

Review Article

Exosomes in development, metastasis and drug resistance of breast cancer

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Transport through the cell membrane can be divided into active, passive and vesicular types (exosomes). Exosomes are nano-sized vesicles released by a variety of cells. Emerging evidence shows that exosomes play a critical role in cancers. Exosomes mediate communication between stroma and cancer cells through the transfer of nucleic acid and proteins. It is demonstrated that the contents and the quantity of exosomes will change after occurrence of cancers. Over the last decade, growing attention has been paid to the role of exosomes in the development of breast cancer, the most life-threatening cancer in women. Breast cancer could induce salivary glands to secrete specific exosomes, which could be used as biomarkers in the diagnosis of early breast cancer. Exosome-delivered nucleic acid and proteins partly facilitate the tumorigenesis, metastasis and resistance of breast cancer. Exosomes could also transmit anti-cancer drugs outside breast cancer cells, therefore leading to drug resistance. However, exosomes are effective tools for transportation of anti-cancer drugs with lower immunogenicity and toxicity. This is a promising way to establish a drug delivery system.

Breast cancer is the most frequently-diagnosed life-threatening cancer and the leading cause of cancer-related death in women.⁽¹⁾ The poor prognosis of most breast cancer patients is due to late diagnosis. Markers of tissues, genes and serum are presently used in diagnosis, but these markers do not work in the diagnosis of breast cancer at an early stage.⁽²⁾ Emerging evidence shows that various factors, including microRNA (miRNA), and proteins participate in the development or progression of breast cancer.^(3–5) Distant metastatic and local recurrent tumors are the main causes of death in the clinic. Signaling pathways, growth factors and miRNA participate in distant metastasis of breast cancer.⁽⁶⁾ De novo and acquired resistance to anticancer agents remains a major obstacle in the treatment of breast cancer.

Transportation between cells and the outside environment can be divided into active, passive and vesicular types. Vesicular transport and especially exosome-mediated transport, which play a vital role in cellular transport, have been studied extensively.⁽⁷⁾ Cell-secreted exosomes communicate with the micro-environment through the delivery of proteins, nucleic acid and other substances. Deregulation of exosome-mediated transport leads to disease development.⁽⁸⁾ Exosomes function as versatile promoters in the tumorigenesis, metastasis and drug resistance of breast cancer. In this review we summarize the current knowledge on the functions of exosomes in the diagnosis,

tumorigenesis, metastasis, microenvironment, drug resistance and therapy of breast cancer.

Exosomes

Exosomes are nano-sized vesicles (diameter 40–100 nm) released upon the fusion of multivesicular bodies with plasma membranes in a variety of mammalian cells.⁽⁹⁾ Exosomes were originally regarded as a form of cell surface molecule removed from reticulocytes. In fact, exosomes exist extensively in body fluids such as blood, urine, ascites and amniotic fluid. The release of exosomes is regulated by some Rab family proteins, including Rab27a and Rab27b.⁽¹⁰⁾ Exosomes consist of lipid bilayer membranes and numerous molecular constituents of their original cells, including proteins and nucleic acid (summarized in Fig. 1).⁽¹¹⁾ Exosomes export many proteins that are tumor promoters or suppressors.⁽¹²⁾ For example, heat shock proteins, p53, phosphatase and tensin homolog and adenomatous polyposis coli, which exist extensively in exosomes, are closely related with tumor development.^(13–16) MiRNA in exosomes account for a majority of circulatory miRNA, which have been investigated as biomarkers in different cancers.⁽¹⁷⁾ These findings indicate that exosomes play a critical role in the development of tumors.

