Review

Roles of Cancer Exosomes in Immunosuppression and Immune Evasion

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Abstract: Extracellular vesicles (EV), including exosomes and microvesicles, are released from various cells and alter recipient cell phenotypes and fates by their biomolecules. Here we review current knowledge about tumor EVs and how they prompt malignant cell communication with tumor-associated cells, such as cancer-associated fibroblasts, tumor endothelial cells, and immune cells. We delineate the major pathways and molecular players that influence each step of cancer initiation, progression, and resistance. Of note, cancer exosomes involve immunosuppression by tumor-associated macrophages, myeloid-derived suppressor cells, and regulatory T cells. Moreover, tumor exosomes can induce the apoptosis of killer T cells and immune checkpoint of dendritic cells and attenuate natural killer cells. An in-depth understanding of EV biology is essential to ensure the clinical development of exosome/EV-based therapeutic products, which will be of benefit to exosome manipulation in cancer management.

Keywords: exosomes; extracellular vesicles; cellular communication; tumor microenvironment; tumor infiltrating lymphocyte; immunosuppression; immune evasion; therapy resistance

1. Introduction

Communication of cancer cells with neighboring and distant cells is crucial for tumor growth and progression. Extracellular vesicles (EVs) are lipid membrane-surrounded vesicles released from cells under physiological and pathological conditions. EVs contain a variety of molecular cargos such as proteins, long and small RNA, DNA, lipid, glycan, minerals, and metabolites [1–9]. Earlier studies classified EVs into exosomes (40–150 nm), microvesicles (100–500 nm), and apoptotic bodies (1–10 μm) based on their mechanisms of biosynthetic pathways and release, while additional types of EVs have been named based on the history of discovery or conceptually, such as oncosomes [10–12], large oncosomes (1–10 μm) [10], stressome, including damaged membrane vesicles [13], matrix vesicles [14], migrasomes [15], exopheres [16] (generated upon neurotoxic stress, ~4 μm), and exomeres (~35 nm) [17,18]. Recent studies have defined the EVs according to the size of vesicles, such as small EVs (sEV), medium EVs (mEV), and large EVs (L-EV). Further, exosomes have been recently classified based on their size: small exosomes (Exo-S) and large exosomes (Exo-L). EVs play trash bag-like roles as cells discard redundant disadvantageous factors, while EVs also mediate trans-cellular communications as their cargos stimulate and reprogram recipient cells through cell signalings or molecular transfer [6,19–22]. Thus, EVs and their cargos are essential for autocrine, paracrine, juxtacrine, and endocrine signals. EVs, including exosomes, are contained in bodily fluids that are sources of biomarkers, such as blood, saliva, cerebrospinal fluid, lymph fluid, sweat, tears, urine,

milk, and seminal fluid. Therefore, EVs can play key roles in cell-to-cell communication in local tissue and between distant organs, individuals, and species [23,24].

2. Exosomes biogenesis and composition

Endocytosis is a dynamic process by which cells internalize macromolecules and surface proteins. Exosomes are endosomal origin vesicles, which take part in paracrine interactions between the cells [25], initially formed as internal luminal vesicles (ILVs) in multivesicular bodies (MVBs) by ESCRT-dependent or ESCRT-independent mechanisms. First, the proteins are transported from the trans-Golgi network (TGN) (e.g., MHC class-II molecules) or internalized from the cellar surface (e.g., activated growth factor receptors). Second, these proteins are ubiquitylated at their cytosolic domains; however, not all proteins require ubiquitinylation to be targeted into the vesicles. After vesicle accumulation, the MVBs have several fates; (i) be directed to the lysosome for degradation (e.g., EGF), (ii) be recycled to the TGN, (iii) or be fused with the plasma membrane resulting in the release of the ILVs known as exosomes [26].

Exosome membranes are enriched in lipids, such as cholesterol, sphingomyelin, and ceramide. The EV contents vary greatly depending on the originating cell. Classical exosome markers, such as tetraspanins (TSPANs; CD9, CD63, CD81, and CD82), heat shock proteins (HSPs), Rabs, Alix, and Annexins, are often lost from exosomes in some pathophysiological conditions but found in other EV types, such as large EVs [3,9,27–30]. DNA and RNAs, including mRNAs, microRNAs, and long noncoding RNAs (lncRNA), are also present in the exosome and other EV types and are crucial players in EV biology [3,8,9,31–36]. Exosomes are internalized by other cells through direct membrane fusion, endocytosis, or cell-type-specific phagocytosis. Exosomes released by tumor cells frequently include oncoproteins linked to different cancer types. The list of proteins found in exosomes is continuously expanding on ExoCarta, a database of exosomal proteins, RNA, and lipids [37] (Table 1).

EV production often correlates with tumor cell transformation, such as epithelial-to-mesenchymal transition (EMT) [13,38–41] and cancer stemness [42,43]. Moreover, recent studies suggest that the EMT progression is correlated with higher PD-L1 expression, immunosuppression, and immune evasion by M2 macrophages, myeloid-derived suppressor cells (MDSC), and regulatory T cells (Treg), whereas the epithelial tumors with lower PD-L1 expression, less Treg and MDSC are susceptible to immune attack by M1 macrophages and killer T cells [44].

