
Extracellular Vesicles (EVs); Basic Science, Clinical Relevance and Applications

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Abstract: All types of cells of eukaryotic organisms produce and release small Nano-vesicles into their extracellular environment. Early studies have described these vesicles as “garbage bags” only to remove obsolete cellular molecules. Valadi and coworkers, in 2007, was the first who discovered the capability of circulating EVs to horizontally transfer functioning gene information between cells. These extra cellular vesicles express components responsible for angiogenesis promotion, stromal remodeling, chemo-resistance, genetic exchange and signaling pathway activation through growth factor/receptor transfer. Extracellular vesicles (EVs) represent an important mode of intercellular communication by serving as vehicles for transfer between cells of membrane and cytosolic proteins, lipids, signaling proteins and RNAs. They contribute to physiology and pathology, and they have a myriad of potential clinical applications in health and disease. Moreover, vesicles can pass the blood-brain barrier and may perhaps even be considered as naturally occurring liposomes. These cell-derived extracellular vesicles not only to represent a central mediator of the disease microenvironment, but their presence in the peripheral circulation may serve as a surrogate for disease biopsies, enabling real-time diagnosis and disease monitoring. In this review, we’ll be addressing the characteristics of different types and the clinical relevance of these extracellular EVs and their potentials as diagnostic markers as well as defining therapeutic options.

Keywords: Extracellular Vesicles (EVs), Exosomes, Horizontal Gene Transfer (HGT), Microvesicles (MVs)

1. Introduction

Transfer of genetic information between cells had been proposed through only two mechanisms: vertical gene transfer, from parent to the next generation, and horizontal gene transfer, induced through bacteriophages [1] or viruses [2]. Recently, another mechanism of horizontal gene transfer has emerged through naturally occurring cell-derived vesicles such as exosomes and microvesicles. Extracellular vesicles are produced constitutively by most, if not all, cell types and, interestingly, contain both, mRNAs and non-coding RNAs such as small regulatory microRNAs (miRNAs) as well as proteins that can be functionally delivered between different cell types and across species [3]. As a result, such vesicles have a significant impact on physiological processes. However, this natural ability of exosomes and microvesicles to transfer genetic information might instead facilitate the spread of disease through the delivery of genetic material

and/or pathogenic proteins [4, 5].

Extracellular Vesicles (EVs) or more accurately nanoparticles, is a term used for vesicles that are released from the plasma membrane under basal conditions or during cell stress [6].

Microvesicles are, not only produced by most cell types, but also present in most, if not all, biological fluids as well as the peripheral blood of healthy individuals in a ratio of ~10(10)/ml of blood [7, 8]. Because of the small size and heterogeneity of vesicles, their detection and classification is challenging [9].

Different types of extracellular vesicles have been identified; exosomes and microvesicles were distinguished unanimously. Apoptotic blebs or bodies had become a separate class [10-13]. Other less commonly used terms for vesicles; “ectosomes,” “membrane particles,” and “exosome-like vesicles” were also described. However, circulating vesicles are likely composed of both exosomes and microvesicles (MVs), and currently available purification

methods do not allow one to fully discriminate between exosomes and MVs and that a single cell type can release both of them in the meantime.

Confusion on the origin and nomenclature of EVs has spread through the literature as well because vesicles with the size of exosomes that bud at the plasma membrane have also been called exosomes [16, 17]. It should be noted that most studies have not clearly defined the origin of EVs under study; therefore, we will mostly refer to EVs rather than MVs or exosomes. A major ongoing challenge is to establish methods that will allow one to discriminate between exosomes and MVs. Differences in properties such as size, morphology, buoyant density, and protein composition seem insufficient for a clear distinction [18].

