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Extracellular Vesicles as Biomarkers and Therapeutic Targets in Cancers

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Abstract

Extracellular vesicles refer to exosomes, apoptotic bodies, microvesicles and large oncosomes, which are membrane bound structures secreted by cells including cancer cells. The pathological role and translational potential of extracellular vesicles (EVs) in cancers are receiving research attention recently. The cargoes of cancer-derived EVs retain the molecular properties of their sources and cancer cells actively release EVs into body fluids that are easy to access. EVs released from cancer cells not only promote cancer progression through the delivery of cancer-associated molecules but also reflect alterations in the state of cancers during therapy. They are considered promising biomarkers for therapeutic response evaluation, especially resistance to therapy and diagnostics. This chapter discusses the various roles of extracellular vesicles in cancers and their potential as therapeutic targets.

Keywords: extracellular vesicles, biomarkers, cancers, therapeutics

1. Introduction

Cancer is described as one of the challenging diseases globally, which accounts for 19 million newly diagnosed cases and over 10 million deaths annually making it the leading cause of death [1]. The burden of cancer incidence and mortality is rapidly growing worldwide [1]. Cancer development in humans is a multistep process, which involves various genetic or epigenetic changes and results in the malignant transformation of the normal cells [2]. Recently the use of proteomics, genomics and bioinformatic techniques has unraveled the intricate interplay of numerous cellular genes and regulatory genetic elements that account for the cancerous phenotypes. Higher mortalities associated with cancers are as a result of the absence of very reliable cancer biomarkers, which could be used to diagnose early cancers, predict prognostics and treatment response as well as detection of biomarkers for drug resistance [3]. The unavailability of good biomarkers is a major hindrance for cancer treatment. Cancer biomarkers are not only important for diagnostic purposes but can also be of great prognostic value. With the identification of the right biomarker the cancer progression and effect of chemotherapeutic drugs can be evaluated in great detail [4]. Again, the presence of resistance to therapy, disease relapse, and individual differences continue to reduce the survival chances of cancer patients and makes the disease

impossible to cure [5]. It is predicted that therapeutic response assessment, especially treatment response prediction, is valuable to guide treatment strategy determinations and provide responsive therapy for better survival [6]. The identification of reliable cancer biomarkers in the management of cancers may play a crucial role in reducing cancer-related mortality.

Cancer biomarkers are biological molecules that suggest the presence of cancer in a patient. They are either produced by the cancer cells or by other non-cancer cells in response to cancer [7]. Cancer biomarkers may be used to identify the presence of cancer and also help determine its stage, subtype, and whether they will respond to therapy [8]. Cancer biomarkers identified from serum are the most desirable form of the biomarkers that can be used for regular personalized screening, diagnosis, establishing prognosis, monitoring treatment, and detecting relapse. Cancer biomarkers can be classified into three main categories: prognostic biomarkers, which allow prediction of the disease course and survival chances; predictive biomarkers; to assess if a patient benefits from a certain treatment; and pharmacodynamic biomarkers, which are used in the clinics to guide personalized drug dosing and response assessment. In recent years, a group of biological molecules receiving research attention due to their potential utility as circulating biomarkers for cancer are the extracellular vesicles.

Extracellular vesicles (EVs) are small, lipid-bound particles containing nucleic acid and protein cargo which are excreted from cells under a variety of normal and pathological conditions [9]. Recent studies indicated that cancer-associated EVs play pivotal roles in constructing favorable microenvironments for cancer cells. They are therefore considered as new and promising biomarkers for many cancer types. EVs secreted from a variety of cancer types, including pancreatic cancer, ovarian cancer, prostate cancer, breast cancer, colorectal cancer, glioblastoma multiforme (GBM) are reported to contain cancer-associated protein markers [10]. The EVs play important roles in the regulation of intercellular communication and cell microenvironment homeostasis and again as important biomarkers of various cancers. As EVs are increasingly revealed to play important roles in cancer development and to carry specific information related to cancer state. In cancer research, growing evidence indicates that EVs possess the ability to promote tumor growth, metastasis, and angiogenesis [11] mediate tumor immune responses [12]; and stimulate chemotherapeutic resistance. Identification and modification of cancer cell-derived extracellular vesicles may allow for the development of novel diagnostic, preventive and therapeutic approaches in cancers. This chapter summarizes the functions of EV's in cancers, their potential as biomarkers and therapeutic targets. It further emphasizes the roles of EV's in cancer prognosis, treatment response and drug resistance.

