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Extracellular Vesicles in Cancer Therapy

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Abstract

Extracellular vesicles (EVs), including a variety of membrane-enclosed nanosized particles carrying cell-derived cargo, mediate a major type of intercellular communication in physiological and pathological processes. Both cancer and non-cancer cells secrete EVs, which can travel to and influence various types of cells at the primary tumor site as well as in distant organs. Tumor-derived EVs contribute to cancer cell plasticity and resistance to therapy, adaptation of tumor microenvironment, local and systemic vascular remodeling, immunomodulation, and establishment of pre-metastatic niches. Therefore, targeting the production, uptake, and function of tumor-derived EVs has emerged as a new strategy for stand-alone or combinational therapy of cancer. On the other hand, as EV cargo partially reflects the genetic makeup and phenotypic properties of the secreting cell, EV-based biomarkers that can be detected in biofluids are being developed for cancer diagnosis and for predicting and monitoring tumor response to therapy. Meanwhile, EVs from presumably safe sources are being developed as delivery vehicles for anticancer therapeutic agents and as anticancer vaccines. Numerous reviews have discussed the biogenesis and characteristics of EVs and their functions in cancer. Here, I highlight recent advancements in translation of EV research outcome towards improved care of cancer, including developments of non-invasive EV-based biomarkers and therapeutic agents targeting tumor-derived EVs as well as engineering of therapeutic EVs.

Keywords

Extracellular vesicles; Exosomes; Tumor microenvironment; Premetastatic niche; Metastasis; Cancer therapy; Drug delivery vehicles; Anticancer vaccines

An introduction to EVs

The past decade has witnessed a rapid growth of research and technology development related to extracellular vesicles (EVs). Secretion of EVs is a fundamental and evolutionarily conserved biological process broadly found from bacteria to humans and in all cell types in a higher organism [1, 2]. EV is an all-inclusive term for a variety of membrane-enclosed nanosized particles carrying cell-derived cargo. Two major types of EVs that have been long recognized are exosomes and microvesicles. In addition, some special types of EVs

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are secreted by certain types of cells or under certain conditions, such as apoptotic EVs and autophagic EVs as well as cancer cell-secreted large oncosomes [3, 4]. Exosomes exhibit a characteristic small size of 40–150 nm and originate from the endosomal system. They are formed in the lumen of an endocytic compartment called multivesicular body (MVB) and are secreted when MVBs fuse with the plasma membrane. Microvesicles with a typical size of 150–1000 nm, as well as large oncosomes that can reach 1–10 μm, are formed and shed through plasma membrane budding and sometimes referred to as large EVs. A subgroup of microvesicles known as arrestin domain-containing protein 1-mediated microvesicles (ARMMs) exhibit a smaller size of 50–200 nm and, together with exosomes, are referred to as small EVs. Recent studies have significantly expanded and refined the catalog of EV subpopulations [4, 5]. Exosomes are further separated into small exosomes with a ~60–80 nm diameter and large exosomes with a ~90–120 nm diameter with different biophysical properties, molecular profiles, and biodistribution patterns [5]. Mitochondria-derived vesicles, sometimes called mitovesicles, are identified as active regulators in mitochondrial function by transferring mitochondrial DNA and proteins [6-8]. Non-membranous extracellular nanoparticles have been discovered, including exomeres and supermeres (named after their identification from supernatant of exomeres), whose characteristics and composition are distinct from exosomes and microvesicles and from each other [5, 9]. Due to the partially overlapping size range, biophysical properties, morphological characteristics, and protein marker expression among different subpopulations of EVs and particles, and also as a result of the limitations of widely used EV purification methods such as differential centrifugation, many studies experiment on a mixed EV population containing exosomes, microvesicles, and non-membranous particles.

Beyond the initially discovered functions of EVs in removal of unwanted proteins [10-12] and antigen presentation [13], a large body of research since the 2000s has established a critical role of EVs in intercellular, inter-tissue, and inter-organ communication [14-20]. EVs contain a blend of RNA (including both mRNA and noncoding RNA species such as miRNA), double-stranded DNA, proteins, lipids, glycoconjugates, and metabolites derived from the parental cell. Enclosed by lipid membranes, cargo of EVs is protected from extracellular degradative enzymes such as proteases and nucleases, allowing long-range transfer across various tissues via blood circulation. Although mechanisms governing cargo sorting into EVs remain to be fully elucidated, it is clear that this process is highly selective, as the levels of many EV cargo molecules are not proportional to their intracellular levels. In consistency with the heterogeneous EV biogenesis pathways, EV cargo molecules such as RNA are not evenly distributed in all EVs. Barman et al. show that RNA is highly enriched in a subpopulation of small EVs with a higher density and these RNA-containing EVs are generated at the endoplasmic reticulum membrane contact sites through the control by VAP-A and its binding partner CERT [21]. Certain sequence motifs are found to control the EV secretion or cellular retention efficiency of miRNAs; different cell types, such as adipocytes, endothelium, liver, and muscle cells, exhibit different preferential use of sorting motifs to form cell type-specific spectra of EV miRNAs [22]. RNA-binding proteins that recognize these sorting motifs, such as Alyref and Fus, are involved in the selective sorting of some EV miRNAs [22]. Several other RNA-binding proteins have also been implicated in selective secretion of EV RNA. Y-box-binding protein 1 binds to and is required for

the exosomal sorting of multiple small noncoding RNA species including miRNA, tRNA, Y RNA, and vault RNA [23, 24]. SYNCRIP, sumoylated hnRNPA2B1, and HuR have been shown to control miRNA secretion in certain cell types [25-27]. The LC3-conjugation machinery has been shown to specify the packaging and secretion of diverse RNA-binding proteins and noncoding RNAs into EVs [28]. In addition, Temoche-Diaz et al. show that metastatic breast cancer cells utilize both selective and non-selective miRNA sorting mechanisms, generating at least two subsets of EVs, and that the Lupus La RNA-binding protein mediates sorting of miR-122, a selectively secreted miRNA [29]. La binds to two motifs in miR-122, leading to the miRNA's sorting into EVs [29]. Compared to non-cancer cells, the secretion levels of EV miR-122 are remarkably higher in breast cancer cells, and cancer cell-secreted miR-122 exerts profound systemic effects and promotes metastasis [30-32]. The La and related RNA-binding proteins (LARPs) are dysregulated in cancer, with most of the LARP family members including La showing an oncogenic effect [33]. It remains to be determined to what degree dysregulation of RNA-binding proteins such as La during malignant transformation alters the miRNA secretion profile as an integral part of cancer pathophysiology. As exemplified by these recent findings, the selective EV cargo sorting mechanisms are specific, diverse, and cellular context-dependent, making them possible targets for pharmacological intervention.

The effects of EVs on recipient cells are also context-dependent, reflecting the multifactorial consequence of cell/tissue-specific adsorption and uptake of EVs, the composition and abundance of functional EV cargo, and the degree of adaptability of recipient cells in response to certain EV cargo. Although our understanding of EVs' interaction with recipient cells and the subcellular re-localization of EV cargo upon internalization is still in its infancy, a variety of mechanisms have been implicated in these processes, including clathrin-dependent endocytosis and clathrin-independent pathways such as phagocytosis, macropinocytosis, and plasma membrane fusion [34]. Different sets of surface proteins on EVs and recipient cells are involved in different EV uptake pathways, granting a layer of specificity in targeted delivery of EV cargo. In some other cases, EV-induced signaling in recipient cells does not require internalization of EVs or their luminal cargo but is triggered by molecular interactions at the interfaces of EVs and recipient cell membranes. For example, EVs from breast cancer cells induce proinflammatory NF-κB signaling in macrophages; this is through activation of Toll-like receptor 2 on macrophages by EV surface proteins and is independent of the luminal contents of EVs [35]. Blocking the uptake or the pathogenic function of EVs in recipient tissues represents another strategy for EV-targeted therapy.

