Review Article

Influence of Microvesicles in Breast Cancer Metastasis and their Therapeutic Implications

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Abstract

Microvesicles are membranous sac structures released from cell surfaces of many eukaryotic cells. Their presence in the blood and urine also signify their potential use as biomarkers for early detection and diagnosis of different diseases. At present, synthesis and release of these vesicles from mammary tumor cells and their role in disease progression requires further research. In this report, correlation of microvesicles along with breast cancer metastasis has been explored. Metastasis is a process of a non-randomized set of events, which begins with a loss of cancer cell adhesion at the primary tumor site. Later on, these cells invade the surrounding tissue and enter into circulation. After compromising host immune response, these cells extravasate and localized at the suitable distant site for a secondary growth. Involvement of microvesicles in modulating this process has also been observed. Microvesicles released from primary cancer cells may carry mRNA, miRNAs, DNA and various proteins. These vesicles may also influence multi drug resistance as observed in breast and leukemia cancer cell lines. A thorough understanding of microvesicles synthesis and their potential implication in metastasis would facilitate the design of novel therapeutic approach for breast cancer.

Keywords: Metastasis, microvesicles, multidrug resistance

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Introduction

Microvesicles (MVs) are membranous sac like structures originating from the plasma membrane of different eukaryotic cells. MVs contain tissue factor (TF) and several other cell surface receptors for molecular cross-talks with extracellular environments.[1–3] MVs may contain mRNA, miRNA, DNA and proteins. Numerous factors such as cancer type, cell cycle and tumor microenvironment influence on vesicle size, composition and release. In recent years, the involvement of these vesicles in regulating the immune response, multi drug resistance, cell apoptosis, angiogenesis and inflammation in different cancers have been observed.[4–8] Membrane surfaces enriched with phospholipid, phosphatidlyserine (PS) and lipid raft (cholesterol and glycosphingolipids) are ideal MV release sites. These regions are responsible for the synthesis of MVs via outward bulging, constriction, budding and release from their respective cells.[9–11] Earlier, it has been identified that MVs differentiate from exosomes on the basis of three factors a) mode of synthesis, b) composition, and c) size of vesicles. Exosomes are formed by invagination of cell membrane rather than membrane bulging outside. Exosomes are smaller than MVs with a size range from 30 nm – 100 nm. MVs size usually ranges from 100 nm – 1μm with a maximum shelf life of up to 1 hour.[12] Exosomes also retain distinct multivesicular bodies (MVD) with the relatively reduced amount of PS as compared to MVs.[13]

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Association of Microvesicles (MVs) with Different Cancer

The prognostic significance of these vesicles in relation to different cancer, including ovarian,[14] gastric,[15] head and neck,[16] brain tumor,[17] and lung cancer,[18] have also been explored. Previously, a distinct protein profiling of MVs released from colon cancer cases had been observed. Involvement of at least 49 proteins released from MVs was correlated with tumorigenesis and metastasis. Data generated from this research not only elucidate the biogenesis of MV but also support the design of therapeutic strategies for colorectal cancer.[19]

Over expression of both FAK (focal adhesion kinase) and EGFR (epidermal growth factor receptor) was significantly correlated with stage-wise cancer progression.[20] Association of both FAK and EGFR in angiogenesis and metastasis of colorectal cancer has also been observed in another study.[5] Due to cancer heterogeneity, a panel of factors responsible for MV germination is still lacking. However, in the subsequent section, an effort to summarize all those molecules influencing MV synthesis and release has been discussed with a prime focus on breast cancer metastasis.

Upstream Factors for Triggering MVs Synthesis

Broadly, factors responsible for MV germination are divided into two distinct but interlinked categories.

