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Exosomes and their role in tumorigenesis and anticancer drug resistance

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ABSTRACT

Exosomes are a class of extracellular vesicles ranging in size from 40 to 100 nm, which are secreted by both cancer cells and multiple stromal cells in the tumor microenvironment. Following their secretion, exosomes partake in endocrine, paracrine and autocrine signaling. Internalization of exosomes by tumor cells influences several cellular pathways which alter cancer cell physiology. Tumor-derived exosomes secreted by cancer or stromal cells can also confer anticancer drug-resistant traits upon cancer cells. These exosomes promote chemoresistance by transferring their cargo which includes nucleic acids, proteins, and metabolites to cancer cells or act as a decoy for immunotherapeutic targets. Depletion of exosomes can reverse some of the detrimental effects on tumor metabolism and restore drug sensitivity to chemotherapeutic treatment. Herein we discuss various approaches that have been developed to deplete exosomes for therapeutic purposes. The natural composition, low immunogenicity and cytotoxicity of exosomes, along with their ability to specifically target tumor cells, render them an appealing platform for drug delivery. The ability of exosomes to mediate autocrine and paracrine signaling in target cells, along with their natural structure and low immunogenicity render them an attractive vehicle for the delivery of anticancer drugs to tumors.

1. Introduction

Exosomes are a heterogeneous class of extracellular vesicles ranging in size from 40 to 100 nm, which are essentially released from all cell types. Exosomes are secreted to the extracellular environment when late endosomal or endosomal multivesicular body (MVB) membranes fuse with the plasma membrane. Involved in numerous biological processes and pathological conditions, exosomes serve as a form of intercellular communication, by delivering their cargo of proteins, lipids, nucleic acids and metabolites to cells in their immediate surroundings as well as at distant organs. All cells in the tumor microenvironment (TME) are known to secrete exosomes, including: cancer cells, fibroblasts and immunocytes. The cargo of these exosomes promotes angiogenesis, modulates stromal reaction and regulates immune response. In addition, exosomes modify signaling pathways of cancer cells, thus promoting tumor progression and drug resistance. In the current review, we describe the mechanisms by which exosomes modulate tumor physiology and drug resistance and discuss strategies to target their activity on tumors.

2. Exosome biogenesis

The term exosome was first used to describe a group of membrane-bound, 30–100 nm vesicles, secreted from reticulocytes during their maturation (Wolf, 1967). Exosomes are one of two broad categories of extracellular vesicles; the other category is microvesicles. Microvesicles (also referred to as ectosomes) are defined as extracellular vesicles formed directly from the plasma membrane by outward budding (Stein and Luzio, 1991). Exosomes are formed as intraluminal vesicles (ILV) in MVBs, by the inward invagination of the plasma membrane (Harding et al., 1983; Johnstone et al., 1987). MVBs, which are components of the endosomal system, can either fuse with lysosomes, leading to the degradation of their cargo, or alternatively, fuse with the plasma membrane (Mobius et al., 2002; Buschow et al., 2009). Fusion with the plasma membrane leads to the release of ILV as exosomes to the extracellular space (Kowal et al., 2014).

The first step in exosome biogenesis is the recruitment of cargo molecules to microdomains that are comprised of specialized lipids and proteins in the MVB membrane (Berson et al., 2001). Cytosolic components are subsequently recruited to these microdomains (Geminard et al., 2004). The endosomal sorting complex required for transport (ESCRT) are protein complexes involved in the recruitment and loading

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of cargo proteins to exosomes and in the later steps of membrane budding. In the canonical pathway, ESCRT 0 and ESCRT I protein complexes participate in the cargo recruitment steps, by recognizing ubiquitin on cargo proteins (Henne et al., 2011). ESCRT II and III protein complexes participate in the later steps of exosome formation, budding and fusion. The proteins Alix Syntenin and Vsp-4 cooperate with the ESCRT proteins in the process of exosome biogenesis. Exosomes can also be formed in an ESCRT-independent manner, as demonstrated by the formation and secretion of exosomes even after depletion of components of the ESCRT complex (Colombo et al., 2013). In oligodendroglial cell lines, for instance, exosome biogenesis is dependent on ceramide formation by sphingomyelinase (Trajkovic et al., 2008). The ceramide-rich domains form spontaneous curvatures that lead to invaginations. Tetraspanins were also implicated in the ESCRT-independent formation of exosomes. Tetraspanins form specialized structural domains in membranes similar to caveolae, by homotypic and heterotypic protein-protein interactions and by associations with lipids including cholesterols and gangliosides (Andreu and Yanez-Mo, 2014). Tetraspanins, specifically CD9, CD81 and CD82, are also involved in the sorting of cargo to exosomes. After interaction with a cognate antigen-specific T cell, MHCII molecules in dendritic cells were incorporated into CD9 containing ILVs that were secreted as exosomes. Unlike lysosomal targeting, this process was independent of MHC II ubiquitination (Buschow et al., 2009; Chairoungdua et al., 2010).

After their secretion, exosomes can act on their cells of origin in an autocrine manner, on cells in their vicinity in a paracrine manner as well as on cells in distant organs. To exert their effect, exosomes may bind to the surface of their target cells and signal through cell surface receptors. Alternatively, they may enter target cells *via* endocytosis or by direct fusion with the plasma membrane of the target cells, and thereby release their cargo. In the sections below, we discuss the effects of exosomes secreted by cancer cells and stromal components, on tumor progression and on the development of resistance to therapy.

3. The role of exosomes in the tumor microenvironment

The TME consists of tumor and stromal compartments. The stroma is heterogeneous in nature, and comprises of the extracellular matrix (ECM) and various cell types. The stromal cellular compartment includes infiltrated immune cells, fibroblasts and vascular cells, all contributing functional and structural support to the tumor (Schiavoni et al., 2013). Constant crosstalk occurs between stromal and tumor cells, and as demonstrated below, a large part of this communication is mediated by extracellular vesicles.

3.1. Immunocyte-tumor communication mediated by exosomes

3.1.1. The effect of tumor-derived exosomes on immunocytes

Immune cells are a major component of the TME (Hanahan and Weinberg, 2011). Tumor-associated macrophages (TAM) promote the progression and metastasis of tumors and are associated with poor prognosis (Schiavoni et al., 2013). Following their recruitment from the circulation, factors in the TME, including tumor-derived exosomes (TDX), drive the differentiation of the macrophages towards an anti-inflammatory M2 phenotype. Transfer of miR-940 by exosomes from hypoxic human epithelial ovarian cancer cells to human macrophages resulted in the polarization of the macrophages to an M2-like phenotype (Chen et al., 2017). Likewise, triple-negative breast cancer-derived exosomes promoted the M2 differentiation of RAW264.4 human macrophages (Piao et al., 2018). The protein cargo of TDX from metastatic mouse colon carcinoma cells polarized bone marrow-derived macrophages towards the M2-like phenotype, by upregulation of MCP-1 and TNFα (Chen et al., 2016). Interestingly, human gastric cancer TDX were reported to increase the transcript levels of pro-inflammatory factors (IL-6, TNF-α and CCL2); however, these inflammatory macrophages also promoted tumor growth and dissemination (Piao et al., 2018).

The presentation of neoplastic cell antigens by dendritic cells (DC) in the draining lymph nodes leads to T cell stimulation (Gardner and Ruffell, 2016). Tumor associated DC were shown to be defective in T cell stimulation (Veglia and Gabrilovich, 2017). The transfer of miR-203 by pancreatic cancer-derived exosomes to neighboring DC resulted in reduced expression of TNF-α and IL-2, which is necessary for DC maturation (Zhou et al., 2014).

