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EXOSOME: EMERGING BIOMARKER FOR CANCER DIAGNOSIS AND THERAPY

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ABSTRACT

Cancer is a leading cause of death worldwide and cancer incidence and mortality are rapidly growing. The prompt diagnosis and treatment for cancer require early screening. There are numerous cancer therapies have been develop such as surgery, radiotherapy, immunotherapy and chemotherapy. These treatment, however, have the ability to kill healthy cells and cause severe side effects. Exosomes are a subset of extracellular vesicles that are secreted by the dynamic, multistep endocytosis process. They transport a variety of functional molecules, including lipids, proteins, nucleic acids (DNA, messenger, and non-coding RNA), and metabolites that aid in intercellular communication. Exosomes have recently been shown to be effective diagnostic, prognostic, and predictive biomarkers. Exosomes have generated a lot of interest in the field of cancer treatment because of these qualities particularly as a biological carrier for certain drugs, inhibitors, antibodies and microRNA. Since specific content within exosomes originate from their cells of origin, this property allows exosomes to serve as valuable biomarkers. This article provides a summary of recent advances in the study of exosomal biomarkers and their role in cancer. We will also discuss the potential use of exosomes as diagnostic and prognostic biomarkers or predictors for various cancer therapeutic strategies. We also provide a brief overview of exosomes formation and function.

INTRODUCTION

Extracellular vesicles are a diverse class of membrane enclosed vesicles distinguished by their cellular origins, sizes and densities. Based on their biogenesis, EVs are divided into three sub-classes: EVs derived from apoptotic cells (50 nm–5 μm) in diameter), plasma membrane-derived microvesicles (also known as "ectosomes") which range in size from (50 nm–1 μm) in diameter) and exosomes (30-150 nanometers). [1] Exosomes are tiny extracellular vesicles that originate from different cell types and serve to mediate cell-to-cell communication. Exosomes contain DNA, proteins, mRNA, circular RNA, micro-RNA, long non-coding RNA etc. They are packaged with a conserved group of proteins, which also includes heat shock proteins (HSP70) and tetraspanins (CD9, CD63, and CD81).These are the primary exosome-specific markers. [2] The Johnstone *et al.* discovered exosomes in the mid-1980s when they discovered that in maturing mammalian reticulocytes, the transferrin receptor and a few other membrane-associated components were shown to be released selectively in circulating vesicles called multivesicular bodies (MVBs), which they named as exosomes. They can be found in most bodily fluids, such as saliva, blood, urine, breast milk, and amniotic fluid, ascitic fluid and hydrothoracic. They are also present in culture medium of most cell types. Exosomes are released from a variety of cell types, including lymphocytes, platelets, epithelial cells, endothelial cells, dendritic cells (DCs), mast cells, mesenchymal stem cells and neurons. [3]

Composition and Formation of Exosomes

Exosomes are made up of DNA, nucleic acids, lipids, and proteins. Exosomes typically contain proteins involved in membrane transport, such as flotillins, annexins and RAB GTPases, MVB-producing proteins such as tumors susceptibility gene 101 proteins (TSG101) and ALIX. Exosomes contain a conserved set of proteins, for instance, tetraspanins, CD9, CD63, and CD81, and heat shock proteins (HSP70). Exosomes contain a variety of nucleic acids, such as mRNA, lncRNA, miRNA, tRNA, snRNA, snoRNA and circRNA. [4] Exosomes are formed by the inward budding of the limited multivesicular body (MVB) membrane, which is generated constitutively from late endosomes. During this process, certain proteins are incorporated into the invaginating membrane, while the cytosolic components are engulfed and enclosed within the intraluminal vesicles (ILVs). The majority of ILVs, known as "exosomes," are released into the extracellular space after fusing with the plasma membrane. [5]

Applications of Exosomes

Figure 1: Applications of Exosomes

Exosomes have various applications as mentioned in above figure 1. In drug delivery, exosomes play a potential role as biological cargo, such as mRNAs, small RNAs, proteins and lipid, across cells. [6] The characteristics of exosomes such as messenger (communicates with neighboring cells) and cell

type specificity makes them an attractive source of biomarker and therapeutic targets. In brain disease models, exosomes membrane markers can be used to identify their cellular origin. [7] In different cancer types, more exosomes are secreted than normal cells which allow them to transfer tumor-associated signaling molecules, such as microRNAs, by fusing exosomes with the target cell membrane. [8, 9] Due to difficulties in isolating exosomes from various body fluids, mesenchymal stem cells (MSCs) derived exosomes have greater therapeutic and regenerative potential. [10] Exosomes have tremendous applications in the field of cancer immunotherapy such as cancer vaccines and as targeted antigen/drug carriers. [11]

Exosomes in Diagnostic:

