



# Exosomes: Small vesicles with big roles in cancer, vaccine development, and therapeutics

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## ABSTRACT

Cancer is a deadly disease that is globally and consistently one of the leading causes of mortality every year. Despite the availability of chemotherapy, radiotherapy, immunotherapy, and surgery, a cure for cancer has not been attained. Recently, exosomes have gained significant attention due to the therapeutic potential of their various components including proteins, lipids, nucleic acids, miRNAs, and lncRNAs. Exosomes constitute a set of tiny extracellular vesicles with an approximate diameter of 30–100 nm. They are released from different cells and are present in biofluids including blood, cerebrospinal fluid (CSF), and urine. They perform crucial multifaceted functions in the malignant progression of cancer via autocrine, paracrine, and endocrine communications. The ability of exosomes to carry different cargoes including drug and molecular information to recipient cells make them a novel tool for cancer therapeutics. In this review, we discuss the major components of exosomes and their role in cancer progression. We also review important literature about the potential role of exosomes as vaccines and delivery carriers in the context of cancer therapeutics.

## 1. Introduction

Exosomes are extracellular vesicles (EVs); approximately 30–100 nm in diameter and with a lipid bilayer membrane. They are secreted by various cell types including cancer cells and are present in biofluids such as blood, cerebrospinal fluid (CSF), and urine [1,2]. Other EVs generally include microvesicles and apoptotic bodies that differ to exosomes in their biogenesis and marker expression [3,4]. Exosomes have been previously considered to be a rubbish bin but a growing number of studies now consider them crucial to intercellular communication and key players in different physiological and pathological processes including cancer [5]. In cancer, they are involved in the induction of angiogenesis, cell migration and proliferation, inflammatory responses, immune suppression, escape from immune surveillance, and metastasis [6,7], as depicted in Fig. 1. Exosomes contain several types of cargo

including proteins, lipids, enzymes, transcription factors, DNA fragments, messenger RNA (mRNAs), micro RNAs (miRNAs), and long non-coding RNAs (lncRNAs). They can transfer these molecules into stromal cells to ensure communication within the microenvironment, modify the recipient cell phenotype to be tumorigenic, and promote primary tumor growth [5–9].

The tumor microenvironment (TME) plays an important role in primary tumor growth and metastasis because cancer cells can establish a strong communication with neighboring and distant cells. The TME contains different elements such as extracellular matrix (ECM), endothelial cells, cancer-associated fibroblasts (CAFs), immune cells, and mesenchymal stem cells (MSCs) [9–13]. Primary tumor cell-derived exosomes are known to induce the transformation of fibroblasts into myofibroblasts that secrete metalloproteinases (MMPs) and in turn degrade the ECM. This degradation results in the release of molecules to

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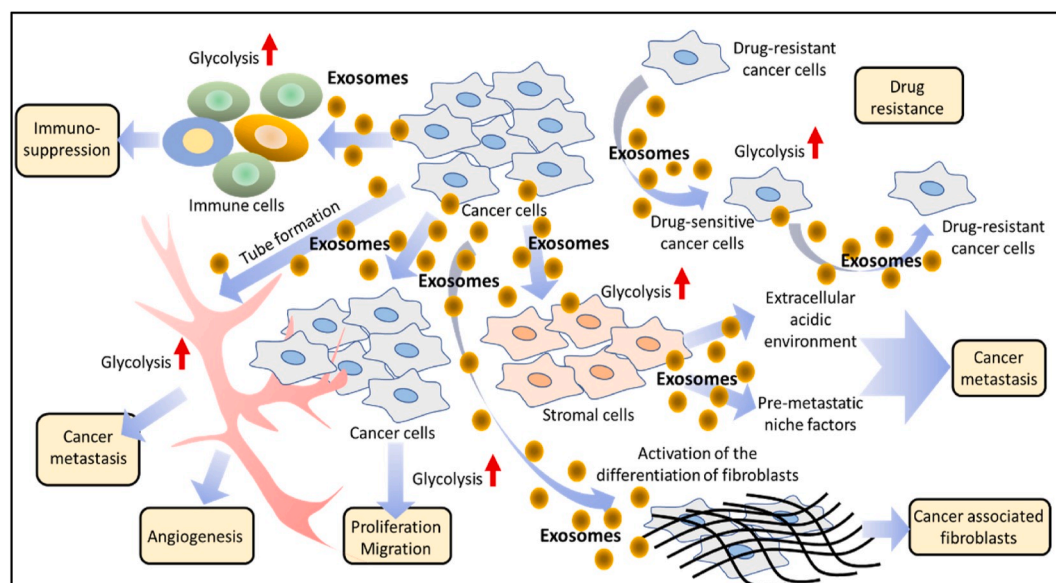
promote invasion of other cells. In addition, these exosomes stimulate the formation of new blood vessels through activation of macrophages in the TME, creating an inflammatory niche. EVs can also induce epithelial-to-mesenchymal transition (EMT), during which epithelial cells lose their cell-cell adhesion and detach from the tumor, promoting the dissemination of cancer cells—one of the hallmarks of metastasis [14–16]. It has been suggested that exosomes secreted by MSC-differentiated adipocytes promote EMT in breast cancer cells through activation of the Hippo signaling pathway. This was confirmed by the phosphorylation of two key transcription factors of this pathway, YAP and TAZ [17]. Other studies have shown that exosomes derived from highly metastatic lung cancer cells and from the serum of patients with late stage lung cancer induce the migration, invasion, and proliferation of human bronchial epithelial cells as well as upregulation of vimentin, a marker associated with EMT and metastasis [18]. Similarly, *in vitro* and *in vivo* analyses confirmed that EMT could be induced by miR-181d-5p that can be transferred from CAF-derived exosomes to breast cancer cells, promoting their proliferation, invasion, migration, and apoptosis suppression through the downregulation of CDX2 and HOXA5 [19].

Tumor cells and the TME are influenced by several conditions such as hypoxia and acidity. Nonetheless tumor cells can adapt to these hostile conditions by remodeling their microenvironment, ensuring tumor progression and metastasis. It has been suggested that one of the mechanisms to remodel the microenvironment and adapt to hypoxia is the production of exosomes by cancer cells. In bladder cancer cells, hypoxia results in the release of exosomes containing high levels of lncRNA-UCA1, an inducer of EMT, that promotes migration and invasion of cancer cells [20]. Similar results have been observed from hypoxic ovarian cancer cells that secrete exosomes enriched with oncogenic protein STAT3. This protein is transferred into recipient cells, regulating the production of Rab7 and Rab27a proteins that stimulate exosome secretion. In addition, STAT3 contributes to cell reprogramming in TME, resulting in a pro-tumorigenic niche and increased cell migration and invasion [21]. Hypoxia affects not only cancer cells but also nearby stromal cells. Studies in lung cancer confirm that exosomes secreted by hypoxic bone-marrow-derived mesenchymal stem cells (BMSCs) in the

tumor microenvironment promote cancer cell invasion and EMT through transfer of miR-193a-3p, miR-210-3p, and miR-5100 from hypoxic BMSCs to cancer cells [22]. Similar results have been obtained in exosomes released by hypoxic colorectal cancer cells. These exosomes transfer Wnt4 mRNA to normoxic colorectal cancer cells, promoting tumor progression and activation of  $\beta$ -catenin signaling to enhance the migration and invasion properties of normoxic cancer cells [23].

Microenvironmental acidity also influences the interaction between tumor cells and neighboring cells in the TME. An acidic microenvironment is a result of upregulated glycolysis that decreases the pH to 6.4–7.3. Studies on melanoma have demonstrated that the acidic microenvironment stimulates a high level of exosome release in the metastatic non-invasive stage, enhancing migration and invasiveness of cancer cells through the transfer of metastasis-promoting molecules. In contrast, this acidity did not influence the release of exosomes from early primary melanoma, suggesting that a low pH does not affect exosome production during the early stages of cancer. Accordingly, the number of exosomes secreted by cancer cells probably indicates the disease development stage [24]. Other studies have shown that some elements of the TME such as MSCs and CAFs can also release exosomes to promote reprogramming of neighboring cells and cancer development. Zhang et al. and Mao et al. demonstrated that MSCs secrete exosomes that increase the invasion of lung cancer cells and promote the development of gastric cancer, respectively [10,22]. Similar results have been observed with exosomes produced by CAFs that can reprogram cancer cell metabolism in pancreatic and prostate cancer [11] and stimulate invasiveness and metastasis in breast cancer [12].