2. Historical Background

Thirty years ago, two papers had been published within a week of each other—reported that, in reticulocytes, transferrin receptors associated with small vesicles are literally jettisoned from maturing blood reticulocytes into the extracellular space [14, 15]. The name “exosome” for these extracellular vesicles was coined a few years later by Rose Johnstone, although the term was initially introduced for vesicles ranging from 40 to 1,000 nm that are released by a variety of cultured cells [19], later, this nomenclature was adopted for 40–100-nm vesicles released during reticulocyte differentiation as a consequence of multivesicular endosome (MVE) fusion with the plasma membrane [20, 21]. One decade later, exosomes were found to be released by B lymphocytes and dendritic cells through a similar route [22, 23].

3. The Main Characteristics of Extracellular Vesicles (EVs)

3.1. Types of EVs

Two common types were distinguished unanimously [i.e., *exosomes* and *microvesicles* (also called shedding vesicles, shedding microvesicles, or nanoparticles)]. A third type; *apoptotic vesicles* (also called apoptotic blebs, or apoptotic bodies) has become a separate class [10, 12]. In addition, “ectosomes,” “membrane particles,” and “exosome-like vesicles” were distinguished on the basis of the physical and chemical characteristics of vesicles, including size, density, appearance in microscopy, sedimentation, lipid composition, main protein markers, and subcellular origin [i.e., originating from intracellular compartments (exosomes) or plasma membranes] [10].

3.2. Isolation and Characterization of EVs

One major challenge in the field is to improve and standardize methods for EV isolation and analysis [25]. Because of their small size, vesicles are below the range of conventional detection methods. Isolation of EVs is mostly from the supernatants of cultured cells grown by performing

differential ultracentrifugation (100000-200000 g). The differences in floatation velocity can be used to separate differently sized classes of EVs [24]. Most size determinations of vesicles are based on the transmission electron microscopy (TEM).

Besides differential centrifugation, *filtration* can be applied to isolate vesicles. Although the pore size of filters is often well defined, increasing forces have to be applied with decreasing pore size, which may result in artifacts [26].

Flow field-flow fractionation (FFFF) [27], a technique that fractionates particles based on differences in their diffusion properties without applying forces equally high to differential centrifugation. Although FFFF is successfully applied to isolate exosomes from human neural stem cells [28], FFFF is not widely applied because it requires extensive optimization of the settings and is relatively expensive.

Characterization of isolated EVs requires complementary biochemical (immune-blotting), mass spectrometry, and imaging techniques. Quickly frozen, vitrified vesicles analyzed by cryo-electron microscopy indeed show that exosomes and other EVs have a perfectly rounded shape [29]. Complementary to electron microscopy, nanoparticle tracking analysis allows determination of the size distribution of isolated EVs based on the Brownian motion of vesicles in suspension [30]. Because conventional flow cannot distinguish between vesicles that are smaller than 300 nm, a novel high resolution flow cytometry-based method has been recently developed for quantitative high throughput analysis of individual (immune-labeled) Nano-sized vesicles [31, 32].

3.3. The Molecular Composition of EVs

Most, if not all, cell types can secrete vesicles both in basal conditions and upon exposure to stress. Microvesicles are nanoparticles that are generated by direct budding of the plasma cell membrane, with a size ranging from 50 to 1000 nm, express surface markers such as integrin- β , CD40 ligand and selectins as well as surface protein receptors that characterize the membrane composition of their cells of origin [33-35]. On the other hand, exosomes are derived from endocytic pathway of different cell types, express contain endosome-associated proteins such as Annexins, Flotillin, Alix, Tsg101 and Tetraspanins, such as CD63, CD81, CD82, CD53, CD9 and CD37 [36-38]. Exosomes from a variety of cells are highly enriched in cholesterol, sphingomyelin, and hexosylceramides, in addition to phosphatidyl-choline and phosphatidyl-ethanolamine. The fatty acids in exosomes are saturated or monounsaturated [39].

