Review

Role of Extracellular Vesicles in Breast Cancer, its Diagnostics and Treatment

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Abstract:
While the risk factors of breast cancer have been extensively studied and recorded, the understanding of the microscopic and nanoscopic mechanisms causing the disease is not yet satisfactory. Recent research in cell biology points to sub-micron sized extracellular vesicles (EVs) which are by now acknowledged to deliver cargo to cells and thereby mediate interaction between them. Investigation of EVs is challenging, as presently there are no golden standard methods for their isolation from bodily fluids or tissues, and their characterization. They are tiny (nano-sized) particles with dynamic identity that require development of new technologically advanced methods. This contribution presents a brief survey of the evidences on various facts that are being collected on breast cancer EVs.

Keywords: Extracellular vesicles; Extracellular vesicles in cancer; Extracellular vesicles in breast cancer; Extracellular particles; Extracellular particles in cancer; Extracellular particles in breast cancer

1. Etiology of breast cancer

Breast cancer is the most often diagnosed and the most prevalent cancer of all types (Luo et al., 2022). Although there are reports on different risk factors, the etiology of cancer is not known. Incidence rate increases with age and saturates after about 55 years of age (Luo et al., 2022). It was found that lifestyle increases the risk for breast cancer incidence, e.g. smoking in premenopausal women (Peñalver-Argüeso et al., 2023), alcohol intake (McDonald et al., 2013), higher intake of sugar (Farvid et al., 2021) and red meat (Lo et al., 2020). Some chemicals were connceted to higher incidence of breast cancer: polutants (White et al., 2023), food aditives (Sellem et al., 2024), heavy metals (Romaniuk et al., 2017), radiation (Preston et al., 2016), drug use disorder (Dahlman et al., 2021), psychological factors (Greer & Morris, 1975), lack of physical activity, and obesity (Hardefeldt et al., 2018). Genetic factors play a significant role in many cases of breast cancer, particularly mutations in the BReast CAncer genes BRCA1 and BRCA2 which are associated with a high risk of developing the disease (Mehrgou et al., 2016). Additionally, various other genetic alterations contributing to breast cancer have been identified (Pal et al., 2024). The reported risk factors indicate the link of cancer incidence with hormonal status (Henderson & Feigelson, 2000). Hormonal factors, especially prolonged exposure to estrogen, are also crucial in the development of breast cancer (Al-Shami et al., 2023). Estrogens promote the growth of breast tissue, which may contribute to the development of cancerous cells, particularly in cases of hormonal imbalance or excessive estrogen production (Yaghjyan & Colditz, 2011). Early menarche (Harris et al., 2024; Lopes et al., 2024), late menopause (Lopes et al., 2024), and hormone replacement therapy (Vinogradova et al., 2020) are all risk factors that increase the likelihood of developing breast cancer.

2. Extracellular vesicles (EVs)

Extracellular vesicles (EVs) are submicron membrane-bound structures released by all cell types into the extracellular space (Yanez-Mo et al., 2015). They are classified into subtypes based on their biogenesis and size, including exosomes, microvesicles (or ectosomes), and apoptotic bodies (Yanez Mo et al., 2015).

EVs have emerged as essential mediators of intercellular communication, carrying diverse molecular cargo, including proteins, lipids, RNA, and DNA (Welsh et al., 2024). This cargo allows EVs to influence a variety of biological processes (Yanez Mo et al., 2015; Welsh et al., 2024). EVs are involved in various disease processes, particularly cancer. They are present in various biological fluids and hold great potential as biomarkers for early cancer detection, disease progression monitoring, and treatment response evaluation (Póvoa & Rodrigues, 2022; Bamanakar et al., 2023). Being crucial for cell-to-cell signaling, they influence tumor growth, metastasis, and immune responses, thus opening new possibilities for diagnosis and therapy (Chang et al., 2021). In breast cancer, EVs hold promise as non-invasive biomarkers detectable in blood and other body fluids for early diagnosis and monitoring (Xu et al., 2024). Ongoing research explores their use for therapeutic applications such as targeted drug delivery due to their ability to transport therapeutic agents directly to tumor cells.

