Exosomes in Triple-Negative Breast Cancer

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Triple-negative breast cancer (TNBC) is the most potent metastatic type of breast cancer that can spread to other body parts. Chemotherapy and surgical intervention are the sole treatments for TNBC, owing to the scarcity of therapeutic targets. Manipulation of the membranes as per the desired targets of exosomes has recently gained much attention as a drug delivery method.

triple-negative breast cancer exosomes biomarkers therapeutics signalling drug-resistant

1. Exosomes Carry Signature Markers from Their Cells of Origin

The number of exosomes released by TNBC (triple-negative breast cancer) cells is more incredible than more significant normal healthy cells. The augmentation of TSAP6, a p53-regulated gene product that governs the exosomal secretion signalling transcription by activating p53, which is frequently aberrantly driven in cancer cells, could alter the rise in exosome shedding from cancer cells. The most associated biomarkers with exosomes are tetraspanins (CD9, CD63, CD81), proteins of the endosome system (TSG101, Rab-GTPase), and heat shock chaperones (HSP70, HSP90). These are all the characterizations of all exosomes [1][2][3].

Exosomes produced by cancer cells have different content than normal cells. The microRNA content in the exosomes extracted from cancer cells has different signature microRNA and acts similar to “fingerprints”, which can identify their origin. Hence, they can also serve as a biomarker. A protein senescence-associated secretory phenotype (SASP) that can enhance tumour growth is one of the constituents of exosomes [4].

Chemotherapeutics induce apoptosis. Some cancer cells experience therapeutic-induced senescence (TIS), which enables them to stay metabolically active but lose their capacity to metastasize. This condition makes them more resistant to chemotherapies. Kavanagh et al. found that exosomes collected from TIS TNBC CAL51 cells had much larger quantities than non-senescent cells. Concerning non-senescent TNBC cells, the researchers found a remarkable increase in the amounts of 142 proteins in exosomes from these TIS cells. Essential proteins, which are all involved in (i) cell proliferation, (ii) ATP depletion, (iii) apoptosis, and (iv) SASP factors, are more prevalent in exosomes from TIS cells, implying that the abolition of these proteins from TIS cells through exosomes permits cancer senescent cells to stay feasible [5].

Exosomes also play an important role as a messenger and can communicate between the recipient cells and donor cells through the microRNA, which they carry, which becomes translated into proteins. Communication can involve immune suppression, microenvironment changes, angiogenesis, immune escape and invasion, and tumour progression and repression.

2. Exosomes for Diagnosis of Triple-Negative Breast Cancer

With the advancement of nano-theragnostics, exosome profiling, diagnosis, and potential disease therapy are possible. A ‘liquid biopsy’ enables the study of EVs and exosomes. It also aids in analysing circulating DNA from tumours and cells from tumours. The exosomes carry specific biomarkers that help to diagnose triple-negative breast cancer. The content of the exosomes is different for patients with triple-negative breast cancer [6]. Proteins that act as biomarkers for TNBC are carcinoembryonic antigen (CEA), survivin, and CA 15-3(cancer antigen 15-3) [7]. The levels of these biomarkers are elevated in patients suffering from TNBC. The microRNA present inside the exosomes also plays a vital role in diagnosing. A specific microRNA identified as a biomarker is miR-373, which is elevated in TNBC patients and further downregulates the expression of estrogen [8]. The Wnt signalling is responsible for metastasis and chemoresistance in TNBC. Myotubularin-related protein 3 is downregulated by miR-1910-3p, which also stimulates the NF-κB and wnt/β-catenin signalling pathways and, in turn, accelerates the development of breast cancer. When combined with the conventional tumour marker CA153, serum miR-1910-3p in exosomes is a potent diagnostic sign that increases the sensitivity of breast cancer detection. In conclusion, miR-1910-3p in serum exosomes may be a new molecular indicator for the detection of breast cancer. Liquid biopsy investigates intra-tumour heterogenicity and the tumour microenvironment, while single-tissue biopsy does not include the microenvironment [9][10] **Figure 1**.

