Molecular Therapy
Oncolytics
Review

The role and application of vesicles in triple-negative breast cancer: Opportunities and challenges

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Extracellular vesicles (EVs) carry DNA, RNA, protein, and other substances involved in intercellular crosstalk and can be used for the targeted delivery of drugs. Triple-negative breast cancer (TNBC) is rich in recurrent and metastatic disease and lacks therapeutic targets. Studies have proved the role of EVs in the different stages of the genesis and development of TNBC. Cancer cells actively secrete various biomolecules, which play a significant part establishing the tumor microenvironment via EVs. In this article, we describe the roles of EVs in the tumor immune microenvironment, metabolic microenvironment, and vascular remodeling, and summarize the application of EVs for objective delivery of chemotherapeutic drugs, immune antigens, and cancer vaccine adjuvants. EVs-based therapy may represent the next-generation tool for targeted drug delivery for the cure of a variety of diseases lacking effective drug treatment.

INTRODUCTION

Breast cancer (BC) has replaced lung cancer to be the malignant tumor with the highest occurrence and deaths in females across the world.[1] Triple-negative breast cancer (TNBC) is identified by hardly expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 and the intracellular and extracellular signaling paths involved in the pathogenesis are more complex. Extracellular vesicles (EVs) especially tumor-derived exosomes (TDEs), play a unique role in the occurrence, development, and therapies of TNBC.[2] In addition to carrying material, transmembrane proteins on the EV surface are also involved in the development of TNBC, such as metastasis. Understanding the complex role between EVs and TNBC is helpful to further develop a new treatment method for TNBC. So we sum up the role of EVs in TNBC from the perspectives of the tumor microenvironment (TME), distant metastasis, drug resistance, and clinical application in this passage.

OVERVIEW OF EVs

EVs are a kind of ordered assembly of molecules with a closed bilayer structure, also known as liposomes.[3] EVs have a complex structure consisting of transmembrane proteins and lipid bilayers wrapped in soluble hydrophilic components and contain substances that originated from the cytoplasm of the donor cell.[4] EVs have also been sorted according to their cell origin. EVs have been sorted into exosomes, microsomes, apoptotic bodies, and oncosomes according to their diameter, mode of abscission, and inclusion.[5] Exosomes are EVs within the scope of 40 to 160 nm in diameter and represent a subtype of EVs formed through the plasma membrane by budding or entosis and exocytosis.[6] Exosomes can be released by almost all kinds of cells and are widely distributed in body fluids.[7] Exosomes contain many cellular components such as DNA, RNA, lipids, metabolites, and cytoplasmic and cell surface proteins.[8,9] Microparticles (MPs) are produced by outbudding off the cell membrane with diameters ranging from 100 to 1,000 nm.[10] Apoptosis also leads to the production of membrane-bound EVs, which are referred to as apoptotic bodies.[11] Studies have indicated that EVs can transport useful materials to healthy cells (e.g., autoantigens).[12,13] Cancer bodies are EVs with a diameter of 1 to 10 μm released by tumor cells.[14] Tumor-associated exosomes are exosomes released by cancer.[15] The bioactive substances in tumor-associated exosomes can influence the recipient cells in two major avenues. The first is the direct contact with the receptor cells. The second is that vesicles enter the receptor cells by plasmalemma fusion and internalization.

EVs IN THE TME

Exosomes from normal cells play a positive part while exosomes from pathological cells play a negative part.[16] Kalluri and LeBleu described the significance of EVs in cell survival, metastasis, metabolic recombination, and angiogenesis.[17] EVs play a significant part in intercellular communication (especially in immune cells) through direct or indirect means, such as receptor-ligand interactions, direct fusion with the plasma membrane, and endocytosis.[18] Attachment of an EV to the target cell can induce signals through receptor-ligand interaction, or lead to transfer of its contents to the cytoplasm.[19] Exosomes secreted by cancer-associated fibroblasts (CAFs) promote proliferation, invasion, migration, and epithelial mesenchymal transformation (EMT) and inhibit apoptosis of BC cells.[20] *In vivo* experiments have indicated that microvesicles released by BC cells can cause transformation and acquisition of malignant features, such as enhanced

https://doi.org/10.1016/j.omto.2023.100752.

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proliferation and survival induction, by transferring tissue glutamin-transferase and fibronectin to surrounding fibroblasts.[21]

EVs IN THE IMMUNE MICROENVIRONMENT

As a communication medium between cancer and immune cells, EVs have been considered to be an important modulator of immune response during tumor progression.[22–24] TDEs can influence anti-tumor activity through delivery of inhibitory cargo.[25] EVs can influence the activation, differentiation or polarization, recruitment, cytokine production, and various other effector functions of innate immune cells through delivery of mediators (bioactive lipids, acute phase proteins, and cytokines), enzymes, and RNAs, thus playing a proinflammatory role, including antigen presentation and activation of immune cells.[26,27]

