), and recent studies indicate that these vesicles may have crucial roles in both physi-

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ological and pathophysiological processes. They have been shown to be involved in intercellular communication (146), coagulation (94), inflammation (49, 96), and tumorigenesis (126) and have an emerging role in the biology of stem cells.

This review provides an overview of the nature of membrane-derived vesicles (EVs), their mechanisms of action, and their possible role in regenerative medicine, as demonstrated by in vitro and in vivo experiments.

*Origins of Extracellular Vesicles*

Two distinct processes responsible for vesicle release from cells have been identified: exosomes are derived from the multivesicular endosomal cell compartment (10, 72, 100, 140, 159), while EVs originate by direct budding from the cell plasma membrane (21, 39, 48, 100, 122, 138). Membrane-vesicle release is initiated by outward budding from the surface of the plasma membrane, followed by a fission event similar to the abscission step observed in cytokinesis (157). Upon release, both types of vesicles, generally referred to as extracellular vesicles [according to the International Society for Extracellular Vesicles (92)], may either circulate in the extracellular space adjacent to the site of release and break down rapidly or may enter into biological fluids (e.g., plasma, urine, milk, and cerebrospinal fluid) and thus reach distant sites.

Apoptotic bodies represent another type of membrane-limited vesicle. These are larger than exosomes and EVs (71), are formed exclusively during the late stage of apoptosis, and contain nuclear material, cellular organelles, and membrane/cytosolic contents (56). Moreover, they expose phosphatydylserine (PS) in the outer leaflet and have a permeable membrane (48).

The different types of vesicles are summarized in Fig. 1. These vesicles may be a promising source of biomarkers for the effects of therapeutic agents in a variety of diseases or as vesicles for drug transport. Different types of vesicles may thus be present simultaneously in the extracellular environment. The present review focuses on membrane-derived EVs.

*Cell Biology of Shedding Vesicles*

*Mechanisms governing vesicle shedding.* Shedding of EVs is considered to be a physiological phenomenon that accompanies cell activation and growth. Many stimuli have been shown to increase vesicle shedding, including hypoxia, oxidative stress, and exposure to shear stress (14, 19, 57, 77, 82, 170). Membrane vesicles are larger than exosomes, being around 100 nm to 2 μm. Despite being generated by analogous processes of budding from the cell surface, they also vary in molecular composition and function depending on the cell of origin. EVs express an array of proteins that differ from those on the surface of the cells from which they originate.

Intracellular factors influence EV shedding, including increased cytosolic calcium ions and degradation of the membrane cytoskeleton (124, 134, 138). Intracellular levels of calcium ions modify the asymmetric phospholipid distribution of the cell membrane via regulation of the specific enzymes flippase, floppase, and scramblase (82). Increased calcium levels inhibit flippase, which promotes the translocation of PS against its electrochemical gradient towards the inner membrane in an ATP-dependent manner (48), and activate scramblase, responsible for the PS shift from the inner to the outer leaflet of the cell membrane bilayer (Fig. 2). Calcium ions also contribute to the reorganization of the cytoskeleton through the activation of cytosolic proteases such as calpain and gelsolin (39). These proteins cut the actin cytoskeleton protein network, allowing membrane budding. The role of the actin cytoskeleton in EV shedding has also been confirmed using cytochalasin D and other drugs that inhibit microfilament polymerization. These agents increase EV formation from platelets, megakaryocytes, and T cells (34, 49, 59). The role of the cytoskeleton in EV formation has also been demonstrated by treating mouse mesoangioblasts with cytochalasin B and nocodazole, an inhibitor of microtubule formation. Microtubule impairment caused a 30% reduction in EV release (30), whereas vesicle shedding was independent of the integrity of the microfilaments. Calcium is not the only second messenger involved in inducing vesicle release, and phorbol ester activation of protein kinase C has also been shown to play a role in various cell types (12, 133, 150).

Distinctive characteristics of EVs include the exposure of PS on their surface (82, 182), although a significant number of EVs released from blood cells do not expose PS on the outer leaflet (149) and the enrichment of proteins associated with membrane lipid rafts (44, 134, 155). Lipid rafts are a subdomain of the plasma membrane that contains high levels of cholesterol and sphingolipids (132, 151). Membrane lipids and proteins sort in these microdomains, allowing specific interactions involved in signal transduction, membrane trafficking, and cytoskeletal organization. The role of lipid rafts in vesicle shedding was demonstrated by depletion of membrane cholesterol (44). Similarly, treatment of mesoangioblasts, a mouse stem cell line, with methyl beta cyclodextrin, which selectively disrupts lipid rafts, significantly reduced vesicle shedding (30) and impaired EV formation in endothelial cells (25).

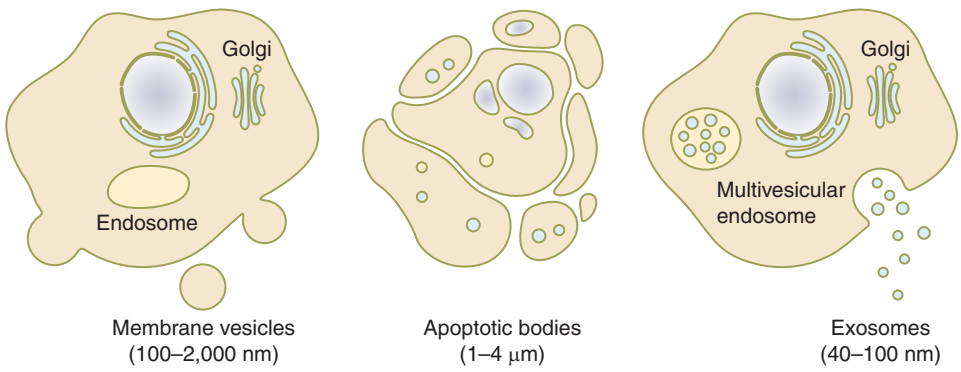


Fig. 1. Origins of various classes of extracellular vesicles. Membrane vesicles are formed by the outward blebbing of the plasma membrane and the subsequent release of the vesicle into the extracellular space. Apoptotic bodies are formed during the late stage of apoptosis. Exosomes are formed from the multivesicular endosomal cell compartment.

