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Chapter

Extracellular Vesicles in Cancer

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Abstract

Extracellular vesicles (EVs) represent a generic term for all the secreted vesicles, which include exosomes, microvesicles, and apoptotic bodies. EVs are key partners in the intercellular communication and play an essential role in multiple physiological and pathological conditions. EVs are shuttles for cargo molecules, such as RNA (mRNA, microRNA, and other noncoding RNAs), DNA, proteins (receptors, transcription factors, enzymes, and extracellular matrix proteins), and lipids. In pathological states, including cancer, EVs might represent either useful biomarkers or can be used for therapeutic purposes. Moreover, in cancer, it was demonstrated that EVs play an essential role in drug resistance. Here, we review the role played by EVs in the most common forms of cancer, with a special focus on ovarian and breast cancers.

Keywords: extracellular vesicles, cancer, biomarker, cargo, therapy

1. Introduction

Extracellular vesicles (EVs) are cell-derived membranous vesicles (from normal or cancerous cells) bearing packages of information within or on their surface. Their content can influence neighboring or remote cells, and therefore, EVs are considered to play an important role in intercellular communication [1]. Different functional molecules (proteins, mRNA, and microRNAs) are transferred between cells with the aid of EVs. The content of EVs is highly variable and dependent of the cell of origin. The EVs in human blood originate from platelets, leukocytes, erythrocytes, endothelial cells, vascular smooth muscle cells, and cancer cells (for review see [2]). It is now widely accepted that extracellular vesicles also represent a potential resource for biomarkers.

The first study suggesting the existence of extracellular vesicles was carried out in 1946 [3]. In a 1967 report, membrane particles derived from activated platelets, termed "platelet dust," were commonly considered as a waste product or cellular debris directly budded from the plasma membrane [4]. Both prokaryotes and higher eukaryotes can release EVs. Different terms are used to describe EVs due to varying methods of isolation and due to the biogenesis mechanism. The terminologies of EVs include microvesicles, dexosomes, texosomes, archaeosomes, argosomes, prostasomes, epididymosomes, and oncosomes [5]. Gradually, while building up knowledge about EVs, a need for its classification emerged and the International Society for Extracellular Vesicles (ISEV) was founded [6]. This society

provided some criteria to classify EVs into three groups: microvesicles (MVs), exosomes, and apoptotic bodies (for details visit www.isev.org). These vesicles are secreted by both normal cells and cancerous cells as means of cell-to-cell communication. Alternatively, they may be prepared artificially from the engineered artificial lipid vesicles called liposomes in which EVs' features, components, or cargos are incorporated and are the most likely to be useful for drug delivery [7].

EVs are actively involved in cell-to-cell communication, inflammation, chronic disease development and progression, pre-metastatic niche formation, and the metastatic organotropism of different tumor types [8]. Tumor-derived EVs (TEVs) have been reported to play major roles in the onset, progression, and metastasis of cancer, including ovarian [9], breast [10], colorectal [11, 12], prostate cancer [13], and melanoma [14–16].

Here, we review knowledge about EVs in cancer, with a focus on breast and ovarian cancers. We discuss the importance of the content of EVs (e.g., nucleic acids, and proteins) in cancer development, metastasis, and drug resistance.

2. The variety of extracellular vesicles

Replacing EVs includes a heterogeneous population of membrane vesicles categorized depending on the mechanism by which they are released from cells. According to their size and mechanisms of biogenesis, EVs can be categorized into three classes: (a) exosomes, (b) ectosomes or shedding microvesicles, and (c) apoptotic bodies [17, 18]. Differentiation criteria are based on their size, content, and by a certain combination of markers (**Figure 1** and **Table 1**). Cancerous cells have been described to release exosomes and ectosomes and some other additional subpopulations of EVs [19].

