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Tumor-derived exosomes: potential biomarker or therapeutic target in breast cancer?

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Running title: exosomes in breast cancer

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Conflict of interest

The authors have no conflict of interest to disclose.

Abstract

Exosomes are released by normal and tumour cells, including those involved in breast cancer, and provide a means of intercellular communications. Exosomes with diameters ranging between 30-150 nm are involved in transferring biological information, via various lipids, proteins, different forms of RNAs, and DNA from one cell to another, and this can result in reprogramming of recipient cell functions. These vesicles are present in all body fluids, e.g., blood plasma/serum, semen, saliva, cerebrospinal fluid, breast milk, and urine. It has been recently reported that these particles are involved in the development and progression of different tumor types, including breast cancer. Furthermore, it has been suggested that exosomes have the potential to be used as drug transporters, or as biomarkers. This review highlights the potential roles of exosomes in normal and breast cancer cells and their potential applications as biomarkers with special focus on their potential applications in treatment of breast cancer.

Key words: breast cancer, exosomes, non-invasive biomarker, therapeutic target

Introduction

Breast cancer is the most common cancer among women and is the most common cause of cancer-related mortality in women globally [Wu et al. 2016]. Breast cancer is pathologically heterogeneous; they may be aggressive tumors with a very poor prognosis, or slow growing indolent tumors with a good prognosis [Tao et al. 2015]. The incidence rates of breast cancer are lower in less developed countries than in more developed countries, whereas the prevalence and related death rates are increasing in both groups of countries [Tamimi 2017]. Based on GLOBOCAN statistics in 2012, 1.7 million breast cancer cases were diagnosed among women, and 0.5 million death from this disease have been recorded globally in 2012 [Wu et al. 2016; Toriola and Colditz 2013]. The lack of understanding of the molecular mechanisms of breast cancer pathogenesis has led to poor clinical outcomes [Kruger et al. 2014; Harris et al. 2015].

Exosomes were first described in relation to their role in excess transferrin receptor exocytosis in sheep cells in vitro [Pan et al. 1985]. These small vesicles (30-100 nm) have been proposed as a means of intercellular communication, and that are now known to be released from various cell types, including dendritic cells, B cells, T cells, endothelial cells, stem cells and especially cancer cells [Yu, Zhang, and Li 2014; Soung et al. 2017]. Exosomes originate from the endosomal membrane compartment and contain a large variety of components such as RNAs and proteins [Inamdar, Nitiyanandan, and Rege 2017]. These latter proteins include membrane associated proteins, such as tetraspanin, CD63, CD81, CD82 and CD9, and cytoplasmic proteins, such as Hsp 90 and Hsp 70, Protein Alix and TSG101. In addition, they include membrane transport and fusion proteins, such as RabGTPases and Annexins. They also contain major histocompatibility complex (MHC) class-I and class-II, FasL and adhesion molecules, metalloproteinases and tissue-specific proteins associated with tumorigenesis and metastasis [Mollaei, Safaralizadeh, and Pouladi 2017]. Exosomes may also contain mRNAs, MicroRNAs, and DNA fragments [Wang et al. 2016].

As their name suggests, tumor-derived (TD) exosomes are vesicles, secreted from tumor cells [Kalluri 2016]. TD exosomes, may contain molecules, that reflect characteristics of the tumor cells from which they are derived; thus, exosomes may be used as biomarkers in the diagnosis of cancer [Roma-Rodrigues, Fernandes, and Baptista 2014; Dijkstra et al. 2014; Aushev et al. 2013]. There is some evidence that TD exosomes may be involved in constructing a microenvironment that supports the spread of a tumor, invasion, angiogenesis and pre-metastatic niche development [Graner, Cumming, and Bigner 2007; Khan et al. 2014]. Moreover, TD exosomes have other emerging characteristics that include; restricting immune control and enhancing chemo-resistance by elimination of chemotherapeutic drugs, which may facilitate tumor growth and metastasis [Wang et al. 2016; Aushev et al. 2013].

Consequently, TD-exosomes may be potential targets for targeted therapy, by their removal or modification [Khalyfa et al. 2016; Zhang and Grizzle 2014]. Because exosomes secrete important mediators, they have been proposed to act as regulators of cancer [Melo et al. 2014]. Exosomes promote cell migration, cancer progression and metastasis by the secretion of growth factors, angiopoietin factors and cytokines from stromal cells, induce proliferation of endothelial cells, and promote angiogenesis in metastatic organs [McGarty 2013]. Exosomes show differential enrichment of proteins with signatures of both identity and abundance of cancer cells [Syn et al. 2017]. Hence, the search for cancer-cell-derived exosomes in a metastatic pattern may determine the mechanism of cancer. In addition, they may be novel biomarkers for the detection of early stage cancer and metastasis; which are new therapeutic strategies for cancer [Soung et al. 2017].