A considerable fraction of tumor CD8+ cytotoxic T cells (CTL) becomes exhausted and unable to exert their cytolytic activity. Head and neck squamous cell carcinoma-derived exosomes mediated the loss of expression of the co-stimulatory molecule CD27/CD28 and attenuated antitumor IFN-γ cytokine production from responder T cells (Maybruck et al., 2017). In addition, TDX from breast and bladder cancer generated high levels of adenosine by ATP hydrolysis; this negatively regulated high-affinity A2A positive T cell functions through the inhibition of responses to IL-2 and TCR ligation (Clayton et al., 2011).

3.1.2. The effect of immune cell-derived exosomes on tumors

Immune cell-derived exosomes are also important contributors to TME immune regulation. By transmission of miR-21-5p and miR-155-5p, exosomes derived from CD163+ (M2) macrophages, isolated from human colon cancer specimens, promoted invasion and migration in SW48 colorectal cancer cells *in vitro*. Moreover, the pre-treatment of SW48 cells with CD163+ macrophages in an experimental metastasis assay using immunodeficient mice, resulted in increased cell invasion and migration (Lan et al., 2018). Similarly, exosomes secreted from murine M2 polarized bone marrow-derived macrophages (BMDM) promoted the migration of mouse gastric carcinoma MFC cells. Mechanistically, BMDM-derived exosomes transmitted apolipoprotein E, which activated the PI3K-Akt signaling pathway in recipient gastric cancer cells (Zheng et al., 2018). In contrast to TAM, DC-derived exosomes (DEX) were shown to promote anti-tumorigenic processes. Murine DEX expressed high levels of transmembrane TNF and of transmembrane and soluble Fas ligand (FasL), which mediated the killing of melanoma, colon adenocarcinoma and squamous cell carcinoma cells (Munich et al., 2012).

Natural killer (NK) cells appear to kill cancer cells also *via* secreted exosomes. The addition of NK-exosomes, enriched with FasL and perforin, to human melanoma cells, had a lethal effect, mediated by FasL. Interestingly, these exosomes had no effect when added to healthy kidney cells (Zhu et al., 2017).

The role of mast cells in the TME is still not clear (Aponte-Lopez et al., 2018). However, internalization of mast cell-derived exosomes by lung cancer cells led to enhanced migration and a high proliferation rate. This effect was mediated by delivery of the mast/stem cell growth factor receptor kit, ultimately leading to increased cyclin D1 expression (Xiao et al., 2014) (Figs. 1 and 2).

3.2. Cancer-associated fibroblast-tumor communication mediated by exosomes

3.2.1. Tumor-derived exosomes affect cancer-associated fibroblast differentiation

As major producers of extracellular matrix, fibroblasts are involved in regulation of tissue remodeling and repair. Cancer-associated fibroblasts (CAF) were differentiated from tissue resident fibroblasts and mesenchymal stem cells, and shown to promote tumor growth (Ohlund et al., 2014). By transmission of telomerase reverse transcriptase (hTERT) mRNA, TDX from T cell leukemia, breast cancer cells, colon carcinoma and chronic myeloid leukemia induced the transformation of fibroblasts to CAF and the formation of tumor-supporting niches (Gutkin et al., 2016).

Proteomics profiling of exosomes derived from malignant mesothelioma (MM) revealed that their cargo is enriched with proteins that function in immune response, angiogenesis and cell transformation

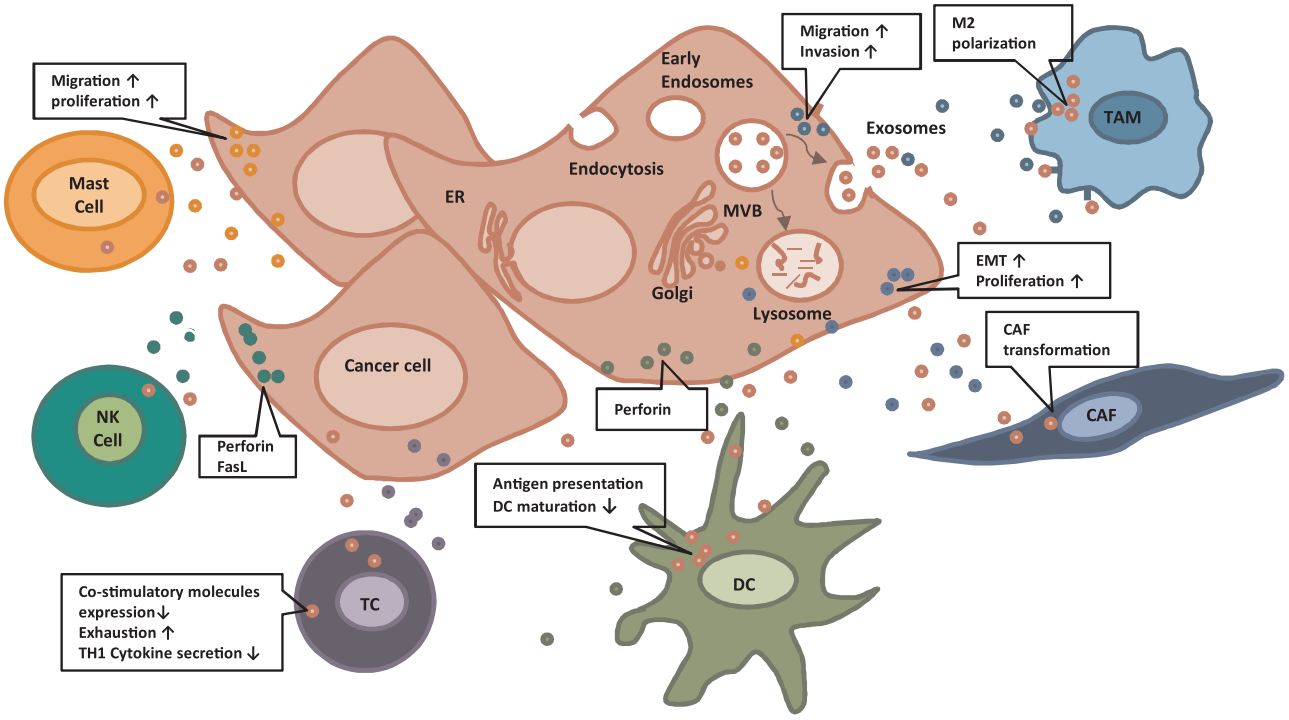

Fig. 1. Exosomes participate in the reciprocal crosstalk between tumor and stromal cells in the tumor microenvironment. At the tumor microenvironment, cancer and stromal cells secrete exosomes that affect cellular processes which promote tumor progression. The figure shows the type of cells and the effect on cancer: TAM – Tumor Associated Macrophages, CAF – Cancer Associated Fibroblasts, DC-Dendritic Cells, TC- T cells, NK – Natural Killer Cells, ER- Endoplasmic Reticulum, MVB– Multivesicular Body, FasL- Fas ligand, EMT- Epithelial to mesenchymal transformation, M2- Macrophages type 2.

