HHS Public Access

Author manuscript

Biochim Biophys Acta. Author manuscript; available in PMC 2018 December 01.

Published in final edited form as:

Biochim Biophys Acta. 2017 December ; 1868(2): 538–563. doi:10.1016/j.bbcan.2017.10.001.

Extracellular vesicles as emerging targets in cancer: recent development from bench to bedside

Kerui Wu[1], **Fei Xing**[1], **Shih-Ying Wu**[1], and **Kounosuke Watabe**[1,*]

[1]Departments of Cancer Biology, Wake Forest University School of Medicine, Winston Salem, NC, USA

Abstract

Extracellular vesicles (EVs) have emerged as important players of cancer initiation and progression through cell-cell communication. They have been recognized as critical mediators of extracellular communications, which promote transformation, growth invasion, and drug-resistance of cancer cells. Interestingly, the secretion and uptake of EVs are regulated in a more controlled manner than previously anticipated. EVs are classified into three groups, (i) exosomes, (ii) microvesicles (MVs), and (iii) apoptotic bodies (ABs), based on their sizes and origins, and novel technologies to isolate and distinguish these EVs are evolving. The biologically functional molecules harbored in these EVs, including nucleic acids, lipids, and proteins, have been shown to induce key signaling pathways in both tumor and tumor microenvironment (TME) cells for exacerbating tumor development. While tumor cell-derived EVs are capable of reprogramming stromal cells to generate a proper tumor cell niche, stromal-derived EVs profoundly affect the growth, resistance, and stem cell properties of tumor cells. This review summarizes and discusses these reciprocal communications through EVs in different types of cancers. Further understanding of the patcment of tumor specific therapeutics. This review will also discuss the translational aspects of EVs and therapeutic opportunities of utilizing EVs in different cancer types.

2. Characteristics and biogenesis of EVs

2.1. EVs are defined as exosomes, microvesicles, and apoptotic bodies

EVs are a family of membrane-wrapped vesicles released from cells to the extracellular space. They are known to transport messages from donor cells to recipient cells, mediating intercellular communications (1). The discovery of EVs can be dated back to 1967 when Peter Wolf identified “Platelet dust” by electron microscopy as a subcellular fraction derived from platelets (2). The first annotation of EVs in cancer patients was reported in 1978 by Friend et al. (3). They described the presence of “rare, pleomorphic membrane-lined particles” in the extracellular space of a cell line established from a patient with Hodgkin’s disease. Two years later, Poste et al. found that fusion of plasma membrane vesicles from a

*Correspondence: Kounosuke Watabe, Ph.D., Department of Cancer Biology, Wake Forest School of Medicine, Winston Salem, NC, USA Tel: 336-716-0231, kwatabe@wakehealth.edu.

highly metastatic melanoma cell line could increase the lung metastasis rate of an otherwise poorly metastatic melanoma cell line (4), suggesting a regulatory role of EVs in recipient cells. However, little attention was paid to these tiny particles until 1983, when two studies showing the recycling of transferrin receptors (TfRs) in reticulocytes through EVs were published consecutively (5, 6). Using labeled antibodies, Johnstone et al. found that TfRs were selectively packaged into vesicles released by cells during the maturation of reticulocytes. Just one month later, Harding et al. reported close connection of endocytosis with the recycling of TfRs in reticulocytes. Their work suggested that transferrin receptors could be either recycled to the membrane through multivesicular endosomes (MVE) or released to the extracellular space in the form of vesicles derived from MVE. More importantly, the balance shifted from recycle to release during the maturation of reticulocytes, indicating a regulatory mechanism for vesicle secretion and cargo sorting. This work provided the first evidence that EVs are an essential part of the biological mechanisms that maintain cellular activity. In 1987, Johnstone redefined “exosome” to refer to these vesicles with a diameter of ~50 nm (7, 8). Thereafter, several studies reported the functional behaviors of EVs in diverse types of cells. In 1996, Raposo et al. found that B lymphocytes secrete major histocompatibility complex (MHC) class II bound exosomes, which induced antigen-specific T cell responses, suggesting their roles in immune system mobilization (9). This pioneering work opened a new era of EV research in immunology. In 2006, it was reported that exosomes and MVs from embryonic stem cells enclosed enriched amount of mRNAs of several pluripotent transcription factors. These mRNAs are engulfed and translated into proteins by hematopoietic progenitor cells to enhance survival and expansion (10). This finding suggests that cells can exchange genetic information through EVs and exert their biological functions. Furthermore, Valadi et al. found that exosomes also contain microRNAs (miRNAs) that can be transferred into recipient cells (11). They also observed packaging of selective nucleic acids into exosomes, which indicated a potential sorting process during exosome loading. Recently, EVs were also found to contain both mitochondrial DNAs and chromosomal DNAs (12–14), which could indicate a novel mechanism of genetic exchange from donor cells to recipient cells. In conclusion, the presence of nucleic acid in EVs provides an opportunity for identifying new diagnostic tools for diseases with genetic mutations, such as cancer (15).

Given the relatively short history of EVs, their nomenclature has yet to be unified. Many different names have been used to refer to these particles, including microparticles, plasma membrane vesicles, membrane blebs, and ectosomes (16). Currently, a widely accepted standard classification separates the EVs into three groups according to their cellular origins: exosomes, MVs, and ABs. Exosomes are generated through the endocytic pathway. Invaginations of late endosomes lead to the formation of MVBs containing small vesicles. Fusion of MVBs with the plasma membrane releases exosomes to the extracellular space. Exosomes are generally saucer-shaped, between 40 and 100 nm in diameter (17). Exosome membrane is a lipid bilayer comprising enriched sphingomyelin and decreased phosphatidylcholine, with a random distribution of phosphatidylethanolamines as compared to the plasma membrane (18). In terms of cargoes carried by exosomes, proteins such as tetraspanins (e.g. CD63, CD9, and CD81), endosome-associated proteins (e.g. Alix and Tsg101) and antigenic peptide binding proteins (e.g., HSC70 and HSP90) have been found

to be enriched in exosomes (19–22), and they are widely used as markers of exosomes (17, 23). However, their expressions are not restricted to exosomes since they can be detected in other vesicles (24, 25), and in some cases, they are more encriched in MVs than exosomes (25, 26). Nucleic acid is another important content carried by exosomes. Functional mRNAs and miRNAs have been reported as important intercellular messengers associated with exosomes (11, 27). Other than RNA, both mtDNA and genomic DNA have been found in exosomes, although there is not sufficient evidence to support the idea that they are actively functional instead of simply passengers (12–15). In addition to exosomes, cells also release EVs by directly budding and fission of the plasma membrane. This class of vesicles, termed MVs, have a diameter ranging from 100–1000 nm (23, 28). MVs have a similar composition of lipids as the plasma membrane but lack the asymmetric distribution seen in the plasma membrane (29). MVs also carry proteins, mRNAs, and miRNAs. Currently, there is no clear evidence indicating a specific marker or cargo associated with MVs compared to exosomes (30). A potential exclusive phenotype of MVs may be a lack of DNA since there is no clear evidence indicating the presence of DNA in MVs except studies with inconsistencies in the nomenclature used to indicate exosomes and MVs. The third category of EVs is ABs. Unlike other EVs, ABs are not released during the normal cellular process. They are only formed by the fragmentation of cells during apoptosis, a process of programmed cell death. As a result, ABs consists of cytoplasm, intact organelles, as well as nuclear fragments (31), which indicates that ABs carry cellular constituents inherited from apoptotic cells. The major significance of ABs is the obliteration of dead cells without ensuing inflammatory reactions since ABs are subsequently phagocytized by other cells. However, it has also been reported that ABs are indeed involved in intercellular communications (32–34).

The field of EV is rapidly developing, and new types of EVs are emerging such as large oncosome (35) and spheresome (36). Large oncosome is a group of EVs with atypically large size (1–10 μm in diameter). Despite the difference in size, large oncosome shares similarities with MV in biogenesis. Similar to MV, large oncosome is also a type of membrane-shedded vesicle that is originated from plasma membrane outward budding. The production of large oncosome is associated with amoeboid migration of cancer cells. The non-apoptotic membrane blebs, which are extruded and retracted to drive the amoeboid movement of cancer cells, were found to budded from palsma membrane to form large oncosomes. Interestingly, biogenesis of MV has also been found to be promoted by RhoA-induced amoeboid phenotype of cancer cells (37). Regradless of these similarities, large oncosome has its unique features. It is specifically generated by cancer cells (38), and its formation can be stimulated by activation of epidermal growth factor receptor and Akt1 (39). Further analysis revealed a link between chromosomal loss of DRF3 and biogenesis of large oncosome. It was found that actin nucleating protein Drf3 could inhibit the release of large oncosome from cancer cells, and chromosomal DRF3 locus is frequently deleted in metastatic prostate cancer (PCa). Another new type of EV is termed spheresome. Spheresome was first identidied in the tissue of gastrointestinal stromal tumors by scanning transmission electron microscopy (36). These vesicles have lipid bilayer membrane with 40–125 nm in diameter. Distinct from exosomes and MVs, spheresomes are not released through fusion of MVBs or plasma membrane budding, and they are released as a cluster wrapped by a large sphere with lipid bilayer membrane. This sphere is around 0.5 to 1.5 μm

in diameter and named multivesicular spheres. Spheresomes are further released by multivesicular spheres in the extracellular space. These findings of new EVs released by cancer cells are particularly exciting as they may reveal novel mechanisms of cancer development and help to identify new biomarkers with high specificity.

2.2. Biogenesis, uptake, and cargo sorting of EVs are tightly regulated

The biogenesis of EVs is tightly controlled by cells through various mechanisms. Exosomes can be generated by the Endosomal Sorting Complex Required for Transport (ESCRT)-dependent or -independent pathways (40). Cancer cells modulate these pathways to facilitate growth and metastasis. For example, metastatic cancer cells were found to deliver angiogenic miR-210 through neutral sphingomyelinase 2 (nSMase2)-dependent exosome biogenesis (41). The MV biogenesis was also found to be well controlled in cancer cells. Tumor cells are capable of switching between MV-secreting phenotype and invadopodia phenotype to facilitate invasion when a different microenvironment was encountered (37). On firm matrices, Rac1 is activated and cancer cells display mesenchymal mode of migration with invadopodia formation, whereas on compliant and deformable matrices, Rac1 is inactivated and RhoA is activated. Cancer cells adopt an amoeboid phenotype with the release of MVs, which is dependent on Rho-mediated regulation of myosin light chain phosphatase. Hypoxia was found to induce the release of both exosomes and MVs (42, 43). HIF induced under hypoxia promotes RAB22A expression, which positively regulates MV formation (42). The uptake of EVs in cancer cells is also found to be tightly regulated. It was shown that the uptake of exosomes in hepatic stellate cells was dependent on integrin $\alpha v\beta 3$, integrin $\alpha 5\beta 1$, or heparan sulfate proteoglycan (HSPG) (44). Mantle cell lymphoma-derived exosomes were found to be selectively incorporated by malignant and non-malignant B-lymphocytes, but not by T lymphocytes or NK cells. This selectivity is mediated by the cholesterol/lipid rafts-dependent endocytosis pathway (45). An important role of lipid rafts is concentrating signaling proteins into this microdomain, one of which is annexin, which was shown to mediate exosome endocytosis (46, 47). Since the regulatory mechanisms of EV biogenesis and uptake have been discussed in detail previously (17, 48–50), this review focuses on cargo sorting in EVs, particularly in exosomes and MVs. ABs are not discussed due to their nature as cell fragments.

Exosomes have been found to selectively incorporate their cargo. For example, as a major component in exosomes, miRNA has been found to be selectively enriched in exosomes. Compared to that in parental cells, the proportion of miRNA is higher in exosomes (51). Different miRNAs can be packaged into exosomes at different efficiencies (52). Astonishingly, cancer cells can take advantage of these sorting mechanisms. Exosomes derived from metastatic cells were shown to have higher enrichment of miRNAs compared to exosomes from non-metastatic cells (53). This may be contributed by a compensatory mechanism in which excess miRNAs were sorted into exosomes depending on the abundance of target genes (54). However, this selective process needs the involvement of machinery proteins capable of sending particular cargoes into exosomes. Currently, such sorting machineries can be divided into four categories: ESCRT-related sorting, lipid-related sorting, membrane protein-related sorting, and others (Figure 1).

a. ESCRT-related sorting—ESCRT-related machineries are important components of endocytic recycling pathways and were found to be involved in exosome biogenesis (55, 56). These components were also reported to involve the cargo sorting into exosomes (Figure 1A). For example, syndecan was found to be sorted into exosomes by binding to syntenin, which formed a complex with ALIX and ESCRT-III (57–59). Heparanase, an enzyme that trims heparan sulfate chains on syndecan, promotes internalization of syndecans by regulating engagements and increasing binding to the complex. This complex is also capable of sorting several other cargoes, including CD63 correlated with exosome secretion and higher loading of proteins such as syndecan-1, VEGF, and HGF (61). Vacuolar Protein Sorting (VPS) proteins are also important components of ESCRT machineries (62). VPS33B was found to regulate the production of total exosomes by interacting with the GDI2/RAB11A/RAB27A pathway. Furthermore, it was found to be associated with loading of selective cargoes, such as thrombopoietin (THPO), and angiopoietin-like proteins (ANGPTLs) into secretory exosomes, while exosomal IGFBP2 was not affected by VPS33B knockdown (63). The small integral membrane protein of the lysosome/late endosome (SIMPLE), a functional partner of ESCRT, was found to reside within the intraluminal vesicles of MVBs and exosomes. Overexpression of SIMPLE increased the exosome yield, as well as the expression of exosomal marker proteins such as CD63 and Alix (64).

b. Lipid-related sorting—Another sorting mechanism is based on lipid raft-based microdomains (Figure 1B). These microdomains are enriched with sphingolipids, cholesterol, cytoplasmic proteins covalently modified by saturated fatty acids, and cell surface proteins attached via a glycosyl phosphatidylinositol anchor (65). These components of lipid raft microdomains were also found in the membranes of exosomes (66). More importantly, the high concentration of sphingolipids resulted in ceramide formation via nSMase, which was shown to be involved in cargo sorting during exosome biogenesis (67). In addition, production of exosomes and secretion of miRNAs was found to be regulated by nSMase2 (68). Kosaka et al. found that knockdown of nSMase2 or treatment with nSMase2 inhibitor decreased both total exosome production and exosomal miRNA expression (41, 68). In neuronal cell GT1–7, the prion protein was found to be selectively sorted into exosomes by nSMase2, while the sorting of the disease associated misfolded prion protein was independent of nSMase2 (69). Furthermore, in colorectal cancer (CRC) cells, the KRAS mutation is involved in differential miRNA sorting from parental cells into exosomes (70). miR-100 level was increased in exosomes derived from KRAS-mutant CRC cells while miR-10b was selectively enriched in wild-type exosomes. A subsequent study revealed that activated KRAS induced Ago2 phosphorylation, resulting in suppressed Ago2 interaction with endosomes and secretion into exosomes (71). With the deficiency in Ago2-based miRNA sorting, an alternative sorting mechanism must be present in KRAS-mutant cells. It was found that nSMase2 inhibitor could decrease miR-100 secretion through exosomes in a mutant KRAS-dependent manner, suggesting the functional link between mutant KRAS and nSMase2 in miRNA sorting. More importantly, there is evidence showing selectivity of nSMase2-mediated miRNA sorting. Inhibition of nSMase2 in KRAS-wild type cells did not cause retention of miR-10b in cells. These results imply distinct mechanisms present in cells for cargo sorting.

c. Membrane protein-regulated sorting—The family of tetraspanin is considered a marker of exosomes, and tetraspanin-enriched microdomains have a role in protein cargo selection in exosome biogenesis. By mass spectrometry, intracellular interactome of tetraspanin-enriched microdomains was shown to have a considerable overlap with protein cargoes in exosomes from human lymphoblasts. When CD81 was knocked out, CD81-associated proteins such as Rac were not present in exosomes (72). Sorting of metalloprotease CD10 into exosomes was also found to be dependent on tetraspanin protein CD9 (73). Moreover, Epstein Barr virus (EBV)-associated oncogene-latent membrane protein 1 (LMP1) was shown to be sorted into exosomes from EBV-infected lymphoblastoid cells in a CD63-dependent manner. The direct interaction between CD63 and LMP1 resulted in endosomal-exosomal trafficking of this protein complex and activation of the NF-κB signaling pathway (74). In addition to tetraspanins, other plasma membrane proteins and membrane trafficking modifications are also known to regulate cargo sorting (Figure 1C). Attachment of plasma membrane anchors could incorporate highly oligomeric cytoplasmic TyA into exosomes and MVs (75). Interestingly, the integral membrane protein CD43 was also shown to direct the sorting of proteins such as Dicer into exosomes (53, 75).

d. Other sorting mechanisms—Several other proteins, including the RNA binding protein heterogeneous nuclear ribonucleoprotein (hnRNP), have been found to be involved in cargo sorting into exosomes (Figure 1D). The sumoylated hnRNPs were revealed to regulate the sorting of miRNAs into exosomes. Sumoylated hnRNPA2B1 recognizes CCGA or CCCU motifs in miRNAs and thus mediates sorting of miRNAs into exosomes derived from T lymphocytes (76). Another hnRNP, hnRNP-Q, was also found to regulate miRNA loading into exosomes in hepatocyte. GGCU, a common sequence motif in miRNA, is recognized by hnRNP-Q and thus sorted into exosomes (77). The sorting of long non-coding RNAs (lncRNAs) is also regulated by hnRNP. In renal cancer, lncARSR was found to be sorted by hnRNPA2B1 into exosomes. Incorporation of exosomes by recipient cells increased the Sunitinib resistance, as lncARSR competitively bound miR-34 and miR-449 to promote AXL and c-MET expression (78). Major vault protein (MVP) was also found to regulate the sorting of miR-193a into exosomes from colon cancer cells (79). Furthermore, Y-box protein 1 was shown to sort selective miRNA populations, including miR-223, into exosomes from HEK293T cells (80). Interestingly, the TfRs were known to be released from exosomes during the maturation of erythrocytes. It was found that the Hsc70 sorts TfRs into exosomes by interacting with the cytosolic domains of the TfRs (81). Nedd4 family-interacting protein 1 (Ndfip1) was also found to increase protein loading into exosomes. Nedd4, Nedd4-2, and Itch, which were absent from exosomes, were detected in exosomes after expression of Ndfip1 in parental cells (82). The modification of cargoes also has a regulatory effect on sorting. Packing of miRNA into exosomes was reported to be related to the 3′ uridylation of miRNAs. By small RNA profiling of B cells and their exosomes, it was found that B cells preferentially sorted 3′ end uridylated miRNAs into exosomes while keeping 3′ end adenylated isoforms in cells (83). Similarly, N-linked glycosylation was also found to regulate the sorting of glycoprotein into exosomes (84). While these sorting-related modifications have been observed, the exact sorting machineries have not been identified, which warrants further investigation of the systemic interactome in exosomal cargo sorting.

e. Cargo sorting in MVs—The vesicle-associated membrane protein 3, a member of the v-SNARE family, was found to regulate the sorting of proteins, including type 1 matrix metalloprotease into MVs through interaction with tetraspanin CD9 (85). Sorting of mRNAs into EVs is related to a zipcode-like 25 nucleotide sequence in the 3′-untranslated region (3′UTR) of mRNAs. This zipcode has a “CTGCC” core on a stem-loop region together with a miRNA-binding site. Incorporation of this zipcode into 3′UTR of one mRNA led to the enrichment of mRNA in EVs (59). The miRNA processor, RNA-induced silencing complex (RISC), was reported to sort miRNA in EVs. Knockout of AGO2, a major functional protein of RISC complex, resulted in differential change of sorting efficiency among endogenous miRNAs (52). Interestingly, Alix, which was found to be involved in cargo sorting in exosome biogenesis, was also shown to bind Ago in EV, resulting in selective miRNA sorting (86). However, these studies on RNA sorting did not distinguish between exosomes and MVs. Therefore, further study is needed to address whether these sorting mechanisms are shared in exosomes and MVs or there is a vesicle type specific mechanism.

2.3. Isolation of EVs

Due to the overlapping sizes among exosomes, MVs, and ABs, and the lack of specific markers to distinguish each type, obtaining EVs with high purity and quality remains a challenge. Currently, there are multiple established approaches for purifying EVs from biofluids or cell culture media (CCM) (Table 1). Depending on the fluid type and the purpose of subsequent analysis, the appropriate selection of isolation method is a key step for accurate assessment of EVs.

a. Differential centrifugation—The most common method for the isolation of EVs is differential centrifugation. By applying differential centrifugal force to the liquid sample, particles are separated based on their different densities or sizes. Differential centrifugation has been widely utilized for isolating exosomes, MVs, and ABs from suspensions. Usually, liquid suspensions are first centrifuged at 300 ×*g* for 10 min to remove cells. Then ABs are isolated as pellets after centrifugation at 2000 ×*g* for 10–20 min (87, 88). The supernatant is collected and then used for MV isolation by centrifugation at 16,500 ×*g* for 20 min (87, 89). To isolate the exosomes, the supernatant is further centrifuged at 120,000 ×*g* for longer than 70 min (87, 89). The pellets from the last step are considered exosomes with some contamination of aggregated proteins and lipoproteins. One additional washing step with PBS is usually applied to further purify the exosomes. In many cases, a 0.22-μm filter is used before proceeding to the exosome isolation step to exclude particles larger than 200 nm in diameter (89). As the most common method for isolating EVs, differential centrifugation has several advantages compared to other methods. It is a very flexible approach that can be used in most circumstances. By changing the rotors and tubes used in the ultracentrifuge, samples with different volumes can be processed. It is in general not limited to the types of the liquid samples. Liquids with low viscosity can be directly centrifuged while those with high viscosity can be diluted before being processed. This protocol of EV isolation has been widely accepted and optimized by many researches. However, the disadvantages of differential centrifugation are also obvious. It requires access to an ultracentrifuge and rotors. The process is relatively time-consuming and labor-intensive. An extra dilution step is needed for samples with high viscosity. Additionally, ultracentrifugation may cause the

damage and aggregation of EVs (88, 90), which may affect the subsequent analysis, such as particle size and number estimate, in EV treatment *in vitro* and *in vivo*. More importantly, isolated exosomes have been reported to be contaminated with aggregated proteins (91).

b. Density gradient centrifugation—To further improve the purity of the exosomes isolated by differential centrifugation, an extra step using sucrose or iodixanol cushion/gradient can be applied (91). Density gradient can greatly improve the purity of isolated exosomes (92). However, it is not able to separate high-density lipoproteins (HDLs) from the exosome sediments (93). Due to the fact that HDLs also carry proteins and miRNAs (94, 95), it is important to exclude the noise from HDLs while studying exosomes.

c. Ultrafiltration—Another common approach to isolate EVs is ultrafiltration, which separates EVs based on their size. Usually, it combines membrane concentrator and size exclusion filters to separate particles with different diameters. The most commonly used filters have pore sizes of 0.8 μm, 0.22 μm, and 0.1 μm. Lobb et al. reported the successful isolation of exosomes from CCM and plasma by using a 100,000 kDa membrane after filtration through 0.22 μm filters (90). Use of low protein binding hydrophilized polyvinylidene difluoride membrane can further increase the yield while decreasing the co-purified proteins (96). While ultrafiltration is less time-consuming compared to ultracentrifugation, it also has several limitations that should be considered. Ultrafiltration potentially decreases the yield of EVs as they can saturate and block the pores of the membrane. The choice of membranes also greatly impacts the quality of the isolated EVs (90). During the filtration steps, high pressure may lead to the leakage of unwanted particles or even rupture of EVs (97).

d. Polymer-based precipitation—Volume-excluding polymers, such as polyethylene glycol (PEG), have been commonly used for virus precipitation. Because of the similarities in biophysical properties between exosomes and viruses, these polymers can also precipitate exosomes (98, 99). Several commercial products, such as ExoQuick (System Biosciences) and Total Exosome Isolation (Thermo Fisher Scientific) also use polymer-based precipitation to isolate exosomes. These products simplify the exosome isolation process with decreases in centrifugation steps and speed. The yields obtained are also higher compared to those by other approaches. However, polymer-based precipitation tends to precipitate larger particles and lipoproteins. Aggregated polymers on the exosomes interfere with subsequent analyses. Furthermore, isolation of EVs from plasma by this approach requires additional thromboplastin treatment.

e. Size-exclusion chromatography (SEC)—Recently, SEC has been reported to be used for EV isolation with high purity and integrity (100–102). SEC separates molecules in solution by their sizes. Using adsorbent materials packed in a column, small molecules are trapped and their passage through the column is impaired. They are eluted in later fractions while large molecules are present in earlier fractions. Several different types of resins have been used in SEC, such as Sepharose CL-2B (100), Sepharose CL-4B (103), and Sephacryl S-400 (101). Compared to other methods, SEC has a significant advantage in its applicability for different types of fluids, particularly for serum and plasma. It also leads to

less lipid and protein contamination in isolated EVs (102, 104). More importantly, it minimally alters the characteristics of the isolates, retaining biologically active and morphologically intact EVs (102, 105). However, SEC also has several disadvantages. As EVs are isolated by serial fractions, pre-optimization is needed to determine the fraction of interest. SEC may also dilute the EVs due to the elution process. Additional concentration, such as centrifugal filtration with 100 kDa membrane, may be needed for subsequent analyses (102).

f. Immunoaffinity—EVs can also be isolated according to their surface markers by immunoaffinity. For example, antibodies targeting CD9, CD63, and CD81 are conjugated onto magnetite beads, which selectively capture EVs expressing these membrane proteins. Commercial products such as MACSPlex Exosome Kit (Miltenyi Biotec) and Exo-Flow (System Biosciences) use this technique for exosome isolation. This approach is especially useful for purifying a subpopulation of EVs derived from a specific type of cells, such as cancer cells (106). However, the heterogeneity in antigen expression and dynamic in epitope masking may affect the isolation efficiency. It is also costly when dealing with large volume of samples.

g. Selection of appropriate isolation method—While various approaches of EVs isolation are available, choosing an appropriate method can greatly impact the consistency of results and the subsequent analyses. Studies have been carried out to compare the approaches in different parameters. For overall yield, volume-excluding polymers, such as miRCURY (Exiqon), ExoQuick (System Biosciences), and Total Exosome Isolation Reagent (Thermo Fisher Scientific), were found to generate higher yield than ultracentrifugation, ultrafiltration (PureExo) and density gradient centrifugation in serum, cell culture media, and milk (107–110). However, despite the improvement in yield, polymer-based precipitation results in compromised purity (107, 108). This is to be considered carefully, especially when the downstream analysis is related to the protein content of the isolated exosomes. It was reported that volume-excluding polymers co-isolated various types of protein factors. In serum, high levels of albumin contamination were found in precipitations (90), while for urine, abundant Tamm–Horsfall glycoprotein (THP), a common protein excreted in ordinary urine, was co-isolated by volume-excluding polymers (111). In studies of the protein content within exosomes, ultracentrifugation and SEC (Izon qEV columns) showed superior results as indicated by particles per μg of protein and expression of exosomal protein markers (90, 102, 107, 111). Density gradient centrifugation (OptiPrep) showed high purity of exosomes and high quality of exosomal proteins, but the yield was low, possibly due to the loss of particles during the centrifugation (90, 107). If a specific subgroup of exosomes is being studied, immunoaffinity capture is also an approach with high-quality protein yield (112). When studying the bioactivity of exosomes or using exosomes as a drug carrier, the isolation method also needs to be carefully optimized. Attention should be paid to the use of volume-excluding polymers since they co-isolate polymers. It was also reported that large aggregates were formed using volume-excluding polymers (113), and toxicity in cell treatment was observed (102), suggesting that they are not suitable for bioactivity assays *in vitro* and *in vivo*. Ultracentrifugation and ultracentrifugation combined with a cushion also generated some degree of aggregation,

while density gradient centrifugation generated exosomes with the highest dispersion, indicating that this approach is superior for maintaining the bioactivity of the exosomes. SEC was also found to generate exosomes with preserved biophysical and functional properties. These exosomes showed less toxicity in cell treatment and better bio-distribution in mice (102, 114), suggesting that SEV isolated exosomes are suitable for *in vitro* and *in vivo* functional assays. mRNA is another important cargo in exosomes that has drawn attention from researchers and clinicians because of its role in exosome-regulated cancer progression. The impact of isolation methods on mRNA carried by exosomes has been studied. Ultracentrifugation could generate high quality of mRNA in exosomes with a reasonable yield (111). Ultracentrifugation combined with a cushion or density gradient centrifugation gave high quality of mRNA, but the yields were compromised compared to other approaches (107, 111). Density gradient centrifugation (OptiPrep) could lead to a distinct mRNA profile of exosome from CCM compared to ultracentrifugation and volume-excluding polymers, possibly due to contamination of the RNA binding proteins (107). Compared to mRNA, small RNA such as miRNA was found to be more enriched in exosomes. Numerous studies have been carried out to study the role of exosomal miRNA in cancer development. However, the method of exosome isolation needs to be optimized and standardized due to its impact on the miRNA profile in exosomes. Ultracentrifugation was found to be a suitable approach for studying most exosomal miRNA due to the high quality and relatively good yield (111, 115). Exosomes isolated by volume-excluding polymers were shown to have low purity, but surprisingly the miRNA content was not affected. The miRNA profiles were consistent between volume-excluding polymers and ultracentrifugation, while volume-excluding polymers outcompeted other methods in yield (111, 115). This suggests the possible use of volume-excluding polymers for miRNA profiling when the bioactivity of exosomes is not required for the subsequent study.