Functions of EVs in cancer

Through selective cargo packaging and delivery, EVs function as a versatile player in the crosstalk between tumor and host to influence every aspect of tumor biology [36-39]. EVs are secreted by both cancer and non-cancer cells, and participate in tumor initiation, progression, and metastasis through short-range and long-range (sometimes systemic) effects on various cell types, as exemplified in the following sections.

Local effects of tumor-derived EVs

Within a solid tumor, EVs can be transferred between any two cells of the same or different types that co-reside in the tumor microenvironment. This paracrine signaling is similar to those mediated by cytokines but has the unique capability to co-transfer multiple functional molecules in a “bulk delivery”, leading to greater potency and diversity of the regulatory effects. Transfer of EVs from more aggressive cancer cells to less aggressive ones usually results in gain of the aggressive phenotype by the latter [40-44], thereby contributing to the spreading and selection of beneficial traits during cancer cell evolution. EVs from cancer cells carrying oncogenic genetic alterations, such as *EGFRvIII* and amplification of *MYC*, inherit these alterations in forms of DNA, RNA, and/or proteins, which can be horizontally transferred to other cancer cells lacking the advantageous feature [15, 45, 46]. In an extreme case, cancer cell-secreted exosomes even enable non-tumorigenic epithelial cells to form tumors [47]. In addition, cancer cell-derived exosomes carry extracellular matrix proteins such as fibronectin, which may promote cancer cell adhesion and directionally persistent movement [48, 49].

EVs also mediate the interplay between cancer cells and non-cancer niche cells such as stromal fibroblasts. Cancer cell-secreted EVs, through EV-encapsulated functional cargo such as TGF-β [50], AKT1 kinase and miRNAs [51, 52], induce activation of normal fibroblast or differentiation of mesenchymal stromal cells towards a myofibroblast phenotype; these stromal cells in turn promote tumor growth and progression by producing cytokines and extracellular matrix proteins [50-53]. EVs from breast cancer cells have also been shown to induce metabolic reprogramming of cancer-associated fibroblasts (CAFs) through miR-105-mediated activation of MYC signaling, which grants CAFs the ability to detoxify metabolic wastes such as lactic acid and ammonium as another way to support sustained tumor growth [54]. Vascular endothelial cells are also well-demonstrated targets of cancer cell-derived EVs. Many EV cargo molecules have been found to promote angiogenesis, such as miR-9 [55], miR-210 [56], ephrin type B receptor 2 [57], angiogenic proteins angiogenin and VEGF [15], and tetraspanin Tspan8/CO-029/D6.1A [58, 59].

On the other hand, EV transfer from non-cancer niche cells to cancer cells also influences tumor growth. Activation of the transcription factor heat shock factor 1 pathway in CAFs leads to increased secretion of EV cargo inhibin subunit beta A and thrombospondin 2, which promote an aggressive gastric cancer phenotype [60]. Upon stimulation by cancer cells, stromal fibroblasts secrete more unshielded RN7SL1 RNA into exosomes, which activate antiviral signaling in cancer cells to promote cancer progression [61, 62]. CAF-secreted exosomes also carry metabolites including amino acids, lipids, and TCA-cycle intermediates, which can fuel cancer cell metabolism to support tumor growth [63]. As such, the mutual exchange of EVs between cancer and stromal cells contribute to a coevolution of different cell types occupying the same niche.

Distant and systemic effects of tumor-derived EVs

Traveling through the circulation, tumor-derived EVs can cross organs to arrive at a distant site, often contributing to establishment of a pre-metastatic niche in preparation for cancer metastasis [36-38, 64-66]. EVs from metastatic breast cancer cells carry miR-105,

which downregulates tight junctions in endothelial monolayers in various organs including tumor, lungs, liver, and brain. The consequent systemic vascular leakiness facilitates distant metastasis of breast tumor cells [67]. In addition, miR-181c in EVs from brain-metastatic breast cancer cells contributes to the destruction of blood-brain barrier to enhance brain metastasis [68]. Melanoma-derived exosomes also induce vascular leakiness and recruit bone marrow progenitor cells to pre-metastatic sites through inducing inflammation factors [69]. It is worth noting that this function of tumor-derived EVs to systemically increase blood vessel permeability may also affect the trafficking of immune cells, pathogens, and other particles including therapeutic agents across the blood vessel walls to indirectly influence tumor progression and therapeutic efficacy.

Many cell types at a pre-metastatic site respond to tumor-derived EVs. Exosomes from pancreatic cancer cells induce TGF-β production in Kupffer cells, which then stimulates hepatic stellate cells to increase fibronectin production and facilitates recruitment of bone marrow-derived cells to the liver, resulting in a tumor-promoting pre-metastatic niche [70]. Breast cancer cells secrete EV-encapsulated miR-122, which suppresses glucose metabolism in lung fibroblasts and astrocytes to allow more extracellular glucose to be used by metastasized cancer cells [30]. A higher level of cell migration-inducing and hyaluronan-binding protein (CEMIP) is detected in exosomes from brain-tropic but not lung- or bone-tropic metastatic cancer cells. $CEMIP^{+}$ exosomes are taken up by brain endothelial and microglial cells to induce endothelial cell branching and inflammation, leading to brain vascular remodeling and metastasis [71]. EVs released by non-cancer cells in a metastatic niche, in turn, can influence metastasized cancer cells. Exosomes secreted by astrocytes in a metastatic brain niche contain miRNA cargo to silence PTEN expression in breast cancer cells metastasized to the brain, which promotes cancer cell survival and proliferation in the brain niche [72].

Cancer cell-secreted EVs also mediate cancer's systemic effects by influencing tissues that are not usually colonized by cancer cells, such as skeletal muscle. Tumor-derived EVs act on muscle cells to promote muscle cell death or induce muscle wasting through EV cargo miRNAs and proteins including Hsp70 and Hsp90 [73, 74]. A recent study by Yan et al. shows that breast cancer-derived EV miR-122 suppresses *O*-linked N-acetylglucosamine (*O*-GlcNAc) protein modification in muscle by targeting *O*-GlcNAc transferase. This results in an increase in the ryanodine receptor 1 proteins and a higher level of cytosolic Ca^{2+}, which in turn activates calpain-mediated cleavage of desmin filaments and myofibrillar destruction, leading to reduced skeletal muscle mass and contractility [31]. EVs from Lewis lung carcinoma (LLC) cells as well as serum from lung cancer patients contain interleukin-6, which activates STAT3 signaling in recipient cells to respectively induce atrophy in myotubes and lipolysis in adipocytes [75]. In addition, EVs from LLC and breast cancer cells also induce adipose tissue browning through parathyroid hormone-related protein-mediated activation of PKA signaling pathway [76] or miRNA-mediated downregulation of ERK1/2-PPARγ and IRS-Glut4 pathways and activation of autophagy [77]. These effects on skeletal muscle and adipose tissue together contribute to cancer cachexia. In turn, EVs from mature but not undifferentiated adipocytes activate HIF-1α signaling in breast cancer cells, leading to a more invasive phenotype in cancer cells and enhanced metastasis [78], which might be more significant in obese settings.

Remarkably, tumor-derived EVs may adapt endocrine tissues as a strategy to broaden and strengthen a systemic effect at the whole-body level. Cao et al. show that breast cancer-derived EV miR-122 suppresses glycolysis and ATP-dependent insulin exocytosis in pancreatic β-cells, leading to enhanced endogenous glucose production, impaired glucose tolerance, fasting hyperglycemia, and increased tumor growth. These effects can be abolished by inhibiting tumor EV secretion, blocking the function of miR-122, and by insulin supplementation [32]. Compared to non-cancer controls, breast cancer patients show higher levels of circulating miR-122, higher fasting glucose, and lower fasting insulin. As such, tumor-derived EVs can impair whole-body glycemic control, which may contribute to tumor progression and a higher incidence of type 2 diabetes in some breast cancer patients. Figure 1 shows a schema of the versatile systemic effects of tumor-derived EVs highlighting these recent findings.