During cancer progression, metastasis is the main cause of cancer death. It involves successive steps such as invasion, intravasation, circulation, extravasation, and proliferation at a distant site [25–27]. Exosomes can influence each step of the process. During invasion, they induce the EMT, reducing adhesion between cells, degrading the ECM, and promoting cell migration. For example, breast cancer cells transfer miR-9 via exosomes into normal fibroblasts (NFs), inducing their change to a CAF phenotype and promoting reorganization of the ECM through expression of metalloproteinases, fibulins and collagens. In addition, normal fibroblasts can release miR-9-containing exosomes to tumor



**Fig. 1. Modulation of various events in the TME via exosomal communication.** Cancer cell-derived exosomes communicate with both autologous cancer cells and heterologous stromal cells, and participate in different biological phenomena including immunosuppression, cancer metastasis, angiogenesis, migration, and proliferation by altering the metabolic status of recipient cells including enhanced glycolysis. Exosomes released from drug-resistant cancer cells are internalized by drug-sensitive cancer cells resulting in augmented glycolysis, causing development of drug resistance in the recipient cells. Further, cancer cell-derived exosomes cause changes in the surrounding microenvironment including development of an acidic extracellular environment, or preparation of pre-metastatic niche, leading to cancer metastasis. Cancer cell-derived exosomes also activate the differentiation of fibroblasts into CAFs.

cells, causing the downregulation of E-cadherin and stimulating tumor cell migration and invasion [28]. In intravasation, exosomes disturb the endothelium to increase vascular permeability and facilitate the entry of tumor cells into blood and lymphatic vessels. *In vitro* and *in vivo* studies on human umbilical vein endothelial cells (HUVECs) treated with exosomes secreted by metastatic breast cancer cells containing thrombospondin-1 (TSP1) have shown increased *trans*-endothelial migration of tumor cells due to the disruption of intercellular junctions, confirmed by a reduction in mRNA expression of junction proteins such as zona occluden-1 (ZO-1) and vascular endothelial cadherin (VE-cadherin) [29]. In the circulation, tumor cells release exosomes that modify the immune system by inhibiting anti-tumor activity of natural killer- and T-cells. In extravasation, exosomes stimulate the production of adhesive molecules, facilitating the adhesion of circulating tumor cells (CTCs) to the blood vessel wall and promoting vascular leakiness and subsequent exit of tumor cells from blood vessels to a new site. For instance, studies of attached hepatocellular carcinoma (HCC) cells demonstrated that they secrete exosomes loaded with SMAD3 protein and mRNA that can be transferred to circulating HCC cells, promoting their adhesion through overproduction of reactive oxygen species and facilitating lung metastasis [30]. Finally, extravasated cells can proliferate at a new distant site and their exosomes can modify the neighboring cells to a pre-metastatic phenotype and recruit bone marrow-derived cells to establish the pre-metastatic niche (PMN) [8, 15,16,31].

## 2. Functional roles of different components of exosomes in cancer

Exosomes carry various functional constituents including proteins, lipids, miRNA, and lncRNA. Different exosomes from different sources carry specific sets of functional constituents. For example, exosomes isolated from the CSF of glioma patients contain a specific protein, epidermal growth factor receptor variant III (EGFRvIII) [32]. Lung cancer exosome-specific protein-1 (LESP-1) has been found specifically in exosomes from the plasma of lung cancer patients [33]. Similarly, a set of 12 specific miRNAs have been found to be augmented in exosomes from lung adenocarcinoma [34]. Among the various constituents of exosomes, we will focus on the major components, namely protein, lipid, miRNA, and lncRNA (Fig. 2).

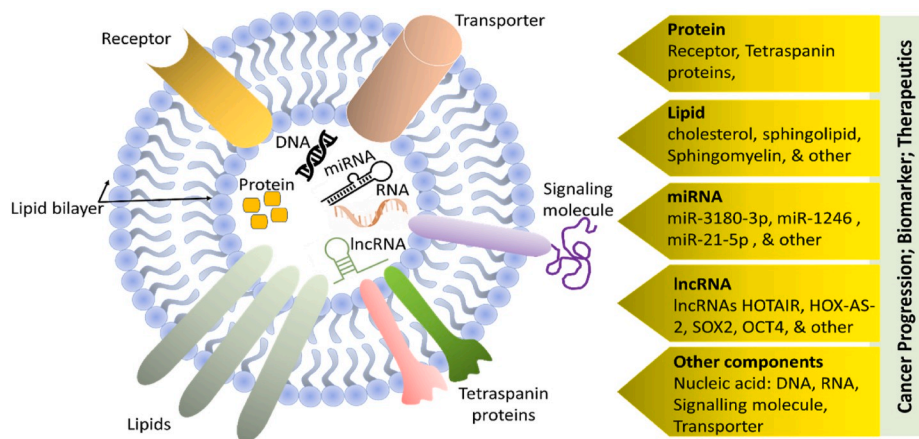
### 2.1. Exosomal protein

Numerous proteins are enriched in exosomes from distinct sources, according to various databases including Vesiclepedia, and ExoCarta,

which are being updated continuously [35,36]. Apart from their own proteins, exosomes also carry proteins derived from their parent cells. Various exosomal proteins originating from cancer cells have been studied as potential biomarkers for diagnostic and prognostic purposes. Importantly, the lipid bilayer of exosomes protects their content from degradation in the blood circulation. Moreover, due to the complex components of blood, proteins expressed by cancer cells are diluted in blood, making them hard to detect in early-stage disease. Nonetheless there are around  $10^9$  exosome particles in each milliliter of human blood. These can be isolated for detection of proteins with a higher sensitivity for the diagnosis and prognosis of cancer [37,38].

Studies have demonstrated that lymphocyte cytosolic protein-1 (LCP1) enriched in exosomes secreted by BMSCs can be transferred to osteosarcoma cells and promote tumor cell proliferation and metastasis *in vitro* and *in vivo* through activation of the JAK2/STAT3 pathway and degradation of Nrdp1 [39]. In non-small cell lung cancer (NSCLC), tumor cells have been shown to secrete high levels of leucine-rich-alpha2-glycoprotein 1 (LRG1) that stimulates proliferation, migration, and invasion of cancer cells. In addition, NSCLC cells transfer LRG1 via exosomes, promoting angiogenesis through the expression of proangiogenic markers such as VEGFA and Ang1, mediated by the TGF- $\beta$  pathway [40]. Analysis of exosomes produced by prostate cancer cells has demonstrated that ITGA3 and ITGB1 proteins influence the behavior of non-cancerous prostate epithelial cells, promoting their migration and invasion. Since these proteins are found in high levels in urine exosomes of cancer patients with metastatic prostate disease, it has been suggested that ITGA3 and ITGB1 can be used in diagnostic tests [41].