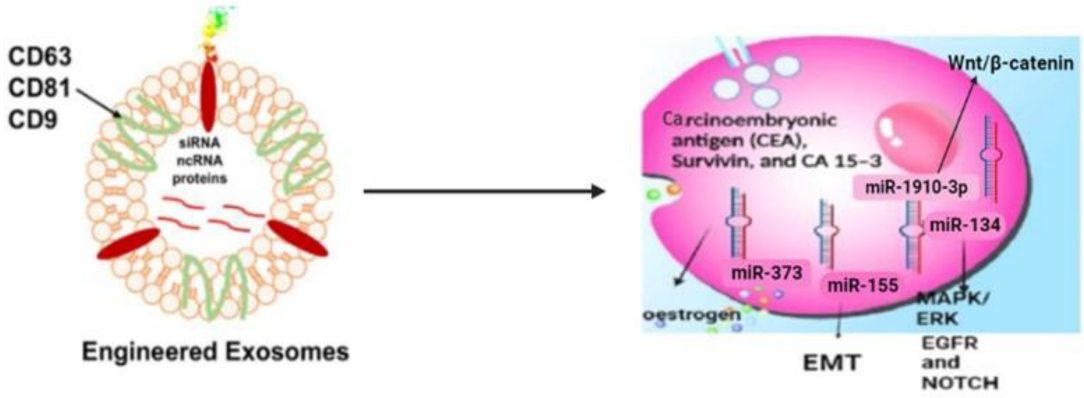

Figure 1. Exosomes as a biomarker for TNBC—showing different protein contents inside exosomes, which are used for identifying TNBC and also in ‘liquid biopsy’. Carcinoembryonic antigen (CEA), survivin, CA 15-3(Cancer antigen 15-3), miR-373, mi-134, miR-1910-3p, and miR-155 are some of the biomarkers (https://biorender.com/, accessed on 30 October 2022).

3. Exosomes Initiating the Epithelial-Mesenchymal Transition in Breast Cancer and TNBC Influencing the Metastasis to

Other Organs

EMT is a process through which normal cells transfer to mesenchymal cells. Mesenchymal cells have the property of invasion and malignancy, leading to the worst prognosis and TNBC migrating to other organs. The EMT transcription factors involved are β-catenin, TWIST, zinc finger protein, SNAIL2 (zinc finger protein SNAI 1), SLUG (SNAI 2 gene), ZEB1 (zinc finger E-box-binding homeobox 1), and ZEB 2. Studies have proven that altering these transcription factors is not enough to cease the epithelial–mesenchymal transition. The exosomes are involved in intercommunication between the cancer cells. The exosomes have various contents, which can regulate the transcription involved in the epithelial-to-mesenchymal transition [11]. The literature, with various reports, shows that exosomes are involved in the epithelial–mesenchymal transition in breast cancer.

Synthesis of exosomes takes place when intercommunication between notch receptors, ligands (JAGs) (jagged protein), and regulators (ADAM 10/17) (A disintegrin and metalloproteinase) occurs. ASPH (aspartate beta-hydroxylase) initiates the Notch cascade to synthesis or release of pro-metastatic exosomes. Normal breast cells have muted ASPH. The activation of ASPH by the NOTCH signalling gives rise to aggressive tumour progression in breast cancer with the help of exosomes [12]. Hippo signalling pathways are essential in restricting organ size. They inhibit oncogenic co-activators such as YAP (yes-associated protein) [13]. Inactivation of the Hippo pathway initiates the TEA (transcriptional enhancer factor) domain transcription factor to bind with the unphosphorylated YAP/TAZ (tafazzin), which translocates it to the nucleus [14]. TEAD (transcriptional enhancer factor domain TEF1)-influences the target genes. The transcription enhances the expression of mesenchymal markers such as Vimentin and N-cadherin and suppresses the expression of epithelial markers including E-cadherin [15]. The YAP gene cross-communicates with transcription factors such as ZEB1, SNAIL, and SLUG, which initiates epithelial–mesenchymal transition in cancer cells. Exosomes from mesenchymal-stem-cell-derived adipocytes enhance Hippo-induced epithelial–mesenchymal transition in breast cancer cells [16].

The Wnt/β-catenin signalling pathway is involved in the epithelial–mesenchymal transition. Specifically, a β-catenin molecule is involved in the epithelial–mesenchymal transition [17][18]. Scientists reported that exosomes containing Wnt ligands could influence Wnt signalling, which is responsible for EMT synthesis. Macrophage-derived exosomes containing Wnt5a initiate the Wnt/β-catenin signalling pathway in breast cancer cells [19]. Exosomal Wnt7a is accountable for the metastasis of breast tumours to the lungs [20]. In human breast fibroblasts, the exosome-mediated release of miR-9 produces cancer-associated fibroblast-like characteristics. Hence, regulating the Wnt ligands encapsulated in exosomes can be reasonable therapeutic control for cancer cells. Modulation of Wnt signalling by proteins derived from exosomes can inhibit metastasis [21].