Table 1. Top 10 proteins identified in exosomes as indicated in the ExoCarta database.

Gene name	Protein name	Number of times identified
CD9	CD9, tetraspanin 29	98
HSPA8	HSC70, Heat shock cognate 71 kDa protein	97
PDCD6IP	Programmed cell death 6-interacting protein	96
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase	95
ACTB	Actin Beta	93
ANXA2	Annexin A2	83
CD63	LAMP-3, CD63, tetraspanin 30	82
SDCBP	Syndecan Binding Protein	78
ENO1	Enolase 1	78
HSP90AA1	HSP90α, Heat Shock Protein 90 Alpha Family Class A Member 1	77

3. Cancer exosomes transfer oncogenic factors to tumor-associated cells

EVs could cause cellular reprogramming and genetic alterations by transferring their cargo contents, such as oncoproteins, lipids, mRNAs, and noncoding RNAs, defined as "oncosomes." Increasing evidence elucidated that tumor cells release exosomes to repro-

gram normal and stromal cells in the tumor microenvironment to provoke tumor initiation, progression, metastasis, and drug resistance. For great examples, mutant KRAS [45] and mutant EGFR [24,45] were found in exosomes and transferred to recipient cells leading to cancer progression [12,46]. Tumor oncosomal MMP3 is transferred to recipient cells and alters transcriptional programs, such as cellular communication network factor 2 (CCN2) expression, in the tumor microenvironment [47,48].

4. Tumor exosomes develop the resistant microenvironment

Individual residence cells in the tumor microenvironnement (TME), each with different biological contributions, interact dynamically to create a unique microenvironment for neoplastic cells. One method of communication between the tumor-associated cells (TACs) delivers exosomes to each other in the TME, which induces phenotypic modifications and remodeling of TACs, causing cancer propagation. Several studies have proved the involvement of cancer exosomes in the modulation of [39]. Along with mediating cell-to-cell communication, tumor exosomes develop cancer therapy resistance [49] (Fig. 1).

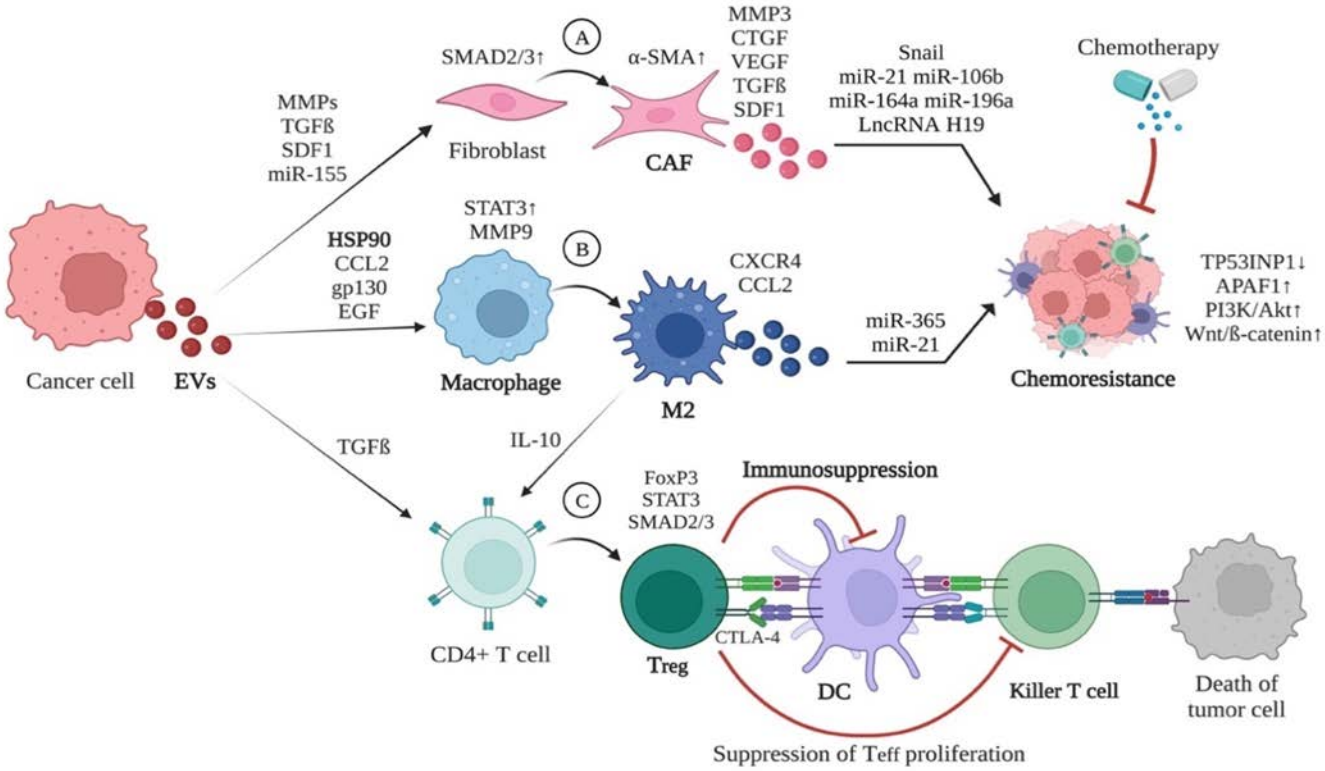

Figure 1. Cancer cells secrete various exosomes/EVs with different cargo, which plays pivotal roles in shaping the tumor-associated cells. Cancer exosomes induce normal fibroblast cells into CAF (A) and polarization of macrophages into M2-type macrophages with an immunosuppressive phenotype (B), ultimately favoring tumor progression and chemoresistance. Cancer exosomes and M2 macrophage-derived exosomes (C) alter the T cell phenotype to immunosupressive Treg cells, which suppress the maturation of DCs and killer T cells.