1. Exosome Proteins as Diagnostic Biomarkers

Exosomes contains a variety of proteins, such as cytosolic proteins as well as origin-specific subsets of proteins. Based on the following characteristics, exosomal proteins from cancer cells are emerging as new biomarkers for cancer monitoring and efficacy assessment:

a) Cancer-related protein, lipid, RNA and DNA in exosomes can be used to test for cancers.

b)

Exosomes are simple to identify clinically due to their small volume, high permeability through the human tissue barrier, and widespread presence in many body fluids

c) The exosome's lipid bilayer membrane structure protects its contents from degradation by enzymes in blood circulation. [12] Exosomal proteins have recently been identified as possible biomarkers for a variety of disorders, including cancer, liver disease, and kidney disease. In 2009, Logozzi et al. reported that plasma CD63+ exosomes are significantly higher in melanoma patients than in healthy controls. [13] Exosome secretion has been reported to be increased in cancer patients; thus, exosomal markers are emerging as attractive targets for cancer detection. [14] Exosome surface proteins from pancreatic ductal adenocarcinoma were studied, and several specific biomarker candidates were discovered (EPCAM, CD151, CLDN4, LGALS3BP, HIST2H2BFand HIST2H2BE). [15] Glypican 1 (GPC1) is one of the most studied surface markers of exosomes derived from pancreatic and colon cancer. [16] The analysis of phosphoproteins in exosomes was of interest, because protein phosphorylation is a common cellular regulatory mechanism for regulating protein activities. Chen et al. discovered more than 100 phosphoproteins in plasma exosomes that are substantially more abundant in breast cancer patients than in healthy people. [17] This study suggests that detecting phosphoproteins in exosomes could help with cancer screening and monitoring. To identify cancer-specific protein signatures of EVs, Hoshin et al. compared the proteomic profiles of EVs derived from tumor tissue and plasma. As a result, they discovered that proteins found in the EVs, such as THBS2, VCAN and TNC could differentiate tumors from normal tissues. Furthermore, they identified a panel of tumor type-specific proteins in the EVs derived from tumor tissue and plasma, which could help in the classification of tumors with unknown primary origin. According to the findings, EV proteins could be used as reliable biomarkers for cancer detection and diagnosis. [18] According to recent emerging evidence, exosomal PD-L1 contributes to immune-suppression, which is a potential predictor of anti-PD-1 therapy in melanoma and Non-Small-Cell Lung Cancer (NSCLC). In heterogeneous and invasive tumor, blood-based exosomal PD-L1expression is an alternative option for tumor biopsy. Besides early diagnosis and prognosis, exosomal proteins are attractive candidates for personalized treatment and post-treatment disease monitoring. [19] Exosomal proteins have the potential to serve as cancer biomarkers, however it is still difficult to detect and measure exosomes in clinical samples. [20] Most recently, in 2013, Yoshioka et al. conducted a comparison of exosomal protein markers in various human cancer types. They discovered that CD63 is more abundant in exosomes derived from malignant cancer cells than those derived from non-cancer cells, further supporting the possibility that exosomal CD63 is a protein marker for cancer. [21] CD81, another tetraspanin family exosomal marker, is essential for hepatitis C attachment and/or cell entry. Furthermore, Welker et al. reported in 2012 that serum exosomal CD81 levels are elevated in patients with chronic hepatitis C and appear to be associated with inflammation and fibrosis severity, implying that exosomal CD81 may be a potential marker for hepatitis C diagnosis and treatment response. [22] Skog et al. discovered glioblastoma-specific epidermal growth factor receptor vIII (EGFRvIII) in serum exosomes isolated from 7 of 25 glioblastoma patients in 2008, implying that exosomal EGFRvIII may provide diagnostic information for glioblastoma. [23]

2. Exosome Nucleic Acids as Diagnostic Biomarkers

Taylor in 2008 demonstrated that eight miRNAs (miR-21, miR-141, miR200a, miR-200c, miR-200b, miR-203, miR-205 and miR-214) previously reported as diagnostic markers for ovarian cancer are found at similar levels in biopsy specimens of ovarian cancer and serum exosomes isolated from the same ovarian cancer patients; however, these exosomal miRNAs could not be detected in normal controls, suggesting that exosomal miRNAs, which are easily attainable, could potentially be used as surrogate diagnostic markers for biopsy profiling. [24]

Rabinowits et al. conducted a similar study in lung adenocarcinoma patients and controls in 2009; comparing circulating levels of tumor-derived exosomes, exosomal small RNA, and specific exosomal miRNAs. They discovered similar miRNA patterns in circulating exosomes and tumour biopsies from lung adenocarcinoma patients, both of which were significantly different from those found in control subjects, implying that circulating exosomal miRNA could be used as a lung adenocarcinoma screening test. [25]

Exosomal miRNAs have also shown promise as biomarkers for esophageal squamous cell cancer diagnosis (ESCC). Tanaka et al. reported in 2013 that the exosomal miR-21 level is higher in serum from patients with ESCC compared to serum from patients with benign tumours without systemic inflammation. Exosomal miRNAs have also been shown to have diagnostic potential for cardiovascular disease and renal fibrosis. [26] Furthermore, a few studies have suggested that, in addition to miRNAs, exosomal mRNAs could be used as biomarkers in clinical diagnostics.