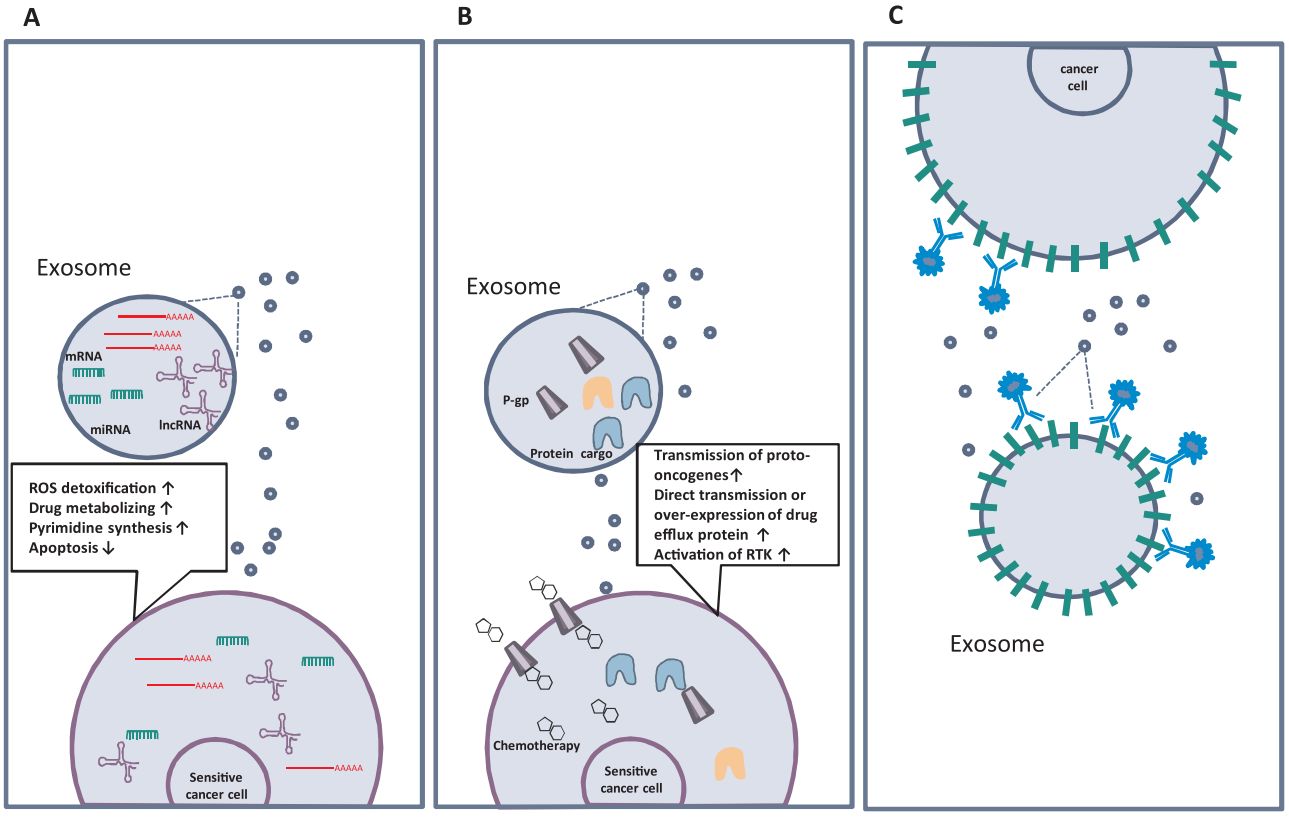

Fig. 2. Mechanisms of promotion of anticancer drug resistance by exosomal cargo. A. Transmission of nucleic acid cargo that elicits gene regulation in the recipient cell. B. Transmission of protein cargo by exosomes induces signaling events inducing overexpression of multidrug efflux transporters in the recipient cell. C. Expression of immune therapy targets by exosomes, directly competing with cancer cells. ROS – reactive oxygen species, RTK- receptor tyrosine kinase.

during cancer progression. MM-derived exosomes were reported to induce the migration of CAF of human and mouse origin by transporting their cargo (Greening et al., 2016). Moreover, constitutively active TGF-β cargo from prostate cancer exosomes and mesothelioma cell-derived exosomes triggered the differentiation of fibroblasts to myofibroblasts (Webber et al., 2010).

3.2.2. The effects of CAF-derived exosomes on tumors

The transfer of miRs -21, -378e and -143 by CAF-derived exosomes to the human breast cancer cell lines BT549, MDA-MB-231 and T47D promoted stemness of the cells, epithelial-mesenchymal transition and anchorage-independent growth (Donnarumma et al., 2017). In prostate cancer, exosomes were shown to regulate metabolism. Specifically, they enhanced proliferation, increased glucose uptake and lactate secretion, and reduced the oxygen consumption rate in prostate cancer cells. The cargo of CAF-derived exosomes contained “off the shelf “metabolites, including lactate, acetate, amino acids, tricarboxylic acid cycle intermediates and lipids that were utilized by the cancer cells (Zhao et al., 2016).

4. The role of exosomes in the emergence of resistance to anti-tumor therapy

A major obstacle in fighting cancer continues to be chemoresistance. Resistance can be inherent to the tumor cells, or emerge after an initial response of the tumor to the treatment (acquired resistance) (Gonen and Assaraf, 2012; Niewerth et al., 2015; Taylor et al., 2015; Zhitomirsky and Assaraf, 2016; Li et al., 2016a; Raz et al., 2016; Bar-Zeev et al., 2017; Gacche and Assaraf, 2018; Leonetti et al., 2019). Multiple mechanisms are involved in the emergence of acquired resistance to therapy in cancer cells, and are discussed elsewhere (Gonen and Assaraf, 2012; Niewerth et al., 2015; Taylor et al., 2015; Zhitomirsky and Assaraf, 2016; Li et al., 2016a; Raz et al., 2016; Bar-Zeev et al., 2017; Gacche and Assaraf, 2018; Leonetti et al., 2019; Binenbaum et al., 2015). Below we discuss how the transmission of extracellular vesicles promotes the development of resistance to therapy in cancer cells.

4.1. Induction of resistance by the transmission of exosomal nucleic acid cargo

The cargo of exosomes was shown to contain several nucleic acid species including miRNA, long non-coding RNA and mRNAs. A large body of evidence currently indicates that exosomal transmission of nucleic acids plays a crucial role in the development of drug resistance in cancer cells (Table 1). In most of the studies described, a drug resistant cell in the TME transmits to a drug-sensitive cancer cell, nucleic acid cargo that results in gene regulation (mostly at the mRNA level). This renders the cell resistant to cancer therapy. Such a mechanism was shown for several types of cancers, including hematological malignancies and colorectal, breast, prostate, liver, lung, ovarian, skin, head and neck, colon, bone and pancreatic cancer (Table 1). For instance, Patel et al., demonstrated that the mere exposure of the pancreatic cancer cell lines MiaPaCa-2 and Colo357 to gemcitabine, the chemotherapeutic agent of choice against pancreatic cancer, caused them to secrete to the medium, exosomes that subsequently increased the IC_{50} of naive cells to gemcitabine from 6.8 and 3.7 μM to 14.5 and 7.7 μM, respectively (Patel et al., 2017). Real-time qPCR analysis demonstrated an increase in the transcript levels of two reactive oxygen species detoxifying enzymes: superoxide dismutase 2 (SOD2) and catalase (CAT); as well as a decrease in the gemcitabine metabolizing enzyme deoxycytidine kinase (dCK). Exosomes from gemcitabine-treated cells contained mRNAs of CAT and SOD2, and miR-155 that reduced the expression of dCK (Patel et al., 2017).