Several studies have also demonstrated that exosomal tetraspanins participate in cancer progression. For example, research on exosomes secreted by pancreatic adenocarcinoma cells revealed that exosomal Cluster of Differentiation 151 (CD151) and Tspan8 promote ECM degradation through their association with proteases and integrins. In addition, these tetraspanins can be transferred to non-metastatic cells, inducing EMT and metastasis in recipient cells [42]. In prostate cancer, exosomes with increased levels of CD151 and low levels of CD9 can stimulate the migration and invasion of non-cancerous prostate cells, leading to metastasis [43]. Other proteins such as integrins  $\alpha_6\beta_4$ ,  $\alpha_6\beta_1$  and  $\alpha_v\beta_5$  have been associated with metastatic organotropism. Based on a study by Hoshino et al.,  $\alpha_6\beta_4$  and  $\alpha_6\beta_1$  in breast cancer cell-derived exosomes promote lung metastasis, while  $\alpha_v\beta_5$  from exosomes produced by pancreatic cancer cells facilitate liver metastasis [44]. Moreover,  $\alpha_v\beta_6$  integrin enriched in exosomes produced by prostate cancer cells can be transferred to surrounding cells, inducing their adhesion and migration, and possibly promoting metastasis [45]. Studies in gastric cancer have revealed that exosomes derived from tumor cells can



**Fig. 2. Schematic representation of the major components of a typical exosome.** An exosome is composed of a lipid bilayer membranous structure that comprises various functional constituents including protein, lipid, miRNA, lncRNA, and other components such as nucleic acids, signaling molecule, and transporters. The exosomal components play a crucial role in cancer progression and act as potential biomarkers. Moreover, they can be employed for therapeutic purposes.

package and deliver EGFR to liver cells, stimulating the activation of HGF and its binding with the c-MET receptor to promote the proliferation of cancer cells and liver metastasis [46]. Another study reported that exosomes from the plasma of head and neck squamous cell carcinoma (HNSCC) patients expressed PD-L1 in their surface that interacts with PD-1 receptor of immune cells, promoting the inhibition of T-cell activity and therefore tumor progression [47].

Some exosomal proteins have been proposed as potential biomarkers for the diagnosis of cancer. Recent studies of serum exosomes from patients with HCC identified 10 differentially expressed proteins (DEPs), namely VWF, TGFB1, LGALS3BP, SERPINC1, HPX, HP, HBA1, FGA, FGG and FGB. Clustering analysis showed proteins were overproduced in patients with HCC and downregulated in healthy controls, suggesting that these DEPs could serve as biomarkers for HCC [48]. Proteomic analysis of plasma exosomes from breast cancer (BC) patients has revealed that proteins such as FAK, MEK1 and fibronectin are expressed in high levels relative to healthy patients and may serve as biomarkers for breast cancer. Additionally, it has been confirmed that low levels of P-cadherin and TAZ are characteristic of stage IIA BC, and IGFR $\beta$  is the main protein in stage I. As a consequence, these proteins are considered possible markers to differentiate stage IIA from I in BC [49]. Other studies based on EV array, have proposed multimarker models to identify lung cancer patients by protein profile analysis of plasma exosomes. For example, a multimarker model with 10 proteins has been suggested, where CD151, TSPAN8 and CD171 are upregulated in exosomes derived from lung cancer patients [50]. Similarly, another study proposed a multimarker model with 30 proteins including CD9, CD63, CD81, and EGFR to detect NSCLC [51].

## 2.2. Exosomal lipid

Lipid composition of exosomes derived from different cells has been studied by various research groups [52]. It has been found that prostate cancer cell-derived exosomes are enriched with high levels of cholesterol, glycosphingolipids, phosphatidylserine, and sphingomyelin [53–56]. Colorectal cancer cell-derived exosomes have been reported to have high levels of cholesterol, glycerides, glycerophospholipids, and sphingolipids [57]. It can be inferred that the change in lipid amount in exosomes can be potentially used for theranostic purposes.

In a hypoxic TME, 3T3-L1 adipocyte-derived exosomes are found to have enzymes associated with lipid synthesis such as acetyl-CoA carboxylase, fatty acid synthase (FASN), and glucose-6-phosphate dehydrogenase. Moreover, hypoxic 3T3-L1 adipocyte-derived exosomes facilitate accumulation of lipid in recipient 3T3-L1 adipocytes [58]. Rat adipocyte-derived exosomes have been found to possess glycosylphosphatidylinositol-anchored proteins that can transfer RNA and promote synthesis of lipid, suggesting that paracrine and endocrine regulation of lipid storage may be a potential target for modulating metabolism [59]. In cell-cell communication, the high level of lipid is favorable for uptake of cancer-derived exosomes by normal cells and for their further change into cancer cells [60–62].

Exosomes have attracted huge attention due to their crucial role in lipid metabolism disorders that are properties of cancer cells and involved in the malignant progression of cancer [63]. It has been shown that in the TME, macrophage-derived exosomes possess bioactive lipids such as prostaglandins PGE1, PGE2, and PGE2 $\alpha$  [64,65]. It has also been found that exosomes derived from lung cancer and prostate cancer cells promote lipid degradation of adipocytes that provides energy for cancer related cachexia and uncontrolled proliferation of cells [66,67].

Several studies have proposed exosomal lipids as promising biomarkers in different cancer types. For example, research on exosomes secreted by ovarian cancer cells SKOV3 revealed that they contain high levels of LPI, LPS, LPG and LPC compared with exosomes derived from HOSEPiC cells. Therefore, these lipids can be used as possible biomarkers for early ovarian cancer detection [68]. Metabolomics analysis using cancer stem cell-derived exosomes and serum exosomes from

patients with malignant melanoma (MM) showed that molecules such as lysophospholipid sphingosine 1-phosphate, palmitoylcarnitine, elaidic-carnitine, phosphatidylcholines, and phosphatidylethanolamines are present at a lower level in MM patients compared with healthy patients, suggesting these lipids as biomarkers for early diagnosis of MM [69]. Studies on pancreatic cancer (PC) have revealed that serum exosomes in cancer patients contain lipids such as lysophosphatidylcholine, phosphatidylcholine and phosphatidylethanolamine that are associated with tumor stage and diameter, suggesting these lipids as important tools to improve the diagnosis of PC [70]. One study of the lipid profile of serum exosomes from NSCLC patients and healthy patients, proposed that it is possible to distinguish between normal, early and late stage NSCLC through clustering analysis using statistical methods, Random Forest (RF) and Least Absolute Shrinkage and Selection Operator (LASSO), highlighting their value in biomarker development based on metabolomics data [71].

## 2.3. Exosomal miRNA

miRNAs are small non-coding RNAs of 18–25 nucleotides in length. They play an important role as post-transcriptional gene regulators by binding to the 3' untranslated region (3'UTR) of target mRNAs, inhibiting mRNA translation or degrading the mRNA, resulting in gene silencing [72–74]. Thus, miRNAs are able to regulate different cellular processes such as differentiation, embryogenesis, proliferation, metabolism, organogenesis, cell cycle, apoptosis, and signaling pathways [75,76]. Since some of these processes are altered in cancer, and miRNA genes are located in chromosomal regions associated with cancer, the expression of miRNAs can be dysregulated in tumor cells, leading to overexpression of oncogenic miRNAs and under-expression of tumor suppressor miRNAs [77,78].

miRNAs are mainly located in the cytosol but they are also packaged as cargo in exosomes where they are protected from degradation by RNase present in different biological fluids [74]. miRNAs can be packaged into exosomes by several sorting processes involving proteins such as RNA-binding proteins (RBPs) and membrane proteins. In the first, heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) interacts with specific motifs of some miRNAs to shuttle them into exosomes. In addition, Argonaute 2 (Ago2) protein, a component of the RNA-induced silencing complex (RISC), participates in miRNA sorting through the KRAS-MEK-ERK signaling pathway. Other proteins such as Y-Box Binding Protein 1 (YBX-1), MEX3C, Major Vault Protein (MVP), and La protein are also involved in sorting of miRNAs into exosomes. Membrane proteins implicated in exosome biogenesis such as caveolin-1, neural sphingomyelinase 2, and vacuolar protein sorting-associated protein 4 (Vps4A) have been suggested as mediators in the sorting of miRNAs [74,79].

