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REVIEW

Formation and role of exosomes in cancer

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Abstract Exosomes offer new insight into cancer biology with both diagnostic and therapeutic implications. Because of their cell-to-cell communication, exosomes influence tumor progression, metastasis, and therapeutic efficacy. They can be isolated from blood and other bodily fluids to reveal disease processes occurring within the body, including cancerous growth. In addition to being a reservoir of cancer biomarkers, they can be re-engineered to reinstate tumor immunity. Tumor exosomes interact with various cells of the microenvironment to confer tumor-advantageous changes that are responsible for stromal activation, induction of the angiogenic switch, increased vascular permeability, and immune escape. Exosomes also contribute to metastasis by aiding in the epithelial-to-mesenchymal transition and formation of the pre-metastatic niche. Furthermore, exosomes protect tumor cells from the cytotoxic effects of chemotherapy drugs and transfer chemoresistance properties to nearby cells. Thus, exosomes are essential to many lethal elements of cancer and it is important to understand their biogenesis and role in cancer.

Keywords Signaling · Immunosurveillance · Fibroblast · Targeted therapy · Multivesicular endosome · Vaccine · ESCRT · Biogenesis

Abbreviations

MVE	Multivesicular endosome
ESCRT	Endosomal-sorting complexes required for transport
MDSC	Myeloid-derived suppressor cells
EMT	Epithelial-to-mesenchymal transition
HIF	Hypoxia-inducible factor
GM-CSF	Granulocyte–macrophage colony-stimulating factor
ASC	Adipose stem cell

Introduction

Despite promising advances, cancer remains the second leading cause of death in the US and new insights into cancer biology are necessary to drive therapeutic innovation [1, 2]. Once viewed as a passive, insignificant appendage to cancer, the microenvironment has moved into the limelight as an integral contributor to cancer suppression [3–5], cancer promotion [6, 7], and drug resistance [8, 9]. It is now recognized that a tumor is really a system, more organ-like than homogenous, with complex interactions between the microenvironment and tumor cells [10]. The tumor microenvironment, or stroma, is composed of

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fibroblasts and the extracellular matrix they deposit, immune cells, and vascular cells [10]. Not only does the microenvironment provide prognostic information since changes in the microenvironment correspond to a better or worse patient outcome, but it also reflects treatment efficacy and has therapeutic potential, since it presents a wealth of new targets [11]. Reviews covering multiple aspects of the tumor microenvironment have been published, including bipolar effects [12], therapeutic targeting [13], immunology [14], and chemoprevention [15]. In this review, we focus instead on an emerging aspect of the tumor microenvironment that enables the crucial communication between the tumor and microenvironment and is implicated in tumor progression, metastasis, and chemoresistance: exosomes (see Box 1 for historical perspective). Tumor progression results from active partnership between the tumor and microenvironment that would be impossible without efficient modes of exchanging information: direct cell-to-cell contact, secretion of signaling molecules, and release of vesicles like exosomes into the extracellular space [16]. In this review, we present the biogenesis of exosomes via multivesicular endosomes (MVEs), the unique contribution they make to cancer progression through interaction with multiple microenvironmental cell types, the role they play in metastasis and chemoresistance, and discuss their application to diagnostics and therapy [17].

Exosome biogenesis in cancer

Although there has been some confusion with regards to exosome identification, exosomes are defined by certain shared characteristics, including size (50–100 nm), density, morphology, and general protein composition (Fig. 1, see Box 2 for use of "exosome" in scientific literature) [18, 19]. Unlike other extracellular vesicles such as microvesicles, ectosomes, and membrane particles, exosomes do not originate by the direct budding or shedding of the plasma membrane (shedding microvesicles reviewed in [20]) [18]. Instead, exosomes are derived from MVEs, commonly referred to as multivesicular bodies (MVBs). Exosome formation occurs when the membrane of the MVE bulges inward and pinches off to create small membranous vesicles within the MVE, packed with cytoplasmic contents, including proteins, RNAs, and recently genomic DNA was also found [21] (refer to [22] for a free database of more than 14,000 biomolecules identified in exosomes). Subsequent exosome secretion occurs when MVEs fuse with the plasma membrane and release their membrane-bound contents (exosomes) into the extracellular environment (Fig. 2). It should be noted that not all MVEs go on to release exosomes; an alternative fate of MVE cargo is degradation by fusion with the lysosome. It has been shown that the presence of high concentrations of the ceramide lipid family appear to help MVE contents escape lysosomal digestion in favor of release as exosomes [23].