Interestingly, extracellular vesicles were demonstrated to contain both mRNA and miRNA. mRNA can be functionally transported and translated into proteins whereas, miRNA can induce translation repression or less often degradation of mRNA of the target cells [40-44]. It is of importance to notice that, in mammals, each mRNA molecule is translated into as many as ~2800 protein molecules [149]. Recently, analysis of RNA from EVs demonstrated that, in addition to mRNA and miRNA, EVs also contain small interfering RNAs (siRNAs) and piwi RNA (piRNAs). The piwiRNA is specifically

characterizes the germ cell line [45, 46]. Many RNAs that were isolated with EVs vary from the RNA profiles of the originating cells [31, 40, 41], suggesting the existence of active sorting mechanisms of RNA molecules and that they are selectively incorporated into EVs [26]. It is well documented that a single miRNA can influence hundreds of mRNA gene transcripts and thereby, it is proposed to be implicated as a key player in virtually every cell process [47]. While the majority of miRNAs are found intracellularly, a virtual number have been detected outside cells. One important mechanism explaining the stability of extracellular RNAs, despite high extracellular RNase activity, is thought to be packing within extracellular vesicles (EVs) which are impermeable to RNases [48]. To date, more than 2000 extracellular miRNA has been discovered in humans, some of them are contained in EVs (10%) and most (90%) are associated with lower-molecular-mass complexes bound to Argonaut 2 (Ago2), nucleophosmin-1 (NPM1) and high density lipoproteins (HDL) [150].

3.4. Biogenesis and Release of EVs

Ribosomes are the factory through which mRNA is translated into non-functioning polypeptides (random coil). Amino acids of the random coil interact with each other to produce a functional three-dimensional protein structure in the endoplasmic reticulum [50]. Only properly folded proteins are then transported through the rough ER-Golgi pathway. In case the process of three dimensional structures produced mal-folded protein, several endoplasmic reticulum chaperone proteins are released to correct misfolded or unfolded proteins; in this reaction glucose, calcium and redox buffers are required for successful protein folding [51]. One major function of chaperones is to prevent newly synthesized polypeptides from aggregation and pass into nonfunctional structures. It is important here to remember that chaperones are not present when the macromolecules have correctly completed the processes of folding [52, 53], which might be, in the future, implicated to discriminate exosomes originating from stressed cells from those from non-stressed cells. Mal-folded proteins are then, sent back to the cytosol in transient complexes that prevents these proteins from secretion through the endosomal pathway [54, 55].

The mechanism of formation of exosomes is the process of the endosomal pathway, including endocytic vesicles, early endosomes, late endosomes; also known as *multivesicular bodies* (MVBs) and lysosomes [56, 57]. The pathway of multivesicular endosomes (MVEs) that are prone to fuse with lysosomes and predestined for lysosomal degradation, differ from the pathway of secretory MVEs predestined to become secreted as exosomes; it can be either: ESCRT-dependent or ESCRT-non-dependent pathways in which exosomal release process might be controlled by natural ceramide biosynthesis enzyme (nSMase2). However, the release is overall modulated by extracellular signals [58, 59].

Endosomal sorting complex responsible for transport (ESCRT) is a four multi-protein complexes assembled within the MVEs: The ESCRT- 0, I and II complexes recognize

membrane proteins at the endosomal membrane, whereas the ESCRT-III complex may be responsible for actual scission of intraluminal vesicles) ILVs [60-63].