Immunomodulatory effects of tumor-derived EVs

Tumor-derived EVs, in most cases, suppress antitumor immunity to promote tumor progression. For example, exosomes from cancer cells have been shown to suppress NK cell function [79] and to trigger tumor-promoting humoral immunity by interacting with B cells [80]. Tumor-derived EVs also suppress T cell function or induce apoptosis of activated T cells through EV cargo CD39, CD73, and Fas ligand [81, 82]. EV-associated programmed death-ligand 1 (PD-L1) suppresses T cell function to facilitate immune escape and promote tumor growth [83, 84]. Although exosomal PD-L1 appears to be resistant to anti-PD-L1 antibody blockade, genetic blockade of exosomal PD-L1 induces systemic antitumor immunity and memory [85]. Tumor-derived EVs induce myeloid-derived suppressor cells in the primary tumor and at metastatic sites to promote tumor growth and metastasis [86, 87]. The effects of tumor-derived EVs on macrophages have also been demonstrated, such as promoting M1/M2 macrophage polarization [88, 89] and activating NF-κB-mediated production of pro-inflammatory cytokines including IL-6 and TNF-α [35].

In a specific context, however, tumor-derived EVs can stimulate antitumor immunity. Moroishi et al. show that EVs from tumors deficient in Hippo pathway induce a type I interferon response to enhance anti-tumor immunity, resulting in tumor destruction in immunocompetent mice [90]. Another study by Plebanek et al. shows that exosomes from poorly metastatic melanoma cells induce immune surveillance by patrolling monocytes, leading to cancer cell clearance at the pre-metastatic niche [91]. Exosomes secreted by melanoma cells overexpressing the heat shock protein HSP70 also carry HSP70 and can activate NK cells to reduce primary and metastatic tumor burdens [92]. Notably, EVs from breast cancer cells treated with topotecan, an inhibitor of topoisomerase I, carry higher levels of DNA, which activates dendritic cells through the cGAS-STING pathway to elicit antitumor immunity [93]. Mechanisms through which nuclear contents, especially genomic DNA, are loaded into EVs are poorly understood but may involve collapse of micronuclei frequently found in cancer cells and subsequent shuttling of released nuclear contents into MVBs [94, 95]. Treatment with genotoxic drugs, which induces formation of micronuclei, also increases the population of EVs containing genomic DNA [95].

Immune cells also secrete EVs to communicate with each other and with cancer cells, as comprehensively discussed in a recent review [96]. EVs secreted by activated macrophages induce the invasiveness of cancer cells by transferring cargo miRNA [97, 98]. Exosomal transfer and exchange of miRNAs between neuroblastoma cells and monocytes contribute to tumor resistance to chemotherapy [99]. ADAM15 in macrophage-derived exosomes, through binding to integrin αvβ3, suppresses vitronectin- and fibronectin-induced cancer cell adhesion, growth, and migration [100]. Antigen-stimulated T cells secrete EVs that contain genomic and mitochondrial DNA, which induces the cGAS-STING pathway and IFN1 response in dendritic cells to grant them resistance to subsequent viral infections [101]. Whether a similar mechanism exists in tumor immunology remains to be determined. As discussed later in this review, EVs derived from immune cells such as dendritic cells have been exploited for cancer therapy.

Effects of EVs in therapy response and resistance

Given that EV secretion is dynamically regulated by the states of secreting cell and by microenvironmental factors and that tumor response to therapies is a behavior at the populational level involving various subsets of cancer and non-cancer cells and their inter-populational crosstalk, it is not surprising that EVs play a critical role in anticancer therapy response and development of drug resistance. Reported mechanisms of EV-mediated chemoresistance include direct efflux of drug from cancer cells, transfer of a drug-resistant phenotype between different subsets of cancer cells, and the pro-survival effect of EVs from non-cancer cells in the tumor microenvironment. Cancer cells can alleviate the intracellular accumulation of chemotherapeutic drugs such as doxorubicin by shedding drug-containing EVs; expression of genes related to EV shedding is correlated with drug resistance in cancer cells [102]. In addition, EVs from drug-resistant cancer cells carry and transfer P-glycoprotein, a drug transporter [103], or regulators of P-glycoprotein (e.g., transient receptor potential channel 5 [104]), to sensitive cancer cells, resulting in spreading of a drug-resistant phenotype and contributing to drug-induced cancer cell evolution.

Conventional anticancer therapies such as chemotherapy are known to alter the composition and abundance of EV cargo. Docetaxel and doxorubicin significantly increase EV secretion of several miRNAs including miR-9-5p, miR-195-5p, and miR-203a-3p, which only occurs in breast cancer cells but not in non-cancer cells. These chemotherapy-induced EV miRNAs, upon uptake by other cancer cells not exposed to chemotherapy, stimulate a cancer stem cell phenotype and promote resistance to therapy [105]. Similarly, other pro-survival EV cargo molecules, such as survivin [106], are induced by chemotherapeutic drugs and transferred between different subsets of cancer cells to help the population survive the treatment. In addition, EV-encapsulated mitochondrial DNA has been shown to influence hormonal therapy-resistant breast cancer cells, promoting their treatment-induced escape from dormancy and leading to hormonal resistance [7].

EVs secreted by non-cancer cells in the tumor microenvironment also contribute to tumor resistance to therapies. CAF-secreted EVs and their miRNA cargo can be transferred to colorectal cancer cells to promote chemoresistance and metastasis [107]. CAFs are found to be intrinsically resistant to chemotherapeutic drug gemcitabine; upon gemcitabine treatment,

CAFs secrete a higher amount of EVs that can promote survival and chemoresistance in pancreatic cancer cells [108]. EVs secreted by CAFs have also been shown to promote therapeutic resistance in cancer cells through unshielded RNA cargo [61, 62]. Overall, these reported mechanisms of EVs in promoting tumor resistance to therapies suggest that simultaneously targeting tumor cells and tumor-released EVs could be a promising strategy to improve tumor response to anticancer therapy and prevent development of drug resistance.

EVs as cancer biomarkers

Virtually all types of cells in the human body can secrete EVs [2]. As a result, EVs in biospecimens such as blood and urine are derived from a mixed source of parental cells. In blood, a majority of circulating EVs are secreted by blood cells, mostly platelets and erythrocytes but also including immune cells, whereas only a small subset of circulating EVs are from other cell types such as endothelial cells, adipocytes, hepatocytes, muscle cells, and cells of the central nervous system [109]. Even EVs derived from bacteria can be detected in human blood especially in patients with intestinal barrier dysfunction [110]. In urine, EVs are originated from kidneys, bladder, prostate (males), and utero-vaginal tract (females) as well as the residing immune cells, bacteria, and yeast. EVs from other distant sites may also pass the glomerular filtration barrier and basement membrane of the kidney to eventually reach the urine [111]. The focus of developing discriminatory biomarkers for cancer diagnosis has been to identify and detect cancer cell-derived EVs and their characteristic cargo, although EVs of non-cancer origins (such as immune cells) reflecting functional parameters of the host may also be associated with cancer development, progression, and clinical outcomes. Assessing EVs that are released by tumor cells into biofluids has all the advantages of liquid biopsy as compared with histological markers, such as being minimally invasive, rapid (allowing real-time result), potentially high throughput, and partially overcoming intra-tumoral heterogeneity. However, like all types of biomarkers, the sensitivity and specificity of EV-based molecular markers remain significant challenges in preclinical and clinical development.