It has been demonstrated that miRNAs packaged in exosomes play an important role in intercellular communication. In cancer, they participate as messengers in the crosstalk between tumor cells and their surrounding microenvironment, promoting tumor growth, cancer progression and resistance to treatment. miRNAs can modulate vascular permeability by inhibiting the expression of proteins that maintain the endothelial junction, thereby promoting cancer metastasis. Nonetheless, they can also stimulate the formation of new blood vessels associated with the tumor. Furthermore, the ECM in the tumor microenvironment can be remodeled by miRNAs secreted by cancer cell-derived exosomes transforming fibroblasts into CAFs, promoting tumor cell migration, and increasing the amount of glucose available for cancer cells. Additionally, exosome-derived miRNAs mediate the communication between cancer cells and immune cells, conferring resistance to chemotherapy and recruiting regulatory T cells (Tregs) to suppress the immune response and evade the host immune system. It has been suggested that metastatic cancer cells secrete exosomes enriched with miRNAs that induce a temporary dormancy stage where cells enter the G<sub>0</sub> phase of the cell cycle and exhibit chemotherapy resistance with a high probability of

recurrence after treatment for many years [74,80].

Several studies have shown that exosome-derived miRNAs play a key role in the progression of different cancer types. Studies in patients with advanced NSCLC and early NSCLC have confirmed that tumor cells secrete exosomes with decreased levels of miR-620 when compared with non-tumor cells of healthy patients, indicating its participation in tumorigenesis and suggesting miR-620 as a diagnostic and prognostic biomarker in NSCLC patients [81]. On the contrary, overexpression of exosomal miR-3180–3p produced by cancer cells suppresses proliferation, migration, and invasion of NSCLC by binding to its target gene, *FOXP4*, that plays an important role in oncogenesis. Accordingly, *in vivo* analysis showed that tumors in mice treated with exosome-derived miR-3180–3p were of small size and low weight [82]. Studies of exosomes released by glioma associated stem cells (GASC) isolated from low-grade glioma demonstrated that they contain oncogenic and onco-suppressive miRNAs that modulate cancer progression and aggressiveness. It has been suggested that miRNAs that are enriched with GASC exosomes such as miR-1246 and miR-4516 can promote glioma immune evasion and tumor progression, respectively. In contrast, miR-451 and miR-150 inhibit glioma cell proliferation, invasion and apoptosis [83]. Recent studies on melanoma showed that human metastatic melanoma cells, which were injected into the brain of mice to simulate melanoma metastasis, released high levels of exosomal miR-224–5p, miR-130a–3p, and miR-21–5p during tumor growth, suggesting these miRNAs as potential biomarkers for melanoma progression. Furthermore, the results demonstrated the presence of tumor suppressor miRNAs in the total plasma, while exosome-derived miRNAs exhibited oncogenic functions. These findings suggest that cancer cells secrete tumor suppressor miRNAs into plasma and package oncogenic miRNAs in exosomes, promoting the interaction between cancer cells and their microenvironment to increase cancer cell survival and prepare a pre-metastatic niche [84].

Four exosome-derived miRNAs, miR-142–5p, miR-150–5p, miR-320a, and miR-4433b–5p, have been identified in serum samples from breast cancer patients. Several analyses showed overexpression of miR-142–5p, miR-320a and miR-4433b–5p when compared with healthy patients. Additionally, the combination of low levels of miR-142–5p and miR-150–5p was associated with advanced tumor grade (grade III) and the combination of low levels of miR-142–5p and miR-320a was associated with a larger tumor (>20 mm). These results highlight the relevance of these miRNAs as biomarkers in breast cancer and support their use to identify different subtypes of this cancer [85]. Recently, studies on cervical cancer have demonstrated that exosomal miR-125a–5p in cancer patients is expressed at a lower level than in healthy patients, suggesting this miRNA as a possible biomarker for cervical cancer diagnosis. Nonetheless decreased levels of miR-125a–5p have been reported in breast, hepatocellular and colon cancer, suggesting that this miRNA is not specific to cervical cancer [86]. A study on ovarian cancer has reported that tumor cells secrete exosomes enriched with miR-141–3p that enters endothelial cells and downregulates the expression of its target, *SOCS5* that in turn plays an important role as a negative regulator of JAK-STAT3 signaling that promotes tumorigenesis. Consequently, upregulation of STAT3 protein expression resulted in overexpression of vascular endothelial growth factor (VEGF) in tumor cells and activated the vascular endothelial growth factor receptor 2 (VEGFR2) in endothelial cells, promoting endothelial cell migration and angiogenesis [87].

Studies in prostate cancer have also revealed the critical role of exosomal miRNAs in disease progression. Exosomes released by PC3 prostate cancer cells have been shown to contain increased levels of miRNA Let-7b. Since Let-7b targets several oncogenes, it can act as a tumor suppressor, affecting cell proliferation, migration, differentiation, and EMT. Thus, packaging of tumor suppressor miRNAs in exosomes produced by cancer cells may be advantageous for their proliferation and guarantee the dissemination of pro-tumorigenic molecules into the microenvironment. Furthermore, the results confirmed that Let-7b was

transferred to monocytic THP-1 cells through exosomes, influencing the polarization of tumor-associated macrophages (TAM) [88]. Other studies have revealed that tumor suppressor miR-26a can regulate the secretion of exosomes in prostate cancer cells by binding its target genes, *SHC4*, *PFDN4*, and *CHORDC1*. As a result, downregulation of miR-26a increased the expression level of target genes, enhancing the release of exosomes in tumor cells and promoting cancer progression. Accordingly, *in vivo* analyses showed that mice with low expression of target genes due to overexpression of miR-26a had smaller tumors with lower weight compared with control mice, suggesting new approaches for prostate cancer treatment [89].

Studies in colon cancer (CC) have identified miRNAs such as Let-7b–3p, miR-139–3p, miR-145–3p, and miR150–3p in exosomes secreted by tumor cells, and these have been proposed as biomarkers to detect early colon cancer. In addition, a comparison between exosomal and plasma miRNAs showed significant differences in their abundance suggesting that free circulating miRNAs have a different biological origin to exosome-derived miRNAs [90]. Similarly, four exosomal miRNAs, miR-19b, miR-21, miR-222 and miR-92a, have been detected in metastatic colorectal cancer (CRC) and consequently proposed as liquid biopsy biomarkers for early detection of metastatic CRC [91]. Recent studies in colorectal cancer (CRC) and peritoneal metastatic cancer, found that exosomal miR-193a and let-7g are negatively correlated. In metastatic cells, miR-193a was downregulated compared with primary CRC cells, while in peritoneal metastatic cells, let-7g was upregulated compared with primary cancer cells. Therefore, low expression of miR-193a and overexpression of let-7g are associated with decreased survival rates in cancer patients [92].

#### 2.4. Exosomal lncRNA

Different noncoding RNAs (ncRNAs) are present in exosomes and include lncRNAs. It has been reported that cancer cell-derived exosomes are enriched with lncRNAs that are taken up by recipient cells resulting in various biological events in a TME. Sequencing has shown that exosomal RNAs provide the intercellular structure of RNAs and revealed selective packing of RNAs into exosomes [93]. Furthermore, it has been established that RNAs secreted from exosomes differ for normal and cancer cells [94]. Additionally, cancer cell-derived exosomes are enriched with specific lncRNA compared with normal cell-derived exosomes, further contributing to the malignant progression of cancer in recipient cells. Notably, cancer cell-derived exosomal lncRNA can serve as a potential diagnostic as well as prognostic biomarker [95,96]. The level of lncRNA in exosomes isolated from various biofluids including serum, cervicovaginal lavage, and urine samples has also been examined by different research groups as possible biomarkers. For example, exosomal lncRNAs such as HOTAIR, HOX-AS-2, MALAT1, SOX2, OCT4, HYMA1, LINC00477, LOC100506688, and OTX2-AS1 are enriched in exosomes isolated from the urine of patients with urothelial bladder cancer [97]. Several lncRNAs including MALAT1, HOTAIR, and MEG3 are differentially expressed in exosomes isolated from cervicovaginal lavage samples of patients with cervical cancer [98]. Nonetheless although exosomal lncRNA has been proposed as a potential cancer biomarker, issues related to sensitivity, specificity, and reproducibility remain a challenge due to the differences in techniques applied for isolation of exosomes.