Extracellular vehicles (EVs)

There are three different mechanisms for EVs being secreted by cells. These are: multi vesicular endosomal cell compartment, cell budding, and apoptotic bodies. Exosomes are released from the multi vesicular endosomal cell compartment into the ECM (extracellular

matrix); it allows them to circulate via the body fluids. Exosomes are small membranous vesicles (30–100 nm), which are secreted from many types of cells; these vesicles are one means of intercellular communication and shuttling of intracellular contents (protein and RNA). Exosomes could therefore be a source of biomarkers that could be used in the diagnosis of a variety of diseases [Raposo and Stoorvogel 2013].

Function and Composition of exosomes (Biogenesis)

Exosomes are formed during the late endosomal phase of cell development; double inward budding of the endosomal membrane initiates the formation of exosomes [Miller and Grunewald 2015]. Multi vesicular bodies produce exosomes through the invagination of their membrane and afterwards they fuse with the cell membrane, in order to secrete these exosomes [Miller and Grunewald 2015].

Exosomes reflect the characteristics of the original cell type, and their contents are selectively loaded. However, the relative amounts of RNA and protein are different from the parent cell. The emergence of exosomes through this inward budding comprises a wide range of surface proteins and bears variety of lipids, nucleic acids such as miRNA's and mRNA's, and proteins [Vader, Breakefield, and Wood 2014]. The exosomes derived from MVB can influence a recipient cell by changing its phenotype and function [Jakobsen et al. 2015].

Potential of EVs as biomarkers

Studies on the potential use of exosomes as biomarkers, were started when it was found that they can transport their load through the circulation to distant cells around the body. Exosomes are found in the blood and urine. In addition, they contain potentially important biomarkers such as tumor-specific proteins [Ogorevc, Kralj-Iglic, and Veranic 2013; Théry et al. 2006], [Nazimek et al. 2015]. Tumor derived exosomes, may be involved in metastasis, invasion and the advancement of the cancer cell [Kahlert and Kalluri 2013]. On the other hand, exosomes enhance angiogenesis in hypoxia and facilitate the metastasis of cancer cells, by modulation of

hypoxia-inducible factors' (HIF) family of transcription factors. Tumor-derived exosomes may prevent immune responses and allow the progression of cancer. They may also have an important role in the cancer cell resistance to chemotherapy.

Exosomes are suggested to be potential biomarkers for cancer diagnosis and assessment of prognosis. They contain various proteins, peptides and tumor specific antigens, or have compounds on their surface that have the capability to target particular cells [Dorayappan et al. 2016; Tran et al. 2015]. The concentration of exosomes in the blood of cancer patients is higher than for healthy subjects; and there is a positive association between malignant tumor growth and these increased numbers [Tang and Wong 2015]. There are also differences in protein and NA (nucleic acid) content in exosomes from patients with and without cancer, and can affect tumor growth by promoting endothelial angiogenic responses [Al-Nedawi et al. 2009].

There is evidence for a role of exosomes released by cancer cells, contributing to disease progression, tumorigenesis, angiogenesis, metastasis, chemo-resistance, pre-metastatic establishment, immune inhibition and ECM remodeling [Andaloussi et al. 2013]. Comparing the exosomes derived from the supernatant derived from cancer cell cultures, or from the serum of cancer patients, with those derived from non-cancerous individuals has shown a significant increase in exosomes in cancerous samples. There is an up-regulation of exosome secretion in cancer cells [Brinton et al. 2015]. TD exosomes have the ability to exchange material between cancer cells and are capable of communicating with various cell types in the periphery. Cancer cells also secrete exosomes that have the potential to reprogram their micro-environment; altering this to facilitate tumor growth and invasion of healthy tissues. The microenvironment consists of the extracellular matrix and stromal cells such as endothelial cells, fibroblasts and inflammatory immune cells, and tumor-associated vasculature [Joyce and Pollard 2009]. Studies have also demonstrated a role of adipose stromal cells (adipocytes), in the development of tumorigenic microenvironment, specifically in obesity-related cancers(38).

Cancer cells release exosomes that may affect fibroblasts, allowing them to organize a more favorable tumor microenvironment by eliciting the TGF β /Smad pathway in target cells [Quail and Joyce 2013; Spill et al. 2016]. In some cases, the exosomal secretion of extracellular matrix metalloproteinase can promote fibroblast remodeling of the TME [Webber et al. 2010; Millimaggi et al. 2007]. In recent studies, TD exosomes have been found to be contributory factors to EMT/ epithelial to mesenchymal transition, causing TMEs to become more able to metastasize and more invasive (41). Oncogenic transmission leading to the process of EMT is likely to be mediated by exosomal cargo transfer which is associated with tumor-driving Epithelial-to-mesenchymal transition [Vella 2014; Greening et al. 2015].