2.1 Exosomes

Exosomes are EVs with multivesicular endosomal origin released by all cell types [33]. Exosomes are found in physiological fluids such as blood and plasma [34, 35], urine [36], cerebral fluid [37], saliva [38, 39], seminal fluid [40], breast milk [41, 42], and amniotic fluid [43, 44]. The presence of EVs has been reported in interstitial spaces since they are released by B cells [45], T cells [46], dendritic cells [47], platelets [48], Schwann cells [49], tumor cells [50], cardiomyocytes [51], endothelial cells [52], stem cells [50], and telocytes [53–55]. Exosomes are able to influence cells from the local environment and also distant target cells, thus regulating intercellular signaling [56]. Their size varies between 30 and 100 nm, and as membrane vesicles, they are delineated by a specific lipid bilayer similar to that of the cells they originate from [57]. Studies have shown that while normal human blood contains about 2000 trillion exosomes, the blood of cancer patients contains a double amount, about 4000 trillion exosomes [57]. In noncancerous cells, exosome secretion was suggested to play a role in cellular homeostasis by removing harmful cytoplasmic DNA of normal cells and in preventing viral hijacking of host cells by excreting viral DNA from cells as shown by Takahashi et al. [58].

The plasma of cancer patients contains different types of exosomes, some released by normal cells and others released by cancerous cells, explaining the heterogeneity in size (30–150 nm) of the exosomal population [59]. Exosomes can be isolated from cancer patients' plasma with a variety of methods [60]. They are based not only on classical techniques such as ultracentrifugation, but also on some modern ones such as size exclusion chromatography [61].