Intercellular communication via exosomes results in the transfer of pro-oncogenic lncRNAs between cells, contributing to the malignant progression of cancer in the TME. For example, ZFAS1, an exosomal lncRNA, has been reported to enhance the migration and proliferation of recipient gastric cancer cells, thereby contributing to progression of gastric cancer [99]. Another study showed that exosomal lncRNA-H19 could augment the proliferation and formation of spheroid of cervical cancer cells [100]. Cancer cell-derived exosomal lncRNAs have also been found to influence tube formation in HUVECs. CD90 positive cancer cell-derived exosomes have been shown to promote adhesion and

tube formation in HUVECs. Importantly, this phenotype is due to exosomal lncRNA-H19 [101]. In another study, exosomal lncRNA-POU3F3 induced migration, proliferation, and angiogenesis of human brain microvascular endothelial cells (HBMEC) in gliomas, suggesting a crucial role for exosomal lncRNAs in angiogenesis and tumor progression.

Several studies have also demonstrated the role of exosomal lncRNAs in development of drug resistance by recipient cells. For example, VLDLR, a HCC-derived exosomal lncRNA, promoted drug resistance of recipient cancer cells [102]. Another study reported that HCC-derived exosomes caused augmented expression of lncRNA-ROR in recipient HepG2 cells. In addition, after knockdown of lncRNA-ROR, TGF $\beta$ -induced drug resistance was reversed in CD133 positive cancer stem like cells [103]. These studies highlight the importance of exosomal lncRNAs in chemotherapy resistance of cancer cells and warrants further investigation.

### 2.5. Other components of exosomes

Exosomes also contain other types of molecules such as DNA, RNA, transcription factors, and enzymes, all of which play key roles in cancer progression. Single-stranded, double-stranded, and mitochondrial DNA have been identified in tumor cell-derived exosomes. Studies of endocrine tumors have demonstrated that serum exosomes from paraganglioma (PGL) and pheochromocytoma (PCC) patients contain double-stranded DNA (dsDNA) with somatic mutations that reflect the mutational status of tumor cells, suggesting exosomal dsDNA as a noninvasive biomarker in the diagnosis of PGL and PCC [104]. It has been shown in breast cancer that CAF-derived exosomes can package and transfer all mitochondrial genome to dormant and metabolically quiescent cancer stem cells (CSCs), restoring their potential and leading to hormone therapy-resistant metastasis [105]. Further, molecules such as miRNA and lncRNA have been reported as the main types of RNA in exosomes. In addition to miRNA and lncRNA, mRNA, rRNA, tRNA, and circRNA have also been identified in exosomes. In pancreatic ductal adenocarcinoma, the presence of PDE8A, a circular RNA, in plasma exosomes and tumor cell-derived exosomes promoted tumor invasion through MACC/MET/ERK or AKT pathways [106]. Studies in malignant melanoma have revealed that exosomes derived from cancer cells and from normal melanocytes have a different mRNA profile. The results showed that mRNAs from melanoma exosomes participate in tumor progression and metastasis and can serve as biomarkers for melanoma [107]. Proteolytic enzymes such as MMPs have also been reported in exosomes. Exosomes derived from hypoxic nasopharyngeal carcinoma cells contain high levels of MMP-13 that can be transferred to normoxic cells to transform them into malignant cells, inducing TME remodeling and metastasis [108]. Studies in NSCLC confirmed that ADAM10 enzyme was enriched in blood exosomes from cancer patients when compared with healthy controls and may therefore serve as a promising biomarker for early detection of NSCLC [109].

Importantly, the research related to RNA in EVs faces various challenges that include the need to standardize a method to isolate EVs, characterization of small amounts of RNA in EVs, and development of methodologies to show functional transport of EV-RNA *in vivo*. These challenges were discussed in a workshop in 2015. Various scientists collaboratively prepared a checklist of experimental features about this issue that should be reported in papers. First published in the year 2013 [110] it was updated in year 2017 [111].

### 3. Targeting exosomal release and uptake for cancer therapeutics

Most pathological events, including cancer, that occur via exosomes involve intercellular communication that in turn involves two major processes: release of exosomes from donor cells and their uptake by recipient cells. Therefore, blocking or inhibiting the release or uptake of

exosomes may be a means by which to inhibit metastasis or malignant progression of cancer. Various *in vivo* and clinical studies have demonstrated the involvement of heparanase/syndecan-1 axis or syndecan heparan sulfate proteoglycans in exosome biogenesis and cancer progression, and may be targeted to alleviate cancer progression [112–114]. Oral squamous cancer metastasis has been reported to be blocked by reducing exosome uptake via heparin [115]. In another study, a therapeutic antibody that could reduce the release of exosomes from tumor, reduced metastasis of breast cancer *in vivo*, suggesting that this method could be potentially useful for cancer therapeutics [116]. Accumulating evidence suggests that an acidic extracellular environment can alter the production of exosomes in cancer cells. Under acidic conditions, melanoma cells release a higher number of exosomes than in the normal physiological environment, suggesting that pH in the TME plays a crucial role for exosomal trafficking in cancer cells [117]. Similar scenarios have been reported in other cancers including osteosarcoma, and breast, colon, and prostate cancer. It is believed that a high release of exosomes in low pH conditions may relieve the accumulation of toxic molecules inside the cells [118]. As an example, proton pump inhibitors have been used to reduce the level of exosomes in cancer models [119]. In addition, RAB27A and RAB27B proteins have been reported to play a crucial role in the formation and release of exosomes since their knock-down inhibited exosome release. Several groups have explored potential inhibitors of RAB27A function [120,121]. Two compounds, Nexinhib4 and Nexinhib20, have been reported to inhibit the release of exosomes by acting on the RAB27A-JFC1 complex [122]. In another study, glioma cell-derived exosomes enriched with monocarboxylate transporter 1 (MCT1) and its chaperon CD147 played a crucial role in the malignant progression of glioma. Knock-down of MCT1 and CD147 reduced the release of exosomes but over-expression increased exosomal release significantly, suggesting that MCT1 and CD147 could be potential anti-cancer targets that can inhibit the secretion of exosomes [123].

After exosomes are released, their uptake by recipient cells is crucial for the further cascade of signaling. This uptake relies on different molecules and glycoproteins on the exosomal membrane as well as the recipient cell [120]. Various exosomal uptake inhibitors have been developed including amiloride, dynasore, chlorpromazine, and heparin [120]. Amiloride targets the sodium/proton exchanger, and inhibits the formation of macropinosome [124,125]. Dynasore specifically inhibits dynamin 2 that is necessary for clathrin-mediated and caveolin-based endocytosis [126]. Heparin blocks the binding of heparin sulfate proteoglycans, which is found on the plasma membrane, thereby acting as an inhibitor of endocytosis [127]. Another drug, Chlorpromazine, inhibits the production of clathrin-coated pits by targeting various receptors including histamine, dopamine, and serotonin, thereby acting as a clathrin-mediated endocytosis inhibitor [120,128]. Evidently, targeting the release of exosomes from donor cells and their uptake by recipient cells offers a potential strategy for therapeutics. Table 1 lists the potential targets associated with release and uptake of exosomes that can be applied for cancer therapeutics.

### 4. Potential of exosomes as a cancer vaccine and cancer immunotherapy

Several studies have aimed to develop vaccines for cancer therapeutics, often referred to as therapeutic cancer vaccine or active specific immunotherapy [140]. Recently, exosomes have been found to possess a tremendous potential for cancer immunotherapy and can be utilized as an effective vaccine against cancer. Fig. 3 shows the comparative effect of exosomes from various cell types, including immune cells, cancer cells, and normal cells, that can be utilized for exosome-based cancer immunotherapy. B cells release exosomes, which carry Major histocompatibility complex (MHC) class II peptide complexes, facilitating the antigen presentation to primed CD4<sup>+</sup> T cells. Exosomes isolated from dendritic cells (DCs), a class of antigen-presenting cell that plays a key

**Table 1**

A list of targets for blocking/stimulation of exosomal release and uptake for cancer therapeutics.

