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].

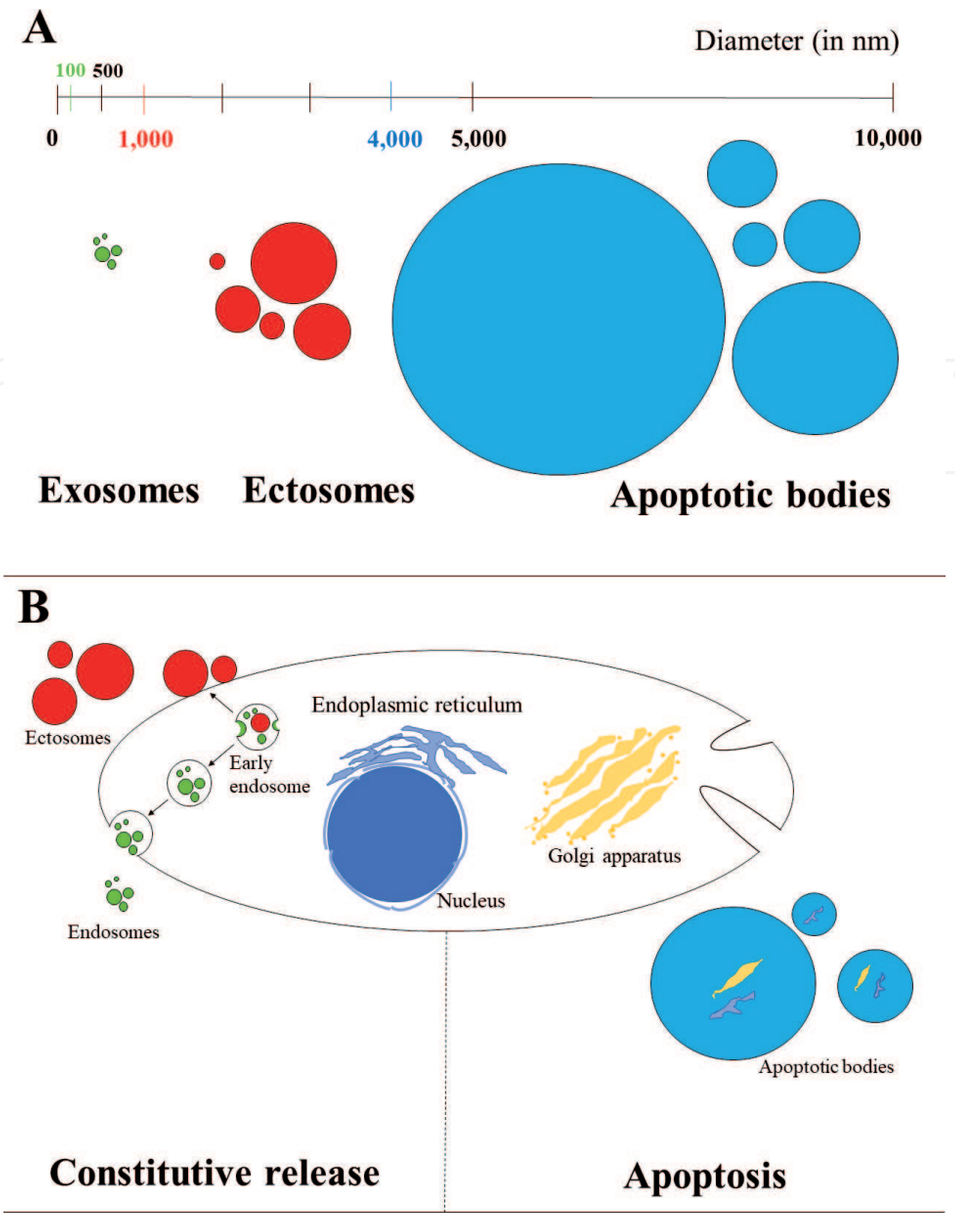


**Figure 1.**
*Classification of EVs based on their diameter (expressed in nm) (A) or on their mechanism of biogenesis (B).*