4.1. Exosomes mediate Tumor-CAF communication to develop chemoresistance

Fibroblasts are major components of tumor stroma, while recent studies evoked the existence of cancer-associated fibroblasts (CAFs). CAFs include myofibroblasts and are differentiated from mesenchymal stem cells (MSCs). Myofibroblasts are a major component of the tumor stroma and mediate angiogenesis, which can be modulated by the cancer exosomes [50,51]. It is worth noting that tumor stroma rich in myofibroblastic cells can maintain tumor growth, vascularization, and metastasis. Webber et al. demonstrated that exosomal TGF-β promotes the differentiation of fibroblasts into myofibroblasts through

the SMAD signaling pathway [26,52]. Cho et al. suggested that breast cancer-derived exosomes stimulate the differentiation of MSCs in adipose tissue into a myofibroblast-like phenotype with a significant increase in α-SMA and other protumorigenic factors, such as VEGF, SDF-1, TGF-β, and CCL5 [53]. Chowdhury et al. proposed that prostate cancer-derived exosomes provoke the MSC differentiation into myofibroblastic cells with an increase in VEGF-A, resulting in proangiogenic functions [54]. Moreover, EGFR-positive tumor-derived exosomes promote angiogenesis by reprograming the tumor endothelial cells (TECs) into VEGF-secretion phenotype [46]. These studies indicated that tumor exosomes play key roles in augmenting CAFs, myofibroblasts, MSCs, and TECs. CAF-derived exosomal microRNA (miRNA, miR) signature supports the communication between tumor cells and other stromal residents in the TME, which promotes cancer progression and therapeutic resistance [55–57]. In esophageal cancer, cisplatin resistance was correlated to exosomal miR-27a/b and its target TGF-β [58]. miR-522 overexpression in CAFs was correlated with cisplatin/paclitaxel resistance of gastric tumor through activation of ubiquitin-specific protease 7 (USP7) / hnRNPA1 axis, inhibiting arachidonate lipoxygenase 15 (ALOX15) and ultimately decreased chemosensitivity [59]. Exosome-enriched miR-196a is transferred from CAF to adjacent tumor cells inducing platinum resistance [60]. Additionally, miR-164a and SNAI1 are delivered directly from CAF to pancreatic cancer cells via exosomes, leading to gemcitabine (GEM) resistance of tumor cells. The resistance can be reversed by treatment with GW4869, an inhibitor of exosome release [61,62]. miR-106b from CAFs was also transferred to cancer cells, which conferred GEM by targeting TP53INP1 (Tumor protein p53 inducible nuclear protein 1) [63]. Besides, miR-21 was reported in GEM-induced chemoresistance [64]. Further, miR-21-rich exosomes released from cancer-associated adipocytes significantly reduced tumor cells' sensitivity to paclitaxel by targeting apoptotic protease activating factor-1 (APAF1) in the ovarian neoplasm microenvironment [65]. Besides, exosome-enriched lncRNA H19 was transferred from CAFs to adjacent colorectal cells, activating the Wnt/ß-catenin signaling pathway and inducing chemoresistance [66,67] (Fig. 1).

4.2. Cancer stem cell-derived exosomes arrange the tumor microenvironment toward tumor progression and immunosuppression

Stem cells were first found in hematopoietic cells and thus designated hematopoietic stem cells (HSCs) [68]. Unequivocal proof of HSCs has given way to the prospective isolation of tissue-specific stem and progenitor cells [69]. Tumors may often originate from the transformation of normal stem cells, and cancer cells may include sub-populations with stem cell phenotypes called cancer stem cells (CSCs), cancer-initiating cells (CICs), or tumor-initiating cells (TICs). Paradoxically, teratoma formation in experimental animals is one of the features of induced pluripotent stem (iPS) cells [70]. Indeed, an increase in the expression of pluripotent stem cell (PSC) markers has been found in CSCs [42,71–73]. Currently defined characteristics of CSCs are cellular aggregation, spheroid formation, tumor initiation, slow cell cycle, entrance into dormancy, chemoresistance, SC marker expression, and pluripotency [42,43,74–76]. Dormant cancer cells within subclones can survive chemotherapy while proliferating subclones are relatively more chemosensitive [73]. Thus, tumors can relapse due to cells surviving after treatment and re-established subclonal diversity [74]. The parental tumors are a source of molecular cargos exported in exosomes and carry various CSC-specific proteins. For instance, in many malignancies, the Wnt/ß-catenin pathway is a key regulator of the CSC phenotype [77,78]. Several studies postulated the possibility of Wnt activation in surrounding tumor cells via absorption of β-catenin-rich exosomes [79,80]. Sheta et al. have postulated the significant role of FGF2 / FGFR signaling in favoring the transformation of normal stem cells into CSCs [81]. Besides, FGF2 in exosomes was proven to regulate stromal function [82,83], suggesting that the exosome released from the surrounding TME may lead to the development of CSCs. Similarly, various CSC-specific molecules are secreted with exosomes [84,85], which include (i) surface receptors (CD133, CD44, CD326/EpCAM), (ii) functional