The consequence of the mechanism of exosome biogenesis is an extracellular particle whose membrane reflects the composition of the MVE, but with the same orientation as the parental cell plasma membrane (Fig. 2). The exosomal membrane is enriched in endosome-related membrane transport and fusion proteins (flotillin, Annexins, GTPases), certain lipids (sphingomyelin, cholesterol, ceramide) and, if from antigen presenting cells, MHC-II. Exosomes also contain endosome-specific tetraspanins (CD9, CD63, CD81, CD82), and MVE biogenesis-related proteins (Alix, TSG101) on their membrane surfaces [24–26]. Beyond their characteristic collection of membrane markers, the molecular content of exosomes can vary significantly based on the physiological conditions and the original cell type [27]. Importantly, the composition of an exosome is not a mere reflection of the donor cell, and it has been shown that the profiles of exosomal cargo can be substantially different from the originating cell, which indicates the existence of a highly controlled sorting process [28]. The exact mechanisms involved in exosome packaging have not been fully delineated, but appear to be similar to how lysosomal-bound MVEs are packaged since endosomal-sorting complexes required for transport (ESCRT) proteins are found in exosomes. [29].

The proteins of the ESCRT pathway divide into four complexes that identify and aggregate ubiquinated proteins in the endosomal membrane (ESCRT-0), cause membrane budding (ESCRT-I, -II), and instigate separation from the membrane (ESCRT-III). However, there is also evidence for ESCRT-independent packaging: MVEs are not obliterated by simultaneous depletion of the subunits belonging to the four ESCRT proteins [29]. These pathways may involve lipids such as sphingosine-1-phosphate (S1P) [30] and the tetraspanin-enriched microdomains [31]. Colombo et al. [29] confirmed that while some exosome production may be ESCRT-independent, at least some exosomes are ESCRT-dependent; they showed that silencing genes of ESCRT-0 (*HRS, STAM1*), ESCRT-I (*TSG101*), or *VPS4B* altered the amount of exosomes produced and their cargo. Insight into the selective sorting of miRNAs into exosomes has recently been reported, involving sumoylated heterogeneous nuclear ribonucleoproteins, mainly hnRNPA2B1, which bind to a subset of miRNAs and control their loading into exosomes [32].

In addition to exosomal packaging, each step of exosome biogenesis—trafficking to the plasma membrane, docking, fusion, and release—appears to be highly