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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## References

[1] Camussi G et al. Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney International. 2010;**78**(9):838-848

[2] Nomura S. Extracellular vesicles and blood diseases. International Journal of Hematology. 2017;**105**(4):392-405

[3] Chargaff E, West R. The biological significance of the thromboplastic protein of blood. The Journal of Biological Chemistry. 1946;**166**(1):189-197

[4] Wolf P. The nature and significance of platelet products in human plasma. British Journal of Haematology. 1967;**13**(3):269-288

[5] Al-Nedawi K. The yin-yang of microvesicles (exosomes) in cancer biology. Frontiers in Oncology. 2014;**4**:172

[6] Gould SJ, Raposo G. As we wait: Coping with an imperfect nomenclature for extracellular vesicles. Journal of Extracellular Vesicles. 2013;**2**:20389

[7] Jo W et al. Microfluidic fabrication of cell-derived nanovesicles as endogenous RNA carriers. Lab on a Chip. 2014;**14**(7):1261-1269

[8] Wendler F, Stamp GW, Giamas G. Tumor-stromal cell communication: Small vesicles signal big changes. Trends Cancer. 2016;**2**(7):326-329

[9] Cheng L et al. A comprehensive overview of exosomes in ovarian cancer: Emerging biomarkers and therapeutic strategies. Journal of Ovarian Research. 2017;**10**(1):73

[10] Lowry MC, Gallagher WM, O'Driscoll L. The role of exosomes in breast cancer. Clinical Chemistry. 2015;**61**(12):1457-1465

[11] Zhou J et al. Tumor-derived exosomes in colorectal cancer progression and their clinical applications. Oncotarget. 2017;**8**(59):100781-100790

[12] Ruiz-Lopez L et al. The role of exosomes on colorectal cancer: A review. Journal of Gastroenterology and Hepatology. 2018;**33**(4):792-799

[13] Pan J et al. Exosomes in diagnosis and therapy of prostate cancer. Oncotarget. 2017;**8**(57):97693-97700

[14] Wozniak M et al. Analysis of the miRNA profiles of melanoma exosomes derived under normoxic and hypoxic culture conditions. Anticancer Research. 2017;**37**(12):6779-6789

[15] Wu Y et al. Melanoma exosomes deliver a complex biological payload that upregulates PTPN11 to suppress T lymphocyte function. Pigment Cell & Melanoma Research. 2017;**30**(2):203-218

[16] Tucci M et al. Exosomes in melanoma: A role in tumor progression, metastasis and impaired immune system activity. Oncotarget. 2018;**9**(29):20826-20837

[17] Cretoiu D et al. Telocytes and their extracellular vesicles-evidence and hypotheses. International Journal of Molecular Sciences. 2016;**17**(8):1322

[18] van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nature Reviews Molecular Cell Biology. 2018;**19**(4):213-228

[19] Lunavat TR et al. Small RNA deep sequencing discriminates subsets of extracellular vesicles released by melanoma cells—Evidence of unique microRNA cargos. RNA Biology. 2015;**12**(8):810-823

[20] Cocucci E, Meldolesi J. Ectosomes and exosomes: Shedding the confusion between extracellular vesicles. Trends in Cell Biology. 2015;**25**(6):364-372

[21] Stein JM, Luzio JP. Ectocytosis caused by sublytic autologous complement attack on human neutrophils. The sorting of endogenous plasma-membrane proteins and lipids into shed vesicles. The Biochemical Journal. 1991;**274**(Pt 2):381-386

[22] Bovellan M et al. Death-associated protein kinase (DAPK) and signal transduction: Blebbing in programmed cell death. The FEBS Journal. 2010;**277**(1):58-65

[23] Escola JM et al. Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes. The Journal of Biological Chemistry. 1998;**273**(32):20121-20127

[24] Lotvall J et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: A position statement from the international society for extracellular vesicles. Journal of Extracellular Vesicles. 2014;**3**:26913