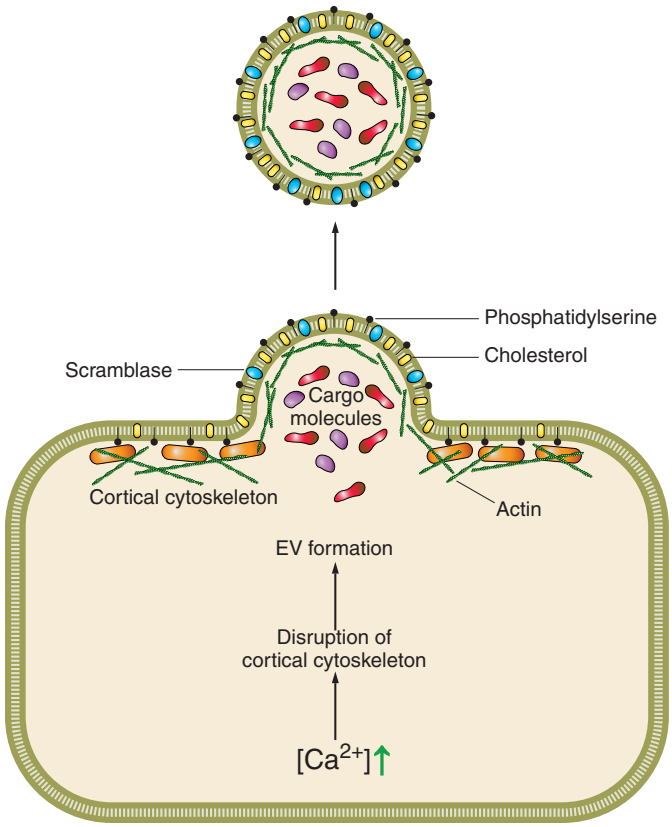


Fig. 2. Proposed mechanisms responsible for membrane-derived vesicle (EV) formation. EV formation was accompanied by an increase in intracellular calcium levels responsible for scramblase activation and phosphatidylserine shift from the inner to the outer membrane leaflet. Disruption of the cortical cytoskeleton was also observed.

*Selective cargo enrichment into membrane vesicles.* Targeting of proteins into shedding vesicles is selective. Specific proteins may be included or excluded from membrane EVs, leading to the expression of proteins arrays that differ from those present on the surface of the cells from which they originated (40, 124). For example, EVs released by polymorphonuclear leukocytes (PMCs) express selectin, integrins, and complement regulators but lack CD14 and FcγRII, which are found on the surface of resting PMCs (60). Moreover, EVs released by a monocytic cell line are loaded with IL-1β (109). Furthermore, intracellular proteins, second messengers, and nucleic acids can be enclosed and specifically sorted into EVs. These examples indicate that the transfer of molecules into EVs is a selective process, allowing the functional properties and roles of EVs to differ from those of their parental cells (44).

*EVs and unconventional protein export.* Some cytosolic proteins (e.g., IL-1β and FGF2) lack exocytosis signals and therefore do not depend on the classical endoplasmic reticulum-Golgi secretory pathway (142). Cells that release these proteins are also able to shed membrane vesicles containing the same proteins, meaning that EV shedding may represent an alternative pathway for the release of these cytosolic proteins (30, 109, 115, 116, 141, 156).

*Release of vesicle components.* Many EVs released from a given cell type are rapidly broken down, thus releasing their cargo into the extracellular space. The breakdown of vesicles upon shedding has consequences for many processes. For example, EVs may contain metalloproteinases responsible for ECM digestion, with consequences for increased tumor cell mobility (150). The breakdown of EVs immediately upon shedding is also important for the release of signaling molecules.

*Interaction of shedding vesicles with target cells.* It is possible that EVs may interact with specific target cells after their release. For example, EVs shed from platelets interact with macrophages and endothelial cells but not with neutrophils (107), while EVs from neutrophils interact with platelets, macrophages, and dendritic cells (55, 60, 136).

Several types of interactions between EVs and target cells have been demonstrated. Interactions may be direct, resulting in EV fusion with the target cell or in its endocytotic uptake, or the interaction may be mediated by receptor binding or may involve surface-receptor transfer (61, 89, 123). Once internalized, EVs can remain segregated within endosomes and finally fuse with lysosomes or may fuse with endosome membranes, thus releasing their contents into the cytoplasm (Fig. 3). In conclusion, EVs are able to interact with their target cells via three different mechanisms: *1*) by fusion and subsequent transfer of their cargo; 2*)* by endocytosis and release of their cargo; and *3*) by binding and signaling (31, 113, 160). EVs may also exert an effect on the ECM through their inclusion of matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9 (30, 108).

## *Functions of Membrane Vesicles*

The functions of extracellular vesicles depend on the phenotype of their parental cells. However, although their cargo reflects the cell from which they were released, selective enrichment of specific molecules has been shown to occur (103).

EVs influence target-cell behavior in several ways. They can act as a signaling complex, transfer membrane receptors between cells, deliver proteins to target cells, and also modify the receiving cells by horizontal transfer of genetic information.

