OncoTargets and Therapy

Dovepress
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REVIEW

The Exosome And Breast Cancer Cell Plasticity

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Abstract: Cancer cell plasticity is the ability of cancer cells to reversibly interchange between distinct cell status, which plays a key role in cancer progression. Cancer cell plasticity is now known to be shaped by the secreted nanoparticles termed exosomes which transport proteins and lipids as well as nucleic acids. These aspects have emerged as key determinants of tumor progression and targeting, with approaches such as immunotherapy showing promise in the clinic. While significant strides have been made in this research area, some very interesting questions still warrant more and deeper investigation. We provide a review of the interplay between exosomes and breast cancer cell plasticity, and the potential implication in metastases and drug-resistance.

Keywords: exosome, breast cancer, cancer cell plasticity

Introduction

Cancer cell plasticity refers to the ability to reversibly interchange between distinct cell status. It includes interconversion of different subtypes of cancer cell pools, activation of facultative cancer stem cells (CSCs), transdifferentiation or dedifferentiation, phenotypic transition of differentiated cells within a tumor to meet the challenges imposed by new microenvironments that accompany metastasis and by therapeutic interventions, and the dramatic habitat changes that accompany metastasis.[1] Breast cancer is the most commonly diagnosed cancer in women worldwide, also the second leading cause of cancer death among women after lung cancer, and accounts for more than 500,000 deaths annually worldwide.[2] It is also a complex heterogeneous disease which differs greatly among different patients (intertumoral heterogeneity) and even within each individual tumor (intratumor heterogeneity).[3] Breast CSCs have differentiation and transdifferentiation abilities. CSCs produce the original lineage cells similar to their normal stem cell counterparts. To promote tumor growth and metastasis in some tissue contexts, CSCs can also transdifferentiate into other lineage cells in addition to recruiting stromal cells from local or distant tissues. Following transformation and progression to malignancy, breast cancer cells do not remain inert but adapt to their systemic and local environment in order to evade death, proliferate and form metastases. This adaptive capacity is a property of cell plasticity. The forging of closer ties between preclinical, translational, and clinical research, together with advances in cancer models and single-cell technologies has revealed an unprecedented level of intra- and inter-tumoral heterogeneity and plasticity, and has started to reveal the pathway via which cancer cells circumvent therapeutic targeting. Cancer cell plasticity is also shaped by the secreted nanoparticles termed exosomes which can transport cellular contents such as proteins and lipids, as well as nucleic acids. Elements of contents of exosomes are now known to regulate cancer progression, tumor

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http://doi.org/10.2147/OTT.S214133

heterogeneity, and therapeutic resistance. While there have been significant strides in this research area, a lot of interesting questions still warrant deeper investigation. This review aimed to provide an in-depth viewpoint of the relations between the exosomes and breast cancer cell plasticity so as to better understand and defeat metastases and drug-resistance.

Plasticity In Histopathology Of Breast Cancer

Breast cancer varies in morphology, immunohistochemical profiles, and histopathological subtypes which have their unique clinical characteristics and individual outcomes. Morphologic plasticity in breast cancer is the representation of the histopathologic heterogeneity.[4] Breast cancer includes multiple histologic types, most are adenocarcinoma and invasive ductal cancer of no special type.[5,6] WHO classification defined 21 distinct histological special types which include invasive lobular carcinoma, apocrine carcinoma, medullary carcinoma, adenoid cystic carcinoma, metaplastic carcinoma, micropapillary carcinoma, mucinous carcinoma, infiltrating ductal carcinoma with osteoclastic giant cells, neuroendocrine carcinoma, tubular carcinoma, invasive cribriform carcinoma, secretory carcinoma, lipid-rich carcinoma, glycogen-rich clear cell carcinoma, and so on. Different pathologic types have different prognosis and outcome following routine systemic therapy. Tubular, mucinous, medullary carcinoma and papillary carcinoma have favorable prognosis and better outcome than classic invasive ductal carcinoma.[6,7] However, histological typing is not enough in clinical management decisions. Histological grade can provide complementary prognostic information which is based on the degree of differentiation, which also highlights the plasticity of breast cancer heterogeneity. The grade is divided into low, intermediate or high based on the morphological parameters, namely the percentage of the tumor arranged in glandular and tubular structures, the degree of nuclear pleomorphism, and the mitotic rate.[8] For decades, the histologic grade has been an important predictor of breast cancer outcome and helped to figure out what treatments might work best.[9,10] In the last decade, gene expression profiling classified breast cancer into 5 intrinsic subtypes (Luminal A, Luminal B, Claudin-low, HER2-enriched, Basal-like) and a Normal Breast-like group.[11–13] Different subtypes of breast cancer differ in incidence, survival and response to treatment.[14–18] The molecular subtype information is complementary to classical clinical-pathological stage, and together can influence patients' outcome and response to the treatment.[19] For example, Luminal A subtype is associated with a low risk of local or regional recurrence,[1,20] basal-like and triple-negative non-basal subtype have higher frequencies of relapse in lung, brain, and distant nodal metastasis than other subtypes.[21] Recent molecular research provides personalized treatment options which are based on significant numbers of publications in genomic profiling of breast cancer. Most molecular studies of breast cancer focuses on just one or two high information content platforms. Actually, breast cancer is a heterogeneous disease which is comprised of multiple distinct subtypes of cells that differ genetically, pathologically, and clinically. Plasticity also means “One tumor, different entities”.