[25] Surman M et al. Deciphering the role of ectosomes in cancer development and progression: Focus on the proteome. Clinical & Experimental Metastasis. 2017;**34**(3-4):273-289

[26] Muralidharan-Chari V et al. ARF6-regulated shedding of tumor cell-derived plasma membrane microvesicles. Current Biology. 2009;**19**(22):1875-1885

[27] Pokharel D et al. Proteome analysis of multidrug-resistant, breast cancer-derived microparticles. Journal of Extracellular Vesicles. 2014;**3**:24384

[28] Minciacchi VR et al. Large oncosomes contain distinct protein cargo and represent a separate functional class of tumor-derived extracellular vesicles. Oncotarget. 2015;**6**(13):11327-11341

[29] Mause SF, Weber C. Microparticles: Protagonists of a novel communication network for intercellular information exchange. Circulation Research. 2010;**107**(9):1047-1057

[30] Gong J et al. Microparticles in cancer: A review of recent developments and the potential for clinical application. Seminars in Cell & Developmental Biology. 2015;**40**:35-40

[31] Crescitelli R et al. Distinct RNA profiles in subpopulations of extracellular vesicles: Apoptotic bodies, microvesicles and exosomes. Journal of Extracellular Vesicles. 2013;**2**:20677

[32] van der Pol E et al. Recent developments in the nomenclature, presence, isolation, detection and clinical impact of extracellular vesicles. Journal of Thrombosis and Haemostasis. 2016;**14**(1):48-56

[33] Abels ER, Breakefield XO. Introduction to extracellular vesicles: Biogenesis, RNA cargo selection, content, release, and uptake. Cellular and Molecular Neurobiology. 2016;**36**(3):301-312

[34] Caby MP et al. Exosomal-like vesicles are present in human blood plasma. International Immunology. 2005;**17**(7):879-887

[35] McDonald MK, Capasso KE, Ajit SK. Purification and microRNA profiling of exosomes derived from blood and culture media. Journal of Visualized Experiments. 2013;**76**:e50294

[36] Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling

of exosomes in human urine. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(36):13368-13373

[37] Bachy I, Kozyraki R, Wassef M. The particles of the embryonic cerebrospinal fluid: How could they influence brain development? Brain Research Bulletin. 2008;**75**(2-4):289-294

[38] Machida T et al. MicroRNAs in salivary exosome as potential biomarkers of aging. International Journal of Molecular Sciences. 2015;**16**(9):21294-21309

[39] Han Y et al. Salivary exosomes: Emerging roles in systemic disease. International Journal of Biological Sciences. 2018;**14**(6):633-643

[40] Hoog JL, Lotvall J. Diversity of extracellular vesicles in human ejaculates revealed by cryo-electron microscopy. Journal of Extracellular Vesicles. 2015;**4**:28680

[41] Lasser C et al. Human saliva, plasma and breast milk exosomes contain RNA: Uptake by macrophages. Journal of Translational Medicine. 2011;**9**:9

[42] Wang X. Isolation of extracellular vesicles from breast milk. Methods in Molecular Biology. 2017;**1660**:351-353

[43] Keller S et al. Body fluid derived exosomes as a novel template for clinical diagnostics. Journal of Translational Medicine. 2011;**9**:86

[44] Ebert B, Rai AJ. Isolation and characterization of amniotic fluid-derived extracellular vesicles for biomarker discovery. Methods in Molecular Biology. 2019;**1885**:287-294

[45] Raposo G et al. B lymphocytes secrete antigen-presenting vesicles. The Journal of Experimental Medicine. 1996;**183**(3):1161-1172

[46] Alvarez V et al. The immunomodulatory activity of extracellular vesicles derived from endometrial mesenchymal stem cells on CD4+ T cells is partially mediated by TGFbeta. Journal of Tissue Engineering and Regenerative Medicine. 2018;**12**(10):2088-2098

[47] Li QL et al. Exvivo experiments of human ovarian cancer ascites-derived exosomes presented by dendritic cells derived from umbilical cord blood for immunotherapy treatment. Clinical Medicine Oncology. 2008;**2**:461-467