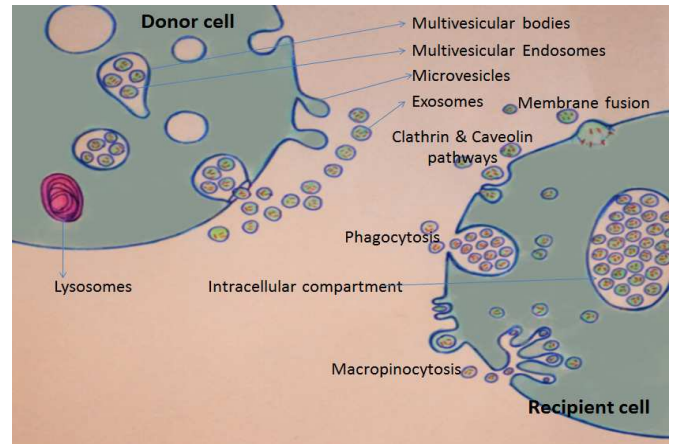


Figure 1. At the “DONOR CELL”: Release of MVs and exosomes. MVs bud directly from the plasma membrane, whereas exosomes are represented by small vesicles of different sizes that are formed as the ILV by budding into early endosomes and MVEs and are released by fusion of MVEs with the plasma membrane. At the “RECIPIENT CELL”: Pathways shown to participate in EV uptake by target cells, including clathrin mediated endocytosis (CME), caveolin-dependent endocytosis (CDE), phagocytosis, macropinocytosis and plasma or endosomal membrane fusion.

The external membrane of the MVBs fuses with the plasma membrane of the cell, resulting in release of their segregated vesicles through the process of exocytosis to the extracellular space. In all human cells, exocytosis can be either constitutive (non- calcium-dependent) or regulated (calcium-dependent), so as extracellular vesicles exocytosis is expected [64].

Disturbances in redox regulation, calcium regulation, glucose deprivation, and viral infection [65, 66] or the over-expression of proteins [67], properly folded or mal-folded, can lead to endoplasmic reticulum (ER) stress. This is a state in which the folding of proteins slows, leading to further accumulation of miRNA species and increase in unfolded proteins. Unfolded protein crowd will be developed, resulting in more slowing of the cell machinery, and finally the unfolded protein response (UPR) develop, with the activation of the signaling pathways that lead to increasing the production of molecular chaperones involved to correct protein folding. This chaperones aim at restoring normal function of the cell by halting protein translation and degrading mis-folded proteins. If these objectives are not achieved within a certain lapse of time or the disruption is massive, the UPR aims towards activation of apoptosis (e.g. p53 pathway activation) [68, 69].

The p53 protein activation respond to a wide variety of stress signals including geno-toxic stress, hypoxia, and even the expression of activated oncogenes [70], which, for instance, could be part of the mis-folded proteins. However, it has been shown that overexpression of TSAP6, a p53-regulated gene and is transcribed in response to stress [54, 55, 71-73]. Thus, the requirement for p53 in exosome production might be through the ability of p53 to up-regulate

TSAP6 transcription. Expression of the TSAP6 gene allows the cells with or without functional p53 to produce exosomes [54, 74]. This finding supports the notion that, cell stress, with or without activation of the apoptotic pathway, can increase the production of exosomes.

3.5. Mechanisms of Action of EVs at the target Cells

The exact mechanisms by which EVs interact with recipient cell membrane and/or delivering their contents of proteins, lipids, and RNAs; is still not fully understood [31]. Target cell specificity for binding of exosomes (or other EVs) is likely to be determined by adhesion molecules and also tetraspanin complexes together with extracellular signals which appear to influence target cell selection in vitro and in vivo [75].

Various mechanisms for EV uptake have been proposed, including clathrin mediated endocytosis (CME), caveolin dependent endocytosis (CDE), phagocytosis, macro pinocytosis and plasma or endosomal membrane fusion [76] (Fig. 1). Regardless the mechanisms; interaction between EVs and recipient cell membrane may include: (i) ligand/receptor binding, (ii) fusion, (iii) internalization of their content or, combination of these [26]. Extracellular vesicles may directly activate the recipient cell by acting as signaling complexes [77, 78]. It worthwhile to mention that during cell exocytosis, proteins embedded in the vesicle membrane fuse and become part of the plasma membrane and the side of the protein that was facing the *inside* of the vesicle now faces the *outside* of the cell. This mechanism is important for the regulation of trans-membrane receptors and transporters [79]. Whether or not this is the case for fusion of exosomal membrane with the target cell membrane, need to be investigated. However, this can reconcile the discrepancy of whether phosphatidyl serine (PS) exposed by EVs [80] or increased due to exposure to shearing forces associated with ultracentrifugation [81].