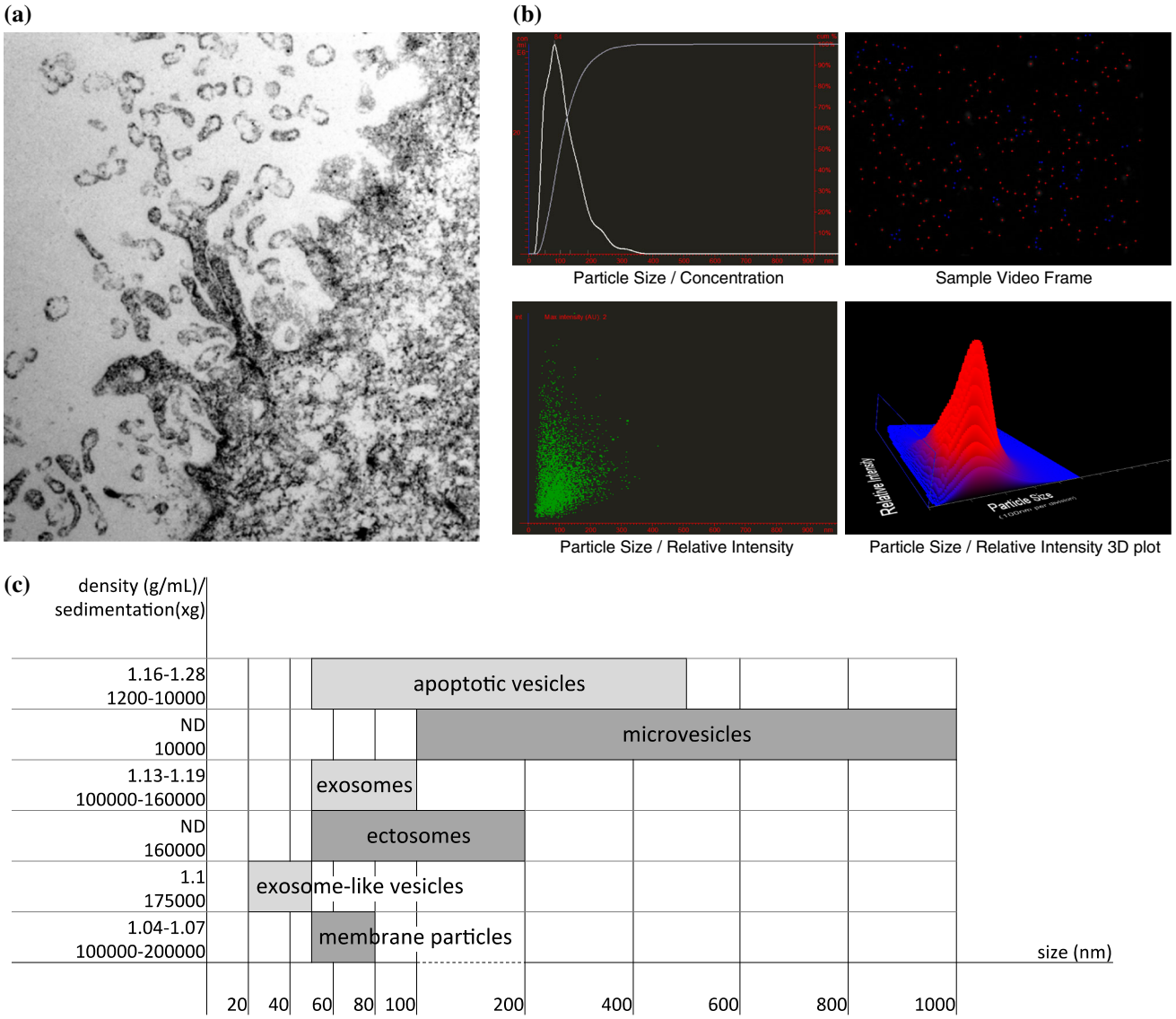

Fig. 1 Exosomes. **a** TEM of plectin-positive pancreatic ductal adenocarcinoma (PDAC) cells with enhanced exosome production. Plectin is necessary for exosome production in PDAC and is aberrantly expressed on the cell surface through an exosome-mediated process (see [39] for details about PDAC exosomes). **b** Nanosight analysis of exosomes isolated from mouse serum using ExoQuick-TC isolation reagent shows the exosome size/concentration, a sample video frame of the exosomes, as well as the exosome size/relative intensity in both a scatter and 3D plot (unpublished image). **c** Exosomes can be differentiated from other secreted vesicles by their size (nm), density in sucrose (g/mL), and sedimentation speed (×g) (numbers from [18])

organized and regulated and, therefore, is potentially altered in cancer. Notably, exosome release tends to be exacerbated in tumor cells as compared to other proliferating cell types, which can be a result of stimulation in response to stressful conditions due to excess growth and cell damage caused by chemotherapeutics. In many cancers, aberrant p53 stimulation results in over-expression of tumor suppressor-activated pathway 6 (TSAP6), which, in turn, increases exosome production [33, 34]. Additionally, heparanase, which is an enzyme up-regulated in many tumor cell lines, has been implicated in exosome secretion [35]. However, tumor cell exosome release is inhibited by previously secreted tumor exosomes that are still present in the microenvironment, creating a balancing negative feedback control loop [36]. The GTPases Rab27a and Rab27b control secretory pathways, including exosome release, probably by regulating the trafficking of secretory vesicles to the plasma membrane, tethering the vesicles to the plasma membrane, and/or helping fuse the vesicles to the plasma membrane [37]. In the absence of Rab27a, other Rab proteins, especially Rab27b, appear to be able to act as compensatory pathways for exosome secretion; Rab2b,

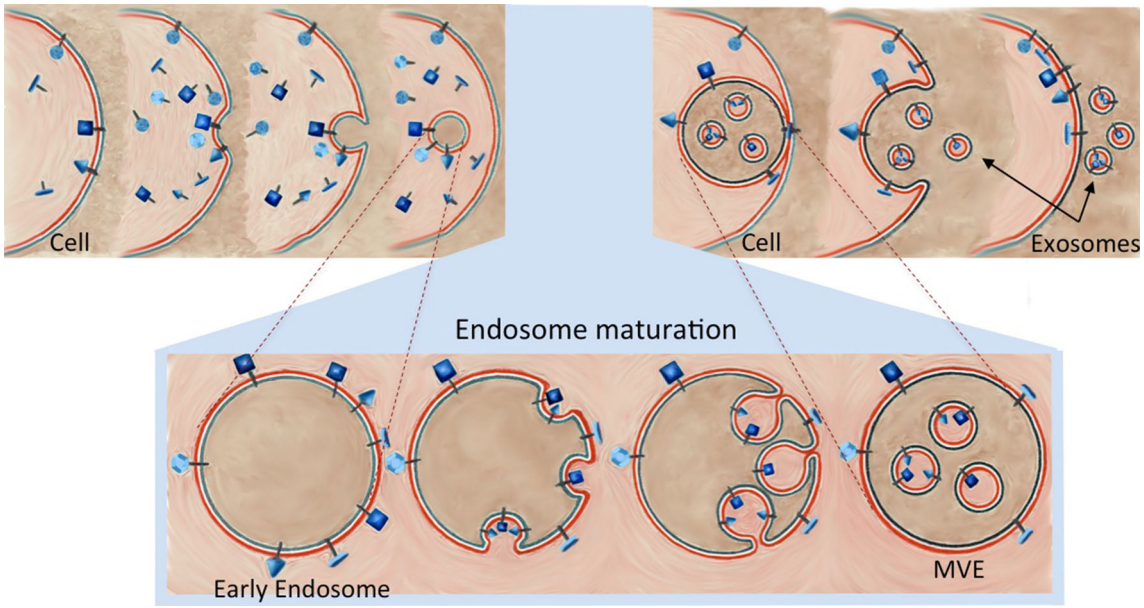

Fig. 2 Exosome Biogenesis. The plasma membrane buds inward, forming a membrane-bound vacuole. This endosome goes through several changes as it matures from an early endosome to a late endosome. Most notably, the endosomal membrane buds inward and pinches off to make membrane-bound vesicles inside the endosome and the endosome is now titled a multivesicular endosome (MVE). The MVE may travel to the lysosome and degrade its contents or it may travel to and fuse with the plasma membrane, releasing its contents, which, once existing outside the cell, are called exosomes (see [26] for more details about MVEs)