Tumor-specific DNA mutations in EVs

Partially owing to the technology readiness for sensitive and specific detection of nucleic acids, EV-associated double-stranded DNA fragments and various RNA species (including mRNA, miRNA, and long noncoding RNA) have been exploited as candidate biomarkers to be detected from biofluids such as blood and urine. Signature genetic and epigenetic alterations, including gene mutation, fusion, and alternative splicing events that are associated with oncogenic signaling or resistance to targeted therapy, are inherited from tumor cells harboring the alterations to secreted EVs. In a highly simplified scenario, presence of such alterations in a total, unfractionated (here defined as not enriched by tumor-specific molecular markers) EV population reflects, possibly in a near-proportional manner, presence of tumor cells carrying the alterations. In non-small cell lung cancer, a combined isolation of exosomal RNA/DNA and cell-free DNA from plasma enables sensitive detection of activating EGFR mutations and $EGFR^{T790M}$ resistance mutation, which are respectively associated with tumor responsiveness to EGFR inhibitors [112]. In

pancreatic cancer, where activating KRAS mutations are found in >90% of cases and often co-occur with genetic alterations of TP53 [113], KRAS mutations and $TP53^{R273H}$ mutation can be detected in circulating exosome-derived DNA [114, 115]. Compared to circulating cell-free DNA, KRAS mutations in exosomal DNA exhibit a higher prevalence especially in patients with early-stage pancreatic cancer [115], suggesting an improved sensitivity for early detection or cancer risk assessment. However, a significant subset of healthy subjects also carry KRAS mutations in exosomal DNA [114, 115], indicating the limitation in cancer screening and diagnosis when this exosomal marker is used alone. This drawback might be alleviated through combination with additional EV-based or non-EV markers with high specificity but suboptimal sensitivity for clinical detection of cancer.

RNA and miRNA markers in EVs

In 2019 the U.S. Food and Drug Administration approved the first exosome-based liquid biopsy test, known as the ExoDx Prostate IntelliScore (EPI) test for prostate cancer detection, which measures RNA levels of three genes (PCA3 and ERG relative to SPDEF) in total EVs isolated from first-catch (non-digital rectal exam) urine samples [116, 117]. ERG overexpression is highly prevalent in prostate cancer as a result of cancer-specific TMPRSS2:ERG gene fusion. TMPRSS2:ERG fusion transcripts, in combination with PCA3, have shown high specificity and sensitivity as urine-based biomarkers for prostate cancer [118, 119]. The success with EPI test represents a strategy to assess previously established tissue or liquid biopsy (such as urine sediments) markers for their compartmentalized presence in EVs to achieve a better performance in cancer diagnosis. However, this strategy is based on the prerequisites that the amount of such biomarker captured in EVs faithfully reflects its abundance in the secreting cell and that the secretion efficiency is relatively constant in tumors from different patients and under different host conditions. In addition, the diverse and heterogeneous cellular origins of EVs in biofluids impose additional challenges when total EVs are used for cancer detection, as those non-cancer cell-derived EVs, either carrying the biomarker or not, would respectively interfere with the specificity or sensitivity of detection.

Other than oncogenic mutations and alterations exclusively found in cancer cells and their EVs, cancer cell-derived EVs also carry different repertoires and altered abundances of cargo RNA, especially some miRNAs, compared to EVs from their normal counterparts. This feature allows development of a much broader group of biomarkers based on their altered (often elevated) levels in tumor-derived EVs. Given that the biogenesis and function of miRNAs can be highly context-dependent, it is not surprising to see different EV cargo miRNAs associated with different types (and subtypes) of cancer. High exosomal levels of miR-10b, miR-21, miR-30c, and miR-181a and low levels of let-7a in blood can differentiate pancreatic cancer from non-cancer controls [120]. For screening of lung cancer, circulating exosomal miR-378a, miR-379, miR-139-5p, and miR-200b-5p can be used to differentiate nodule from non-nodule, whereas miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100, and miR-154-3p can further detect lung adenocarcinoma from the nodule population [121]. Exosomal miR-1246 and miR-21 are detected at significantly higher levels in the blood of breast cancer patients compared to healthy controls [122]. In addition, higher levels of circulating EV miR-122 are detected in breast cancer patients

using either CD9/CD63/CD81-based total EV isolation from serum or enrichment for circulating EVs expressing CD24, a previously proposed marker for breast cancer cell-derived EVs [32, 123]. Even by testing RNA extracted directly from serum without EV enrichment, a higher miR-122 level in circulation can predict poor response to anticancer therapy and development of metastasis in breast cancer patients [124]. Altered secretion of miRNAs into the EVs may also be associated with certain oncogenic mutations in cancer cells. For example, KRAS mutation status in colorectal cancer cell lines is associated with different EV miRNA secretion pattern, including a higher level of miR-10b in wild-type EVs and a higher level of miR-100 in mutant KRAS EVs [125]. Theoretically, EV-based DNA and RNA biomarkers can potentially enable detection of all types of cancer including those without signature genetic alterations, as dysregulated gene expression is a common hallmark of cancer. Due to their low but still detectable presence in some non-cancer EVs, the specificity of "level-based" biomarkers could be lower than those genetic alterations unique to cancer cells. This limitation may be alleviated by multiplex detection of several markers to increase the specificity. In addition, multiplex marker detection and generation of a multi-marker score may also serve as a partial solution to challenges associated with tumor heterogeneity, by enabling a "mix-and-match" customizable combination of biomarkers to detect cancer cells with varying degrees of genetic diversity.

Protein markers in EVs

In addition to DNA and RNA, EV-associated proteins are also exploited as biomarkers in cancer detection and prognosis. There has been a long-standing interest in identifying EV proteins that are highly specific to the secreting cancer cells, especially those on the outer surface of EVs for direct detection and isolation from blood and other biospecimens. Using mass spectrometry, Melo et al. identify glypican-1 (GPC1), a cell surface proteoglycan, to be expressed on EVs from MDA-MB-231 breast cancer cells but not those from non-cancer cells. The team further shows that higher levels of $GPC1^+$ circulating EVs are detected in 75% of breast cancer patients and all pancreatic cancer patients studied compared to the baseline levels in healthy subjects [126]. Furthermore, levels of $GPC1^+$ circulating EVs can distinguish pancreatic cancer cases from healthy controls and benign pancreatic disease with high specificity and sensitivity, and can reflect pancreatic cancer burden [126]. Peinado et al. report that a melanoma-specific protein, tyrosinase-related protein-2, is detected in circulating EVs from patients with stage III-IV melanoma at significantly higher levels compared to healthy controls and predicts disease progression in patients with stage III melanoma [69]. Another seminal study by Hoshino et al. shows that the integrin expression pattern on tumor-derived EVs at least partially determines their selective uptake by organ-specific cells and contributes to the preparation of pre-metastatic niche [127]. In cancer patients, levels of certain integrins on circulating EVs are associated with organ-specific metastasis: higher exosomal ITGβ4 levels are found in patients with lung metastasis regardless of tumor type and in breast cancer patients prior to clinically detectable lung metastasis, whereas higher exosomal ITGαv levels are associated with liver metastasis [127]. As for brain metastasis, Rodrigues et al. show that the cell migration-inducing and hyaluronan-binding protein is higher in exosomes from patients with brain metastasis and can predict brain metastasis and patient survival [71]. A comprehensive proteomic profiling of extracellular vesicles and particles (EVPs) has been carried out by Hoshino et al. using

patient-derived specimens, identifying pan-EVP markers as well as tumor-type-specific EVP proteins in tissue explants and plasma [128]. These protein markers may be used for cancer detection and classification of tumors of unknown primary origin.