The Biological Characteristics Of Exosome In Breast Cancer

An important “cross-talk” between cancer cells and its surrounding microenvironment is fundamental, just like “inter-sectional crosstalk of seed and soil”. Cells secrete extracellular vesicles (EVs) into their local environment or body fluids such as saliva, urine, serum, as well as cerebrospinal fluid, and so on. Exosomes are small EVs (30–100 nm in diameter) compared with microvesicles (50–1000 nm in diameter) and apoptotic bodies (1–5 μm in diameter).[22] The exosomes have pleiotropic functions in pathological and physiological processes, are novel mediators of cell-cell or cell-environment communication and activate signaling pathways in cells when they fuse or interact.[23,24] Exosomes can fuse with multivesicular bodies through the plasma membrane after being secreted into the extracellular environment. Exosomes can be isolated by techniques such as ultracentrifugation, ultrafiltration and immunoprecipitation technologies which all exploit the characteristics of exosomes, such as their size, density, shape, and surface proteins, to aid their isolation.[25–27] The contents of exosomes may vary depending on cell of origin, status of activation and cell fate, but they have some particular contents, especially those involving vesicle biogenesis and intracellular sorting. They contain the proteins which are involved in membrane transport and fusion (flotillin, GTPases, annexins), tetraspanins family proteins (CD9, CD63, CD81 and CD82), heat-shock proteins (Hsp 60, Hsp70, Hsp90, HSPA5 and CCT2), proteins involved in biogenesis of multivesicular bodies such as TSG101 or ALIX, and lipid-bound proteins which account in part for the increased membrane rigidity relative to parent cell membranes.[28–35] Previous proteomic research indicated

the exosomal proteome from MDA-MB-231 cells is distinct compared with MCF7. Periostin, integrin-β1, β-catenin, and N-Cadherin were enriched in the MDA-MB-231-derived exosomes compared with MCF7.[36] The tetraspanins family members (CD9, CD63, CD81 and Tetraspanin-14 antigens) are increased in the exosomes from MCF-7 compared with those from MDA-MB-231.[37]

The Effect Of Exosome On The Dialog Between Breast Cancer Cells And Stromal Cells

The cancer surrounding stroma is the tumor-nourishing compartment in the tumor microenvironment responsible for the process of carcinogenesis and advancement. The stroma is composed of the extracellular matrix, endothelial cells, fibroblasts, adipocytes, and cells of the immune system which regulate the behavior of and co-evolve with tumor cells.[38,39] The long-known "seed and soil" hypothesis for carcinogenesis and metastasis postulates that the appropriate host microenvironment (the soil) and the optimal growth of tumor cells are reciprocal.[40] The cancer cells and their microenvironment interact reciprocally as intimate partners during the progression of breast cancer.[41] Stromal cells provide matrix components or soluble factors that increase cancer cell survival and growth, which also promotes phenotypic plasticity in cancer cells, helping to acquire a more aggressive phenotype and influences treatment response.[42–44] Previous research indicated that RNA within exosomes transferred from stromal to breast cancer cells can activate STAT1-dependent antiviral signaling which is involved in the antiviral/NOTCH3 pathways in NOTCH signaling in breast cancer.[44] Both Notch pathway and antiviral/interferon signaling are known to regulate the maintenance of normal and cancer stem-like cells in cancer therapy resistance.[45] And breast cancer cells' exosome can destroy the tight junctions of vascular endothelial cells which are involved in the process of metastasis.[46] Cancer-associated fibroblasts (CAFs) are major stromal components which affect all aspects of tumor evolution, they build up and remodel the ECM structure through secretion of growth factors, cytokines, and chemokines.[47–49] The miRNA from the breast cancer exosome has been implicated in the intercellular crosstalk also. The breast-cancer-secreted, extracellular-vesicle-encapsulated miR-105 can mediate metabolic reprogramming of CAFs via MYC signaling. These CAFs in turn promote breast cancer growth by conditioning the shared metabolic environment. miR-105-reprogrammed CAFs promote glutamine and glucose metabolism to nourish adjacent breast cancer cells with sufficient nutrients, thus detoxifying metabolites under extreme metabolic conditions.[50] The exosomal G protein-coupled receptor, sphingosine-1-phosphate receptor 2 derived from MDA-MB-231, can promote CAFs' proliferation via activating ERK signaling.[51] The MMP-2 and MMP-9 in cancer cell exosomes can degrade components of the extracellular matrix and facilitate the aggressive cancer cells to invade surrounding tissue.[52]