Every approach of EV isolation described above (Table 1) has its advantages and disadvantages. Selecting an approach appropriate for the samples and optimizing the method are important for accurate assessment for downstream analyses. Combination of different approaches can be used to further improve the quality of EVs. For example, when additional filtration is combined with volume-excluding polymers, the purity of isolated exosomes is further improved. A modified protocol using ExoQuick (with DL-dithiothreitol to reduce THP, increased reagent used, and increased centrifuge speed) was also reported to increase the quality of miRNA, mRNA, and protein in isolated exosomes (111). As a burgeoning subject in cancer research, the study of EVs also adds to the improvement of isolation methods. For example, System Biosciences Inc. provides updated kits such as ExoQuick-PLUS and ExoQuick-LP for removal of contamination carried by volume-excluding polymers alone, although the efficiency of these products and the quality of isolates need to be further tested. Other more advanced devices such as microfluidic platforms (see section 4) are being developed and have great potential for the easy isolation and assessment of EVs, especially for clinical use. With the optimizing and standardizing of isolation methods, new biological functions of these small particles may be further disclosed.

3. Roles of EVs in cancer progression

3.1. EVs act as carriers of macromolecules for intercellular communication between cancer cells

In recent years, the number of publications on the role of EVs in cancer development and progression has exponentially increased. Cancer cells have been found to release higher quantities of EVs compared to normal cells, and more EVs can be detected in the circulation of cancer patients (22, 116, 117). Oncogenic components have been found in the EVs released by cancer cells and they trigger various signaling pathways in recipient cells once incorporated. One major approach for EVs to affect cancer progression is to innately transfer pathogenic components between cancer cells. Conferring these components, which include proteins, mRNA, miRNA, DNA, and lipids, phenocopies behaviors from aggressive cancer cells to more indolent cancer cells (Figure 2 and Table 2).

a. Transfer of protein—The active proteins carried by EVs can be directly transferred into the new host cell where they perform functional bioactivities. In PCa, higher activity of metalloproteinases and increased oncogenic signaling molecules were found in hypoxic cancer cells by LC/MS/MS (118). Exosomes from these PCa cells under hypoxia enhance invasiveness and stemness of naïve PCa cells by transferring molecules targeting the epithelial adherens junction pathway. In breast cancer, Hsp90α carried by invasive cells can be conferred to other breast cancer cells via exosomes. In the recipient cells, hsp90α interacts with plasminogen activator, possibly through the bridge of annexin II, and activates plasminogen, which increases cancer cell mobility (119). Other than the delivery of cytoplasmic molecules, the membrane-bound proteins on EVs can also serve as ligands for the receptors expressed on the recipient cells. In the breast cancer, membrane-bound EDIL-3 on exosomes from donor cancer cells was shown to bind and activate the integrin-FAK signaling cascade to promote cell invasion and accelerate lung metastasis (120). Furthermore, exosomes from high-grade bladder cancer cells were found to contain increased levels of protein EDIL-3. This protein could also activate the EGFR signaling in the recipient cells, promoting the migration of cancer cells (121). However, in this case, it is unknown whether EDIL-3 on the exosome membrane directly activates EGFR, or if it needs to be incorporated and recycled as a free ligand. Other than promoting invasion and migration, transfer of protein contents between cancer cells through EVs also confers drug resistance. P-glycoprotein (P-gp), a transporter protein associated with the efflux of cancer therapy drugs (122), is a typical example that was found to be involved in EV-mediated phenocopy of drug resistance in cancers. The docetaxel-resistant PCa cells expressed increased levels of MDR-1/P-gp (123). MDR-1/P-gp could be carried and transported by exosomes to non-resistant cells where they induce the drug resistance, motility, invasion, proliferation, and anchorage-independent growth (124). In breast cancer, it was found that docetaxel-resistance could be transferred between MCF7 breast cancer cells through P-gp-containing exosomes (125). Besides exosomes, MVs also deliver pathogenic signaling molecules between cancer cells. EGFRvIII was found to be shared between glioma cells through MVs (126). Acquisition of EGFRVIII in recipient cells resulted in the transfer of oncogenic activity such as activation of MAPK and Akt, increase of VEGF and Bcl-x(L) levels, decrease of p27 levels, and induction of spindle-like morphology and anchorage-

independent growth. Breast cancer cells under hypoxia were found to have increased expression of RAB22A, which promoted the biogenesis of MVs. These MVs could be incorporated by naïve cancer cells and promote focal adhesion formation, invasion, and metastasis of the recipient cells (42).

b. Transfer of RNA—Intercellular exchange of RNAs is another approach of trait transfer between cancer cells. It was found that small RNAs ranging from 18–28 nt were particularly enriched in exosomes (127). The most abundant small RNAs found in exosomes were miRNA and rRNA (127, 128). Transfer of miRNAs by exosomes can result in translational repression by 3′UTR binding in recipient cancer cells (129). In breast cancer, enriched miR-10b was secreted in exosomes from metastatic cells. Non-metastatic cells could uptake these exosomes and increase the invasive ability through miR-10b-induced HOXD10 suppression (130). Drug resistance can also be modulated by exosomal miRNAs. MiR-221/222 derived from tamoxifen-resistant MCF7 breast cancer cells can be transferred to sensitive cells through exosomes. In the recipient cells, the miRNAs enhanced tamoxifen resistance by targeting the expression of P27 and ERα (131). MiR-222, together with miR-100 and miR-30, could also confer chemoresistance to adriamycin and docetaxel between breast cancer cells by targeting tumor suppressor genes such as PTEN (132). The transfer of miRNAs between cancer cells has also been discovered in other types of cancers, including hepatocellular cancer (133), oral squamous cell carcinoma (43), esophageal cancer (134), and ovarian cancer (135). In general, these transferred miRNAs act as oncogenic factors that target tumor suppressor genes and induce aggressive phenotypes in recipient cells.

Other than small RNAs, lncRNA is another category of non-coding RNAs (ncRNAs) found to be carried by EVs. LncRNA in exosomes derived from cancer cells often harbors enriched miRNA seed regions (136). In renal cell carcinoma, exosome-mediated transmission of lncARSR, which acts as a sponge for miR-34 and miR-449, can confer sunitinib resistance to sensitive cells (78). In breast cancer, tamoxifen-resistant cancer cells had increased expression of the lncRNA UCA1, which was sorted into exosomes. Uptake of exosomal UCA1 induced tamoxifen resistance in sensitive cancer cells (137). Even though the detailed mechanism was not revealed in this study, UCA1 was previously found to induce aggressive phenotype and drug resistance by suppression of p27 (138) or acting as sponge for miR-143 (139) and miR-204-5p (140).

Transfer of ncRNAs may modulate the gene expression in an indirect way, while delivery of mRNA could directly result in the increased translation of this transcript. It was reported that mRNA transferred by exosomes is active and can be translated into functional proteins in the recipient cells (11
). Jurkat T Cells were found to contain a specific splice variant (GlnRS $^{\Delta iABD}$) of GlnRS mRNA in exosomes and this transcript variant can be translated into a protein product (141). A study from Breakefield's lab also indicates that exosomes and MVs from glioblastoma contain RNAs and could promote the growth of human U87 glioma cells (142). In glioma, mRNA, miRNA, and proteins were found to be selectively packaged into exosomes and MVs, which showed a promoting effect on tumor growth (142).

c. Transfer of macromolecules by ABs—Currently, there is no clear evidence indicating that ABs mediate the conferring of pathogenic components between cancer cells. However, they are also known to carry functional molecules such as mRNAs, miRNAs, DNA, and proteins that can trigger signaling pathways in recipient cells (143–146). Further studies are needed to decipher the role of ABs in intercellular communication between cancer cells.

3.2. EVs facilitate intercellular communication between cancer cells and microenvironmental cells to promote tumor progression

TME has a significant role in the development and metastasis of cancer cells (27, 147, 148). In general, TME is composed of extracellular matrix, fibroblasts, adipocyte, blood vessels, lymphatic vascular networks, and other associated tissue cells (147, 149). The interaction between cancer and TME cells is an essential process for the survival of cancer cells. The crosstalk between cancer cells and TME can be established through direct cell-cell contact or secreted factors (150). EVs, by carrying ample biologically active molecules, were also found to mediate the distant communication between cancer and TME cells (Figure 2 and Table 3) (151). Transfer of cellular components from cancer cells to TME cells helps the establishment of a niche for cancer growth. On the other hand, TME-derived EVs are also utilized by cancer cells to reinforce the growth, survival, and mobility of cancer cells.

a. Cancer-associated fibroblasts (CAFs)—Fibroblasts, the most abundant cells in connective tissues, form the tissue framework by secreting extracellular matrix components (152). Recently, Nabet et al. reported that the activated NOTCH-MYC signaling by breast tumor cells in fibroblasts induced CAFs, which have increased amount of unshielded RNAs of *RN7SL1* (153). These RNAs are secreted by CAFs through exosomes, acting as damage-associated molecular patterns that bind to pattern recognition receptors in recipient cells. This signaling cascade then drives an inflammatory response in immune cells and promotes growth, metastasis, and therapy resistance in cancer cells. CAFs also secrete CD81-containing exosomes to promote autocrine Wnt-planar cell polarity signaling that induces protrusive activity and motility of breast cancer cells (154). Breast cancer cells were also found to be capable of transforming the fibroblasts through MVs. These MVs contained tissue transglutaminase and fibronectin, which could activate the AKT/ERK signaling pathway in fibroblasts, thus leading to anchorage-independent growth and enhanced survival capability (155). In PCa, exosomes derived from cancer cells promoted differentiation of fibroblasts to myofibroblasts (156, 157). This effect was mediated by membrane-bound TGFβ on the exosomes through SMAD-dependent signaling. Interestingly, soluble TGFβ was not able to trigger differentiation of fibroblasts, suggesting differential roles of membrane TGFβ from exosomes and soluble TGFβ. On the other hand, CAFs could also support PCa growth under nutrient-stressed conditions by directly transferring intact metabolites such as amino acids (158). Exosome derived from CAFs could also confer drug resistance. It was found that CAFs are resistant to gemcitabine in pancreatic cancer, and gemcitabine treatment increased exosome release from CAFs. These exosomes could endow drug resistance to pancreatic cancer cells by transferring Snail and miR-146a (158). Other than exosomes and MVs, the interaction between cancer cells and fibroblasts has also been reported to be mediated by ABs (32). ABs from transformed rat embryonic fibroblasts

delivered oncogenes to mouse embryonic fibroblasts, resulting in aneuploidy and oncogenic change. Besides exosomes, MVs and ABs, large oncosomes also carry oncogenic messages mediating the communication between cancer cells and fibroblasts (159). In PCa, large oncosomes derived from cancer cells were shown to contain active AKT1. Uptake of large oncosome by fibroblasts leads to activation of MYC, reprograming the fibroblast to support angiogenesis and tumor growth. The interactions between cancer cells and fibroblast cells have also been reported in other cancers, such as melanoma and glioma (155, 160, 161) (Table 3).

b. Mesenchymal stem cells (MSCs)—MSCs are another major component of stromal cells. They are known to differentiate into mesenchymal tissues such as bone, cartilage, and fat (162). In some aspects, MSCs share many similarities with fibroblasts, such as cell surface markers and pluripotency (163). Similar to fibroblasts, MSCs greatly impact cancer progression by interacting with cancer cells (164), and EVs play important roles in mediating this interaction. For example, in breast cancer, exosomes derived from MSCs contain miR-16, which targets the expression of VEGF in breast cancer cells upon uptake (165). Low VEGF production from breast cancer cells resulted in decreased angiogenesis. MSCs were originally identified in bone marrow (166), where they were known to be quiescent but metabolically active (167). These MSCs, together with hematopoietic stem cells, form a pre-metastatic niche for cancer cells (167–169). One function of this niche is the transfer of the quiescent trait to cancer cells by EVs. As in breast cancer, MSC-derived exosomes delivered miR-222/223 to promote cancer cell quiescence and drug resistance (170). Targeting miR-222/223 sensitized breast cancer cells to carboplatin-based therapy. MiR-23b, another exosomal miRNA secreted by bone marrow MSCs, was also found to induce dormancy in metastatic breast cancer cells by targeting MARCKS (171). The communication between bone marrow-derived MSCs and cancer cells is bidirectional. Not only do cancer cells take in exosomes, they also secrete exosomes to educate MSCs in bone. In PCa, exosomes from cancer cells were shown to trigger MSC differentiation into pro-angiogenic and pro-invasive myofibroblasts by the carried TGFβ. However, soluble TGFβ could not induce the same change in MSCs (172). Aside from bone marrow-derived MSCs, adipose-derived MSCs were also found to interact with cancer cells through EVs. Exosomes secreted by cancer cells facilitate tumor growth by inducing MSC differentiation into tumor-associated myofibroblasts through the activation of the SMAD dependent pathway (173, 174), which is similar to that found in bone marrow-derived MSCs (172). Furthermore, PCa cell-associated exosomes can promote cancer progression by directly inducing neoplastic reprogramming of adipose-derived MSCs and the transformed adipose-derived MSCs have PCa-like mesenchymal-to-epithelial transition (MET) morphology and cellular markers, such as CK8, CK5/18, and the PCa-specific marker, AMACR (175). This change is possibly caused by the trafficking of oncogenic miRNAs, transcripts, and proteins into MSCs.

c. Endothelial cells—Endothelial cells are another type of TME cells that have been widely reported to interact with cancer cells. In pancreatic cancer, Tspan8 contributed to the selective sorting of angiogenic cargoes, such as CD106 and CD49d into exosomes. Uptake of these exosomes by endothelial cells resulted in increased angiogenesis (176). In renal cell carcinoma, exosomes from CD105-positive cancer stem cells had the ability to induce

activation of endothelial cells and to promote angiogenesis and lung metastasis *in vivo* (177). In glioma, exosomes and MVs promoted angiogenesis by directly transferring RNA and proteins into endothelial cells (142). Moreover, EVs derived from glioma cells were reported to carry angiogenic factors such as EGFRvIII and TF/VIIa, which can be incorporated by endothelial cells (142, 178).

d. Tissue-specific microenvironmental cells—Other than microenvironmental cells found to communicate with various types of cancer cells, cancer cells also interact with tissue-specific cell types to support growth and metastasis of cancer cells. When breast cancer cells reach the brain, they interact with brain microenvironment cells, such as astrocytes. Both direct contact and soluble factors were reported as the patterns of interaction (179–181), and exosomes were also found to be involved in this communication. Astrocytes were shown to secrete miR-19a-containing exosomes to breast cancer cells in the brain (182). This miRNA directly binds the 3′UTR of PTEN mRNA and decreases the expression of this tumor suppressor. The loss of PTEN resulted in an increase of CCL2 secretion from cancer cells, which recruited myeloid cells to enhance cancer cell growth. In pancreatic cancer, exosomes from cancer cells were selectively incorporated by Kupffer cells in the liver. Macrophage migration inhibitory factor carried by exosomes induced release of TGFβ from Kupffer cells, which promoted fibronectin deposition and subsequently recruited bone marrow-derived macrophages and neutrophils in the liver, forming the pre-metastatic niche (183). In another study, exosomes and MVs from pancreatic cancer were also found to be engulfed by muscle cells (184). The miRNA in the EV, miR-21, activated the Toll-like 7 receptor to promote apoptosis of myoblasts, which led to cancer cachexia.

Reprograming TME cells by tumor cells through cell-cell communication has been frequently observed in multiple malignancies. Interestingly, this communication seems to be established by cancer cells though modulation of membrane compositions on EVs. Breast and pancreatic cancer cell lines that metastasize to different organs produced exosomes harboring organotropic integrins so that these exosomes could induce pre-metastatic niches at the preferred sites (185). Lipid composition also contributes to selective uptake of exosomes in recipient cells (186). Exosomes from mantle cell lymphoma patients were selectively untaken by B-lymphocytes mediated by a cholesterol-dependent pathway, while no apparent internalization in T lymphocytes or NK cells was observed (45). Besides delivery of bioactive molecules to trigger signaling pathways in recipient cells, EVs also bridge cancer cells and microenvironment cells as chemoattractants. Exosomes from chronic myelogenous leukemia cells were found to be chemoattractant for endothelial cells (187).Under non-apoptotic doses of hypoxia and irradiation, MVs derived from lung cancer cells were shown to attract endothelial cells (160). Furthermore, MVs released by platelets were found to attracted lung cancer cells (188). EVs also mediated the interaction between cancer cells and diverse environmental cells such as platelets, adipocytes, bone marrow cells, and immune cells (189–192). A summary of these interactions in different types of cancers mediated by exosomes, MVs, and ABs is presented in Table 3.

4. Translational applications of EVs in cancer

4.1. EV as a biomarker for cancer diagnosis

a. Level of EVs as a diagnostic marker—Emerging evidence suggests various translational utility of EVs. Cancer cells secrete more EVs than normal cells (193, 194), and consequently, patients with cancer have higher levels of EVs compared to healthy individuals (195–200). With the increased level of EVs in circulation and the packaging of cancer-related molecules, these vesicles may serve as promising biomarkers for cancer diagnoses. The number of EVs in the blood has been proven to be an indicator for multiple types of cancers. While cancer cells secrete more EVs compared to normal cells, higher grade cancer cells secrete more EVs than lower grade cancer cells (143–145). In colorectal cancer, the level of exosomes in cancer patients is statistically higher than that in healthy controls and it is positively correlated with the level of CEA. Colorectal cancer patients with poorly differentiated tumors and shorter overall survival have increased numbers of exosomes in the plasma (199). The level of MVs is also found to be an indicator for PCa (198). Prostasomes, MVs secreted by normal and malignant prostate acinar cells, were found to be elevated in the blood of PCa patients. Their numbers were 2.5- to 7-fold higher compared with that in the benign group, and were higher in patients with high-grade (Gleason scores more than 7) than in those with low grade (Gleason scores less than 6) PCa. In PCa, the number of ABs from cancer tissue also correlated with the disease progression (201), suggesting the poteintal of AB as biomaker. Other than these typical EVs, the newly identified EV, large oncosome, has also been shown to be a specific marker for cancer diagnosis (38). In mouse model of prostate cancer, large oncosomes were detected in both tumor tissues and circulation. More importantly, the abundance of large oncosome was found to be correlated with tumor progression in both human and mouse. These findings suggest that the level of EVs can serve as circulating biomarkers for cancer diagnosis and prognosis.

b. EV-derived ncRNA as a diagnostic marker—Tumor cells also produce distinct EVs compared to normal cells, and cancer cell derived-EVs inherit content from cancer cells. Because of the enwrapped cancer-specific molecules, these EVs may be used as an indicator for differential diagnosis of cancers. miRNA is the most studied biomarker in EVs due to the high enrichment of small RNA in these particles. It was reported that EVs contribute to the majority of miRNAs found in serum and saliva (202, 203). In PCa, 12 exosomal miRNAs were found to be differentially expressed between PCa patients and healthy donors. While the level of only one miRNA, miR-181a-2*, was significantly decreased in PCa patients, levels of other miRNAs were increased in the blood (204). Among the increased miRNAs, miR-107 and miR-574-3p were also found to be at higher concentration in urine exosomes of PCa patients compared to that in healthy individuals, indicating that these exosomes may serve as non-invasive diagnostic makers for PCa. The authors also found that levels of miR-141 and miR-375 in exosomes were increased in PCa patients with recurrences compared to those in non-recurrent patients. In colorectal cancer, seven miRNAs, including let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, and miR-23a, were found to be upregulated in tumor-bearing patients compared to those in healthy individuals (205). The levels of these seven miRNAs were decreased after surgical

resection of tumors in the patients. Compared to known tumor markers such as CEA and CA19-9, these seven miRNAs were more sensitive for identifying tumor presence, suggesting their potential use for early detection of colorectal cancer. In addition to the presence of specific miRNAs, the total miRNA amount in lung cancer was also reported to be increased in cancer cell-derived exosomes compared to that in control, indicating the diagnostic utility of miRNA levels in exosomes (206). LncRNA is another category of ncRNA wrapped in EVs. The level of certain lncRNAs was also found to be changed in the blood of cancer patients. Level of long intergenic non-protein coding RNA 152 (LINC00152) was found to be elevated in plasma of gastric cancer patients compared to those in healthy control groups (207). It had a sensitivity of 48.1% and specificity of 85.2% in the diagnosis of gastric cancer.

c. EV-derived mRNA as a diagnostic marker—Besides ncRNAs, coding RNA transcripts are also present in EVs. In PCa, tumor-specific mRNAs were detected in exosomes from the urine of patients. The mRNAs of PSA and TMPRSS2:ERG were found in the exosomes from the urine of newly diagnosed but untreated PCa patients. However, they were not present in exosomes from the urine of patients receiving androgen deprivation therapy or patients with an impaired/nonfunctional prostate (208). Surprisingly, no prostasomes (MVs) were present in urine from PCa patients, while no exosomes were found in the urine of healthy donors, possibly due to the increased biogenesis of exosomes in the maligned tissue. In another study, mRNAs of ERG (including TMPRSS2: ERG), PCA3, and SPDEF in urine exosomes plus standard of care (SOC), which includes prostate-specific antigen (PSA) level, age, race, and family history, were found to more accurately discriminate PCa with Gleason scores higher than 7 from lower grade PCa or benign lesions (209). Combination of exosomal RNA analysis with SOC increased the value of receiver operating characteristic curve (AUC) to 0.77, compared to 0.66 for SOC alone and 0.61 for PSA alone. This strategy has been commercialized by Exosome Diagnostics and is available for clinical diagnosis as a non-invasive screening system of PCa, named ExoDx™ Prostate(IntelliScore).

d. EV-derived DNA as a diagnostic marker—DNA mutation is a hallmark of cancer, which makes exosome-derived DNA an excellent tool for identifying cancer. Exosomes were found to harbor double-stranded DNA (dsDNA) (15). Melanoma cell-derived exosomes were found to have the *BRAF(V600E)* mutation in dsDNA, consistent with the parental cell lines. In non-small cell lung cancer (NSCLC), the dsDNA in exosomes derived from several cell lines were found to have *EGFR* mutations. In NOD/SCID mice, when cancer cells were implanted, the same DNA mutations in the cancer cells could be identified in circulating exosomes. Similarly, exosomes from pancreatic cancer cell lines and patient serum carry genomic DNA spanning all chromosomes with mutated *KRAS* and *P53* (210). The inherited DNA mutation in exosomes suggests they may serve as precise biomarkers for early identification of cancer. The genetic information provided by dsDNA in exosomes may also provide a guide for targeted therapy in cancer.

e. EV-derived protein as a diagnostic marker—Proteins carried by EVs can also serve as biomarkers in cancer. In ovarian cancer, claudin-4 was expressed in both cancer

cells and cancer cell-shed exosomes (211). The exosomal claudin-4 had a sensitivity of 51% in ovarian cancer detection compared to 71% of CA125. This suggests exosomal claudin-4 could potentially complement CA125 as a diagnostic for ovarian cancer. In pancreatic cancer, glypican-1 (GPC1) was found to be specifically enriched in exosomes (212). GPC1 in serum exosomes could distinguish cancer patients from healthy donors. Furthermore, the level of GPC1+ exosomes also correlated with tumor burden and survival of pancreatic cancer patients. Tumors carrying specific KRAS mutations could also be detected in GPC-1+ exosomes, indicating its role as a prediction and prognostic biomarker for pancreatic cancer. Combination of different components within exosomes could result in high sensitivity in cancer detection. In pancreatic cancer, cancer-initiating cell markers (CD44v6, Tspan8, EpCAM, MET, and CD104) had a sensitivity of 0.96 and a specificity of 0.86, while pancreatic cancer-associated exosomal miRNAs had a sensitivity of 0.81 and specificity of 0.94. A combination of both testing approaches achieved a sensitivity of 1 (213).

f. Future perspectives of EV-based diagnosis—A Large number of EVs can be released from a single cancer cell, suggesting that EV is a signal amplifier for cancer detection. More importantly, they inherit the disease markers from parental cancer cells and can be easily accessed from blood, urine, or saliva. These properties of EVs make them the perfect targets for tracing cancer cells. EV-based diagnosis enables the possibility of cancer identification at a very early stage. Continuous efforts are underway to identify EVs as diagnostic markers. A summary of biomarkers associated with cancer cell-derived EVs is shown in Table 4. Various research institutions and companies, such as Exosome Diagnostics, are actively working on the development of EV-based diagnosis. One successful innovation is the ExoDx Prostate(IntelliScore) system for PCa screening. As aforementioned, compared to the conventional SOC method, IntelliScore achieves higher sensitivity and specificity in detecting high-grade PCa without the need of invasive tissue collection. A study involving 774 patients to test the IntelliScore system using urine exosomes was published before the commercial launch of the system (209). The company also provides the lung cancer diagnostic system ExoDx Lung(ALK) as a tool to detect EML4-ALK fusion transcript in serum exosomes, while other systems including ExoDx Lung(T790M) and ExoDx Lung(EGFR) will be available in late 2017. These serum exosome-based diagnostic tools can provide guidance for targeted therapy in lung cancer patients. The efficiency of these strategies is currently being validated in clinical trials (NCT03236675).