The lncRNAs MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) and ID4 (inhibitor of DNA-binding 4) cause an upregulation of the circular RNA circ 0076611 in exosomes released by TNBC. This circular RNA circ 0076611, released from the exosomes of TNBC cells, interacts with MYC (proto-oncogenes) and VEGFA (vascular endothelial growth factor A) mRNAs, further initiating cell proliferation and migration of TNBC cells [22]. Exosomes secreted by the TNBC cell line containing MMP-1 (Matrix Metallopeptidase 1) interact with the PAR1(protenase-activated receptor 1), a G protein receptor that initiates the EMT, which enables the breast cancer tumour to

metastasize to the lungs [23]. miR-939 in exosomes downregulates the expression of VE-cadherin, destroys the barrier function of endothelial monolayers, and initiates tumourigenesis in breast cancer [24]. miR-155 is upregulated in TNBC, induces epithelial–mesenchymal transition, and plays an essential role in resistance [25][26] **Figure 2**.

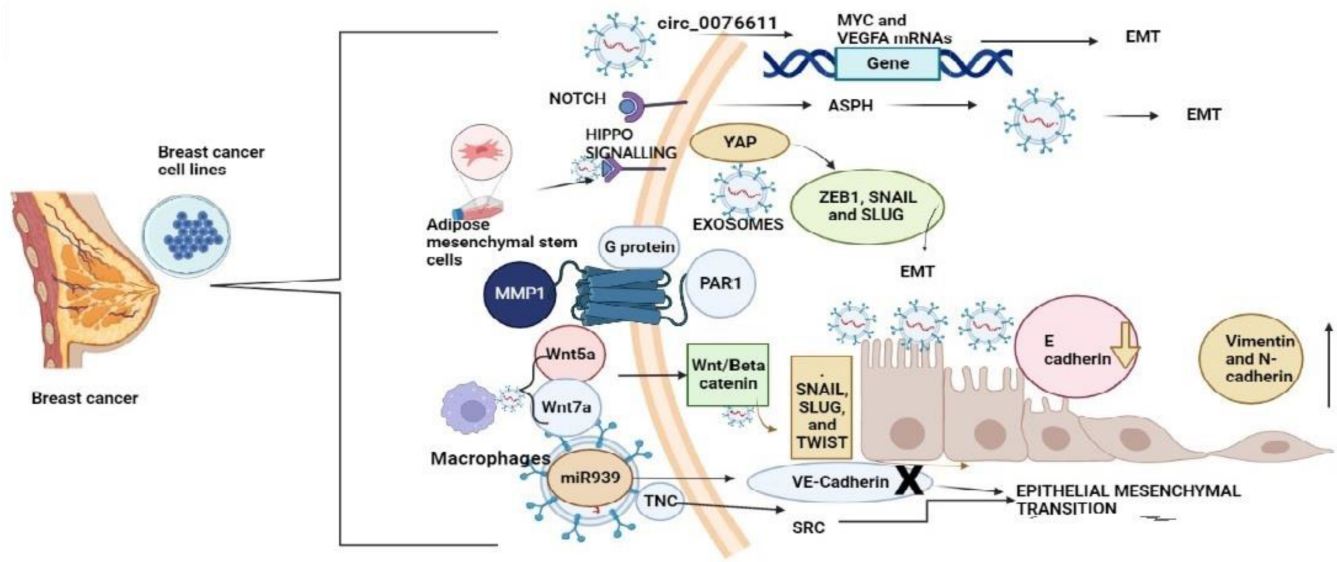

Figure 2. Exosomes targeting different pathways in TNBC and breast cancer leading to epithelial–mesenchymal transition of breast tumours to other parts of the body (exosomes have circ_0076611 target MYC and VEGFA genes and initiate epithelial–mesenchymal transition). NOTCH signalling initiates ASPH in breast cancer cell lines and triggers epithelial–mesenchymal transition. Exosomes extracted from adipose mesenchymal stem cells inhibit Hippo signalling and initiate the TEA domain transcription factor to bind with the unphosphorylated YAP/TAZ, which translocates it to the nucleus. TEAD-influenced target gene transcription enhances the expression of mesenchymal markers such as Vimentin and N-cadherin and suppresses the expression of epithelial markers including E-cadherin. The YAP gene cross-communicates with transcription factors such as ZEB1, SNAIL, and SLUG, which initiates epithelial–mesenchymal transition in cancer cells. MMP-1 interacts with PAR1, a G protein receptor that initiates the EMT. Macrophage-derived exosomes containing Wnt5a and Wnt7a activate Wnt/β signalling, which, in turn, activates SNAIL, SLUG, and TWIST and initiates EMT. miR-939 in exosomes downregulates the expression of VE-cadherin, destroys the barrier function of endothelial monolayers, and initiates tumourigenesis in breast cancer. (Created with the help of Biorender).

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