MACROPHAGE

Necrotic apoptosis EV phagocytosis by macrophages can regulate cytokine and chemokine secretion.[28] After EV uptake, interferon (IFN) β induces interferon stimulator genes in the recipient macrophages through IFN-α/β receptors and can induce differentiation of monocytes into macrophages. High expression of this feature is related to T cell infiltration and perpetuates patient existence.[29,30] Macrophage growth factor CSF-1 binds to TNBC-derived EV to promote the differentiation of M2 macrophages.[31] TDEs can regulate macrophages that express programmed cell death ligand 1 (PD-L1). Therefore, they are supposed as promising predictive landmarks of tumor progression and potential marks for therapeutic immune tolerance.[32]

NATURAL KILLER CELLS

Studies have shown that exosome pretreatment of mouse cancer cells can reduce the quantity of natural killer (NK) cells.[33] Liu et al., after exosome pretreatment with mouse BC cell lines, found that not only the number of NK cells in mice was reduced, but also the cytotoxicity was impaired.[34] Experiments with BC cell line MDA231 have shown that exosomes can significantly prevent interleukin (IL)-2-induced NK cell proliferation, leading to immune escape and cancer progression.[35]

T CELLS

T cell-derived exosomes show efficient antitumor responses through stimulating CD8+ T cells and strengthening cytotoxicity.[36] EVs derived from tumor cells inhibit the antitumor ability of T cells and block the genesis of EVs, thus promoting the activation, proliferation, and antitumor capacity of T cells.[37]

REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS) take part in REDOX response *in vivo* as modulators of cell function and signal transduction.[38] The increase of ROS greatly downregulates the expression of exosomal miR-155-5p secreted by tumor; in other words, ROS can induce the downregulation of EVs that produce a favorable TME for tumor.[39]

TUMOR-ASSOCIATED FIBROBLASTS

Exosomes derived from CAFs cause increased expression of PD-L1 through miR-92 notably promote apoptosis of T cells and block the function of NK cells, thereby inhibiting immune cell function in BC.[40] ATP-binding box (ABC) transporters regulate the intracellular levels of assorted cytokines and chemokines by regulating the transmembrane transport of cytokines in the tumor immune microenvironment (TIME) through EVs, and control the distinction, removal, and function of immune cells to affect the TIME.[41,42]

Besides, EVs carrying tumor cell-derived PD-L1 may interact with T cells producing programmed death 1 (PD-1), thereby reducing the response of TNBC patients to immune checkpoint blocking drugs. Lee et al. demonstrated in cellular animal experiments that macitentan improves the antitumor immune response by inhibiting the secretion of these EVs.[43]

EVs IN METABOLIC MICROENVIRONMENT

The Warburg effect is a major characteristic of carcinoma metabolism, where tumor cells still resort to lactate glycolysis even under conditions of adequate oxygen.[44] Compared with normal tissues, TNBC samples showed significant upregulation of oxidative phosphorylation.[45] MicroRNAs (miRNAs) contained in EVs in the microenvironment of BC can alter the metabolic level of cancer cells. For example, BC-derived vesicles miR-122 can be transferred to stromal cells of neutrophils in the brain and lungs to alter glucose metabolism, thereby improving the metastasis efficiency of BC cells.[46] Morrissey et al. demonstrated that TDEs augment PD-L1 expression by way of metabolic reprogramming led by NF-κB-dependent glycolysis, and lead to increased glucose uptake through TDE signaling of NF-κB.[47]

TDEs restrain mitochondrial oxidative phosphorylation and augment the transformation of pyruvate to lactic acid. Lactate acid feeds back NF-κB pathway and arguments PD-L1 ulteriorly, thereby polarizing macrophages into an immunosuppressive phenotype TDEs[47] (Figure 1). In BC, CAF-derived exosomes can act as a molecular "sponge" to up-control the expression of the PKM pathway, restrain mitochondrial oxidative phosphorylation, augment glycolysis carboxylation, and enhance multiplication of BC cells.[48]

In addition, macropinocytosis refers to the formation of an endocytic vesicle driven by actin on the plasma membrane that deforms the plasma membrane.[49,50] By stimulating the giant pinocytosis pathway, autophagy-inhibited cancer cells meet their energy requirements through degradation of proteins external to the lysosome, such as albumin or extracellular matrix components.[51,52] Tu et al. found that the central Rab GAP cascade of protein PripA-TbcrA complex promotes macropinocytosis.[53]

EVs IN VASCULAR REMODELING

Hypoxia has been indicated to further angiogenesis pathways by secretion of cancer-associated EVs.[54] Exosome-promoted