Several components of the TME were shown to transfer drug resistance traits through the use of exosomes, including CAF, cancer-associated adipocytes (CAA) and TAM. Our laboratory recently found that in pancreatic ductal adenocarcinoma (PDAC), TAM-derived exosomes (TDE) specifically target cancer cells in the TME. Delivery of exosomal miR-365 to the cancer cells resulted in an increase in the IC_{50} to gemcitabine, and a decrease in apoptosis. Inhibition of miR-365 with a specific anti-Mir *in vivo* and *in vitro* reversed the resistance to the drug. Mechanistically, we found that TDE and miR-365 increased the levels of pyrimidines in the cancer cells. This increase in pyrimidine levels, specifically dCTP, can compete with gemcitabine, which is a dCTP analogue (Binenbaum et al., 2018). Similarly, an increase in dCTP signals the cell that there is enough pyrimidine products, which upregulates the enzyme that inactivates gemcitabine, namely cytidine deaminase. CAF- and CAA-derived exosomes were demonstrated to induce paclitaxel (PTX) resistance in SKOV3 ovarian cancer cells, by the delivery of miR-21. Internalization of miR-21 by the cancer cells resulted in the downregulation of its target, APAF1, a protein that binds to cytochrome C and dATP to form an apoptosome. Degradation of APAF1 inhibited activation of caspases 9 and 3, and the development of drug resistance (Au Yeung et al., 2016).

4.2. Induction of drug resistance by exosomal protein cargo

One of the mechanisms contributing to drug resistance is the extrusion of cytotoxic drug substrates out of the cells by the ATP-dependent multidrug transporter P-glycoprotein (P-gp) (Li et al., 2016a; Genovese et al., 2017; Borgnia et al., 1996; Bar-Zeev et al., 2016). The MCF-7/ADM human breast cancer cell line expresses high levels of P-gp. It displays 630-fold resistance to doxorubicin, 146-fold to PTX, 56-fold to epirubicin, 5-fold to vincristine and 127-fold to mitoxantrone, compared with the parental line MCF-7/WT. Upon treatment with doxorubicin, the drug did not accumulate in the nucleus in MCF-7/ADM cells as expected, but was secreted out of the cells *via* exosomes. The observation of high levels of P-gp in the exosomes suggests direct transfer of the P-gp protein by the exosomes. Internalization of P-gp-containing exosomes resulted in the transfer of the drug resistance traits upon drug sensitive cells, accompanied by an increase in P-gp levels (Lv et al., 2014). The transient receptor potential channel 5 (TrpC5) is a protein that regulates P-gp. TrpC5 was implicated in the increased levels of exosome secretion observed in MCF-7/ADM cells. Moreover, internalization of MCF-7/ADM exosomes by MCF-7/WT cells resulted in the induction of doxorubicin resistance in these cells. MCF-7/ADM exosomes were shown to be enriched with TrpC5. The internalization of TrpC5-containing exosomes led to Ca^{2+} influx through TrpC5. This resulted in the nuclear translocation of NFAT and the upregulation of the MDR gene product, P-gp. This increase in P-gp was abolished with the TrpC5-specific blocking antibody, T5E3 (Ma et al., 2014). UCH-L1 protein was also shown to upregulate P-gp expression. MCF-7/ADM derived exosomes were enriched with both P-gp and UCH-L1 proteins. Treatment of MCF7/WT cells with the UCH-L1 specific inhibitor LDN-57444 prevented the development of drug resistance after the internalization of MCF-7/ADM-derived exosomes (Ning et al., 2017).

Transmission of proto-oncogenes *via* exosomes is an additional molecular mechanism of acquired drug resistance in cancer. RNA seq of glioma tumors indicated that 7.7% of grade III astrocytomas and 15% of secondary glioblastoma tumors carried the oncogenic fusion gene PTPRZ1–MET (ZM). High levels of MET and phospho-MET were detected in exosomes isolated from glioblastoma cell lines (U118, LN18) and from primary cultures carrying the ZM fusion. Internalization of exosomes from ZM-fusion bearing cells (ZM exosomes) increased MET and phospho-MET levels in the recipient U87 and N3 cells, and triggered the activation of MET signaling. Treatment of ZM exosome-U87 and N3 cells with the alkylating agent temozolomide resulted in upregulation of pro-apoptotic proteins and downregulation of anti-apoptotic proteins, thus promoting temozolomide resistance. Exosome release and the transfer of temozolomide resistance were abrogated when ZM was silenced in U118 cells (Zeng et al., 2017).

Table 1
Exosomal nucleic acid cargo implicated in the development of drug resistance in cancer.