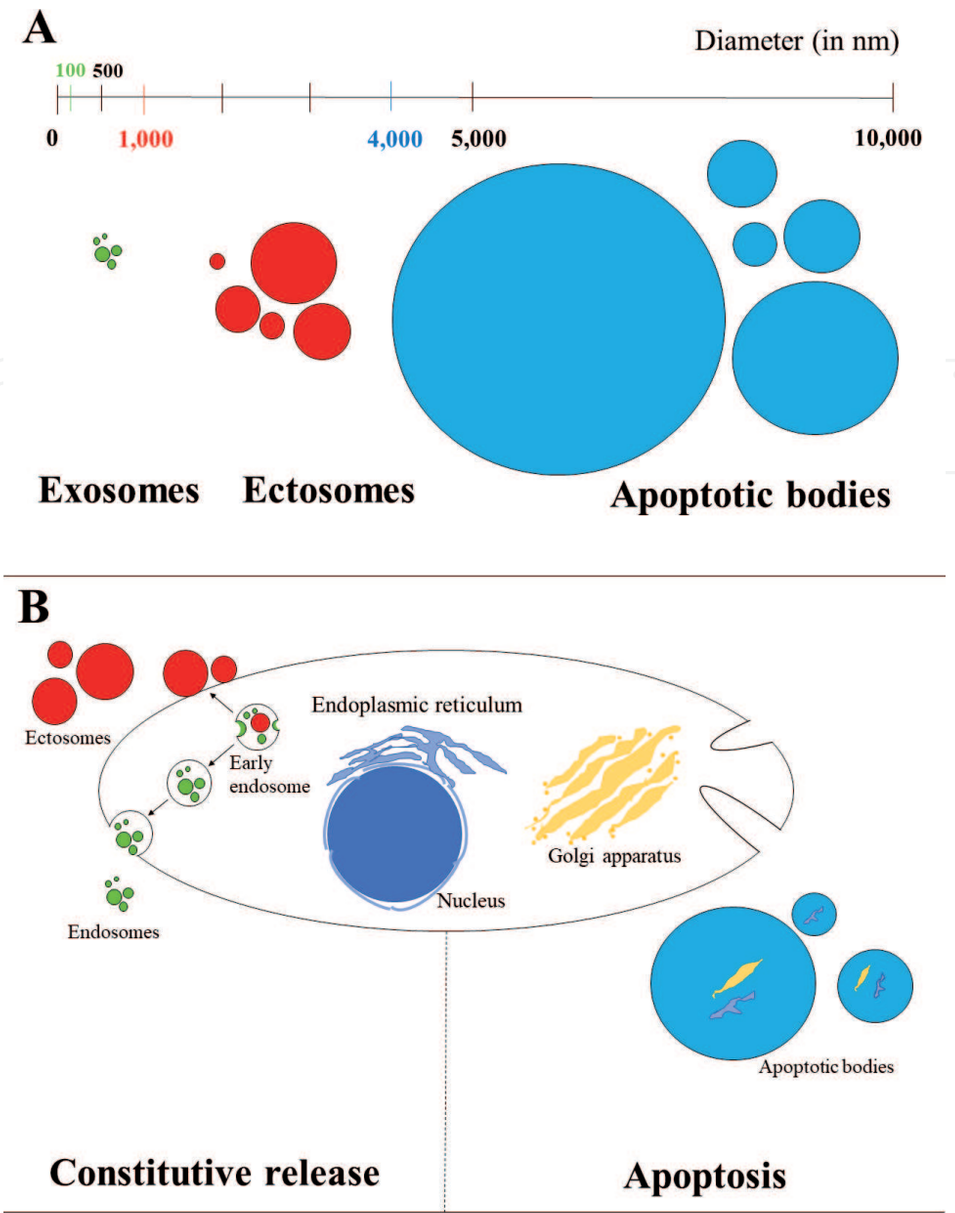

Figure 1.
Classification of EVs based on their diameter (expressed in nm) (A) or on their mechanism of biogenesis (B).

Tumor cell-derived exosomes are able to promote inflammation and are able to compromise innate immunity by delivering different signals, which affect the proliferation, apoptosis, cytokine production, and reprogramming of T cells [62].

2.2 Ectosomes

Ectosomes are a heterogeneous vesicle population, ranging in diameter between 100 and 1000 nm. Discovered in approximately the same time as exosomes, in 1990s, ectosomes did not attract the same interest as the study of exosomes. While the interest in exosomes reached the maximum between 2008 and 2010, the ectosomes had its peak in 2012 [20, 63].

Ectosomes are known under different names, which might be misleading (**Table 1**), while ecto is a prefix that means outwardly, externally, and is therefore suggestive of their way of forming. The mechanism of formation of ectosomes differs greatly from that of exosomes, as well as their cargo molecules. Ectosome formation does not require exocytosis. Ectosomes are formed by direct outward

	Exosomes	Ectosomes	Apoptotic bodies
Size	30–100 nm	100–1000 nm	500–4000 nm
Sedimentation rate	100,000–120,000×g	16,000–20,000×g	5000–16,000×g
Biogenesis	Endosomal pathway, accumulated within the multivesicular bodies, exocytosis [20]	Generated directly from the plasma membrane by shedding [21]	Cell fragmentation Blebbing or zeiosis—bulge of membrane by increasing the surface area through tearing [22]
Types of generation	Constitutive	Regulated	Regulated
Filtration	20–200 nm	>200 nm	>1000 nm
Intracellular storage	Yes	No	No
Marker proteins	CD 9, CD63 and CD61, tetraspanins, HSP70, HSP90, Alix, Rab5a/b [23–25]	TyA and C1a, ARF6 and VCAMP3, β1 integrins, selectins, CD40, MMP, lineage markers, and ezrin [26–28]	Calreticulin, TSP and C3b, and histones [29, 30].
Content	Proteins, cholesterol, ceramide, noncoding RNA, mRNA, miRNA, and cytosol [31]	Proteins, phosphatidylserine, cholesterol, mRNA, miRNA, and cytosol [31]	Proteins, phosphatidylserine, DNA, rRNA, and cytosol [18]
Organelles	No	No	Yes
Alternative names	Prostasomes, tolerosomes, dexosomes, nanovesicles, exosome-like vesicles, and others [18, 32]	Nanoparticles, microparticles, microvesicles, shedding vesicles, shedding bodies, exovesicles, secretory vesicles, and oncosomes [18]	Apoptotic blebs [18]
Impact on the immune system	Immunostimulators	Immunosuppressors	Immunosuppressors

Table 1.
Classification of EVs based on size and their biogenesis.

budding of the plasma membrane in specialized microdomains of the plasmalemma, the phenomenon known as microvesicle shedding [29]. They are released both by cells in normal resting state and by cells upon stimulation. Ectosome fusion with the plasma membrane of a recipient cell is followed by changes in antigens, enzymes, and other proteins in a specific site of plasmalemma, while their content release into the cytoplasm can alter the recipients' cell gene expression [64, 65]. Tumor-derived ectosomes were shown to have immunosuppressive properties by inducing the chemotaxis of granulocytes, lymphocytes, and monocytes due to several chemokines (e.g., particularly IL-8) transported in ectosomes [66].