2. Overview and biogenesis of extracellular vesicles

As membranous vesicles, many cell types in the human body release EVs and cancer cells actively secrete EVs even during the early phase of the disease. Another interesting characteristic of EVs is that its contents are protected from degradative enzymes in body fluids [13]. Based on various characteristics, ranging from size, biogenesis, content, cell of origin, morphology, EV are categorized into four main classes: endosomal-derived small exosomes (Exo) (30–150 nm), plasma membrane-derived middle-sized microvesicles (MV) (100–1000 nm), and large oncosomes (LO) (1000–10,000 nm), as well as apoptotic bodies (500–4000 nm) that are released from dying cells [14].

2.1 Exosomes

Typically, exosomes are about 30–150 nm in diameter and are generated via an endosomal route [15–17]. Exosomes are generated through the endosomal network. This is a compartment, which is membranous in nature and aids in the sorting and direction of intraluminal vesicles such as cell surface membranes and lysosomes to their specific destinations. It is known that exosomal vesicles are formed during an inward budding of early endosomal limiting membrane, which develops into multivesicular bodies in the process [17–19]. When late endosomal membranes invaginate, intraluminal vesicles (ILVs) are formed within the larger multivesicular bodies [20]. It is during this process that the molecules carried by exosomes including proteins, lipids and nucleic acids are incorporated into the invaginating membrane while the components of the cytosol are engulfed by the ILVs [21]. They are liberated into the surrounding body fluids when the multivesicular bodies fuse with plasma membrane. The general function of these early endosomes and multivesicular bodies are endocytic and transportation of the cell's material. These include storage, recycling, transport, protein sorting and release of these materials [16].

2.2 Apoptotic cell-derived extra cellular vesicles

Apoptotic cell-derived extracellular vesicles (ApoEVs) are subcellular and membrane bound in nature. They are produced when cells are undergoing senescence. Further they can be derived from various cell types including endothelial cells, osteoblasts, precursor cells, stem cells and immunocytes [22]. Basically, three major steps are involved in ApoEVs formation. Firstly, there is a prerequisite step which involves cell surface membrane blebbing [23], which is then followed by projections of apoptotic membrane such as apoptopodia, beaded apoptopodia and microtubule spikes which releases 10–20 ApoEVs [24] and lastly the final formation of ApoEVs. Several factors have been shown to present the regulatory function on the generation of ApoEVs, these includes Rho-associated kinase (ROCK1) [25, 26] and Myosin-Light Chain Kinase (MLCK) [27]. Specifically, MLCK is known to enhance nuclear material packaging into ApoEVs, thus molecules that could inhibit caspases, MLCK and ROCK1 are also able to downregulate the production of ApoEVs [28]. Orlando et al., report that formation of blebs which is the first stage in ApoEVs formation are mediated by the presence of actomyosin which increases cell contraction leading to elevated hydrostatic pressure [29]. Researchers have unraveled that ApoEVs are key messengers released by dying cells to regulate processes including cell clearance, tissue homeostasis, pathogen dissemination and immunity thereby implicating them as therapeutic targets and diagnostic purposes.

2.3 Microvesicles

Microvascular vesicles are derived from myriad cell types surfaces [19]. Unlike ApoEVs, which are generated via indiscriminate surface blebbing or exosomes, which are derived intracellularly within MVBs, microvesicles are formed through active interaction between cytoskeletal protein contraction and the redistribution of phospholipids. Aminophospholipid translocases closely regulate an uneven distribution of the phospholipids in the plasma membrane leading to the formation of micro-domains [30–32]. Specifically, the plasma membrane budding process is induced by translocation of phosphatidylserine to the outer-membrane

leaflet [33, 34]. The process is completed via actin–myosin interactions which cause cytoskeletal structures to contract. This ensures the release of nascent microvesicles into the extracellular space via the direct outward blebbing and breaking off of the plasma membrane [35, 36]. After blebbing, there is a distinct localization of plasma membrane lipids and proteins which informs the rigidity and curvature of the membrane [37, 38]. In addition to the redistribution of membrane lipids and proteins, there is a selective redistribution of the components of microvesicles' cargo for specific microvesicles enrichment [39]. MVs carry proteins, such as enzymes, growth factors, growth factor receptors, cytokines and chemokines. They also carry lipids, and nucleic acids, including mRNA, miRNA, ncRNA, and genomic DNA [40]. MVs have been detected in the circulation of patients with several cancers, such as lung, breast, prostate ovarian, gastric cancer and colorectal cancer [41, 42]. They have been identified to contribute to tumorigenesis, progression of cancer cells, evasion of apoptosis by tumor cells, and induction of angiogenesis. The tumor-promoting role of MV in tumor mediated exosome communication largely depends on their bioactive cargo. It is believed that the shuttling of tumor-specific proteins to the surrounding cells influence tumor growth. This is achieved through the transfer of oncogenic traits between tumor cells, which result in enhanced tumor growth, and progression [43]. MVs are recently receiving research attention as potential biomarkers because tumor cells are able to constitutively release large amounts of MVs bearing tumor-specific antigens into the bloodstream and other bodily fluids [35]. Researchers have proposed many uses of MV in cancers. Others believe that MVs can be useful for disease staging as well as evaluate the response to therapy by permitting an accurate assessment of a patient's responsiveness and personalization of treatment [44].