Intrinsic Factors

Involvement of ARF6 (ADP ribosylation factors 6) in invadopodia formation was frequently observed in breast cancer cell lines.[21,22] ARF6 triggers protease release via an ERK mediated pathway to alter cell morphology. These proteases were released in MV packaging from cancer cells in the vicinity as observed in both *in vitro* and *in vivo* models.[23] Furthermore, the specialized proportion of cholesterol, PS and phosphatidylethanolamine (PE) also regulate vesicle formation. These components mediate membrane bending, aggregate amphipathic moieties in the lipid

bilayer, and tensile strength required for MV synthesis,[24] Cytoskeleton proteins (actin and myosin) rearrangements also induce a tensile strength for MVs release.[23] Three enzymes (flippases, floppases, and scramblase) are found responsible for phosopholipids mobility within the membrane. A prognostic association of these enzymes in colorectal patients had also been reported.[24] However, their involvement in breast cancer metastasis requires further research. Hence, MVs formation is influenced by the activation and regulation of several intrinsic factors.

Extrinsic Factors

Extrinsic factors regulate MVs synthesis as observed in various cancer cell lines. Higher number of MVs was released from chemo-insensitive cell lines when compared with chemo-sensitive lines. Earlier excessive release of cisplastin (CDDP) along with MRP2, ATP7A, and ATP7B proteins in CDDP resistant human ovarian cells was observed.[25] Similarly, release of chemotherapeutic drugs in chemo-resistant cells belonging to breast, colon, leukemia, ovary, prostate origin in MVs, have studied.[26] These resistant cells use MVs to cargo various apoptotic factors, along with chemotherapeutic drugs destined for cancer cells death. This phenomenon is further explained in subsequent sections of effect of MV on drug resistant. Cancer cells also release pro-apoptotic factors (like caspase 3) in intercellular space as a self-protective mechanism using these vesicles. This phenomenon of caspase-3 inactivaion is observed in MCF-7 cells lacking caspase-3 expression.[27] Hence, cancer cells secrete MVs as a defense mechanism against pro-apoptotic signals and as a drug resistant response.

Involvement of MVs in Breast Metastasis

Cancer metastasis is a set of non-randomized events starting from cancer cell proliferation, release from the primary site, loss of cell adhesion, invasion across the basement membrane, motility, localization at distant sites, and proliferation at secondary sites. Identification and role of MVs at different stages of metastasis are discussed in the subsequent section.

Effect of MVs in Cell Transformation

Cancer cells trigger oncogenic response either in the vicinity or at distant site via released vesicles. MVs are also responsible for the transformation of epithelial cells and fibroblasts by as observed in MDA-MB-231 and U87. After exposure to MVs both aforementioned cell types showed anchorage independent growth and enhanced survival.[28] Similar findings of non-aggressive cell transformation to aggressive state, were also observed in glioma under the influence of EGFRVIII enclosed MVs. Cell transformation is also influenced by several other factors including mitogen activated protein kinase, and TFs.[29] Furthermore, TFs also impart a salient role in cancer cell proliferation. TF retaining MVs release is regulated by loss of p53 inactivation, or internalization of E-cadherin receptor, as well as hypoxia and k-ras activation.[30] Apart from cancer cell proliferation, TF also significantly triggers thrombosis formation, ultimately leading to around 30% venous thromboembolism related deaths in cancer.[31,32] Thromobus formation is relatively a less frequent event in breast cancer affected patients with a worse prognosis.[32] However, promiscuous interactions of MV usually lead to cell transformation and increase the emboli formation in cancer metastasis.

Effect of MVs on Cell Adhesion and Invasion

MVs play a vital role in extracellular matrix degradation (ECM) as frequently observed in highly invasive breast cancer cell lines. Increased MMP-2 (matrix metallo proteinase-2) and uPA (urokinase plasminogen activator) expression were detected using gelatin and casein zymography methods.[33] Over expression of uPA in breast tumors was also significantly correlated with poor prognosis.[34] These tumor cells also secrete another molecules, termed as cath-D (cathepsin D) enclosed in these vesicles. Once in intercellular space cath-D cleaves pro-apoptotic proteins, and induces degradation of ECM.[5,35] Thus, vesicles are the key players responsible for not only transporting protease outside the cell but, also to regulate cell invasiveness. Role of integrin (β) presents on MVs membrane has also facilitate cancer cell adhesion with extracellular cellular matrix (ECM).[23] The summarized list of proteins identified in these vesicles, in context to breast cancer metastasis has been mentioned in Table 1.