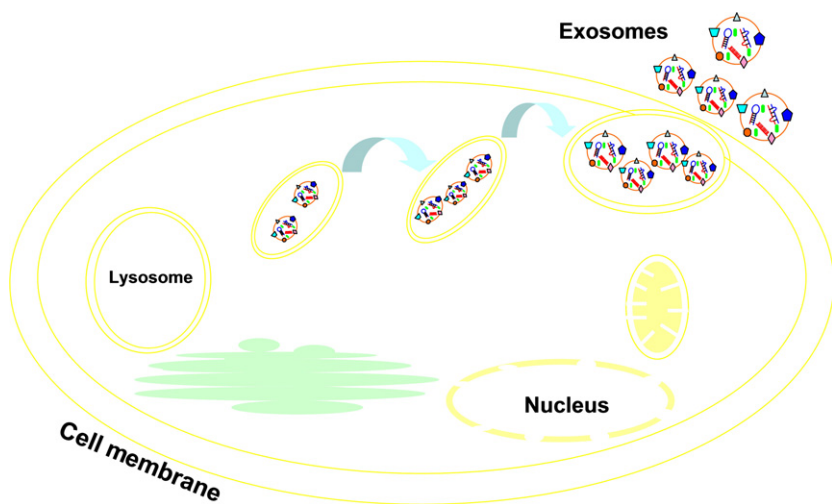


Fig. 1. Exosomes consist of lipid bilayer membranes and numerous molecular constituents of their original cells, including proteins and nucleic acid material. The triangle, squares and circles on the membrane of exosome represent different surface molecules, such as glycoprotein and CD molecules.

Roles of Exosomes in Diagnosis of Breast Cancer

Diagnosis of breast cancer depends on imaging, biomarkers and pathology. Biomarkers are major method for breast cancer screening and early diagnosis. Molecular markers of breast cancer are classified into tissue, genetic and serum markers. Tissue markers include estrogen receptors (ER), progesterone receptors (PR), human epidermal growth factor-2 (HER-2), p53 and Ki-67.⁽²⁾ Negative results in ER and PR, HER-2 gene amplification, p53 mutation and increased Ki-67 status assays are significantly correlated with poorer prognosis.^(18–20) Serum markers cover a wide range, including carcinoembryonic antigen, CA 15.3, BR 27.29, MCA, CA 549-muc-1 family and oncoproteins.⁽²⁾ However, none of the markers above can be used in the diagnosis of breast cancer at an early stage.

Saliva is used as a noninvasive method to detect cancers at an early stage, including breast, pancreatic and oral cancers.^(21–23) As reported, salivary biomarkers (transcriptomic and proteomic signatures) are high-specificity and high-sensitivity discriminators for detection of early breast cancer.⁽²¹⁾ Exosomes derived from breast cancer (exo-BCa) can interact with salivary gland cells to alter the composition of the secreted exosomes both transcriptomically and proteomically.⁽²⁴⁾ Distal breast cancer communicates with salivary glands through secretion of exosomes. In addition, the salivary biomarkers partly result from the exo-BCa-delivered proteins and mRNA. Monitoring the mRNA and protein expressions of salivary biomarkers among persons at high risk of breast cancer serves as a new efficient way for detection of breast cancer. Similarly, pancreatic cancer-derived exosomes could also lead to secretion of salivary biomarkers.⁽²⁵⁾ The levels of serum exosomal Survivin (an inhibitor of apoptosis protein) and its splice variants are significantly higher in breast cancer patients compared to those who are disease-free for 5 years.⁽²⁶⁾ Exosomes could play an important role as biomarkers of early breast cancer. This result may serve as a diagnostic and/or prognostic marker in early breast cancer patients.

Exosomes in Breast Cancer Tumorigenesis

It is generally supposed that cancers may originate from cancer stem cells which possess stem-like self-renewing ability and are the initial cellular components within a tumor that facilitate tumor progression.⁽²⁷⁾ Breast cancer may arise from breast cancer stem cells which are able to proliferate extensively and generate differentiated breast cancer cells.⁽²⁸⁾ The differen-

tiated breast cancer cells are characterized by the ability of anti-apoptosis and cell cycle dysregulation.