In addition to detecting cancer of various stages and predicting tumor progression, EV-based biomarkers could also predict patient response to therapy. Chen et al. report that EVs secreted by metastatic melanomas carry PD-L1, which suppresses the function of CD8 T cells to promote tumor growth. Levels of circulating exosomal PD-L1 in patients with metastatic melanoma exhibit a varying degree of changes during the course of anti-PD-1 therapy; a greater increase in exosomal PD-L1 during early phase of the treatment stratifies clinical responders from non-responders [83]. Another study generates an EV-score based on four circulating EV proteins (ARG1/CD3/PD-L1/PD-L2) to predict gastric cancer response to immune checkpoint inhibitor-based immunotherapy and monitor disease progression during with treatment [129]. Collectively, these important findings suggest that EV cargo molecules may be developed as specific and precise biomarkers for cancer risk assessment, early diagnosis, tumor burden monitoring, prediction of therapeutic response, as well as prediction of metastasis and/or detection of micro-metastasis to guide clinical decision making and win the critical time window for primary and secondary prevention treatment. Commonly used strategies to identify EV-based cancer biomarkers are summarized in Figure 2.

EVs as therapeutic targets

As the versatile functions of tumor-derived EVs have started to be recognized in all aspects of tumor-host interaction, targeting tumor-initiated intercellular communication mediated by EVs has become an attractive strategy in cancer therapy. Current approaches mainly aim at suppressing tumor secretion of EVs, recipient cell uptake of tumor-derived EVs, and function of specific EV cargo. These approaches are summarized in Figure 3 and discussed below.

Targeting EV secretion at the origin

Given that EV secretion is not unique to cancer cells and that EVs secreted by normal cells participate in many physiological functions, a major challenge in targeting EV secretion as a cancer therapy is how to specifically block EV biogenesis and secretion pathways in cancer cells without affecting EV secretion by normal cells. To address this the field continues to require extensive basic research on cellular pathways and genes involved in the production of various types of EVs in both cancer and normal cells. Although these pathways may be shared by cancer and normal cells, it is hoped that cancer cells' secretion of EVs, or a subpopulation of EVs with tumor-promoting function, is more dependent on a potentially druggable mechanism compared to normal cells, thereby creating a dosing window for specific targeting of cancer cells. Several strategies have been tested in preclinical models. Lipid profiling demonstrates a similarity between exosomes and lipid rafts, with remarkable enrichment of ceramide and cholesterol compared to total cellular membrane. Sphingolipid ceramide induces exosome biogenesis by triggering budding of exosomes into the lumen of MVBs, whereas GW4869, a chemical inhibitor of the neutral sphingomyelinase 2

(nSMase2) that controls the biosynthesis of ceramide, attenuates exosome secretion in oligodendroglial cells [130]. Kosaka et al. report use of GW4869 to suppress the secretion of exosomal cargo including miRNAs and proteins without significantly altering the composition of exosomes using HEK293 and COS-7 non-cancer cells [131]. The group further shows that nSMase2 is expressed at higher levels in cancer cells compared to non-cancer cells, and that its expression level correlates with level of exosome secretion and the metastatic potential of cancer cells [56]. Based on these findings, GW4869 is used as an *in vivo* treatment to suppress EV secretion especially by tumor cells. In a colorectal cancer model, cancer-cell-derived EVs are shown to promote tumor growth by inducing cancer cell proliferation and inhibiting apoptosis, whereas intra-tumoral injection of GW4869 suppresses tumor growth [132]. However, the off-target effects of GW4869 remain to be elucidated and additional knowledge is needed to clarify to what degree GW4869 influences EV secretion by normal cells and the diverse physiological functions mediated by EV communication.

Several other compounds targeting different pathways have been shown to suppress secretion of different EV subpopulations at least in cell line models. Datta et al. perform a high-throughput screening of pharmacological compounds to identify those modulating exosome biogenesis and/or release by prostate cancer cells [133]. This strategy identifies tipifarnib, along with several other compounds, as a potent inhibitor of exosome biogenesis and/or secretion. Tipifarnib, a farnesyl transferase inhibitor, decreases the abundances of key components in ESCRT-dependent (Alix) and ESCRT-independent (nSMase2) exosome biogenesis as well as and in exosome transport (Rab27a). The study also identifies several azole compounds, including the antifungal agent ketoconazole, as inhibitors of EV production [133]. Dimethyl amiloride, an inhibitor of the H^+/Na^+ and Na^+/Ca^{2+} channels, suppresses EV secretion by tumor cells both *in vitro* and *in vivo* [87]. Due to the established role of EV secretion as one way to remove anticancer drugs from tumor cells, targeting tumor EV secretion has also been explored as a strategy to improve efficacy of chemotherapeutic drugs. In this regard, indomethacin, calpeptin, chloramidine, bisindolylmaleimide-I, and cannabidiol have all been shown to enhance tumor response to anticancer drugs through their proposed effect on blocking EV secretion and increasing drug retention within tumor cells [134-138]. As some of these compounds are clinically approved for other indications, repurposing drugs previously approved for cancer or non-cancer diseases could be a promising adjunct therapeutic strategy to suppress cancer cell EV secretion, which is expected to overcome EV-mediated drug resistance and block other tumor-promoting effects of EVs.

Targeting EV action at the destination

In addition to targeting the origin of EV production, efforts have also been made to target the destination of tumor-derived EVs by suppressing their cellular uptake. Dynasore, an endocytosis inhibitor that targets dynamin, is used to suppress EV uptake in various types of cells including fibroblasts and endothelial cells, where it blocks EV-mediated effects [54, 139]. Some tumor-derived EVs can adapt normal niche cells to facilitate their cellular uptake. Ortiz et al. show that melanoma-derived EVs suppress type I interferon signaling and expression of cholesterol 25-hydroxylase (CH25H) in normal cells. As CH25H can

restrict EV uptake, this tumor EV-mediated adaptation further facilitates EV uptake and full establishment of a pre-metastatic niche [140]. By screening selected agents that interfere with lipid membrane fusion, the anti-hypertensive drug reserpine is found to inhibit EV uptake by normal cells, block pre-metastatic niche formation, and suppress metastasis [140]. Recent studies show that EV uptake and internalization by recipient cells depend on cell-surface heparan sulfate (HS) proteoglycans (HSPGs), which vary in composition across tissues and in tumors. This route of EV uptake depends on the structural specificity of intact HS chains as well as the overall sulfation and negative charge [141-143]. Internalization of EVs from glioma cells is significantly blocked by enzymatic depletion of cell-surface HSPGs or inhibition of HSPG biosynthesis in recipient cells [141]. The cell-surface adsorption and uptake of EVs can be inhibited by free heparin and other low molecular weight HS mimetics in a dose-dependent manner, whereas the closely related chondroitin sulfate has no effect [141, 144]. Heparin has been used as an experimental approach to block tissue uptake of EVs and EV-mediated effects in a mouse model of breast cancer [145]. As a natural product, heparin has shown an excellent safety profile and is traditionally used as an anticoagulant. Therefore, heparin and non-anticoagulant derivatives may hold promise as therapeutic agents blocking the uptake of at least a significant fraction of tumor-derived EVs.

Targeting EV transportation en route

Between the origin and destination, EVs may also be targeted "*en route*" in the circulation. In patients with end stage kidney disease, hemodialysis using Fresenius FX-800 filters results in reductions in circulating submicron particles including those <40 nm, 40–100 nm, and 100–1000 nm in size. Total Annexin V^+ EVs, as well as those expressing markers of endothelial ($CD144^+$), platelet ($CD41^+$), and leukocyte ($CD45^+$) origins, are all reduced following hemodialysis [146]. Affinity hemodialysis using specialized devices such as Hemopurifier, which has been shown to reduce the systemic load of viruses in patients with hepatitis C virus [147], has been proposed as a strategy to specifically deplete a subset of EVs from the blood [148]. An ongoing phase I clinical trial (ClinicalTrials.gov Identifier: NCT04453046) will evaluate use of Hemopurifier to deplete immunosuppressive exosomes in combination with pembrolizumab in patients with advanced and/or metastatic squamous cell carcinoma of the head and neck. As the blood filtration devices can be designed to include different affinity agents, they can be potentially used to selectively remove EVs from various cell origins including various types of cancer cells and may be particularly effective in preventing metastasis and suppressing some immunomodulatory effects mediated by circulating EVs.