Oncosomes are a particular type of ectosomes, excessively large, which can even reach 1000 nm, characteristic to advanced cancers. There is a confusion in the use of these terms in the literature and that is why we thought to treat oncosomes as a particular category of ectosomes. The generic name of ectosomes can include oncosomes, while the name of oncosomes excludes ectosomes released from normal cells. Oncosomes content is adapted to serve cancer metabolism, so they will contain enzymes involved in glucose, glutamine, and amino acid metabolism. Furthermore, oncosomes are enriched in proteins, which have a

role in cell migration, angiogenesis, and cancer progression and metastasis [28]. Oncosomes allow intercellular transfer of oncogenes, hence the motivation to be considered as existing biomarkers in the blood or plasma of patients for the detection of cancer [67].

2.3 Apoptotic bodies

Apoptotic bodies (ApoBDs) are the largest type of extracellular vesicles (typically 1–5 μm in diameter) visible during an apoptotic process. Kerr in 1972 proposed the term "apoptotic body" [68]. ApoBDs are released as blebs of cells undergoing apoptosis and consist of cytoplasm, organelles with or without a nuclear fragment. It has also been shown that ApoBDs can harbor proteins, lipids, DNA, rRNA, organelles, and cytosol [18]; this is the reason why the disassembly of an apoptotic cell into ApoBDs can mediate intercellular communication and may contribute to the development of various disease states [69]. These bodies are then phagocytosed by macrophages or neoplastic cells and degraded within phagolysosomes. Their formation has been proposed to play an important role in the clearance of apoptotic cells by phagocytes. Different cell types can generate ApoBDs via different mechanisms [70]. These ApoBDs can be classified based on cell-type-specific surface markers and content. Jiang et al. showed that ApoBDs share the same surface markers as their cell of origin; this is the reason why apoptotic bodies are very different and can be divided into specific subclasses [70].

ApoBD occurs spontaneously in untreated malignant neoplasms, and is implicated in both physiological involution and atrophy of various tissues and organs. Pathological settings include inflammation [71], autoimmunity [72–74], viral infection [75], and tumorigenesis because they participate in the horizontal transfer of oncogenes due to their nuclear material content from the dying cells [76].

3. Extracellular vesicles and tumor microenvironment

Tumor masses are composed of cancer cells and stromal cells, in which one include mesenchymal cells, fibroblasts and immune cells, and extracellular matrix (ECM) components. All these cells emit EVs and participate in the creation of a unique tumor nanoenvironment. EVs are capable of horizontal transfer of bioactive content to interact with cells in the tumor microenvironment. These interactions can include fusion of the EV with the plasmalemma of the recipient cell or endocytosis of the EVs [77]. EVs represent the bidirectional way of interaction between stromal and cancer cells as a mean to exchange information and modify the tumor microenvironment. Therefore, the content of these vesicles is of great significance in the evolution of the cancer, since it was shown to modulate the complex signaling networks that facilitate tumor progression [78].

3.1 Nucleic acids

Circulating DNA can be found in free form or contained in EVs and is thought to be the future in cancer diagnosis and treatment monitoring. This will be possible because the DNA fragments contained in EVs are relatively intact (average 15 kbp) by comparison with the circulating cell-free one (average 130 bp) due to the protection offered by the lipid bilayer [79]. Vagner et al. showed in a very recent study that the majority of the extracellular DNA is contained in large oncosomes, rather than in exosomes, both in vitro and in the patients' plasma and has all cancer-specific genomic alterations [80]. The majority of the DNA contained

in tumor-derived exosomes is double stranded and represent the whole genomic DNA, suggesting its usefulness in identifying mutations present in parental tumor cells, as it was indicated by Thakur et al. [81]. Wyatt et al. showed that cancer-derived DNA is sufficient to identify the DNA alterations from metastatic tissue and is very important because it integrates somatic information from more than one metastatic lesion [82].

The presence of retrotransposons, cDNAs, and ncRNAs has also been reported in EVs and appears to be a unique feature of tumor cells [83]. Several studies reported a correlation between increased retrotransposon activity and tumorigenesis [84]. For example, LINE-1 hypomethylation in various human cancers was intensively studied since it is considered to be an early event in tumorigenesis and to be linked with the induction of proto-oncogenes [85]. Loss of LINE-1 methylation was found to associate with more aggressive progression of colorectal cancer [86]. Moreover, LINE-1 hypomethylation level can be considered as an important epigenetic process, which became a potential prognostic factor for ovarian multistep carcinogenesis [87].

miRNAs represent potential candidates responsible for influencing the tumor microenvironment; however, little is known about the mechanism by which they produce changes in the transcriptome of target cells [88].