Despite the great promise of EV-based diagnosis, efficient capture of cancer cell-derived EVs apart from normal cell-derived EVs is still challenging. Conventional methods of EV isolation are time-consuming and labor-intensive, preventing the broad application of EV-based diagnosis in clinical practice. Thus, development of new approaches aimed at efficient isolation of EVs is emergent. Currently, microfluidic platforms are being actively developed and optimized for EV isolation and analysis. These devices are usually in a chip format and have built-in components for capturing EVs by filtration (214) or immunoaffinity (215, 216). In addition to isolating EVs, these devices have integrated components for further analysis of EVs. One of the integrated functions is the quantification of EVs (214, 216) since the

number of EVs is an important biomarker of cancer. In addition, determining the content of EVs is also very valuable for diagnosis and prognosis, and microfluidic chips have been developed to profile the molecular markers carried by EVs by Hakho Lee and Ralph Weissleder. They reported the iMER platform, which captures EVs, and performs on-chip RNA isolation and profiling (217). They validated this device on detection of MGMT and APNG, the drug-resistance markers in glioblastoma multiforme (GBM). More importantly, the mRNA levels in EVs detected by iMER correlated with those in cancer cells, indicating the potential of iMER in predicting drug response in GBM patients. They also developed nPLEX to profile the protein expression in exosomes from cancer patients (218). This nPLEX chip has a lattice of 44 × 32 nanoholes per sensing unit, with a nanohole diameter of 200 nm. Facilitated by antibodies, theses nanoholes specifically capture exosomes and binding can be monitored by measuring either wavelength shifts in the light spectrum or intensity changes at a fixed wavelength. By modifying the capture antibodies, this chip is able to quantitatively detect exosome proteins. Meanwhile, IBM also developed a new lab-on-a-chip/nano-DLD technology aimed at rapid cancer diagnostics with automated exosome isolation. Other than the small microfluidic chips that could be used for diagnosis of a specific type of cancer, more integrated systems aimed at comprehensive screening of cancers by EVs are being developed. As the leader in the liquid biopsy market, Exosome Diagnostics developed an integrated exosome analysis system, Shahky™, for exosomal protein capture and quantitative analysis. They also recently announced the launch of MedOncAlyzer 170, which examines both exosomal RNA and circulating tumor DNA. By combining both exosomal RNA and ctDNA, this system could identify cancers in both early and late stages. The mutations in COSMIC, a catalogue of 8,2000 somatic mutations in oncogenic variants of 170 cancer-related genes, could be detected in a plasma sample less than 0.5 mL. With high sensitivity and specificity, these integrated systems could be powerful tools for disease diagnosis, and particularly complementing current diagnostics for the early stages of cancer. The landscape of cancer diagnosis will undergo rapid change with the involvement of EV-based techniques.

4.2. Therapeutic applications of EVs in Cancer

a. Target EVs in cancer—Therapeutic applications of EVs have also been actively explored. Consistent with the nature of increased secretion from malignant cells, more tumor-promoting roles of EVs have been revealed compared to tumor-suppressive roles. Thus, direct targeting of EVs has been tested for the treatment of cancer. Several drugs have been found with the ability to inhibit biogenesis of exosomes in multiple studies (Table 5). Of them, GW4869 is widely used to eliminate exosomes. Sphingomyelinase mediates the formation of ceramide from sphingomyelin and ceramide is essential for the budding of intraluminal vesicles (ILVs) into MVBs (67). GW4869, as an inhibitor of nSMase2, was shown to decrease the biogenesis of exosomes in several cell lines (130, 219, 220). Similar to GW4869, other nSMase2 inhibitors, including spiroepoxide, glutathione, and manumycin-A (67, 221), were also found to inhibit the exosome biogenesis in various reports. However, inhibition of nSMase2 did not lead to exosome suppression in all cell lines (222), suggesting an alternative regulation of exosome biogenesis in different cell types. Another important regulator of exosome biogenesis is calcium (223). It was found in a hematopoietic cell line, K562, that monensin could enlarge MVBs and increase the

production of exosomes. This effect was achieved by the increase of Ca^{2+} as the Na+/H+ exchanger, monensin, reversed activity of the Na^{+}/Ca^{2+} exchanger (224). Furthermore, transferrin, which was reported to increase the intracellular Ca^{2+} level, was also able to induce exosome release in K562 cells. Interestingly, dimethyl amiloride, an inhibitor of the H+/Na+ and Na+/Ca^{2+} exchangers, was found to inhibit exosome release in K562 cells. Other than nSMase2 and calcium, exosome biogenesis was also found to be related with several other signaling pathways. Brefeldin A-inhibited guanine nucleotide-exchange protein (BIG)2 was found to be associated with the release of type I tumor necrosis factor receptor carried by exosomes from human vascular endothelial cells (225). Use of brefeldin A has been reported to successfully decrease exosome production from cells (119, 221). The regulatory effect of BIG2 on exosome release is dependent on two distinct mechanisms. One is through the activation of class I ADP-ribosylation factors (ARFs) 1 and 3 (168), while another is through the role of BIG2 as a kinase-anchoring protein for RIIβ that localizes protein kinase A signaling in exosome trafficking (226). In addition, ARF 6 was found to be associated with exosome biogenesis, particularly for syntenin-positive exosomes (58). ARF6 induces budding of ILVs in MVBs through its effector phospholipase D2 (PLD2) and treatment of PLD2 inhibitor CAY10594 on MCF7-suppressed exosome production (58). However, ARF6 was also found to regulate shedding of MVs from cancer cells (227). The specificity of CAY10594 needs to be further addressed to clarify its role in exosomes and MVs. While the above reagents target the biogenesis of exosomes, the uptake of EVs can also be suppressed to block the oncogenic roles of EVs on recipient cells. Disruption of actin cytoskeletal filaments by Cytochalasin D or Lantrunculin A has been shown to suppress exosome uptake (228). Treatment with heparin, a competitive inhibitor of cell surface receptors dependent on HSPG coreceptors, decreased exosome uptake in bladder cancer cells (229). However, these reagents also inhibit endocytosis in target cells, and therefore, the possible negative side effects of these reagents should be considered. Other than exosomes, drugs targeting MV release have also been developed. While the biogenesis of MV occurs by out-budding of the plasma membrane, constituents of membranes have been explored for their roles in this process. Actin is a cytoskeleton protein positively involved in membrane dynamics, cell mobility, and contraction, and it is essential for MV biogenesis. Inhibition of actin-related proteins suppressed the release of MVs from cells. One of the MV inhibitors with this mechanism of action is calpeptin (230, 231), an inhibitor of calpain. Calpain-mediated destabilization of the cortical actin cytoskeleton is essential for MV biogenesis. Another important regulator of actin filaments is RhoA. Inhibition of RhoA pathway by targeting the factor downstream of RhoA, Rho-associated, coiled-coil containing protein kinase, resulted in a decrease of MV release (232, 233). Due to the shared membrane trafficking machineries or regulatory pathways, some reagents could lead to inhibition of both exosomes and MVs. For example, exosome release was found to be regulated in a calcium-dependent manner (223); however, calcium also regulates phospholipid redistribution, a process related to MV production (234). Calcium chelator EGTA was found to inhibit both exosome and MV production from cells (223, 235). Currently, these EV-inhibiting reagents are limited to research use only. In addition to inhibition of EV release, these reagents also induce off-target changes, as many of them are major regulators of cell functions. The translational implication of EV-targeted reagents requires further understanding of the regulatory pathways specific to each EV.

b. EV-based cancer vaccine

Cancer cell-secreted EVs could also be used for vaccine immunotherapy. It was found that both tumor cell-derived EVs and immune cell-derived EVs have a role in immune activation. More importantly, antigen-presenting EVs from B lymphocytes and dendritic cell containing MHC class I and class II complexes could also stimulate both CD4+ and CD8+ T cells (9, 236, 237). Other than presenting antigens directly, EVs have also been found to transfer antigenic peptides to antigen-presenting cells (APCs) (238), or exchange functional peptide-MHC complexes between APCs (236), thus mediating the indirect antigen presenting process. For example, exosomes released from tumor cells could be recognized and engulfed by dendritic cells for antigen presentation, which is capable of inducing potent CD8+ T cell-dependent antitumor effects (239). These exosomes also contain enriched immunostimulatory factors such as tetraspan proteins CD63 and CD82, which is a possible reason for the superior anti-tumor effect compared to that of whole dendritic cells (240). Exosome released from dendritic cells could act as signal extender by transmitting antigen to other dendritic cells (241). These findings suggest that exosomes could be used as a cell-free vaccine for immunotherapy in cancer patients (Figure 3). In a phase I clinical study of cancer vaccine using dendritic cell-derived exosomes (dexosome), tumor-specific antigens-MAGE peptides were loaded into dexosomes from NSCLC patients (242). These dexosomes were given back to patients and were well-tolerated. More importantly, these vaccines activated immune response and induced long term stable disease in some patients. A similar study was carried out in 15 late stage melanoma patients. MAGE 3 peptides were loaded onto autologous DC derived-exosomes and then used as cancer vaccine. The dexosome-based cancer vaccine was well-tolerated, and in 4 patients some extent of therapy response was observed; in particular, partial response was observed in 1 patient (243). However, in this trial, no significant T cell response was observed. These two studies suggest the safety and feasibility of using exosomes as cell-free cancer vaccines. Furthermore, an intriguing finding in both trials was the increase of NK activity following immunization, suggesting dexosomes contained stimulatory factors for innate immune cells (242, 243). To further verify the safety and immunotherapeutic effect of dexosomes, a phase II clinical trial was performed in 22 late stage NSCLC patients after first line chemotherapy (244). The dexosomes in this study was termed second generation since it was optimized to boost both NK cell and T cell response. To achieve this purpose, the exosomes were isolated from IFN-γ matured DC before antigen loading. These second generation dexosomes were well tolerated and 7 patients experienced stabilized disease during 4 months, which didn't meet the primary endpoint that 50% patients achieve progression-free survival by 4 months post-chemotherapy. However, dexosome-enhanced NK cell response was observed in patients with prolonged survival and it correlated with the expression of MHC II and BAG6, which are known to be activators of NK cells, on the dexosomes (245–247). However, there was no clear evidence of activation of tumor-specific T cell response in patients, which might be caused by several reasons. One may be the immunosuppressive phenotype associated with these patients since they were previously vaccinated with metastatic NSCLC. Other than dexosomes, exosomes from other sources have also been shown to have immunomodulatory functions. Tumor cell-derived exosome (TEX) was also found to be immunogenic with antigen presenting functions (239). It was reported to trigger tumor specific DC and T cell responses *in vitro* and *in vivo* in multiple types of cancers (248, 249). However, use of TEX

in a clinical setting has its limitation due to the source of cancer cells. In some cases of cancers, ascites could be an alternative source of TEX. In a phase I clinical trial in 40 advanced CRC patients, exosomes from ascites were shown to induce tumor-specific antitumor cytotoxic T lymphocyte response when combined with GM-CSF treatment (250). Similar to other reports, treatment with exosomes in CRC patients was well-tolerated. However, ascites is limited by the types of cancer and clinical presentation. Moreover, TEX was also found to be immunosuppressive in some cases (251, 252). With these complications, especially oncogenic compositions in TEX, its use as a cancer vaccine needs further investigation to verify its feasibility and safety. Exosome-based immunotherapy requires the loading of antigen, which can be achieved in both indirect and direct ways (242). In the indirect approach, peptides are added into DC culture, so they can be loaded onto MHC proteins expressed on the cell surface. Later antigen-presenting exosomes could be purified from the CM of these peptide-treated DC (253). In the direct loading approach, antigen-presenting exosomes are generated by acid elution. DC-derived exosomes are mixed with peptide in acetate buffer of pH 5.1 and assembly of MHC and peptide is achieved by neutralizing the buffer to pH 7.0 (253).

c. EV-based drug delivery system

Another therapeutic application of EVs includes use as a drug delivery system (Figure 3). As drug carriers, exosomes outcompete ABs and MVs. There are several advantages associated with exosome-based drug carriers. First, their size distribution is smaller and more homogenous compared to that of MVs and ABs. The advantage in size enables better biodistribution and bioavailability of exosomes in the human body. Escape of lung clearance and permeability across the blood-brain barrier were reported for exosome-based drug carriers (200, 254). In addition, exosomes could achieve targeted delivery compared to synthetic nanoparticles. Exosomes could recognize and thus deliver cargoes to specific types of cells by the ligands or receptors expressed on the membrane. One of these bridges is the tetraspanin family, which is widely expressed on exosome membrane and acts as an exosome marker (255). Tetraspanin 8, an organizer of microdomains in the membrane, was found to be expressed in a rat pancreatic cancer cell line and associated with integrin α4 (256). Expression of tetraspanin 8-integrin α4 complex resulted in selective uptake of exosomes by endothelial and pancreas cells, with CD54 as the ligand. Other integrins were also found to be associated with organotropic metastasis of multiple cancers (185). Integrin β_4 was associated with lung metastasis while integrin β5 was associated with cancer liver metastasis. Targeted delivery of exosomes from different cell lines to different organs mediated by integrin receptors resulted in pre-metastatic niches responsible for organotropic metastases. In another study, MUC1 was expressed on the exosome from breast milk and responsible for the targeted delivery to monocyte-derived dendritic cells via the CD209 receptor in the recipient cells (257). Other than the natural targeting proteins carried by exosomes, artificial targeting components can be easily engineered onto exosomes. While for synthetic nanoparticles, targeting components must be loaded directly, in exosomes they can be assembled using either direct or indirect approaches. As mentioned in dexosome, peptides can be loaded onto either exosomes or parental cells (242, 253). Compared to synthetic nanoparticles, the approach of modification of exosomes through parental cells is much easier and cost efficient. Ectopic expression of targeting components can be

introduced into parental cells that generate a significant number of engineered exosomes. For example, a vector coding targeting peptides fused with a transmembrane protein, such as Lamp2b, was introduced into parental cells to enhance the targeted delivery (258). Secondary, the source of exosome is abundant. Drug carrier exosomes have been reported to be isolated from various types of cells, including HEK-293 cells, immature DC, MSCs, and cancer cells (259–263). Furthermore, the exosome-based drug carrier is less immunogenic compared to synthetic nanoparticles. Different therapeutic reagents including protein, siRNA, miRNA, and chemicals can be incorporated into exosomes via electroporation, chemical-based transfection, modification of parental cells, or direct incubation (264), which increases the bioactivity and achieve targeted delivery in patients. It was reported that exosomal delivery of curcumin increased the stability and the blood concentration of the drug, inducing specific delivery to inflammatory cells (261). As a follow up of this work, James Graham Brown Cancer Center initiated two phase I clinical trials to test the therapeutic effect of plant exosomes on head and neck cancer as well as colon cancer (NCT01668849, NCT01294072).

5. Discussion

EVs are emerging targets for cancer diagnosis and therapy. The physiological and pathological roles of EVs are under active study. However, successful translation from bench to bedside still needs a deeper understanding of these particles that were previously considered cell debris. However, current studies of EVs still lack the standardization of isolation and analysis. A primary reason is a lack of the specific markers of exosomes and MVs, which hampers accurate demarcation of their identities. Exosome makers, such as tetraspanins, could also be found in MVs (19), and past studies may lack the accurate authentication of the particles studied, which is a potential cause of controversial results from different publications. With various approaches now available, the isolation methods have been shown to have a great impact on subsequent analysis (111). Thus, standardization of the isolation method is critical for an accurate assessment of EVs. In this review, we discussed the advantages and disadvantages of currently available isolation techniques and their impact on subsequent analyses, which we hope will be helpful to standardize the isolation protocols in this research field, although the identity of isolated particles still needs to be verified due to the variations in morphologies of EVs. While exosomes usually have diameters ranging from 40–100 nm, ones with diameters larger than 100 nm have also been identified (250). Different subpopulations of exosomes were discovered with diverse sizes, cargoes, and morphologies (19, 24, 265–267). These findings suggest that classification of EVs requires combinatory analyses of sizes, makers, and shapes.

When quantifying the RNA content in exosomes and MVs, especially small RNAs, the normalization of data may be a critical point. Currently, there are three strategies to normalize qRT-PCR readings of small RNA in EVs. They can be normalized to average Cq values of all arrays. While this approach has the highest accuracy, it needs a large number of arrays and is more suitable for global gene profiling. Another strategy is to normalize the expression to endogenous reference genes. However, there is no well accepted “housekeeping” transcript for small RNA in EVs. Selection of endogenous reference genes needs optimization and verification on a case-by-case basis. The third approach is using an

exogenous spike-in synthetic oligonucleotide. This strategy is very controversial. It could provide an estimate of efficiency in RNA extraction and reverse transcription. However, for using them for normalization, a fixed amount of spike-in needs to be added into exactly the same amount of RNA from different samples, which is not practical. Furthermore, this approach cannot compensate the variations in RNA quality resulted from sample handling before the spike-in is added (268). Thus, normalization of small RNAs from EVs requires great caution, and selection of reference genes needs to be carefully determined and verified. Another concern is the EV concentration used in the functional study. The exosome concentration in plasma has been reported to be 10^9–10^{11} exosome/ml (269, 270). When translated into protein, serum and plasma have the concentrations at 50–200 μg/ml while freeze-thaw can increase the concentration to 50–500 μg/ml (269, 271). Thus, exosome treatment should ensure that the concentration used is within the physiological range.

While the translational application of EV as a diagnostic tool has been rapidly developed and established, the therapeutic application of EVs still lags behind. Even though exosome targeted therapy has been shown to have an anti-cancer effect in specific cases in pre-clinical models (182, 272), efficacy and safety in clinical use need further study. Currently, there is no available drug that can be used to safely and specifically inhibit EV biogenesis in patients. More investigation is needed to picture the layout of the machineries involved in EV biogenesis, cargo sorting, and EV uptake. Understanding how cancer cells coordinate these machineries to generate EVs with tumor-promoting effect could provide the opportunities to find specific regulators involved in EV-associated cancer development, which helps the development of drugs targeting EVs in cancer without significant side effects. A potential direction is looking at the different subpopulations among the same type of EVs. Cancer cells were found to secrete different subpopulations of exosomes harboring different cargoes and functions (273, 274). Deciphering the differences between these subpopulations is necessary for fully understanding the roles of cancer cell derived exosomes. It is also crucial to distinguish cancer-promoting exosomes and cancer supportive exosomes since both have been identified in cancer cells. This would help in identifying specific targets for exosome-targeted therapy. It also helps in identifying the appropriate population of tumor cell-derived exosomes that can be used as a tumor vaccine. At the same time, understanding the differences between EVs from same cells would also optimize the efficacy of a dexsome-based vaccine. It was reported that MHC II was more enriched on larger EVs (100–200 nm) compared to that on the smaller EVs (30–50 nm) (56), which might be a cause of the failure of exosome-based cancer vaccine in inducing T cell-specific responses (243, 244). Selection of right EV population could enhance the immunostimulatory effect of these vaccines, enabling their better clinical use. Currently, more studies are conducted on roles of exosomes in cancer, and less emphasis is placed on MVs and ABs. However, MVs and ABs are also known to impact cancer progression, and have regulatory pathways distinct from those of exosomes (275, 276). Therefore, understanding differential roles of exosomes, MVs, and ABs in cancer is equally important, which is likely to reveal novel diagnostic and therapeutic opportunities.

EVs as drug carriers are also being actively explored. Exosomes from stem cells have been identified with therapeutic effect in tissue repair, immune modulation, and anti-inflammation (277). They were also found to be candidates for carriers of anti-cancer drugs. RoosterBio

and Exopharm recently announced the Exomere project that aims at developing clinical-grade stem cell-derived EVs for therapeutics. Another company, MDimune, is developing BioDrone™, an anti-cancer drug delivery system based on EVs. With more active studies on EVs and rapid development in technology, this field of study may change the landscape of the fight against cancer in the near future.

Acknowledgments

This work was supported by NIH grant R01CA173499,R01CA185650 and R01CA205067 (K.W). The figures were produced, in part, by using Servier Medical Art.

Abbreviations

EV(s)	extracellular vesicle(s)
MV(s)	microvesicle(s)
AB(s)	apoptotic bodie(s)
TME	tumor microenvironment
TfRs	transferrin receptors
MVE	multivesicular endosomes
MHC	major histocompatibility complex
miRNAs	microRNAs
PCa	prostate cancer
ESCRT	Endosomal Sorting Complex Required for Transport
nSMase2	neutral sphingomyelinase 2
HSPG	heparan sulfate proteoglycan
VPS	Vacuolar Protein Sorting
THPO	thrombopoietin
ANGPTLs	angiopoietin-like proteins
SIMPLE	small integral membrane protein of the lysosome/late endosome
CRC	colorectal cancer
EBV	Epstein Barr virus
LMP1	latent membrane protein 1
hnRNP	heterogeneous nuclear ribonucleoprotein
lncRNAs	long non-coding RNAs

MVP	Major vault protein
Ndfip1	Nedd4 family-interacting protein 1
3′UTR	3′-untranslated region
RISC	RNA-induced silencing complex
CCM	cell culture media
HDLs	high-density lipoproteins
PEG	polyethylene glycol
SEC	size-exclusion chromatography
THP	Tamm–Horsfall glycoprotein
ncRNAs	non-coding RNAs
CAFs	Cancer-associated fibroblasts
MSCs	mesenchymal stem cells
MET	mesenchymal-to-epithelial transition
LINC00152	long intergenic non-protein coding RNA 152
SOC	standard of care
PSA	prostate-specific antigen
dsDNA	double-stranded DNA
NSCLC	non-small cell lung cancer
GPC1	glypican-1
GBM	glioblastoma multiforme
ILVs	intraluminal vesicles
BIG	Brefeldin A-inhibited guanine nucleotide-exchange protein
ARF(s)	ADP-ribosylation factor(s)
PLD2	phospholipase D2
PLA	proximity ligation assay

References

1. Maas SL, Breakefield XO, Weaver AM. Extracellular Vesicles: Unique Intercellular Delivery Vehicles. Trends Cell Biol. 2017; 27(3):172–188. DOI: 10.1016/j.tcb.2016.11.003 [PubMed: 27979573]
2. Wolf P. The nature and significance of platelet products in human plasma. Br J Haematol. 1967; 13(3):269–88. [PubMed: 6025241]

3. Friend C, Marovitz W, Henie G, Henie W, Tsuei D, Hirschhorn K, Holland JG, Cuttner J. Observations on cell lines derived from a patient with Hodgkin's disease. Cancer Res. 1978; 38(8): 2581–91. [PubMed: 78764]
4. Poste G, Nicolson GL. Arrest and metastasis of blood-borne tumor cells are modified by fusion of plasma membrane vesicles from highly metastatic cells. Proc Natl Acad Sci U S A. 1980; 77(1): 399–403. [PubMed: 6928631]
5. Pan BT, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. Cell. 1983; 33(3):967–78. [PubMed: 6307529]
6. Harding C, Heuser J, Stahl P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. J Cell Biol. 1983; 97(2):329–39. [PubMed: 6309857]
7. Trams EG, Lauter CJ, Salem N Jr, Heine U. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. Biochim Biophys Acta. 1981; 645(1):63–70. [PubMed: 6266476]
8. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem. 1987; 262(19):9412–20. [PubMed: 3597417]
9. Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, Geuze HJ. B lymphocytes secrete antigen-presenting vesicles. J Exp Med. 1996; 183(3):1161–72. [PubMed: 8642258]
10. Ratajczak J, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P, Ratajczak MZ. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. Leukemia. 2006; 20(5):847–56. DOI: 10.1038/sj.leu.2404132 [PubMed: 16453000]
11. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007; 9(6):654–9. DOI: 10.1038/ncb1596 [PubMed: 17486113]
12. Guescini M, Guidolin D, Vallorani L, Casadei L, Gioacchini AM, Tibollo P, Battistelli M, Falcieri E, Battistin L, Agnati LF, Stocchi V. C2C12 myoblasts release micro-vesicles containing mtDNA and proteins involved in signal transduction. Exp Cell Res. 2010; 316(12):1977–84. DOI: 10.1016/j.yexcr.2010.04.006 [PubMed: 20399774]
13. Guescini M, Genedani S, Stocchi V, Agnati LF. Astrocytes and Glioblastoma cells release exosomes carrying mtDNA. J Neural Transm (Vienna). 2010; 117(1):1–4. DOI: 10.1007/s00702-009-0288-8 [PubMed: 19680595]
14. Cai J, Han Y, Ren H, Chen C, He D, Zhou L, Eisner GM, Asico LD, Jose PA, Zeng C. Extracellular vesicle-mediated transfer of donor genomic DNA to recipient cells is a novel mechanism for genetic influence between cells. J Mol Cell Biol. 2013; 5(4):227–38. DOI: 10.1093/jmcb/mjt011 [PubMed: 23580760]
15. Thakur BK, Zhang H, Becker A, Matei I, Huang Y, Costa-Silva B, Zheng Y, Hoshino A, Brazier H, Xiang J, Williams C, Rodriguez-Barrueco R, Silva JM, Zhang W, Hearn S, Elemento O, Paknejad N, Manova-Todorova K, Welte K, Bromberg J, Peinado H, Lyden D. Double-stranded DNA in exosomes: a novel biomarker in cancer detection. Cell Res. 2014; 24(6):766–9. DOI: 10.1038/cr.2014.44 [PubMed: 24710597]
16. van der Pol E, Boing AN, Gool EL, Nieuwland R. Recent developments in the nomenclature, presence, isolation, detection and clinical impact of extracellular vesicles. J Thromb Haemost. 2016; 14(1):48–56. DOI: 10.1111/jth.13190 [PubMed: 26564379]
17. Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol. 2002; 2(8):569–79. DOI: 10.1038/nri855 [PubMed: 12154376]
18. Laulagnier K, Motta C, Hamdi S, Roy S, Fauvelle F, Pageaux JF, Kobayashi T, Salles JP, Perret B, Bonnerot C, Record M. Mast cell-and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. Biochem J. 2004; 380(Pt 1):161–71. DOI: 10.1042/BJ20031594 [PubMed: 14965343]
19. Bobrie A, Colombo M, Krumeich S, Raposo G, Thery C. Diverse subpopulations of vesicles secreted by different intracellular mechanisms are present in exosome preparations obtained by differential ultracentrifugation. J Extracell Vesicles. 2012; 1doi: 10.3402/jev.v1i0.18397