Exosomes stimulated by hypoxia, heparanase and other factors are associated with angiogenesis of breast cancer, which is the most significant part of breast cancer tumorigenesis.^(29,30) Exosomes promote the formation of tumor blood vessels that support tumor growth and extension. Exo-BCa are proved to increase proliferation and reduce apoptosis of the surrounding normal breast cells. Exo-BCa can restrain immunological responses through regulating the expression of the NKG2D receptor by effector cells, which promotes breast cancer immune evasion.⁽³¹⁾ The NKG2D-positive cells are considerably reduced in a dose-dependent way when exo-BCa is cultured with fresh peripheral blood leukocytes. The reduction of T cells may be one way for breast cancer cells to escape from immune recognition and attack. Exosomes derived from heparanase-high breast cancer cells could enhance the spread of cancer cells.⁽²⁹⁾ Heparanase-regulated exo-BCa is associated with enhanced tumor growth and angiogenesis. Exosomes-transported miRNA and proteins could promote neoplastic transformation and widely participate in different stages of breast cancer development.^(3,32,33) One way by which exosomes promote tumorigenesis is to convert tumor microenvironment to permissive niches. In what follows, we will elaborate on the tumor microenvironment.

Exosomes in Microenvironment, Invasion and Metastasis of Breast Cancer

It is generally recognized that both tumor cells and their microenvironment contribute to tumor progression and metastasis. The significance of tumor microenvironment is enhanced by the inclusion of microenvironment in “hallmarks of cancer.”^(34,35) The tumor microenvironment is comprised of stromal cells, soluble factors, extracellular matrix, signaling molecules, hypoxia and mechanical cues (e.g. exosomes).⁽³⁶⁾ Stromal cells are comprised of fibroblasts, vascular system cells and immune cells, while soluble factors include growth factors, hormones, cytokines and chemokines.^(37,38) Combinations of tumor microenvironment factors can support tumor progression by helping tumor cells to escape from host immunity and drug treatments, and offer niches for metastasis.⁽³⁹⁾ Hypoxia facilitates the release of exosomes by breast cancer cells and, therefore, hypoxic breast cancer cells may release more exosomes into the microenvironment to promote tumor

survival.⁽³⁰⁾ Exo-BCa could lead to induction of reactive oxygen species and autophagy when interacting with primary human mammary epithelial cells (HMEC).⁽⁴⁰⁾ HMEC–exosome interactions also induce the phosphorylation of ATM, H2AX, Chk1 and p53, indicating the induction of DNA damage repair responses and stabilization of p53. The consequential transformation of microenvironment is more suitable for cancer survival. In what follows, we will discuss the role of exosomes (an important part of microenvironment) in invasion and metastasis of breast cancer.

Most breast cancer-induced deaths are due to high invasiveness and distant metastasis of breast cancer. No effective solution can cure advanced breast cancer. Researchers are still trying to understand the drivers of migratory cells.⁽⁴¹⁾ The roles of exosomes in invasion and metastasis of breast cancer are gradually being clarified. Amphiregulin exosomes enhance the invasiveness of recipient breast cancer cells, and exosomes contribute to breast cancer invasion through the epidermal growth factor receptor ligand signaling.⁽⁴²⁾ Exosomes promote cell invasion in breast cancer through the transfer of miR-10b, which is overexpressed in MDA-MB-231 breast cancer cells as compared to non-malignant breast cells.⁽⁴³⁾ In addition, exosomes derived from MDA-MB-231 cells could induce the invasion of non-malignant HMEC. Exosomes modulate tumor microenvironment by delivering miRNA. Tumor-associated macrophages promote the invasion and metastasis of breast cancer through exosome-mediated delivery of invasion-potentiating miRNA to breast cancer.⁽⁴⁴⁾