[48] Contursi A et al. Platelets in cancer development and diagnosis. Biochemical Society Transactions. 2018;**46**(6):1517-1527

[49] Fevrier B et al. Cells release prions in association with exosomes. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(26):9683-9688

[50] Console L, Scalise M, Indiveri C. Exosomes in inflammation and role as biomarkers. Clinica Chimica Acta. 2019;**488**:165-171

[51] Vrijsen KR et al. Cardiomyocyte progenitor cell-derived exosomes stimulate migration of endothelial cells. Journal of Cellular and Molecular Medicine. 2010;**14**(5):1064-1070

[52] Lai RC et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Research. 2010;**4**(3):214-222

[53] Song D et al. Telocytes and lung disease. Histology and Histopathology. 2016;**31**(12):1303-1314

[54] Cretoiu D, Cretoiu SM. Telocytes in the reproductive organs: Current understanding and future challenges. Seminars in Cell & Developmental Biology. 2016;**55**:40-49

[55] Cretoiu D et al. Telocytes heterogeneity: From cellular morphology to functional evidence. Seminars in Cell & Developmental Biology. 2017;**64**:26-39

[56] Bei Y et al. Circulating exosomes in cardiovascular diseases. Advances in Experimental Medicine and Biology. 2017;**998**:255-269

[57] Kalluri R. The biology and function of exosomes in cancer. The Journal of Clinical Investigation. 2016;**126**(4):1208-1215

[58] Takahashi RU, Miyazaki H, Ochiya T. The roles of microRNAs in breast cancer. Cancers (Basel). 2015;**7**:598-616

[59] Willms E et al. Cells release subpopulations of exosomes with distinct molecular and biological properties. Scientific Reports. 2016;**6**:22519

[60] Muller L et al. Isolation of biologically-active exosomes from human plasma. Journal of Immunological Methods. 2014;**411**:55-65

[61] Hong CS et al. Isolation of biologically active and morphologically intact exosomes from plasma of patients with cancer. Journal of Extracellular Vesicles. 2016;**5**:29289

[62] Gao L et al. Tumor-derived exosomes antagonize innate antiviral immunity. Nature Immunology. 2018;**19**(3):233-245

[63] Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. The Journal of Cell Biology. 2013;**200**(4):373-383

[64] Muralidharan-Chari V et al. Microvesicles: Mediators of extracellular communication during cancer progression. Journal of Cell Science. 2010;**123**(Pt 10):1603-1611

[65] Pollet H et al. Plasma membrane lipid domains as platforms for vesicle biogenesis and shedding? Biomolecules. 2018;**8**(3):94

[66] Baj-Krzyworzeka M et al. Tumour-derived microvesicles contain interleukin-8 and modulate production of chemokines by human monocytes. Anticancer Research. 2011;**31**(4):1329-1335

[67] Meehan B, Rak J, Di Vizio D. Oncosomes–large and small: What are they, where they came from?. Journal of Extracellular Vesicles. 2016;**5**:33109

[68] Kerr JF, Wyllie AH, Currie AR. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. British Journal of Cancer. 1972;**26**(4):239-257

[69] Atkin-Smith GK et al. A novel mechanism of generating extracellular vesicles during apoptosis via a beads-on-a-string membrane structure. Nature Communications. 2015;**6**:7439

[70] Jiang L et al. Determining the contents and cell origins of apoptotic bodies by flow cytometry. Scientific Reports. 2017;**7**(1):14444

[71] Berda-Haddad Y et al. Sterile inflammation of endothelial cell-derived apoptotic bodies is mediated by interleukin-1alpha. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(51):20684-20689

[72] Schiller M et al. Autoantigens are translocated into small apoptotic bodies during early stages of apoptosis. Cell Death and Differentiation. 2008;**15**(1):183-191

[73] Cocca BA, Cline AM, Radic MZ. Blebs and apoptotic bodies are B cell autoantigens. Journal of Immunology. 2002;**169**(1):159-166