20. Dozio V, Sanchez JC. Characterisation of extracellular vesicle-subsets derived from brain endothelial cells and analysis of their protein cargo modulation after TNF exposure. J Extracell Vesicles. 2017; 6(1):1302705.doi: 10.1080/20013078.2017.1302705 [PubMed: 28473883]

21. Pook M, Tamming L, Padari K, Tiido T, Maimets T, Patarroyo M, Juronen E, Jaks V, Ingerpuu S. Platelets store laminins 411/421 and 511/521 in compartments distinct from alpha- or dense granules and secrete these proteins via microvesicles. J Thromb Haemost. 2014; 12(4):519–27. DOI: 10.1111/jth.12513 [PubMed: 24450402]

22. Logozzi M, De Milito A, Lugini L, Borghi M, Calabro L, Spada M, Perdicchio M, Marino ML, Federici C, Iessi E, Brambilla D, Venturi G, Lozupone F, Santinami M, Huber V, Maio M, Rivoltini L, Fais S. High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients. PLoS One. 2009; 4(4):e5219.doi: 10.1371/journal.pone.0005219 [PubMed: 19381331]

23. Lee Y, El Andaloussi S, Wood MJ. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum Mol Genet. 2012; 21(R1):R125–34. DOI: 10.1093/hmg/dds317 [PubMed: 22872698]

24. Willms E, Johansson HJ, Mager I, Lee Y, Blomberg KE, Sadik M, Alaarg A, Smith CI, Lehtio J, El Andaloussi S, Wood MJ, Vader P. Cells release subpopulations of exosomes with distinct molecular and biological properties. Sci Rep. 2016; 6:22519.doi: 10.1038/srep22519 [PubMed: 26931825]

25. Menck K, Bleckmann A, Wachter A, Hennies B, Ries L, Schulz M, Balkenhol M, Pukrop T, Schatlo B, Rost U, Wenzel D, Klemm F, Binder C. Characterisation of tumour-derived microvesicles in cancer patients' blood and correlation with clinical outcome. J Extracell Vesicles. 2017; 6(1):1340745.doi: 10.1080/20013078.2017.1340745 [PubMed: 28804596]

26. Comelli L, Rocchiccioli S, Smirni S, Salvetti A, Signore G, Citti L, Trivella MG, Cecchettini A. Characterization of secreted vesicles from vascular smooth muscle cells. Mol Biosyst. 2014; 10(5): 1146–52. DOI: 10.1039/c3mb70544g [PubMed: 24626815]

27. Wu K, Sharma S, Venkat S, Liu K, Zhou X, Watabe K. Non-coding RNAs in cancer brain metastasis. Front Biosci (Schol Ed). 2016; 8:187–202. [PubMed: 26709907]

28. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013; 200(4):373–83. DOI: 10.1083/jcb.201211138 [PubMed: 23420871]

29. Kastelowitz N, Yin H. Exosomes and microvesicles: identification and targeting by particle size and lipid chemical probes. Chembiochem. 2014; 15(7):923–8. DOI: 10.1002/cbic.201400043 [PubMed: 24740901]

30. Lawson C, Vicencio JM, Yellon DM, Davidson SM. Microvesicles and exosomes: new players in metabolic and cardiovascular disease. J Endocrinol. 2016; 228(2):R57–71. DOI: 10.1530/JOE-15-0201 [PubMed: 26743452]

31. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007; 35(4):495–516. DOI: 10.1080/01926230701320337 [PubMed: 17562483]

32. Bergsmedh A, Szeles A, Henriksson M, Bratt A, Folkman MJ, Spetz AL, Holmgren L. Horizontal transfer of oncogenes by uptake of apoptotic bodies. Proc Natl Acad Sci U S A. 2001; 98(11): 6407–11. DOI: 10.1073/pnas.101129998 [PubMed: 11353826]

33. Singh P, Goel H, Husain M, Lan X, Mikulak J, Malthotra A, Teichberg S, Schmidtmayerova H, Singhal PC. Tubular cell HIV-entry through apoptosed CD4 T cells: a novel pathway. Virology. 2012; 434(1):68–77. DOI: 10.1016/j.virol.2012.09.009 [PubMed: 23040891]

34. Berda-Haddad Y, Robert S, Salers P, Zekraoui L, Farnarier C, Dinarello CA, Dignat-George F, Kaplanski G. Sterile inflammation of endothelial cell-derived apoptotic bodies is mediated by interleukin-1alpha. Proc Natl Acad Sci U S A. 2011; 108(51):20684–9. DOI: 10.1073/pnas.1116848108 [PubMed: 22143786]

35. Minciacchi VR, Freeman MR, Di Vizio D. Extracellular vesicles in cancer: exosomes, microvesicles and the emerging role of large oncosomes. Semin Cell Dev Biol. 2015; 40:41–51. DOI: 10.1016/j.semcdb.2015.02.010 [PubMed: 25721812]

36. Junquera C, Castiella T, Munoz G, Fernandez-Pacheco R, Luesma MJ, Monzon M. Biogenesis of a new type of extracellular vesicles in gastrointestinal stromal tumors: ultrastructural profiles of

spheresomes. Histochem Cell Biol. 2016; 146(5):557–567. DOI: 10.1007/s00418-016-1460-5 [PubMed: 27373548]

37. Sedgwick AE, Clancy JW, Olivia Balmert M, D'Souza-Schorey C. Extracellular microvesicles and invadopodia mediate non-overlapping modes of tumor cell invasion. Sci Rep. 2015; 5:14748.doi: 10.1038/srep14748 [PubMed: 26458510]
38. Di Vizio D, Morello M, Dudley AC, Schow PW, Adam RM, Morley S, Mulholland D, Rotinen M, Hager MH, Insabato L, Moses MA, Demichelis F, Lisanti MP, Wu H, Klagsbrun M, Bhowmick NA, Rubin MA, Freeman MR, D'Souza-Schorey C. Large oncosomes in human prostate cancer tissues and in the circulation of mice with metastatic disease. Am J Pathol. 2012; 181(5):1573–84. DOI: 10.1016/j.ajpath.2012.07.030 [PubMed: 23022210]
39. Di Vizio D, Kim J, Hager MH, Morello M, Yang W, Lafargue CJ, True LD, Rubin MA, Adam RM, Beroukhim R, Demichelis F, Freeman MR. Oncosome formation in prostate cancer: association with a region of frequent chromosomal deletion in metastatic disease. Cancer Res. 2009; 69(13): 5601–9. DOI: 10.1158/0008-5472.CAN-08-3860 [PubMed: 19549916]
40. Kowal J, Tkach M, Thery C. Biogenesis and secretion of exosomes. Curr Opin Cell Biol. 2014; 29:116–25. DOI: 10.1016/j.ceb.2014.05.004 [PubMed: 24959705]
41. Kosaka N, Iguchi H, Hagiwara K, Yoshioka Y, Takeshita F, Ochiya T. Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic microRNAs regulate cancer cell metastasis. J Biol Chem. 2013; 288(15):10849–59. DOI: 10.1074/jbc.M112.446831 [PubMed: 23439645]
42. Wang T, Gilkes DM, Takano N, Xiang L, Luo W, Bishop CJ, Chaturvedi P, Green JJ, Semenza GL. Hypoxia-inducible factors and RAB22A mediate formation of microvesicles that stimulate breast cancer invasion and metastasis. Proc Natl Acad Sci U S A. 2014; 111(31):E3234–42. DOI: 10.1073/pnas.1410041111 [PubMed: 24938788]
43. Li L, Li C, Wang S, Wang Z, Jiang J, Wang W, Li X, Chen J, Liu K, Li C, Zhu G. Exosomes Derived from Hypoxic Oral Squamous Cell Carcinoma Cells Deliver miR-21 to Normoxic Cells to Elicit a Prometastatic Phenotype. Cancer Res. 2016; 76(7):1770–80. DOI: 10.1158/0008-5472.CAN-15-1625 [PubMed: 26992424]
44. Chen L, Brigstock DR. Integrins and heparan sulfate proteoglycans on hepatic stellate cells (HSC) are novel receptors for HSC-derived exosomes. FEBS Lett. 2016; 590(23):4263–4274. DOI: 10.1002/1873-3468.12448 [PubMed: 27714787]
45. Hazan-Halevy I, Rosenblum D, Weinstein S, Bairey O, Raanani P, Peer D. Cell-specific uptake of mantle cell lymphoma-derived exosomes by malignant and non-malignant B-lymphocytes. Cancer Lett. 2015; 364(1):59–69. DOI: 10.1016/j.canlet.2015.04.026 [PubMed: 25933830]
46. Koumangoye RB, Sakwe AM, Goodwin JS, Patel T, Ochieng J. Detachment of breast tumor cells induces rapid secretion of exosomes which subsequently mediate cellular adhesion and spreading. PLoS One. 2011; 6(9):e24234.doi: 10.1371/journal.pone.0024234 [PubMed: 21915303]
47. Valapala M, Vishwanatha JK. Lipid raft endocytosis and exosomal transport facilitate extracellular trafficking of annexin A2. J Biol Chem. 2011; 286(35):30911–25. DOI: 10.1074/jbc.M111.271155 [PubMed: 21737841]
48. Mulcahy LA, Pink RC, Carter DR. Routes and mechanisms of extracellular vesicle uptake. J Extracell Vesicles. 2014; 3doi: 10.3402/jev.v3.24641
49. Akers JC, Gonda D, Kim R, Carter BS, Chen CC. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. J Neurooncol. 2013; 113(1):1–11. DOI: 10.1007/s11060-013-1084-8 [PubMed: 23456661]
50. Tian T, Zhu YL, Hu FH, Wang YY, Huang NP, Xiao ZD. Dynamics of exosome internalization and trafficking. J Cell Physiol. 2013; 228(7):1487–95. DOI: 10.1002/jcp.24304 [PubMed: 23254476]
51. Goldie BJ, Dun MD, Lin M, Smith ND, Verrills NM, Dayas CV, Cairns MJ. Activity-associated miRNA are packaged in Map1b-enriched exosomes released from depolarized neurons. Nucleic Acids Res. 2014; 42(14):9195–208. DOI: 10.1093/nar/gku594 [PubMed: 25053844]
52. Guduric-Fuchs J, O'Connor A, Camp B, O'Neill CL, Medina RJ, Simpson DA. Selective extracellular vesicle-mediated export of an overlapping set of microRNAs from multiple cell types. BMC Genomics. 2012; 13:357.doi: 10.1186/1471-2164-13-357 [PubMed: 22849433]
53. Melo SA, Sugimoto H, O'Connell JT, Kato N, Villanueva A, Vidal A, Qiu L, Vitkin E, Perelman LT, Melo CA, Lucci A, Ivan C, Calin GA, Kalluri R. Cancer exosomes perform cell-independent

microRNA biogenesis and promote tumorigenesis. Cancer Cell. 2014; 26(5):707–21. DOI: 10.1016/j.ccell.2014.09.005 [PubMed: 25446899]

54. Squadrito ML, Baer C, Burdet F, Maderna C, Gilfillan GD, Lyle R, Ibberson M, De Palma M. Endogenous RNAs modulate microRNA sorting to exosomes and transfer to acceptor cells. Cell Rep. 2014; 8(5):1432–46. DOI: 10.1016/j.celrep.2014.07.035 [PubMed: 25159140]
55. Grant BD, Donaldson JG. Pathways and mechanisms of endocytic recycling. Nat Rev Mol Cell Biol. 2009; 10(9):597–608. DOI: 10.1038/nrm2755 [PubMed: 19696797]
56. Colombo M, Moita C, van Niel G, Kowal J, Vigneron J, Benaroch P, Manel N, Moita LF, Thery C, Raposo G. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. J Cell Sci. 2013; 126(Pt 24):5553–65. DOI: 10.1242/jcs.128868 [PubMed: 24105262]
57. Roucourt B, Meeussen S, Bao J, Zimmermann P, David G. Heparanase activates the syndecan-syntenin-ALIX exosome pathway. Cell Res. 2015; 25(4):412–28. DOI: 10.1038/cr.2015.29 [PubMed: 25732677]
58. Ghossoub R, Lembo F, Rubio A, Gaillard CB, Bouchet J, Vitale N, Slavik J, Machala M, Zimmermann P. Syntenin-ALIX exosome biogenesis and budding into multivesicular bodies are controlled by ARF6 and PLD2. Nat Commun. 2014; 5:3477.doi: 10.1038/ncomms4477 [PubMed: 24637612]
59. Bolukbasi MF, Mizrak A, Ozdener GB, Madlener S, Strobel T, Erkan EP, Fan JB, Breakefield XO, Saydam O. miR-1289 and "Zipcode"-like Sequence Enrich mRNAs in Microvesicles. Mol Ther Nucleic Acids. 2012; 1:e10.doi: 10.1038/mtna.2011.2 [PubMed: 23344721]
60. Latysheva N, Muratov G, Rajesh S, Padgett M, Hotchin NA, Overduin M, Berditchevski F. Syntenin-1 is a new component of tetraspanin-enriched microdomains: mechanisms and consequences of the interaction of syntenin-1 with CD63. Mol Cell Biol. 2006; 26(20):7707–18. DOI: 10.1128/MCB.00849-06 [PubMed: 16908530]
61. Thompson CA, Purushothaman A, Ramani VC, Vlodavsky I, Sanderson RD. Heparanase regulates secretion, composition, and function of tumor cell-derived exosomes. J Biol Chem. 2013; 288(14): 10093–9. DOI: 10.1074/jbc.C112.444562 [PubMed: 23430739]
62. Hurley JH, Emr SD. The ESCRT complexes: structure and mechanism of a membrane-trafficking network. Annu Rev Biophys Biomol Struct. 2006; 35:277–98. DOI: 10.1146/annurev.biophys.35.040405.102126 [PubMed: 16689637]
63. Gu H, Chen C, Hao X, Wang C, Zhang X, Li Z, Shao H, Zeng H, Yu Z, Xie L, Xia F, Zhang F, Liu X, Zhang Y, Jiang H, Zhu J, Wan J, Wang C, Weng W, Xie J, Tao M, Zhang CC, Liu J, Chen GQ, Zheng J. Sorting protein VPS33B regulates exosomal autocrine signaling to mediate hematopoiesis and leukemogenesis. J Clin Invest. 2016; 126(12):4537–4553. DOI: 10.1172/JCI87105 [PubMed: 27797340]
64. Zhu H, Guariglia S, Yu RY, Li W, Brancho D, Peinado H, Lyden D, Salzer J, Bennett C, Chow CW. Mutation of SIMPLE in Charcot-Marie-Tooth 1C alters production of exosomes. Mol Biol Cell. 2013; 24(11):1619–37. S1–3. DOI: 10.1091/mbc.E12-07-0544 [PubMed: 23576546]
65. Calder PC, Yaqoob P. Lipid rafts–composition, characterization, and controversies. J Nutr. 2007; 137(3):545–7. [PubMed: 17311937]
66. de Gassart A, Geminard C, Fevrier B, Raposo G, Vidal M. Lipid raft-associated protein sorting in exosomes. Blood. 2003; 102(13):4336–44. DOI: 10.1182/blood-2003-03-0871 [PubMed: 12881314]
67. Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brugger B, Simons M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science. 2008; 319(5867):1244–7. DOI: 10.1126/science.1153124 [PubMed: 18309083]
68. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. J Biol Chem. 2010; 285(23):17442–52. DOI: 10.1074/jbc.M110.107821 [PubMed: 20353945]
69. Guo BB, Bellingham SA, Hill AF. The neutral sphingomyelinase pathway regulates packaging of the prion protein into exosomes. J Biol Chem. 2015; 290(6):3455–67. DOI: 10.1074/jbc.M114.605253 [PubMed: 25505180]

70. Cha DJ, Franklin JL, Dou Y, Liu Q, Higginbotham JN, Demory Beckler M, Weaver AM, Vickers K, Prasad N, Levy S, Zhang B, Coffey RJ, Patton JG. KRAS-dependent sorting of miRNA to exosomes. Elife. 2015; 4:e07197.doi: 10.7554/eLife.07197 [PubMed: 26132860]
71. McKenzie AJ, Hoshino D, Hong NH, Cha DJ, Franklin JL, Coffey RJ, Patton JG, Weaver AM. KRAS-MEK Signaling Controls Ago2 Sorting into Exosomes. Cell Rep. 2016; 15(5):978–87. DOI: 10.1016/j.celrep.2016.03.085 [PubMed: 27117408]
72. Perez-Hernandez D, Gutierrez-Vazquez C, Jorge I, Lopez-Martin S, Ursa A, Sanchez-Madrid F, Vazquez J, Yanez-Mo M. The intracellular interactome of tetraspanin-enriched microdomains reveals their function as sorting machineries toward exosomes. J Biol Chem. 2013; 288(17): 11649–61. DOI: 10.1074/jbc.M112.445304 [PubMed: 23463506]
73. Mazurov D, Barbashova L, Filatov A. Tetraspanin protein CD9 interacts with metalloprotease CD10 and enhances its release via exosomes. FEBS J. 2013; 280(5):1200–13. DOI: 10.1111/febs.12110 [PubMed: 23289620]
74. Verweij FJ, van Eijndhoven MA, Hopmans ES, Vendrig T, Wurdinger T, Cahir-McFarland E, Kieff E, Geerts D, van der Kant R, Neefjes J, Middeldorp JM, Pegtel DM. LMP1 association with CD63 in endosomes and secretion via exosomes limits constitutive NF-kappaB activation. EMBO J. 2011; 30(11):2115–29. DOI: 10.1038/emboj.2011.123 [PubMed: 21527913]
75. Shen B, Wu N, Yang JM, Gould SJ. Protein targeting to exosomes/microvesicles by plasma membrane anchors. J Biol Chem. 2011; 286(16):14383–95. DOI: 10.1074/jbc.M110.208660 [PubMed: 21300796]
76. Villarroya-Beltri C, Gutierrez-Vazquez C, Sanchez-Cabo F, Perez-Hernandez D, Vazquez J, Martin-Cofreces N, Martinez-Herrera DJ, Pascual-Montano A, Mittelbrunn M, Sanchez-Madrid F. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat Commun. 2013; 4:2980.doi: 10.1038/ncomms3980 [PubMed: 24356509]
77. Santangelo L, Giurato G, Cicchini C, Montaldo C, Mancone C, Tarallo R, Battistelli C, Alonzi T, Weisz A, Tripodi M. The RNA-Binding Protein SYNCRIP Is a Component of the Hepatocyte Exosomal Machinery Controlling MicroRNA Sorting. Cell Rep. 2016; 17(3):799–808. DOI: 10.1016/j.celrep.2016.09.031 [PubMed: 27732855]
78. Qu L, Ding J, Chen C, Wu ZJ, Liu B, Gao Y, Chen W, Liu F, Sun W, Li XF, Wang X, Wang Y, Xu ZY, Gao L, Yang Q, Xu B, Li YM, Fang ZY, Xu ZP, Bao Y, Wu DS, Miao X, Sun HY, Sun YH, Wang HY, Wang LH. Exosome-Transmitted lncARSR Promotes Sunitinib Resistance in Renal Cancer by Acting as a Competing Endogenous RNA. Cancer Cell. 2016; 29(5):653–68. DOI: 10.1016/j.ccell.2016.03.004 [PubMed: 27117758]
79. Teng Y, Ren Y, Hu X, Mu J, Samykutty A, Zhuang X, Deng Z, Kumar A, Zhang L, Merchant ML, Yan J, Miller DM, Zhang HG. MVP-mediated exosomal sorting of miR-193a promotes colon cancer progression. Nat Commun. 2017; 8:14448.doi: 10.1038/ncomms14448 [PubMed: 28211508]
80. Shurtleff MJ, Temoche-Diaz MM, Karfilis KV, Ri S, Schekman R. Y-box protein 1 is required to sort microRNAs into exosomes in cells and in a cell-free reaction. Elife. 2016; 5doi: 10.7554/eLife.19276
81. Geminard C, Nault F, Johnstone RM, Vidal M. Characteristics of the interaction between Hsc70 and the transferrin receptor in exosomes released during reticulocyte maturation. J Biol Chem. 2001; 276(13):9910–6. DOI: 10.1074/jbc.M009641200 [PubMed: 11133993]
82. Low LH, Chow YL, Li Y, Goh CP, Putz U, Silke J, Ouchi T, Howitt J, Tan SS. Nedd4 family interacting protein 1 (Ndfip1) is required for ubiquitination and nuclear trafficking of BRCA1-associated ATM activator 1 (BRAT1) during the DNA damage response. J Biol Chem. 2015; 290(11):7141–50. DOI: 10.1074/jbc.M114.613687 [PubMed: 25631046]
83. Koppers-Lalic D, Hackenberg M, Bijnsdorp IV, van Eijndhoven MA, Sadek P, Sie D, Zini N, Middeldorp JM, Ylstra B, de Menezes RX, Wurdinger T, Meijer GA, Pegtel DM. Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes. Cell Rep. 2014; 8(6):1649–58. DOI: 10.1016/j.celrep.2014.08.027 [PubMed: 25242326]
84. Liang Y, Eng WS, Colquhoun DR, Dinglasan RR, Graham DR, Mahal LK. Complex N-linked glycans serve as a determinant for exosome/microvesicle cargo recruitment. J Biol Chem. 2014; 289(47):32526–37. DOI: 10.1074/jbc.M114.606269 [PubMed: 25261472]

85. Clancy JW, Sedgwick A, Rosse C, Muralidharan-Chari V, Raposo G, Method M, Chavrier P, D'Souza-Schorey C. Regulated delivery of molecular cargo to invasive tumour-derived microvesicles. Nat Commun. 2015; 6:6919.doi: 10.1038/ncomms7919 [PubMed: 25897521]

86. Iavello A, Frech VS, Gai C, Deregibus MC, Quesenberry PJ, Camussi G. Role of Alix in miRNA packaging during extracellular vesicle biogenesis. Int J Mol Med. 2016; 37(4):958–66. DOI: 10.3892/ijmm.2016.2488 [PubMed: 26935291]

87. Szatanek R, Baran J, Siedlar M, Baj-Krzyworzeka M. Isolation of extracellular vesicles: Determining the correct approach (Review). Int J Mol Med. 2015; 36(1):11–7. DOI: 10.3892/ijmm.2015.2194 [PubMed: 25902369]

88. Jeppesen DK, Hvam ML, Primdahl-Bengtson B, Boysen AT, Whitehead B, Dyrskjot L, Orntoft TF, Howard KA, Ostenfeld MS. Comparative analysis of discrete exosome fractions obtained by differential centrifugation. J Extracell Vesicles. 2014; 3:25011.doi: 10.3402/jev.v3.25011 [PubMed: 25396408]

89. Crescitelli R, Lasser C, Szabo TG, Kittel A, Eldh M, Dianzani I, Buzas EI, Lotvall J. Distinct RNA profiles in subpopulations of extracellular vesicles: apoptotic bodies, microvesicles and exosomes. J Extracell Vesicles. 2013; 2doi: 10.3402/jev.v2i0.20677

90. Lobb RJ, Becker M, Wen SW, Wong CS, Wiegmans AP, Leimgruber A, Moller A. Optimized exosome isolation protocol for cell culture supernatant and human plasma. J Extracell Vesicles. 2015; 4:27031.doi: 10.3402/jev.v4.27031 [PubMed: 26194179]

91. Thery C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol. 2006; :22. Chapter 3, Unit 3. doi: 10.1002/0471143030.cb0322s30 [PubMed: 18228490]

92. Zhang Z, Wang C, Li T, Liu Z, Li L. Comparison of ultracentrifugation and density gradient separation methods for isolating Tca8113 human tongue cancer cell line-derived exosomes. Oncol Lett. 2014; 8(4):1701–1706. DOI: 10.3892/ol.2014.2373 [PubMed: 25202395]

93. Yuana Y, Levels J, Grootemaat A, Sturk A, Nieuwland R. Co-isolation of extracellular vesicles and high-density lipoproteins using density gradient ultracentrifugation. J Extracell Vesicles. 2014; 3doi: 10.3402/jev.v3.23262

94. Desgagne V, Guerin R, Guay SP, Corbin F, Couture P, Lamarche B, Bouchard L. Changes in high-density lipoprotein-carried miRNA contribution to the plasmatic pool after consumption of dietary trans fat in healthy men. Epigenomics. 2017; 9(5):669–688. DOI: 10.2217/epi-2016-0177 [PubMed: 28470118]

95. Heinecke JW. The protein cargo of HDL: implications for vascular wall biology and therapeutics. J Clin Lipidol. 2010; 4(5):371–5. DOI: 10.1016/j.jacl.2010.08.005 [PubMed: 20975842]