*Membrane vesicles as signaling complexes during development.* EVs play an important role in developmental signaling and morphogenesis in multicellular organisms (70). The formation of morphogen gradients is essential for tissue patterning, and morphogens are generally released from producing cells and spread through adjacent tissues. For example, some cells express developmental gradients during tissue differentiation by secreting specific proteins such as Hedgehog, Wingless, or Decapentaplegic (101). Greco et al. (70) demonstrated that many morphogens were tightly associated with the cell membrane and were released via morphogen-enriched vesicles, thus creating a morphogen gradient. However, it has recently been demonstrated that particles similar to EVs, and named nodal vesicular parcels (NVPs), are also involved in development of the mammalian left-right axis. NVPs are released by node cells in response to FGF signaling and contain morphogens such as sonic hedgehog and retinoic acid. The NVPs are transferred to the left side of the embryo through nodal flow generated by the rotation of specific cilia (75) (Fig. 4).

*Role of EVs in receptor transfer into target cells.* The phenomenon of receptor transfer has been best studied in platelet-derived membrane vesicles, which transfer platelet-

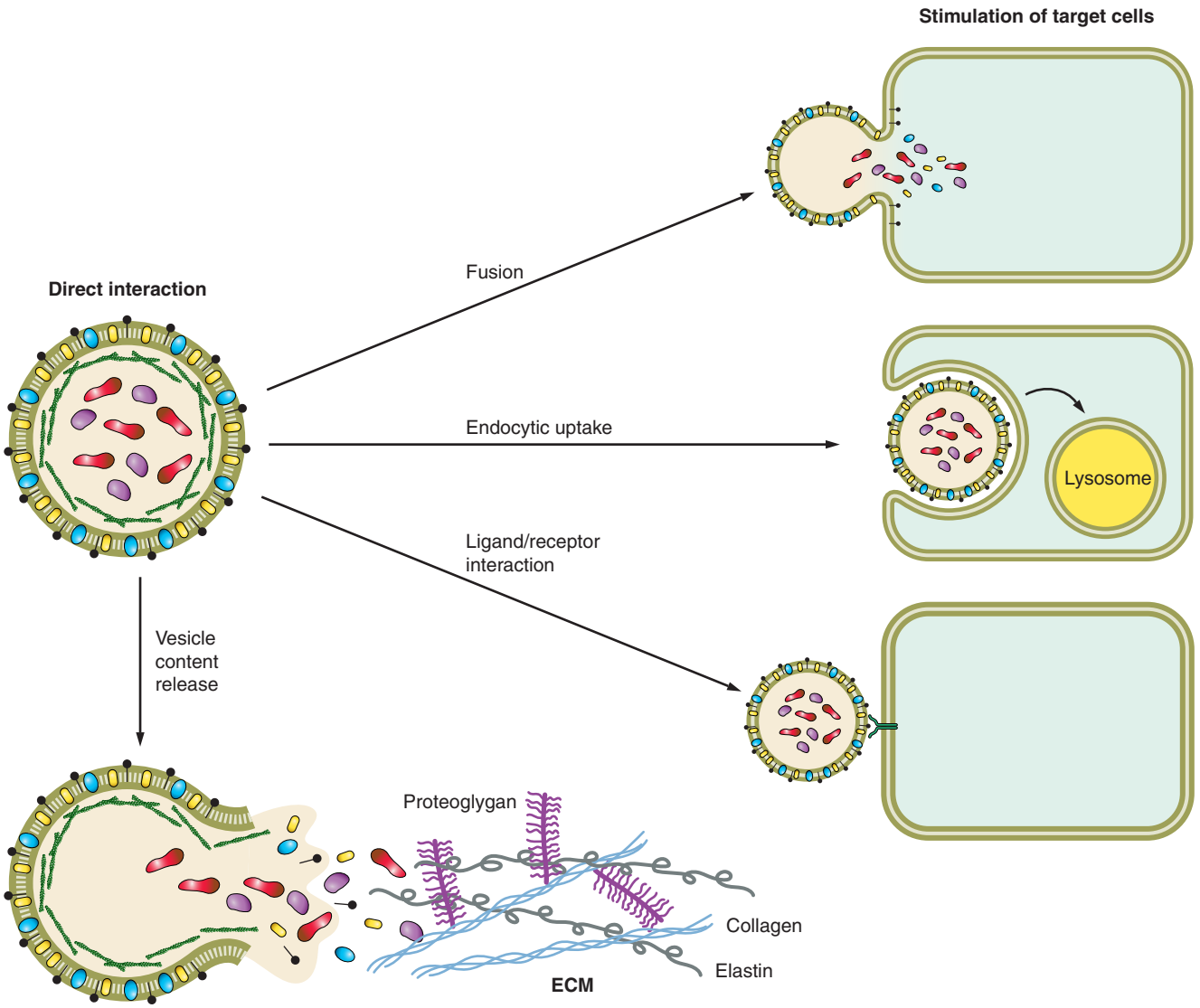


Fig. 3. Mechanisms whereby EVs achieve their biological effects. EVs may activate cell signaling by physical ligand/receptor interactions or by fusing with their target cells and transferring their contents. They may also be endocytosed by the target cells or may release their contents into the extracellular space. ECM, extracellular matrix.

specific adhesion molecules to hematopoietic cells (11, 89). EV-mediated transfer has also been described for monocyte/macrophage tissue factor, which can be transferred from leukocyte-derived EVs to the surface of activated platelets, in a lipid-raft-dependent mechanism (44).