Rab5a, Rab9a, Rab27a, and Rab27b have all been shown to decrease exosome secretion in cervical cancer cells [38]. Similarly, knockdown of plectin in three types of pancreatic cancer cells decreased exosome production about fivefold [39]. Another Rab protein, Rab11, has been shown to regulate the exosome pathway of a leukemia cell line by influencing calcium-dependent fusion of MVEs with the plasma membrane [40, 41]. Recently, invadopodia have emerged as a docking site for exosomes, promoting cancer invasion. In fact, exosome secretion and invadopodia formation appear to be interdependent; inhibition of exosome biogenesis affects invadopodia formation and stability and, conversely, inhibition of invadopodia formation greatly decreases exosome secretion [42].

Exosomes and the tumor microenvironment

Tumor progression, metastasis, and chemoresistance all depend on the ability of the tumor and its microenvironment to communicate. Exosomes are a unique form of transferring information both locally and to a distant site. Cells of the microenvironment release exosomes to affect each other and the tumor cells. Meanwhile, tumor cells release exosomes to reprogram their surroundings to be tumor permissive and even tumor promoting. Here we discuss the evidence of how exosomes influence each major type of stromal cell—cancer-associated fibroblasts, vascular endothelial cells, and immune cells—as well as how exosomes accelerate metastasis and chemoresistance.

Cancer-associated fibroblasts

Fibroblasts become "activated" as they progress through changes during the neoplastic process, including changes in morphology and protein expression, with disorganized and uncontrolled growth as well as increased production of collagen and stimulation of hyaluronate synthesis [43]. These activated fibroblasts can both hinder and promote tumor growth and progression, depending on the molecular state of the tumor epithelial cells, and are certainly capable of accelerating growth and promoting tumor cell invasion [6, 44–48]. Cancer exosomes have been shown to trigger such fibroblast transformation through the TGFβ/Smad pathway and elicit effects unique from soluble TGFβ [49–51]. It has even been proposed that the stroma may go beyond promoting pre-existing tumors to causing tumorigenicity in adjacent cells through oncogenic signals [43]. Co-cultures of tumor fibroblasts with non-transformed epithelial cells caused immature, pleomorphic epithelial cells with enlarged nuclei and aberrant mitosis. Cells also increased in their rate of proliferation, lost polarity, and had altered cell cycle protein expression; p53, PCNA, Ki67and cytokeratin expression were increased, whereas p21 nuclear expression and Bcl2 were decreased [52].