Using fluorescent proteins as an exosome marker, Suetsugu *et al.* (2013) observe that exosomes produced by breast cancer cells move to other cancer cells and normal lung tissue cells in orthotopic nude mouse breast cancer models.⁽⁴⁵⁾ This direct evidence proves that exosomes transfer from breast cancer cells to stroma at metastatic sites. Adipose tissue-derived mesenchymal stem cells (MSC) could be transformed into myofibroblasts via a Smad-mediated pathway after treatment with breast cancer exosomes.⁽⁴⁶⁾ Tumor-associated myofibroblasts play a key role in tumor cell metastasis by participating in tumor angiogenesis and forming matrix-remodeling proteins within tumor microenvironment.⁽⁴⁷⁾ Fibroblast-secreted exosomes facilitate the protrusive activity and motility of breast cancer cells via Wnt-planar cell polarity signaling.⁽⁴⁸⁾ This study reveals that exosomes from stroma in tumor microenvironment play a critical role in breast cancer metastasis. In addition, MSC-derived exosomes contribute to migration of the breast cancer cell line MCF-7 through the Wnt signaling pathway.⁽⁴⁹⁾ Breast cancer cells interact with MSC to promote breast cancer migration. Exosome-mediated transfer of miR-105 can destroy the tight junctions and the integrity of endothelial monolayers, thereby promoting the metastatic progression of breast cancer.⁽⁵⁰⁾ MiR-105 overexpression in local breast cancer cells enhances vascular permeability and induces metastasis, but these effects are reduced by the suppression of miR-105 in metastatic breast cancer.

Figure 2 presents a summary diagram of exosomes interacting with salivary gland cells and NKG2D-positive cells, and acting in microenvironment, invasion and metastasis of breast cancer.

Exosomes in Breast Cancer Resistance

De novo and acquired resistances to radiation, chemotherapy or targeted therapies are significant challenges in the treatment of breast cancer.⁽⁵¹⁾ Emerging evidence indicates that cancer

stem cells or cancer-initiating cells may contribute to radiation resistance of breast cancer.^(52,53) Deregulation of tumor-associated miRNA, proteins and signal transduction pathways is involved in breast cancer chemoresistance.^(54,55) Trastuzumab resistance is partly induced by cancer-associated fibroblasts through increasing cancer stem cells and activating multiple pathways in HER2-positive breast cancer.⁽⁵⁶⁾ The findings above suggest that various factors participate in development of breast cancer resistance.

Exosomes transfer RNA and proteins to mediate the communication between stromal cells and cancer cells, which can influence treatment response. Exosomes transferred from stromal to breast cancer cells contribute to chemotherapy and radiation resistance through antiviral and NOTCH3 pathways.⁽⁵⁷⁾ These exosomes increase the interferon-related DNA damage resistance signature and enhance the transcription of NOTCH target genes, thereby promoting the resistance through expanding the spectrum of therapy-resistant breast cancer cells. Release of exosomes can be promoted by hypoxia and exosomes that are associated with the radiation resistance of tumor cells under hypoxic conditions.^(30,58) Chronic hypoxia can alter DNA damage repair pathways and thereby induce DNA replication errors and genetic instability, which contribute to radiation resistance.⁽⁵⁹⁾ Therefore, hypoxia-induced radiation resistance may partly be due to the delivery of exosomes. Exosomes from drug-resistant breast cancer cells transmit chemoresistance through the delivery of p-gp and miRNA.^(60,61) Drug-sensitive variant MCF-7 cell line (MCF-7/S) can acquire drug resistance in the presence of exosomes from docetaxel (DOC/exo)-resistant MCF-7 breast cancer cells.⁽⁶⁰⁾ Meanwhile, p-gp expression of MCF-7/S increases and is influenced by the dose of exosomes. These results point to the fact that docetaxel-resistant breast cancer cells assimilate other breast cancer cells to acquire chemoresistance via exosome delivery of p-gp. After coculture with DOC/exo, the general resistance of MCF-7/S is enhanced and levels of some miRNAs are distinctively increased, and thereby, the general resistance of MCF-7/S is enhanced after coculture.⁽⁶¹⁾ In addition, DOC/exo is capable of altering gene expression in MCF-7/S. Accumulation of anti-cancer drugs in exosomes/vesicles that shed out of cancer cells is a drug efflux mechanism involved in drug resistance.⁽⁶²⁾ Docetaxel resistance is related to the enhancement of exosome secretion in a prostate cancer model, probably due to docetaxel efflux through exosomes.⁽⁶³⁾ However, this hypothesis has not been proven in any breast cancer model, and should be verified through further studies. Figure 3 presents a summary diagram of exosomes in breast cancer resistance.