[74] Tran HB et al. Subcellular redistribution of la/SSB autoantigen during physiologic apoptosis in the fetal mouse heart and conduction system: A clue to the pathogenesis of congenital heart block. Arthritis and Rheumatism. 2002;**46**(1):202-208

[75] Singh P et al. Tubular cell HIV-entry through apoptosed CD4 T cells: A novel pathway. Virology. 2012;**434**(1):68-77

[76] Bergsmedh A et al. Horizontal transfer of oncogenes by uptake of apoptotic bodies. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**(11):6407-6411

[77] Sadallah S et al. Microparticles (ectosomes) shed by stored human platelets downregulate macrophages and modify the development of dendritic cells. Journal of Immunology. 2011;**186**(11):6543-6552

[78] Minciacchi VR et al. MYC mediates large oncosome-induced fibroblast reprogramming in prostate cancer. Cancer Research. 2017;**77**(9):2306-2317

[79] Diehl F et al. Circulating mutant DNA to assess tumor dynamics. Nature Medicine. 2008;**14**(9):985-990

[80] Vagner T et al. Large extracellular vesicles carry most of the tumour DNA circulating in prostate cancer patient plasma. Journal of Extracellular Vesicles. 2018;**7**(1):1505403

[81] Thakur BK et al. Double-stranded DNA in exosomes: A novel biomarker in cancer detection. Cell Research. 2014;**24**(6):766-769

[82] Wyatt AW et al. Concordance of circulating tumor DNA and matched metastatic tissue biopsy in prostate cancer. Journal of the National Cancer Institute. 2017;**110**(1):djx118

[83] Balaj L et al. Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. Nature Communications. 2011;**2**:180

[84] Wiemels JL et al. Chromosome 12p deletions in TEL-AML1 childhood acute lymphoblastic leukemia are associated with retrotransposon elements and occur postnatally. Cancer Research. 2008;**68**(23):9935-9944

[85] Hur K et al. Hypomethylation of long interspersed nuclear element-1 (LINE-1) leads to activation of proto-oncogenes in human colorectal cancer metastasis. Gut. 2014;**63**(4):635-646

[86] Sunami E et al. LINE-1 hypomethylation during primary colon cancer progression. PLoS One. 2011;**6**(4):e18884

[87] Pattamadilok J et al. LINE-1 hypomethylation level as a potential prognostic factor for epithelial ovarian cancer. International Journal of Gynecological Cancer. 2008;**18**(4):711-717

[88] Ohshima K et al. Let-7 microRNA family is selectively secreted into the extracellular environment via exosomes in a metastatic gastric cancer cell line. PLoS One. 2010;**5**(10):e13247

[89] Choi DS et al. The protein interaction network of extracellular vesicles derived from human colorectal cancer cells. Journal of Proteome Research. 2012;**11**(2):1144-1151

[90] Demory Beckler M et al. Proteomic analysis of exosomes from mutant KRAS colon cancer cells identifies intercellular transfer of mutant KRAS. Molecular & Cellular Proteomics. 2013;**12**(2):343-355

[91] Tauro BJ et al. Oncogenic H-ras reprograms Madin-Darby canine kidney (MDCK) cell-derived exosomal proteins

following epithelial-mesenchymal transition. Molecular & Cellular Proteomics. 2013;**12**(8):2148-2159

[92] Al-Nedawi K et al. Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**(10):3794-3799

[93] Taraboletti G et al. Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. The American Journal of Pathology. 2002;**160**(2):673-680

[94] Giusti I et al. Cathepsin B mediates the pH-dependent proinvasive activity of tumor-shed microvesicles. Neoplasia. 2008;**10**:481-488

[95] Subra C et al. Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. Journal of Lipid Research. 2010;**51**(8):2105-2120

[96] Haraszti RA et al. High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources. Journal of Extracellular Vesicles. 2016;**5**:32570

[97] Kim CW et al. Extracellular membrane vesicles from tumor cells promote angiogenesis via sphingomyelin. Cancer Research. 2002;**62**(21):6312-6317