96. Merchant ML, Powell DW, Wilkey DW, Cummins TD, Deegens JK, Rood IM, McAfee KJ, Fleischer C, Klein E, Klein JB. Microfiltration isolation of human urinary exosomes for characterization by MS. Proteomics Clin Appl. 2010; 4(1):84–96. DOI: 10.1002/prca.200800093 [PubMed: 21137018]

97. Sharma S, Rasool HI, Palanisamy V, Mathisen C, Schmidt M, Wong DT, Gimzewski JK. Structural-mechanical characterization of nanoparticle exosomes in human saliva, using correlative AFM, FESEM, and force spectroscopy. ACS Nano. 2010; 4(4):1921–6. DOI: 10.1021/nn901824n [PubMed: 20218655]

98. Rider MA, Hurwitz SN, Meckes DG Jr. ExtraPEG: A Polyethylene Glycol-Based Method for Enrichment of Extracellular Vesicles. Sci Rep. 2016; 6:23978.doi: 10.1038/srep23978 [PubMed: 27068479]

99. Weng Y, Sui Z, Shan Y, Hu Y, Chen Y, Zhang L, Zhang Y. Effective isolation of exosomes with polyethylene glycol from cell culture supernatant for in-depth proteome profiling. Analyst. 2016; 141(15):4640–6. DOI: 10.1039/c6an00892e [PubMed: 27229443]

100. Boing AN, van der Pol E, Grootemaat AE, Coumans FA, Sturk A, Nieuwland R. Single-step isolation of extracellular vesicles by size-exclusion chromatography. J Extracell Vesicles. 2014; 3doi: 10.3402/jev.v3.23430

101. Baranyai T, Herczeg K, Onodi Z, Voszka I, Modos K, Marton N, Nagy G, Mager I, Wood MJ, El Andaloussi S, Palinkas Z, Kumar V, Nagy P, Kittel A, Buzas EI, Ferdinandy P, Giricz Z. Isolation of Exosomes from Blood Plasma: Qualitative and Quantitative Comparison of

Ultracentrifugation and Size Exclusion Chromatography Methods. PLoS One. 2015; 10(12):e0145686.doi: 10.1371/journal.pone.0145686 [PubMed: 26690353]

102. Gamez-Valero A, Monguio-Tortajada M, Carreras-Planella L, Franquesa M, Beyer K, Borras FE. Size-Exclusion Chromatography-based isolation minimally alters Extracellular Vesicles' characteristics compared to precipitating agents. Sci Rep. 2016; 6:33641.doi: 10.1038/srep33641 [PubMed: 27640641]
103. Xu R, Fitts A, Li X, Fernandes J, Pochampally R, Mao J, Liu YM. Quantification of Small Extracellular Vesicles by Size Exclusion Chromatography with Fluorescence Detection. Anal Chem. 2016; 88(21):10390–10394. DOI: 10.1021/acs.analchem.6b03348 [PubMed: 27689436]
104. Blans K, Hansen MS, Sorensen LV, Hvam ML, Howard KA, Moller A, Wiking L, Larsen LB, Rasmussen JT. Pellet-free isolation of human and bovine milk extracellular vesicles by size-exclusion chromatography. J Extracell Vesicles. 2017; 6(1):1294340.doi: 10.1080/20013078.2017.1294340 [PubMed: 28386391]
105. Hong CS, Funk S, Muller L, Boyiadzis M, Whiteside TL. Isolation of biologically active and morphologically intact exosomes from plasma of patients with cancer. J Extracell Vesicles. 2016; 5:29289.doi: 10.3402/jev.v5.29289 [PubMed: 27018366]
106. Mizutani K, Terazawa R, Kameyama K, Kato T, Horie K, Tsuchiya T, Seike K, Ehara H, Fujita Y, Kawakami K, Ito M, Deguchi T. Isolation of prostate cancer-related exosomes. Anticancer Res. 2014; 34(7):3419–23. [PubMed: 24982349]
107. Van Deun J, Mestdagh P, Sormunen R, Cocquyt V, Vermaelen K, Vandesompele J, Bracke M, De Wever O, Hendrix A. The impact of disparate isolation methods for extracellular vesicles on downstream RNA profiling. J Extracell Vesicles. 2014; 3doi: 10.3402/jev.v3.24858
108. Yamada T, Inoshima Y, Matsuda T, Ishiguro N. Comparison of methods for isolating exosomes from bovine milk. J Vet Med Sci. 2012; 74(11):1523–5. [PubMed: 22785357]
109. Lane RE, Korbie D, Anderson W, Vaidyanathan R, Trau M. Analysis of exosome purification methods using a model liposome system and tunable-resistive pulse sensing. Sci Rep. 2015; 5:7639.doi: 10.1038/srep07639 [PubMed: 25559219]
110. Helwa I, Cai J, Drewry MD, Zimmerman A, Dinkins MB, Khaled ML, Seremwe M, Dismuke WM, Bieberich E, Stamer WD, Hamrick MW, Liu Y. A Comparative Study of Serum Exosome Isolation Using Differential Ultracentrifugation and Three Commercial Reagents. PLoS One. 2017; 12(1):e0170628.doi: 10.1371/journal.pone.0170628 [PubMed: 28114422]
111. Alvarez ML, Khosroheidari M, Kanchi Ravi R, DiStefano JK. Comparison of protein, microRNA, and mRNA yields using different methods of urinary exosome isolation for the discovery of kidney disease biomarkers. Kidney Int. 2012; 82(9):1024–32. DOI: 10.1038/ki.2012.256 [PubMed: 22785172]
112. Tauro BJ, Greening DW, Mathias RA, Ji H, Mathivanan S, Scott AM, Simpson RJ. Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. Methods. 2012; 56(2):293–304. DOI: 10.1016/j.ymeth.2012.01.002 [PubMed: 22285593]
113. Yamashita T, Takahashi Y, Nishikawa M, Takakura Y. Effect of exosome isolation methods on physicochemical properties of exosomes and clearance of exosomes from the blood circulation. Eur J Pharm Biopharm. 2016; 98:1–8. DOI: 10.1016/j.ejpb.2015.10.017 [PubMed: 26545617]
114. Nordin JZ, Lee Y, Vader P, Mager I, Johansson HJ, Heusermann W, Wiklander OP, Hallbrink M, Seow Y, Bultema JJ, Gilthorpe J, Davies T, Fairchild PJ, Gabrielsson S, Meisner-Kober NC, Lehtio J, Smith CI, Wood MJ, El Andaloussi S. Ultrafiltration with size-exclusion liquid chromatography for high yield isolation of extracellular vesicles preserving intact biophysical and functional properties. Nanomedicine. 2015; 11(4):879–83. DOI: 10.1016/j.nano.2015.01.003 [PubMed: 25659648]
115. Rekker K, Saare M, Roost AM, Kubo AL, Zarovni N, Chiesi A, Salumets A, Peters M. Comparison of serum exosome isolation methods for microRNA profiling. Clin Biochem. 2014; 47(1–2):135–8. DOI: 10.1016/j.clinbiochem.2013.10.020 [PubMed: 24183884]
116. Riches A, Campbell E, Borger E, Powis S. Regulation of exosome release from mammary epithelial and breast cancer cells - a new regulatory pathway. Eur J Cancer. 2014; 50(5):1025–34. DOI: 10.1016/j.ejca.2013.12.019 [PubMed: 24462375]

117. Muralidharan-Chari V, Clancy JW, Sedgwick A, D'Souza-Schorey C. Microvesicles: mediators of extracellular communication during cancer progression. J Cell Sci. 2010; 123(Pt 10):1603–11. DOI: 10.1242/jcs.064386 [PubMed: 20445011]

118. Ramteke A, Ting H, Agarwal C, Mateen S, Somasagara R, Hussain A, Graner M, Frederick B, Agarwal R, Deep G. Exosomes secreted under hypoxia enhance invasiveness and stemness of prostate cancer cells by targeting adherens junction molecules. Mol Carcinog. 2015; 54(7):554–65. DOI: 10.1002/mc.22124 [PubMed: 24347249]

119. McCready J, Sims JD, Chan D, Jay DG. Secretion of extracellular hsp90alpha via exosomes increases cancer cell motility: a role for plasminogen activation. BMC Cancer. 2010; 10:294.doi: 10.1186/1471-2407-10-294 [PubMed: 20553606]

120. Lee JE, Moon PG, Cho YE, Kim YB, Kim IS, Park H, Baek MC. Identification of EDIL3 on extracellular vesicles involved in breast cancer cell invasion. J Proteomics. 2016; 131:17–28. DOI: 10.1016/j.jprot.2015.10.005 [PubMed: 26463135]

121. Beckham CJ, Olsen J, Yin PN, Wu CH, Ting HJ, Hagen FK, Scosyrev E, Messing EM, Lee YF. Bladder cancer exosomes contain EDIL-3/Del1 and facilitate cancer progression. J Urol. 2014; 192(2):583–92. DOI: 10.1016/j.juro.2014.02.035 [PubMed: 24530986]

122. Amin ML. P-glycoprotein Inhibition for Optimal Drug Delivery. Drug Target Insights. 2013; 7:27–34. DOI: 10.4137/DTI.S12519 [PubMed: 24023511]

123. O'Neill AJ, Prencipe M, Dowling C, Fan Y, Mulrane L, Gallagher WM, O'Connor D, O'Connor R, Devery A, Corcoran C, Rani S, O'Driscoll L, Fitzpatrick JM, Watson RW. Characterisation and manipulation of docetaxel resistant prostate cancer cell lines. Mol Cancer. 2011; 10:126.doi: 10.1186/1476-4598-10-126 [PubMed: 21982118]

124. Corcoran C, Rani S, O'Brien K, O'Neill A, Prencipe M, Sheikh R, Webb G, McDermott R, Watson W, Crown J, O'Driscoll L. Docetaxel-resistance in prostate cancer: evaluating associated phenotypic changes and potential for resistance transfer via exosomes. PLoS One. 2012; 7(12):e50999.doi: 10.1371/journal.pone.0050999 [PubMed: 23251413]

125. Lv MM, Zhu XY, Chen WX, Zhong SL, Hu Q, Ma TF, Zhang J, Chen L, Tang JH, Zhao JH. Exosomes mediate drug resistance transfer in MCF-7 breast cancer cells and a probable mechanism is delivery of P-glycoprotein. Tumour Biol. 2014; 35(11):10773–9. DOI: 10.1007/s13277-014-2377-z [PubMed: 25077924]

126. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, Rak J. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat Cell Biol. 2008; 10(5):619–24. DOI: 10.1038/ncb1725 [PubMed: 18425114]

127. Huang X, Yuan T, Tschannen M, Sun Z, Jacob H, Du M, Liang M, Dittmar RL, Liu Y, Liang M, Kohli M, Thibodeau SN, Boardman L, Wang L. Characterization of human plasma-derived exosomal RNAs by deep sequencing. BMC Genomics. 2013; 14:319.doi: 10.1186/1471-2164-14-319 [PubMed: 23663360]

128. Fiskaa T, Knutsen E, Nikolaisen MA, Jorgensen TE, Johansen SD, Perander M, Seternes OM. Distinct Small RNA Signatures in Extracellular Vesicles Derived from Breast Cancer Cell Lines. PLoS One. 2016; 11(8):e0161824.doi: 10.1371/journal.pone.0161824 [PubMed: 27579604]

129. Thind A, Wilson C. Exosomal miRNAs as cancer biomarkers and therapeutic targets. Journal of Extracellular Vesicles. 2016; 5 doi: Artn3129210.3402/Jev.V5.31292.

130. Singh R, Pochampally R, Watabe K, Lu Z, Mo YY. Exosome-mediated transfer of miR-10b promotes cell invasion in breast cancer. Mol Cancer. 2014; 13:256.doi: 10.1186/1476-4598-13-256 [PubMed: 25428807]

131. Wei Y, Lai X, Yu S, Chen S, Ma Y, Zhang Y, Li H, Zhu X, Yao L, Zhang J. Exosomal miR-221/222 enhances tamoxifen resistance in recipient ER-positive breast cancer cells. Breast Cancer Res Treat. 2014; 147(2):423–31. DOI: 10.1007/s10549-014-3037-0 [PubMed: 25007959]

132. Chen WX, Liu XM, Lv MM, Chen L, Zhao JH, Zhong SL, Ji MH, Hu Q, Luo Z, Wu JZ, Tang JH. Exosomes from drug-resistant breast cancer cells transmit chemoresistance by a horizontal transfer of microRNAs. PLoS One. 2014; 9(4):e95240.doi: 10.1371/journal.pone.0095240 [PubMed: 24740415]

133. Kogure T, Lin WL, Yan IK, Braconi C, Patel T. Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. Hepatology. 2011; 54(4):1237–48. DOI: 10.1002/hep.24504 [PubMed: 21721029]

134. Liao J, Liu R, Shi YJ, Yin LH, Pu YP. Exosome-shuttling microRNA-21 promotes cell migration and invasion-targeting PDCD4 in esophageal cancer. Int J Oncol. 2016; 48(6):2567–79. DOI: 10.3892/ijo.2016.3453 [PubMed: 27035745]

135. Au Yeung CL, Co NN, Tsuruga T, Yeung TL, Kwan SY, Leung CS, Li Y, Lu ES, Kwan K, Wong KK, Schmandt R, Lu KH, Mok SC. Exosomal transfer of stroma-derived miR21 confers paclitaxel resistance in ovarian cancer cells through targeting APAF1. Nat Commun. 2016; 7:11150.doi: 10.1038/ncomms11150 [PubMed: 27021436]

136. Ahadi A, Brennan S, Kennedy PJ, Hutvagner G, Tran N. Long non-coding RNAs harboring miRNA seed regions are enriched in prostate cancer exosomes. Sci Rep. 2016; 6:24922.doi: 10.1038/srep24922 [PubMed: 27102850]

137. Xu CG, Yang MF, Ren YQ, Wu CH, Wang LQ. Exosomes mediated transfer of lncRNA UCA1 results in increased tamoxifen resistance in breast cancer cells. Eur Rev Med Pharmacol Sci. 2016; 20(20):4362–4368. [PubMed: 27831634]

138. Huang J, Zhou N, Watabe K, Lu Z, Wu F, Xu M, Mo YY. Long non-coding RNA UCA1 promotes breast tumor growth by suppression of p27 (Kip1). Cell Death Dis. 2014; 5:e1008.doi: 10.1038/cddis.2013.541 [PubMed: 24457952]

139. Tuo YL, Li XM, Luo J. Long noncoding RNA UCA1 modulates breast cancer cell growth and apoptosis through decreasing tumor suppressive miR-143. Eur Rev Med Pharmacol Sci. 2015; 19(18):3403–11. [PubMed: 26439035]

140. Bian Z, Jin L, Zhang J, Yin Y, Quan C, Hu Y, Feng Y, Liu H, Fei B, Mao Y, Zhou L, Qi X, Huang S, Hua D, Xing C, Huang Z. LncRNA-UCA1 enhances cell proliferation and 5-fluorouracil resistance in colorectal cancer by inhibiting miR-204-5p. Sci Rep. 2016; 6:23892.doi: 10.1038/srep23892 [PubMed: 27046651]

141. Wang F, Xu Z, Zhou J, Lo WS, Lau CF, Nangle LA, Yang XL, Zhang M, Schimmel P. Regulated capture by exosomes of mRNAs for cytoplasmic tRNA synthetases. J Biol Chem. 2013; 288(41):29223–8. DOI: 10.1074/jbc.C113.490599 [PubMed: 24003230]

142. Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol. 2008; 10(12):1470–6. DOI: 10.1038/ncb1800 [PubMed: 19011622]

143. Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, Hristov M, Koppel T, Jahantigh MN, Lutgens E, Wang S, Olson EN, Schober A, Weber C. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. Sci Signal. 2009; 2(100):ra81.doi: 10.1126/scisignal.2000610 [PubMed: 19996457]

144. Hasselmann DO, Rappl G, Tilgen W, Reinhold U. Extracellular tyrosinase mRNA within apoptotic bodies is protected from degradation in human serum. Clin Chem. 2001; 47(8):1488–9. [PubMed: 11468248]

145. Holmgren L, Szeles A, Rajnavolgyi E, Folkman J, Klein G, Ernberg I, Falk KI. Horizontal transfer of DNA by the uptake of apoptotic bodies. Blood. 1999; 93(11):3956–63. [PubMed: 10339505]

146. Lleo A, Zhang W, McDonald WH, Seeley EH, Leung PS, Coppel RL, Ansari AA, Adams DH, Afford S, Invernizzi P, Gershwin ME. Shotgun proteomics: identification of unique protein profiles of apoptotic bodies from biliary epithelial cells. Hepatology. 2014; 60(4):1314–23. DOI: 10.1002/hep.27230 [PubMed: 24841946]

147. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008; 27(45):5904–12. DOI: 10.1038/onc.2008.271 [PubMed: 18836471]

148. Sharma S, Xing F, Liu Y, Wu K, Said N, Pochampally R, Shiozawa Y, Lin HK, Balaji K, Watabe K. Secreted Protein Acidic and Rich in Cysteine (SPARC) Mediates Metastatic Dormancy of Prostate Cancer in Bone. Journal of Biological Chemistry. 2016; 291(37):19351–19363. [PubMed: 27422817]

149. Wang M, Zhao J, Zhang L, Wei F, Lian Y, Wu Y, Gong Z, Zhang S, Zhou J, Cao K, Li X, Xiong W, Li G, Zeng Z, Guo C. Role of tumor microenvironment in tumorigenesis. J Cancer. 2017; 8(5):761–773. DOI: 10.7150/jca.17648 [PubMed: 28382138]

150. Nefedova Y, Landowski TH, Dalton WS. Bone marrow stromal-derived soluble factors and direct cell contact contribute to de novo drug resistance of myeloma cells by distinct mechanisms. Leukemia. 2003; 17(6):1175–82. DOI: 10.1038/sj.leu.2402924 [PubMed: 12764386]

151. Tkach M, Thery C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. Cell. 2016; 164(6):1226–32. DOI: 10.1016/j.cell.2016.01.043 [PubMed: 26967288]

152. Mao Y, Keller ET, Garfield DH, Shen K, Wang J. Stromal cells in tumor microenvironment and breast cancer. Cancer Metastasis Rev. 2013; 32(1–2):303–15. DOI: 10.1007/s10555-012-9415-3 [PubMed: 23114846]

153. Nabet BY, Qiu Y, Shabason JE, Wu TJ, Yoon T, Kim BC, Benci JL, DeMichele AM, Tchou J, Marcotrigiano J, Minn AJ. Exosome RNA Unshielding Couples Stromal Activation to Pattern Recognition Receptor Signaling in Cancer. Cell. 2017; 170(2):352–366 e13. DOI: 10.1016/j.cell.2017.06.031 [PubMed: 28709002]

154. Luga V, Zhang L, Viloria-Petit AM, Ogunjimi AA, Inanlou MR, Chiu E, Buchanan M, Hosein AN, Basik M, Wrana JL. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. Cell. 2012; 151(7):1542–56. DOI: 10.1016/j.cell.2012.11.024 [PubMed: 23260141]

155. Antonyak MA, Li B, Boroughs LK, Johnson JL, Druso JE, Bryant KL, Holowka DA, Cerione RA. Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells. Proc Natl Acad Sci U S A. 2011; 108(12):4852–7. DOI: 10.1073/pnas.1017667108 [PubMed: 21368175]

156. Webber J, Steadman R, Mason MD, Tabi Z, Clayton A. Cancer exosomes trigger fibroblast to myofibroblast differentiation. Cancer Res. 2010; 70(23):9621–30. DOI: 10.1158/0008-5472.CAN-10-1722 [PubMed: 21098712]

157. Webber JP, Spary LK, Sanders AJ, Chowdhury R, Jiang WG, Steadman R, Wymant J, Jones AT, Kynaston H, Mason MD, Tabi Z, Clayton A. Differentiation of tumour-promoting stromal myofibroblasts by cancer exosomes. Oncogene. 2015; 34(3):290–302. DOI: 10.1038/onc.2013.560 [PubMed: 24441045]

158. Richards KE, Zeleniak AE, Fishel ML, Wu J, Littlepage LE, Hill R. Cancer-associated fibroblast exosomes regulate survival and proliferation of pancreatic cancer cells. Oncogene. 2016; doi: 10.1038/onc.2016.353

159. Minciacchi VR, Spinelli C, Reis-Sobreiro M, Cavallini L, You S, Zandian M, Li X, Mishra R, Chiarugi P, Adam RM, Posadas EM, Viglietto G, Freeman MR, Cocucci E, Bhowmick NA, Di Vizio D. MYC Mediates Large Oncosome-Induced Fibroblast Reprogramming in Prostate Cancer. Cancer Res. 2017; 77(9):2306–2317. DOI: 10.1158/0008-5472.CAN-16-2942 [PubMed: 28202510]

160. Wysoczynski M, Ratajczak MZ. Lung cancer secreted microvesicles: underappreciated modulators of microenvironment in expanding tumors. Int J Cancer. 2009; 125(7):1595–603. DOI: 10.1002/ijc.24479 [PubMed: 19462451]

161. Zhao XP, Wang M, Song Y, Song K, Yan TL, Wang L, Liu K, Shang ZJ. Membrane microvesicles as mediators for melanoma-fibroblasts communication: roles of the VCAM-1/VLA-4 axis and the ERK1/2 signal pathway. Cancer Lett. 2015; 360(2):125–33. DOI: 10.1016/j.canlet.2015.01.032 [PubMed: 25661735]

162. Rosenbaum AJ, Grande DA, Dines JS. The use of mesenchymal stem cells in tissue engineering: A global assessment. Organogenesis. 2008; 4(1):23–7. [PubMed: 19279711]

163. Haniffa MA, Collin MP, Buckley CD, Dazzi F. Mesenchymal stem cells: the fibroblasts' new clothes? Haematologica. 2009; 94(2):258–63. DOI: 10.3324/haematol.13699 [PubMed: 19109217]

164. Ridge SM, Sullivan FJ, Glynn SA. Mesenchymal stem cells: key players in cancer progression. Mol Cancer. 2017; 16(1):31.doi: 10.1186/s12943-017-0597-8 [PubMed: 28148268]

165. Lee JK, Park SR, Jung BK, Jeon YK, Lee YS, Kim MK, Kim YG, Jang JY, Kim CW. Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating VEGF

expression in breast cancer cells. PLoS One. 2013; 8(12):e84256.doi: 10.1371/journal.pone.0084256 [PubMed: 24391924]

166. Pontikoglou C, Deschaseaux F, Sensebe L, Papadaki HA. Bone marrow mesenchymal stem cells: biological properties and their role in hematopoiesis and hematopoietic stem cell transplantation. Stem Cell Rev. 2011; 7(3):569–89. DOI: 10.1007/s12015-011-9228-8 [PubMed: 21249477]
167. Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, Scadden DT, Ma'ayan A, Enikolopov GN, Frenette PS. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature. 2010; 466(7308):829–34. DOI: 10.1038/nature09262 [PubMed: 20703299]
168. Feldman LJ, Vallone VBF, Choi H, Labovsky V, Martinez LM, Bordenave RH, Milovic V, Jaimovich G, Batagelj E, Dimase F, Villafane AR, Chasseing NA. Bone Marrow Mesenchymal Stem Cells: Pre-Metastatic Niche For Breast Cancer. Blood. 2013; 122(21)
169. Decker AM, Jung Y, Cackowski F, Taichman RS. The role of hematopoietic stem cell niche in prostate cancer bone metastasis. J Bone Oncol. 2016; 5(3):117–120. DOI: 10.1016/j.jbo.2016.02.005 [PubMed: 27761370]
170. Bliss SA, Sinha G, Sandiford OA, Williams LM, Engelberth DJ, Guiro K, Isenalumhe LL, Greco SJ, Ayer S, Bryan M, Kumar R, Ponzio NM, Rameshwar P. Mesenchymal Stem Cell-Derived Exosomes Stimulate Cycling Quiescence and Early Breast Cancer Dormancy in Bone Marrow. Cancer Res. 2016; 76(19):5832–5844. DOI: 10.1158/0008-5472.CAN-16-1092 [PubMed: 27569215]
171. Ono M, Kosaka N, Tominaga N, Yoshioka Y, Takeshita F, Takahashi RU, Yoshida M, Tsuda H, Tamura K, Ochiya T. Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. Sci Signal. 2014; 7(332):ra63.doi: 10.1126/scisignal.2005231 [PubMed: 24985346]
172. Chowdhury R, Webber JP, Gurney M, Mason MD, Tabi Z, Clayton A. Cancer exosomes trigger mesenchymal stem cell differentiation into pro-angiogenic and pro-invasive myofibroblasts. Oncotarget. 2015; 6(2):715–31. DOI: 10.18632/oncotarget.2711 [PubMed: 25596732]
173. Cho JA, Park H, Lim EH, Lee KW. Exosomes from breast cancer cells can convert adipose tissue-derived mesenchymal stem cells into myofibroblast-like cells. Int J Oncol. 2012; 40(1):130–8. DOI: 10.3892/ijo.2011.1193 [PubMed: 21904773]
174. Cho JA, Park H, Lim EH, Kim KH, Choi JS, Lee JH, Shin JW, Lee KW. Exosomes from ovarian cancer cells induce adipose tissue-derived mesenchymal stem cells to acquire the physical and functional characteristics of tumor-supporting myofibroblasts. Gynecol Oncol. 2011; 123(2):379–86. DOI: 10.1016/j.ygyno.2011.08.005 [PubMed: 21903249]
175. Abd Elmageed ZY, Yang Y, Thomas R, Ranjan M, Mondal D, Moroz K, Fang Z, Rezk BM, Moparty K, Sikka SC, Sartor O, Abdel-Mageed AB. Neoplastic reprogramming of patient-derived adipose stem cells by prostate cancer cell-associated exosomes. Stem Cells. 2014; 32(4):983–97. DOI: 10.1002/stem.1619 [PubMed: 24715691]
176. Nazarenko I, Rana S, Baumann A, McAlear J, Hellwig A, Trendelenburg M, Lochnit G, Preissner KT, Zoller M. Cell surface tetraspanin Tspan8 contributes to molecular pathways of exosome-induced endothelial cell activation. Cancer Res. 2010; 70(4):1668–78. DOI: 10.1158/0008-5472.CAN-09-2470 [PubMed: 20124479]
177. Grange C, Tapparo M, Collino F, Vitillo L, Damasco C, Deregibus MC, Tetta C, Bussolati B, Camussi G. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. Cancer Res. 2011; 71(15):5346–56. DOI: 10.1158/0008-5472.CAN-11-0241 [PubMed: 21670082]
178. Svensson KJ, Kucharzewska P, Christianson HC, Skold S, Lofstedt T, Johansson MC, Morgelin M, Bengzon J, Ruf W, Belting M. Hypoxia triggers a proangiogenic pathway involving cancer cell microvesicles and PAR-2-mediated heparin-binding EGF signaling in endothelial cells. Proc Natl Acad Sci U S A. 2011; 108(32):13147–52. DOI: 10.1073/pnas.1104261108 [PubMed: 21788507]
179. Xing F, Kobayashi A, Okuda H, Watabe M, Pai SK, Pandey PR, Hirota S, Wilber A, Mo YY, Moore BE, Liu W, Fukuda K, Iiizumi M, Sharma S, Liu Y, Wu K, Peralta E, Watabe K. Reactive astrocytes promote the metastatic growth of breast cancer stem-like cells by activating Notch

signalling in brain. EMBO Mol Med. 2013; 5(3):384–96. DOI: 10.1002/emmm.201201623 [PubMed: 23495140]