Exosomes in Breast Cancer Therapeutics

Surgical treatment, chemotherapy, radiotherapy, hormone therapy and target therapy are currently recognized as effective methods for treatment of breast cancer. Rapidly-dividing cells, such as breast cancer cells, hair follicle cells, gastrointestinal tract cells and bone marrow cells, are targets of chemotherapy and radiotherapy.⁽⁶⁴⁾ Hormone therapy has been used in patients with positive estrogen receptor and progesterone receptor.⁽⁶⁵⁾ However, the therapeutic effects and palliative/unpredictable responses of the two treatments are limited by the side effects on normal cells. Drug resistance is also responsible for treatment failure. Thus, more attention should be devoted to research on novel therapies for breast cancer. In what follows, we discuss the role of exosomes in breast cancer therapeutics.

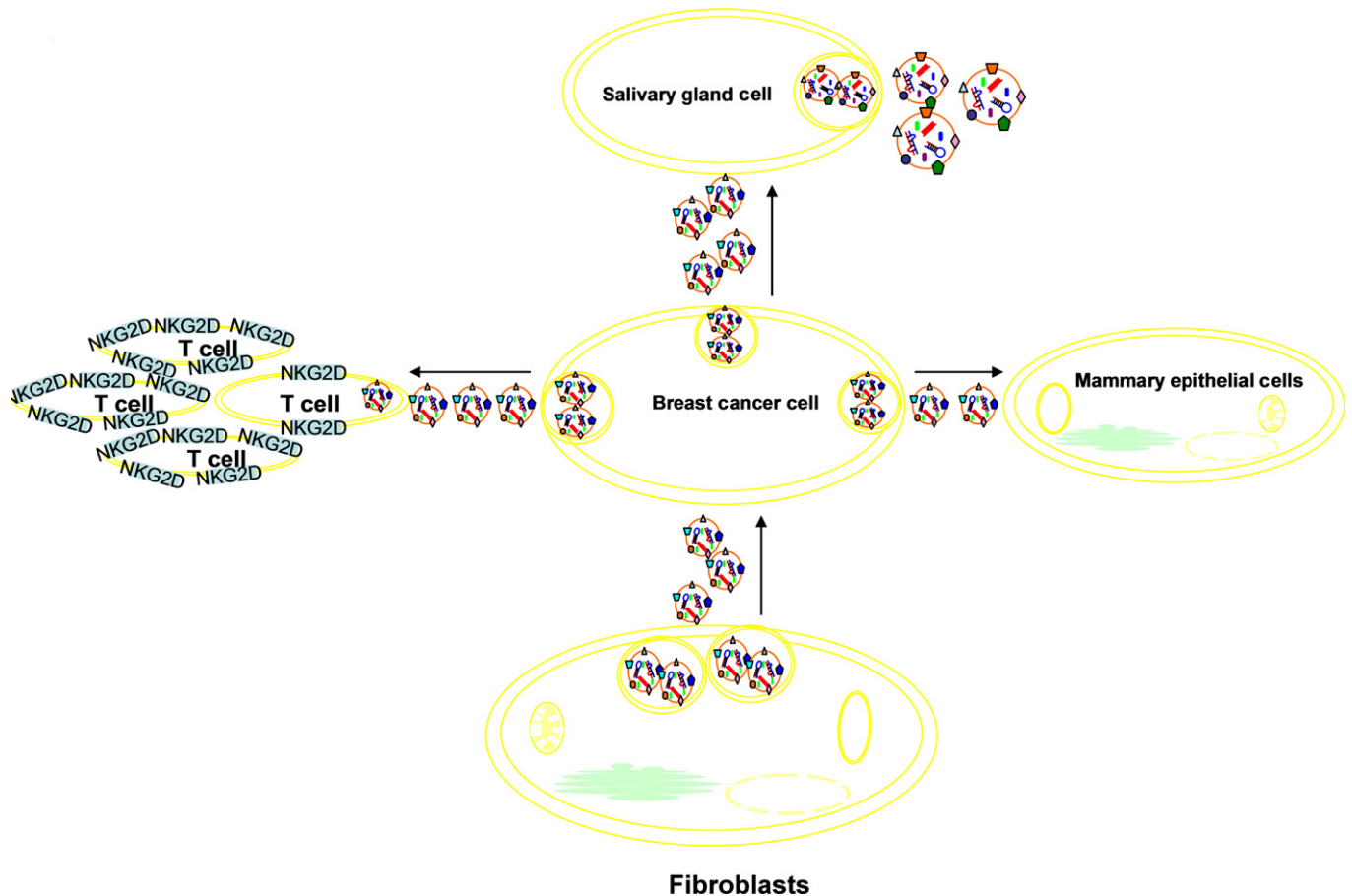