enzymes (ALDH, MMPs), and (iii) pluripotency/stem cells factors (Oct4), which facilitate communication between cancer cells and the TME [38,48,76,86,87]. The dependency of such stromal cells highlights the involvement of miR-155-rich exosomes in reprogramming normal adjacent fibroblasts into CAFs [88]. Uptake of miR-155 by fibroblasts may account for the dramatic repression of Tumor Protein P53 Inducible Nuclear Protein 1 (TP53INP1) in pancreatic stromal cells. Further, gastric cancer-derived exosomes usually carry TGF-β[89] that activates the Smad pathway conducive to generating functional CAFs [89]. Further, CSCs-derived exosomes can induce immunosuppression. $EGFR^+$ and $HER2^+$ exosomes are often released from CSCs [90,91]. These receptors can stimulate the monocyte MAPK signaling pathway, which promotes the development of TAMs [92,93]. CSCs-derived exosomes have been found as carriers of miRNAs associated with ECM remodeling. miRNA-105, found in exosomes from breast cancer stem cells, directly alters endothelial tight junctions and raises the permeability of tumor blood arteries by targeting endothelial tight junction protein ZO-1 [94]. Besides, tumor endothelial cells (TECs) movement and the creation of early vascular lumens are induced by the interaction between the miR-92a found in exosomes produced by K562 tumor cells and the proangiogenic protein integrin-α5 [95]. From here, CSC-exosomes are engaged in regulating the tumor microenvironment (Fig. 2).

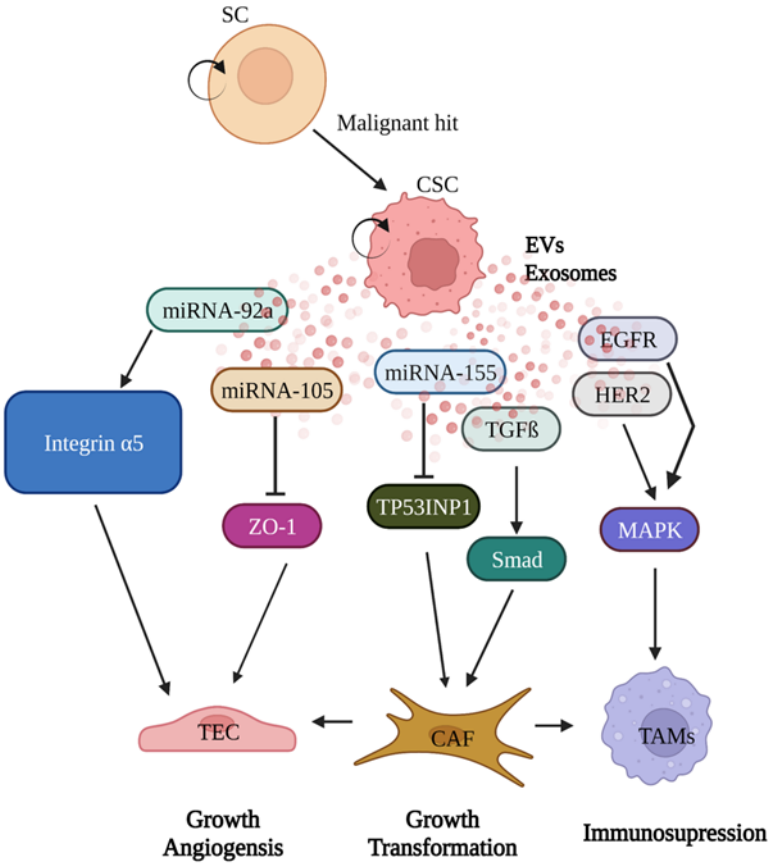

Figure 2. Potential roles of CSCs-derived exosomes/EVs in tumors. HER2 and EGFR are abundant in CSC-exosomes and activate the MAPK signaling pathway in monocytes, which in turn induce the development of TAMs and promote immunosuppression. Exosomes rich in TGFβ and miR-155 promote the production of CAFs, which aid in the development of the TME. Exosomes containing miR-105 reduce ZO-1 expression in TECs, increasing tumor blood vascular permeability in the TME. Additionally, exosomes that carry miR-92a interact with the proangiogenic protein integrin-α5 to promote the migration of TECs and the early formation of vascular lumens, thereby encouraging angiogenesis.