[98] Beloribi S et al. Exosomal lipids impact notch signaling and induce death of human pancreatic tumoral SOJ-6 cells. PLoS One. 2012;**7**(10):e47480

[99] Vancheri C et al. The lung as a privileged site for the beneficial actions of PGE2. Trends in Immunology. 2004;**25**(1):40-46

[100] Xiang X et al. Induction of myeloid-derived suppressor cells by tumor exosomes. International Journal of Cancer. 2009;**124**(11):2621-2633

[101] Kumar V et al. The nature of myeloid-derived suppressor cells in the tumor microenvironment. Trends in Immunology. 2016;**37**(3):208-220

[102] Prima V et al. COX2/mPGES1/PGE2 pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells. Proceedings of the National Academy of Sciences of the United States of America. 2017;**114**(5):1117-1122

[103] Ayob AZ, Ramasamy TS. Cancer stem cells as key drivers of tumour progression. Journal of Biomedical Science. 2018;**25**(1):20

[104] Ye J et al. The cancer stem cell niche: Cross talk between cancer stem cells and their microenvironment. Tumour Biology. 2014;**35**(5):3945-3951

[105] Allinen M et al. Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell. 2004;**6**(1):17-32

[106] Donnarumma E et al. Cancer-associated fibroblasts release exosomal microRNAs that dictate an aggressive phenotype in breast cancer. Oncotarget. 2017;**8**(12):19592-19608

[107] Chiquet-Ehrismann R et al. Tenascins in stem cell niches. Matrix Biology. 2014;**37**:112-123

[108] Minn AJ et al. Genes that mediate breast cancer metastasis to lung. Nature. 2005;**436**(7050):518-524

[109] Enriquez VA et al. High LIN28A expressing ovarian cancer cells secrete exosomes that induce invasion and migration in HEK293

cells. BioMed Research International. 2015;**2015**:701390

[110] Nakamura K et al. Clinical relevance of circulating cell-free microRNAs in ovarian cancer. Molecular Cancer. 2016;**15**:48

[111] Chen Y et al. Aberrant low expression of p85alpha in stromal fibroblasts promotes breast cancer cell metastasis through exosome-mediated paracrine Wnt10b. Oncogene. 2017;**36**(33):4692-4705

[112] Melo SA et al. Cancer exosomes perform cell-independent microRNA biogenesis and promote tumorigenesis. Cancer Cell. 2014;**26**(5):707-721

[113] Lee JE et al. Identification of EDIL3 on extracellular vesicles involved in breast cancer cell invasion. Journal of Proteomics. 2016;**131**:17-28

[114] Choi D et al. Extracellular vesicle communication pathways as regulatory targets of oncogenic transformation. Seminars in Cell & Developmental Biology. 2017;**67**:11-22

[115] Sleeman JP. The metastatic niche and stromal progression. Cancer Metastasis Reviews. 2012;**31**(3-4):429-440

[116] Chin AR, Wang SE. Cancer tills the premetastatic field: Mechanistic basis and clinical implications. Clinical Cancer Research. 2016;**22**(15):3725-3733

[117] Liu Y, Cao X. Characteristics and significance of the pre-metastatic niche. Cancer Cell. 2016;**30**(5):668-681

[118] de la Fuente A et al. M-Trap: Exosome-based capture of tumor cells as a new technology in peritoneal metastasis. Journal of the National Cancer Institute. 2015;**107**(9):djv184

[119] Szajnik M et al. Exosomes in plasma of patients with ovarian carcinoma: Potential biomarkers of tumor progression and response to therapy. Gynecology & Obstetrics. 2013;**S4**:3

[120] Maia J et al. Exosome-based cell-cell communication in the tumor microenvironment. Frontiers in Cell and Development Biology. 2018;**6**:18

[121] Zhang Y, Wang XF. A niche role for cancer exosomes in metastasis. Nature Cell Biology. 2015;**17**(6):709-711