Fig. 2. Exosomes derived from breast cancer (exo-BCa) interact with salivary gland cells, altering the composition of their secreted exosomes, which could be biomarkers of early breast cancer. Exo-BCa reduce NKG2D receptor expression of T cells to protect breast cancer cells from immune recognition and attack. Exo-BCa could induce the invasion ability of non-malignant human mammary epithelial cells. Exosomes secreted by fibroblasts in microenvironment facilitate breast cancer cells' protrusive activity and motility.

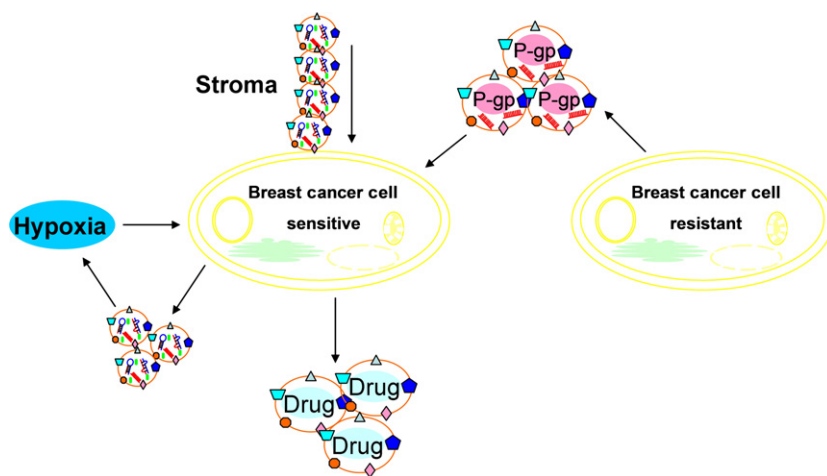
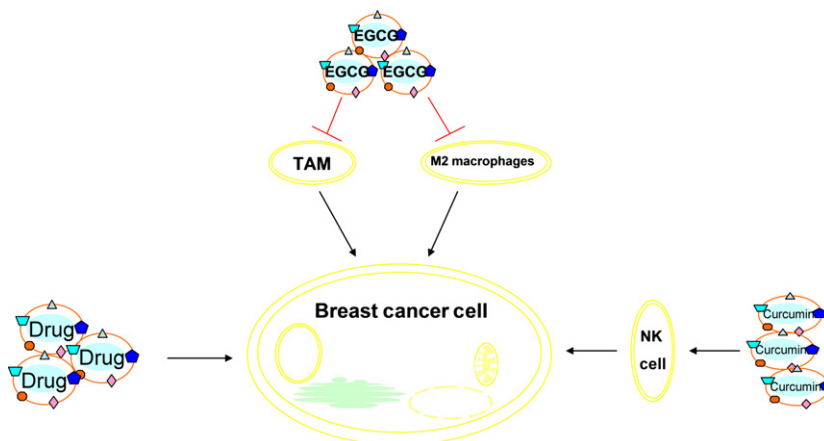


Fig. 3. Exosomes lead to transferred from stroma to breast cancer cells contributed to chemotherapy and radiation resistance. Release of exosomes is promoted by hypoxia and exosomes are associated with radiation resistance under hypoxic conditions. Exosomes from drug-resistant breast cancer cells transmit chemoresistance through delivering p-gp and miRNA. Accumulation of anticancer drugs in exosomes/vesicles that shed out of breast cancer cells is a drug efflux mechanism involved in drug resistance.