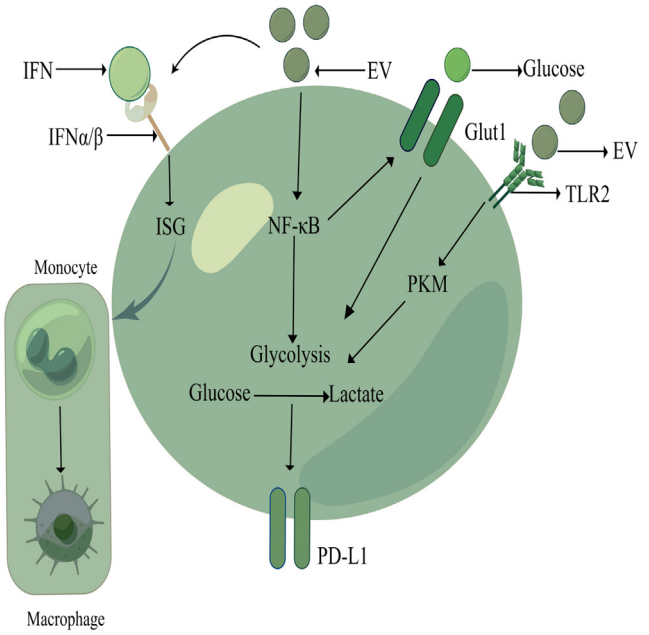

Figure 1. Role of exosomes in tumor microenvironment

After EV uptake, interferon (IFN) β induces IFN stimulating genes in recipient macrophages via IFN α/β receptors, and it can induce monocytes to differentiate into macrophages, which can extend the life span of cancer patients. TDE increases PD-L1 expression through NF-κB-dependent glycolytic-led metabolic reprogramming, and promotes glucose to lactate conversion through the PKM pathway via TLR2, the lactic acid is increased, lactic acid further feeds back NF-κB, thereby increasing PD-L1, thereby polarizing macrophages to an immunosuppressive phenotype.

angiogenesis is mediated by up-control of protease-activated receptor 2 in epithelial cells.[55] BC-derived EVs contain nucleoside diphosphokinase B, whose expression and phosphotransferase activity are enriched in EVs, promoting vascular endothelial cell migration and destroying monolayer integrity.[56] Delta-like 4 (Dll4), Notch ligand, Dll4-containing exosomes mediate Dll4-Notch signaling and can cross collagen matrix and bind to endothelial cells leading to increased cell motility.[57] Exosomes act as "sponges" that absorb miR-106 and control the expression of angiogenic factor that activate signaling in mesenchymal and endothelial cells, thereby promoting angiogenesis.[58,59] Exosomes activate AMPK signaling through a typical forward signaling pathway dependent on ligin Ephrin A1.[60] VEDG is a main angiogenic factor in BC.[61] Overexpression of VEGF often occurs prior to the invasive growth of BC cells.[62] Interleukin-3 promotes angiogenesis through EVs. The experimental results showed that targeted blocking of EVs could not only induce tumor vessel regression of TNBC cells, but also increase tumor cell apoptosis and decrease cell activity and migration ability.[63]

EVs IN DISTANT METASTATIC IMPLANTATION

Zomer et al. demonstrated that EVs enhanced the migration and metastasis capability of tumor through the Cre-LoxP system.[64] It was found that the EVs derived from TNBC modified the mechanical properties of MCF7 cells, a kind of non-metastatic TNBC cells, by reducing cell rigidity and rearranging cytoskeleton, make it metastatic.[65]

CAF-DERIVED EVs

By regulating CD44 expression, CAF-derived EVs transfer circHIF1A into BC cells during hypoxia and strengthen the plasticity of tumor stem cells by regulating CD44 expression.[66] Chen et al. demonstrated that miR-500a-5p in EVs released by CAF promotes the proliferation and metastasis of BC cells through reducing the expression of ubiquitin-specific peptidase 28.[67] Exosome-mediated miR-9 is up-control in several BC cell lines and has been recognized as a metastasis-promoting miRNA, affecting the characteristics of breast fibroblasts and increasing the conversion to CAF phenotype to promote tumor growth and metastasis.[68,69]

TUMOR CELL-DERIVED EVs

BC cells can enhance the arousal of fibroblasts through secreting EVs containing abundant Survivin protein.[70] TDEs were shown to promote TNBC tumorigenesis, metastasis, and migration via the miR-637/Akt1 axis.[71] In the BC microenvironment, miR-10b in vesicles can urge the invasion of non-metastatic cancer cells through inhibiting protein levels of target genes, such as KLF4 and HOXD10.[72,73] After co-culturing two TNBC cell lines with dissimilar levels of glycolytic activity, Kang found that in the process of transforming the phenotypes of other subtypes into invasive phenotypes induced by invasive cancer cells, EVs carry key protein of phosphorylated PKM2 to activate glucose metabolism.[74]

PRE-TRANSLATIONAL NICHE

As a microenvironment fitting for the existence and growth of tumor in the distal metastatic site, the pre-metastatic niche can also be reshaped through miRNA in EVs. For example, miR-186 and miR-205 in EVs were found to increase the formation of niches in liver, thus providing a beneficial environment for the growth of metastatic cancer cells.[75–77]

CHEMOTHERAPY

Although chemotherapy may reduce tumor size, it may increase the danger of metastasis.[78] Doxorubicin (DXR) therapy upregulates the secretion of primary breast tumor EVs, and intake of these DXR-EVs in other organs (mainly the lung) to initiate the pre-metastatic niche and create suitable conditions for the colonization of metastatic BC cells.[79]