2.4 Large oncosomes

Large oncosomes (LO) are atypically large (1–10 μm diameter) cancer-derived extracellular vesicles (EVs), originating from the shedding of membrane blebs and associated with advanced disease [45]. They contain proteins and nucleic acids [46]. Proteins such as caveolin-1 and metalloproteinases 2–9 (MMP2, 9) and GTPase ADP-ribosylation factor 6 (ARF6) are reported to be contained in LO [45]. LO contain miRNA, mRNA and DNA, which transmit signaling complexes between cell and tissue compartments. They can propagate oncogenic information, including transfer of signal transduction complexes, across tissue spaces. Compared to other EVs such as exosomes and MV, LO remains a poorly characterized EV type. LO exerts some functional effects varying on different cells from a direct proteolytic activity to the activation of pro-tumorigenic signals into different types of target cells including other tumor cells or cells of tumor microenvironment [47]. LO has been identified in highly migratory and invasive prostate cancer cells [48]. Recent studies have found that LO can contribute to tumor progression because they are able to degrade directly ECM in vitro [45]. Other researchers again have revealed that they have the ability to establish a tumor growth-supporting environment. This they believe is through the export of specific oncogenic cargo to other tumor or stromal cells [49]. Prostate cancer cell–derived oncosomes contain bioactive MMP9 and MMP2 and exhibit proteolytic activity on gelatin. This suggests that they could be a means to focally concentrate proteases that facilitate migration of tumor cells, thus promoting metastasis [50]. Considering their atypical size and their specific release from cancer cells, LO are promising source of both diagnostic and prognostic markers in cancers.

3. Extracellular vesicles in the pathology of cancers

The importance and the role played by the tumor microenvironment on tumor development and progression has been established in recent years [51]. EVs are known to influence the tumor microenvironment either through a direct impact on the tumor or from a distant site which promote future metastasis of circulating cancer cells [51]. Due to these characteristics, key processes involved in cancer developments such as angiogenesis, thrombosis, oncogenic transfer, immune modulation and pre-metastatic niche formation have seen an up-regulation of EVs [52–57]. Compared to non-malignant cells, tumor cells are known to release higher amounts of EVs. In this regard increased levels ESCRT components as well as heparanase and syntenin have been expressed in various cancers [58–60]. Specifically, in colorectal cancer and pancreatic carcinoma, hyperactivity of RalB has been observed and in non-small-cell lung cancer YKT6 overexpression coupled with elevated Rho-ROCK signaling expressed in various type of cancers may contribute to EVs generation in tumor cells [61–64]. On the basis that tumorigenesis occurs due to accumulation of genetic alterations, the metastatic traits of EVs are expressed through the transfer of their oncogenic cargo. Tumor derived EVs through the co-transfer of protein crosslinking enzymes (tissue transglutaminase) and fibronectin, are able to import transformed characteristics of cancer cells on to recipients endothelial cells and fibroblast [57]. Both the cell-intrinsic and environmental signals may influence EV release in tumor cells. EVs production in tumor cells may be induced by the activation of H-RAS^{v12} and EGFRvIII oncogenic signal pathways [65–67]. Again, the level (de)regulation of the machinery, which aid in plasma membrane fusion could also influence the release of EVs in tumor cells. For example, it has been demonstrated that EV secretion could be enhanced when PKM2 (a glycolytic enzyme associated with the Warburg effect) is over expressed leading to phosphorylating tSNARE SNAP23 [68]. Also SRC, a proto-oncogene, through the phosphorylation of the cytosolic domains of syntenin and syndecan is able to stimulate the syntenin exosome biogenesis pathway [69]. On the other hand, in some cancers such as colon cancer cells, mutant proto-oncogene, KRAS could be transferred via EVs to increase the population of recipients colon cancer cells expressing the wild-type KRAS [70]. Further an increase in levels of tissue factor (TF) bearing EVs are known to mediate thrombosis occurrence in cancer patients. Available evidence indicates a possible role of tumor-derived EVs in thrombosis occurrence among cancer subjects [54]. Specifically, P-selectin glycoprotein ligand-1 (PSGL-1) and TF have been implicated in cancer associated thrombosis [71]. In mice with induced pancreatic tumor, formation of thrombosis was high compared to cancer free mice [72]. A major hallmark of tumor growth and development is increased angiogenesis. That is to say for the development of the tumor beyond its minute size an adequate supply of oxygen and nutrients is essential for its survival. Thus, numerous studies have established that besides the cell's intrinsic mechanisms, the release and regulation of exosomes and microvesicles could be due to enhanced prevailing hypoxic microenvironmental conditions [73–75]. In hypoxic glioma cells, an induction of a pro-angiogenic process mediated by derived EVs was able to influence the vasculature surrounding cell [55]. Another specific example where EVs promotes angiogenesis is reported in squamous carcinoma. It was reported that in A431 squamous carcinoma cells, angiogenesis was induced as a results of a direct transfer oncogenic epidermal growth factor receptor (EGFR) from the derived EVs to endothelial cells [76].