Targets	Source of exosomes	Targeting agent	Functionality	Ref.
Calcium (Ca <sup>2+</sup> )	Breast cancer cells	Munc13-4	Calcium stimulates exosome secretion through upregulation of Munc13-4 protein in metastatic cells	[129]
Previous presence of exosomes in the environment	Normal mammary epithelial cells (HMEC B42)	-	Exosomes from HMEC B42 cells inhibit exosome secretion of breast cancer cells	[130]
Differentiation of cancer cells	Colorectal cancer cells (HT29)	Sodium butyrate (NaBu)	Colorectal Cancer cell differentiation induced by NaBu promote increased secretion of exosomes and their expression of CD133	[131]
Tetraspanin-6 (TSPN6)	Breast cancer cells (MCF-7)	SCD4-FL and SDC4-CTF proteins	High levels of TSPN6 inhibit exosome secretion due their binding with SDC4-FL and SDC4-CTF proteins	[132]
Integrin beta 3 (ITGB3)	Breast cancer cells (MDA.MB.231)	Dynamin and focal adhesion kinase (FAK)	ITGB3 on the surface of target cell interacts with HSPGs in exosome membrane promoting dynamin and FAK expression to induce exosome uptake	[133]
Time of incubation and exosome concentration	Bladder cancer cells (SW780)	Heparin	Long incubation times and high exosome concentrations increase the uptake process. Heparin treatment can block the exosome uptake	[134]
Dynamin2	Erythroleukemia cells (K562) and HTLV-transformed T-cells leukemia cells (MT4)	Knockdown of dynamin2 (Dyn2)	Cellular internalization of exosomes via phagocytosis is inhibited with the knockdown of dynamin2	[135]
Preferential uptake, time of incubation and exosome concentration	Pancreatic cancer cells (PANC-1)	-	PANC-1 cells prefer uptake their own exosomes rather than exosomes derived from other cells. The exosome uptake is time- and dose- dependent	[136]
Preferential uptake	Mesenchymal stem cells	-	Placental mesenchymal stem cells make selective uptake of exosomes secreted by the same type of cells	[137]
Preferential uptake, clathrin-dependent endocytosis, phagocytosis and macropinocytosis	Ovarian cancer cells (SKOV3)	Inhibitors such as chlorpromazine, cytochalasin D and EIPA	SKOV3 cells internalize preferentially exosomes derived by them. Treatment with chlorpromazine, cytochalasin D and EIPA significantly decrease exosome uptake	[138]
Preferential uptake	Fibrosarcoma cells (HT1080) and cervical cancer cells (HeLa)	-	HT1080 and HeLa cells uptake preferentially exosomes from the same origin	[139]

role in the adaptive immune system, carry either MHC class I- or MHC class II- peptide complex, facilitating the recognition of CD4<sup>+</sup> or CD8<sup>+</sup> T cells. Upon exposure to pathogens, macrophages release exosomes that carry antigens related to pathogens, facilitating DC maturation and release of pro-inflammatory cytokines. Apart from immune cells, cancer cell-derived exosomes can cause immune suppression or activation. Interestingly, normal cell-derived exosomes show various immune-modulatory activities, facilitating normal physiological activities including fertilization and pregnancy.

Importantly, DCs facilitate tumor immunity through their capacity to uptake and express tumor antigens, making them crucial targets for immunotherapy against cancer. Nevertheless the anti-tumor effect of DCs is unsatisfactory because of their poor immunogenicity, low uptake of antigens, and activation of T cells [141]. Recently, it has been reported that exosomes from DCs have potential implications in antigen presentation [142]. DCs release large quantities of exosomes that induce efficient antitumor effects, since DC-derived exosomes are enriched with MHC I, MHC II, heat-shock proteins (HSP), and CD86 that can stimulate the activation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells [143,144]. Interestingly, the simultaneous stimulation of IL-2 and exosomal CD80 results in the expression of exosomal peptide MHC I, causing CD8<sup>+</sup> T cell proliferation that induces better anti-tumor activity *in vivo* [145]. Moreover, DC-derived exosomes can activate CD8<sup>+</sup> and CD4<sup>+</sup> T cells and can elicit anti-tumor activity via exosomal CD80 and IL-2 *in vivo* [146,147]. Another study demonstrated that exosomes isolated from  $\alpha$ -fetoprotein-expressing DCs could stimulate mice with hepatocellular cancer to synthesize IFN- $\gamma$ -expressing CD8<sup>+</sup> T cells accompanied by augmented IFN- $\gamma$  and IL-2 and reduced CD25+Foxp3+Tregs, IL-10 and TGF- $\beta$  [148]. Even though DC-derived exosomes enriched with MHC have been thought to elicit a T cell response, other studies have demonstrated that they can elicit a T cell response independent of the MHC if whole antigens are present [149]. Conclusively, DC-derived exosomes are equipped with the ability to elicit an immune response.

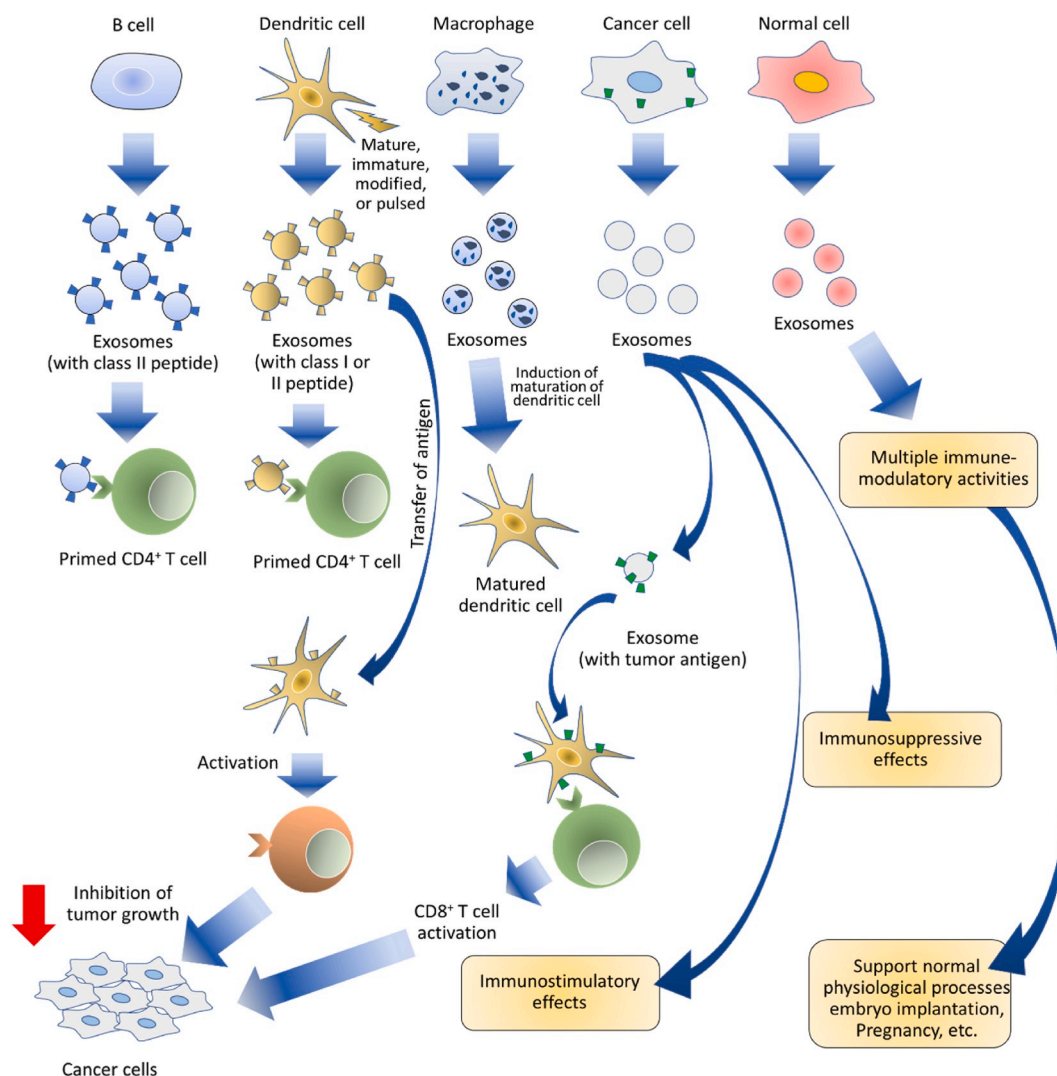
B cell lymphoma cell-derived exosomes (BL-EXO) have also been studied for their potential application as vaccines. It has been found that BL-EXO can cause clonal expansion of T cells, and is able to increase the secretion of IL-6 and TNF- $\alpha$ , and reduce the expression of

immunosuppressive cytokines, IL-4 and IL-10 [150]. Nonetheless BL-EXO have also been reported to cause apoptosis of CD4<sup>+</sup> T cells through MHC II and FasL [151]. BL-EXO exposed to heat shock are enriched with more HSP60 and HSP90, show an augmented level of immunogenic molecules, and are able to activate CD8<sup>+</sup> T cells to elicit anti-tumor activity [152].