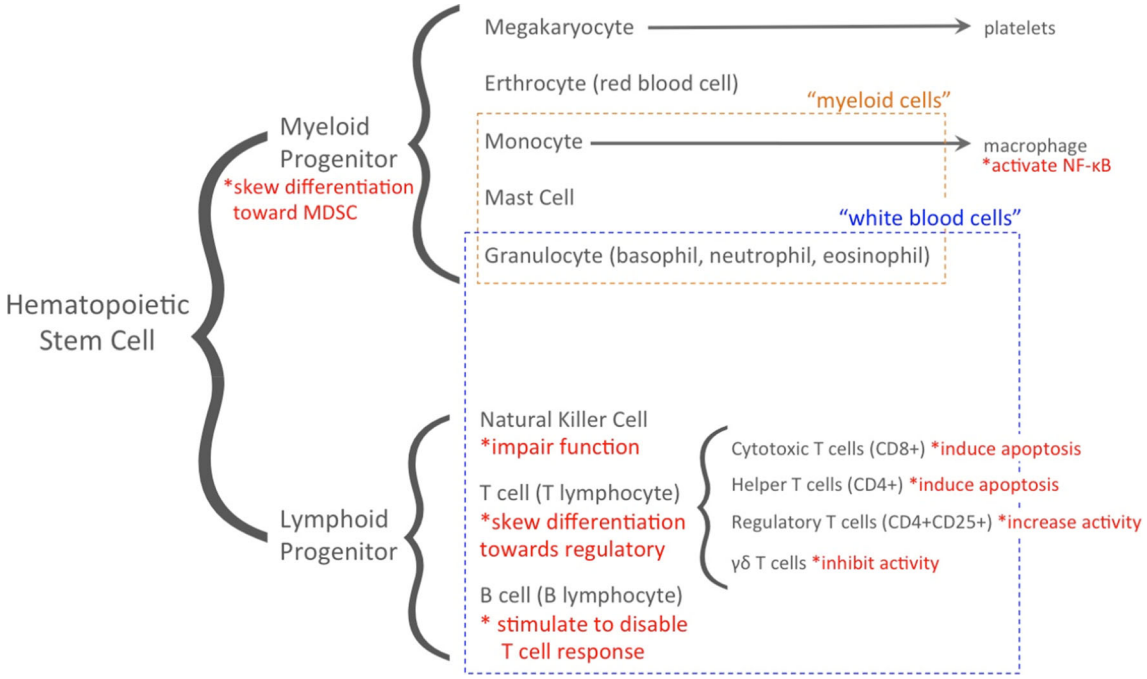

Fig. 3 Role of exosomes in immune escape. This immune cell lineage groups cell types in *dashed boxes* that are commonly classified as myeloid cells or white blood cells. Highlighted in *red* with *asterisks* are the immune escape mechanisms conferred to each immune cell type by tumor-derived exosomes (functions from [60–81])

Vascular endothelial cells

Tumors must have access to nutrients, oxygen, and waste removal to grow beyond a few cubic millimeters and to accomplish this, the tumor cells must access the host vasculature and divert blood to the tumor [53, 54]. Creation of a tumor blood supply requires induction of the angiogenic switch, in other words sufficient increase of pro-angiogenic factors to overcome anti-angiogenic factors [54]. Many soluble factors such as VEGF contribute to flipping this angiogenic switch [55]. In addition, exosomes have also been shown to play a role in increasing angiogenesis [55]. As hypoxia is sensed throughout the tumor, carcinoma cells secrete exosomes into the microenvironment to initiate signaling and flip the angiogenic switch to ensure adequate oxygenation [55, 56]. Hypoxia-induced proteins secreted in the tumor exosomes are taken up by the normal host endothelial cells, where the exosomal cargo stimulates new tubule formation, eventually creating a network of blood vessels to supply that area [57]. Endothelial cells that have taken up hypoxic tumor exosomes start releasing growth factors and cytokines that stimulate pericytes via the PI3 K/AKT pathway [58]. Moreover, exosomes transfer miR-105 to endothelial cells, increasing vascular permeability by damaging tight junctions [59].

Immune cells

The immune system responds to cancer through both innate and adaptive immunity, the latter of which involves antigen-presentation. Hematopoietic stem cells from the bone marrow give rise to blood cells, including immune cells (Fig. 3). Dendritic cells are a separate class of immune cells that claim multiple origins and facilitate communication between the innate and adaptive components [60, 61]. Exosomes produced by immune cells and those produced by tumor cells have opposite effects. Thus, in the initial stages of cancer, the host immune system may stifle tumor progression; however, growing tumors activate suppressive pathways and eventually manage to evade immune surveillance and exosomes are involved in both responses [60]. Exosomes produced by immune cells ignite an anti-tumor immune response. Mast cell exosomes induce dendritic cell differentiation and activate T and B cells and dendritic cell exosomes sensitize other immune cells to tumor antigens [62, 63]. In contrast, tumor-derived exosomes participate in immune escape by (1) increasing the influencing myeloid progenitor cell differentiation, (2) decreasing T cell proliferation and effector functions, and (3) cancelling the natural cytotoxic responses mediated by natural killer cells (Fig. 3) [64, 65].

The first part of exosome participation in immune escape involves myeloid cell precursor differentiation. Tumor exosome uptake by myeloid progenitor cells inhibits differentiation into dendritic cells and instead channels them into myeloid-derived suppressor cell (MDSC) differentiation, causing MDSCs to accumulate in tumor tissue, primary and secondary lymphoid organs, and blood [66, 67]. This creates a dual pro-tumor effect: dendritic cells are no longer present to display antigens to simulate the host immune system to act against the tumor and MDSCs supply the tumor with growth- and angiogenesis-stimulating molecules and factors [65–67]. Such exosome-induced accumulation of MDSCs is MyD88 dependent and requires tumor exosomal expression of TGFβ and prostaglandin E2 (PGE2) [65, 68, 69]. While toll-like receptor 2/Stat3 has also been shown to be essential for tumor exosomes' effect on MDSCs, additional studies revealed that this might be a cell culture artifact [70]. Additionally, MDSCs create a powerful immunosuppressive effect because they deplete arginine and produce nitric oxide to decrease T cell functionality [71].

Second, exosomes dysregulate T cells, by impeding proliferation, decreasing differentiation into helper T cells, increasing differentiation into regulatory T cells, and varying the levels of cytokines in stimulated T cells [72]. In particular, tumor exosomes impair T cell response to IL-2, which inhibits differentiation of naïve T cells into Th1 and Th17 helper T cells. Th1 helper T cells affect macrophages, cytotoxic T cells, and helper T cells; whereas Th17 helper T cells affect neutrophils, B cells, and helper T cells [73]. Tumor exosomes also deplete cytotoxic T cell populations by inducing apoptosis via FasL and deplete mature Th1 populations by inducing apoptosis via galectin-9 [74, 75]. Moreover, tumor exosomes stimulate B cells to disable helper T cells so that the immune system is less responsive to tumor antigen presentation [76, 77].