After being endocytosed, EVs may be distend to the endosomal pathway targeted to lysosomes for degradation or release their functionally active nucleoproteins load inside the recipient cell [40]. mRNA can be translated in the recipient cells via ribosomes, whereas miRNAs and siRNA are known to regulate more than 80% of all protein-encoding genes through gene silencing effect [82-84].

It is therefore conceivable that EV may play a critical role in signaling mechanisms essential for normal biological functions, as well as disseminating pathogenesis through interaction with recipient cells.

4. Functions of EVs

It has become evident that EVs are important factors involved in a wide range of physiological and pathological processes. As the apical/baso-lateral membrane distribution is lacking in all blood cells including immune cells, transient exposure time and interaction between EVs and circulating blood cells would have been expected, at least theoretically, to be some folds higher than that of organ fixed endothelial cells. This may explain the wide range of involvement of immune cells in a diversity of cytopathic effects of exosomes. For this

reasons it would be expected that blood cells, including immune cells, are, at least chronologically, the first to interact with the circulating EVs. Extracellular vesicles have since been demonstrated to be released by a variety of cells of the immune system, including dendritic cells, macrophages, B cells, T cells, and NK cells [80]. These extracellular vesicles have been demonstrated to be key mediators/regulators of normal immune responses [85]. So, Douglas Taylor was right to say “one can view tumors as a “*cyber-terrorist*” using these extracellular vesicles to elicit aberrant immune regulation” [26].

4.1. Extracellular Vesicles Effects on the Immune Systems

4.1.1. EVs as Immune-Suppressants

By transporting ligands and receptors, exosomes can orchestrate cell growth and development, and modulate the immune system. Activated T cells and peripheral blood mononuclear cells release vesicles exposing Fas ligand (FasL), a death receptor ligand, which may have immune regulatory function [86]. During first-trimester, trophoblast cells from a pregnant uterus release exosomes exposing FasL, which are capable of inducing Fas-mediated T-cell death, suggesting that exosomes contribute to this immune privilege [87, 88]. Moreover, epithelial cells from ovarian cancer release FasL-exposing microvesicles that are capable of inducing T-cell apoptosis [89]. Exosomes isolated from pleural effusion of patients with mesothelioma down-regulate NKG2D expression of NK cells and CD8⁺ cells via a transforming growth factor (TGF)- β -dependent mechanism, indicating that NKG2D may be one of the targets for exosome mediated immune evasion [90, 91]. Moreover, exosomes have the ability to prevent allergic sensitization in allergic asthmatic patients [93]. Immature or suppressive DCs reduce adaptive immune activation by inducing T cell apoptosis and thus promoting a tolerogenic immune response as seen in murine models of transplantation and autoimmune diseases. Suppressive exosomes may also influence the balance between pro-inflammatory and anti-inflammatory effector T cells inducing T helper Th17/Th1 cells to differentiate into Th2 and Foxp3⁺ regulatory T cells [92]. For this reason, exosomes could potentially be used to avoid graft rejection or as a treatment of autoimmune diseases. Moreover, the latent membrane protein-1 (LMP-1) of Epstein-Barr virus (EBV), which is exposed on exosomes from EBV-infected cells, inhibits the proliferation of peripheral blood mononuclear cells. This mechanism is thought to be relevant in development of EBV-associated tumors, such as nasopharyngeal carcinoma and Hodgkin's disease, by allowing tumor cells to evade the immune system [93]. Taken together, exosomes are likely to orchestrate the efficacy of the immune system by a whole set of different mechanisms important not only for normal development but also for tumor development.