EPITHELIAL MESENCHYMAL TRANSFORMATION

FUS/circEZH2/KLF5/feedback loops contribute to metastasis of CXCr4-induced breast cancer to liver by enhancing EMT.[80] EVs transfer miR-221 to recipient cells, thereby promoting EMT.[81] In BC, the pluripotent factors Lin28A and Lin28B promote lung metastasis by inhibiting the pre-metastatic niche through EVs.[82,83]

BRAIN METASTASIS

Although the BBB (blood-brain barrier) does not allow passive entry of anything sized larger than 400 D into the brain, EVs can mediate

BC metastasis to the brain.[84,85] EVs induce endothelial cells in the BBB to phagocytose and absorb EVs, a standard biological pathway referred to as subendocytosis.[86,87] Studies have shown that EVs migrate to and colonize the cerebral vascular niches in the brain to induce proinflammatory states and promote the colonization of cancer cells in the brain.[88]

LIVER METASTASIS

The liver is one of the most common organs for TNBC metastasis and is related with prognosis and survival.[89,90] Circulating cancer cells and bone marrow resident cancer cells migrate as "metastatic seeds" along the circulation to organs, causing liver metastases.[91,92] Aspartate β hydroxylase can initiate the organ metastasis of BC cells by directing the synthesis and secretion of EVs rich in metalloproteinases.[93] The EVs isolated from TNBC patients contained higher levels of transforming growth factor β 1 (TGFβ1). TGFβ1 can upregulate fibronectin in sinusoidal endothelial cells. It is an extracellular matrix protein that promotes adhesion between cancer cells and the liver microenvironment.[94]

EVs RELEASED BY MACROPHAGE

In lung adenocarcinoma, EVs released by M2 macrophage boost the formation of TME and strengthen the invasion and metastasis ability of tumor cells through capacity delivering miR-942.[95,96] In colorectal cancer, EVs activate the large tumor suppressor kinase 2-yes associated protein-matrix metalloproteinase 7 axis, exosome miRNA-106b-5p activates EMT cancer cells and interacts with TAM, and exosome miR-934 promotes the occurrence of liver metastasis by inducing macrophage M2 polarization.[97–99]

SURFACE MEMBRANE PROTEIN

MPs and EVs are different subtypes of EVs. Three tumor progression-related EV surface membrane proteins (CD9, Slc29a1) are present on both surfaces, which enable the binding of EVs to specific receptor cells. Experiments on mice have indicated that reduced expression of these surface membrane proteins reduces the lung tropism of metastasized TNBC cells.[100] CD81, which is a kind of tetraspanin transmembrane protein on the EVs, has been newly identified as a factor of TNBC dryness and metastasis. Their extracellular regions have been found to interact with CD44 in human and mouse models, so as to promote TNBC lung metastasis.[101]

Besides, it was shown that the EVs derived from circulating cancer endothelial cells promoted the formation of new blood vessels in TNBC lung metastasis and depleted T cells. That is to say, the EVs could not only promote the growth of lung metastases, but also cause extensive immunosuppression.[102]

EVs IN MEDIATING DRUG ACTION

There are two main effects of EVs in TNBC. First EVs participate in drug resistance; second by virtue of their properties, they can act as carriers of drugs for therapeutic purposes.

EVs IN MEDIATED RESISTANCE

EVs can assist drug efflux pumps to transport products from drug-resistant cancer cells to drug-sensitive cells, or help maintain tumor cells healthy by effecting inhibitory substances through EVs.[103,104] Lehuédé et al. found that tumor-associated adipocytes increased the amount of EVs released by cancer cells and promoted the direct efflux of Adriamycin, rendering the BC cells resistant to Adriamycin.[105] EVs directly participate in drug efflux through various drug transporters in a targeted manner.[106] P-glycoprotein is a transporter on the ABC on EV membranes, which can use ATP as energy to export various compounds, including various anticancer drugs, thereby enhancing drug resistance.[107,108] BC resistance protein can promote the transfer of chemotherapy drugs through the PI3K-Akt signaling pathway, and enhance the resistance of cancer cells to mitoxantrone.[109] Xia et al. demonstrated the involvement of TAM in conferring resistance to most TNBCs by secreting EVs containing non-coding RNAs (ncRNAs), including circular RNAs (circRNAs), long non-coding RNAs (lncRNAs), and miRNAs.[110] ncRNAs carried by EVs released by CAFs can enhance chemotherapy resistance through controlling signaling pathways (PI3K/AKT and ER) via target organs or targeted ion channels CAFs.[111] EVs can restrain the migration and proliferation of T cells and differentiation of macrophages, thus participating in cancer immune escape.[112] Mixed lineage kinase 4 has been shown to promote the chemoresistance of TNBC by modulating pro-survival response to DNA damage therapy.[113] Histone chaperone proteins regulate cell proliferation and chemical resistance in TNBC by the YAP1/NDRG1 transcriptional axis.[114] Lin et al. found that ubiquitin-specific protease 7 induced chemical resistance of TNBC through deubiquitination and stabilization of ABCB1.[115] Activation of the mitochondrial apoptosis pathway in tumor cells is an important pathway of cell death after chemotherapy, and changes in this pathway can induce the progression of drug resistance in cancer cells.[116] EVs released by tumor can “trap” anti-PD-L2 antibodies, leading to a decrease in drug concentration in the body, and faster clearance of the bound anti-PD-L1 antibodies. This results in insufficient time for drug action, impairing the anti-tumor ability and subsequent drug resistance.[117]