RNAs are a major important cargo incorporated into EVs. Cancer cells promote an increase in the release of EVs containing varying amount and types of proteins and RNAs compared to normal cells [77, 78]. There exist an EV-RNA mediated crosstalk within tumors and also between tumors and stroma which could modify the malignant behavior of cancer cells [79]. EV-RNAs derived from tumor may be implicated in the devolvement of oncogenic, pro-angiogenic, and pro-metastatic processes as well as stromal cell differentiation in the tumor microenvironment. Also it is known that normal and tumor cells subpopulations are likely to be driven towards malignant phenotypes aided by tumor derived EVs [79]. Some EV-RNAs are known to actively mediate proliferation, migration, invasion, apoptosis, dormancy and therapy resistance of cancer cells. There seems to be a dual function of EV-RNAs in cancer pathology. Whiles some are known to promote the malignant characteristics of cancer cells, it also possible for some EV-RNAs to inhibit the malignant characteristics of cancer cells. In this regard various studies have reported the ability of EV-RNAs to inhibit mechanisms that favor tumor growth. In order to establish homeostasis, various non tumor cells can produce miRNAs which could suppress the malignant phenotypes of adjacent cancerous cells [80]. This is due to the fact that a natural competition exists between cancerous and adjacent non-cancerous cells during the development of cancer [81, 82]. In hepatocellular carcinoma, EV-miRNAs released from liver stem cells were able to promote apoptosis whiles inhibiting cell proliferation *in vitro and in vivo* [83]. Again, in pancreatic ductal adenocarcinoma cells, tumor-associated stroma cells derived EV-miR-145 inhibited cancer cell viability whiles promoting apoptosis [84]. Similarly, EV-miE-145 derived from adipose tissue-derived mesenchymal stem cells promoted apoptosis and inhibited proliferation in prostate cancer cells [85]. Another important tumor modulatory role influenced by EVs is the immune system modulation [86–88]. Examples of cancer derived EVs in immune-modulation have been reported in the peripheral circulation of oral squamous carcinoma patients in which Fas ligand positive EVs were able to induce apoptosis of effector cytotoxic T cells [89]. Other studies have demonstrated that various Treg regulatory mechanisms such as Treg expansion promotion, Treg induction, Treg suppressor functional upregulation and others have been promoted by cancer-derived EVs (**Figure 1**) [90].

Figure 1. Tumor cells release EV-RNAs. These EV-RNAs mediate many functions including sustaining proliferation, migration, invasion and metastasis, evading growth suppression, dormancy and therapy resistance of tumor cells, which promote growth.

4. Extracellular vesicles in cancer metastasis

The release and (de)regulation of cancer EVs and their cargo critically influence the crosstalk between tumor and stromal in the tumor microenvironment, adjacent normal cells and even distant (pre-) metastatic areas. Various stages of cancer metastasis especially the epithelial-mesenchymal transition (EMT) stage are influenced by cancer stromal cell derived EVs [91]. Mesenchymal stromal cells (MSC) are very important in the cancer stromal EMT induction [92]. There is ample evidence to show that certain components of the cargo carried by MSC-derived EVs could promote cancer metastasis by stimulating, inducing and promoting EMT. Specifically, it was shown that in breast cancer cells, EVs generated from adipose-tissue MSCs could activate the Wnt signaling pathway thus promoting cancer cell migration [93]. Again it has been demonstrated that EVs generated from human umbilical cord MSCs promoted EMT through Extracellular signal-regulated kinase (1/2) (ERK) signaling pathway with subsequent promotion of invasion and migration of breast cancer cells [94]. Also in lung cancer, EVs derived human umbilical cord MSCs promoted EMT and when TGF- β in the MSCs were knocked down EMT was inhibited [95].