Targeting specific EV cargo

As a result of the partially conserved mechanisms underlying EV biogenesis, tissue-specific homing, and cellular uptake, targeting EVs as a whole will continue to encounter the issue of lacking cancer specificity. Meanwhile, targeting specific EV cargo that can mediate critical pathways in tumor-induced host cell adaptations allows a more specific and functionally defined blockade of EVs' effects. This may also allow individualization of therapy based on the cargo composition and function of tumor-derived EVs, which are dependent on the contexts of tumor and host cells including the genetic and epigenetic makeup and functional

state of the cells. Targeting mechanisms controlling cancer-specific sorting of certain cargo molecules into the EVs, such as the RNA-binding proteins responsible for the secretion of some EV-enriched miRNAs, could be a strategy to block cargo loading in EV-secreting tumor cells. This may have additional effects on tumor cells by causing an accumulation of the cargo molecules inside of the secreting tumor cells. In addition, direct functional blockade of EV cargo molecules, or inhibition of their downstream pathways in recipient cells, can also be employed to specifically suppress selected functions of EVs. For example, intraperitoneal administration of an oligonucleotide inhibitor of miR-122 has been shown to block the systemic effects of breast tumor-secreted EV miR-122 [30, 32]; the therapeutic effect can also be achieved by restoring target gene expression in tissues affected by tumor-secreted miR-122 [31, 32]. Targeting EV-mediated intercellular communication between tumor and host is expected to be more effective when combined with surgical resection of the tumor and/or cytotoxic anticancer therapies, when tumor-derived EVs as well as the EV-secreting tumor cells are simultaneously targeted.

EVs as delivery vehicles for therapeutic agents

Another clinical utility of EVs, often derived from normal cells and modified to gain additional features, is as delivery vehicles for anticancer drugs or vaccines. Although some proof-of-concept studies use model cell lines such as HeLa cells to produce EVs for demonstration of therapeutic manipulations [149], it is widely recognized that EVs from cancer cells and oncogene-transformed cells exhibit pathogenic capacities due to known and unknown mechanisms. Therefore, normal native cells are exploited as donors for therapeutic EVs in many studies. Mesenchymal stem cells (MSCs) and dendritic cells (DCs) are popular choices due to the feasibility to obtain from patients for *in vitro* expansion and manipulations as an autologous EV therapy, which would minimize the immunogenicity of therapeutic EVs. EVs derived from blood and tumor cells are also studied in certain settings.

As targeted delivery vehicles for anticancer drugs

MSCs are multipotent cells that can differentiate into various cell types and can be isolated from various sources including bone marrow or adipose tissue, making them a promising tool for cell-based therapies [150]. Bone marrow-derived MSCs (BM-MSCs) are recruited to primary and metastatic tumors, where they exert context-dependent functions to either promote or suppress tumor progression [151]. An established function of MSC-derived EVs is immune modulation, an important beneficial effect seen in MSC-based cell therapy. MSC-EVs mediate immunosuppressive and anti-inflammatory effects through transfer of immunomodulatory effectors or signal molecules to regulate immune cells such as macrophages and T cells [152]. Unmodified MSC-EVs may also suppress tumor growth through their anti-angiogenic effect. In a mouse model of breast cancer, MSC-EVs are shown to be taken up by cancer cells, downregulate the expression of vascular endothelial growth factor in cancer cells through EV cargo miR-16, and suppress tumor angiogenesis and tumor growth [153]. A study comparing the biodistribution of various EVs shows that unmodified EVs from primary mouse DCs and human MSCs, similar to EVs from other tested mouse and human cells, accumulate mainly in liver, spleen, gastrointestinal (GI) tract, and lungs in mice following intravenous administration. However, DC-EVs show higher

accumulation in spleen and lower accumulation in liver, whereas MSC-EVs show higher accumulation in liver and lower accumulation in GI tract [154].

The surface of EVs can be decorated to create or enhance tissue-specific delivery. For example, brain-targeting therapeutic EVs have been generated by engineering EV-producing DCs to express Lamp2b fused to the neuron-specific rabies viral glycoprotein peptide [155], or by directly conjugating a cyclo(Arg-Gly-Asp-d-Tyr-Lys) peptide onto EV surface [156]. A seminal study by Pi et al. demonstrates use of arrow-shaped RNA for directional control in engineering therapeutic EVs. HEK293T cell-derived EVs engineered this way to display various ligands are able to specifically deliver therapeutic siRNA into tumor cells and suppress tumor growth [157]. In this study, EVs displaying an aptamer binding to prostate-specific membrane antigen or EGFR and loaded with survivin siRNA are shown to respectively inhibit prostate or breast tumor growth, whereas folate-displaying and survivin siRNA-loaded EVs inhibit colorectal tumors [157]. Blood has also been shown as an abundant and safe source of therapeutic EVs. Blood-derived exosomes can be engineered to serve as a versatile combinatorial delivery system, for example by loading with chemotherapy drug doxorubicin, miRNA inhibitor or therapeutic siRNA, and decorations to enhance tumor accumulation and endosome escape [158, 159].

Despite the undesired pathogenic functions of tumor cell-derived EVs, these EVs display enhanced targeting and/or uptake efficiency by the parental tumor [160]. For example, systemic administration of EVs from HT1080 cells are more effective than those from Hela cells in homing to HT1080 tumor and, when loaded with chemotherapy drug, enhance drug retention in tumor tissues and potentiate anti-tumor effects [161]. However, another study finds that intravenously-injected, unmodified tumor-derived EVs experience rapid clearance and minimal tumor accumulation, whereas intratumorally delivered EVs indeed exhibit enhanced accumulation in tumor tissues compared to control liposomes [162]. A recent study takes advantage of the membrane fraction of EVs from hepatocellular carcinoma cells to generate membrane hybrid lipid nanovesicles. These vesicles exhibit a homing specificity to the parental tumor cells and can bypass the endosomal degradation pathway to improve the delivery efficiency of therapeutic siRNA [163]. Overall, it is a valid strategy to manipulate tumor cell-derived EVs, especially those from the autologous tumor, by loading therapeutic cargo and meanwhile depleting pathogenic cargo as a personalized EV therapy targeting the parental tumor as well as its metastases.

As cell-free anticancer vaccines

DCs are professional antigen-presenting cells that can induce primary and secondary immune responses. DCs derived from patients and pulsed with tumor-associated antigens have been tested as anticancer vaccines in clinical trials. Due to the widespread immunosuppression within tumors, DC-based vaccines are being evaluated as combinatorial treatment for T cell-targeting immunotherapies [164]. Similarly, EVs derived from DCs, which could be derived from autologous monocytes, are also candidates for cell-free anticancer vaccines through their ability to target and stimulate the immune system. DC-EVs inherit the antigen-presenting property from parental DCs, and contain functional Major Histocompatibility Complex (MHC) class I/II and T-cell costimulatory molecules [165,

166]. Early studies show that DC-EVs pulsed with tumor peptides can prime specific cytotoxic T lymphocytes and suppress tumor growth in a mouse tumor model [166]. More recent data from preclinical models and clinical trials show that DC-EVs exert potent effects on modulating innate immune responses such as triggering natural killer (NK) cell activation and possibly transferring functional peptide-loaded MHC class I/II complexes to DCs [167]. A phase II clinical trial has tested the clinical benefit of EVs derived from IFN-γ-maturated DCs and loaded with MHC class I/II-restricted cancer antigens as maintenance immunotherapy after induction chemotherapy in patients with advanced non-small cell lung cancer. Although the primary endpoint of progression-free survival is not reached, the study confirms the capacity of these DC-derived EVs to boost NK cell-mediated antitumor immunity [168].