CLINICAL APPLICATION OF EVs

Due to high biocompatibility, low immunogenicity, intrinsic cell targeting, and ease of chemical and genetic manipulation, vesicles are considered effective vectors for biotherapy (Figure 2).

EVs AS TRANSPORT CARRIERS

Based on EV secretion and gene transfer by macrophages, nanoparticles can be used for delivery of active drugs to cells.[118] Nanoparticles of tumor exosomes are effective carriers for chemotherapeutic agents.[119] Disease-related miRNA mimics or inhibitors can be loaded into the EVs of patients by using the carrier properties of exosomes and their capability to cross the BBB for therapeutic purposes.[120] circRNA-CREIT disrupts the stability of PKR protein and its overexpression significantly increases chemo-induced apoptosis, thus it can

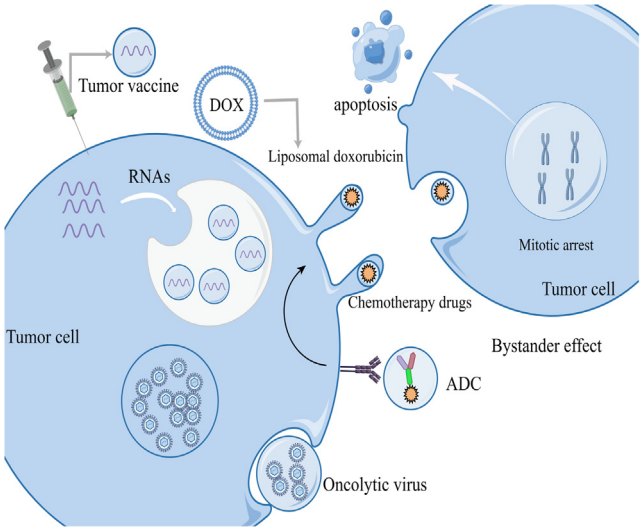

Figure 2. Vesicles as drug loading route for tumor treatment

At present, the clinical application of vesicles is mainly reflected in tumor vaccine, liposome drug, ADC drug, and oncolytic virus and so on. Tumor vaccine is a kind of biological immunotherapy that contains DNA, RNA polypeptide, and other tumor antigens by vesicles. Liposome drugs including liposome paclitaxel, liposome Adriamycin, and liposome carboplatin have been used in clinical treatment. ADC drugs are loaded with vesicles, which transport therapeutic drugs targeted to tumor cells, and can enhance tumor killing ability through the bystander effect. In oncolytic virus therapy, the vesicles transport the virus into the tumor cells for therapeutic purposes by replicating and spreading the virus from the tumor cells to death.

be packed into EVs to significantly strengthen the chemosensitivity of TNBC through exosomal delivery of stress-inhibiting particles.[121]

ANTIBODY-DRUG CONJUGATES

Antibody-drug conjugates (ADCs) have been approved for targeted tumor therapy.[122] These are designed to selectively deliver payloads to target tumor cells and mitigate the adverse effects on non-malignant cells.[123] ADC drugs are internalized for intracellular processing and payload release.[124] Most ADCs exhibit cytotoxic effects on adjacent cancer cells that lack target antigen expression through their ability to cross cell membranes with a payload.[125] ADC target antigens are usually expressed on EVs to some extent.[126] EVs can deliver ADC to adjacent tumor cells that lack antibody target proteins, resulting in the bystander effect that can enhance the anticancer effectiveness of ADC.[127] TROP2 target ADC drugs (Goxstuzumab) developed for TNBC have been utilized in clinical practice.[128] The progress in the research and development of other ADC drugs is summarized in Table 1.

LIPOSOMAL DRUGS

Liposomal drugs are also an embodiment of the clinical application of vesicles, such as paclitaxel, liposomal Adriamycin, and liposomal carboplatin.[129–132] Chemical or biological modification of EVs through genetic or chemical modification can alter or enhance their therapeutic ability.[133,134] Tumor cell-derived MPs carry chemotherapy drugs to reverse M2 macrophages into M1 macrophages by activating lysozyme somatic pigment P2 and nuclear hnRNPA1B450, thereby enhancing antitumor immunity.[135]