5. Extracellular vesicles in biomarkers in cancer diagnosis

EVs have gained extensive attention as promising biomarkers for cancer diagnostics. Characteristics of EV include their relatively short-lived or highly labile in the cytoplasm of donor cells make them a stable biomarker cargo, be it protein, lipid, nucleic acid. In cancer these molecules can be reflective of both the tumors presence and also of cancer staging. Some studies have demonstrated that biomolecules in serum or plasma exosomes are of great value for tumor diagnosis including long non-coding RNAs (lncRNAs), miRNAs, and proteins [96]. Some other important properties of EVs which make researchers believe they represent cancer biomarkers are: (a) most of EVs populations are shed from all cell types in the organism; (b) molecular determinants contained in EVs are dependent on cells/tissues of origin; (c) however the specific EVs cargo (i.e. proteins, miRNAs) is not always coupled to the overexpression in the cells of origin; (d) molecular cargos in EVs can be affected by microenvironment conditions such as inflammation, oxygen deprivation, and metabolic balance; (e) EVs size may affect their content. In several cancers, including ovarian cancer, it has been demonstrated that the expression of a specific subset of miRNAs may potentially be used in clinical practice, for example, for screening or early diagnosis to evaluate the response to therapeutic treatments. EVs in blood and urine of prostate cancer patients contain unique prostate-cancer specific contents that are biomarkers of prostate cancer [97, 98]. EVs are proving to be valuable diagnostic biomarker in pancreatic cancer; flow cytometry coupled with mass spectrometry analysis of exosome glypican-1 can distinguish benign disease from early and late stage cancer [99]. Again, the detection of DEL-1 on circulating EVs facilitated early-stage breast cancer diagnosis and discrimination of breast cancer from benign breast disease [100]. EV-survivin is proposed to be useful in breast cancer diagnosis [101]. Kibria et al. also suggested that EV-CD47 may be a possible breast cancer biomarker [102].

6. Extracellular vesicles as therapeutic targets in cancers

Communication between cells in a tumor microorganism is largely via chemokines, cytokines, or growth factors [10]. These notwithstanding, EVs from cells in the tumor microenvironment are also noted to facilitate such communications owing to their role in tumor progression [103]. EVs are endogenous vesicles whose composition and function makes them attractive vehicles for the delivery of therapeutic agents to target cells. They have experienced increasing attention in recent years since studies into their roles demonstrated their importance as therapeutic nanomaterials. Compared to some existing synthetic or traditional carriers, EVs are considered more suitable for use as nanovesicles due to their characteristic properties of being intrinsically biocompatible, low immunogenicity and toxicity and their ability to cross-physiological barriers such as the blood-brain barrier. In addition, they have biodegradable and modification abilities and have the capability to escape clearing actions of the immune system [104, 105]. The first report of a successful therapeutic application of EVs was reported by Alvarez-Erviti et al. [106] in 2011. In that study, modified exosomes were exploited and a transfer of siRNAs was made into the brain of mice, which resulted in a knock down of the targeted gene. Supporting this hypothesis, a study by Saari et al. observed the delivery of chemotherapeutics to recipient cells and these were subsequently released into intracellular milieu to give rise to an increased cytotoxic bioactivity. The chemotherapeutics were noted to have been loaded by tumor-cell derived EVs [107]. There are three main approaches that are utilized by EVs in their role as therapeutic agents that include elimination of EVs in circulation, inhibition of secretion and disruption of the absorption of EVs.

The elimination of EVs secreted by cancer cells has been one of EV-targeting therapeutic strategies. The first report of the use of this target therapeutic approach was by Marleau et al., [108]. In the study, a hemofiltration system that was capable of targeting EVs from cancer cells by specifically aiming at human epidermal growth factor receptor 2 (HER-2) on the surface of EVs was proven [108]. This targeting of HER-2 which results in the selective elimination of cancer derived-EVs could be very valuable for cancer treatment [109].

A number of studies have focused on other strategies that block EV secretion. Inhibition of intraluminal vesicles formation and release of EVs by the fusion of MVBs to the plasma membrane have been achieved by the use of a sphingomyelinase inhibitor drug, GW4869 [110, 111]. Again, the inhibition of EV production and the transfer of miR-210-3p have reportedly been achieved by the attenuation of neutral sphingomyelinase 2 (nSMase2). nSMase2 is known to control the synthesis of ceramide and suppresses angiogenesis and metastasis in breast cancer xenograft model [112]. Conversely, EV secretion from prostate cancer cells was not inhibited by the downregulation of nSMase2. Meanwhile, nSMases have been revealed in normal neural cells [113, 114]. Their presence in these normal cells indicates the inhibition of some other fundamental pathways. Cancer specific mechanisms of EV secretion are therefore very crucial in the establishment of the role of EVs as cancer therapeutic targets. Quite recently, a group of researchers have identified a number of activators and inhibitors of EV production from prostate cancer cells [115]. This implies a clear understanding of cancer specific mechanism of EV production is required in identifying cancer-specific therapies mediated by targeted EVs.