4.3. Tumor exosomes induce endothelial cells angiogenesis, extravasation, and intravasation

Tumor endothelial cells (TECs) line tumor-associated blood vessels and assuring the passage of nutrients into tumor tissues [96]. TECs are abnormal in morphology, function, and gene expression [97]. TECs support tumor cells disseminating to the distal sites via extravasation and preserve them from anoikis, thereby promoting tumor metastasis [98]. TECs can also release angiocrine factors, such as VEGF, to support tumor progression [99,100]. Abnormal characteristics of TECs are caused by the tumor microenvironment, such as hypoxia that promotes the production of VEGF and increases vascular permeability and genetic instability in TECs [101]. While TECs adhere to the endothelia of venules, they will enter circulation, exit the bloodstream, position themselves upon distance endothelium surfaces, and subsequent metastatic growth [102,103]. Phosphatidylserine, the inner bilayer of the intact cellular membrane, and P-selectin glycoprotein ligand-1 (PSGL1) are considered to work together, promoting the exosome to adhere to the endothelium [103]. The phenotypic alterations of TECs were led by EVs that contain growth factors and receptors, such as VEGF and its receptor VEGFR1 [104,105], SDF1 / CXCL12 [106,107], FGF-4 [108], EGF [109], adrenomedullin [110], and TSP-1 [111]. CXCR4, a receptor for SDF1, is overexpressed in TECs, while a CXCR4 antagonist (plerixafor, also known as AMD3100) induced tumor angiogenic inhibition-triggered necrosis (TAITN) in head and neck squamous cell carcinoma (HNSC) [106]. TAITN reduced TECs that supplies oxygen to tumor cells, whereby the loss of TECs induced hypoxia [106]. Thus, chemokine signaling plays a key role in tumor angiogenesis, a novel therapeutic target. Tumor-derived exosomes are related to tumor growth and metastasis of HNSC and induce angiogenesis by reprogramming TECs [107]. Exosomal WNT4 from colorectal cancer stimulated β-catenin nuclear translocation in endothelial cells, which improved tumor growth and angiogenesis [112,113]. On the other hand, human liver stem cells (HLSC) derived EVs inhibited tumor angiogenesis since the HLSC-EVs possessed specific microRNAs, which targeted and downregulated proangiogenic genes. LncRNAs contained in exosomes can promote tumor angiogenesis. Exosomes released by $CD90^+$ liver cancer cells promoted angiogenesis and adhesion of endothelial cells by providing lncRNA H19 [114]. LncRNA H19 also promoted angiogenesis in glioblastoma [115]. Exosomes derived from lung cancer cells contained the lncRNA growth arrest-specific 5 (lncRNA GAS5), up-regulating PTEN expression and inhibiting the PI3K/AKT phosphorylation, thereby increasing angiogenesis [116]. These studies indicate that tumor exosomes stimulate TECs to promote angiogenesis and metastasis.

4.4. Tumor-macrophage communication via exosomes for acquiring immunosuppression and chemoresistance

Macrophages are generally divided into the pro-inflammatory M1-type and immunosuppressive M2-type. M1-polarized macrophages possess antitumor activity, whereas M2-polarized macrophages promote tumor growth [117]. Tumor-associated macrophages (TAMs) are often M2-like phenotypes and are considered key participants in cancer progression via the production of numerous growth factors, cytokines, and extracellular matrix (ECM) remodeling molecules for stimulating cancer growth, migration, and angiogenesis [118]. Indeed, tongue cancer EVs stimulate macrophage polarity into M2-type, while HSP90 partially mediates the TAM polarization in HNSC [38]. Breast cancer-derived exosomal glycoprotein 130 (gp130) activates the IL-6 / STAT3 pathway in macrophages [119], consequently increasing macrophage survival and inducing the expression of several genes associated with tumorigeneses, such as IL-10, CXCR4, and CCL2 [119,120]. Each cytokine has a specific role in regulating tumor immune surveillance. IL-10 induces immunosuppressive effects by modulating dendritic cells and cytotoxic T cells [121], while CXCR4 is associated with proangiogenic and immunosuppressive phenotypes [120]. IL-6 and CCL2 (also called MCP-1: monocyte chemoattractant protein 1) are associated with TAM polarization [122]. These immunosuppressive effects are inhibited by adding a GP130 inhibitor to the cancer-derived exosomes [117]. Zheng et al. showed

that macrophage-derived exosomal miR-21 enhanced the PI3K/Akt signaling pathway, inhibited apoptosis by downregulating PTEN, and induced resistance to cisplatin in gastric cancer cells [123]. Likewise, Binenbaum et al. demonstrated that miR-365 transferred by M2 macrophage-derived exosomes increased the tri-phospho-nucleotide pool in pancreatic cancer cells and activated cytidine deaminase, which eventually conferred GEM resistance and supported tumor cells proliferation [124] (Fig. 1).