3.2 Proteins

Proteins exported in EVs are signaling molecules that interfere in a whole series of processes such as cell metabolism, cell invasion and growth, angiogenesis, and mRNA processing [89]. Among these, it is worth mentioning that the epidermal growth factor receptor vIII (EGFRvIII), mutant Ras family members, or c-Met have been proposed as cancer biomarkers [90, 91]. Other proangiogenic regulators, such as VEGF and bFGF, are harbored in EVs shed from cancerous cells promoting new blood vessel formation [92].

Moreover, EVs also transfer proteases such as MMP-2, MMP-9, and MT1-MMP and become responsible for the partial degradation of the extracellular matrix [93]. The content is released in an acidic environment after the vesicle is stabilized in the extracellular matrix with the aid of β1 integrin adhesion molecules [94].

3.3 Lipids

Naturally, EVs also contain a lipid component, which consists of the main membrane lipids: sphingomyelin, phosphatidylserine, and glycosphingolipids and cholesterol, but they also carry polyunsaturated fatty acids PUFAs, mainly arachidonic acid and linoleic acid [95, 96]. Sphingomyelin, as a component of the EVS, was firstly reported by Kim et al. who described its angiogenic properties [97] . In addition, it has been shown that the lipid content of exosomes suppresses critical cancer survival pathways such as notch leading to cancer cell death of human pancreatic tumoral SOJ-6 cells [98]. Moreover, other important signaling mediators, such as prostaglandins, arachidonic acid, phospholipase A2, and phospholipase C and D, are also found in EVs [99]. The prostaglandins found in breast-cancer-derived exosomes, such as PGE2, are responsible for promoting tumor growth by inducing the release of pro-inflammatory cytokines such as IL-6 and VEGF, which induce the accumulation of myeloid-derived suppressor cells capable to differentiate into macrophages in the tumor microenvironment [100, 101]. PGE2 indeed makes the connection between cancer and macrophages and can promote tumorigenesis by enhancing the expression of programmed cell death protein ligand 1 (PD-L1) responsible for tumor escape from immune system during cancer progression [102].

Cancer stem cells (CSCs) are held directly responsible to promote cancer initiation and progression. Also, there are several studies showing their importance in therapy resistance, recurrence, and metastasis [103]. CSCs themselves do not exist as a static population, their stemness being supported by the mesenchymal stem cells, endothelial cells, fibroblasts, or immune cells by paracrine signaling [104]. For example, in breast cancer, the overexpression of the chemokines CXCL14 and CXCL12 in myoepithelial cells and myofibroblasts favors the metastasis [105]. Cancer-associated fibroblasts (CAF) release exosomes, which induce the stemness of breast cancer cell lines, developing an aggressive cancer cell phenotype [106]. Also, the ECM molecules are relevant for breast cancer colonization, which contribute to the control of CSC. In this sense, tenascin C, a protein in the ECM, contributes in the formation of the stem niche by protecting CSC from immune surveillance [107]. In breast cancer, high levels of tenascin C are associated with poor clinical outcome in breast cancer due to lung cancer metastasis [108].

4. Role of extracellular vesicles in tumorigenesis

The role of EVs in tumorigenesis was described in various types of cancer, including ovarian [109–111] and breast cancers [112, 113]. EVs undergo several alterations in tumorigenesis, including changes in their biogenesis, release rate and/or protein content, incorporation of oncogenic and mutant macromolecules, mediated release of genomic DNA, and uptake of tumoral cells [114]. The transfer of DNA between apoptotic tumoral cells and other cells is important in tumorigenesis. In vitro, it was shown that apoptotic bodies derived from cancer cells are responsible for triggering the expression of oncogenes in fibroblasts due to the information contained in tumor-derived EVs [76].