*Ability to change neighboring-cell destiny by horizontal transfer of RNA.* Recent attention has focused on the capacity of EVs to induce epigenetic changes in target cells. The occurrence of epigenetic changes in coculture conditions has been well-documented (3, 28, 29), and this phenomenon could be explained by the transfer of genetic information between the cells. EVs have recently been shown (137) to alter the phenotype of neighboring cells, making them resemble the EV-producing cells. EVs can transfer not only surface determinants and cytoplasmic proteins but also mRNA and microRNA (miRNA), which is recognized as a regulatory signal in cell-to-cell communication (172, 180). miRNAs are a class of small noncoding RNAs of 19–23 nucleotides long, which are known to modulate gene expression by translational inhibition, or by promoting the degradation of target mRNAs (97). Intercellular communication based on miRNAs includes three processes. *1*) miRNAs must be selectively packaged into vectors. Current evidence suggests that the export profile is not representative of the parent cell and is distinct in terms of both abundance and content (166, 173, 180), and specific pathways have been found to regulate cellular miRNA release (58, 119, 127, 131, 171, 173). *2*) miRNAs must be protected from circulating RNAses and transferred to targeted recipient cells. *3*) miRNAs must retain the ability to control gene expression by regulating target-mRNA turnover (84). Transferred miRNAs use the cellular machinery to reduce target-gene expression, and consequently alter cellular phenotype.

The release of nucleic acids in vesicles protects them from plasma ribonucleases, and EVs may thus represent a new mechanism of genetic exchange between cells (46, 52, 138,

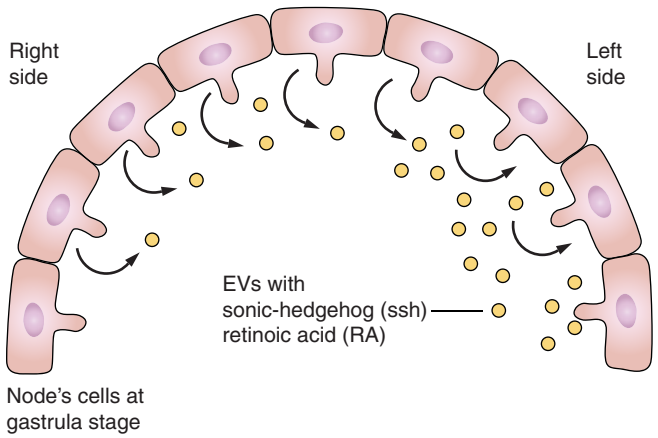


Fig. 4. Role of EVs during development. EVs containing the morphogens sonic-hedgehog and retinoic acid are involved in the determination of left-right axis during embryo development.

179), allowing different cell types to influence the behavior of other cells.

*Protective role of membrane vesicles.* EVs have demonstrated a protective role against both internal and external stressors. For example, platelets incubated with complement complex release EVs containing that complex. Vesicle shedding also protects platelets against complement-induced lysis (152). In addition, EVs may be responsible for the release of potentially harmful molecules such as chemotherapeutics, caspases, and oxidized phospholipids. For example, membrane vesicles from viable endothelial cells contain caspase-3, and inhibition of their release triggers both cell apoptosis and detachment (1, 2), strongly suggesting that vesicle release protects the cells against the intracellular accumulation of high levels of caspase-3. Moreover, the release of death signals can induce apoptosis in neighboring cells (144).

*EVs contain bioactive lipids.* EVs also contain bioactive lipids, including sphingosine 1-phosphate and arachidonic acid, which induce several biological responses in target cells (16, 17, 139). EVs released by platelets may activate endothelial cells, polymorphonuclear leukocytes, and monocytes (15, 16, 17, 120). EV-contained lipids may also stimulate cytokine secretion and tissue-factor expression in endothelial cells, inhibit apoptosis of PMCs, and induce chemotaxis in some cell lines (16, 22, 49, 114).

### *Tumor Cell-Derived EVs: an Example of Vesicles in Disease*

Aberrant levels of EVs are observed in some diseases, and their numbers, cellular origins, and composition are disease/state dependent. For example, cancer cells have been found to shed large numbers of vesicles, both in vivo and in vitro (6, 45, 65). In addition, the environment of many tumors is highly enriched in membrane vesicles released not only by proliferating tumor cells but also by infiltrating cells (e.g., macrophages and neutrophils). EVs shed from tumor cells (tEVs) facilitate the transfer of soluble proteins, nucleic acids, transmembrane proteins, chemokine receptors, tissue factor, and receptor tyrosine kinases (4) and are involved in many aspects of tumor progression.

*Invasive growth and metastasis.* ECM DEGRADATION. ECM invasion is an essential process for tumor growth (79), and tEVs are involved in this process by virtue of their protease content. They contain or expose proteases including MMPs such as MMP-2 and MMP-9, as well as their zymogens, and urokinase-type plasminogen activators (uPA). MMPs degrade basement-membrane collagens, while uPA is responsible for converting plasminogen into plasmin, a serine protease that degrades many components of the ECM and also activates MMP zymogens. The release of MMPs through EVs thus promotes tumor invasion and metastasis formation (8, 51, 64, 65) (Fig. 5*A*).

ANGIOGENESIS. Several studies demonstrated that tEVs stimulate the secretion of proangiogenic factors by stromal cells and facilitate the proliferation of endothelial cells, thus promoting angiogenesis and allowing tumor growth. tEVs contain mRNA encoding growth factors such as VEGF and hepatocyte growth factor, which they transfer into monocytes, thus inducing the production of these growth factors and thereby stimulating angiogenesis (12, 13). Angiogenesis is also induced by EV-mediated miRNA and mRNA transfer in endothelial cells, which stimulates tubule formation in endothelial cells by modifying the translational profile of the cells (153) (Fig. 5*C*).

METASTASIS. tEVs are also responsible for the horizontal propagation of oncogenes and their associated transforming phenotypes. Al-Newadi et al. (4) demonstrated intercellular transfer through tEVs of the truncated form of the epidermal growth factor receptor from glioma cells to other glioma cells lacking this receptor, after which the recipient cells became transformed and showed modified expression of various epidermal growth factor receptor target genes.