Exosomes that are endogenous nano-sized membrane vesicles can be used to carry drugs with low immunogenicity and toxicity.⁽⁶⁶⁾ Exosomes after modification to target breast cancer cells could be used to deliver doxorubicin (dox).⁽⁶⁷⁾ Purified exosomes loaded with dox via electroporation, produced by mouse immature dendritic cells to reduce immunogenicity and toxicity, are highly efficient at targeting and dox delivery, leading to significant inhibition of breast cancer without obvi-

ous toxicity. As reported, exosomes derived from epigallocatechin gallate are able to inhibit infiltration of tumor-associated macrophage and polarization of M2 macrophages, thereby suppressing breast cancer growth.⁽⁶⁸⁾ Exosomes have the potential to worsen the tumor microenvironment for cancer growth. Immunity escape is an important characteristic of breast cancer. Curcumin-pretreated exo-BCa could reverse immune suppression of NK cell activation, which may account

Fig. 4. Exosomes derived from epigallocatechin gallate were able to suppress breast cancer growth by inhibiting tumor-associated macrophage infiltration and M2 macrophage polarization. Exo-BCa pretreated with curcumin could reverse immune suppression of NK cell activation. Exosomes loaded with anti-cancer drugs showed highly efficient targeting and drug delivery, leading to significant inhibition of breast cancer without obvious toxicity and immunogenicity.



for anti-cancer properties of curcumin. In other words, exosomes are able to transmit anti-tumor substances to recover the surveillance of immune system. Exosomes released by adenoviral vector (AdV_{HER2})-transfected dendritic cells proved capable of inducing HER2-specific cytotoxic T lymphocyte responses and protective immunity against trastuzumab-resistant breast cancer *in vitro*.⁽⁶⁹⁾ This novel HER2-T_{EXO} vaccine may offer a better therapeutic choice for HER2-positive breast cancer patients, especially trastuzumab-resistant patients. Nevertheless, there are also some unsolved problems in the clinical application of exosomes. The acquisition of plenty of exosomes is inconvenient owing to the low number of exosomes. Thus, researchers must seek better methods to produce plenty of exosomes without great cost. The way in which exosomes should enter the body is also controversial. Intravenous injection is convenient but the exosomes should be targeted to breast cancer cells, which is a challenge for exosome design. Local injection avoids the problem of targeted delivery design, but it faces new problems. Whether locally injected exosomes are absorbed effectively and cover all breast cancer cells remains unresolved. Figure 4 presents a summary diagram of exosomes in breast cancer therapeutics.

Conclusions and Prospects

Exosomes are nano-sized vesicles that mediate inter-cellular and intra-cellular communication. There is growing evidence

that exosomes play an essential role in the process of pathological states. Exosomes could function as biomarkers of various cancers, including breast cancer. The contents and quantity of exosomes released by breast cancer cells change compared to normal breast cells. Dysregulation of exosomes in body fluid indicates that diseases may lack sensitivity and specificity. Exosomes are potential carriers of drugs targeting breast cancer cells. In this article, we review studies on how exosomes act as a drug delivery tool *in vitro*. However, there is a long way to go before exosomes can be utilized to establish a drug delivery system *in vivo*. Further studies are required to better understand the role of exosomes in the occurrence of breast cancer. In conclusion, we summarize the main roles of exosomes in the occurrence and progression of breast cancer, and provide a new perspective on the drug delivery system.

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Disclosure Statement

The authors have no conflict of interest to declare.

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