4.5. Cancer exosomes induce immunosuppressive Tregs and apoptosis of killer T cells

It has been suggested that tumor-infiltrating lymphocytes (TILs) include tumor-reactive lymphocytes and tumor antigen-specific lymphocytes. A therapy in which tumor-reactive T cells in TILs are expanded, cultured, and infused is being attempted. At the same time, it is suggested that many cells negatively regulate the antitumor immune response, such as regulatory T cells (Tregs), in TILs. Killer T cells, also called cytotoxic T lymphocytes (CTLs), are a group of $CD3^+$ $CD8^+$ T cells that exhibit cytotoxicity specifically to cells presenting antigen peptides on MHC class I molecules on the target cell surface. Killer T cells recognize antigen peptides and secrete cytotoxic granules that contain perforin and granzymes. Perforin polymerizes on the target cell membrane to form pores, and granzymes, which belong to serine proteases, invade the target cells through the pores and induce apoptosis of the target cells. Activated killer T cells are considered the master regulator of the antitumor immune response. A growing body of studies has reported the significance of $CD4^+$ helper T cells in the generation and maintenance of effective cytotoxic and memory $CD8^+$ T cells, known as $CD4^+$ T-cell help. This phenomenon optimizes the expansion, trafficking, and effector function of $CD8^+$ T cells, thereby potentiating immune-mediated tumor destruction [125–127]. Cancer cell-derived exosomes suppress these T cells, which are more sensitive to the suppressive effects of tumor exosomes than other immune cells. These immunosuppressive effects of cancer exosomes involve the induction of apoptosis, inhibition of proliferation and differentiation, and dysfunctionality of T cells. Yang et al. demonstrated that exosomes from ovalbumin peptide (OVA)-expressing melanoma suppressed OVA-specific immune response [128]. Several studies showed tumor exosomes induce T cell apoptosis through FasL, TNF, and galectin-9, located on the EV surface [129–132]. Furthermore, PTEN of tumor exosomes appeared to regulate the PI3K/AKT pathway, leading to AKT dephosphorylation and increasing the expression of pro-apoptotic BAX and decreasing anti-apoptotic Bcl-2, Bcl-xL, and MCL-1 (myeloid leukemia cell differentiation protein) in activated killer T cells [133–135]. Additionally, administration of GL26 glioblastoma exosomes to mice was associated with a reduction in the number of killer T cells and a decline in the IFN-γ and granzyme expression [136]. Clayton et al. reported that extracellular ectonucleotidases CD39 and CD73 contribute to rising adenosine levels in the tumor microenvironment by dephosphorylating exogenous ATP and 5′AMP to form adenosine and hence attenuating the T cell function [137]. Regulatory T cells (Tregs) are an immunosuppressive subset of $CD4^+$ T cells and negatively impact the immune response. TGF-β1 and IL-10 in exosomes stimulate the differentiation of $CD4^+$ $CD25^-$ T cells into Tregs and foster the Tregs proliferation by increasing the phosphorylated SMAD2/3 and STAT3 [138]. These studies demonstrated that cancer exosomes suppress killer T cells through activating pro-apoptotic signals and promoting differentiation of T cells into Tregs, immunosuppressive T cells (Fig. 2).

4.6. Tumor exosomes potentiate immunosuppressive roles of MDSCs

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells unable to differentiate into dendritic cells (DCs), macrophages, or granulocytes. MDSCs are one of the main drivers of immunosuppression in the tumor microenvironment, as they exhibit a strong suppressive capacity against T cells and NK cells antitumor activity, recruiting immunosuppressive Tregs and creating a microenvironment favorable for immunosuppression and tumor progression. Therefore, an increased

MDSCs frequency and activity were positively correlated with tumor progression and recurrence and negatively correlated with the immunotherapy efficacy and clinical outcomes [139]. Xiang et al. reported that tumor exosomes stimulated MDSC differentiation through TGF-β and prostaglandin E2 (PGE2) in vivo. Tumor exosomes also induce the expression of Cox2, IL-6, VEGF, and arginase-1 in the accumulating MDSCs. Blocking the tumor exosomal PGE2 and TGF-β activities disrupted the stimulatory effect of these exosomes on MDSC and attenuated MDSC-mediated immunosuppression [140]. It was recently shown that a chemokine CCL bound to cancer exosomes determines uptake by CXCR-expressing cells [141], and resident stroma-secreted chemokine CCL2 recruits MDSCs in the tumor microenvironment [142]. These findings suggested that tumor exosomes potentiate the immunosuppressive roles of MDSCs in regulating NK cells and T cells, whereas blocking immunosuppressive cytokines on the tumor exosomes attenuates the unfavorable immunosuppression by MDSCs.

4.7. Tumor exosomes downregulate a killing factor on natural killer cells

Natural Killer (NK) cells have abilities to kill tumor cells and virus-infected cells without prior sensitization. NK group 2 member D (NKG2D) protein is a type-II transmembrane receptor expressed on NK cells and killer T cells. In NK cells, NKG2D mediates the direct killing of target cells, whereas, in $CD8^+$ killer T cells, it acts as a costimulatory receptor leading to activation of the T-cell receptor (TCR) and T-cell effector function [143,144]. Lundholm et al. found that exosomes from human prostate cancer express ligands for NKG2D on their surface, which selectively decreases the expression of the receptor NKG2D on NK and $CD8^+$ killer T cells in a dose-dependent manner, leading to impairing the cytotoxic function of these killer cells and promoting the tumor immune escape [143]. Clayton et al. demonstrated that human prostate cancer cell exosomes (derived from PC-3 and DU-145 cell lines) express NKG2D ligands on their surface that downregulated NKG2D expression in effector lymphocytes [145]. Exosomal TGF-β might be involved in NKG2D downregulation because cell activity and NKG2D expression were restored by using TGF-β neutralizing antibody [146]. These studies indicated that NKG2D on the surface of NK cells is crucial for killing tumor cells, whereas tumor exosomes often express NKG2D-ligand that downregulates NKG2D on NK cells.