Focusing on a lung cancer model, Ratajczak et al. (138) demonstrated that tumor cells increased or decreased their secretion of tEVs in response to microenvironmental changes. tEVs released from this kind of tumor play a role in regulating the biology of the endothelium, by inducing phosphorylation of MAPKp42/44 and AKT, similar to platelet EVs. They also stimulate stromal fibroblasts by inducing proangiopoietic factors such as IL-8, VEGF, IL-11, and MMP-9. Importantly, the authors demonstrated that, in addition to cross talk between tumor cells and the microenvironment, feedback signals to tumor cells from stromal fibroblasts were stimulated by their secreted EVs (177). Similar results were obtained by Castellana et al. (33) in prostatic tumors.

*Cellular survival.* ESCAPE FROM APOPTOSIS. Cells release EVs as a protective mechanism against intracellular stress (85). In the same way, tumor cells may release EVs containing caspase-3, thus preventing its intracellular accumulation, which leads to cell death by apoptosis. tEVs may also contribute to cell survival by preventing the intracellular accumulation of chemotherapic drugs (143, 148), thus making cells resistant to chemotherapy (Fig. 5*B*).

ESCAPE FROM IMMUNE SURVEILLANCE. EVs released from many cancer cells expose Fas ligand, the ligand of the death receptor Fas, which induces T-cell apoptosis and inhibits the function of adaptive immune cells (7, 81, 94), thus allowing cancer cells to escape from immunosurveillance. Apoptosis of circulating T cells appears to be a generalized phenomenon in various types of cancers and has been observed in melanoma, ovarian, and breast carcinomas and in head and neck cancer (18, 54, 76) (Fig. 5*D*). Moreover, tEVs also target antigen-presenting cells. Valenti and coworkers demonstrated that tEVs fused with the plasma membrane in monocytes, thereby

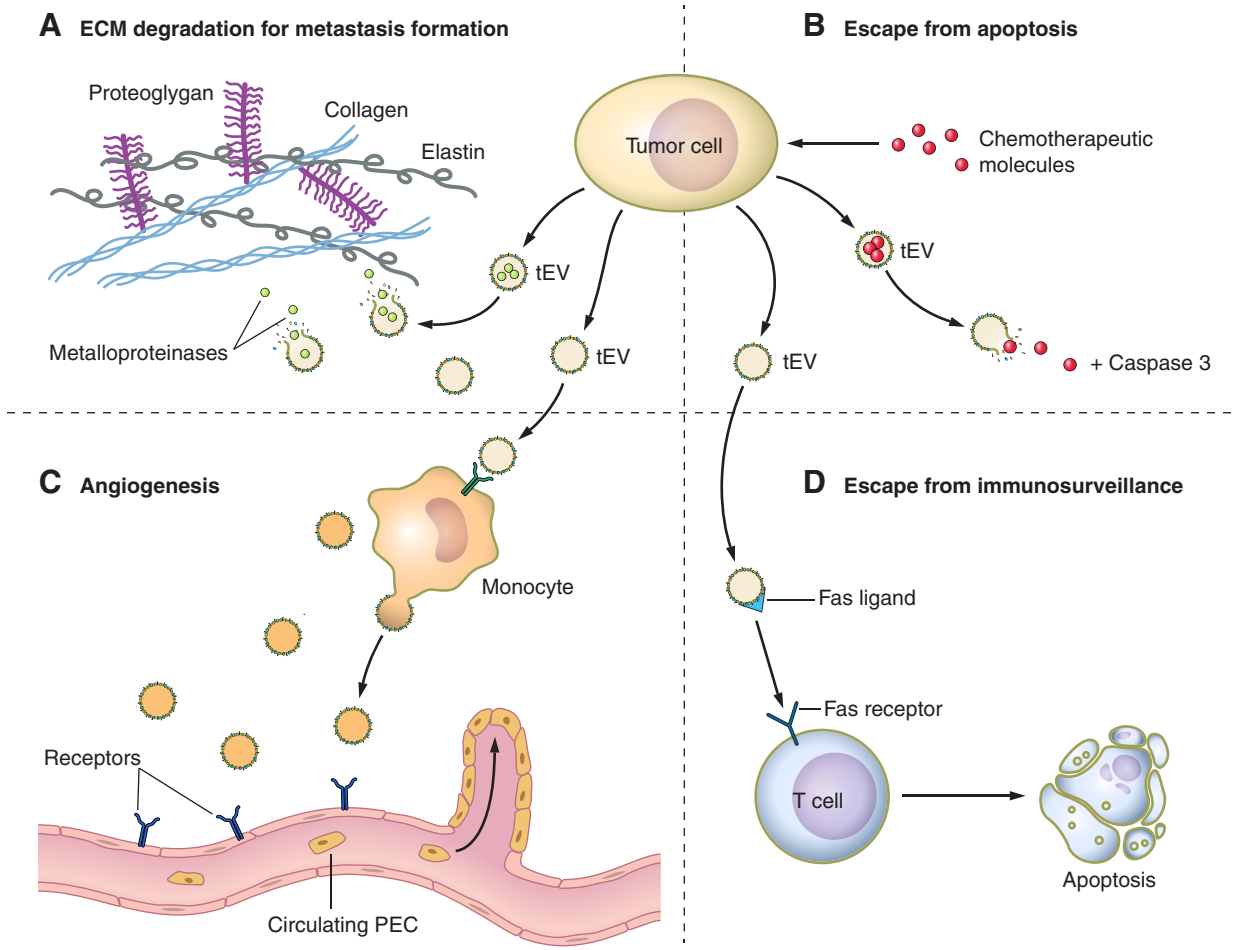


Fig. 5. Tumor-derived EVs (tEVs) influence several aspects of cancer progression. *A*: EVs shed from tEVs contain metalloproteinases that are responsible for matrix degradation, facilitating cancer invasiveness. *B*: tEVs allow to tumour cells to survive to chemotherapy and apoptosis by drug and caspase 3 efflux respectively. *C*: tEVs stimulate the secretion of proangiogenic factors by stromal cells and facilitate the proliferation of endothelial cells, thus promoting angiogenesis and allowing tumor growth. Angiogenesis is also influenced by mRNA and micro (mi)RNA release through tEVs. *D*: tEVs released from many cancer cells expose Fas ligand, which induces T-cell apoptosis and inhibits the function of adaptive immune cells thus allowing cancer cells to escape from immunosurveillance.

impairing their differentiation into antigen-presenting cells (167) and aiding evasion of the immune response (4).