3. Mechanisms of EV formation

3.1. Endosomal Pathway (Exosome Formation)

Exosomes, a subtype of EVs, are generated through the Endosomal Sorting Complexes Required for Transport (ESCRT) - dependent and -independent mechanisms (Teng & Fussenegger, 2020). Initially, the plasma membrane invaginates, forming early endosomes that mature into late endosomes, also known as multivesicular bodies (MVBs) (Yanez Mo et al., 2015). The inward budding of the endosomal membrane leads to the formation of intraluminal vesicles (ILVs) within MVBs. These ILVs eventually fuse with the plasma membrane, releasing exosomes into the extracellular space (Yanez Mo et al., 2015). The ESCRT machinery plays a critical role in sorting and packaging cargo into ILVs, while the tetraspanin family of proteins, such as CD63, also contribute to this process in an ESCRT-independent manner (Teng & Fussenegger , 2020).

3.2. Budding from the Plasma Membrane (Microvesicle Formation)

Microvesicles, also called ectosomes, are formed through direct outward budding of the plasma membrane (Kralj-Iglič et al., 2020). This process is driven by the reorganization of the actin cytoskeleton and the activation of specific lipid signaling pathways (Romer et al., 2010). The local accumulation of phosphatidylserine (PS) on the inner leaflet of the membrane and the interaction with various membrane-binding proteins promote membrane curvature, leading to the formation and release of microvesicles (Record et al., 2018). Unlike exosomes, microvesicles do not involve endosomal intermediates but are directly shed from the cell surface.

3.3 Apoptotic Body Formation

Apoptotic bodies are larger EVs formed during the programmed cell death (apoptosis) process (Yanez Mo et al., 2015, Kralj-Iglic et al., 2020). As cells undergo apoptosis, the plasma membrane begins to bleb, and fragments of the dying cell, including cytoplasm, organelles, and nuclear fragments, are packaged into apoptotic bodies. These larger vesicles are then released into the extracellular space, and they typically serve to eliminate cellular debris in an organized manner, facilitating immune system clearance (Boada - Romero et al., 2020).

3.4 Regulatory Factors

The mechanisms of EV formation are complex and involve multiple pathways that enable cells to release distinct types of vesicles into the extracellular space. Exosome formation through the endosomal pathway, microvesicle shedding from the plasma membrane, and apoptotic body formation during cell death are all critical processes in cellular communication. The formation and release of EVs are regulated by various factors, including cellular stress, environmental signals, and disease states. Key signaling pathways, such as those involving RABS (small GTPases), phosphoinositides, and tetraspanins, play critical roles in regulating vesicle biogenesis (Rädler et al., 2023). Additionally, cellular conditions like hypoxia, senescence, oncogene activation, oxidative stress (Chiaradia et al., 2021), inflammation (Ammirata et al., 2024) can influence the quantity and cargo of EVs, thereby modulating their functions in both physiological and pathological contexts.

In contrast to exosomes, microvesicles form through a different process that involves direct outward of the plasma membrane (Kralj-Iglič et al., 2020). This pathway, often referred as ectosome formation, is driven by the rearrangement of the cytoskeleton, specifically actin filaments, and the activation of various lipid signaling pathways (Meldolesi, 2018). These processes induce membrane curvature, leading to the shedding of vesicles from the cell surface. Microvesicles are typically larger than exosomes, with diameters ranging from 100 to 1000 nm.

3. Methods of EV isolation from bodily fluids

Ultracentrifugation: This standard technique uses high-speed centrifugation to separate EVs based on their density. It is efficient but time-consuming and requires precise optimization of speed and duration. High centrifugal forces can also damage EVs, affecting their integrity and functionality (Božič et al., 2019). Size-Exclusion Chromatography (SEC): SEC separates EVs by size as they pass through a porous matrix, offering higher purity compared to ultracentrifugation, however, it is slower and may require optimization for specific sample types (Clos-Sansalvador et al., 2022). Density Gradient Centrifugation (DGC): DGC separates particles through a gradient of different densities, providing high purity, particularly in complex samples, however, it is complex, time-consuming, and requires careful handling (Clos-Sansalvador et al., 2022). Additional techniques like ultrafiltration, immunoaffinity capture (Welsh et al., 2024) and microfluidic analysis (Chen et al., 2024) are emerging for EV isolation from smaller samples.

4. Methods of EV isolation from tissues

Isolating EVs from tissues is complex due to the challenge of extracting them from the extracellular matrix (Crescitelli et al., 2021). Successful isolation has been reported from

various tissues including adipose tissue (Sabio and Crewe, 2023), tumors (Swatler et al., 2024), placenta (Zabel et al., 2020), and brain (Metamoros-Angles et al., 2024). In the central nervous system (CNS), EVs released by nerve cells play roles in both normal and pathological processes (Gassama et al., 2021). Protocols for isolating EVs from tissue interstitial fluids have been developed only recently (Guerrero-Alba et al., 2024). The MISEV2023 guidelines, updated by the International Society for Extracellular Vesicles (ISEV)(Welsh et al., 2024), offer standardized practices for isolating and characterizing EVs from solid tissues. These guidelines emphasize: Clarification of terminology to ensure consistent use of EV definitions; Experimental design to ensure reliable and reproducible results; Method flexibility, allowing researchers to select methods suitable for their research needs. EVs from tissues are typically separated based on biophysical properties like size, density, and surface composition (Welsh et al., 2024).