4.8. Tumor exosomes involve the immune checkpoint by stimulating dendritic cells to express PD-L1

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) derived from bone marrow and play a central role in initiating the immune response. Upon capturing the antigens with their recognition receptors, DCs undergo maturation and travel to lymph nodes, where DCs present the captured antigens to naïve T cells for their activation and polarization, establishing links between innate and adaptive responses [147–149]. A recent study indicated that exosomes from Lewis lung carcinoma (LLC) cells inhibited the DCs maturation and cytokine production, suppressed the differentiation of bone marrow precursors into $CD11c^+$ DCs, and induced apoptosis in DCs [150]. The LLC-derived exosomes up-regulated PD-L1 expression on DCs; thus, PD-L1 blockade immune checkpoint inhibitors (ICI) such as Nivolumab (marketed as Opdivo) significantly reversed the immunosuppressive effect of LLC exosomes on DCs. Moreover, Yang et al. showed that treating the DCs with tumor exosomes induced TGF-β production in DCs [128]. Thus, tumor exosomes involve the immune checkpoint by stimulating dendritic cells to express PD-L1.

5. Exosomal oncoproteins and oncolipid that enhance tumor progression and metastasis

The tumor microenvironment comprises a diverse range of cells, such as endothelial cells, fibroblasts, and immune cells. Direct interaction between tumor cells and their environment is necessary for cancer progression by enhancing angiogenesis, metastasis, and

suppressing tumor immunity [102]. Growing evidence indicates that cancer cell-derived exosomes transfer oncogenic proteins and nucleic acids that modulate the activity of recipient cells and promote tumor initiation, invasion, and metastasis. Matrix / moonlighting metalloproteinases (MMPs), especially MMP3 and MMP9, are protumorigenic cargos of EVs in cancer [151]. Notably, MMP3 in colon cancer EVs plays key roles in tumorigenesis and metastasis [47,48,76]. MMP3 in exosomes are transferred into recipient cell nuclei and trans-activate protumorigenic genes, such as cellular communication network factor 2 (CCN2) and HSPs [48,152–154]. A part of small EV subpopulations, exomeres, contains beta-galactoside a2, 6-sialyltransferase 1 (ST6Gal-I), and amphiregulin (AREG) [155]. ST6Gal-I is transported from exomeres to recipient cells, triggering metastasis [156,157]. Besides, exosomes enriched in HSPs can play cytoprotective and anti-apoptotic roles in tumors and tumor-associated cells [13,151,158]. Metastatic tongue cancer exosomes abundantly contained members of the HSP family, such as TRAP1, HSP90α, HSP90β, HSP105, and HSP70s [27]. Besides, HSP90α is released in EV-free forms upon hypoxia and can promote tumorigenesis [87]. Cell division control 37 (CDC37) is an intracellular cochaperone of HSP90 and plays protumorigenic roles in cancer [27,128,159]. The triple targeting of CDC37, HSP90α, and HSP90β inhibited protumorigenic exosomes in tongue and prostate cancer [38]. Nevertheless, extracellular HSPs can play immunogenic and immunosuppressive roles depending on the immune cells and their receptors that detect HSPs [160,161]. Redundant lipids are released from cells through the release of exosomes and cholesterol efflux pump proteins. One of such pumps overexpressed in metastatic cancer cells was adenosine triphosphate (ATP)-binding cassette G1 (ABCG1), which co-overexpressed with ABCG2, a drug efflux pump found in CSCs [43]. The targeted silencing of ABCG1 led to exosome lipid accumulation and triggered tumor cell death. These facts suggest that cancer cells can often release redundant toxic lipids, whereas loss of the ABCG1 pump could trigger the accumulation of redundant toxic lipids leading to tumor cell death. Macrophages play key roles in cholesterol transport from peripheral blood vessels to the liver. Therefore, TAMs may play key roles in metabolizing redundant and toxic lipids released by tumor cells.

6. Prognostic biomarkers in exosomes

Given the presence of special contents in exosomes reflecting the unique qualities and condition of the cells or tissues from whence they originated, there is a great interest in identifying exosome contents using transcriptomics and proteomics techniques [162–165]. Consequently, exosomes can be evaluated as potential sources of cancer diagnostic markers. For instance, Ahadi and co-workers profiled the lncRNAs content of exosomes derived from five different prostate cancer cell lines and identified a list of statistically significant expressed lncRNAs enriched within prostate cancer exosomes [166,167]. By comparing the proteomic contents of metastatic versus non-metastatic breast cancer, Vardaki et al. identified periostin as a candidate marker of localized disease or lymph node metastasis [167]. Metastatic tongue cancer cells-derived EVs abundantly contained members of HSPs such as TRAP1, HSP90α, HSP90β, HSP105, and HSP70s compared to less metastatic parental cells [27]. Further, Proteoglycan glypican-1 (GP1)-localized to exosome membranes was a possible marker for patients with pancreatic disease [168]. Also, miRNAs enriched in exosomes serve as tumor markers. For a great instance, in patients who experienced ovarian cancer, eight microRNA types (miR-21, miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-205, and miR-214)-positive exosomes were significantly distinct from profiles observed in healthy control patients, suggesting an effective way to screening asymptomatic ovarian cancer patients [169]. The effectiveness of exosomes as biomarkers depends on the enrichment of the markers within the exosome that would otherwise make up a very small amount of the secretome [170]. Salivary exosomes enriched with miR-1246 and miR-464 were investigated as candidate biomarkers for pancreaticobiliary tract cancer [171]. Along the same line, circulating exosomes from glioblastoma patients showed unique signatures of EGFRvIII mRNA, which can serve the role of