Cargo	Cancer type	Cell line	Therapy	Resistance conferring molecule name	Target gene	Ref #
mRNA	Non-small cell lung cancer	HBEC	Gemcitabine, cisplatin	ZEB1 mRNA	NI	(Lobb et al., 2017)
	Ovarian cancer	Ovarian cells	Cisplatin	DNA methyltransferase 1	NI	(Cao et al., 2017)
	Colon cancer	HCT116 and BJ-5ta fibroblasts	Oxaliplatin	ΔNp73	NI	(Soldevilla et al., 2014)
	Osteosarcoma	MG 63	Doxorubicin	MDR-1 mRNA, P-glycoprotein	NI	(Torreggiani et al., 2016)
LNC RNA	HER2+ breast cancer	SKBR-3 and BT474	Trastuzumab	lncRNA-SNHG14	Bcl-2/apoptosis regulator BAX signaling pathway	(Dong et al., 2018)
	ER positive breast cancer	MCF-7, LCC2	Tamoxifen	lncRNA UCA1	NI	(Dong et al., 2018)
	Hepatocellular cancer	HepG2, Hep3B, PLC/PRF-5 and Huh-7, MzChA-1	Sorafenib, camptothecin, and doxorubicin	lnc-VLDLR	ATP-binding cassette, subfamily G member 2	(Takahashi et al., 2014)
	Non-small cell lung cancer	HCC827 and HCC4006	Erlotinib	lncRNA RP11-838N2.4	Forkhead box protein O1	(Zhang et al., 2018b)
	Renal cell carcinoma	7Su3rd and ACSu3rd	Sunitinib	lncARSR	binding miR-34/miR-449; regulate AXL and c-MET expression	(Qu et al., 2016)
miRNA	Chronic myeloid leukemia	K562 and K562\G01 (res)	Imatinib	miR-365.	BAX and cleaved caspase-3	(Min et al., 2018)
	Breast cancer	MDA-MB, HMLE cells, MCF-7 and MCF-10A	Docetaxel epirubicin or gemcitabine	MiR-1246	Cyclin-G2	(Li et al., 2017)
	Breast cancer	MCF-7/Adr (drug resistance). drug-sensitive variant (MCF-7/S).	adriamycin	miR-222	NI	(Yu D-D and Zhang, 2016)
	Triple-negative breast cancer	Hs578Ts(i)8 cells	Anti-Hsp90 drugs	miR-134	STAT5B	(O'Brien et al., 2015)
	ER positive breast cancer	MCF-7	Tamoxifen	miR-221/222	P27 and ERα	(Wei et al., 2014)
	Prostate cancer	PC3-TXR and DU145-TXR	Paclitaxel	hsa-miR3176, -141-3p, -5004-5p, -16-5p,-3915, -488-3p, -23c, -3673 and -3654	Androgen receptor, phosphatase and tensin homolog, Hub gene T-cell factors/lymphoid enhancer-binding factors 4	(Li et al., 2016c)
	Cervical cancer	HepG2, Hela cells	Cisplatin	miR-106a/b	SIRT1	(Raji et al., 2017)
	Non-small cell lung cancer	PC-9GR cells	Gefitinib	miR-214	NI	(Zhang et al., 2018c)
	Non-small cell lung cancer	H827. HCC827	Gefitinib	miR-21	Phosphorylated-protein kinase B	(Jing et al., 2018)
	Lung cancer	A549	Cisplatin	miR-100-5p	mammalian target of rapamycin (mTOR)	(Qin et al., 2017)
	Pancreatic ductal adenocarcinoma	Panc1, MiaPaCa2, and PSN1	Gemcitabine	miR-155	TP53INP1	(Mikamori et al., 2017)
	Pancreatic ductal adenocarcinoma	KPC cell line and murine peritoneal macrophages	Gemcitabine	miR-365	NI	(Binenbaum et al., 2018)
	Pancreatic cancer	MiaPaCa and Colo-357	Gemcitabine	miR-155-mediated	DCK	(Patel et al., 2017)
	Pancreatic ductal adenocarcinoma	Fibroblast → panc1, AsPC1	Gemcitabine	mir146a and snail mRNA	NI	(Donnarumma et al., 2017)
	Ovarian cancer	Primary CAA and CAF→ A2780, ALST OVCA432, OVCA433, OVCAR5, eyA8, HeyA8-MDR, SKOV3ip, SKOV3-TR and SKOV3	Paclitaxel	miR21	APAF1	(Au Yeung et al., 2016)
	Ovarian cancer.	M2- THP-1→ HeyA8, SKOV3-ip1, A2780, HeyA8-MDR, SKOV3-TR, A2780-CP20, HIO180	Paclitaxel	miR-1246	Cav1-gene	(Kanlikilicer et al., 2018)
miRNA	Ovarian cancer	A2780 PEO1 and PEO4 OC	Paclitaxel	miR-433	cyclin-dependent kinase 6, MAPK14, E2F3 and CDKN2A	(Weiner-Gorzel et al., 2015)
	Ovarian cancer	OVCAR5, OVCAR8 and IGROV1 cells CP70 →A2780	Cisplatin	miR-21-3p	NAV3	(Pink et al., 2015)
	Oral squamous cell carcinoma	HSC-3-Res and SCC-9-Res → HSC-3 and SCC-9	Cisplatin	miR-21	PTEN and PDCD4	(Liu et al., 2017)
	Glioma	U87MG →SHG-44 cells	Temozolomide	miR-221	DNM3 gene	(Yang et al., 2017)
	Neuroblastoma	human monocytes →SK-N-BE(2) or CHLA-255	Cisplatin	miR-155	TERF1	(Challagundla et al., 2015)
	Gastric cancer cells	Murine macrophages→ MFC,MGC-803	Cisplatin	miR-21	PTEN/PI3K/AKT signaling pathway	(Zheng et al., 2017)

Treatment of melanoma patients bearing the BRAF activating mutation V600E with BRAF kinase inhibitors (vemurafenib and dabrafenib) results in tumor regression, followed by rapid development of drug resistance. One mechanism for the development of resistance is the upregulation of receptor tyrosine kinases (RTK), which induced the activation of the PI3K- Akt signaling pathway. Exosomes from the PLX4720 BRAF inhibitor-resistant melanoma cell line LM-MEL-64R3, but not the parental cell line, were rich in the RTK PDGFRβ. The transmission of exosomal PDGFRβ to PLX4720 responsive cells activated the PI3K/AKT pathway and resulted in the development of resistance (Vella et al., 2017).

4.3. Exosomes serve as decoy targets for immunotherapeutics

Rituximab is an anti-CD-20 monoclonal antibody used for treatment of patients with malignant B-cell lymphoma. Upon binding to its cognate antigen, CD20, rituximab induces a cytolytic effect in lymphoma cells by direct induction of apoptosis, complement-dependent cytolysis and antibody-dependent cellular cytotoxicity. Myeloid neoplasms, which are particularly aggressive hematological malignancies, were found to express high levels of the protein ATP-binding cassette (ABC) transporter A3. This leads to increased production and secretion of exosomes, and the potential for drug resistance. Exosomes that were purified from primary lymphoma specimens and from aggressive B-cell lymphoma cell lines (Su-DHL-4, Balm-3, OCI-Ly1) were found to express high levels of CD20 and to bind and deplete rituximab from the growth medium. Moreover, 3 h after the administration of rituximab to patients, more than half the antibody in their serum was bound to exosomes, and thus was not soluble and available to attack lymphoma cells. Exosomal expression of anti-CD20 antibodies led to the depletion of complement components from the serum. This resulted in the inhibition of complement dependent cytolysis. That study provides evidence that exosomes provoke resistance to immunotherapy, by direct competition with antigen antibody binding (Aung et al., 2011). Another example of exosome interference with immune therapy is the surface expression of human epidermal receptor (HER) by exosomes derived from cancers that overexpress HER, such as cancers of the breast, pancreas, brain and the gastrointestinal tract. Breast cancer exosomes that express Her2 compete with breast cancer cells for herceptin binding, an antibody directed to Her2, thus limiting its availability and diminishing its antitumor efficacy (Ciravolo et al., 2012).

5. Potential modalities to overcome the pro-tumorigenic effcts of exosomes

The evidence presented above supports the notion that exosomes play a detrimental role in many cancers and enhance the development of drug resistance. This is the rationale for the development of treatments that will abolish these inhibitory effects of exosomes. Four major strategies can be taken to overcome these deleterious effects of exosomes: (1) inhibition of exosome biogenesis and secretion, (2) inhibition of exosome uptake by target cells, (3) depletion of exosomes from the circulation, and (4) targeting exosomal cargo. Notably, while several studies support the detrimental role of exosomes in the development of drug resistance in cancer, a number of studies demonstrated that increased secretion of exosomes by tumors can result in drug sensitization, usually by the export of MDR efflux pumps out of tumor cells. Moreover, exosomes were shown to be involved in the generation of an anti-tumor immune response, often carrying tumor antigens. Therefore, the benefits and risks of exosome depletion should be weighed for each type of cancer.

5.1. Inhibition of exosome biogenesis and secretion

Several studies have attempted to sensitize tumors to therapy by using pharmacological agents that are known to interfere with exosome biogenesis and/or secretion (Fig. 3A). For example, Uchini et al. investigated the capability of several pharmacological agents to inhibit extracellular vesicle release from prostate cancer cells while maintaining high cell viability. The compounds Cl-amidine and bisindolylmaleimide-I decreased the levels of both large extracellular vesicles and exosomes. The combination of Cl-amidine or bisindolylmaleimide-I with the chemotherapeutic agent 5-FU increased apoptosis levels in two prostate cancer cell lines, PC3 and MCF-7 (Kosgodage et al., 2017). Following their entry into tumor cells, anthracyclines that are used for treatment of leukemia and lymphomas exit the nucleus and are exported out of the cells by exosomes. The ABC transporter A3 (ABCA3) was found to be essential for exosome secretion and for the development of drug resistance. Treatment of diffuse large B-cell lymphoma cell lines with the cyclooxygenase inhibitor, indomethacin, reduced the levels of ABCA3 and exosome biogenesis. This resulted in longer accumulation of the drugs doxorubicin and pixantrone in the nuclei of the tumor cells and increased sensitivity to these drugs (Koch et al., 2016). The anti-histamine drug ketotifen reduced exosome secretion in three cancer cell lines: HeLa (cervix carcinoma), MCF7 (breast cancer) and BT549 (breast cancer). Pre-treatment of these cell lines with ketotifen resulted in their sensitization to doxorubicin (DOX) treatment (Khan et al., 2018). Cyclophosphamide is an alkylating agent that induces a T cell-dependent anti-tumor response by eliminating regulatory T cells from the TME. Tumor-derived exosomes were shown to induce immunosuppressive functions in myeloid-derived suppressor cells (MDSC), thereby reducing the anti-tumor effect of cyclophosphamide. Depletion of exosomes using dimethyl amiloride (DMA), in three cancer models: EL4 thymoma, TS/A mammary carcinoma and CT26 colon carcinoma, inhibited the activation of MDSC suppressor functions. *In vivo*, the treatment of EL4 thymoma, TS/A mammary carcinoma and CT26 colon carcinoma tumor with a combination of DMA and cyclophosphamide resulted in smaller tumors and increased survival (Chalmin et al., 2010).