The Metabolism Plasticity And Exosome

An emerging hallmark of cancer is the altered metabolism, cancer cells experience complex metabolic rearrangement to sustain cancer growth by changes in metabolic pathways in biosynthetic processes and energy production.[53] The metabolic plasticity is paralleled by the metabolic interactions that occur between distinct tumor cell populations within the tumor, as well as between stoma and tumor.[54] Cancer cells of various origins displayed distinct metabolic strategies, and different tumor cell subtypes within a particular type of cancer can metabolically adapt due to distinct metabolic strategies.[55–59] The metabolic remodeling can satisfy the biosynthetic demand to support their abnormal proliferation and dissemination in nutrient-deprived and poorly oxygenated microenvironment.[60] Breast cancer metabolism heavily relies on aerobic glycolysis and glutamine catabolism to support cancer cell growth.[61–64] The different subtypes of breast cancer have different metabolisms. The triple-negative breast cancers (TNBC) typically related with the Warburg and mixed type, luminal type has obvious reverse Warburg and metabolic null type, estrogen receptor-positive breast cancers may rely on oxidative phosphorylation.[7,55,57–59,65] And the hormonal therapy can abrogate oxidative phosphorylation generating self-renewal-deficient cancer cells in luminal breast cancer.[66] Notch signaling was enhanced to promote self-renewal of CSCs that display high glycolytic activity and aggressive hormone-independent tumor growth in vivo.[67–69] The Warburg type and the mixed type correlated with higher Ki-67 labeling indices which accompany high ATP synthase and glutaminase expression in stroma.[55] TNBC cells have special metabolic characteristics manifested by high glucose uptake, increased lactate production, and low mitochondrial respiration which is correlated with attenuation of mTOR pathway and decreased expression of p70S6K.[65] According to Warburg's hypothesis, cancer cells are dominated by aerobic glycolysis as main mode of increased uptake of glutamine, glucose and aerobic glycolysis instead of

more efficient oxidative phosphorylation. Cancer cells rely heavily on glucose and convert it to pyruvate through glycolysis rapidly. The glycolytic enzymes are commonly identified in the content of breast cancer exosome, such as enolase, aldolase, fructose bisphosphatase 1, triosephosphate isomerase, phosphoglycerate kinase, GADPH, and so on.[70,71] They are also intrinsically associated with oncogenic switch, resistance to chemotherapy and radiotherapy.[72] Moreover, microRNA (miRNA) or long noncoding RNA (lncRNA) transferred by exosome is emerging as important regulators of cellular metabolism. The exosomal miR-155 of breast cancer cells triggers cancer-associated cachexia to promote metastasis on the catabolism of adipocytes and muscle cells via PPARγ. It promotes the beige/brown differentiation, remodeled resident adipocytes' metabolism through downregulating the expression of PPARγ[73] Other research indicated that cancer-cell-secreted exosomal miR-122 can restrain glucose utilization through suppressing glycolytic enzyme pyruvate kinase in niche cells of pre-metastatic niches, which can reprogram energy metabolism to accommodate the massive energy needs of cancer cells during metastatic growth.[74] The exosomal HIF-1α-stabilizing lncRNA from tumor-associated macrophages inhibits glycolysis and apoptosis resistance of breast cancer cells.[75] The breast cancer cells can communicate through direct or indirect contact, such as the secretion of exosomes, to adapt to the shifting condition, metabolic cause lower glucose concentration and higher acidity subsequently suppressing infiltrated immune cells, contributing to cancer immune evasion and cancer aggressiveness.