Another study showed that exosomes released from plasmacytoma cells are enriched with tumor antigens and HSP70 protein that have been utilized as a vaccine. Following vaccination in mice, CTLs were produced and anti-tumor immunity was induced [153]. Mesothelioma cell-derived exosomes have been reported to be a source of antigen for DC-based immunotherapy with their use resulting in higher survival rates of tumor-bearing mice [154]. Notably, although tumor-derived exosomes can produce an anti-tumor response, they can also cause immunosuppression and hinder cancer immunotherapy. Therefore, it is crucial to decipher the immune-stimulating mechanism of exosomes so that they can be utilized as a carrier of antigens and adjuvants of cancer vaccines [155].

## 5. Exosome-based delivery systems for cancer therapeutics

Chemotherapy in the form of systemically infused antitumor drugs is the most common treatment to inhibit cancer progression. Nonetheless survival rates are sub-optimal and adverse effects include induction of drug resistance and severe side effects such as asthenia, cardiomyopathy, alopecia, and vomiting. Additionally, several chemotherapeutic drugs have a high non-specific toxicity, low solubility, poor bioavailability, rapid clearance and low intratumoral delivery [139,156–159]. For these reasons, new strategies for cancer targeted therapy such as drug delivery systems (DDSs) have been developed. These systems aim to enhance the efficacy and specificity of drugs and decrease their toxicity and side effects. DDSs can attach to or encapsulate therapeutic molecules for transfer into recipient cells. A few examples of synthetic DDSs are liposomes, ligand-conjugated nanoparticles, paramagnetic nanoparticles, micelles, dendrimers, nanocapsules, nanosponges, carbon nanotubes, and ultrasound microbubbles. DDSs have multiple advantages such as controlled composition, high loading capacity, large



**Fig. 3. Potential application of exosomes in immunotherapy and cancer vaccines.** Schematic representation showing the cascade of events followed by release of exosomes from B cells, DCs, macrophages, cancer cells, and normal cells, that can be employed in strategies for exosome-based cancer immunotherapy.

scale production, and low cost. Despite the benefits of these artificial platforms their application is hindered by several factors including adverse immunogenic reactions, toxicity, low biocompatibility, and interaction with plasma proteins that promote clotting, inflammation, and endothelium damage [160–162]. Liposomes are the most studied synthetic DDSs and contain an aqueous core surrounded by a lipid bilayer that can encapsulate hydrophilic or hydrophobic molecules, respectively. Since they are similar to the cell membrane, they have high biocompatibility, produce negligible toxicity, and can be efficiently internalized by tumor cells. Nevertheless, their rapid clearance by the reticuloendothelial system (RES) and their accumulation in the spleen and liver do not allow them to effectively reach target tissues. Although some liposomal formulations such as Doxil, DaunoXome, Depocyt, Myocet, Mepact, and Marqibo have been approved by the Food and Drug Administration (FDA) and are being marketed, disadvantages such as short half-life, sensitivity to sterilization, low stability in blood circulation, and low reproducibility limit the development of drug delivery strategies based on liposomes [157,161,163,164].

Recently, several studies have proposed the use of exosomes as potential DDSs for therapeutic application in cancer to overcome the problems associated with synthetic DDSs. These EVs serve as efficient vehicles for tumor-targeted delivery because their biological origin reduces the immune response, they can cross biological barriers including

the blood brain barrier (BBB) [165–168], and can transfer their cargo to nearby or distant cells. In addition there are adhesion proteins on their surface, and they are small in size with excellent stability, biocompatibility and safety [156,161,162,169]. Nonetheless the presence of five critical features favours their use in nanomedicine to achieve desirable anticancer effects following systematic administration. They have a prolonged circulation in blood vessels due to their ability to avoid phagocytosis by the RES, increased tumor accumulation, deep tumor penetration through ECM degradation, efficient cellular internalization, and an ability of intracellular drug release [169].

Exosomes can be secreted by different cell types but the most used ones in drug delivery are those derived from mesenchymal stem cells, tumor cells, immune cells, and food. MSCs can be isolated from different human tissues and can differentiate into several types of cells. These cells and their exosomes have therapeutic potential because of their antitumor activity, and their ability to suppress inflammation and to repair injured tissue. Furthermore, MSC-derived exosomes are characterized by minimal immunogenicity and can be produced on a large scale at low cost [156,170–173]. Tumor cells produce high quantities of exosomes with homing abilities, tumor-specific antigens on their surface and important roles in cancer progression from early stages to metastasis. Several studies have confirmed that tumor-derived exosomes loaded with drugs may decrease the number of cancer cells and enhance



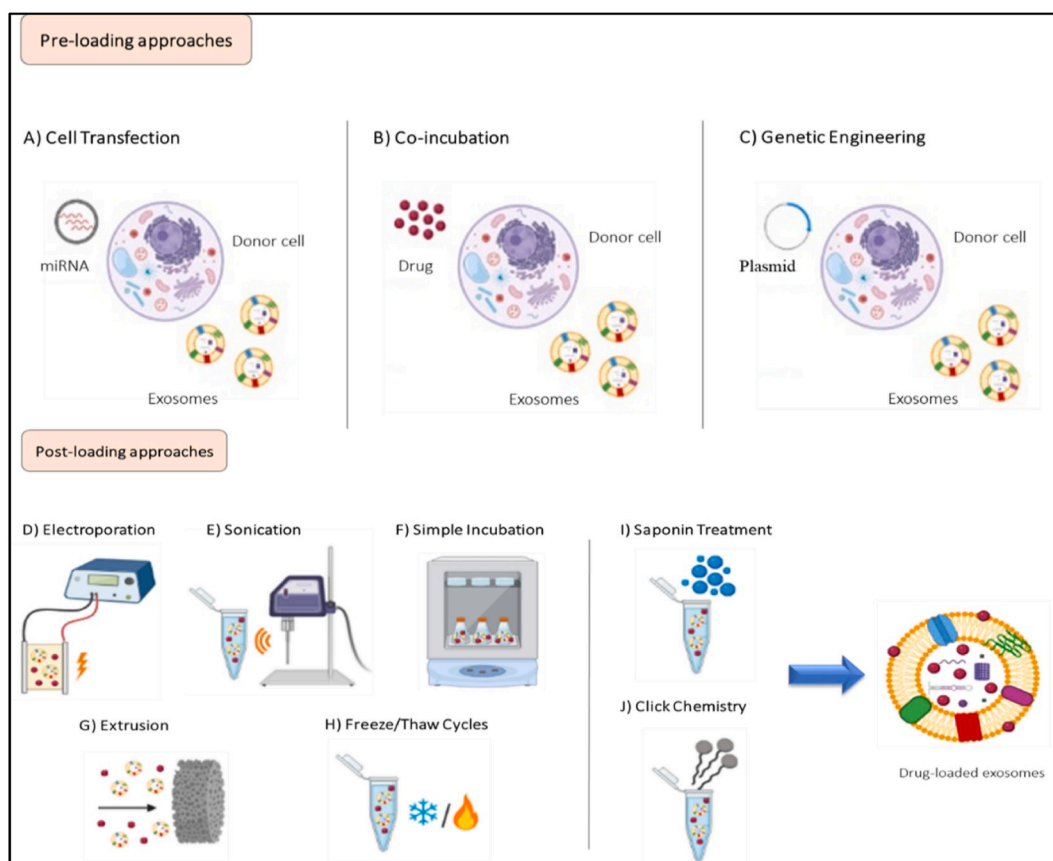
patient survival [156,158,159]. Exosomes secreted by immune cells such as macrophages and DCs have immunomodulatory roles and can inhibit tumor growth when they are loaded with chemotherapeutic drugs that can accumulate efficiently in cancer cells [156,172,174,175]. Food-derived exosomes are mainly isolated from milk and plants. In spite of their excellent stability under several conditions, ability for large scale production, and efficient loading of hydrophilic and hydrophobic molecules, more studies are needed to evaluate their toxicity [157,160,163,172,176,177].