180. Wu K, Fukuda K, Xing F, Zhang Y, Sharma S, Liu Y, Chan MD, Zhou X, Qasem SA, Pochampally R, Mo YY, Watabe K. Roles of the cyclooxygenase 2 matrix metalloproteinase 1 pathway in brain metastasis of breast cancer. J Biol Chem. 2015; 290(15):9842–54. DOI: 10.1074/jbc.M114.602185 [PubMed: 25691572]
181. Xing F, Liu Y, Sharma S, Wu K, Chan MD, Lo HW, Carpenter RL, Metheny-Barlow LJ, Zhou X, Qasem SA. Activation of the c-Met pathway mobilizes an inflammatory network in the brain microenvironment to promote brain metastasis of breast cancer. Cancer Research. 2016; 76(17): 4970–4980. [PubMed: 27364556]
182. Zhang L, Zhang S, Yao J, Lowery FJ, Zhang Q, Huang WC, Li P, Li M, Wang X, Zhang C, Wang H, Ellis K, Cheerathodi M, McCarty JH, Palmieri D, Saunus J, Lakhani S, Huang S, Sahin AA, Aldape KD, Steeg PS, Yu D. Microenvironment-induced PTEN loss by exosomal microRNA primes brain metastasis outgrowth. Nature. 2015; 527(7576):100–4. DOI: 10.1038/nature15376 [PubMed: 26479035]
183. Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, Becker A, Hoshino A, Mark MT, Molina H, Xiang J, Zhang T, Theilen TM, Garcia-Santos G, Williams C, Ararso Y, Huang Y, Rodrigues G, Shen TL, Labori KJ, Lothe IM, Kure EH, Hernandez J, Doussot A, Ebbesen SH, Grandgenett PM, Hollingsworth MA, Jain M, Mallya K, Batra SK, Jarnagin WR, Schwartz RE, Matei I, Peinado H, Stanger BZ, Bromberg J, Lyden D. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. Nat Cell Biol. 2015; 17(6):816–26. DOI: 10.1038/ncb3169 [PubMed: 25985394]
184. He WA, Calore F, Londhe P, Canella A, Guttridge DC, Croce CM. Microvesicles containing miRNAs promote muscle cell death in cancer cachexia via TLR7. Proc Natl Acad Sci U S A. 2014; 111(12):4525–9. DOI: 10.1073/pnas.1402714111 [PubMed: 24616506]
185. Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, Molina H, Kohsaka S, Di Giannatale A, Ceder S, Singh S, Williams C, Soplop N, Uryu K, Pharmer L, King T, Bojmar L, Davies AE, Ararso Y, Zhang T, Zhang H, Hernandez J, Weiss JM, Dumont-Cole VD, Kramer K, Wexler LH, Narendran A, Schwartz GK, Healey JH, Sandstrom P, Labori KJ, Kure EH, Grandgenett PM, Hollingsworth MA, de Sousa M, Kaur S, Jain M, Mallya K, Batra SK, Jarnagin WR, Brady MS, Fodstad O, Muller V, Pantel K, Minn AJ, Bissell MJ, Garcia BA, Kang Y, Rajasekhar VK, Ghajar CM, Matei I, Peinado H, Bromberg J, Lyden D. Tumour exosome integrins determine organotropic metastasis. Nature. 2015; 527(7578):329–35. DOI: 10.1038/nature15756 [PubMed: 26524530]
186. Smyth TJ, Redzic JS, Graner MW, Anchordoquy TJ. Examination of the specificity of tumor cell derived exosomes with tumor cells in vitro. Biochim Biophys Acta. 2014; 1838(11):2954–65. DOI: 10.1016/j.bbamem.2014.07.026 [PubMed: 25102470]
187. Taverna S, Flugy A, Saieva L, Kohn EC, Santoro A, Meraviglia S, De Leo G, Alessandro R. Role of exosomes released by chronic myelogenous leukemia cells in angiogenesis. Int J Cancer. 2012; 130(9):2033–43. DOI: 10.1002/ijc.26217 [PubMed: 21630268]
188. Janowska-Wieczorek A, Wysoczynski M, Kijowski J, Marquez-Curtis L, Machalinski B, Ratajczak J, Ratajczak MZ. Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer. Int J Cancer. 2005; 113(5):752–60. DOI: 10.1002/ijc.20657 [PubMed: 15499615]
189. Yang M, Chen J, Su F, Yu B, Su F, Lin L, Liu Y, Huang JD, Song E. Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells. Mol Cancer. 2011; 10:117.doi: 10.1186/1476-4598-10-117 [PubMed: 21939504]
190. Peinado H, Aleckovic M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, Hergueta-Redondo M, Williams C, Garcia-Santos G, Ghajar C, Nitadori-Hoshino A, Hoffman C, Badal K, Garcia BA, Callahan MK, Yuan J, Martins VR, Skog J, Kaplan RN, Brady MS, Wolchok JD, Chapman PB, Kang Y, Bromberg J, Lyden D. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat Med. 2012; 18(6):883–91. DOI: 10.1038/nm.2753 [PubMed: 22635005]
191. Sagar G, Sah RP, Javeed N, Dutta SK, Smyrk TC, Lau JS, Giorgadze N, Tchkonia T, Kirkland JL, Chari ST, Mukhopadhyay D. Pathogenesis of pancreatic cancer exosome-induced lipolysis in

adipose tissue. Gut. 2016; 65(7):1165–74. DOI: 10.1136/gutjnl-2014-308350 [PubMed: 26061593]

192. Janowska-Wieczorek A, Marquez-Curtis LA, Wysoczynski M, Ratajczak MZ. Enhancing effect of platelet-derived microvesicles on the invasive potential of breast cancer cells. Transfusion. 2006; 46(7):1199–209. DOI: 10.1111/j.1537-2995.2006.00871.x [PubMed: 16836568]
193. Xu Y, Zhang Y, Wang L, Zhao R, Qiao Y, Han D, Sun Q, Dong N, Liu Y, Wu D, Zhang X, Huang N, Ma N, Zhao W, Liu Y, Gao X. miR-200a targets Gelsolin: A novel mechanism regulating secretion of microvesicles in hepatocellular carcinoma cells. Oncol Rep. 2017; 37(5):2711–2719. DOI: 10.3892/or.2017.5506 [PubMed: 28440466]
194. Redzic JS, Kendrick AA, Bahmed K, Dahl KD, Pearson CG, Robinson WA, Robinson SE, Graner MW, Eisenmesser EZ. Extracellular vesicles secreted from cancer cell lines stimulate secretion of MMP-9, IL-6, TGF-beta1 and EMMPRIN. PLoS One. 2013; 8(8):e71225.doi: 10.1371/journal.pone.0071225 [PubMed: 23936495]
195. Steinbichler TB, Dudas J, Riechelmann H, Skvortsova II. The role of exosomes in cancer metastasis. Semin Cancer Biol. 2017; 44:170–181. DOI: 10.1016/j.semcancer.2017.02.006 [PubMed: 28215970]
196. Sandvig K, Llorente A. Proteomic analysis of microvesicles released by the human prostate cancer cell line PC-3. Mol Cell Proteomics. 2012; 11(7):M111 012914.doi: 10.1074/mcp.M111.012914
197. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol. 2008; 110(1):13–21. DOI: 10.1016/j.ygyno.2008.04.033 [PubMed: 18589210]
198. Tavoosidana G, Ronquist G, Darmanis S, Yan J, Carlsson L, Wu D, Conze T, Ek P, Semjonow A, Eltze E, Larsson A, Landegren UD, Kamali-Moghaddam M. Multiple recognition assay reveals prostasomes as promising plasma biomarkers for prostate cancer. Proc Natl Acad Sci U S A. 2011; 108(21):8809–14. DOI: 10.1073/pnas.1019330108 [PubMed: 21555566]
199. Silva J, Garcia V, Rodriguez M, Compte M, Cisneros E, Veguillas P, Garcia JM, Dominguez G, Campos-Martin Y, Cuevas J, Pena C, Herrera M, Diaz R, Mohammed N, Bonilla F. Analysis of exosome release and its prognostic value in human colorectal cancer. Genes Chromosomes Cancer. 2012; 51(4):409–18. [PubMed: 22420032]
200. Wang J, Zheng Y, Zhao M. Exosome-Based Cancer Therapy: Implication for Targeting Cancer Stem Cells. Front Pharmacol. 2016; 7:533.doi: 10.3389/fphar.2016.00533 [PubMed: 28127287]
201. Aihara M, Truong LD, Dunn JK, Wheeler TM, Scardino PT, Thompson TC. Frequency of apoptotic bodies positively correlates with Gleason grade in prostate cancer. Hum Pathol. 1994; 25(8):797–801. [PubMed: 7520020]
202. Gallo A, Tandon M, Alevizos I, Illei GG. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. PLoS One. 2012; 7(3):e30679.doi: 10.1371/journal.pone.0030679 [PubMed: 22427800]
203. Tanaka Y, Kamohara H, Kinoshita K, Kurashige J, Ishimoto T, Iwatsuki M, Watanabe M, Baba H. Clinical impact of serum exosomal microRNA-21 as a clinical biomarker in human esophageal squamous cell carcinoma. Cancer. 2013; 119(6):1159–67. DOI: 10.1002/cncr.27895 [PubMed: 23224754]
204. Bryant RJ, Pawlowski T, Catto JW, Marsden G, Vessella RL, Rhees B, Kuslich C, Visakorpi T, Hamdy FC. Changes in circulating microRNA levels associated with prostate cancer. Br J Cancer. 2012; 106(4):768–74. DOI: 10.1038/bjc.2011.595 [PubMed: 22240788]
205. Ogata-Kawata H, Izumiya M, Kurioka D, Honma Y, Yamada Y, Furuta K, Gunji T, Ohta H, Okamoto H, Sonoda H, Watanabe M, Nakagama H, Yokota J, Kohno T, Tsuchiya N. Circulating exosomal microRNAs as biomarkers of colon cancer. PLoS One. 2014; 9(4):e92921.doi: 10.1371/journal.pone.0092921 [PubMed: 24705249]
206. Rabinowits G, Gercel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal microRNA: a diagnostic marker for lung cancer. Clin Lung Cancer. 2009; 10(1):42–6. DOI: 10.3816/CLC.2009.n.006 [PubMed: 19289371]

207. Li Q, Shao Y, Zhang X, Zheng T, Miao M, Qin L, Wang B, Ye G, Xiao B, Guo J. Plasma long noncoding RNA protected by exosomes as a potential stable biomarker for gastric cancer. Tumour Biol. 2015; 36(3):2007–12. DOI: 10.1007/s13277-014-2807-y [PubMed: 25391424]
208. Nilsson J, Skog J, Nordstrand A, Baranov V, Mincheva-Nilsson L, Breakefield XO, Widmark A. Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. Br J Cancer. 2009; 100(10):1603–7. DOI: 10.1038/sj.bjc.6605058 [PubMed: 19401683]
209. McKiernan J, Donovan MJ, O'Neill V, Bentink S, Noerholm M, Belzer S, Skog J, Kattan MW, Partin A, Andriole G, Brown G, Wei JT, Thompson IM Jr, Carroll P. A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial Biopsy. JAMA Oncol. 2016; 2(7):882–9. DOI: 10.1001/jamaoncol.2016.0097 [PubMed: 27032035]
210. Kahlert C, Melo SA, Protopopov A, Tang J, Seth S, Koch M, Zhang J, Weitz J, Chin L, Futreal A, Kalluri R. Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. J Biol Chem. 2014; 289(7):3869–75. DOI: 10.1074/jbc.C113.532267 [PubMed: 24398677]
211. Li J, Sherman-Baust CA, Tsai-Turton M, Bristow RE, Roden RB, Morin PJ. Claudin-containing exosomes in the peripheral circulation of women with ovarian cancer. BMC Cancer. 2009; 9:244.doi: 10.1186/1471-2407-9-244 [PubMed: 19619303]
212. Melo SA, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, LeBleu VS, Mittendorf EA, Weitz J, Rahbari N, Reissfelder C, Pilarsky C, Fraga MF, Piwnica-Worms D, Kalluri R. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature. 2015; 523(7559):177–82. DOI: 10.1038/nature14581 [PubMed: 26106858]
213. Madhavan B, Yue S, Galli U, Rana S, Gross W, Muller M, Giese NA, Kalthoff H, Becker T, Buchler MW, Zoller M. Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. Int J Cancer. 2015; 136(11):2616–27. DOI: 10.1002/ijc.29324 [PubMed: 25388097]
214. Liang LG, Kong MQ, Zhou S, Sheng YF, Wang P, Yu T, Inci F, Kuo WP, Li LJ, Demirci U, Wang S. An integrated double-filtration microfluidic device for isolation, enrichment and quantification of urinary extracellular vesicles for detection of bladder cancer. Sci Rep. 2017; 7:46224.doi: 10.1038/srep46224 [PubMed: 28436447]
215. Zhao Z, Yang Y, Zeng Y, He M. A microfluidic ExoSearch chip for multiplexed exosome detection towards blood-based ovarian cancer diagnosis. Lab Chip. 2016; 16(3):489–96. DOI: 10.1039/c5lc01117e [PubMed: 26645590]
216. Fang S, Tian H, Li X, Jin D, Li X, Kong J, Yang C, Yang X, Lu Y, Luo Y, Lin B, Niu W, Liu T. Clinical application of a microfluidic chip for immunocapture and quantification of circulating exosomes to assist breast cancer diagnosis and molecular classification. PLoS One. 2017; 12(4):e0175050.doi: 10.1371/journal.pone.0175050 [PubMed: 28369094]
217. Shao H, Chung J, Lee K, Balaj L, Min C, Carter BS, Hochberg FH, Breakefield XO, Lee H, Weissleder R. Chip-based analysis of exosomal mRNA mediating drug resistance in glioblastoma. Nat Commun. 2015; 6:6999.doi: 10.1038/ncomms7999 [PubMed: 25959588]
218. Im H, Shao H, Park YI, Peterson VM, Castro CM, Weissleder R, Lee H. Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor. Nat Biotechnol. 2014; 32(5): 490–5. DOI: 10.1038/nbt.2886 [PubMed: 24752081]
219. Richards KE, Zeleniak AE, Fishel ML, Wu J, Littlepage LE, Hill R. Cancer-associated fibroblast exosomes regulate survival and proliferation of pancreatic cancer cells. Oncogene. 2017; 36(13): 1770–1778. DOI: 10.1038/onc.2016.353 [PubMed: 27669441]
220. Hu Y, Yan C, Mu L, Huang K, Li X, Tao D, Wu Y, Qin J. Fibroblast-Derived Exosomes Contribute to Chemoresistance through Priming Cancer Stem Cells in Colorectal Cancer. PLoS One. 2015; 10(5):e0125625.doi: 10.1371/journal.pone.0125625 [PubMed: 25938772]
221. Mittelbrunn M, Gutierrez-Vazquez C, Villarroya-Beltri C, Gonzalez S, Sanchez-Cabo F, Gonzalez MA, Bernad A, Sanchez-Madrid F. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. Nat Commun. 2011; 2:282.doi: 10.1038/ncomms1285 [PubMed: 21505438]
222. Phuyal S, Hessvik NP, Skotland T, Sandvig K, Llorente A. Regulation of exosome release by glycosphingolipids and flotillins. FEBS J. 2014; 281(9):2214–27. DOI: 10.1111/febs.12775 [PubMed: 24605801]

223. Savina A, Furlan M, Vidal M, Colombo MI. Exosome release is regulated by a calcium-dependent mechanism in K562 cells. J Biol Chem. 2003; 278(22):20083–90. DOI: 10.1074/jbc.M301642200 [PubMed: 12639953]

224. Nassar-Gentina V, Rojas E, Luxoro M. Rise in cytoplasmic Ca2+ induced by monensin in bovine medullary chromaffin cells. Cell Calcium. 1994; 16(6):475–80. [PubMed: 7712541]

225. Islam A, Shen X, Hiroi T, Moss J, Vaughan M, Levine SJ. The brefeldin A-inhibited guanine nucleotide-exchange protein, BIG2, regulates the constitutive release of TNFR1 exosome-like vesicles. J Biol Chem. 2007; 282(13):9591–9. DOI: 10.1074/jbc.M607122200 [PubMed: 17276987]

226. Islam A, Jones H, Hiroi T, Lam J, Zhang J, Moss J, Vaughan M, Levine SJ. cAMP-dependent protein kinase A (PKA) signaling induces TNFR1 exosome-like vesicle release via anchoring of PKA regulatory subunit RIIbeta to BIG2. J Biol Chem. 2008; 283(37):25364–71. DOI: 10.1074/jbc.M804966200 [PubMed: 18625701]

227. Muralidharan-Chari V, Clancy J, Plou C, Romao M, Chavrier P, Raposo G, D'Souza-Schorey C. ARF6-regulated shedding of tumor cell-derived plasma membrane microvesicles. Curr Biol. 2009; 19(22):1875–85. DOI: 10.1016/j.cub.2009.09.059 [PubMed: 19896381]

228. Svensson KJ, Christianson HC, Wittrup A, Bourseau-Guilmain E, Lindqvist E, Svensson LM, Morgelin M, Belting M. Exosome uptake depends on ERK1/2-heat shock protein 27 signaling and lipid Raft-mediated endocytosis negatively regulated by caveolin-1. J Biol Chem. 2013; 288(24):17713–24. DOI: 10.1074/jbc.M112.445403 [PubMed: 23653359]

229. Franzen CA, Simms PE, Van Huis AF, Foreman KE, Kuo PC, Gupta GN. Characterization of uptake and internalization of exosomes by bladder cancer cells. Biomed Res Int. 2014; 2014:619829.doi: 10.1155/2014/619829 [PubMed: 24575409]

230. Atanassoff AP, Wolfmeier H, Schoenauer R, Hostettler A, Ring A, Draeger A, Babiychuk EB. Microvesicle shedding and lysosomal repair fulfill divergent cellular needs during the repair of streptolysin O-induced plasmalemmal damage. PLoS One. 2014; 9(2):e89743.doi: 10.1371/journal.pone.0089743 [PubMed: 24587004]

231. Jorfi S, Ansa-Addo EA, Kholia S, Stratton D, Valley S, Lange S, Inal J. Inhibition of microvesiculation sensitizes prostate cancer cells to chemotherapy and reduces docetaxel dose required to limit tumor growth in vivo. Sci Rep. 2015; 5:13006.doi: 10.1038/srep13006 [PubMed: 26302712]

232. Abid Hussein MN, Boing AN, Sturk A, Hau CM, Nieuwland R. Inhibition of microparticle release triggers endothelial cell apoptosis and detachment. Thromb Haemost. 2007; 98(5):1096–107. [PubMed: 18000616]

233. Li B, Antonyak MA, Zhang J, Cerione RA. RhoA triggers a specific signaling pathway that generates transforming microvesicles in cancer cells. Oncogene. 2012; 31(45):4740–9. DOI: 10.1038/onc.2011.636 [PubMed: 22266864]

234. Bucki R, Bachelot-Loza C, Zachowski A, Giraud F, Sulpice JC. Calcium induces phospholipid redistribution and microvesicle release in human erythrocyte membranes by independent pathways. Biochemistry. 1998; 37(44):15383–91. DOI: 10.1021/bi9805238 [PubMed: 9799499]

235. Stratton D, Lange S, Inal JM. Pulsed extremely low-frequency magnetic fields stimulate microvesicle release from human monocytic leukaemia cells. Biochem Biophys Res Commun. 2013; 430(2):470–5. DOI: 10.1016/j.bbrc.2012.12.012 [PubMed: 23237811]

236. Thery C, Duban L, Segura E, Veron P, Lantz O, Amigorena S. Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes. Nat Immunol. 2002; 3(12):1156–62. DOI: 10.1038/ni854 [PubMed: 12426563]

237. Hao S, Bai O, Li F, Yuan J, Laferte S, Xiang J. Mature dendritic cells pulsed with exosomes stimulate efficient cytotoxic T-lymphocyte responses and antitumour immunity. Immunology. 2007; 120(1):90–102. DOI: 10.1111/j.1365-2567.2006.02483.x [PubMed: 17073943]

238. Vincent-Schneider H, Stumptner-Cuvelette P, Lankar D, Pain S, Raposo G, Benaroch P, Bonnerot C. Exosomes bearing HLA-DR1 molecules need dendritic cells to efficiently stimulate specific T cells. Int Immunol. 2002; 14(7):713–22. [PubMed: 12096030]

239. Wolfers J, Lozier A, Raposo G, Regnault A, Thery C, Masurier C, Flament C, Pouzieux S, Faure F, Tursz T, Angevin E, Amigorena S, Zitvogel L. Tumor-derived exosomes are a source of shared

tumor rejection antigens for CTL cross-priming. Nat Med. 2001; 7(3):297–303. DOI: 10.1038/85438 [PubMed: 11231627]

240. Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, Ricciardi-Castagnoli P, Raposo G, Amigorena S. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. Nat Med. 1998; 4(5):594–600. [PubMed: 9585234]
241. Andre F, Chaput N, Schartz NE, Flament C, Aubert N, Bernard J, Lemonnier F, Raposo G, Escudier B, Hsu DH, Tursz T, Amigorena S, Angevin E, Zitvogel L. Exosomes as potent cell-free peptide-based vaccine. I. Dendritic cell-derived exosomes transfer functional MHC class I/peptide complexes to dendritic cells. J Immunol. 2004; 172(4):2126–36. [PubMed: 14764678]
242. Morse MA, Garst J, Osada T, Khan S, Hobeika A, Clay TM, Valente N, Shreeniwas R, Sutton MA, Delcayre A, Hsu DH, Le Pecq JB, Lyerly HK. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. J Transl Med. 2005; 3(1):9.doi: 10.1186/1479-5876-3-9 [PubMed: 15723705]
243. Escudier B, Dorval T, Chaput N, Andre F, Caby MP, Novault S, Flament C, Leboulaire C, Borg C, Amigorena S, Boccaccio C, Bonnerot C, Dhellin O, Movassagh M, Piperno S, Robert C, Serra V, Valente N, Le Pecq JB, Spatz A, Lantz O, Tursz T, Angevin E, Zitvogel L. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of thefirst phase I clinical trial. J Transl Med. 2005; 3(1):10.doi: 10.1186/1479-5876-3-10 [PubMed: 15740633]
244. Besse B, Charrier M, Lapierre V, Dansin E, Lantz O, Planchard D, Le Chevalier T, Livartoski A, Barlesi F, Laplanche A, Ploix S, Vimond N, Peguillet I, Thery C, Lacroix L, Zoernig I, Dhodapkar K, Dhodapkar M, Viaud S, Soria JC, Reiners KS, Pogge von Strandmann E, Vely F, Rusakiewicz S, Eggermont A, Pitt JM, Zitvogel L, Chaput N. Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC. Oncoimmunology. 2016; 5(4):e1071008.doi: 10.1080/2162402X.2015.1071008 [PubMed: 27141373]
245. Reiners KS, Topolar D, Henke A, Simhadri VR, Kessler J, Sauer M, Bessler M, Hansen HP, Tawadros S, Herling M, Kronke M, Hallek M, Pogge von Strandmann E. Soluble ligands for NK cell receptors promote evasion of chronic lymphocytic leukemia cells from NK cell anti-tumor activity. Blood. 2013; 121(18):3658–65. DOI: 10.1182/blood-2013-01-476606 [PubMed: 23509156]
246. Nakayama M, Takeda K, Kawano M, Takai T, Ishii N, Ogasawara K. Natural killer (NK)-dendritic cell interactions generate MHC class II-dressed NK cells that regulate CD4+ T cells. Proc Natl Acad Sci U S A. 2011; 108(45):18360–5. DOI: 10.1073/pnas.1110584108 [PubMed: 22042851]
247. Jiang YZ, Couriel D, Mavroudis DA, Lewalle P, Malkovska V, Hensel NF, Dermime S, Molldrem J, Barrett AJ. Interaction of natural killer cells with MHC class II: reversal of HLA-DR1-mediated protection of K562 transfectant from natural killer cell-mediated cytolysis by brefeldin-A. Immunology. 1996; 87(3):481–6. [PubMed: 8778037]
248. Bu N, Li QL, Feng Q, Sun BZ. Immune protection effect of exosomes against attack of L1210 tumor cells. Leuk Lymphoma. 2006; 47(5):913–8. DOI: 10.1080/10428190500376191 [PubMed: 16753878]
249. Rao Q, Zuo B, Lu Z, Gao X, You A, Wu C, Du Z, Yin H. Tumor-derived exosomes elicit tumor suppression in murine hepatocellular carcinoma models and humans in vitro. Hepatology. 2016; 64(2):456–72. DOI: 10.1002/hep.28549 [PubMed: 26990897]
250. Dai S, Wei D, Wu Z, Zhou X, Wei X, Huang H, Li G. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. Mol Ther. 2008; 16(4):782–90. DOI: 10.1038/mt.2008.1 [PubMed: 18362931]
251. Muller L, Mitsuhashi M, Simms P, Gooding WE, Whiteside TL. Tumor-derived exosomes regulate expression of immune function-related genes in human T cell subsets. Sci Rep. 2016; 6:20254.doi: 10.1038/srep20254 [PubMed: 26842680]
252. Haderk F, Schulz R, Iskar M, Cid LL, Worst T, Willmund KV, Schulz A, Warnken U, Seiler J, Benner A, Nessling M, Zenz T, Gobel M, Durig J, Diederichs S, Paggetti J, Moussay E, Stilgenbauer S, Zapatka M, Lichter P, Seiffert M. Tumor-derived exosomes modulate PD-L1 expression in monocytes. Sci Immunol. 2017; 2(13)doi: 10.1126/sciimmunol.aah5509