4.1.2. EVs as Immune-Stimulants

EVs are used by cells not only to suppress the immune system but also to present antigens and stimulating the

immune reaction. Human intestinal epithelial cells release exosomes exposing MHC class I and MHC class II molecules at both the apical and basolateral sides, suggesting a possible involvement of exosomes in the transcellular transport of antigens from the lumen of the gut to immune cells [94, 95]. The initiation of T-cell-mediated antitumor immune responses requires uptake, processing, and presentation of tumor antigens by DCs. Exosomes from mouse tumor cells transfer tumor antigens to DCs in vivo [96]. Exosomes exposing MHC class II-antigen complexes are secreted from antigen-loaded DCs when these cells contact antigen-specific CD4 T cells. This secretion is preceded by accumulation of intraluminal vesicles exposing both MHC class II and CD9 in MVEs [97]. Mature dendritic cells also secrete exosomes showing functional peptide-bearing MHC class I and II molecules on their membranes that can directly bind to T-cell receptors and activate CD4⁺ or CD8⁺ T cells inducing an adaptive immune response [98, 99].

4.2. Other Clinically Relevant Functions of EVs

4.2.1. Role of EVs in Inflammation

Cell-derived vesicles from stressed cells can trigger the production of pro-inflammatory cytokines by activating targeted cells independent of the presence or absence of previous infections. This activation results in expression and production of tissue factors and interleukins [100]. The elegant experiment of Deng and his colleagues shows that EVs derived from adipose tissue when intravenously injected into wild type C57BL/6 mice resulted in development of insulin resistance, whereas injection into TLR-4 knockout B6 mice the insulin resistance was not marked due to the effect of increased levels of TNF- α and IL-6 [101]. Based on this experiment, the gradual progressive dissemination of insulin resistance throughout the body may be attributed, at least in part, to EVs released from inflamed adipose tissues which may open the gate for a new modality of treatment for insulin resistance type 2 diabetic patients [102]. Another example is that extracellular vesicles from human atherosclerotic plaques have been shown to mediate the functional transfer of ICAM-1 to endothelial cells of neighboring healthy cells, thereby promoting the adhesion of monocytes and trans-endothelial migration [103]. Thus, plaque EVs may further facilitate atherosclerotic plaque progression. Moreover, the ability of EVs to modulate the inflammatory response is not limited to blood. Autologous EVs from synovial fluid and EVs from T cells, monocytes, and platelets trigger the production and release of interleukins 6 and 8, matrix metallo-proteases, monocyte-chemotactic proteins 1 and 2, vascular endothelial growth factor (VEGF), and ICAM-1 by synovial fibroblasts, indicating that these vesicles may enhance the destructive activity of these fibroblasts in rheumatoid arthritis [104].

4.2.2. Extracellular Vesicles and Viruses

Viruses use vesicles for infection and survival. HCV entry receptors may partly contribute to exosome uptake even in the absence of viral envelope or core proteins [105]. HCV entry

receptors CD81 and SR-BI are also known to localize in lipid rafts of exosomes [106], supporting a hypothetical role of these receptors in exosome uptake by the neighboring healthy hepatocytes. The cytopathic effect of HCV infection differs between complete virus genome and exosome mediated hepatocyte infection (reinfection). While complete free virus genome mostly produces cell lysis and apoptosis, exosomes mediated infection or reinfection, can produce an antibody-resistant cell-to-cell transmission route and establish a productive infection even in the absence of viral envelope or core proteins [107-109]. Moreover, in membranoproliferative (mesangiocapillary) glomerulonephritis (MPGN) associates HCV, mesangial expansion is largely attributed to macromolecular deposits (EVs) and to lesser extent the direct pathogenetic effect of the virus [110], supporting the hypothetical role of exosome in transmitting HCV genetic information to the kidney [111]. Recently, it has been shown that exosomes released from HIV-infected cells contain negative regulatory factor, which induces apoptosis of uninfected cells though these cells do not contain the RNA of the virus itself (bystander effect) [112], besides, EVs produced in response to HIV virus are more infectious to CD4 T cells than cell-free viral particles [113]. Moreover, human herpes virus 4 or EBV encodes RNAs that can be transferred from infected to uninfected cells by EVs [114-116]. The uptake of EVs from EBV-infected cells by epithelial cells results in activation of growth-stimulating signaling pathways and can manipulate the growth characteristics of neighboring cells [117, 118].