[122] Yang N et al. The role of extracellular vesicles in mediating progression, metastasis and potential treatment of hepatocellular carcinoma. Oncotarget. 2017;**8**(2):3683-3695

[123] Dhondt B et al. Function of extracellular vesicle-associated miRNAs in metastasis. Cell and Tissue Research. 2016;**365**(3):621-641

[124] Robado de Lope L et al. Tumour-adipose tissue crosstalk: Fuelling tumour metastasis by extracellular vesicles. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 2018;**373**(1737):20160485

[125] Maji S et al. Exosomal annexin II promotes angiogenesis and breast cancer metastasis. Molecular Cancer Research. 2017;**15**(1):93-105

[126] Nawaz M et al. Extracellular vesicles in ovarian cancer: Applications to tumor biology, immunotherapy and biomarker discovery. Expert Review of Proteomics. 2016;**13**(4):395-409

[127] Liang B et al. Characterization and proteomic analysis of ovarian cancer-derived exosomes. Journal of Proteomics. 2013;**80**:171-182

[128] Yokoi A et al. Malignant extracellular vesicles carrying MMP1 mRNA facilitate peritoneal

dissemination in ovarian cancer. Nature Communications. 2017;**8**:14470

[129] Samuel P et al. Cisplatin induces the release of extracellular vesicles from ovarian cancer cells that can induce invasiveness and drug resistance in bystander cells. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 2018;**373**(1737):20170065

[130] Yu DD et al. Exosomes in development, metastasis and drug resistance of breast cancer. Cancer Science. 2015;**106**(8):959-964

[131] Gomes FG et al. Breast-cancer extracellular vesicles induce platelet activation and aggregation by tissue factor-independent and -dependent mechanisms. Thrombosis Research. 2017;**159**:24-32

[132] O'Brien K et al. Exosomes from triple-negative breast cancer cells can transfer phenotypic traits representing their cells of origin to secondary cells. European Journal of Cancer. 2013;**49**(8):1845-1859

[133] Li J et al. Claudin-containing exosomes in the peripheral circulation of women with ovarian cancer. BMC Cancer. 2009;**9**:244

[134] Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecologic Oncology. 2008;**110**(1):13-21

[135] Yin J et al. Secretion of annexin A3 from ovarian cancer cells and its association with platinum resistance in ovarian cancer patients. Journal of Cellular and Molecular Medicine. 2012;**16**(2):337-348

[136] Jin Y et al. Annexin A3 is a potential predictor of platinum resistance in epithelial ovarian cancer patients in a prospective cohort. Journal of Cancer. 2015;**6**(7):678-685

[137] Hamrita B et al. Proteomics-based identification of alpha1-antitrypsin and haptoglobin precursors as novel serum markers in infiltrating ductal breast carcinomas. Clinica Chimica Acta. 2009;**404**(2):111-118

[138] Hannafon BN et al. Plasma exosome microRNAs are indicative of breast cancer. Breast Cancer Research. 2016;**18**(1):90

[139] Di Modica M et al. Breast cancer-secreted miR-939 downregulates VE-cadherin and destroys the barrier function of endothelial monolayers. Cancer Letters. 2017;**384**:94-100

[140] Kuvibidila S, Rayford W. Correlation between serum prostate-specific antigen and alpha-1-antitrypsin in men without and with prostate cancer. The Journal of Laboratory and Clinical Medicine. 2006;**147**(4):174-181

[141] Khan S et al. Plasma-derived exosomal survivin, a plausible biomarker for early detection of prostate cancer. PLoS One. 2012;**7**(10):e46737

[142] Huang X et al. Characterization of human plasma-derived exosomal RNAs by deep sequencing. BMC Genomics. 2013;**14**:319

[143] Huang X et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. European Urology. 2015;**67**(1):33-41

[144] Soekmadji C et al. Extracellular vesicles for personalized therapy decision support in advanced metastatic cancers and its potential impact for prostate cancer. Prostate. 2017;**77**(14):1416-1423