GENETIC ENGINEERING

Current studies have developed different EV labeling and imaging methods that facilitate the use of EVs in genetic engineering.[136] EVs with antibody targeting groups and immunoregulatory proteins displayed on the membrane surface were genetically engineered to not only activate T cells to kill epidermal growth factor receptor (EGFR)-positive TNBC cells, but also induce antitumor immunity.[31] Silva et al. suggested that the new EVs sorting protein could enrich the target protein substance into vesicles thus widening the prospects for the usage of EVs as delivery carriers for more complicated therapeutic proteins.[137] Cas9 EVs modified by genetic engineering can be used as a novel gene editing tool.[138] EVs can also serve as multifunctional ribonucleoprotein delivery vectors for effective and secure gene editing.[139]

TUMOR VACCINE

mRNAs coated with lipid nanoparticles can be used as tumor vaccines, protein replacement therapies, and gene editing components for rare genetic diseases.[140–142] Human neutrophil elastase and Hylton alcohol (TLR3 agonist) were loaded into alpha-whey protein engineered EVs released by TNBC to produce dendritic cell (DC) vaccines that were effective in both mouse experiments and patient trials.[143] Preclinical models and mouse experiments demonstrated that combination with anti-PD-L1 ligand-dependent corepressor (LCOR)-mRNA which was delivered to tumor cells via EVs to restore LCOR expression, could overcome TNBC resistance and eliminate cancer metastasis.[144]

EVs IN COMBINATION WITH OTHER TREATMENTS

Tian et al. found that the combined use of vesicles loaded with small interfering RNA targeting PD-L1 and radiotherapy notably inhibited tumor growth and improved mouse survival.[145] EVs secreted by gamma-δT cells were found to effectively destroy nasopharyngeal carcinoma cells as well as boost T cell activity in the immunosuppressed condition. Moreover, it showed a synergistic effect with radiotherapy.[146] Recently, Yu et al. found that exosomes inhibit docetaxel resistance in lung adenocarcinoma by chelating ubiquitin-specific protease 5 to destabilize upstream transcription factor 1 proteins.[147]

EVs AS BIOMARKERS

EVs can be applied as functional biomarkers for diagnosis and prognostic assessment.[148] The transmembrane proteins CD147 and A33 contained in fecal EVs were dissimilar between colorectal tumor patients and healthy people. Thus, it is a novel non-invasive marker for colorectal cancer screening and prognosis.[149] Liquid biopsy is a minimally invasive and repeatable method for tumor diagnosis and treatment.[150] EVs have advantages such as easy access, stable structure, and rich information constracted with circulating tumor cells and circulating tumor DNA.[151] Jafari et al. found that EVs can be utilized

Table 1. ADC drugs

Drug	Target spot	Stage	Number	Identifier
Sacrituzumab	TROP-2	II	51	NCT04230109
Govitecan				
Datopotamab	TROP-2	I		NCT03401385
Deruxtecan		II	118	NCT05460273
PF-06664178	TROP-2	I	31	NCT02122146
ZEN003694		I	75	NCT02711956
Talazoparib	TROP-2	II	179	NCT03901469
SKB264	TROP-2	I/II	430	NCT04152499
Enfortumab Vedotin	Nectin-4	II	288	NCT04225117
PF-06650808	Notch3	I	40	NCT02129205
PF-06647263		I	60	NCT02078752
PF-06647020		I	138	NCT02222922
PF-06263507		I	26	NCT01891669
BAY94-9343		I	12	NCT02485119
Cofetuzumab Pelidotin	PTK7	I	18	NCT03243331
AVID100		I	49	NCT01891669
CAB-RC48-ADC	EGFR	I/II	49	NCT01891669
SAR566658	Ror2	I/II	420	NCT03504488
RC48-ADC	CA6	II	23	NCT02984683
RC108		II	64	NCT05331326
SKB264		I	32	NCT05331326
MRG002		II	95	NCT05445908
ARX788		II	66	NCT04742153
DS-8201a		I	106	NCT03255070
ADCT-502		I	75	NCT03366428
ADCT-301	HER-2	I	21	NCT03125200
PF-06804103		I	78	NCT03621982
A166	HER-2		95	NCT03284723
XB002		I/II	49	NCT03602079
BAY94-9343		I	524	NCT04925284
TORL-2-307-ADC		I	12	NCT02485119
TORL-1-23		I	70	NCT05156866
CAB-AXL-ADC		I	70	NCT05103683
FDA018-ADC		II	240	NCT05103683
TR1801-ADC		I	78	NCT05174637
F0002-ADC		I	40	NCT03859752
OBT076		I	23	NCT03894150
HS-20089		I	150	NCT04064359
STRO-002		I	177	NCT05263479
FOR46		I	160	NCT03748186
MYTX-011		I	56	NCT03575819
SYD1875		I	150	NCT05652868
REGN5093-M114		I	31	NCT04202705
SOT102		I/II	81	NCT04982224
STI-6129		I/II	269	NCT05525286
FDA022-BB05		I/II	64	NCT05565807
		I	107	NCT05564858

as a non-invasive tool of diagnosis for glioblastoma.[152] The use of EVs can improve the sensitivity of detection of EGFR mutations in plasma from patients with lung cancer.[153] Thanks to the characteristics and metabolic analysis of EVs, there are clear differences between patients with early gastric cancer and healthy control group, which provides a new idea for monitoring early gastric cancer.[154] Other aspects of EVs as markers of diagnosis and therapy and prognostic screening for tumor are shown in Table 2.