4.2.3. Tumor Growth, Metastasis, and Angiogenesis and EVs

One of the molecular mechanisms underlying the intercellular transfer of metastatic activity is the transfer of an oncogenic growth factor receptor or their ligand. The ability of exosomes to promote metastasis and angiogenesis can be increased when exosomes are released under hypoxic conditions (i.e., conditions that many tumors encounter when growing) [119]. Cancer cells can release vesicles containing the Fas-associated death domain, a key adaptor protein that transmits apoptotic signals and becomes lost in many cancer cells [120]. In addition, exosomes may protect tumor cells from entering or accumulation of antitumor drugs, thereby possibly contributing to (multi) drug resistance. For instance, exosomes from HER2-overexpressing breast cancer cell lines, or exosomes present in serum from patients with breast cancer, capture the humanized antibody trastuzumab, thereby reducing the effective concentration of this anticancer drug [121]. Vesicles may also contribute to drug resistance by exchanging drug transporters between cells. There are other ways in which vesicles promote tumor growth, including the release of active matrix-degrading enzymes, stimulation of angiogenesis [123], and production of TF [124]. Vesicles can expose phosphatidylserine (PS), a negatively charged phospholipid to which (activated) coagulation factors can bind and assemble in the presence of calcium ions, thereby promoting coagulation [125, 126]. Cell-derived vesicles from tumors are strongly pro-coagulant [127]. This activity was due

to the exposure of TF, the initiator of coagulation [128].

4.2.4. Neurodegenerative Disorders

Parkinson's disease is characterized by intracellular aggregates of α -synuclein, the Lewy bodies, in dopaminergic neurons. Alvarez-Ervit and colleagues showed that α -synuclein released from cells over expressing this protein is efficiently transferred to recipient normal cells through exosomes [129]. Moreover, Surgucheva showed that another member of the synuclein family, γ -synuclein, secreted from neuronal cells into exosomes can be transmitted to glial cells, thus promoting the aggregation of intracellular proteins [130]. Alzheimer's disease (AD); characterized by extracellular aggregates of beta amyloid peptides known as amyloid plaques [131], exosome-associated-amyloid peptides that may be involved in plaque formation playing a significant role in the pathogenesis and the progression of AD, besides, tau proteins are mainly secreted through exosomes in vitro and in vivo [133].

4.2.5. Cardiovascular Disorders and EVs

Exosome-mediated cellular communication is also involved in the pathogenesis of cardio-vascular disease. Serum levels of miR-1 and miR-133a are localized inside exosomes and are significantly increased in patients with acute myocardial infarction and angina pectoris [134]. Furthermore, circulating platelet-derived exosomes from septic patients induced myocardial dysfunction in isolated heart and papillary muscle preparations in a nitric-oxide dependent mechanism [134]. Extracellular vesicles from human atherosclerotic plaques have been shown to mediate the functional transfer of ICAM-1 to endothelial cells of neighboring healthy cells, thereby promoting the adhesion of monocytes and trans-endothelial migration [103]. Thus, plaque EVs may further facilitate atherosclerotic plaque progression. Exosomes enriched in miR-143/145; regulated by KLF2 (*Krüppel-like factor 2*), a key transcription factor able to mediate an athero-protective endothelial phenotype generated by shear stress. They showed that these miRNAs are transferred into smooth muscle cells (SMCs) acting on miRNA targets thus preventing SMC de-differentiation [135].