5. Methods of EV characterization

Western blotting, Nanoparticle Tracking Analysis (NTA), and Electron Microscopy (EM) are commonly used to analyze EV composition, number density, and morphology, respectively (Welsh et al., 2024). Despite these challenges, EVs show promise for identifying new biomarkers that could enable early cancer detection (Póvoa & Rodrigues, 2022; Möller & Salomon, 2023). Advanced technologies, such as mass spectrometry and sequencing, enable the identification of potential biomarkers and the improvement of diagnostic tools (Pocsfalvi et al., 2016). Advances in techniques such as nonlinear optical microscopy, which uses endogenous contrasts, have enhanced the ability to analyze EVs' biochemical and functional properties (Sorrells et al., 2024). Advanced deconvolution methods can be used to assess the EV transcriptome and compare expression profiles with different cell types (Larsen et al., 2024). Cancer cell-derived EVs carry molecules like messenger RNA and micro RNA, which affect signaling pathways and protein expression in target cells, supporting tumor growth and spread (Thery et al., 2018). However, quantifying EVs remains challenging, as many methods rely on indirect markers or particle counts, which may not always be specific to EVs (Atlantis Bioscience, 2024).

6. Types and treatments of breast cancer

Carcinomas are tumors that start in the epithelial cells that line organs and tissues throughout the body. According to the type of cells in the breast that become cancer cells, we distinguish ductal carcinoma (originating in the milk ducts) and lobular carcinoma (originating in the glands in the breast that make milk). If the cancer remains in the duct, it is called ductal carcinoma in situ; if it expended in other breast tissue, it is called invasive or infiltrating breast cancer. Cancer cells carry estrogene (ER) and progesterone receptors (PR) and can be accordingly stimulated by the hormones (HR) (Orrantia-Borunda et al., 2022). Some have increased quantities of the human epidermal growth factor receptor-2 (HER2) protein which also stimulates growth of the cancer cells (Orrantia-Borunda et al., 2022). The cancer cells are acquired from the tumor and tested for ER, PR and HER2 to be assigned positive or negative for the particular type. Recently, additional markers are considered such as different micro RNAs and gene mutants (Orrantia-Borunda et al., 2022).

Primary treatment of breast cancer is surgery combined with chemotherapy, targeted therapy and/or radiation therapy; in surgery, a part or entire breast is removed, usually together with nearby lymph nodes to test whether the cancer has spread (Trayes & Cokenakes, 2021). Chemotherapy includes oral or intravenous drugs to kill the cancer cells. Radiation therapy is used to shrink tumor before surgery and after surgery to kill the possibly remaining cancer cells. Targeted therapy includes endocrine therapy to diminish the amount of hormones that could stimulate the growth of the cancer cells. Antibody drug conjugates (ADCs) contain monoclonal antibodies and cytotoxic substances to deliver these drugs to the targets: cancer cells with specific surface antigens. In this way the treatment is more effective and the risk of systemic toxicity is lower than in conventional chemotherapy. This method is particularly promising for HER2-low and triple (ER, PR and

HER2) negative breast cancer which previously lacked effective treatments. (Mark et al., 2023).

7. Effect of radiation on EVs of cancer cells and radiation induced bystander effect

About half of the cancer treatments involve radiotherapy (Huber st al., 2024). At the cellular level radiotherapy should cause DNA destruction in cancer cells (Jassi et al., 2024) by inducing changes in DNA repair and causing cell cycle arrest and apoptosis. As key mediators of cell communication, also EVs play an important role in these processes (Szatmári et al., 2019). EVs are selectively targeting cells and can modulate the effects of radiation (Ripoll-Viladomiu et al., 2024), promote immune and inflammatory responses (Huang et al., 2018) and transmit microRNAs (e.g. miR-603) to influence the response of cancer cells in glioblastomas (Ramakrishnan et al., 2020). Radiotherapy affects not only cancer cells but also surrounding normal tissue. The radiation-induced bystander effect describes the changes in non-irradiated cells induced by the signals from irradiated cells. Communication between the irradiated and non-irradiated cells via radiation-influenced EVs creates indirect radiation exposure which affects adaptation to radiation (Jassi et al., 2024).