The third arm of immunosurveillance concerns the cancelling of cytotoxic responses mediated by natural killer cells. Negating natural killer cells' killing capacity represents a mode of immunosuppression independent of the effect of regulatory T cells [73]. Unlike T and B cells, natural killer cells do not depend on MHC to respond to antigens and instead scan a cell and activate if the cell is missing self-identifiers or engages activating receptors, such as the NKG2D immunoreceptor, which recognizes self-proteins that are rarely expressed on healthy cells, but frequently are expressed by cells stressed by infection, tumorigenesis, or damage [78]. To escape this mode of destruction, cancer cells release NKG2D-ligand-expressing exosomes, essentially packaging destruction tags into exosomes to remove them from the cancer cell membrane [78]. These NKG2D-ligand-containing exosomes also reduce the amount of NKG2D in natural killer cells' membranes and lessen natural killer cell cytotoxicity in an NKG2D-independent way [79]. Exosome immunomodulation has been exclusively covered in recent reviews [80, 81].

Other roles of exosomes in cancer: metastasis and chemoresistance

Metastasis

The stage at diagnosis dictates patient prognosis with the presence of distal metastatic disease being almost universally fatal. For many cancers, the process of metastasis is initiated by the tumor cells undergoing an epithelial-to-mesenchymal transition (EMT) making them capable of migrating and gaining access to the vascular or lymphatic channels, where they can establish a metastasis or circulate to a different organ and produce a metastasis there [82]. Exosome composition changes with gain of metastatic capacity, becoming enriched in EMT proteins and other proteins that help coordinate metastatic efforts between the primary tumor and microenvironment [83]. Additionally, exosomes influence surrounding microenvironment cells to aid in metastasis. For example, when macrophages engulf tumor exosomes, NF-κB is activated, causing secretion of pro-inflammatory cytokines that have been linked to metastasis development [84]. Furthermore, a hypoxic tumor microenvironment induces expression of factors (HIFs) that are linked to metastasis and poor prognosis in patients [85]. HIFs have been implicated in the hypoxia-induced increase in exosome production mentioned in relation to angiogenesis. In breast cancer, exosomes secreted by hypoxic tumor cells stimulated focal adhesion formation, invasion into the extracellular matrix, and metastasis to the lungs [86]. While migratory capability is essential to metastasis, reaching a new organ is not sufficient for establishment of a metastasis; rather, the tumor cell must reach a niche that has the correct conditions for growth and where a new metastatic cell-niche microenvironment crosstalk is created [87]. Both HIFs and tumor exosomes have also been implicated in formation of a premetastatic niche [88, 89]. Upon exposure to tumor exosomes, adipose stem cells (ASC) have been shown to be recruited to the metastatic effort [90]. In a study by Abd Elmageed, et al. [90], ASC from prostate cancer patients were primed with exosomes isolated from prostate cancer cells and then transplanted into mice. These ASC became genetically unstable after exposure to the tumor exosomes, underwent reverse EMT and oncogenic transformation, then developed into tumors; thus, it is hypothesized that ASC in cancer patients may circulate to a new location and follow the same pattern to establish metastases [90].

Chemoresistance

Often, even after surgery, radiation, and chemotherapy, a small number of drug-resistant cancer cells will remain and cause recurrence. In breast cancer, for example, recurrent cancer strikes the vast majority of patients [91]. Since tumor cells are a heterogeneous population, the level of chemoresistance varies from tumor cell to tumor cell. As one mechanism of drug resistance, residual tumor cells will transmit resistance properties via exosomes to sensitive cells, creating a larger population of cells that are unaffected by cancer treatments [92]. Chen et al. [92] compared adriamycin- and docetaxel-resistant breast cancer cell lines to sensitive cell lines, establishing certain miRNAs (*miR-100*, *miR-222*, *miR-30a*, *miR-17*) that appear to be transferred intracellularly to confer resistance. Once transferred to sensitive cells, these miRNAs alter the cell cycle distribution and affect apoptosis pathways to decrease drug susceptibility. Docetaxel resistance has also been studied in prostate cancer, where the conferred resistance was found to be partly attributable to transfer of MDR-1/P-gp, a P-glycoprotein transporter protein that is over-expressed in drug resistant tumors [93]. High levels of other transporter proteins, including MRP2, ATP7A, and ATP7B, have been found in exosomes from cisplatin-resistant ovarian cancer cells [94]. In addition to these transporter proteins, multiple genes (*PI3 kinase, rho GTPases, annexins, XIAP*) whose products function in lysosome formation and the lysosome protein LAMP1 have also been implicated in cisplatin sensitivity [94]. Other studies have revealed that modulating the chemosusceptibility of other cells is not the only means by which exosomes increase chemoresistance. In fact, tumor cells can also package chemotherapy drugs into exosomes to protect the tumor cell from cytotoxic effects [94, 95]. Therefore, disabling exosome-mediated elimination could increase drug efficacy. With these exciting advances, it should be noted that drug resistance is a multifaceted problem of which exosomes likely play only a contributing part. Still, the discovery of the ability of exosomes to affect chemoresistance has led researchers to suggest the need for more comprehensive profiling of the molecular contents of exosomes to deduce the key contributors to the spreading of drug resistance [92, 93].