253. Qazi KR, Gehrmann U, Domange Jordo E, Karlsson MC, Gabrielsson S. Antigen-loaded exosomes alone induce Th1-type memory through a B-cell-dependent mechanism. Blood. 2009; 113(12):2673–83. DOI: 10.1182/blood-2008-04-153536 [PubMed: 19176319]

254. Li X, Tsibouklis J, Weng T, Zhang B, Yin G, Feng G, Cui Y, Savina IN, Mikhalovska LI, Sandeman SR, Howel CA, Mikhalovsky SV. Nano carriers for drug transport across the blood-brain barrier. J Drug Target. 2017; 25(1):17–28. DOI: 10.1080/1061186X.2016.1184272 [PubMed: 27126681]

255. Andreu Z, Yanez-Mo M. Tetraspanins in extracellular vesicle formation and function. Front Immunol. 2014; 5:442.doi: 10.3389/fimmu.2014.00442 [PubMed: 25278937]

256. Rana S, Yue S, Stadel D, Zoller M. Toward tailored exosomes: the exosomal tetraspanin web contributes to target cell selection. Int J Biochem Cell Biol. 2012; 44(9):1574–84. DOI: 10.1016/j.biocel.2012.06.018 [PubMed: 22728313]

257. Naslund TI, Paquin-Proulx D, Paredes PT, Vallhov H, Sandberg JK, Gabrielsson S. Exosomes from breast milk inhibit HIV-1 infection of dendritic cells and subsequent viral transfer to CD4+ T cells. AIDS. 2014; 28(2):171–80. DOI: 10.1097/QAD.0000000000000159 [PubMed: 24413309]

258. Hung ME, Leonard JN. Stabilization of exosome-targeting peptides via engineered glycosylation. J Biol Chem. 2015; 290(13):8166–72. DOI: 10.1074/jbc.M114.621383 [PubMed: 25657008]

259. Ohno SI, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, Fujita K, Mizutani T, Ohgi T, Ochiya T, Gotoh N, Kuroda M. Systemically Injected Exosomes Targeted to EGFR Deliver Antitumor MicroRNA to Breast Cancer Cells. Mol Ther. 2013; 21(1):185–191. DOI: 10.1038/mt.2012.180 [PubMed: 23032975]

260. Shtam TA, Kovalev RA, Varfolomeeva EY, Makarov EM, Kil YV, Filatov MV. Exosomes are natural carriers of exogenous siRNA to human cells in vitro. Cell Commun Signal. 2013; 11:88.doi: 10.1186/1478-811X-11-88 [PubMed: 24245560]

261. Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, Barnes S, Grizzle W, Miller D, Zhang HG. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. Mol Ther. 2010; 18(9):1606–14. DOI: 10.1038/mt.2010.105 [PubMed: 20571541]

262. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol. 2011; 29(4):341–5. DOI: 10.1038/nbt.1807 [PubMed: 21423189]

263. Xin H, Li Y, Buller B, Katakowski M, Zhang Y, Wang X, Shang X, Zhang ZG, Chopp M. Exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. Stem Cells. 2012; 30(7):1556–64. DOI: 10.1002/stem.1129 [PubMed: 22605481]

264. Johnsen KB, Gudbergsson JM, Skov MN, Pilgaard L, Moos T, Duroux M. A comprehensive overview of exosomes as drug delivery vehicles - endogenous nanocarriers for targeted cancer therapy. Biochim Biophys Acta. 2014; 1846(1):75–87. DOI: 10.1016/j.bbcan.2014.04.005 [PubMed: 24747178]

265. van der Vlist EJ, Arkesteijn GJ, van de Lest CH, Stoorvogel W, Nolte-'t Hoen EN, Wauben MH. CD4(+) T cell activation promotes the differential release of distinct populations of nanosized vesicles. J Extracell Vesicles. 2012; 1doi: 10.3402/jev.v1i0.18364

266. Palma J, Yaddanapudi SC, Pigati L, Havens MA, Jeong S, Weiner GA, Weimer KM, Stern B, Hastings ML, Duelli DM. MicroRNAs are exported from malignant cells in customized particles. Nucleic Acids Res. 2012; 40(18):9125–38. DOI: 10.1093/nar/gks656 [PubMed: 22772984]

267. Tauro BJ, Greening DW, Mathias RA, Mathivanan S, Ji H, Simpson RJ. Two distinct populations of exosomes are released from LIM1863 colon carcinoma cell-derived organoids. Mol Cell Proteomics. 2013; 12(3):587–98. DOI: 10.1074/mcp.M112.021303 [PubMed: 23230278]

268. Schwarzenbach H, da Silva AM, Calin G, Pantel K. Data Normalization Strategies for MicroRNA Quantification. Clin Chem. 2015; 61(11):1333–42. DOI: 10.1373/clinchem.2015.239459 [PubMed: 26408530]

269. Muller L, Hong CS, Stolz DB, Watkins SC, Whiteside TL. Isolation of biologically-active exosomes from human plasma. J Immunol Methods. 2014; 411:55–65. DOI: 10.1016/j.jim.2014.06.007 [PubMed: 24952243]

270. Alamri Y, Vogel R, MacAskill M, Anderson T. Plasma exosome concentration may correlate with cognitive impairment in Parkinson's disease. Alzheimers Dement (Amst). 2016; 4:107–108. DOI: 10.1016/j.dadm.2016.08.002 [PubMed: 27722197]

271. Szajnik M, Derbis M, Lach M, Patalas P, Michalak M, Drzewiecka H, Szpurek D, Nowakowski A, Spaczynski M, Baranowski W, Whiteside TL. Exosomes in Plasma of Patients with Ovarian Carcinoma: Potential Biomarkers of Tumor Progression and Response to Therapy. Gynecol Obstet (Sunnyvale). 2013; (Suppl 4):3.doi: 10.4172/2161-0932.S4-003 [PubMed: 24466501]

272. Matsumoto A, Takahashi Y, Nishikawa M, Sano K, Morishita M, Charoenviriyakul C, Saji H, Takakura Y. Accelerated growth of B16BL6 tumor in mice through efficient uptake of their own exosomes by B16BL6 cells. Cancer Sci. 2017; doi: 10.1111/cas.13310

273. Smith ZJ, Lee C, Rojalin T, Carney RP, Hazari S, Knudson A, Lam K, Saari H, Ibanez EL, Viitala T, Laaksonen T, Yliperttula M, Wachsmann-Hogiu S. Single exosome study reveals subpopulations distributed among cell lines with variability related to membrane content. J Extracell Vesicles. 2015; 4:28533.doi: 10.3402/jev.v4.28533 [PubMed: 26649679]

274. Oksvold MP, Kullmann A, Forfang L, Kierulf B, Li M, Brech A, Vlassov AV, Smeland EB, Neurauter A, Pedersen KW. Expression of B-cell surface antigens in subpopulations of exosomes released from B-cell lymphoma cells. Clin Ther. 2014; 36(6):847–862 e1. DOI: 10.1016/j.clinthera.2014.05.010 [PubMed: 24952935]

275. Caivano A, Del Vecchio L, Musto P. Do we need to distinguish exosomes from microvesicles in hematological malignancies? Leukemia. 2017; doi: 10.1038/leu.2017.205

276. Muhsin-Sharafaldine MR, Saunderson SC, Dunn AC, Faed JM, Kleffmann T, McLellan AD. Procoagulant and immunogenic properties of melanoma exosomes, microvesicles and apoptotic vesicles. Oncotarget. 2016; 7(35):56279–56294. DOI: 10.18632/oncotarget.10783 [PubMed: 27462921]

277. Pashoutan Sarvar D, Shamsasenjan K, Akbarzadehlaleh P. Mesenchymal Stem Cell-Derived Exosomes: New Opportunity in Cell-Free Therapy. Adv Pharm Bull. 2016; 6(3):293–299. DOI: 10.15171/apb.2016.041 [PubMed: 27766213]

278. O'Brien K, Rani S, Corcoran C, Wallace R, Hughes L, Friel AM, McDonnell S, Crown J, Radomski MW, O'Driscoll L. Exosomes from triple-negative breast cancer cells can transfer phenotypic traits representing their cells of origin to secondary cells. Eur J Cancer. 2013; 49(8):1845–59. DOI: 10.1016/j.ejca.2013.01.017 [PubMed: 23453937]

279. Menck K, Scharf C, Bleckmann A, Dyck L, Rost U, Wenzel D, Dhople VM, Siam L, Pukrop T, Binder C, Klemm F. Tumor-derived microvesicles mediate human breast cancer invasion through differentially glycosylated EMMPRIN. J Mol Cell Biol. 2015; 7(2):143–53. DOI: 10.1093/jmcb/mju047 [PubMed: 25503107]

280. Qu JL, Qu XJ, Zhao MF, Teng YE, Zhang Y, Hou KZ, Jiang YH, Yang XH, Liu YP. Gastric cancer exosomes promote tumour cell proliferation through PI3K/Akt and MAPK/ERK activation. Digestive and Liver Disease. 2009; 41(12):875–880. DOI: 10.1016/j.dld.2009.04.006 [PubMed: 19473897]

281. Boelens MC, Wu TJ, Nabet BY, Xu B, Qiu Y, Yoon T, Azzam DJ, Twyman-Saint Victor C, Wiemann BZ, Ishwaran H, Ter Brugge PJ, Jonkers J, Slingerland J, Minn AJ. Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways. Cell. 2014; 159(3):499–513. DOI: 10.1016/j.cell.2014.09.051 [PubMed: 25417103]

282. Gernapudi R, Yao Y, Zhang Y, Wolfson B, Roy S, Duru N, Eades G, Yang P, Zhou Q. Targeting exosomes from preadipocytes inhibits preadipocyte to cancer stem cell signaling in early-stage breast cancer. Breast Cancer Res Treat. 2015; 150(3):685–95. DOI: 10.1007/s10549-015-3326-2 [PubMed: 25783182]

283. Zhao H, Yang L, Baddour J, Achreja A, Bernard V, Moss T, Marini JC, Tudawe T, Seviour EG, San Lucas FA, Alvarez H, Gupta S, Maiti SN, Cooper L, Peehl D, Ram PT, Maitra A, Nagrath D. Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism. Elife. 2016; 5:e10250.doi: 10.7554/eLife.10250 [PubMed: 26920219]

284. Rana S, Malinowska K, Zoller M. Exosomal tumor microRNA modulates premetastatic organ cells. Neoplasia. 2013; 15(3):281–95. [PubMed: 23479506]

285. Hood JL, San RS, Wickline SA. Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. Cancer Res. 2011; 71(11):3792–801. DOI: 10.1158/0008-5472.CAN-10-4455 [PubMed: 21478294]

286. Hong BS, Cho JH, Kim H, Choi EJ, Rho S, Kim J, Kim JH, Choi DS, Kim YK, Hwang D, Gho YS. Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. BMC Genomics. 2009; 10:556.doi: 10.1186/1471-2164-10-556 [PubMed: 19930720]

287. Huang X, Yuan T, Liang M, Du M, Xia S, Dittmar R, Wang D, See W, Costello BA, Quevedo F, Tan W, Nandy D, Bevan GH, Longenbach S, Sun Z, Lu Y, Wang T, Thibodeau SN, Boardman L, Kohli M, Wang L. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. Eur Urol. 2015; 67(1):33–41. DOI: 10.1016/j.eururo.2014.07.035 [PubMed: 25129854]

288. Gabriel K, Ingram A, Austin R, Kapoor A, Tang D, Majeed F, Qureshi T, Al-Nedawi K. Regulation of the tumor suppressor PTEN through exosomes: a diagnostic potential for prostate cancer. PLoS One. 2013; 8(7):e70047.doi: 10.1371/journal.pone.0070047 [PubMed: 23936141]

289. Duijvesz D, Burnum-Johnson KE, Gritsenko MA, Hoogland AM, Vredenbregt-van den Berg MS, Willemsen R, Luider T, Pasa-Tolic L, Jenster G. Proteomic profiling of exosomes leads to the identification of novel biomarkers for prostate cancer. PLoS One. 2013; 8(12):e82589.doi: 10.1371/journal.pone.0082589 [PubMed: 24391718]

290. Khan S, Jutzy JM, Valenzuela MM, Turay D, Aspe JR, Ashok A, Mirshahidi S, Mercola D, Lilly MB, Wall NR. Plasma-derived exosomal survivin, a plausible biomarker for early detection of prostate cancer. PLoS One. 2012; 7(10):e46737.doi: 10.1371/journal.pone.0046737 [PubMed: 23091600]

291. Corcoran C, Rani S, O'Driscoll L. miR-34a is an intracellular and exosomal predictive biomarker for response to docetaxel with clinical relevance to prostate cancer progression. Prostate. 2014; 74(13):1320–34. DOI: 10.1002/pros.22848 [PubMed: 25053345]

292. Lau C, Kim Y, Chia D, Spielmann N, Eibl G, Elashoff D, Wei F, Lin YL, Moro A, Grogan T, Chiang S, Feinstein E, Schafer C, Farrell J, Wong DT. Role of pancreatic cancer-derived exosomes in salivary biomarker development. J Biol Chem. 2013; 288(37):26888–97. DOI: 10.1074/jbc.M113.452458 [PubMed: 23880764]

293. Cazzoli R, Buttitta F, Di Nicola M, Malatesta S, Marchetti A, Rom WN, Pass HI. microRNAs derived from circulating exosomes as noninvasive biomarkers for screening and diagnosing lung cancer. J Thorac Oncol. 2013; 8(9):1156–62. DOI: 10.1097/JTO.0b013e318299ac32 [PubMed: 23945385]

294. Ohshima K, Inoue K, Fujiwara A, Hatakeyama K, Kanto K, Watanabe Y, Muramatsu K, Fukuda Y, Ogura S, Yamaguchi K, Mochizuki T. Let-7 microRNA family is selectively secreted into the extracellular environment via exosomes in a metastatic gastric cancer cell line. PLoS One. 2010; 5(10):e13247.doi: 10.1371/journal.pone.0013247 [PubMed: 20949044]

295. Escrevente C, Keller S, Altevogt P, Costa J. Interaction and uptake of exosomes by ovarian cancer cells. BMC Cancer. 2011; 11:108.doi: 10.1186/1471-2407-11-108 [PubMed: 21439085]

296. Hessvik NP, Overbye A, Brech A, Torgersen ML, Jakobsen IS, Sandvig K, Llorente A. PIKfyve inhibition increases exosome release and induces secretory autophagy. Cell Mol Life Sci. 2016; 73(24):4717–4737. DOI: 10.1007/s00018-016-2309-8 [PubMed: 27438886]

297. Montermini L, Meehan B, Garnier D, Lee WJ, Lee TH, Guha A, Al-Nedawi K, Rak J. Inhibition of oncogenic epidermal growth factor receptor kinase triggers release of exosome-like extracellular vesicles and impacts their phosphoprotein and DNA content. J Biol Chem. 2015; 290(40):24534–46. DOI: 10.1074/jbc.M115.679217 [PubMed: 26272609]

298. Kosgodage US, Trindade RP, Thompson PR, Inal JM, Lange S. Chloramidine/Bisindolylmaleimide-I-Mediated Inhibition of Exosome and Microvesicle Release and Enhanced Efficacy of Cancer Chemotherapy. Int J Mol Sci. 2017; 18(5)doi: 10.3390/ijms18051007

299. Truman JP, Al Gadban MM, Smith KJ, Jenkins RW, Mayroo N, Virella G, Lopes-Virella MF, Bielawska A, Hannun YA, Hammad SM. Differential regulation of acid sphingomyelinase in macrophages stimulated with oxidized low-density lipoprotein (LDL) and oxidized LDL immune

complexes: role in phagocytosis and cytokine release. Immunology. 2012; 136(1):30–45. DOI: 10.1111/j.1365-2567.2012.03552.x [PubMed: 22236141]

300. Hu G, Yao H, Chaudhuri AD, Duan M, Yelamanchili SV, Wen H, Cheney PD, Fox HS, Buch S. Exosome-mediated shuttling of microRNA-29 regulates HIV Tat and morphine-mediated neuronal dysfunction. Cell Death Dis. 2012; 3:e381.doi: 10.1038/cddis.2012.114 [PubMed: 22932723]
301. Del Conde I, Shrimpton CN, Thiagarajan P, Lopez JA. Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. Blood. 2005; 106(5): 1604–11. DOI: 10.1182/blood-2004-03-1095 [PubMed: 15741221]
302. Penet MF, Abou-Hamdan M, Coltel N, Cornille E, Grau GE, de Reggi M, Gharib B. Protection against cerebral malaria by the low-molecular-weight thiol pantethine. Proc Natl Acad Sci U S A. 2008; 105(4):1321–6. DOI: 10.1073/pnas.0706867105 [PubMed: 18195363]
303. El-Assaad F, Wheway J, Hunt NH, Grau GE, Combes V. Production, fate and pathogenicity of plasma microparticles in murine cerebral malaria. PLoS Pathog. 2014; 10(3):e1003839.doi: 10.1371/journal.ppat.1003839 [PubMed: 24651155]

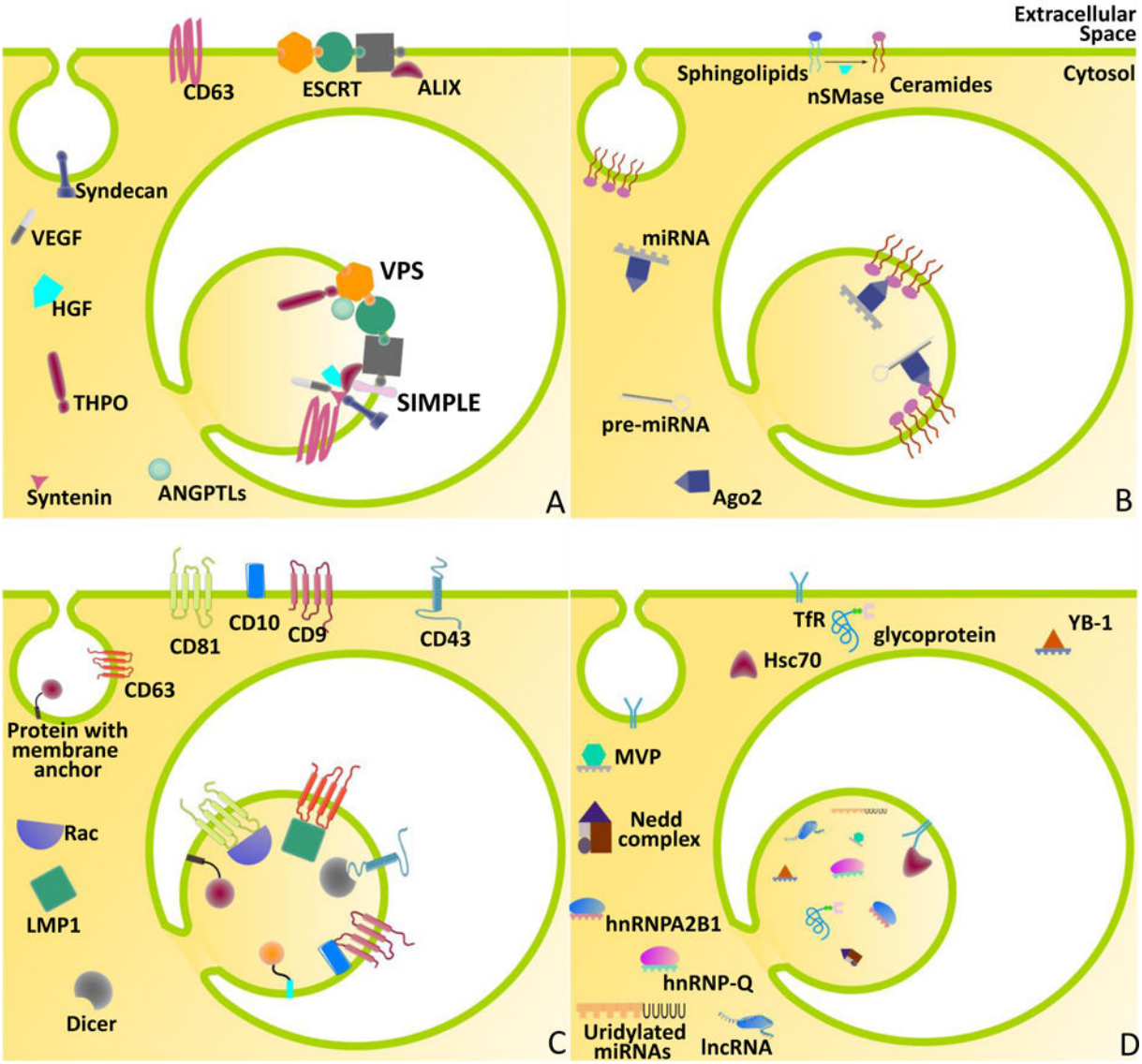

Figure 1. Pathways of cargo sorting in exosomes

A, ESCRT-related sorting. Proteins belonging to ESCRT family regulate cargo sorting from cells to exosomes. Binding of syndencan to ALIX-ESCRT complex results in incorporation of syndecan, VEGF and HGF. SIMPLE affects CD63 and ALIX expression in exosome. VPS proteins controls loading of selective cargoes such as THPO and ANGPTLs. B, Lipid-related sorting. The nSMase catalyzes ceramide formation from sphingolipids. Ceramide is associated with Ago2 and miRNA sorting. C, Membrane protein-regulated sorting. Tetraspanins and integral membrane proteins regulate exosomal cargo sorting. Membrane anchors also control the selectivity of exosomal cargo. D, Other sorting mechanisms. Other proteins (such as hnRNPs) and modification (uridylation of miRNA and N-linked glycosylation of protein) also regulate cargo sorting.

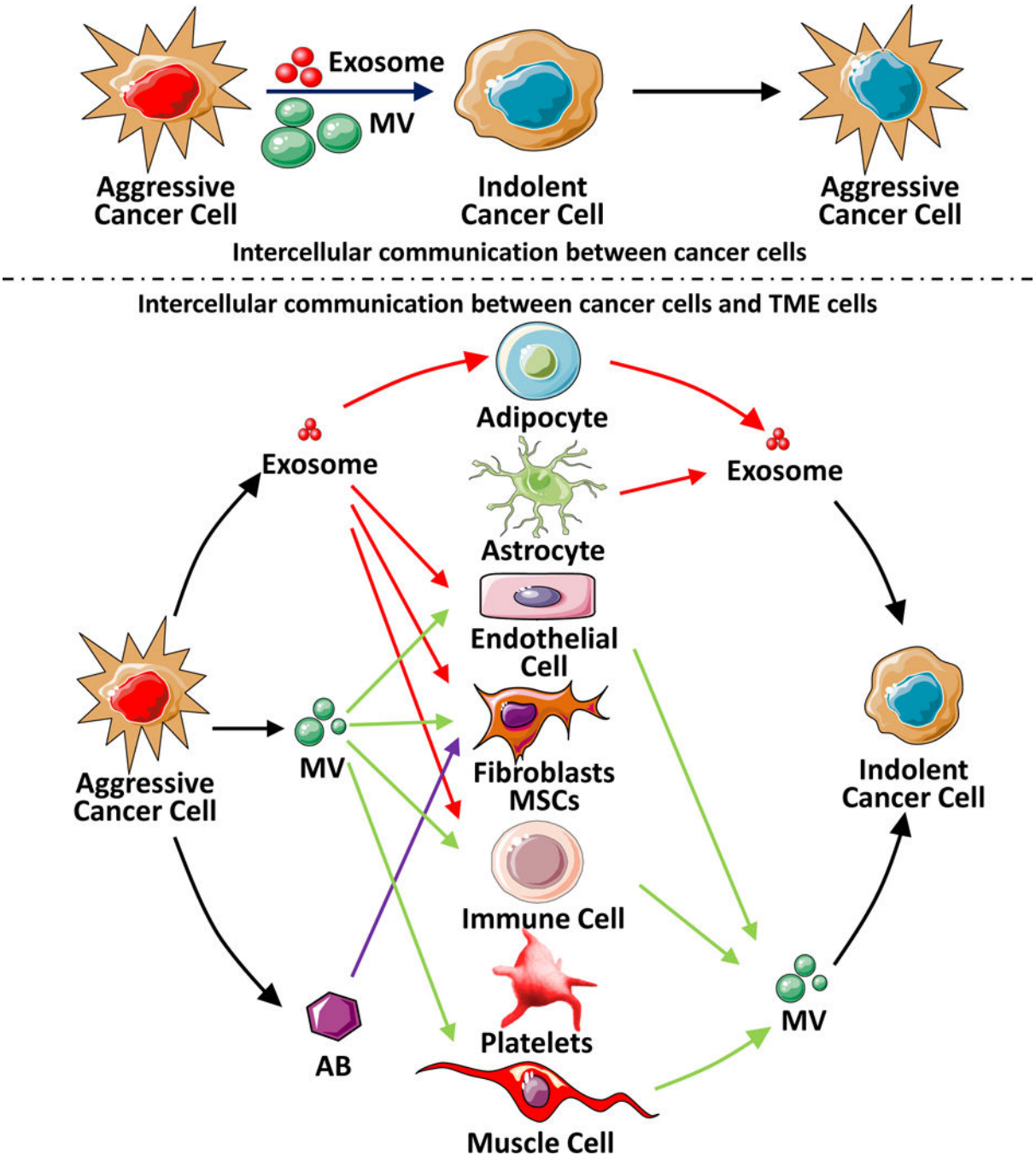

Figure 2. Intercellular communication through EVs

EVs-mediated intercellular communication occurs between cancer cells. Aggressive cancer cells secrete exosomes and MVs containing oncogenic factors to transform indolent cancer cells into aggressive phenotypes. EVs also mediate the intercellular communication between cancer cells and TME cells. EVs derived from cancer cells modulate TME cells thus generating a favorable microenvironment for the growth and metastasis of cancer cells. TME cells could also secrete EVs to affect cancer cells, inducing aggressive phenotypes in indolent cancer cells.