Ongoing efforts are currently directed to develop new compounds that inhibit exosome biogenesis and secretion. By interaction with the chaperone mortalin, the HIV-1 Nef protein is sorted into exosomes. Delivery of a biotinylated peptide (PEG-SMRwt-Clu) that blocks this interaction to cells abolished exosome release and arrested the proliferation of MCF-7 (estrogen responsive) and MDA-MD-231 (estrogen non-responsive) breast cancer cells. In addition, treatment of MCF-7 cells with PEG-SMRwt-Clu resulted in their sensitization to PTX and cisplatin (Huang et al., 2017). To promote the drug discovery of new compounds that inhibit exosome biogenesis and secretion, Datta et al. developed a quantitative high throughput screening platform. The prostate cancer cell line C4-2B was screened against a total of 4580 compounds, of which 3300 were approved and registered and 1280 were annotated active compounds. The cell line was transfected with CD63 –GFP, and compounds that affected the GFP signal were further validated by exosome quantification. The screen resulted in five validated compounds that inhibited exosome secretion in cancer cells. Two of the compounds discovered were clinically approved: the antifungal drug ketoconazole and the farnesylation inhibitor tipifarnib. The approval of these compounds will enable rapid progression in their development as therapies (Datta et al., 2018).

5.2. Inhibition of exosomes internalization by target cells

The mechanism by which exosomes are internalized by cancer cells appears to be diverse, and to be dependent both on the source of exosomes and the type of target cells. Mechanisms of exosomes uptake include membrane fusion, clathrin and caveolin-mediated endocytic pathways involving specific receptor ligand interactions, and stimulation of macropinocytosis (Mulcahy et al., 2014; French et al., 2017). The complexity of these processes and interactions warrant additional research, however, conceptually, targeting exosomes uptake may prove to be an efficient tool to overcome the pro-tumorigenic effects of

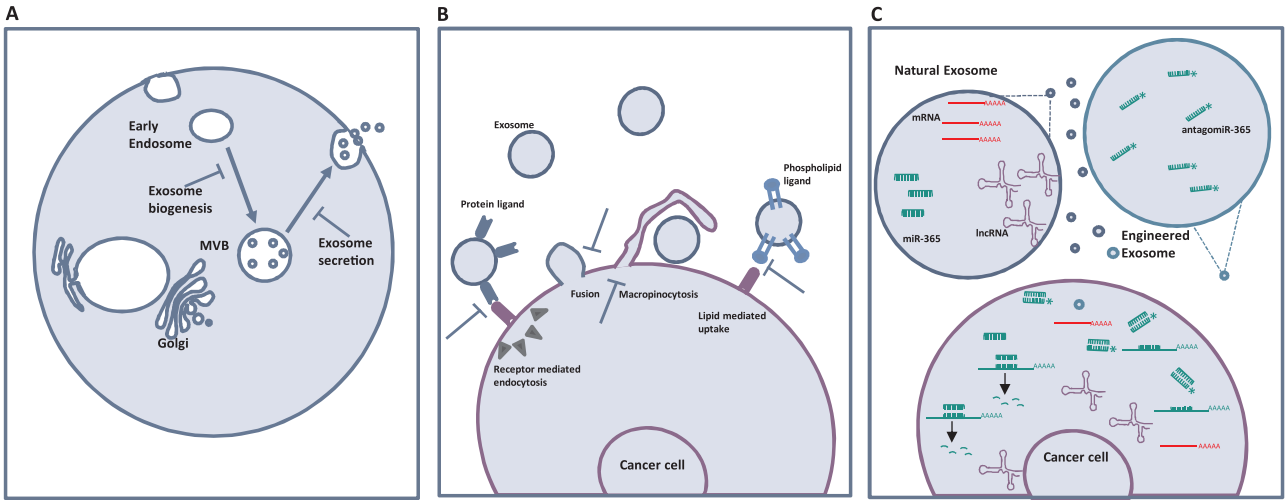

Fig. 3. Potential modalities to overcome the deleterious effects of exosomes on tumor progression. A. Inhibition of exosomes biogenesis and secretion. B. Inhibition of exosomes internalization by target cells. C. Targeting of exosomal cargo (*e.g.*, macrophage-derived exosomes deliver miR-365 that induce gemcitabine resistance). Engineered exosomes carrying an inhibitor to miR-365 (antagomiR-365) can reverse the effect of miR-365.

exosomes (Fig. 3B). Exosomes uptake can be inhibited by blocking the interaction between lipids on exosomes surface and specialized membrane domains (lipid rafts) on the target cells. Exosomal phosphatidyl serine was shown to be required for exosome internalization by cervical cancer cells (Al-Nedawi et al., 2009). Pre-treatment of the exosomes with Annexin V resulted in inhibition of their internalization. A growing list of proteins including tetraspanins, integrins, immunoglubolins, glycoproteins and lectins were shown to interact with receptors on target cells, leading to their internalization by clathrin- or caveolin-mediated endocytic pathways (Mulcahy et al., 2014). These specific-protein-protein interactions can be targeted in order to block internalization of exosomes. Lastly, exosomes uptake can be inhibited using macrocytosis inhibitors targeting actin organization (Rac1 activation) or micropinosome formation (phosphoinositide 3-kinase) (Yuan et al., 2017; Nakase et al., 2015).

5.3. Depletion of exosomes from the circulation

In the previous section we described how the binding of rituximab and herceptin to exosomes decreases their efficacy and cytotoxicity by making them unavailable to bind to lymphoma cells. Inhibition of exosome release by the exosome biogenesis inhibitors, rapamycin and indomethacin, resulted in increased complement-dependent cytolysis levels of lymphoma cells by rituximab. Therefore, depletion of exosomes from the circulation could augment immune therapies. One promising method to deplete exosomes from circulating blood is extracorporeal hemofiltration. A clinical trial that removed particles smaller than 120 KDa from the blood of cancer patients (probably including exosomes) by blood ultrapheresis resulted in the shrinking of tumor size in 6 of 16 patients (Lentz, 1989). ADAPT™ (adaptive dialysis-like affinity platform technology) is a device under development by Aethlon Medical, Inc. (San Diego, CA, USA). This device works similar to dialysis machines. It comprises hollow-fiber cartridges that separate the plasma, and an outer capillary space that enables mobilization of affinity molecules, like antibodies. The company is currently developing a device with immobilized HER2 antibody to capture Her2+ breast cancer exosomes and inhibit disease progression (Marleau et al., 2012).