ONCOLYTIC VIRUS THERAPY

Oncolytic virus treatment for cancer is an active area of research.[162] Oncolytic virus therapy entails transporting the virus to tumor cells through vesicles where they replicate and spread resulting in the death of cancer cells.[163] In a clinical trial of pediatric high-grade glioma therapy with herpes simplex virus type 1 (HSV-1) G207 was found to significantly improve median survival.[164] Oncolytic reovirus selectively induces autophagy in KRAS-mutated colorectal cancer and further promotes apoptosis.[165] The successful application of oncolytic viruses in other cancer species also boosted the study of the role of oncolytic viruses in TNBC. At present, the study has entered phase 1–2 clinical trials. The clinical trial conducted by Soliman et al. preliminarily confirmed that oncolytic virus therapy enhanced the efficacy of neoadjuvant chemotherapy in TNBC patients (ClinicalTrials.gov ID: NCT02779855).[166] Two mumps virus (MuV) isolates, MUV-UA and MUV-UC, strongly killed a set of established human BC cell lines *in vitro*, significantly extending the survival of nude mice with MD-MB-231 tumor xenografts, and showed significant killing activity against BC patient-derived xenograft cell lines grown as 3D organoids, including from patients resistant to anthracycline and taxane chemotherapy.[167]

EVs IN COMBINATION WITH OTHER TREATMENTS

Cell-derived EVs carry proteins, RNAs, and lipids, which are involved in intercellular communication, regulation of inflammation, and promotion of vascular repair to urge wound healing in diabetic patients.[168] In the mouse experimental model, vesicles secreted by cardiosphere-derived cells were found to mediate anti-inflammatory, immunomodulatory and anti-myocardial fibrosis, improve cardiac function, and inhibit arrhythmia.[169] Experiments in rats have shown that EVs containing LncRNA TUG1 can be potential therapeutic targets for myocardial infarction.[170] In terms of assessing endometrial receptivity, biopsies of EVs originating from the endometrium can avoid endometrial injury associated with traditional biopsy methods.[171] Brain-derived EVs released by the central nervous system have been considered as diagnostic and prognostic biomarkers for Alzheimer's disease.[172] In animal studies of acute kidney injury,

Table 2. Tumor-derived exosomes as diagnostic biomarkers

Cancer	Source	Biomarker	Number	Reg. NO
Gastric	Plasma	Exosome	80	NCT01779583
Cancer	Serum	MiR-15b-3p		Wei et al., 2020[155]
Hepatocellular	Serum	miRNA-21		Lee et al., 2019[156]
Carcinoma	Urine	Phospholipid		ChiCTR2200065653
Colorectal	Serum	Has-circ-0004771		Pan et al., 2019[157]
Cancer	Blood	EXOSCOL01	80	NCT04394572
Non-small cell	Plasma	CD63; EGFR;		Fan et al., 2020[158]
Lung Cancer	Blood	CD8+; EGFR		ChiCTR2200061697
	Plasma		150	NCT05587114
	Serum	LncRNA	1000	NCT03830619
Small Cell	Plasma	Non-coding RNA		ChiCTR2200064246
Lung Cancer				
Prostatic	Urine	MiR-196a		Rodríguez et al., 2017[159]
Cancer				
Cholangiocarcinoma	Plasma	NcRNA	80	NCT03102268
Ovarian Cancer	Plasma	CA125; CD24	90	Im et al., 2014[160]
Breast Cancer	Blood	CA153	90	Wang et al., 2017[161]
	Plasma		100	NCT01344109
Osteosarcoma	Blood	RNA	50	NCT03108677
Clear cell	Urine	CD9+	74	NCT04053855
Renal Cancer				
Thyroid	Urine	Thyroglobulin	30	NCT05463107
Cancer	Urine	Galectin −3		NCT03488134
	Saliva	HPV		NCT02147418
Oropharyngeal Cancer				

stem cell-derived EVs were found to improve renal function and inflammatory response status, and reduce apoptosis.[173] Mesenchymal stromal cell-derived EVs reduce inflammation in lung diseases such as pulmonary fibrosis, asthma, and chronic obstructive pulmonary disease, regulate immunity, and improve organ function.[174] EVs released by mesenchymal stem cells mediate signal transduction through paracrine pathways to regulate systemic inflammation and promote liver repair.[175] Implantation of mRNA encoding extracellular matrix α1I collagen from human dermal fibroblasts EVs was found to reduce dermal wrinkles in mice.[176] Experiments on mice show that timely treatment of waste in the body can delay aging, which provides new ideas for anti-aging in humans.[177] Liu et al. demonstrated the participation of EVs in the humoral coordination of bacterial infection.[178] EVs bind to circulating iron-containing proteins, regulate systemic iron metabolism, and prevent bacteria from obtaining iron, leading to bacteriostatic effects.[178]