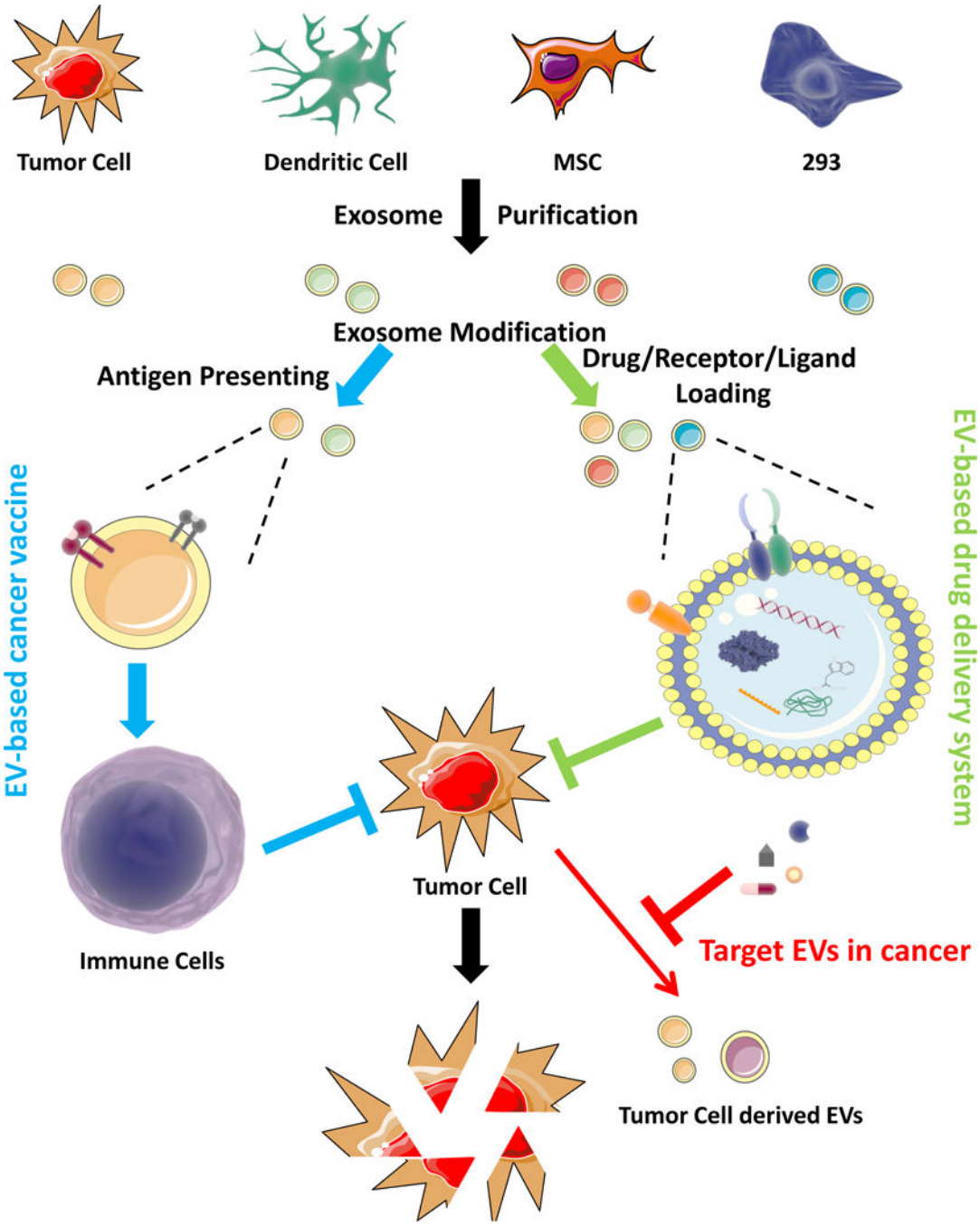

Figure 3. Therapeutic Applications of EVs

Exosomes purified from tumor cells and dendritic cells are used as tumor vaccine. By loading tumor specific antigens to exosomes, they can activate and mobilize immune cells to kill tumor cells. Exosomes derived from tumor cells, dendritic cells, MSCs and HEK293 cells can be used as drug carrier for cancer theray. Specifc ligands or receptors are expressed in exosomes, if not, can be introduced into exosomes to achieve targeted delivery. Loading

drugs into exosomes results in better biodistribution, stablility and less side effects. Inhibition of EV biogenesis by drugs is also reported to suppress the tumor growth.

Table 1

Methods to Isolate EVs

Exosome isolation method	Mechanism	Advantage	Disadvantage	Most suitable Application	Examples
Differential centrifugation	Distinct molecules in biofluid are separated by differential centrifugal force.	As the most common approach used to isolate EVs, it is cost effective and highly flexible in the scale of production. It is able to separate ABs, MVs, and exosomes from biological fluid.	It requires specialized instruments such as ultracentrifuges and rotors. The isolation efficiency is subject to change based on different instruments. The multi-step process is also relatively time consuming. Biological fluid with high viscosity needs dilution before ultracentrifugation.	Protein, mRNA, miRNA	NA
Density gradient centrifugation	Combining ultracentrifuge and density gradient could exclude molecules with different densities from EVs.	Density gradient further purifies EVs from co-precipitation such as protein aggregates, apoptotic bodies, or nucleosomal fragments.	Other than shared disadvantages with ultracentrifugation, density gradient centrifugation needs extra washing steps. It doesn't separate certain lipoproteins and virus vesicles since they share the similar densities.	Functional study; Protein; mRNA; miRNA	OptiPrep
Ultrafiltration	Using membranes with different pore sizes to seperate EVs.	This method is able to enrich EVs from small biofluid volume. The procedure is relatively easy and quick.	Causing retention of exosomes because of membrane -protein binding or saturation of membrane pores. Using force filtration potentially leads to artificial vesicles or vesicle rupture.	NA	PureExo; DiagExo
Polymer-based precipitation	Using volume-excluding polymers to wrap and aggregate Evs, which could be precipitated at low speed centrifugation.	Specialized instruments are not needed. The process of isolation is easy to handle. It is high-throughput and efficient for EV isolation.	It is compromised in purity with contaminants such as lipoproteins. The presence of polymers in precipitation might affect following analyses.	miRNA	miRCURY; ExoQuick and Total Exosome Isolation Reagent
Size exclusion chromatography	SEC is based on a column of beads with different pore sizes. EVs are separated and collected as molecules with different hydrodynamic radii pass through the column at different rates.	Usually only single step is needed to isolate EV by SEC from biofluid. The integrity and biological activity of the molecules isolated are minimally altered during the process of SEC.	SEC doesn't concentrate EVs. Optimization and re-equilibration are needed before running, which limit the efficiency of SEC.	Functional study; Protein; mRNA; miRNA	Izon qEV SEC
Immunoaffinity capture	EVs are isolated based on antibodies recognizing antigens expressed on the surface of vesicles.	It can be used to isolate total EVs or a specific group of EVs expressing unique antigen.	Immunoaffinity capture is not suited for large sample volume. Heterogeneity in antigen expression and dynamic in epitope masking affect the isolation efficiency. The specific antibody needs to be available.	Subgroup analysis	MACSPlex Exosome Kit; Exo-Flow
Microfluidic device	Using distinct or combined technologies such as immune affinity, SEC and filtration to study the EVs in biofluid at microscale.	Small sample volume needed, high effeciency, lower cost in sample analysis and high sensitivity.	The cost of device is high. It still needs development and optimization to be a clinical tool.	Clinical diagnosis	iMER; nPLEX; NanoDLD

Table 2

EVs act as carriers of macromolecules for intercellular communication between cancer cells

Type of cancer	Type of EVs	Key molecules	Role	Reference
Bladder Cancer	Exosome	EDIL-3	Exosomes from high grade bladder cancer cells promote migration of bladder cancer cells by activation of EGFR.	(121)
Prostate Cancer	Exosome	MDR-1/P-gp	Exosomes from docetaxel-resistant prostate cancer cells confer docetaxel-resistance to non-resistant cells, inducing the motility, invasion, proliferation and anchorage-independent growth.	(124)
		metalloproteinases, TGF-β2, TNF1α, IL6, TSG101, Akt, ILK1, and β - catenin	Exosomes from prostate cancer cells under hypoxia enhance the invasiveness and stemness of naive PCa cells by targeting of epithelial adherens junction pathway.	(118)
Breast cancer	Exosome	miR-10b	Metastatic breast cancer cells secrete miR-10b containing exosomes to non-metastatic cells to promote the invasion by targeting HOXD10 and KLF4.	(130)
		miR-221/222	Exosomal miR-221/222 derived from tamoxifen resistant MCF7 targets P27 and ERa in recipient cells to enhanced tamoxifen resistance.	(131)
		miR-100, miR-222 and miR-30a	Exosomes from adriamycin and docetaxel resistant cells confer chemoresistance by transferring miRNAs to sensitive cells.	(132)
		Lnc UCA11	Exosomal lnc UCA1 confers tamoxifen resistance from resistant cells to sensitive cells.	(137)
		EDIL-3	EDIL3 containing exosomes enhance cell invasion and accelerates lung metastasis *in vivo* through integrin-FAK signaling cascade.	(120)
		Hsp90α	Hsp90α from invasive cancer cell derived exosomes activates plasmin to increase cancer cell mobility.	(119)
		P-gp	Docetaxel-resistance could be transferred between MCF7 breast cancer cells through Pgp contained exosomes.	(125)
		Unknown	Exosomes from aggressive cancer cells transfer phenotypic traits to less aggressive cancer cells.	(278)
	MV	EMMPRIN	EMMPRIN from tumor cells derived MVs activates p38/MAPK pathway to promote cancer invasion.	(279)
		RAB22A	MVs from breast cancer cell under hypoxia promote focal adhesion formation, invasion, and metastasis in naive breast cancer cells.	(42)
Renal cell carcinoma	Exosome	LncARSR	Exosome-mediated transmission of lncARSR, which acts as a sponge for miR-34 and miR-449, can confer sunitinib resistance to sensitive cells.	(78)
Gastric cancer	Exosome	Unkown	Gastric cancer exosomes promote cancer cell proliferation by activation of PI3K/Akt and MAPK/ERK pathways.	(280)
Hepatocellular cancer	Exosome	miR-584, miR-517c, miR-378, etc.	HCC cell-derived exosomes suppress TAK1 expression in recipient cells to enhance transformed cell growth.	(133)
Glioma	Exosome and MV	mRNA, miRNA and angiogenic proteins	Glioblastoma derived EVs confer transcripts and angiogenic proteins to promote the tumor growth.	(142)
	MV	EGFRvIII	Cancer cells share EGFRvIII through MVs, leading to the transfer of oncogenic activity	(126)

Type of cancer	Type of EVs	Key molecules	Role	Reference
Oral squamous cell carcinoma	Exosome	miR-21	Exosome derived hypoxic OSCC cells increase the invasion and migration of normoxic cells by transferring miR-21.	(43)

Table 3

EVs facilitate intercellular communication between cancer cells and microenvironmental cells to promote tumor progression

Type of cancer	Type of EVs	Interacting Cells	Role	Reference
Breast cancer	Exosome	Astrocyte	Astrocyte-derived exosomes transfer miR-19a to inhibit PTEN in cancer cell to support cancer growth in brain.	(182)
		Fibroblast	Breast cancer cells trigger NOTCH-MYC signaling in CAF to increase RNAs of *RN7SL1*. Exosomal *RN7SL1* acts as damage-associated molecular patterns to modulate immune cells and cancer cells.	(153)
		Fibroblast	Fibroblasts secrete CD81 containing exosomes to promote autocrine Wnt-planar cell polarity signaling that induces protrusive activity and motility of breast cancer cells.	(154)
		Stroma	Noncoding transcripts and transposable elements in stromal exosomes activate STAT1 through pattern recognition receptor RIG-I and expand therapy-resistant tumor-initiating cells.	(281)
		Mesenchymal Stem Cells	Mesenchymal stem cell-derived exosomes deliver miR-16 to inhibit VEGF expression in cancer cells, leading to decreased angiogenesis.	(165)
		Bone marrow mesenchymal stem cell	Mesenchymal stem cell-derived exosomes deliver miR-222/223 to promote quiescence and drug resistance. Targeting miR-222/223 sensitized breast cancer cells to carboplatin-based therapy.	(170)
			Exosomal miR-23b induces cancer cell dormancy by targeting MARCKS	(171)
		Adipose tissue derived mesenchymal stem cells	Cancer cell-derived exosomes induce adipose tissue derived MSCs into tumor-associated myofibroblasts, which release tumor-promoting factors. This process is mediated by phosphorylation of SMAD2.	(173)
		Pre-adipocyte	Preadipocyte-derived exosomes promote tumorigenesis and cancer stem cell properties. Shikonin inhibits cancer growth by targeting SOX9 signaling through miR-140.	(282)
		Macrophages	IL-4 activated macrophages transfer miR-223 to promote the invasion of breast cancer cells via targeting the Mef2c-b-catenin pathway.	(189)
	MV	Normal epithelial cells/Fibroblast	MVs derived from MDAMB231 cells induce transformation of normal epithelial and fibroblast cells by transferring tissue transglutaminase and fibronectin.	(155)
		Platelet	MVs from platelets transfer integrin CD41 to the surface of breast cancer cells and enhance their adhesion to endothelial cells; MVs also promote invasion by up-regulation of CXCR4, p42/44, AKT, and MMPs.	(192)
Prostate cancer	Exosome	Adipose-derived mesenchymal stem cells	Prostate cancer cell-derived exosomes induce tumorigenic reprogramming of	(175)

Type of cancer	Type of EVs	Interacting Cells	Role	Reference
			adipose stem cells by trafficking of oncogenic factors and downregulating tumor suppressors.	
		Bone-marrow mesenchymal stem cell	Exosome TGFβ trigger mesenchymal stem cell differentiation into myofibroblasts, which support tumor cell growth.	(172)
		Fibroblasts	Exosomal TGFβ induces fibroblast differentiation to myofibroblasts, which are pro-angiogenic and tumor-promoting.	(156, 157)
		Cancer-associated fibroblast	CAF-derived exosomes promote tumor growth under nutrient stressed conditions by delivering intact metabolites.	(283)
		Lymph node stroma cells and lung fibroblasts	Exosomal miR-494 and miR-542-3p target cadherin-17 to increase matrixmetalloproteinase expression in niche cells.	(284)
	Large oncosome	Fibroblasts	Large oncosomes derived from prostate cancer cells contain active AKT1. Internalization of large oncosome by fibroblasts activates MYC and reprograms fibroblast to support angiogenesis and tumor growth.	(159)
pancreatic cancer	Exosome	Adipocyte	Exosomal adrenomedullin from cancer cell induce lipolysis through p38 and ERK1/2, which causes weight loss of cancer patients.	(191)
		Endothelial Cell	Uptake of Tspan8-CD49d containing exosome induces several angiogenesis-related genes to enhance endothelial cell proliferation.	(176)
		Kupffer cells	Exosome from pancreatic ductal adenocarcinomas induces the release of transforming growth factor ß by KCs and fibronectin production by hepatic stellate cells, which are critical to recruit macrophage for the formation of pre-metastatic niche.	(183)
		Cancer-associated fibroblast	CAFs exposed to gemcitabine significantly increase the release of exosomes, which contain chemoresistance-inducing factor Snail and miR-146a.	(158)
	Exosome and MV	muscle cell	tumor-derived EVs induce apoptosis of skeletal muscle cells through delivery of miR-21	(184)
ovarian cancer	Exosome	adipose tissue derived mesenchymal stem cells	Cancer cell-derived exosomes induce myofibroblasts like phenotype in adipose tissue derived MSCs, through SMAD2 or AKT phosphorylation.	(174)
		Lymph Node Cells	Exosomes go to lymph node and induce premetastatic niche by inducing expression of factors responsible for cell recruitment, matrix remodeling, and angiogenesis.	(285)
Melanoma	Exosome	Bone marrow cells	Melanoma cell derived exosomes educate bone marrow progenitors by transferring MET. Targeting Rab27A could decrease cancer growth and metastasis.	(190)
	MV	Fibroblasts	MVs from melanoma cells induce VCAM-1 in tumor-associated fibroblast,	(161)

Type of cancer	Type of EVs	Interacting Cells	Role	Reference
			which is involved in melanoma cell attachment to fibroblast.	
Renal cell carcinoma	Exosome	Endothelial cells	Exosomes from CD105-positive cancer stem cells contain angiogenic mRNAs and miRNAs to promote lung endothelial cells, establishing the pre-metastatic niche.	(177)
Lung cancer	MV	Endothelial cells; Stroma fibroblasts	Lung cancer cells secrete more MVs under non-apoptotic doses of hypoxia and irradiation. These MVs activate and chemoattract fibroblasts and endothelial cells, increasing metastasis of lung cancer cells	(160)
		Platelet	Platelet derived MVs transfer integrin CD41 to promote growth, invasion and angiogenesis of lung cancer cells. These MVs also chemoattract cancer cells.	(188)
Colorectal cancer	Exosome	Endothelial cells	CRC cell-derived exosomes transport cell cycle-related mRNAs to endothelial cells to promote angiogenesis.	(286)
Bladder Cancer	Exosome	Endothelial cells	Exosomes from high grade bladder cancer contain EDIL-3, which promotes angiogenesis, and migration of endothelial cells and bladder cancer cells.	(121)
Glioma	Exosome and MV	Endothelial cells	GBM Cancer cells under hypoxia release TF/VIIa containing exosomes to activate hypoxic endothelial cells. Glioblastoma exosomes and MVs can deliver functional RNA to HBMVECs to promote angiogenesis.	(142, 178)
	MV	normal fibroblasts	MVs derived from U87 glioma cells induce transformation of normal fibroblasts by transferring tissue transglutaminase and fibronectin.	(155)
Transformed fibroblast	AB	Fibroblast	Apoptotic bodies from transformed rat embryonic fibroblasts deliver oncoaenes to mouse embryonic fibroblasts.	(32)

Table 4

Diagnostic Value of EVs in Cancer

Type of cancer	Marker	Type of EVs	Conclusions	Source	Reference
Colorectal Cancer	Level of circulating exosomes	Exosome	Level of exosomes in cancer patients is statistically higher than in healthy controls. It correlates with CEA and poorly differentiated tumors and shorter overall survival.	Plasma; CCM	(199)
	let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, and miR-23a	Exosome	Serum exosomal levels of seven miRNAs are significantly higher in CRC patients, cell lines compared to healthy controls.	Serum; HCT116, HT-29, RKO, SW48, and SW480	(205)
Prostate Cancer	miRNAs	Exosome and MV	12 miRNAs are differentially expressed in prostate cancer patients compared with controls; Among them, miR-107 and miR-574-3p can serve as urine marker. 11 miRNAs, including miR-141 and miR-375, are higher in prostate cancer patients with metastases compared with those without metastases.	Plasma; Serum; Urine	(204)
	miR-1290 and miR-375	Exosome	Plasma exosomal miR-1290 and miR-375 are associated with poor overall survival of CRPC patients.	Plasma	(287)
	Frequency of apoptotic bodies	AB	The level of apoptotic bodies in tumor tissue is positively correlated with Gleason grade.	Tumor tissue	(201)
	mRNA of ERG, PCA3, and SPDEF	Exosome	ExoDx Prostate IntelliScore gene signature in urine exosome plus SOC could increase the sensitive of detecting PCa with Gleason scores higher than 7 in a non-invasive way.	Urine	(209)
	mRNA of PCA-3 and TMPRSS2:ERG	Exosome	The mRNAs of PCA-3 and TMPRSS2:ERG in urine exosome can be used as the biomarkers for diagnosis and prognosis.	Urine	(208)
	PTEN	Exosome	Prostate cancer cells down-regulate PTEN by incorporating PTEN into exosomes, which can be used as a blood biomarker for diagnosis.	plasma and DU145, PC-3	(288)
	PDCD6IP, FASN, XPO1 and ENO1	Exosome	By using high performance LC-FTMS, exosomal proteins PDCD6IP, FASN, XPO1 and ENO1 are discovered as new candidate biomarkers for prostate cancer.	PC346C and VCaP	(289)
	Survivin	Exosome	Prostate cancer patients express higher level of Survivin in blood exosome compared to BPH and healthy controls. Exosomal Survivin levels are also higher in patients with relapse on chemotherapy.	Plasma; serum	(290)
	miR-34a	Exosome	Decreased exosomal miR-34a indicates higher resistance to Docetaxel in prostate cancer.	22Rv1, DU145, and PC3	(291)
			The prostasomes in the serum can be detected by a sensitive and specific		
	Level of prostasomes	MV	assay-proximity ligation assay (PLA). By PLA, higher level of prostasomes can be detected in prostate cancer patients compared to healthy donors. The amount of prostasome in serum is also correlated to prostatectomy Gleason scores.	Plasma	(198)
	Level of large oncosomes	Large oncosome	Large oncosome could be detected in human tumor tissues, but not in benign compartments. In mouse model of prostate cancer, large oncosome	Plasma; Tumor tissue	(38)

Type of cancer	Marker	Type of EVs	Conclusions	Source	Reference
			could be identified in tumor tissues and circulation. The abundance of large oncosome correlated with tumor progression.		
Ovarian cancer	Claudin-4	Exosome	Claudin-4-containing exosomes can be detected in the serum of ovarian cancer patients. Claudin-4 and CAI 25 are strongly correlated.	Plasma; Cell lines	(211)
Esophageal Squamous Cell Carcinoma	miR-21	Exosome	Exosomal miR-21 is significantly higher in patients with ESCC and it is positively correlated with disease progression.	Serum	(203)
Pancreatic cancer	CD44v6, Tspan8, EpCAM, MET and CD104; miR-1246, miR-4644, miR-3976 and miR-4306	Exosome	Pancreatic cancer patients have higher CD44v6, Tspan8, EpCAM, MET, CD104, miR-1246, miR-4644, miR-3976 and miR-4306 in serum exosome. Combined evaluation of miRNAs and protein in exosome for diagnosis achieves high sensitivity and specificity.	Serum; Cell lines	(213)
	Transcripts of Apbb1ip, Daf2, Foxp1, Incenp, BCO31781, and Gng2;	Exosome	Transcriptomic biomarkers of Apbb1ip, Daf2, Foxp1, Incenp, BCO31781, and Gng2 are up-regulated in both serum and saliva-derived exosomes of pancreatic tumor-bearing mice.	Salivary and serum from mouse	(292)
	Genomic DNA	Exosome	Serum exosomes of pancreatic cancer patients carry genomic DNA spanning all chromosomes with mutated KRAS and P53.	Serum; T3M-4 and Panc-1	(210)
	Glypican-1	Exosome	Glypican-1 positive serum exosomes distinguish pancreatic cancer patients from healthy controls. The level of glypican-1 positive serum exosomes correlates with tumor burden and the survival of patients.	Serum	(212)
Multiple cancers	dsDNA	Exosome	DsDNA is present in exosome, representing the whole genomic DNA from cancer cell. Exosomal dsDNA can be used to identify mutations and cancers.	Plasma; Cell lines	(15)
Lung cancer	Level of circulating exosomes and exosomal miRNAs	Exosome	Lung cancer patients have increased level of circulating exosome. MiR-17-3p, miR-21, miR-106a, miR-146, miR-155, miR-191, miR-192, miR-203, miR-205, miR-210, miR-212, hsa-miR-214 are upregulated in NSCLC.	Plasma	(206)
	miRNAs	Exosome	MiR-378a, miR-379, miR-139-5p, and miR-200b-5p are up-regulated in patients with lung adenocarcinomas or lung granulomas. Expression of exosomal miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100, and miR-154-3p can be used to distinguish lung adenocarcinomas from lung granulomas.	plasma	(293)
Melanoma	CD63, Caveolin-1	Exosome	Plasma exosomes expressing CD63 or caveolin-1 are higher in melanoma patients compared to healthy donors. Caveolin-1+ plasma exosomes have higher sensitivity compared to CD63+ plasma exosomes.	Plasma; Me501 and MeBS	(22)
Gastric cancer	Let-7	Exosome	Exosomal let-7 miRNA family is up-regulated in metastatic gastric cancer cell line.	Cell lines	(294)
	LINC00152	Exosome	Exosomal LINC00152 is higher in plasma of gastric cancer patients compared to healthy controls.	Plasma	(207)

Table 5

Drugs targeting EV

Drug	Target	Direction	Mechanism of Action	Reference
dimethyl amiloride	Exosome	Inhibition	inhibitor of the H+/Na+ and Na+/Ca2+ exchangers	(223)
Transferrin	Exosome	stimulation	transferrin receptor (increase cytosolic Ca2+)	(223)
Monensin	Exosome	stimulation	a Na+ ionophore (increase cytosolic Ca2+)	(223)
GW4869/spiroepoxide/glutathione/manumycin-A brefeldin A	Exosome	Inhibition	target nSMase2 for ceramide formation	(67, 221, 222)
	Exosome	Inhibition	Inhibitor of BIG2	(226)
CAY10594	Exosome	Inhibition	Inhibitor of PLD2	(58)
chlorpromazine	Exosome	Inhibition (uptake)	block clathrin-mediated endocytosis	(295)
Cytochalasin D/Lantrunculin A	Exosome	Inhibition (uptake)	disrupt actin cytoskeletal filaments	(228)
Heparin	Exosome	Inhibition(uptake)	Inhibitor of cell surface receptors dependent on HSPG co-receptors	(229)
apilimod	Exosome	stimulation	PIKfyve	(296)
CI-1033/PF-00299804	Exosome	stimulation	ErbB/EK	(297)
Cl-amidine	Exosome and MV	Inhibition	Inhibit protein deimination of cytoskeletal actin	(298)
Bisindolylmaleimide-I	Exosome and MV	Inhibition	protein kinase C inhibitor preventing the externalization of phosphatidylserine	(298)
Imipramine	Exosome and MV	Inhibition	Inhibitor of acid sphingomyelinase aSMase	(298, 299)
EGTA	Exosome and MV	inhibition	calcium chelation	(223, 235)
MßCD	Exosome and MV	Inhibition	membrane cholesterol depletion	(300, 301)
D-pantethine	MV	Inhibition	blocks the translocation of the aminophospholipid phosphatidylserine from the inner to the outer membrane leaflet	(302, 303)
Y-27632	MV	Inhibition	Target RhoA mediated action mobilization and MV formation	(232, 233)
calpeptin	MV	inhibition	inhibitor of calpain mediated destabilization of cortical actin cytoskeleton	(230, 231)