5.4. Targeting exosomal cargo

When the mechanisms by which exosomes exert drug resistance in cancer cells are known, targeting the cargo of these exosomes may be more practical than blocking exosome secretion systematically. Sunitinib is a receptor tyrosine kinase inhibitor used as the mainstay drug for patients with advanced renal cell carcinoma (RCC). Resistance to sunitinib is also transmitted *via* tumor-derived exosomes. It should be emphasized that molecular mechanisms of sunitinib resistance that are exosome-independent were recently described including lysosomal sequestration due to its hydrophobic weak base nature (Zhitomirsky and Assaraf, 2016, 2015; Zhitomirsky and Assaraf, 2017; Gotink et al., 2011). A long noncoding RNA (lncRNA) lncARSR, enriched in RCC TDE was shown to promote sunitinib resistance. Systemic administration of locked nucleic acid- targeting lncARSR to an orthotopic xenograft model of RCC resulted in restoration of responsiveness to sunitinib (Qu et al., 2016). Our laboratory recently found that exosomes derived from TAM promote resistance to gemcitabine, the workhorse drug for the treatment of many cancers. Treatment of PDAC cells with macrophage-derived exosomes increased the cellular miR-365 in PDAC cells and resistance to the drug (Binenbaum et al., 2018). The delivery of miR-365 antagomiR *in vitro* resulted in sensitization to gemcitabine treatment (Fig. 3C). Immune transfer of macrophages transfected with miR-365 antagomiR to KPC orthotopic tumors, which were induced in Rab27 a/b knockout mice (an exosome-free environment), resulted in increased survival after gemcitabine treatment, compared with immune transfer of macrophages transfected with control oligo. Increased survival was also observed following the immune transfer of macrophages isolated from a Rab27 a/b knockout mouse. This indicates that exosome secretion is required for the transfer of miR-365. These results emphasize the importance of mechanistic studies that enable targeting the emerging mechanism discussed here.

6. Exosomes as drug delivery systems

Efficient delivery of therapeutic agents and specificity to malignant tissues are the main pillars for successful cancer therapies, especially chemotherapy (Li et al., 2016b). Several of the newer drug candidates such as proteins and nucleic acids are highly unstable *in vivo*. This poses substantial challenges to successful therapeutic outcomes. Currently, the preferred pharmaceutical vehicles for drug delivery are phospholipid membrane vesicles and liposomes. Despite considerable progress in the design and efficacy of synthetic liposomes as drug delivery vehicles, the ideal liposome remains elusive (Ha et al., 2016; Lai et al., 2013).

6.1. Advantages of exosomal delivery for cancer therapy

Considerable efforts are being invested to develop exosomes as a novel nanoscale delivery platform (Tan et al., 2013). Several intrinsic characteristics make exosomes attractive as nanocarriers for drug delivery. Compared with synthetic liposomes, exosomes are biocompatible and biodegradable, have low toxicity and do not elicit acute immune reactions. Exosomes carry the required fusogenic properties and uptake machinery, and demonstrate higher specificity to cancer cells than liposomes of a similar size. Exosomes tend to accumulate more in tumor tissues containing abnormally formed blood vessels than in normal tissues. Specificity can be further enhanced by engineering exosomes with tumor-targeting proteins, peptides or antibodies for precise targeted drug delivery. In contrast to liposomes, which are rapidly cleared from the bloodstream by mononuclear phagocytes, exosomes are stable in biological fluids (Wang et al., 2016; Ohno et al., 2016; Batrakova and Kim, 2015; Katakowski and Chopp, 2016).

A critical step in the utilization of exosomes as nanocarriers is the development of an efficient cargo loading method. To date, the exosome loading approaches that have been tested are electroporation, passive diffusion, loading of the cargo to parental cells (by incubation, transfection or overexpression) and isolation of secreted exosomes, sonication, extrusion, as well as freeze and thaw cycles (Luan et al., 2017; Johnsen et al., 2014). The physical and chemical properties of the cargo mostly dictate its loading efficiency. Therefore, the optimal loading method should be empirically tested for each substrate (Batrakova and Kim, 2015; Luan et al., 2017).

Another important decision is the choice of exosome-producer cells, since this can impact the function, distribution and immunogenicity of the exosomes. Cells should be selected that produce large quantities of exosomes (Colao et al., 2018). Accordingly, mesenchymal stem cells (MSCs) and bovine milk were found to be good scalable sources of exosomes (Colao et al., 2018; Yeo et al., 2013; Munagala et al., 2016).

6.2. Exosomes as delivery systems for chemotherapy

Several pre-clinical studies used exosomes to successfully deliver the anti-cancer drugs PTX and DOX. These studies demonstrated efficient loading and delivery to lung, pancreatic and prostate cancer cells *in vitro* and *in vivo*. Exosomes demonstrated superior delivery of PTX compared with liposomes and the free drug (Munagala et al., 2016; Kim et al., 2016; Pascucci et al., 2014; Saari et al., 2015). Importantly, exosomes successfully delivered PTX to MDR cancer cells overexpressing P-glycoprotein (Kim et al., 2016). The specificity of exosomes can be further enhanced by engineering them with tumor-targeting proteins. The addition of an anisamide-polyethylene glycol (AA-PEG) moiety that targeted exosomes to the sigma receptor (overexpressed in lung cancer cells) further enhanced the specificity and efficient accumulation of PTX-loaded exosomes in lung cancer metastases (Kim et al., 2016). Another advantage of exosomes is their ability to deliver soluble drugs across the blood–brain barrier. Injection of brain endothelial cell-derived exosomes, loaded with PTX and DOX, into the circulation in zebrafish embryos resulted in the accumulation of these drugs in the brain, unlike free PTX. Moreover, U-87 MG tumors that were induced in the brain ventricle of zebrafish embryos shrunk following treatment with DOX delivered by exosomes (Yang et al., 2015). When exosomes were used to deliver DOX in mice bearing breast cancer tumors and high grade serous ovarian cancer, systemic toxicity of the drug decreased, and tolerability increased. Histopathological analyses of heart tissue demonstrated vacuoles and moderate myofibril disorganization in mice treated with 6 mg/kg of free DOX, but not in mice treated with DOX delivered by exosomes (Hadla et al., 2016). Systemic toxicity can be further reduced by the expression of tissue targeting moieties on the exosome surface (Tian et al., 2014; Li et al., 2018; Qi et al., 2016).

6.3. Exosomes as delivery systems for gene therapy

Despite the therapeutic potential of nucleic acid drugs, their clinical application is limited. This is due in part to a lack of appropriate delivery systems. Exosomes may present an ideal vector for the delivery of therapeutic nucleic acids.

Exosomal delivery of SiRNA: Several pre-clinical studies used exosomes to deliver siRNA directed against oncogenes. Hepatocyte growth factor (HGF) stimulates the mitogenic ability of hepatocytes and is upregulated in various tumors. Exosomes isolated from HEK-293 cells transfected with HGF siRNA reduced HGF and VEGF protein levels and inhibited proliferation and migration of gastric cancer cells. *In vivo*, lower vessel density was observed in tumors of mice in which siRNA was delivered by exosomes than in mice treated with free siRNA (Zhang et al., 2018a). Similar results were observed in pancreatic cancer with exosomes derived from fibroblasts loaded with kras-siRNA. The delivery of siRNA to pancreatic cancer cells was superior by exosomes than by liposomes. *In vivo*, tumor growth was blunted with exosomes to a much higher extent than with liposomes (Kamerkar et al., 2017).