Reports into the role of EVs have shown that the process of anti-melanoma is facilitated by EVs released by natural killer cells [116]. Similarly, the abundance of histocompatibility complex classes I and II from dendritic cells are capable of

triggering other immune system cell types and also activate antitumour immune responses [117]. The use of these traditional methods in obtaining EVs for direct use as cancer therapeutic targets are not without challenges. Indistinct production mechanisms, low product yield, and the high probability of obtaining EV contents that stand the chance of mutation are a few of such challenges faced by these methods. The intrinsic properties of EVs, however, makes the engineering of these nanoparticles for the purpose of drug delivery to target cells a more favorable approach for cancer management. Engineering parental cells to shed EVs with a particular cargo or loading it directly can achieve encapsulating of therapeutic cargoes into EVs. This has been utilized in breast cancer and leukemia cell studies by Usman et al., [118] in the delivery of RNA drugs by RBC-derived EVs (RBCEVs) which showed an improved miRNA inhibition and CRISPR-Cas9 genome editing with no known cytotoxicity. Other studies on the engineering of EVs include research using mesenchymal stem cells in the overexpression of MiR-379 to obtain MiR-379-rich EVs which functions to subdue metastatic breast cancer development [119].

7. Role of extracellular vesicles in cancer prognosis and treatment response

An increasing amount of research has established that EVs are present in every human biological fluid including lymphatic and seminal fluids, bile, urine, breast milk, ascites, cerebrospinal fluid, saliva and blood, making these fluids a good source for many liquid biopsy approaches [120–122]. An increase in the rate of release of EVs on the account of cellular activation and/or during pathologic conditions may be considered an indication of a possible pathologic condition [123–125]. Real-time cancer treatment response and monitoring can be done using cancer-derived components obtained from these body fluids. The components include EVs, microRNA, circulating tumor cells (CTC), circulating cell-free tumor DNA, long non-coding RNA and EVs [126]. During the development and treatment of cancer conditions, the state of the cell is revealed by the level of active secretion of EVs, which provide timely information on the changing dynamics of the cell [18, 127]. Cancer-derived components like miRNAs obtained through liquid biopsy inhibits mRNA degradation by binding to coding sequences, 5'untranslated region (UTR), or 3'UTR of target mRNAs leading to the inhibition of mRNA degradation or translation [128]. When miRNAs bind to target mRNAs, the mRNA level as well as protein expression are essentially regulated. This means, circulating EVs are latent tools that are utilized in the quest to find a way of monitoring changes in tumor cells during treatment.

A number of studies have reported the relationship between EVs and cancer treatment response. The presence of immune checkpoints and the application of the blocking of these points by some drugs have been exploited in novel anti-cancer treatment regimens [129]. Research into the capacity of EVs as a regulating tool for checkpoint therapy has contributed immensely to the growing need of the essence of monitoring immunotherapy. Anti-tumor immunity and related expressions can be suppressed by programmed cell death 1 ligand (PDL-1) and the identification of these ligands on EVs has shown the potential for use as biomarkers in tumor patients [130]. In a syngeneic mouse melanoma model in C57BL/6 mice and B16-F10 cells experiment by Chen et al. [131]. Analysis of PDL-1 expression proved the application of EVs as a potential monitoring tool in PDL-1 therapy in melanoma patients. PDL-1 expression was either present or knocked down in these models and the levels of tumor-infiltrating CD8⁺ T-lymphocytes was significantly reduced in the PDL-1

expressing group compared those knocked down. A positive correlation which varied all through anti-PDL-1 therapy was observed of interferon- γ and the level of EV associated PDL-1 during the analysis of patients with metastatic melanoma [131].