In ovarian or breast cancer, investigating the content of EVs might give important informations on tumorigenesis. To detail, exosomes released by IGROV1 ovarian cancer cells (with high content of RNA-binding proteins, such as LIN28A or LIN28B), but not by OV420 ovarian cancer cells, were taken up by HEK293 cells, contributing to the tumor development [109]. Moreover, in hypoxia conditions, SKOV3 human epithelial ovarian cancer cells release exosomes with high content of miR-940 that stimulate the M2 macrophage phenotype, and in turn, M2 subtype macrophages stimulate the tumor cell migration and proliferation [111]. The majority of circulating miRNA, packed in EVs, can be used as biomarkers in ovarian cancer, but their use is not only limited for diagnosing the existence of the cancer, but also being reliable markers for monitoring the tumor histology, stage, or the patient outcome [110].

The content of EVs released from two human breast cancer cell lines, MCF-7 (less invasive) and MDA-MB-231 (more invasive), was analyzed, and approximately, 270 proteins were identified [113]. In circulating EVs, epidermal growth factor-like repeats and discoidin I-like domains 3 (EDIL3) are the extracellular matrix (ECM) protein that was described to play a critical role in tumorigenesis by the activation of integrin-focal adhesion kinase (FAK) signaling cascade [113]. Breast-cancer-derived EVs (e.g., exosomes) present a cell-independent microRNA biogenesis from pre-miRNAs (like Dicer, AGO2, or TRBP) to mature miRNAs [112]. In particular, exosomes detected in the cells and sera of patients affected by breast cancer were shown to stimulate tumorigenesis in nontumoral epithelial cells by a Dicer-dependent mechanism [112]. It was also demonstrated that in the breast tumor microenvironment, there is a downregulation of the tumor suppressor p85α, which is clinically relevant in tumorigenesis, and the mechanism involves the loss of p85α expression in stromal fibroblasts promoting breast cancer progression by the epithelial-to-mesenchymal transition [111].

5. Extracellular vesicles and metastatic niches

Tumor microenvironment was described to undergo series of molecular and cellular changes to form the metastatic-designated sites, called pre-metastatic niche [115, 116]. The formation of pre-metastatic niche requires the cross talk between primary tumor-derived components, and the microenvironment of the host stromal components and of the tumor-mobilized bone-marrow-derived cells [117].

Interestingly, the role of EVs in metastatic niches can be exploited in novel therapeutic approaches. Indeed, technologies based on exosomes, separated from the ascitic fluid of ovarian cancer patients, embedded in a 3D scaffold metastatic trap, were successfully tested in murine models of ovarian metastasis in order to improve survival [118]. Numerous studies indicated that tumor-derived exosomes might play a role in promoting angiogenesis and modulation of the immune system [119, 120]. Moreover, exosomes derived from cancerous tumor are capable of remodeling the surrounding parenchyma, thus supporting tumor progression and the generation of the pre-metastatic niche [121, 122].

6. Role of extracellular vesicles in metastasis

EVs have been described to play an essential role in the local and distant communication between cancer cells and their environment and in contributing to the progression of metastasis [123]. Although the function of EVs in metastasis is not completely understood, studies show that miRNAs isolated from EVs are actively involved in complex metastatic processes, including local invasion, angiogenesis, immune modulation, metastatic niche preparation, colonization, and dormancy [123].

EVs play an essential role in the tumor metastasis by ensuring the cross talk between tumor and the adipose tissue, and obesity was described to influence the metastatic behavior of tumors, especially in melanoma, breast, and ovarian cancers [124].

In breast cancer, metastatic exosomes creating a facilitating local environment for metastasis was demonstrated, and annexin II contained in these exosomes contributes to this process by promoting angiogenesis [125].

7. Tumor-derived extracellular vesicles in ovarian cancer

Nawaz et al. have recently done an extensive review on the role of EVs in ovarian cancer and concluded that the gaining of new insights into these mechanisms would contribute to the identification of new biomarkers among the ovarian-cancer-derived EVs and to the development of efficient EVs-based immunotherapies [126]. Proteomic analysis of exosomes derived from two human ovarian cancer cell lines (i.e., OVCAR-3 and IGROV1) indicated the presence of 2230 proteins, 1017 proteins being common for both cell lines, 380 proteins being newly reported compared to the ExoCarta database, and some of them being associated with tumorigenesis and metastasis and might represent promising biomarkers [127].