EVs have also been engineered to target specific pathways in immune cells such as the STING (STimulator of InterferoN Genes) pathway. A recent study uses HEK293 cells to generate EVs that are subsequently loaded with cyclic dinucleotide (CDN) agonists of STING. The resultant EVs, known as exoSTING, exhibit enhanced potency and antitumor activity compared to free CDN STING agonists. Upon intra-tumoral injection, exoSTING induces CD8 T cell-dependent systemic anti-tumor immunity, enhances local IFN-γ induction, and suppresses tumor growth in multiple tumor models [169]. In a model of liver cancer, intravenously administered exoSTING also exhibits potent anti-tumor activity, but this may depend on high liver uptake of EVs from the circulation [169]. Based on these results, intratumorally administered exoSTING is currently in phase I clinical trial (ClinicalTrials.gov Identifier: NCT04592484) as a potential anticancer therapy.

Unanswered questions and challenges

EVs mediate a much broader range of pathophysiological processes than what has been studied. For example, it remains to be seen how EVs mediate the inter-kingdom communication between cancer cells, especially those of the GI tract, and non-human cells such as gut bacteria and parasites. The potential EV-mediated exchange of genetic and cellular materials between human and foreign cells in human body and the role of this cross-species interaction in physiology and human diseases is an intriguing direction. In addition, those relatively less studied functional components of EVs, such as lipids and glycans, have just started to be recognized as important effectors in human diseases. It is recently found that gut commensal bacteria secrete membrane vesicles that can activate peripheral cGAS-STING signaling by delivering bacterial DNA into host cells, promoting host resistance to systemic viral infections; this beneficial effect is suppressed by use of antibiotic [170]. Whether this mechanism is implicated in tumor immunology and response to immunotherapy warrants further investigation. Another recent study shows that hydrolysis of phospholipids in EBV lymphoma-derived EVs generates immunoregulatory lipid mediators and facilitates macrophage uptake of aggregated EVs to promote lymphoma growth [171]. Advancements in other rapidly growing research fields such as microbiome and lipidomics are expected to boost future growth of the fundamental research and clinical applications of EVs.

In addition to the all-time need for improved specificity and sensitivity, EV-based biomarkers also face the issue of lacking standard operating procedures, which could cause inconsistency in test results. This is partially due to the diverse EV purification and testing methods used at the current stage. Because of the high degree of heterogeneity in EVs' molecular composition and function, it is critical to define the characteristics of EV subpopulations that are used as biomarkers or targeted in a therapy. Between total EVs that can be isolated from biospecimens using a more straightforward method and certain EV subpopulations (such as those derived from tumor cells) that might provide higher accuracy and precision in detection of EV-based biomarkers, a balance will need to be reached. Increasing efforts are made to characterize EVs at the single particle level, which will tremendously advance our understanding of EV diversity. A recent study using single-EV analysis shows that only ~40% of EVs secreted by pancreatic cancer cells expressing $KRAS^{mut}$ and/or $P53^{mut}$ proteins are also positive for these markers; in patients with early pancreatic cancer, $KRAS^{mut}$ and $P53^{mut}$ proteins are detected in <0.1% of total EVs in the plasma [172]. How single-EV analysis may transform the discovery and application of EV biomarkers remains to be seen.

The value of EVs in cancer therapy is far beyond a new bottle for old wine. Although testing previously established tissue-based molecular markers for their prevalence in EVs and packaging existing anticancer agents into therapeutic EV vehicles are widely pursued, many studies compare the performance of EV-based biomarkers or therapeutics to the non-EV versions and show the superiority of EV-based approach. For biomarker detection, EVs improve the spatial resolution by defining the compartmental localization of the biomarkers in liquid biospecimens with the potential to predict the tissue origin and homing tropism of the EVs and their parental cancer cells. For targeted delivery, EVs enable customized and individualized combinations of therapeutics that may act in a synergistic manner to reduce the minimum effective dose and toxicity. Compared to other delivery vehicles, both autologous and non-autologous EVs show some advantages such as higher biocompatibility and lower immune clearance when administered systemically. However, lack of a thorough understanding of the contents, spatial organization, and heterogeneity of EVs could impose challenges on quality control, safety, efficacy, and manufacturing costs. Future studies are expected to address these concerns to accelerate use of EVs as a standard approach in cancer prevention and treatment.

Closing remarks

We are in the midst of one of the fastest growing fields of research, with EV-oriented concepts, knowledge, and methodology rapidly integrating into all disciplines of biomedical science and technology. While basic science research will continue to elucidate outstanding questions to expand the repertoire and improve the accuracy, specificity, and efficacy of EV-based clinical applications, the field has quickly moved into the era of preclinical development and clinical validation. Our knowledge on EV biogenesis, classification, nomenclature, purification methodology, and compositional and functional diversity is quickly evolving, which can be reflected by the exponentially growing number of publications and frequent updates in the guidelines of minimal information for studies of EVs [173]. With this consideration, the goal of this review is to summarize the rationales

and representative strategies in EV-associated therapeutic development by discussing recent findings, with the hope to invite new disciplines and investigators into the field. Many recent reviews have comprehensively summarized the up-to-date significant accomplishments in understanding and harnessing EVs in cancer care; the reader is referred to those review articles, such as [174-177], for a more thorough listing of EV therapeutics in cancer and other human diseases. Due to space limitation, many excellent studies are not discussed in this review. However, here I would like to acknowledge all EV researchers for their contributions to shape our current view of EVs and quicky drive the field towards improved care of cancer.

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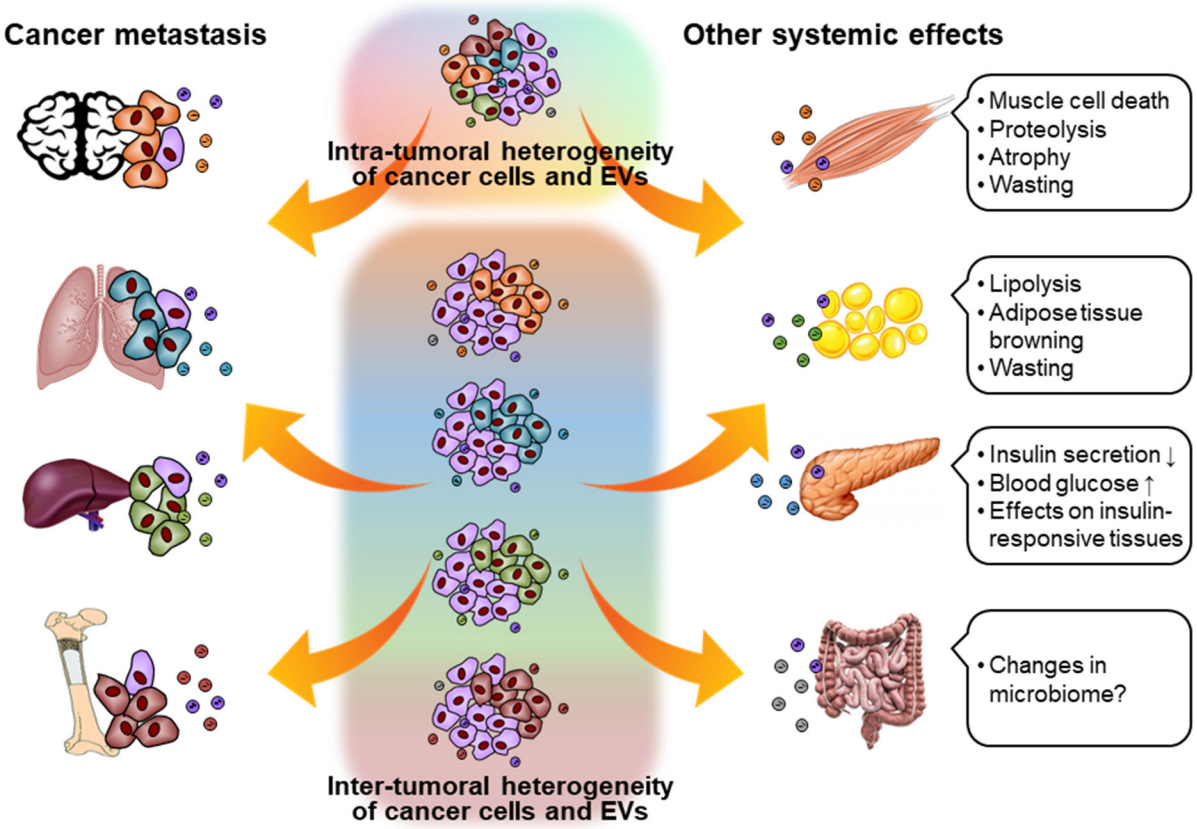