8. Clinical studies on EVs in breast cancer diagnostics

Recent studies have focused on the lipid composition of plasma-derived EVs as diagnostic biomarkers for breast cancer. Using mass spectrometry-based lipidomics, researchers identified significant alterations in lipid classes, including phosphatidylcholines and sphingomyelins, in EVs from breast cancer patients compared to healthy controls. These distinct lipid profiles demonstrated high accuracy in differentiating cancer patients from non-cancer individuals, highlighting their potential as a non-invasive diagnostic tool for early detection and disease stratification (Nishida-Aoki et al., 2020). There are various molecular mechanisms by which EVs promote brain metastasis in breast cancer (Sakamoto et al., 2023): EVs deliver bioactive molecules, such as microRNAs and proteins, to recipient cells, thereby regulating signal transduction and protein expression levels; EVs from brain metastatic breast cancer cells disrupt the blood-brain barrier by altering tight junctions between endothelial cells and promoting tumor cell infiltration into the brain parenchyma; EVs influence astrocyte function, contributing to the establishment of a pre-metastatic niche that supports tumor growth (Sakamoto et al., 2023). Mizenko et al. (2024) analyzed 471 clinical trials performed from 2000 to 2022 to evaluate the status and challenges of EV-based diagnostics and therapies. They found that 70% of trials focus on diagnostics, with cancer (breast and lung) as the primary targets, while 18% explore therapeutic applications, particularly those using mesenchymal stromal cell-derived EVs for inflammatory, respiratory, and neurological disorders. Ultracentrifugation was the most common EV isolation method (31%), and RNA sequencing was the leading characterization tool (36%). However, only 36% of trials fully reported EV isolation/characterization protocols. Most trials were conducted in North America (42%) and Asia (36%), underscoring regional research dominance (Mizenko et al., 2024). The authors emphasize the need for standardized methodologies to address EV heterogeneity and enhance clinical translation. Zhang et al. (2023) assessed EVs in 80 patients with varying stages of the disease. The study demonstrated that the presence of specific EV markers correlated with tumor progression, offering a non-invasive approach for real-time monitoring (Zhang et al., 2023). Challenges such as standardizing isolation and characterization methods remain, with future research aiming to address these limitations to facilitate clinical translation.

9. Predictive value of EVs

Effects of EVs in cancer include angiogenesis, epithelial–mesenchymal transition, extracellular matrix remodelling, and immune escape (Tao and Guo, 2020). The predictive value of EVs lies in their ability to reflect real-time changes in the tumor microenvironment (Xu et al., 2024). Tumor-derived EVs contain a variety of biomolecules, including proteins, lipids, and nucleic acids, which reflect the molecular characteristics of the tumor,

providing valuable insights into disease progression and therapeutic response (Zhang et al., 2023; Xu et al., 2024). EVs are suggested to play crucial roles in intercellular communication, promoting tumor growth, metastasis, and drug resistance (Schwarzenbach & Gahan, 2020). They offer several advantages over traditional biomarkers, including their ability to capture tumor heterogeneity and their potential to track both primary tumors and metastatic sites (Lee et al., 2023; Vinik et al., 2020). The protein content of EVs can serve as a tool for monitoring therapeutic response and detecting early relapse in metastatic breast cancer (Tian et al., 2021; Zhou et al., 2021). Studies have identified specific EV proteins that correlate with breast cancer progression, recurrence, and drug resistance, suggesting their potential for personalized treatment strategies (Serretiello et al., 2024; Tian et al., 2021). Recent advancements in proteomics and advanced analytical techniques, such as mass spectrometry, have enabled the identification of tumor-specific EV biomarkers, offering further insights into the molecular underpinnings of breast cancer (Bandu et al., 2024; Muttiah et al., 2024). The integration of EV analysis with other biomarkers, including circulating tumor DNA and small RNAs, enhances the sensitivity and specificity of early detection and monitoring of therapeutic efficacy (Koi et al., 2020; Rayamajhi et al., 2024).

10. Conclusions

EVs hold substantial promise as non-invasive biomarkers for breast cancer diagnosis, metastasis monitoring, and treatment response prediction. Their role in tumor biology, combined with advances in detection technologies, makes them a valuable tool for precision oncology and personalized cancer therapy. The potential use of EVs as delivery vehicles for targeted therapies opens new avenues for breast cancer treatment, with ongoing research exploring their role in immuno-oncology and drug delivery (Asleh et al., 2023; Wang et al., 2021).

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