Exosomes can be administered via intravenous, intraperitoneal, subcutaneous, intranasal, and oral routes. Although intravenous injection is the most used way to deliver drug-loaded exosomes into target cells, they are rapidly eliminated from the circulation and are stored in the spleen and liver when administered intravenously, hindering the drug accumulation in tumor tissues. Drug-loaded exosomes can also be locally administered by intraperitoneal and subcutaneous routes. An intranasal route can be used to deliver drugs into the central nervous system, while exosomes derived from milk or plant extracts can be administered orally [178].

Several methods have been reported for loading therapeutic molecules into exosomes such as pre-loading and post-loading approaches. These occur during exosome biogenesis and after exosome isolation, respectively. In the pre-loading approach, therapeutic molecules are packaged into cells via transfection, co-incubation, or genetic

engineering, and then are loaded into exosomes produced by them using cellular sorting machinery. The post-loading approach involves the incorporation of molecules directly into the exosomes after their isolation through physical and chemical mechanisms that induce the formation of transient pores in the lipid bilayer to increase membrane permeability, so promoting the uptake of the therapeutic agents. Electroporation, sonication, simple incubation, extrusion, freeze-thaw cycles, click chemistry, and saponin treatment are examples of post-loading techniques, as shown in Fig. 4 [158,160,162,163,179,180].

Chemotherapeutic drugs such as paclitaxel (PTX) and doxorubicin (Dox) have been encapsulated in exosomes to treat different cancer types. For instance, studies demonstrated that PTX incorporated by SR4987 MSCs and transferred to exosomes secreted by them had an anti-proliferative effect against CFPAC-1 cells and inhibited pancreatic tumor growth [173]. High levels of cytotoxicity were observed in 3LL-M27 lung carcinoma cells due to the accumulation of significant amounts of macrophage-derived exosomes loaded with PTX (exoPTX) in comparison with synthetic nanoparticles. Moreover, PTX packaged into exosomes produced toxicity in MDR cells after being administered intranasally in mice with lung metastasis, confirming their efficacy to treat resistant tumors [181]. Similarly, an exosomal formulation based on vectorized AA-PEG exosomes containing PTX (AA-PEG-exoPTX) to deliver the drug into target cells efficiently decreased lung metastasis and increased survival in a mouse model [174]. On the other hand,



**Fig. 4. Various methods of loading cargoes in exosomes for therapeutic delivery.** In pre-loading methods, therapeutic molecules are incorporated into donor cells before the production of exosomes. During exosome biogenesis, incorporated molecules are packaged within exosomes to be used for therapeutic purposes. miRNA, siRNA, and mRNA can be loaded into parent cells through transfection (A). Moreover, some parent cells can uptake drug molecules by passive diffusion when they are co-incubated (B). Engineered exosomes can be produced through introduction of plasmids into parent cells to induce the expression of therapeutic molecules in exosomes (C). In post-loading methods, drug molecules are loaded directly into exosomes after isolation through physical and chemical methods. Physical methods such as electroporation (D), sonication (E), simple incubation (F), extrusion (G), and freeze/thaw cycles (H) increase exosome membrane permeability to uptake drug molecules. Chemical methods such as saponin treatment (I) and click chemistry (J) can also be used. Saponin treatment promotes the formation of pores due to its interaction with membrane cholesterol, while click chemistry enables the binding of drug molecules to the external surface of the exosome membrane. Created with BioRender.com.

Dox-loaded exosomes engineered to enhance the expression of DARPins ligands were preferentially internalized by HER2<sup>+</sup> breast cancer cells when compared with HER2<sup>-</sup> cells, confirming the targeted drug delivery ability of exosomes [182]. Similarly, Tian et al. engineered immature DCs (imDCs) via transfection to express the glycoprotein 2b (Lamp2b) bound with iRGD specific to  $\alpha v$  integrin. Exosomes produced by these cells were loaded with Dox and efficiently delivered to breast cancer cells with  $\alpha v$  integrins on their membrane. As a consequence, the targeted exosomes inhibited tumor growth without producing cardiotoxicity [175]. Studies of fibrosarcoma cell-derived exosomes loaded with Doxil (D-exo) and incubated with fibrosarcoma cells and cervical cancer cells showed preferential uptake by their parent cells, increasing the drug concentration inside the tumor and reducing tumor size and cardiac toxicity [139]. Other drugs such as aspirin and celastrol also can be incorporated into exosomes for cancer therapy. A new strategy was proposed to encapsulate aspirin into exosomes derived from colorectal and breast cancer cells, transforming it into a nanostructure amorphous with hydrophobic and hydrophilic properties. Encapsulation of the amorphous aspirin could improve its dissolution and its anticancer activity [183]. Other studies have suggested an exosomal formulation with exosomes derived from milk loaded with celastrol (CEL), a plant terpenoid with antitumor properties. This formulation showed an anti-proliferative activity against NSCLC cells, induction of apoptosis, and strong inhibition of NF- $\kappa$ B when compared with free CEL [176]. Despite the significant advances in the development of exosome-based targeted delivery systems, more studies are needed to validate their clinical application.

Interestingly, members of the ISEV and the European Cooperation in Science and Technology (COST) program of the European Union, namely European Network on Microvesicles and Exosomes in Health and Disease (ME-HaD) provide a summary of the latest developments and information about EV-based therapies. They emphasize that the clinical use of EVs demands the classification of EV-based therapies in accordance with various prevailing regulatory systems. Notably, it is crucial to determine whether EVs can be viewed as active drug constituents or key drug delivery vehicles. To achieve an effective and impregnable clinical translation of EV-based therapies, collaboration is required of clinicians, scientists, and authorized regulatory bodies [184].

## 6. Concluding remarks

The components of exosomes, which largely depend on their cell of origin, are carried to recipient cells and show various functions in physiological as well as pathological conditions including cancer progression. Most events are guided by each of these exosomal components via autologous or heterologous uptake. Interestingly, the release of exosome is significantly enhanced in different diseases including cancer compared with the normal condition. Various scientists have attempted to identify specific target molecules that are responsible for the increased release of exosomes. In addition, various drugs have been discovered for inhibiting the release or uptake of pro-oncogenic exosomes in TME that can be utilized as novel cancer therapeutics. Another emerging area of research related to exosomes that has gained considerable attention is their application in immunotherapy to develop potential vaccines against cancer. Various cells have been employed for isolating exosomes to serve as cancer immunotherapy such as B cells, DCs, macrophages, cancer cells, and normal cells although each of these exosome sources possesses different advantages and disadvantages for developing vaccines against cancer. One of the important requirements for the cargoes of exosomes to show functional phenotypes in the recipient cells is their delivery by exosomes with their ability of inter-cellular communication. This ability to carry functional components has been widely utilized for delivery of therapeutics to target cancer cells. Overall, exosomes are tiny vesicles with multiple functions. They have the ability to halt disease progression and, depending on the source and

their constituents, may serve as an important therapy for cancer.

## Declaration of competing interest

None.

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