Role Of Exosome In Plasticity In CSCs Of Breast

The hallmark feature of CSCs is reported to be self-renewal, and CSCs can differentiate into multiple subpopulations of cells within tumors.[76,77] CSCs can regenerate tumors which recapitulate the heterogeneity of primary tumor from which they were isolated following orthotopic transplantation into mice. However, CSCs also induce resistance to anticancer therapy. The plasticity of the bidirectional conversion between non-CSCs to CSCs status is so complicated. The plasticity of CSCs refers to both reversible mesenchymal transitions and acquisition of stemness traits, which induce metastatic dissemination and development of resistance to treatments. The exosome derived from CSCs (CSC-exo) contained self-renewal promoting regulatory miRNAs, stemness specific proteins, and survival factors which can regulate tumor microenvironment and maintain tumor heterogeneity.[78] The CSCs reside in CSCs niches, which is a distinct protective microenvironment which regulates stemness, proliferation, and therapeutic resistance.[79,80] The exosomal miRNAs of breast CSCs can promote the aggressiveness of cancer cells through nearby immune cells via interaction with toll-like receptors to up-regulate secretion of TNFα and IL-6 secretion.[81] And the dietary chemopreventive compound sulforaphane could promote exosomal miR-140 secretion of breast CSCs which prevents stemness in recipient cells in in vivo rat breast cancer models. The breast cancer stem cell exosome can modulate CSCs niche which is a vital aspect of exosome signaling in cancer. Lee et al revealed that exosome of osteogenic differentiating human adipose-derived stem cells can promote the drug resistance of breast CSCs by reprogramming of tumorigenic CSCs into non-tumorigenic cells, increasing the expression of osteogenic-related genes and decreasing the expression of drug-resistance genes such as ATP binding cassette transporter, the breast cancer gene family and the ErbB gene family.[82] Stemness-related molecules can be transferred from breast CSCs to non-CSCs by exosome, which leads non-CSCs to regain stemness phenotype. CSC-exo induced dynamic or transient tumor plasticity in the tumor microenvironment.[83] It has also been investigated as potential therapeutic agents, so targeting the CSC-exosome transfer may have great potential for breast cancer therapy. The transcription factor ZEB1 and H3K27me3 histone modifications involved in the plasticity that the normal and CSC-like cells can arise de novo from more differentiated cell types and that hierarchical models of mammary stem cell biology should encompass bidirectional interconversions between stem and nonstem compartments.[84] The poised chromatin at ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity; the therapies targeting non-CSCs-to-CSCs plasticity should offer improved clinical outcome for breast cancer patients.[30,85,86] The interactions of CSCs and their surrounding microenvironment affect breast cancer cell malignancy directly and leads to tumor initiation, epithelial-to-mesenchymal transition (EMT), mesenchymal-to-epithelial transition (MET), metastasis, and therapeutic resistance. Recent single-cell studies in breast cancer have suggested that metastases derive from CSCs accompanied with increased MYC expression and CDK inhibition, which differentiate and undergo a switch from dormancy into proliferation as they colonize and produce more advanced metastatic tumors.[87] The stemness of hybrid epithelial/mesenchymal state in breast cancer is associated with poor survival; the plasticity to transition between EMT and MET can be the target to improve breast

cancer patient survival independent of breast cancer-subtype.[88,89] Due to most of current CSCs surface markers of breast appear to be present on embryonic or adult stem cells, and they are rarely expressed on normal breast tissue cells, e.g., CD10 and CXCR4.[90] In the future, multiple-antibody coated exosomes will need to be engineered to improve their CSCs targeting efficiency and to eradicate the CSCs and tumor plasticity, ideally.