In some specific cancer studies, König et al., [132] analyzed EV concentration and circulating tumor cells in breast cancer patients as a marker for the close observation, monitoring and prediction of prognosis in primary and locally advanced breast cancer. Analysis of the cells and EVs were done before and after the administration of neoadjuvant chemotherapy (NACT) prior to a surgical procedure. Patients' response to NAC is an early indication of the efficacy of subsequent systemic therapy. The overall after-NACT response is a strong prognostic factor for the risk of reoccurrence [133]. Patients with a pathologic complete response (pCR) after NACT have a significant higher overall as well as disease-free survival (OS, DFS) than their counterpart patients with residual invasive disease [134]. Studies have shown that before the administration of NACT during therapy, there is an overall an increase in EV concentration, which is linked to lymph node infiltration, while the after-NACT elevation of EV concentration is associated with reduced three-year progression-free and overall survival. This means, the analysis of EVs together with CTC analysis is a promising tool in the assessment of residual disease and the monitoring of therapy and disease outcome [132]. Other studies have used EVs in diverse ways with respect to their role in treatment response and prognosis. The first exosome-based liquid biopsy test, ExoDx™ Prostate IntelliScore (Exosome Diagnostics, Inc., Waltham, MA, USA), was approved by the Food and Administration Authority (FDA) in 2019 to analyze the exosomal RNA for the biomarkers PCA3, TMPRSS2:ERG, and SPDEF on urine specimen [135]. The prostate specific antigen available in this approach is an effective diagnostic and prognostic tool and the monitoring of this antigen together with digital rectal examination is utilized in men who have gone through a definitive therapy for localized cancer of the prostate. Again, in non-metastatic prostate cancer patients undergoing radiotherapy, a higher concentration of circulating EVs have been detected by Nano tracking analysis as a means of monitoring treatment response [136]. The study proposed a possible radiation specific induction resulting from the upregulation of hsa-miR-21-5p and hsa-let-7a-5p, both of which are specific miRNAs related to prostate cancer and radiotherapy [137]. This is further supported by the observation of altered expression of blood extracted EVs and their miRNA cargoes in the monitoring of prostate cancer radiotherapy response [138]. A high expression of some specific miRNAs before radiotherapy were noted to be an indication of better therapeutic outcomes [138]. More applications of the role of EVs in the cancer are recorded for cancer conditions such as glioblastoma [139], colorectal [140], liver [141], and non-solid cancers [142].

8. The role of extracellular vesicles in drug resistance in cancers

Due to an improved effectiveness of cancer therapies lately, there have been an increase in the survival rate of diagnosed cases [143]. Some tumors however, remain non-responsive to available treatment regimen resulting in patients going through relapse. Many cancer drugs work by causing damage in the DNA of dividing cells, which eventually result in their apoptotic death. Research has shown that some cells gain the ability to effectively repair the damage caused to them or lose the capacity to recognize apoptotic signals which renders them less capable of submitting to programmed cell death [144, 145]. Such cells become more likely to grow resistant.

The failure of treatment in general and for that matter cancer treatment could occur through various ways. It could happen through drug metabolism alterations, or changes in the efflux and/or absorption of drugs from target cells. In addition to this, the ability of drugs to induce mutations and the inhibition of cellular apoptotic pathways are all ways by which drug resistance could occur. Once acquired, the multidrug resistance of cancer drugs can lead to resistance to other drugs of different structural make-up or target.

Cancer cells have specific characteristic genetic make-up together with varying expressions of tumor suppressor genes and oncogenes. This makes them respond distinctively to various drugs. Interactions exist between host and tumor microenvironment together with changes in these genetic factors which contribute to drug resistance [146, 147]. Drug resistance represents a daunting challenge in the treatment of cancer patients. There are two types of drug resistance: *de novo* drug resistance, which refers to the insensitivity of cancer cells to chemotherapy before receiving drug treatment, and acquired resistance, which refers to the acquired drug resistance of cancer cells after being treated [148]. Understanding drug resistance has not been an easy task because of how complex and challenging their supporting molecular mechanisms are [149–152]. In fact, the source of resistance of a drug in a person may be very different from that of another individual because of the variations in fundamentals of different cellular processes. Playing an important role in drug resistance are extracellular vesicles. They mediate cancer drug resistance such that cells that secrete more of these vesicles show higher level of resistance than those that secrete less [153].

Recently, there are many studies concerning the role and effects of EVs in disease control and drug resistance. They have been identified to show a profound role in the development of chemo-insensitivity and drug resistance [148]. The study into their characteristics increased after they were discovered to be involved aspects of cancer progression including in proliferation, tumorigenesis, angiogenesis, and invasiveness [154–156]. In cancer drug resistance analysis, therapeutic targets are very much implicated in the development of resistance. The mediation of drug resistance by EVs takes place through a number of mechanisms. One is by the reduction of the effective concentration of cytotoxic drugs at target sites through the behavior of EVs acting as a pathway for the sequestration of such drugs. That is, resistance can arise when there is an up-regulation of vesicles that export drugs from cells or a reduction in those carriers that import drugs into the cells [157–161]. The results of such changes are the alteration in the concentrations of chemotherapeutics at the active sites. These vesicles may also act as decoys, carrying membrane proteins and capturing monoclonal antibodies intended to target receptors at the cell surface. They can also mediate cross-talk between cancer cells and stromal cells in the tumor microenvironment, leading to tumor progression and acquisition of therapeutic resistance. Apart from their role in drug resistance within a cell, EVs can transfer the resistance from a cell to another cell [162].