4.2.6. Autoimmune Disorders and EVs

Synovial fluid obtained from patients with RA contain APO2L/TRAIL that has been associated with exosomes in synovial fibroblasts appears to be low compared to that found in the synovial fibroblast of control patients [137]. Martinez-Lostao and co-workers have recently demonstrated that tethering APO2L/TRAIL to the liposome membrane may substantially reduce synovial hyperplasia and inflammation in rabbit knee joints [136]. It has been recently reported that inappropriate clearance of apoptotic vesicles is considered to be the primary cause of developing systemic autoimmune diseases [138].

5. Diagnostic Potentials of EVs

Recently, evidences are accumulating supporting the

promising potential of EVs as disease biomarkers. Exosomes are stable, disease-specific, disease characterizing, and can also predict prognosis [139]. Temporal changes in exosomal nucleoprotein contents have been demonstrated to accurately predict disease recurrence and overall patient survival [140]. Moreover, the proteomic and genomic profiles of circulating exosomes provide a real-time monitor of therapeutic response. By correlating these circulating markers with real-time clinical parameters, circulating exosomes profile can be considered a "liquid biopsy" of cells [141].

Prostate-surface antigen (PSAs) had been the first urinary EVs biomarker recognized. It is associated with exosomes in urine of patients with prostate cancer [142]. Furthermore, exosomes in cerebrospinal fluid of patients with Alzheimer's disease contain phosphorylated Thr181, which is an established biomarker of this disease [143]. Significant increase in the levels of microRNAs has also been observed in extracellular vesicles from patients with ovarian cancer, compared to the non-malignant samples is another evidence of the potential diagnostic role of EVs [141].

Most of studies are focusing on selection of one or a few EVs miRNAs as disease biomarkers, whereas, every single miRNA has numerous potential targets and also many miRNAs can target the same protein. Moreover, miRNAs usually target proteins from one or more related pathways. All these factors need to be considered to unveil the role of miRNA as diagnostic biomarkers to facilitate and fasten shifting of EVs from bench to bed side.

6. Therapeutic Potentials of EVs

Exosomes have a specific cell tropism, according to their characteristics, which can be used to target them to specific tissues and/or organs through a mechanism similar to a cellular signaling based response [144]. Moreover, exosomes nanoparticles can be engineered and/or loaded with several molecules (drugs, small molecules and nanoparticles) and targeted to specific organs, and therefore can be used for the delivery of therapeutic agents [145]. Transfection of an expression vector and loading of modified vesicles with BACE1 siRNA has been efficiently investigated, to retard the production of unfolded amyloid proteins, in animal and preclinical trials to deliver siRNA into the mouse brain of Alzheimer's disease with promising results [146]. Ohno *et al.* showed that systemically injected exosomes targeted to EGFR-expressing breast cancer cells may deliver Let-7a antitumor miRNA (antagomirs), and thus represent a vehicle for conveying drugs to tumors [147]. It has recently been demonstrated that extracellular vesicles or microvesicles (MVs) released from different mesenchymal stem cells are an integral component of cell-to-cell communication network involved in tissue regeneration [148, 149], and therefore may contribute to the paracrine action of MSCs. Because of their potential therapeutic application in acute tissue injury of different organs (heart, kidney, lung and liver), cell-free MSCs extracellular vesicles are currently used in clinical trials of a wide range of diseases [150].

7. Conclusion

During the past decade, the interest of physicians and molecular biologist in EVs has expanded logarithmically. EVs are abundantly released by most cells and present in body fluids, carry RNAs, and show regulatory functions that represent a perfect tool for early diagnosis and therapeutic vehicle for a wide range of diseases. Although the clinical application of EVs remains years away, the significance of their diagnostic and therapeutic potential is not an issue for debate. Deciphering the molecular mechanisms of EVs biogenesis and function as well as more accurate and standardized purification methods is required for the implementation of EVs in clinical practice. To help coordinate these enormous challenges, the International Society for Extracellular Vesicles (ISEV) was launched in 2011.

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Conflict

We, the authors of this review, declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research. We fully declare that no financial or other potential conflict of interest.

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