Additionally, matrix metalloproteinase-1 (MMP1) might be a very good biomarker for the ovarian cancer due to its overexpression in ascites-derived EVs in correlation with the degree of malignancy and the low prognosis for the ovarian cancer patients [128]. Moreover, the peritoneal dissemination of ovarian cancer is facilitated by malignant EVs containing MMP1 derived from the ascites of patients, and EVs were demonstrated to induce apoptosis in mesothelial cells [128].

The mechanisms of drug resistance development also involve the release of EVs from ovarian cancer cells upon exposure to drug (i.e., cisplatin) and induce invasiveness [129].

8. Breast-cancer-derived extracellular vesicles

In breast cancer, EVs can play two essential roles "diagnosis biomarkers" or "therapeutic targets." Thus, breast cancer induces the release of exosomes from salivary glands, being potential markers for early diagnosis [130]. Interestingly, EVs serve as a cargo not only for nucleic acids and proteins, but also for anticancer drugs. Considering the critical contribution of EVs in facilitating tumorigenesis, metastasis, and drug resistance [130], they could be considered as potential therapeutic targets in breast cancer.

Moreover, the analysis of EVs can help to distinguish the "degree of aggressiveness" in breast cancer. To detail, EVs derived from the aggressive human breast cancer MDA-MB-231 cell line, but not from the less invasive human breast cancer MCF-7 cell line, were demonstrated to induce platelet activation and aggregation by tissue factor-independent and tissue factor-dependent procoagulant activities [131]. EVs have been demonstrated to be involved in the cross talk between neighboring cancer cells and to transmit phenotypic aggressive traits from one cell to another. To date, EVs released by Hs578Ts(i)8 triple-negative breast cancer cells were able to increase the invasion, proliferation, and migration characteristics of Hs578T cells [132].

9. Extracellular vesicles as biomarkers—new diagnostic tools

In different body fluids, especially plasma and serum, EVs biomarkers have been detected with great clinical value in various types of cancer, **Table 2**.

The protein content of the EVs can be potentially used in the early detection of cancer as suggested in a pilot study by Smalley et al. [151]. The plasma levels of exosomal proteins represents an important biomarker that discriminates between ovarian cancer patients and normal ones, and their values correlate with the stage of the disease [119]. Among exosomal proteins, TGF-β1 and MAGE3/6 can be used as reliable biomarkers to discriminate between benign and malignant ovarian tumors, or to ascertain the efficacy of chemotherapy [119]. Although epithelial cell adhesion molecule (EpCAM) was demonstrated to promote epithelial-mesenchymal transition in advanced stages of endometrial cancer [152], studies indicated that EpCAM is not a robust biomarker to classify exosomes derived from benign and malignant ovarian tumors [134] or to detect early stages of the pathology [153]. Besides EpCAM, several exosomal proteins were identified to be overexpressed in ovarian cancer, including proliferation cell nuclear antigen (PCNA), tubulin beta-3 chain (TUBB3), epidermal growth factor receptor (EGFR), apolipoprotein E (APOE), claudin 3 (CLDN3), fatty acid synthase (FASN), ERBB2, and L1CAM (CD171) [127]. Additionally, claudin-4, but not claudin-3, is a valuable biomarker in the peripheral blood of ovarian cancer patients with almost 98% specificity [133]. Exosomal proteins can also represent important biomarkers for the evaluation of efficacy of therapies. Thus, annexin A3 can be employed for early detection of the resistance to platinum-based therapy in ovarian cancer patients [135, 136].

In breast cancer, several studies identified various exosomal miRNAs as potential biomarkers correlated with tumor malignancy degree and prognosis. Indeed, exosomal miR-21 and miR-1246 had higher levels in plasma of breast cancer

Biomarkers of EVs	Sample	Types of cancer	Reference
TGF-beta1, MAGE3/6, and Claudin-4	Plasma	Ovarian cancer	[119, 133]
EpCAM and annexin A3	Serum		[134–136]
Alpha-1-antitrypsin and haptoglobin precursors	Serum	Breast cancer	[137]
miR-21, miR-939, miR-373, and miR-1246	Plasma		[58, 138, 139]
miR-1290 and miR-375	Plasma	Prostate cancer	[140–144]
Survivin, CD9+, CD63+, and alpha-1-antitrypsin			
IL-8 and TGF-beta mRNAs	Plasma	Glioma	[60]
miR-21	CSF		[145]
miR-1246, miR-4644, miR-3976, and miR-4306	Serum	Pancreatic cancer	[146]
CD44v6, Tspan8, EpCAM, and CD104			
Alpha-1-antitrypsin, and histone H2B1K	Urine	Urothelial carcinoma	[147]
long coding RNA CRNDE-h	Serum	Lymph node and distant metastasis of colorectal cancer	[148]
miR-21	Plasma	Esophageal cancer	[149]
miR-19a l	Serum	Colorectal cancer	[150]