Applications of exosomes to cancer diagnostics and therapy

Biomarkers

The inherent properties of exosomes make them ideal candidates for facilitating cancer diagnosis and prognosis, as well as the prediction and monitoring of therapeutic response. Because exosomes are readily accessible in nearly all bodily fluids (blood, saliva [24], urine [96], breast milk [97], ascites [98], amniotic fluid [99], bronchoalveolar fluid [100], cerebrospinal fluid [101], seminal fluid [102], synovial fluid [103]), they offer the key advantage of non-invasive testing opportunities [104]. Notably, analysis of circulating exosomes may be a powerful tool to facilitate early cancer detection, since a solid tumor is not required for testing. The mere fact that exosome production is increased in cancer allows for exosome analysis to be useful for cancer detection and assessment of disease progression. Indeed, studies have demonstrated elevated levels of exosomes in the plasma of cancer patients as compared to control groups and even noted a positive correlation between the abundance of tumor exosomes and cancer stage in the case of ovarian cancer [105, 106]. Perhaps more importantly, however, is the fact that exosomes are packed full of biologically active molecules that reflect the pathological state of the host cell, making exosomes a reservoir of potential diagnostic and prognostic markers. Further, exosomes offer an *enriched* source of biomarkers (a result of the concentration of highly selected molecules during exosomal packaging), which would otherwise constitute a mere fraction of the total proteome/transcriptome of bodily fluids, and even of the tumor cells [107].

A number of studies have identified tumor-specific markers in circulating exosomes in a plethora of cancers, including elevated levels of claudin-3 in ascites-derived exosomes of colorectal cancer patients [108], the known prostate cancer mRNA biomarkers PCA-3 and TMPRSS2:ERG in urine exosomes [109], as well as the tumor-specific gene variant *EGFRvIII* in serum exosomes of glioblastoma patients [57]. In addition to proteins and mRNAs, circulating microRNAs (miRNAs) have gained substantial attention with regard to being highly promising biomarker candidates. In a report by Taylor et al. [106] it was demonstrated that malignant ovarian cancers could be reliably distinguished from benign disease based on the levels of eight specific exosomal miRNAs. Other exosomal miRNAs associated with specific cancers include urine-derived exosomal miR-107 and miR-574-3p in prostate cancer [110], plasma-derived exosomal miR-141 and miR-195 in breast cancer [111], and serum-derived miR-21 in glioblastoma [57] as well as esophageal squamous cell carcinoma [112]. All of the aforementioned exosomal miRNAs are present at elevated levels in cancer patients as compared to normal control groups. It should be noted that many studies investigating circulating miRNAs as cancer biomarkers are not necessarily exosomal in origin, although it is believed that the encapsulation of miRNAs into exosomes is a primary mechanism by which miRNAs exist stably in the extracellular environment.

Vaccines

Exosomes are uniquely suited for drug or gene delivery because they are naturally biocompatible, stable while circulating in the blood, and capable of crossing the blood brain barrier [113]. Dendritic cell-derived exosomes (dexosomes) have been successfully engineered to target helper T cells to stimulate cytotoxic T cell proliferation, influence T cell differentiation, and create an anti-tumor environment [114]. Dexosomes have entered clinical trials for colorectal cancer, metastatic melanoma, and non-small cell lung cancer. In a phase I clinical trial, dexosomes isolated from ascites combined with granulocyte–macrophage colony-stimulating factor (GM–CSF), which increases immunity induction, were nontoxic and able to incite an antitumor cytotoxic T cell response; without GM–CSF, dexosomes were insufficient to produce a response [115]. In a separate phase I clinical trial, dexosomes purified from cell culture and pulsed with tumor peptides displayed low toxicity and some tumor regression was observed, supporting continuation to a phase II clinical trial [116]. Likewise, dexosomes generated only grade 1–2 adverse effects and were able to stabilize disease during a phase I clinical trial for non-small cell lung cancer patients [117]. Reviews have been recently published for exosomes as cancer vaccines [113] and dexosome immunotherapy [118].

Conclusion

With all of the important functions of exosomes to tumors, the study of exosomes is of vital importance for understanding cancer mechanisms. Although many studies have been published, we have just begun to understand the biogenesis from MVEs, especially in the context of cancer when exosome production is increased. Exosomes appear to have both an ESCRT-dependent and -independent means of sorting that enriches exosomes secreted from tumors with proteins that affect the three major stromal cell types; tumor exosomes can activate fibroblasts to increase cancer aggression, have altered cargo content under hypoxic conditions to stimulate pro-angiogenic signals in endothelial cells, and promote immune escape through MDSCs, T-cells, and natural killer cells [29, 30]. Because exosomes affect metastasis through many different routes, including EMT proteins, pro-inflammatory cytokines, and HIFs, therapeutic intervention of exosomes signifies a new approach to controlling tumor growth and spread. Furthermore, by disabling tumor-enabled communication, it may be possible to abrogate spread of chemoresistant properties.