Figure 1.

The versatile systemic effects of tumor-derived EVs. EVs secreted by cancer cells travel to and influence normal cells at a distant organ. This leads to establishment of a pre-metastatic niche to facilitate cancer metastasis. On the other hand, recent studies have started to demonstrate other systemic effects of tumor-derived EVs that influence organs and tissues beyond a potential metastatic site, such as skeletal muscle, adipose tissues, and pancreatic islets. The diverse distant effects of tumor-derived EVs can be mediated by different EV subpopulations from the same tumor reflecting intra-tumoral heterogeneity of cancer cells and EVs, or by EVs from tumors with different characteristics reflecting inter-tumoral heterogeneity.

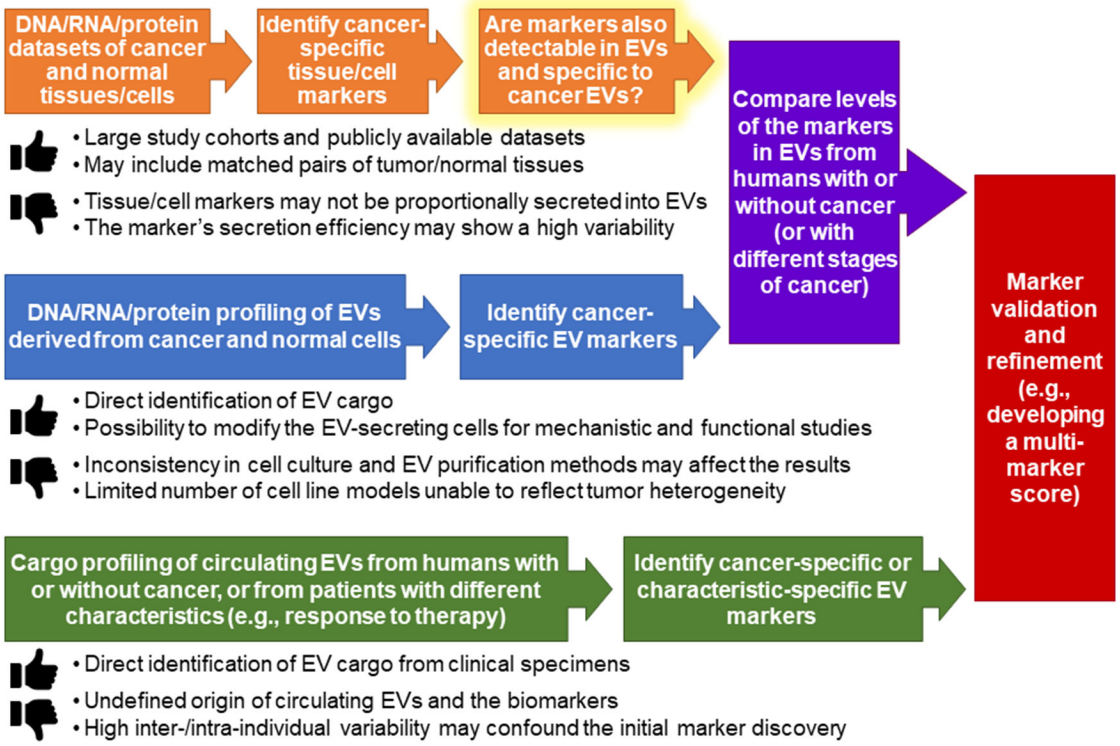

Figure 2.

Common strategies to identify EV-based cancer biomarkers. Identification of EV-based cancer biomarkers often employs at least one of the three summarized strategies. Each strategy has its advantages and limitations, some of which are listed in the figure.

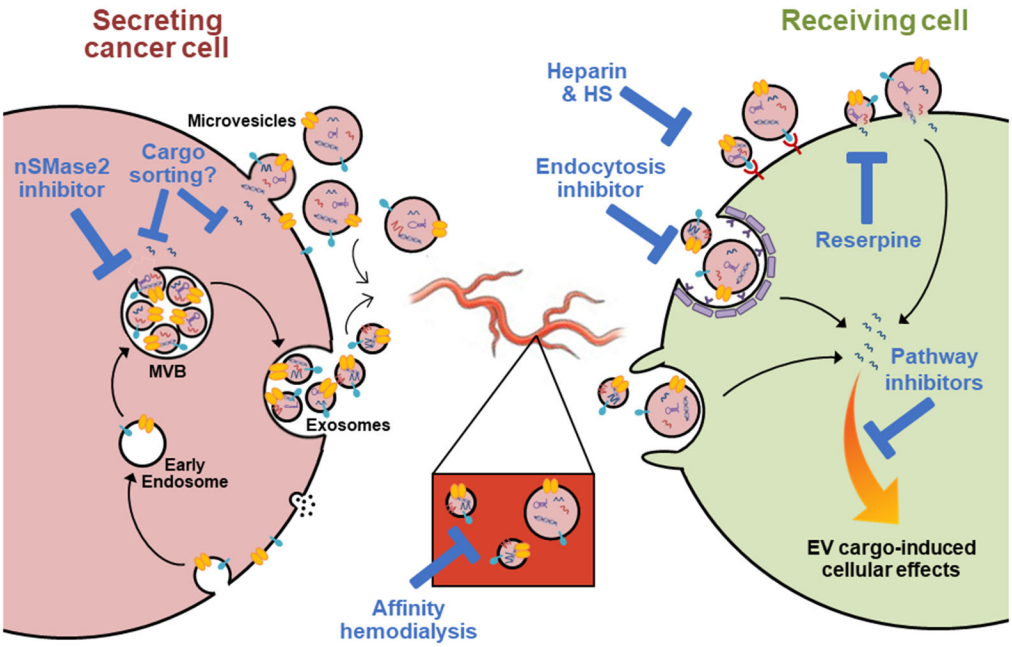

Figure 3.

EV-targeted therapies. Various approaches have been developed or proposed to block EV-mediated effects in cancer. These include suppressing tumor cell secretion of EVs by nSMase2 inhibition, depleting a specific subpopulation of EVs from the circulation by affinity hemodialysis, blocking recipient cell uptake of EVs by endocytosis inhibition, by free heparin and HS, and by reserpine, and inhibiting functional EV cargo by targeting their sorting into EVs or their downstream pathway in recipient cells. Additional miscellaneous compounds that have been shown to suppress EV secretion are described in the text.