[145] Shi R et al. Exosomal levels of miRNA-21 from cerebrospinal fluids associated with poor prognosis and tumor recurrence of glioma patients. Oncotarget. 2015;**6**(29):26971-26981

[146] Madhavan B et al. Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. International Journal of Cancer. 2015;**136**(11):2616-2627

[147] Lin SY et al. Proteome profiling of urinary exosomes identifies alpha 1-antitrypsin and H2B1K as diagnostic and prognostic biomarkers for urothelial carcinoma. Scientific Reports. 2016;**6**:34446

[148] Liu T et al. Exosomal long noncoding RNA CRNDE-h as a novel serum-based biomarker for diagnosis and prognosis of colorectal cancer. Oncotarget. 2016;**7**:85551-85563

[149] Liao J et al. Exosome-shuttling microRNA-21 promotes cell migration and invasion-targeting PDCD4 in esophageal cancer. International Journal of Oncology. 2016;**48**(6):2567-2579

[150] Matsumura T et al. Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. British Journal of Cancer. 2015;**113**(2):275-281

[151] Smalley DM et al. Isolation and identification of potential urinary microparticle biomarkers of bladder cancer. Journal of Proteome Research. 2008;**7**(5):2088-2096

[152] Hsu YT et al. EpCAM-regulated transcription exerts influences on nanomechanical properties of endometrial cancer cells that promote epithelial-to-mesenchymal transition. Cancer Research. 2016;**76**(21):6171-6182

[153] Zhang W et al. Characterization of exosomes derived from ovarian cancer cells and normal ovarian epithelial cells by nanoparticle tracking analysis. Tumour Biology. 2016;**37**(3):4213-4221

[154] Dumitru A, Toader DO, Cretoiu SM, Cretoiu D, Suciu N, Radu BM. Alterations in Calcium Signaling Pathways in Breast Cancer, Calcium and Signal Transduction. Rijeka: IntechOpen; 2018

[155] Xu M et al. A temporal examination of calcium signaling in cancer- from tumorigenesis, to immune evasion, and metastasis. Cell & Bioscience. 2018;**8**:25

[156] Keup C et al. RNA profiles of circulating tumor cells and extracellular vesicles for therapy stratification of metastatic breast cancer patients. Clinical Chemistry. 2018;**64**(7):1054-1062

[157] Sadovska L, Eglitis J, Line A. Extracellular vesicles as biomarkers and therapeutic targets in breast cancer. Anticancer Research. 2015;**35**(12):6379-6390

[158] Konig L et al. Elevated levels of extracellular vesicles are associated with therapy failure and disease progression in breast cancer patients undergoing neoadjuvant chemotherapy. Oncoimmunology. 2017;**7**(1):e1376153

[159] Zitvogel L et al. Eradication of established murine tumors using a novel cell-free vaccine: Dendritic cell-derived exosomes. Nature Medicine. 1998;**4**(5):594-600

[160] Zech D et al. Tumor-exosomes and leukocyte activation: An ambivalent crosstalk. Cell Communication and Signaling: CCS. 2012;**10**(1):37

[161] Shen C et al. Antileukaemia immunity: Effect of exosomes against NB4 acute promyelocytic leukaemia

cells. The Journal Of International Medical Research. 2011;**39**:740-747

[162] Morse MA et al. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. Journal of Translational Medicine. 2005;**3**(1):9

[163] Escudier B et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: Results of thefirst phase I clinical trial. Journal of Translational Medicine. 2005;**3**(1):10

[164] Dai S et al. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. Molecular Therapy. 2008;**16**(4):782-790

[165] Munoz JL et al. Delivery of functional Anti-miR-9 by mesenchymal stem cell-derived exosomes to glioblastoma multiforme cells conferred chemosensitivity. Molecular Therapy--Nucleic Acids. 2013;**2**:e126

[166] Tian Y et al. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials. 2014;**35**(7):2383-2390

[167] Kalani A et al. Curcumin-primed exosomes mitigate endothelial cell dysfunction during hyperhomocysteinemia. Life Sciences. 2014;**107**(1-2):1-7