The potential of exosomes to contain biomarkers of carcinogenesis, therapeutic response, and disease progression, has led to profiling the cargo of various types of exosomes and careful isolation/purification is necessary to yield unadulterated results [119]. Exosomes would be ideal biomarkers because they can be collected in a non-invasive procedure, and reflect the current state of the tumor. Exosomes derived from immune cells and engineered to stimulate an anti-tumor immune response are naturally advantageous for drug or gene delivery [113]. Since exosomes are involved in so many of the processes that make cancer dangerous, it will be important to consider how they impact cancer biology in developing new therapies.

Given the fact that exosomes from discrete cancers could be quite unique in terms of biological activity, a thorough interrogation of isolated and purified exosomes from molecularly to genomically well-characterized cancers is of paramount importance. Combinatorial data sets of proteomic, transcriptomic, metabolomic and lipidomic data are needed to define cancer exosomes as a function of metastasis and virulence. Mass spectrometry-based metabolomics and lipidomic analyses are beginning to expand our understanding of exosomal biochemistry and biophysics. Even though there are multiple studies defining the lipid content of exosomes (reviewed in [120]) that note elevated levels of disaturated phospholipids, sphingolipids and cholesterol, very few lipidomic studies on defined cancer cell populations [121] or immunological cells from the tumor microenvironment [122] are available. Beyond defining new biomarker or signaling elements with MS-based lipidomic and metabolomic studies that utilize well-characterized patient cells, these types of studies will begin to redefine the biophysical properties that underlie exosomal generation, release, and fusion in the tumor microenvironment. Critical to these types of studies are the validation of new bioengineering techniques to improve the yield of isolating circulating cancer-derived exosomes from exosomes released from non-transformed cells. New microfluidic platforms that utilize immunoaffinity-, magnetic- or electrical field-based separations offer the potential to isolate these discrete exosome populations [123–125].

Box 1: historical perspective on exosome formation

In the 1970s, plasma membrane fragments were being isolated from human bodily fluids as well as cultured cells [27, 126, 127]. However, it was not until 1983 that two teams of researchers from the laboratories of Stahl and Johnstone independently discovered what we now term exosomes [128, 129]. The actual title of "exosome" was applied by the Johnstone group to these vesicles in 1987 [130]. Although "exosome" had previously been used by

Trams et al. to describe small enzyme-containing vesicles that they observed being released from both normal and cancerous cells in culture that they postulated to have a role in communication, these vesicles do not fit the current definition of exosome [25, 131]. It was later discovered that the intracellular origin of exosomes is multivesicular endosomes (MVEs) that fuse with the plasma membrane and release their contents into the extracellular space; the connection between exosomes and MVEs lead scientists to believe that exosomes were an additional means of waste removal from cells [132, 133]. Indeed, immunological cell waste, including Major Histocompatability Complex (MHC), was found to be discarded via exosomes, but Raposo et al. [134] re-popularized the idea that exosomes were more than garbage receptacles and further postulated that exosomes could function in antigen presentation and stimulate a T cell response. Presently, exosomes are even categorized as a novel mechanism of cell-to-cell communication, particularly between tumor and stromal cells [135, 136]. For a more in-depth look at the history and nomenclature of exosomes, see [131, 137].

Box 2: use of "exosome" in scientific literature

It is important to recognize many different systems of classification of small, secreted vesicles have been used and, thus, terminology cannot necessarily be relied upon. Typically, it is agreed that exosomes are about 50–100 nm; sediment at 100,000–160,000$\times g$ or float on a sucrose gradient of 1.13–1.19 g/mL; look like a flattened sphere (transmission electron microscope, TEM), round trilobed structure with a central depression (2nN amplitude modulated—atomic force microscopy, AM–AFM), or round bulging sphere (field emission scanning electron microscope, FESEM). To ensure that research articles are indeed referring to exosomes as now defined, a few precautions should be taken:

- careful search of the parameters used by the authors to classify the described vesicles
- analysis of purification methods–since some are more robust than others, many false positive identifications of exosome proteins have arisen from analysis of contaminated samples [119]
- many papers describe exosomes but incorrectly refer to them as some other type of vesicle, so it may be easy to overlook valuable exosome research
- while some noted the presence of DNA as a difference between apoptotic vesicles and exosomes, others later concluded that exosomes also contain DNA [22, 138]
- sometimes LAMP1 or LAMP2 is used to differentiate exosomes from other secretory vesicles; however, because of their lysosomal escape, some exosomes lack these lysosomal proteins [139–141].

For a more detailed analysis, refer to [137], which reviews the inconsistencies in nomenclature for exosomes.

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