Cancer exosomes are potentially strong mediators with a natural ability for modulating the behavior of surrounding cells. This enables metastasis to take place by conditioning the environment, colonizing and facilitating cancer cells migration. In a series of experiments, Sung et al., found that exosome secretion was associated with directional cell movement and persistent migration of cancer cells[Sung et al. 2015].Using live-cell imaging Sung et al found that the inhibition of exosome biogenesis led to the disruption of directional cancer cell migration. Moreover, reactivating the biogenesis pathways led to the re-establishment of cell transportation and movement. Thus, cancer exosomes are capable of secreting and delivering, essential ECM molecules to drive the migration of cancer cells by adjusting cell adhesion with an effect on integrin [Peinado et al. 2012].Valenzuela et al., have reported that exosomes secreted from tumor cell lines contain survivin, cIAP1, cIAP2, and XIAP; which all have an inhibitory effect on apoptosis (IAPs) [Valenzuela et al. 2015].

The effects of cancer exosomes on the immune system appear to be at least two fold. They can promote the immune response to the tumor, or enhance immunosuppressive functions that support tumorigenesis. Cancer cell-derived exosomes can induce apoptosis of CD8⁺ T cells by means of the death receptor pathway [Peng, Yan, and Keng 2011].They may also be

responsible for T cell dysregulation by supporting regulatory T cells proliferation while inhibiting the proliferation of effector T cells [Miller and Grunewald 2015].

Exosomes and Breast cancer: Diagnosis

Some studies have reported that exosomes extracted from the saliva, may be used for diagnosing early Breast cancer^[48]. Exosomes derived from breast cancer (exo-Breast cancer) appear to be able to interact with salivary gland cells and modify the configuration of the secreted exosomes by changing transcriptional activity [Zhang et al. 2016]. Furthermore, particular exo-Breast cancer proteins and mRNAs could be found in saliva [Zhang and Grizzle 2014; Lau and Wong 2012]. It is possible that, controlling mRNA and protein expressions may be a feasible way of protecting individuals at high risk of breast cancer. Another investigation reported a relationship between exosomal survivin expression and breast cancer. In this study, patients were compared with controls that were disease free for 5 years, these patients had a significant increase in the serum survivin level (specifically survivin-2B). Thus, measuring serum survivin-B2 could be another way of monitoring the breast cancer risk [Khan et al. 2011]. Recently, Roberg-Larsen et al. found a large population of particular extracellular vesicles named HG-NV (Homogenous nano vesicle/Huang-Ge-nano vesicle) in a breast cancer cell line [Roberg-Larsen et al. 2017]. Zhang et al. showed that some specific proteins and RNAs exist in breast cancer cells derived from HG-NVs. These may be used as potential biomarkers for breast cancer diagnosis [Zhang et al. 2016].

Additionally, Melo et al., reported that the cell surface proteoglycan, GPC1 (glypican-1), may be a specific cancer biomarker. According to the results of this study, in 75% of patients with breast cancer, the levels of exosomes with GPC1 on their surface (GPC1+) are higher, than for healthy controls [Melo et al. 2015].

Exosomes and Breast cancer: a potential therapeutic option

To date, there are four common therapeutic options for breast cancer: chemotherapy, radiotherapy, endocrine therapy and surgical excision; although, the results of these treatments is often poor, with a high risk of relapse and side effects, including venous thrombus, leukaemia, neurotoxicity and cardiotoxicity [Overmoyer 2015; Feng et al. 2014].

Immune promoting attributes of exosomes may allow them to be used as cellular vehicles as novel drug delivery tools, or vaccines for cancer immune therapy. These vaccines, allow the presentation of tumor antigens to the immune system that produces an effective immune responses against the tumor [Koido et al. 2011]. Because TD exosomes contain various tumor antigens, they may participate in antigen presentation to activate T cells such as dendritic cells and appear to be a feasible cancer vaccine [Cho et al. 2005; Tan, De La Peña, and Seifalian 2010]. Many nano carriers have been developed into drug delivery systems, because of the ease of application, little likelihood of toxicity and sustained effect, because of their lack of elimination by the reticuloendothelial system. As natural carriers, exosomes are safe and effective for targeted tumor drug delivery or therapy [Tian et al. 2014]. Ohno et al., have reported that exosomes can effectively deliver let-7a to breast cancer cells with epidermal growth factor receptors; GE11 peptide that binds to epidermal growth factor receptor-positive exosomes are an appropriate vehicle for delivering drugs to tumors' that express epidermal growth factor receptor [Ohno et al. 2013].

Conclusion

Exosomes are the very small EVs that appear to have important roles in the progression and metastasis of cancer. By transporting specific contents such as lipids, proteins, and transferring RNA, they may be able to mediate intercellular communication in TME. There is growing evidence that exosomes can be utilized as biomarkers for breast cancer. Furthermore, exosomes

have the potential to be potent drug transporters, allowing the targeted delivery of therapeutic agents to the sites of tumor cells. Whilst, further work is needed, exosomes, could be used as a non-invasive and effective mechanism to fight cancer in the future.

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