Stem cells, like tumor cells, have also recently been shown to release large numbers of membrane vesicles.

*Stem Cell Niche and EVs*

Interactions between stem cells and the microenvironment have been suggested to play a critical role in determining stem cell phenotype. Indeed, stem cell behavior, in particular the balance between self-renewal and differentiation, is controlled by the integration of intrinsic genetic factors with extrinsic cues supplied by the surrounding microenvironment, known as the stem cell niche (90, 104, 121). The stem cell niche includes discrete and dynamic functional domains that influence stem cell behavior, thus regulating tissue homeostasis under diverse physiological (development and aging) and pathological (injury and disease) conditions (Fig. 6). In addition to interactions between stem cells and the niche, stem cells within a common niche also interact with each other.

The concept of the stem cell niche arose from the observation that spleen-derived hematopoietic stem cells displayed decreased proliferative potential compared with bone marrow-derived hematopoietic stem cells (147). Implicit in this model is the prediction that the removal of stem cells from their niche results in loss of their identity and self-renewal capacity. Stem cell numbers can thus be limited by the availability of niches that release signals for self-renewal and survival, and the niche provides a mechanism for balancing the production of stem cells and progenitor cells. In vivo, the stem cell niches create specific microenvironments consisting of soluble and surface-bound signaling factors, cell-cell contacts, stem cell niche-support cells, ECM, and the local mechanical microenvironment. All these niche components are integrated with inputs that regulate physicochemical cues, such as metabolites, oxygen, and hormones (130). The role of the niche is particularly important in tissue repair by directing repair via reiteration of developmental programs that expand or specify stem cells.

In conclusion, the term "niche" coined by Schofield (147), comprises the following characteristics: *1*) it is a defined anatomic location; *2*) it is required for stem cell expansion; *3*) it preserves stem cells by limiting their differentiation; *4*) it is

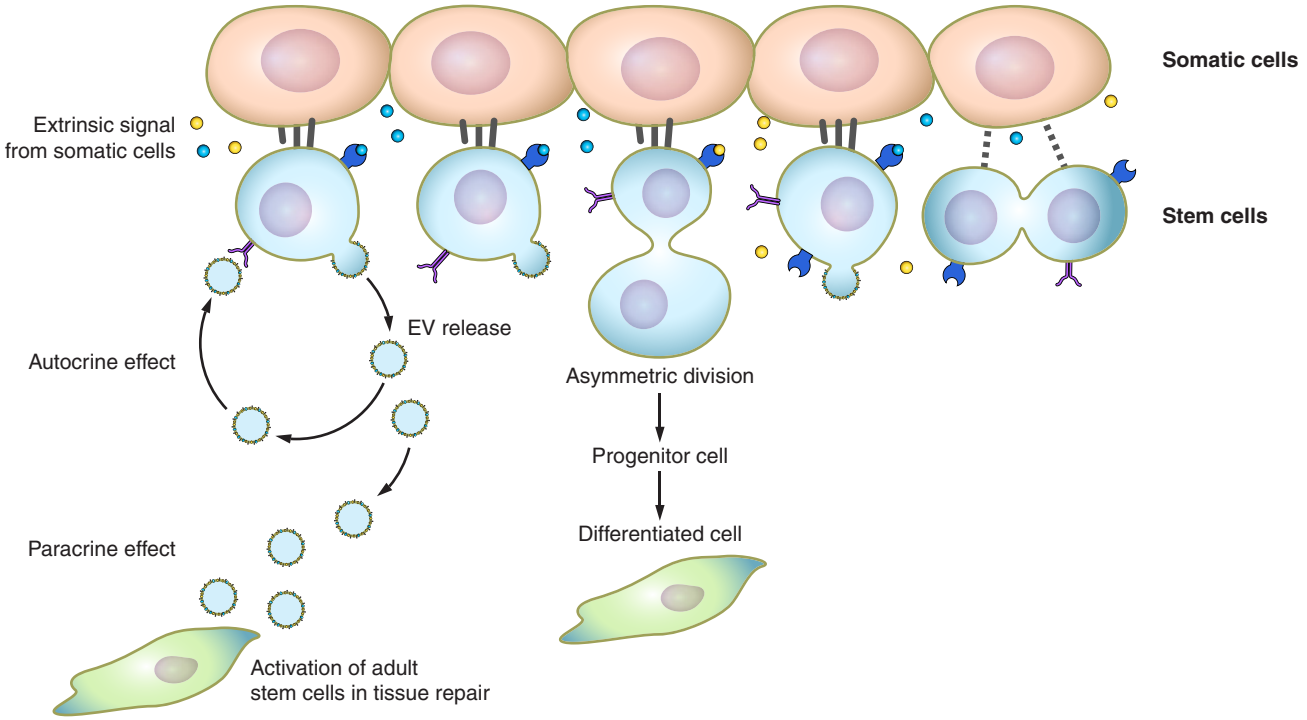


Fig. 6. EVs are able to balance between stem cell self-renewal and differentiation. Under physiological condition EVs present in the niche regulate the balance between stem cells self renewal and differentiation. Under pathological condition (injury and disease), EVs released from injected stem cells (e.g., mesenchymal stromal stem cells) exert a paracrine effect on damaged tissue resident stem cells and/or somatic cells to restore tissue function.

limited in size and limits stem cell numbers; and *5*) it is capable of reverting daughter cells to a stem cell fate (145).