Table 2.
Biomarkers contained in EVs relevant in different types of cancer.

patients compared to control patients [138]. Additionally, high levels of exosomal miR-939 were associated with low outcome in patients with triple-negative breast cancer [139], and high levels of exosomal miR-373 were identified in triple-negative, estrogen–receptor- and progesterone-receptor-negative, breast cancer patients [58]. Moreover, an extensive proteomics analysis identified alpha1-antitrypsin and haptoglobin precursors as novel biomarkers in the serum of patients with infiltrating ductal breast carcinomas [137].

The release of EVs has a calcium-dependent mechanism, and alterations in calcium signaling have been described in tumorigenesis, metastasis, or drug resistance in various types of cancer, including breast and ovarian cancers [154, 155]. Therefore, more attention should be paid to the calcium-dependent signaling cascades in different cancer stages in direct relationship with the cell-to-cell communication mechanisms of EVs in order to identify novel specific and reliable biomarkers.

10. Therapeutic roles of extracellular vesicles in cancer

EVs have a big potential for cancer therapy monitoring (**Table 3**). These are described as secreted lipid bilayer-enclosed lumens and are claimed to be valuable reservoirs of liquid biopsy biomarker [156]. EVs (mainly EVs-associated proteins and microRNAs) are proved to be the biomarkers in breast cancer diagnosis [157, 158].

Source of EVs	Therapeutic effect	Reference
Tumor peptide-loaded dendritic cells-derived exosomes	Immunotherapy—because they suppress tumor growth	[159]
EVs from the rat pancreatic adenocarcinoma cell line BSp73ASML	Adjuvant therapy in immunotherapy	[160]
Tumor antigen containing EVs	Activates an antitumor response against OVA-transfected BL6–10 melanoma cells	[160]
EV vaccine derived from colorectal cancer (NB4 cell—a human acute promyelocytic leukemia cell line)	Activates CTLs through self-derived dendritic cell activation	[161]
EVs from self-derived dendritic cells	Immunotherapy in cases of unresectable nonsmall-cell lung cancer	[162]
EVs from autologous self-derived dendritic cells	Metastatic melanoma patients	[163]
EVs from ascites in combination with granulocyte macrophage colony-stimulating factor	Immunotherapy in colorectal cancer	[164]
miR-9 in mesenchymal stem cell-derived EVs	Chemosensitive in glioblastoma multiforme cells	[165]
iRGD-Exos-doxorubicin	Suppressed breast tumor growth in an MDA-MB-231 tumor-bearing nude mouse model	[166]
Curcumin-primed EVs from a mouse brain endothelial cell line	Treating endothelial cell dysfunction during hyperhomocysteinemia in vitro	[167]

Table 3.
EVs and their role in therapeutic approaches in cancer.

11. Integrative overview

EVs play an essential role in cellular communication both in physiological and pathological conditions. In pathological conditions, EVs have been implicated in cancer, spreading of viruses or other pathogens, altered immune response, development of neurodegenerative diseases, etc. In cancer, EVs ensure the cross talk between tumoral cells or between tumoral cells and nontumoral cells, and enable the development of multiple processes, including tumorigenesis, pre-metastatic niche formation, metastasis, and drug resistance. In ovarian and breast cancers, the involvement of EVs in all these processes of tumor evolution has been described and the analysis of EVs content is particularly useful for identifying biomarkers of the disease per se and, moreover, for the stage of the pathology evolution. However, there are still technical limitations for separation and/or analysis of EVs, and in clinical practice, the standardization of EVs-based reproductible protocols is required urgently. Considering the presence of EVs in such a variety of body fluids and tissues, an important conclusion is to consider EVs both as biomarkers and potential therapeutic targets (especially for immunotherapies) and to exploit them in the next future to improve the outcome of cancer patients.

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