Exosomal delivery of RAD51 siRNA successfully reduced the protein level of this DNA damage-repair protein in fibrosarcoma and cervical adenocarcinoma cell lines. This resulted in increased apoptosis levels after the induction of DNA double-strand breaks (Shtam et al., 2013). Similarly, the delivery of PLK-1 siRNA by human embryonic kidney and mesenchymal stem cell-derived exosomes to bladder cancer cells successfully knocked down PLK-1 protein expression and substantially decreased their proliferation (Greco et al., 2016).

Exosomal delivery of miRNAs: miRNA is a short form of non-coding RNA found in eukaryotic cells. Since miRNA molecules are a component of exosome cargo, the use of exosomes to deliver therapeutic miRNA is logical. Several studies reported successful loading and delivery of miRNA cargo by exosomes and subsequent targeting of tumor promoting genes in cancer cells, resulting in suppression of tumor growth. These include the delivery of Let-7a tumor suppressor miRNA to EGFR expressing breast cancer xenografts, by exosomes expressing the EGFR binding molecule GE11 (Ohno et al., 2013); the use of MSC-derived exosomes to deliver miRNA-143 to human osteosarcoma cells (Shimbo et al., 2014); and the delivery of miR-146b to glioma cells and tumors developed in the rat brain (Katakowski et al., 2013), and miR-122 to hepatocellular carcinoma (HCC) cells (Lou et al., 2015). Our laboratory successfully transfected tumor-derived macrophages with miR-365 antagomiR. Transfer of the macrophages to mice bearing orthotopic pancreatic tumors delayed the development of miR-365-dependent gemcitabine resistance and increased mice survival (Binenbaum et al., 2018).

6.4. The role of exosomes in cancer vaccines

An additional application of exosomes for therapeutic intervention is tumor vaccination. Systemic injection of classically activated macrophage (M1)-derived exosomes resulted in homing to macrophages and DC in the draining lymph nodes, and the induction of a proinflammatory immune response. These observations encouraged researchers to utilize exosomes as an adjuvant to anti-cancer vaccines. Combining M1 exosomes with a lipid calcium phosphate nanoparticle-encapsulated Trp2 anti-cancer vaccine elicited a robust T cell immune response. This resulted in antigen-specific killing efficiency that was superior to that of the anti-cancer vaccine alone (Cheng et al., 2017). Tumor antigen-adjuvant co-delivery by exosomes is an effective approach to preferentially elicit anti-tumor immunity. The delivery of tumor antigens by murine melanoma B16BL6 cell exosomes, complexed with CpG DNA to the DC cell line DC2.4, resulted in enhancement of antigen presentation by these cells. Immunization of mice bearing melanoma tumors with B16BL6 CpG exosomes resulted in a strong intra-tumoral immunization effect, which led to CD4+ and CD8 + T cell-mediated inhibition of tumor growth (Morishita et al., 2016).

Similarly, the administration of engineered exosomes, purified from mouse colorectal cells enriched with the immunoregulatory factors MHC-1 and IL-15R, together with CpG adjuvant to mice bearing hepatic and colorectal tumors, promoted the infiltration of tumors by CD4+ and CD8α+ cells and significantly decreased tumor volumes (Yang et al., 2018).

Targeting antigens to exosomes can improve the immunogenicity and therapeutic efficacy of viral-based immunotherapies. Modified vaccinia Ankara virus, encoding the tumor-associated antigen prostatic acid phosphatase (PAP), which was fused to the lactadherin C1C2 domain and targeted to exosomes, resulted in higher antibody titers against PAP than mice that were treated with viruses encoding wild-type PAP (Rountree et al., 2011). Injection of DC treated with mesothelioma TDX into a mouse model bearing MM tumors resulted in increased numbers of CD4 + T and CD8 + T cells, and in DC infiltration to the tumors; and improved survival compared to mice receiving necrotic tumor lysate-loaded DC (Mahaweni et al., 2013). Moreover, exosomes from HCC antigen-modified DC could be used as cell-free vaccines. Exosomes purified from DC expressing α feto-protein, an HCC antigen to mice with HCC, triggered T-cell-mediated immune response that suppressed tumor growth (Lu et al., 2017).

The promising pre-clinical results described above have led to several clinical trials that mainly used patient autologous exosomes. A Phase I clinical trial investigated the safety and feasibility of vaccination with autologous exosomes from DC, in patients with stage III/IV MAGE3+ metastatic melanoma. To generate DC DEX, patients underwent leukapheresis. Dex were then isolated from DC and loaded with MAGE3 antigenic peptides. Patients were vaccinated with four doses of Dex intradermally and subcutaneously. The treatment was tolerable in all patients. Of the 15 patients who received treatment, the single patient who exhibited a minor response continued with the treatment for 24 months (every three weeks); the disease was stable for 24 months. The researchers were not able to detect CD4+ and CD8+ T cells specific for the MAGE3 antigens in the peripheral blood (Escudier et al., 2005). A similar trial investigated the feasibility of vaccination with DEX, loaded with MAGE antigens, of 13 patients with advanced non-small cell lung cancer (NSCLC). Of three patients tested, one demonstrated MAGE specific CTL responses, and two of four patients displayed increased NK activity after vaccination (Morse et al., 2005). A phase II clinical trial investigated whether IFN-γ-maturated DC (IFN-γ-Dex) could improve the rate of progression-free survival at 4 months post platinum-based chemotherapy. The trial enrolled 22 non-resectable NSCLC patients. The results showed no clear induction of adaptive immune responses that could be monitored after IFN-γ-Dex injections. The therapy failed to show evidence of antigen-specific T cell immune responses in patients (Besse et al., 2016). A phase I clinical trial that included 40 colorectal cancer patients investigated the ability of autologous ascites-derived exosome (Aex) treatment, with and without granulocyte-macrophage colony-stimulating factor (GM-CSF), to enhance CTL mediated anti-tumor responses. The group that received exosomes in combination with GM-CSF demonstrated a favorable cytotoxic T cell response directed against the tumor, a response that was not seen in patients treated with Aex alone (Dai et al., 2008).

7. Concluding remarks

One of the most devastating problems in cancer treatment is the rapid development of drug resistance. In recent years, exosomes have emerged as major conveyers of cell to cell communication. Most studies of exosomes in the TME reported mainly pro-tumorigenic effects, regardless of whether the exosomes are secreted from stromal or tumor cells. Numerous mechanisms that promote the development of drug resistance in cancer have been described. Here, we described how exosomes play a role in the generation of therapy resistance, and even more profoundly, in the transmission of drug resistance traits between cells. The concerted efforts by the scientific community in an attempt to shed light on the basic molecular processes involving exosome biogenesis, secretion and uptake by target cells is beginning to pay off. We can now harness this knowledge to interfere with the detrimental effects of exosomes, halt them and even utilize them to our benefit. Moreover, the proof of concept studies described above show the great promise that exosomes have as nano-delivery vehicles for cancer therapeutics. Several of the natural attributes of exosomes, namely their biocompatibility and their ability to specifically target tumors and cross anatomic barriers render them ideal for drug delivery. Future efforts should be invested to develop technologies to produce large quantities of exosomes; or alternatively, to develop artificial exosomes that will successfully mimic natural exosomes, based on exosome lipid and protein compositions.

Declaration of competing interest

The authors declare that there are no conflicts to disclose.

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