a "liquid-biopsy (such as blood collection)" rather than a "surgical tissue-biopsy" for glioblastoma detection [2]. This evidence implies that exosomes produced from bodily fluids could be a very informative and least noninvasive cancer detection tool. However, there are still challenges with using exosomes as biomarkers. For instance, (a) The heterogeneity / diversity of exosome population in biofluids is the first issue [172]. Different protein / RNA expression patterns and profiles may result from heterogeneous exosomes, leading to false negatives or positive errors in prognosis and diagnosis. (b) The second problem is the lack of a universal, verified biomarker for all malignancies, leading to co-isolation and impurity of harvested exosomes. (c) The third challenge is the difficulty of isolating and purifying exosomes [172,173]. Therefore, there was a huge disparity across clinical studies for identifying tumor-derived exosome biomarkers, and more research is required to evaluate the viability of using exosomes to diagnose cancer.

7. Therapeutic application of exosomes as delivery systems

As demonstrated by an expanding body of investigations, exosomes possess special characteristics, which make them ideal for delivering anticancer agents over conventional drug delivery vectors like liposomes, e.g., in vivo circulatory stability, high efficiency [174,175], and actively cross biological barriers [176]. Therefore, researchers have tried two strategies of loading exosomes with therapeutic compounds [177]: a direct loading of selective agents on the lumen or surface of exosomes and another indirect loading technique that uses co-culture with therapeutic agents to load agents into exosomes via the endosomal pathway or plasma membrane shedding, or genetic manipulation of the cells to express active molecules on their exosomes. Exosomal protein composition and lipid content might affect their propensity to target particular organs [178]. For instance, different types of integrins can modify the pharmacokinetics of exosomes and enhance their organ-tropic accumulation in the brain, lungs, or liver [179]. In addition, it was shown that pancreatic cells preferentially ingested exosomes harboring tetraspanin-8 in association with integrin-α4 [180]. EV lipids can also influence how well they are absorbed, e.g., phosphatidylserine has been linked to the absorption of EVs by macrophages [181]. In clinical trials for the tissue-specific delivery of biotherapeutics, exosomes are a potential delivery vector. Despite advantages, it has been shown that some obstacles are related to the drug delivery efficiency of exosomes [182–184], such as the lack of standards for isolation and purification and difficulty in preservation. Additionally, producer cell engineering methods for cargo loading might further customize exosomes for targeted distribution, which presents some challenges in terms of selecting dependable and secure source cells with a high level of exosomes production potential as well as selecting an efficient and cautious administration method for delivering exosomes into the site of tumor cells.

8. Conclusion

We presented evidence for EVs / exosomes involving cancer progression, metastasis, and therapy resistance. Of note, tumor exosomes involve immunosuppression and immune evasion by acting on M2 macrophages, MDSC, and Tregs. Moreover, cancer exosomes induce the apoptosis of killer T cells and immune checkpoint of dendritic cells and attenuate NK cells. EVs from tumors include a diverse range of biomolecules that influence local and distant tissue function, establishing cancer pathology via exosome cargo. Besides, due to their nanoscale size and non-proliferative nature, EVs are safe and practical for the development of new therapies. While more research is needed to develop logistical clinical diagnostics and therapeutics, exosomes appear to be important mediators, carrying promising biomarkers, and potential medicinal agents in cancer management.

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Abbreviations:

αSMA	α-smooth muscle actin
CCL	C-C motif chemokine ligand
CTL	Cytotoxic T lymphocytes
CXCR	Chemokine receptor
DC	Dendritic cell
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
ESCRT	Endosomal sorting complexes required for transport
EV	Extracellular vesicle
HLSC	Human liver stem cells
HNSC	Head and neck squamous cell carcinoma
IFN	Interferon
IL	Interleukin;
ILV	Internal luminal vesicle
lncRNA	Long noncoding RNA
MCL-1	Myeloid cell leukemia-1
MHC class-II	Molecules major histocompatibility complex class-II
MMP	Matrix / moonlighting metalloproteinase
MV	Microvesicle
MVB	Multivesicular body
OVA	Ovalbumin peptide
PI3K	Phosphatidylinositol 3-kinase
PKB/Akt	Protein kinase B
PTEN	Phosphatase and tensin homolog
SDF-1	Stromal cell-derived factor 1
STAT	Signal transducer and activator of transcription
TAC	Tumor-associated cell
TAITN	Tumor angiogenic inhibition triggered necrosis
TAM	Tumor-associated macrophage
TEC	Tumor endothelial cell
TIL	Tumor infiltrating lymphocytes
TGF-β	Transforming growth factor-beta
TME	Tumor microenvironment
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor

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