9. The role of EV proteins and RNA transfers in cancer drug resistance

Although cargoes of EVs are passively packaged into EVs, evidence have shown the existence of selective packaging as well [163]. Major components of EVs include protein that contribute to determining the destination of EVs and also influence the phenotype of recipient cells [163]. Vesicular protein transfers between cells constitute a potential mechanism of action of these effects. An example is the transfer of an ATP

binding cassette called P-glycoprotein (P-gp), which has been reported to mediate resistance in recipient cells during their transfer between cells. In other instances, it is the expression of P-gp that becomes induced in receiver cells after a different kind of protein is delivered [164]. For example, the transfer of TrpC5 protein to recipient cells by adriamycin resistant MCF7 cells through EVs is known to stimulate the translocation of NFATc3 protein resulting in the transcriptional activation of MDR1 (ABCB1) promoter [165]. The characteristics and pathways by which drug-sensitive cells acquire resistance from EVs containing P-gp have been investigated quite extensively. The process of transfer of cancer traits from drug resistant cells to drug sensitive cells is dependent on characteristics of donor cell. While EVs from cells of leukemia transfer P-gp to malignant and non-malignant cells, those from drug resistant breast cancer cells transfer P-gp to malignant recipient cells only [166]. These findings demonstrated that P-gp transfer by EVs are potentially tissue selective and are likely associated with the cell of origin of EVs rather than their possible relation to a particular feature of recipient cell membrane [166]. In another study, de-Souza et al., [167] explored the selectivity of P-gp transfer and found no discrimination in relation to cell-type. In their study, EVs from drug-resistant leukemia cells could transfer P-gp to drug-sensitive lung and breast cancer cells. Altogether, these findings indicate the debatable issue of the selectivity of EV cargo and therefore require further investigation.

EVs can also carry non-coding RNAs such as miRNAs, lncRNAs, and circRNAs which are noted to mediate cell to cell transfer of resistance [164, 168]. These RNAs have been found to be associated with cancer progression and their deregulation is noted to support drug resistance in tumors of diverse origins [169–171]. Through the transmission of active biomolecules to neighboring cells, these various RNAs induce drug resistance in recipient cells. The transmitted biomolecules regulates certain genes together with their corresponding signaling pathways [168].

miRNAs contributes to the progress of chemoresistance by influencing the genes that are involved in cell cycle, cell proliferation and survival, apoptosis and immunity [172]. They regulate the genes by inhibiting the translation of mRNA. miRNAs have been reported in several studies to play a role in EV mediated chemoresistance. For instance, several miRNAs have been identified to be involved in the transfer of gemcitabine resistance. Gemcitabine is a chelator of DNA that gets activated by deoxycytidine kinase. EVs secreted by macrophages associated with tumors and having miR-365 cargoes have been identified to induce resistance of pancreatic ductal adenocarcinoma cells in the treatment of gemcitabine. The concentration of triphosphate nucleotides (NTPs) in the recipient cells become increased by miRNAs and the result is competitive interaction between activated gemcitabine and the increased levels of NTPs which efficiently reduces the efficacy of gemcitabine [173]. In another study, abundance of miR-1246 was observed to have been present in EVs secreted by paclitaxel resistant ovarian cancer cells. The transfer of this miRNA upregulated the expression of ABCB1 and inhibited the expression of Cav1 to facilitate paclitaxel efflux and in the process promoting drug resistance phenotype in recipient cells [174]. Conversely, EVs secreted by cancer-associated fibroblasts were found to contain miR-106 when exposed to gemcitabine. Resistance of pancreatic cancer cells (AsPC-1) against the treatment of gemcitabine has been found to be associated with the uptake of miR-106 enriched EVs [175].

Several studies have also made efforts to demonstrate the link between specific EV-transferred miRNAs and drug resistance. Through the EV-mediated transfer of miR-21, drug resistance has been found to be induced in MCF7 cells after they

were co-cultured with EVs from multidrug-resistant chronic myeloid leukemia cell lines [167], Again, cisplatin resistance in lung cancer cells by miR-96 [176], Adriamycin resistance in breast cancer by miR-222 [148], and EVs' miR-155 mediated gemcitabine resistance in pancreatic cancer cells [177] have been reported. Further studies found exosomal miR-19b mediated oxaliplatin-resistance in SW480 colorectal cancer cells [178], tamoxifen-resistance in ER-positive breast cancer MCF7 cells by miR221/222 [179].

10. Conclusion

The EVs are secreted from various types of cells and are regulated by physiological conditions and other pathological conditions including cancers. EVs are considered to be attractive resources for cancer biomarker development. More research to identify potential biomarkers should be performed. This may provide more clues for elucidating the biological functions of EVs in cancer development as well as predicting the disease progression. These researches about the EVs will again offer valuable information to increase our understanding into the pathology of cancer and provide the novel ways to advance the diagnosis and prognosis of cancers.

Acknowledgements

This work was supported by China Postdoctoral Science Foundation (Grant No. 2018M642187).

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