Stem cells are defined functionally as cells with the capacities to self-renew and to differentiate (154, 174). Depending on their developmental stage and origin, stem cells are classified as embryonic or adult stem cells. Adult stem cells are involved in tissue homeostasis and repair after wounding throughout the lifetime of an ogranism. When partially committed as a result of stimulation to differentiate along a defined lineage, they are also referred to as progenitor cells, which are early descendants of stem cells that can only differentiate and can no longer self-renew. Following engraftment, stem cells receive cues from the local environment that induce cell differentiation. For instance stem cells differentiated into nerve cells in murine models of neural ischemia and demyelinating lesions, while stem cells localized to mouse brain tumors remained in a quiescent and undifferentiated state (32).

Stem cells, especially embryonic stem cells, have recently been shown to be an abundant source of EVs (53, 92, 158). It was hypothesized that these EVs may be involved in the self-renewal and expansion of pluripotent or multipotent stem cells in vitro, and Ratajczak et al. (138) demonstrated that embryonic stem cell-derived EVs were able to induce changes in hematopoietic progenitor cells (138). In particular, these vesicles were shown to contain Wnt-3, a factor involved in stem cell expansion (63, 70, 74), and they were also enriched in the pluripotent stem cell transcription factor Oct-4. In addition, RNAse treatment showed that EVs contained not only the protein Oct-4 but also its mRNA, which can be translated within the target cells (138). Similarly, Deregibus et al. (46) demonstrated that EVs derived from human endothelial progenitor cells were able to trigger angiogenesis in human vascular endothelial cells by horizontal transfer of mRNA. Several subsequent studies indicated that mRNAs delivered by EVs could be translated into the corresponding proteins by the target cells (23, 24, 47, 73, 166). In addition to mRNAs, EVs derived from stem cells can also transfer miRNAs, and the discovery that embryonic stem cells release abundant EVs containing miRNAs suggests a role for EVs in signaling within the stem cell niche (179).

The role of these EVs has been demonstrated in pathological conditions, and it has been suggested that EVs mediate a bidirectional exchange of genetic information between stem cells and injured cells (27). mRNAs and miRNAs delivered from injured cells may reprogram the phenotype of the stem cells to induce them to acquire specific features of the tissue (137). In contrast, EVs released from stem cells may induce the de-differentiation of cells surviving injury, resulting in cell cycle reentry and tissue self-repair by delivering specific mRNAs and miRNAs (47). Administration of heterologous multipotent mesenchymal stromal stem cells (MSCs) confers a protective role against acute kidney injury and increases renal function in chronic kidney disease (29, 62). Interestingly, it has been demonstrated that MSCs play a paracrine role and that homing is not an absolute requirement for the beneficial effect of MSC-based therapy. In particular, it has been demonstrated that EVs derived from human MSCs stimulate proliferation and inhibit apoptosis of tubular epithelial cells in vitro (41). These results suggest that tubular cell regeneration and proliferation are stimulated by mRNA transfer, and the therapeutic potential of MSC-derived EVs has thus been investigated by several groups in recent years (see Ref. 92).

EVs of endothelial origin, released from ischemic muscle, were able to induce the differentiation of bone marrow-derived

progenitor cells (BM-MNCs) into endothelial cells both in vitro and in vivo and subsequently to promote postnatal vasculogenesis. This interaction is mediated by PS, which is instrumental in the internalization of EVs by BM-MNCs. Similarly, Aliotta et al. (3) demonstrated that murine bone marrow cells expressed genes for lung-specific proteins after incubation with injured lung-conditioned medium containing EVs with specific lung mRNA.

### *Adult Stem Cells: Their Role in Regenerative Medicine*

MSCs are currently the most widely studied stem cells, and in 2010 there were more than 100 registered clinical trials using these cells (9). MSCs were originally thought to home and engraft in injured tissues, where they differentiated to replace damaged cells. However, it has since been demonstrated that <1% of transplanted cells reach the target tissue and differentiate. Inefficiencies in MSC homing were confirmed by their transient localization after intravenous injection to organs such as the lung, spleen, liver, and kidney (80, 93). The positive effects of MSC transplantation were demonstrated to result from their ability to release trophic mediators responsible for the cell therapeutic effects (31, 36, 83, 106, 162, 169).

One of the best described roles of EVs in adult stem cell biology is their paracrine effect on different kinds of stem cells after acute myocardial infarction. To date, the use of adult stem cells, such as BM-MNCs, bone marrow cells, hematopoietic stem cells, endothelial progenitor cells, MSCs, and cardiac stem cells, has been associated with an overall improvement of cardiac function in this pathology. Several studies reported that the three main mechanisms of adult stem cell action in heart repair were cardiomyocyte regeneration, vasculogenesis, and paracrine actions, the involvement of which was first reported for MSCs by Gnecchi and colleagues (66, 67, 95). These paracrine factors (e.g., IL-1β, MMP-2, MMP-9, and FGF-2; for a complete list see Ref. 68) are released through membrane vesicles or exosomes (98), as previously demonstrated in other cell lines (26, 82). The possible use of exosomes for the treatment of cardiovascular diseases was demonstrated by Lai et al. (98) in a mouse model (for a review, see Ref. 99). The released factors may promote angiogenesis, dampen inflammatory responses, enhance the survival of parenchymal cells, and support endogenous stem cell progenitors. (For recent reviews on the paracrine mechanisms of MSC-based therapy, see Refs. 105, 117, 178.) A positive effect was also observed in liver regeneration (73, 41). MSCs have been clinically tested in relation to their role in bone repair and regeneration and have been used to treat osteogenesis imperfecta (78, 102, 128). Ciapetti et al. (38) suggested that the beneficial effect of these cells relied on their ability to release trophic signals such as growth factors and cytokines that accelerate the regeneration process. Gatti et al. (62) recently reported that EVs purified from MSC-conditioned medium were sufficient to protect against tubular injury in rats. MSCs have also been used to treat neuronal injury and neurodegenerative diseases. In these cases, the reparative potential of the stem cells could depend on their ability to release several kinds of growth factors, the secretion of which was increased when MSCs were exposed to injured-brain lysate in vitro (37). Moreover, in vitro experiments also demonstrated that MSCs possessed the ability to transdifferentiate into neuronal-like cells (163, 164). Importantly, EVs have recently been shown to be secreted by neural stem/precursor cells (87, 91, 112, 135).