Effect of MVs on Cancer Cells Motility and Localization

After extracellular degradation, cancer cells traverse through the intercellular spaces forming an amoeboid phenotypic appearance. This epithelial to mesenchymal transition (EMT) is influenced by regulators like transcription factors snail, SIP1, twist and vesicles.[36] MVs modulate immune response, epithelial mesenchymal transition, localization at distant sites via genetic and protein

Table 1. Proteins retrieved from microvesicles and their role in breast cancer metastasis

Molecules	Effect on Cancer Metastasis	References
ARF6	Release of microvesicle from parental cells	Hashimoto, et al.[21]
Lipid raft	Phosphatidlyserine and associated factors influence microvesicle formation, blugging constriction on cell membrane	Martins, et al.[48]
FAK, EGFR	Significant correlation with tumor stage and grading	Galindo-Hernandez, et al.[20]
Caspase 3 Cathepsin-D	Transport of pro-apoptotic factors from tumour cells to outside	Wesierska-Gadek, et al.[27] Masson, et al.[35]
Tissue factor	Results in thrombus formation in cancer patients	Owens and Mackman,[31]
Integrins	Cancer cells adhesion with basement membrane	Muralidharan-Chari, et al.[23]
MMPs	Involved in cancer cell invasion and degradation of extracellular matrix	Ginestra, et al.[49]
uPA	Involved in cancer cell induce degradation of extracellular matrix	Annecke, et al.[34]
CD45	Potential to be used as biomarker	Toth, et al.[39]
CD44	Induces chemokines based suppression of cells proliferation	Jaiswal, et al.[40]
p-glycoprotein MRP1	Responsible for chemoresistance in breast cancer therapy	Roseblade, et al.[45]

transformation to recipient cells. Their interaction with circulating monocytes also inhibit their differentiation into antigen presenting cells as observed in melanoma and colorectal cancers.[37] Involvement of these vesicles in suppressing breast cancer affected patients immunity is an area that requires further research.

Use of MVs as Biomarker

Numerous surface receptors on MVs membrane make them a suitable choice to be used as a diagnostic tool for cancer detection. Abundance of CD44, CD63, FasL, HLA, carcinoembryonic antigen (CEA), CA15-3 and CD45 along with disease progression have been observed in several published reports.[2,38,39] However, no significant correlation of annexin V, endothelial cell-derived microparticle and von Willebrand factor antigen (vWF) with disease prognosis has been reported so far.[39] This study suggests CD45 associated vesicles, as a potential biomarker for breast cancer. The cell adhesion protein (CD44) is exclusively present on the breast cancer cell-derived MVs as compared to leukemic cell-derived MVs.[40] Anti-tumor effect of human monocytes was also suppressed by CD44 as observed in later studies. Hyaluronan and CD44 mediated interaction between cancer cells and monocytes led to reduction of tumor necrosis factor (TNF), IL10, and IL12p40.[41] These vesicles assist in escaping host immunity, suppression of cell apoptosis, and tumor restriction. Hence, certain receptors present on breast cancer cell-derived MVs may be used as potential biomarkers.

Effect of MVs on Drug Resistant

The role of MVs in impeding drug resistant using two important proteins belonging to ABC (ATP Binding Cassette superfamily) is worthwhile to mention here. These are termed as p-glycoprotein and MRP1 (Multi drug Resistant associated Protein 1). Being membrane transporters, these molecules influence a wide range of unrelated drugs and are categorized in MDR (multi drug resistant) pool.[42] A majority of chemotherapeutic drugs are exported in extracellular space by these aforementioned proteins. They were found to be responsible for packaging and releasing of drugs.[41,42] MVs act as cargo, to either transport these drugs in the vicinity or to non-treated cells at distant locations.[43,44]

Future Trends Related to MVs

The implication of MVs in transporting various biomolecules, using a non-genetic route can be explored for therapeutic targeting. According to recent reports, MVs are the key factors determining the ultimate fate of drug trafficking across malignant and non-malignant cells as observed in leukemia and breast cell lines.[45–50] In spite of restricting MVs formation, two core limitations related to target specificity and limited shell life need to be addressed. Deciphering this biological pathway and designing a useful approach would be a valuable addition and effective alternate mode of treatment for breast cancer.

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Conflict of interests

The author declares no conflict of interest with any institute.

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