Effects Of Exosome In EMT/MET Plasticity Of Breast Cancer Cells

EMT is a biologic process defined as the loss of epithelial characteristics and the acquisition of mesenchymal phenotype. Epithelial cells undergo multiple biochemical changes such as a loss of the epithelial traits of tight cell-cell adhesion and apico-basal polarization and a gain of invasiveness, enhanced migratory capacity, elevated resistance to apoptosis, and greatly increased production of ECM components. The reverse process of EMT is the transition from motile, multi-polar or spindle-shaped mesenchymal cells to the epithelial cells, i.e., MET. The EMT/MET plasticity has been observed preclinically and clinically, whether any of these phenotypic transitions are indispensable for metastatic outgrowth remains unclear. It is involved in various pathophysiological processes including migration, treatment resistance and metastasis of breast cancer. Breast cancer cells acquire the increased motility and invasiveness along with EMT and re-epithelialize to form a metastatic solid mass under MET.[91,92] Under EMT, cancer cells lose their polarity and cell-cell junctions and turn into a low proliferation state with increased migratory and invasion capabilities which are strongly associated with activation of Zeb (zinc finger and homeodomain proteins Zeb 1 and 2), Snail (zinc finger proteins Snail and Slug), and Twist (basic helix-loop-helix proteins E12, E47, Twist1, Twist 2 and Id) pathways.[93,94] Once the cancer cells have reached the distant premetastatic niche, the reverse process takes place. It is a process called MErT (mesenchymal to epithelial reverting transition) which can return tumor cells to a high proliferative state and enables formation of macrometastases.[30,86] The phenotypic plasticity that enables the crossover of EMT/MErT is necessary for tumor metastasis. EMT/MET plasticity implies switching on/off a set of genes which is mainly orchestrated by specific "master" transcription factors,[95,96] miRNAs and lncRNAs. Twist1, ZEB1, ZEB2, Snail1 and Slug, as key EMT-inducing transcription factors, are involved in breast cancer metastasis through different signaling cascades such as serine/threonine-specific protein kinase (Akt), wingless-related integration site (Wnt), signal transducer and activator of transcription 3 (STAT3), and mitogen-activated protein kinase (MAPK) pathways, by repressing epithelial-related genes.[97–100] Exosomes contain active proteases capable of ECM degradation and remodeling by selectively and directly binding to the ECM-binding motif present on exosomal surface adhesion proteins.[101] Exosome biogenesis is enhanced by invadopodia and drives invasive behavior in cancer cells including breast cancer.[102] The exosomal miRNA secreted by breast cancer cells can enhance cell motility of normal fibroblasts and in turn is able to stimulate tumor cell migration by modulating its direct target, E-cadherin.[103] The normal hepatic niche-derived exosome can modulate MET process during seeding and suppression of tumor growth once the breast cancer cells have reached the liver.[104] Previous results indicated that the plasticity of EMT/MET phenotypes of breast cancer cells can be modulated by exosomes; primary cancer cells preserved their own niche and gave cells with aggressive traits necessary to colonize other free niches by exosome.[96] The communication resulted in relevant plasticity changes of gene expression of recipient cells in addition to microenvironment alterations. Exosome biogenesis is observed in immune cells, mesenchymal stem cells, neurons, fibroblasts, endothelial cells (ECs), and epithelial cells. Breast cancer cells secrete exosomes with specific capacity for cell-independent miRNA biogenesis, while normal cells lack this ability. Exosomes derived from cancer cells and serum from patients with breast cancer contain the RISC loading complex proteins, TRBP, Dicer and AGO2, which process precursor microRNAs into mature miRNAs.[105] The exosomes transferred from stromal to breast cancer cells can expand therapy-resistant breast cancer cells; RNA within exosomes stimulates the pattern recognition receptor RIG-I to activate STAT1-dependent antiviral signaling.[30]

Plasticity-Based And Exosome Therapy For Breast Cancer

Breast cancer treatment includes surgery, chemotherapy, hormone therapy, radiation therapy, and targeted therapy. Plasticity in breast cancer cells within the same tumor is a reason for therapeutic resistance or later relapse because of genetic change, environmental differences, and reversible changes in cell properties. Some strategies target the tumorigenic cells as a result of minority populations of CSCs as they contribute to tumor growth and disease progression, while most other cancer cells have little or no capacity to drive tumor growth.[106,107] A key question raised regarding the plasticity within the same

tumor or among breast cancer patients is whether multiple pathways are important and whether they should be targeted simultaneously. Therapy failure may also contribute to tumor cell plasticity. The exosome has a close relationship with cancer cells' plasticity, so how can we make full use of plasticity-based and exosome therapy for breast cancer? First, CD47, HER-2, miR-21 and miR-1246 breast cancer patients' exosomal biomarkers, exosome-carrying TRPC5 and GSTP1 correlated with chemotherapy resistance, TRPC5, NEUROD1, HTR7, NANOG, HOXC and KISS1R in exosome were related with PFS, DFS or OS of breast cancer.[108] Second, the proposed targeting of the phenotypic plasticity will prove beneficial and to eradicate the exosome induced the key transcription factors involved in the alternation of EMT-MErT and non-CSCs-to-CSCs is providing new potential avenues for targeting the properties associated with cancer cell plasticity. Currently, post-translational modifications such as Ubiquitin and Ubiquitin-like modifiers in exosome were proposed to alter exosomal protein in cancer therapy.[109] Strategies to destroy the release of exosomes and exosome-mediated plasticity can potentially be exploited therapeutically in the future, including ESCRT (endosomal sorting complexes required for transport)-dependent and independent systems, tetraspanins and lipid-dependent mechanisms.[110] Third, as exosomes can mediate cell-to-cell communication, exosomes may be exploited as drug delivery vehicles with long-term safety and natural ability to carry intercellular nucleic acids and therapeutic molecules across membranes difficult to cross, such as BBB.[111] More research is needed.

Disclosure

The authors report no conflicts of interest in this work.

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