Based on these data, it is possible to postulate a paracrine/autocrine hypothesis that extends the traditional concept of the stem cell niche to include the influence of stem cell-released factors on the microenvironment, modulating both stem cell biology and tissue response. In conclusion, stem cells are able to release membrane vesicles containing active mediators with a paracrine effect on the stem cell niche and also on adjacent parenchymal and stromal cells under both normal and pathological conditions. In the latter case, these paracrine factors may enhance cell survival and activate endogenous mechanisms of repair and regeneration. Finally, stem cell-released factors may exert autocrine effects on the cells themselves, thus influencing cell survival, self-renewal, and cell growth (43, 125, 175).

Experimental data suggest that EVs could be considered as biomarkers of disease statuses or could be used to monitor the behavior of neural progenitors (82, 83).

### *Future Perspectives*

The data summarized in this review suggest that EVs and exosomes may have clinical applications. EVs and exosomes have many characteristics that make them ideal drug-delivery vehicles: they can contain both proteins and genetic material; are well-tolerated in the body, as demonstrated by their presence in all biological fluids; and are able to cross the plasma membrane to release their contents within target cells. Finally, they have the intrinsic ability to home to target tissues and can be modified to enhance cell-type specific targeting (5). For example, Katsuda et al. (92) proposed a general strategy for the clinical application of MSC-derived EVs. The first step involves the isolation of MSCs from either the patient or an allogenic donor (92). These cells have the advantage of poor immunogenicity, thus facilitating the use of allogenic donors (165). The isolated cells are then expanded, during which process they may be manipulated and modified, to produce modified EVs. Thirdly, the EVs released into the MSC medium are collected. This hypothesis opens novel therapeutic opportunities, given that the harvested EVs can also be modified by the addition of mRNA or miRNA (5) or by loading with therapeutic drugs (181). Exosomes can also be loaded, either in vivo during their biogenesis or in vitro after their purification (100), although the in vivo drug loading of both EVs and exosomes requires an in-depth understanding of their biogenesis. The final step is administration of EVs to the patients. The route of administration depends on the target disease; for example, EVs administered intravenously (5) or intranasally (181) cross the blood-brain barrier and can deliver the cargo directly into the brain. In vivo studies have already demonstrated the beneficial effects of intravenously injected exosomes in tissue repair (10).

In conclusion, EVs and exosomes released from stem cells mimic the effect of the cells, suggesting a potentially valuable role in regenerative medicine. It is therefore essential to study the mechanisms responsible for microparticle release and the selective enrichment of paracrine factors and RNAs. The presence of selected miRNAs within MSC EVs has been demonstrated by Collino et al. (41) who confirmed the exis-

tence of mechanisms controlling cargo sorting in MSC-derived EVs.

### *Advantages and Disadvantages*

Most cell types have been shown to release either EVs or exosomes into the extracellular environment. The inclusion of proteins and nucleic acids within EVs protects them from the extracellular environment, allowing the long-range exchange of information. EVs and exosomes demonstrate several possible advantages over stem cells in terms of their use in regenerative medicine. Importantly, they are more stable and induce stronger signaling and are produced in higher concentrations than stem cells. Additionally, EVs and exosomes as delivery vehicles possess an intrinsic homing ability relative to other synthetic particles, thus avoiding their unwanted accumulation in organs other than the target tissue. They demonstrate no inherent toxicity, are not associated with any long-term maldifferentiation of engrafted cells or tumor generation (161), and carry no apparent risk of aneuploidy (26) or immune rejection following in vivo allogenic administration (24). The progenerative effects mediated by EVs depend on their enrichment in bioactive lipids, the delivery of proteins that improve cell function, and the presence of both mRNA and miRNA, which may function to regulate cellular processes such as cell survival. In addition, it is possible to manipulate cells and introduce drugs into them, indicating that EVs and exosomes represent promising candidate drug-delivery vehicles with the ability to carry both hydrophobic and hydrophilic drugs.

In terms of their disadvantages, vesicle shedding may be disadvantageous in certain pathologies, such as cancer. Tumor cells release many EVs that are involved in tumor progression and may contain proteases involved in ECM degradation, thus promoting metastasis formation (11, 60, 77, 79). tEVs may also be involved in the regulation of cell survival by altering the tumor immunological response by releasing membrane antigens (4) and thereby imparting a growth advantage to the malignancy. They can also contain caspase-3, thus preventing its accumulation and inhibiting cell death by apoptosis. EVs may also contribute to cell survival by preventing the accumulation of chemotherapeutic drugs (143, 148).

In conclusion, a better understanding of the properties of EVs is required. In particular, further studies are needed to clarify *1*) the stimuli and pathways regulating the assembly of bioactive molecules within vesicles, *2*) the signals triggering their release, *3*) the receptors responsible for conferring their target specificity, and *4*) their diagnostic potential in diseases.

## GRANTS

This research was supported by grants from the University of Palermo and PhD funding.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

## AUTHOR CONTRIBUTIONS

Author contributions: G.T. and F.G. drafted manuscript; G.T., R.T., G.S., and F.G. approved final version of manuscript; R.T. and F.G. edited and revised manuscript; G.S. prepared figures.

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