SUMMARY AND OUTLOOK

BC, especially TNBC, is a malignant cancer with high incidence and lacking prognosis. Due to the lack of related receptors, chemotherapy is the mainstay of therapy for TNBC. Although PARP inhibitors or immunotherapy can be used in some cases of TNBC, treatment needs are still far from being met. In recent years, preclinical and clinical studies have indicated promising prospects for use of EVs for treatment of various types of cancer and aging-related diseases. EVs were earlier thought to act as "garbage bags," carrying metabolic waste and apoptotic components. However, there is growing evidence that EVs play a significant part in intercellular crosstalk through transfer of materials. TDEs play a critical part in inducing host immunosuppression. These EVs can transport antitumor drugs from tumor cells via their own membrane proteins, thereby interfering with immunotherapy and inducing chemotherapeutic resistance. EVs of cancer cells after genotoxic stress and epigenetic drug therapy or radiotherapy enhance antitumor effects. The special components of the EVs originating from tumor cells and the specific membrane proteins make them new biomarkers. Unlike conventional cancer testing, liquid biopsies are inexpensive, repeatable, minimally invasive, relatively acceptable to patients, and easier to apply for large-scale cancer screening. EVs (mainly exosomes) are also used for drug and gene delivery. For example, through genetic engineering modification, antibody coupling, and genetic or chemical modification, the characteristics of vesicle lipid or membrane-specific proteins can be used to

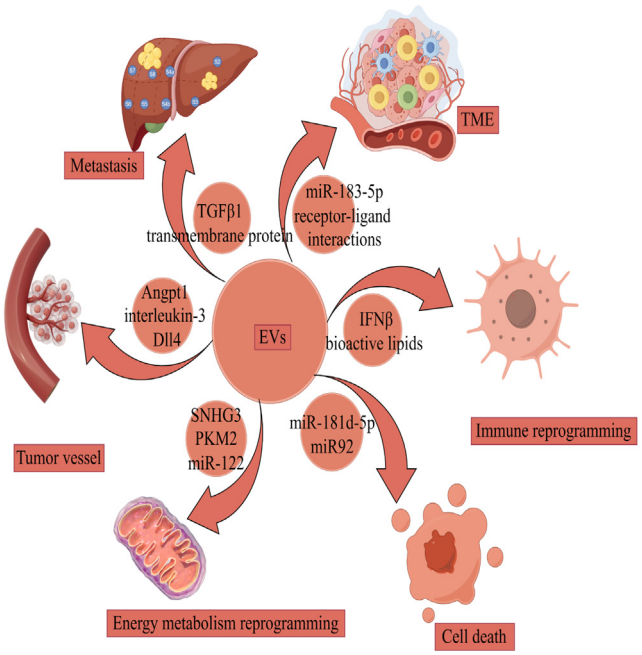

Figure 3. Role of EVs in the genesis and development of TNBC

The EVs from TNBC contained higher levels of transforming growth factor β 1. This factor can upregulate fibronectin on sinusoidal endothelial cells and promote adhesion of cancer cells to the liver microenvironment. EVs carrying bioactive substances participate in the change of TME, and play a bridge role in the process of tumor development. EVs shuttle through the immune system to regulate the proliferation and differentiation of various immune cells, which facilitates the escape of tumor cells from immune surveillance. TDEs inhibit mitochondrial oxidative phosphorylation, increase glycolytic carboxylation, and enhance TNBC cell proliferation. EVs act as "sponges" that absorb and regulate the expression of angiogenic factors, thereby promoting tumor angiogenesis.

selectively enter tumor cells, thus inhibiting the development of cancer and achieving anticancer effect. EVs containing immune checkpoint components can be used as new targets for immunotherapy. However, further research is required for in-depth characterization of the molecular mechanisms by which EVs regulate immunity, metabolism, and drug resistance. Moreover there is a need to further refine the methods for the extraction, purification, and storage of EVs; in addition, their potential effects on the host immune system also need to be confirmed. Understanding the pathological effects and mechanisms of EV-mediated TNBC will further enhance the role of immunotherapy and other precision therapies in the treatment of cancer (Figure 3).

ACKNOWLEDGMENTS

Throughout the writing of this dissertation I have received a great deal of support and assistance. I would first like to thank my supervisor, Xi-He Zhao, whose expertise was invaluable in formulating the manuscript. Her insightful feedback pushed me to sharpen my thinking and brought my work to a higher level. I would like to acknowledge my teammates, Chun-Yan Yan and Meng-Lu Zhao, for their wonderful collaboration and patient support. Figures were created by Figdraw (www.figdraw.com). This study was funded by the National Natural Science Foundation of China (No.81802760, 81702402), Science and Technology Project of Liaoning (No.20170520027), and 345 talent plan project of Shengjing Hospital (X.-H.Z., Lei Liu).

AUTHOR CONTRIBUTIONS

Conception and design: X.-H.Z.; Administrative support: X.-H.Z.; Collection and assembly of data: All authors; Manuscript writing: All authors; Final approval of manuscript: All authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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