3. Other bio-fluids derived exosomes as diagnostic biomarkers

There is growing evidence that bodily fluids other than serum and urine can be used as a source of exosomes in diagnostic. According Palanisamy et al. (2010) human saliva contains hundreds of stable mRNA core transcripts that could be used as a resource for illness diagnoses. [27] In 2013, Lau et al. demonstrate that saliva exosomes may provide discriminatory biomarkers for pancreatic cancer. [28] Another body fluid that has been researched as a potential source of diagnostic exosomal markers is amniotic fluid. Exosomes were extracted from amniotic fluid by Keller et al. in 2007, proving for the first time that fetal exosomes are present in amniotic fluid indicating the possibility of using amniotic fluid exosomes for early prenatal diagnosis. [29] In 2008, Gilad et al. reported that miRNAs associated with the human placenta (miR-526a, -527, -515-5p, and -R521) are detectable in both pregnant women's serum and amniotic fluid and are correlated with pregnancy stage. [30] Further

research in these areas should broaden the range of applications for exosomal biomarkers.

Table 1: Exosomal RNAs/ Biomarkers for cancer diagnosis

Disease	Associated RNAs/Bio-markers	Role in disease	References
Malignant Cancer	CD63	High level in malignant cancer cells	[13]
Chronic Hepatitis C	CD81	plays a critical role in hepatitis C attachment and/or cell entry	[22]
Gliablastoma	Epidermal Growth Receptor VIII	provide diagnostic information for glioblastoma	[23]
Ovarian Cancer	miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, miR-214	used as surrogate diagnostic markers for biopsy profiling	[24]
Esophageal Squamous Cell Cancer	miR-21	Elevated level in serum from ESCC patients than benign tumor patients	[26]
Pancreatic Ductal Adenocarcinoma	CLDN4, EPCAM, CD151, LGALS3BP, HIST2H2BE, and HIST2H2BF	Exosomes surface biomarkers specific for pancreatic ductal adenocarcinoma	[15]
Colon and pancreatic cancer	Glypican 1 (GPC1)	One of the most investigated exosomes surface marker	[16]
Tumor Screening & Monitoring	VCAN, TNC, and THBS2	Distinguish tumors from normal tissues, help to classify tumors of unknown primary origin and diagnosing the cancer type	[18]
Melanoma/ Non-Small-Cell Lung Cancer	PDL1	contributes to immunosuppression and predictor for anti-PD-1 therapy	[19]

Role of exosomes in Cancer

Exosomes were initially thought to be cellular waste and thus received little attention. However, the analysis of proteins, lipids and nucleic acids isolated from exosomes has improved our understanding of the function of exosomes in epigenetic regulation and intercellular communication. Its role in cargo trafficking along with its differential expression is associated with the disrupted homeostasis and provides an opportunity to defend against different diseases like cancer. [31]

According to Huang et al. (2019), prostate cancer-related transcript 1 (PCAT1) was found in esophageal squamous cell carcinoma (ESCC) cell-derived exosomes and promoted tumors cell growth through exosomes. Additionally, the level of exosomes in the serum of ESCC patients was higher than that of healthy volunteers. In oesophageal squamous cell carcinoma, long non-coding RNA PCAT1, a novel serum-based biomarker, promotes cell proliferation by sponging miR-326. According to Liu X. et al. (2019), miR-501 targeted the BH3-like motif protein in gastric cancer, promoting carcinogenesis and chemoresistance. Exosomes induce cancer initiation and progression; these harmful processes can be avoided by inhibiting exosomes production or uptake by target cells. As a result, exosomes are becoming useful tools for cancer diagnosis and treatment. [32] Exosomes from pancreatic cancer cells were shown to initiate cell transformation by inducing mutations in NIH/3T3 recipient cells. [33] Exosomes derived from breast cancer and prostate cancer cells induce neoplasia through transfer of their miRNA cargo [34] miR-125b, miR-130, miR-155, as well as HRas and Kras mRNAs in exosomes from prostate cancer cells, participate in neoplastic reprogramming and tumor formation of adipose stem cells. [35] Breast cancer exosome-derived miR-122 suppresses pyruvate kinase and subsequent glucose uptake in the lungs, which promotes metastasis. [36] Although RNA shielded by proteins prevents it from being recognized as pathological RNAs that would otherwise elicit inflammatory responses, breast cancer cells induce the accumulation of unshielded RN7SL1 (RNA component of signal recognition particle 7SL1) RNA in Cancer-associated fibroblasts (CAF) exosomes, which ultimately produces a pro-inflammatory response when delivered to immune cells and leads to increased tumors growth and metastasis in mice. [37] Exosomes, through the circulating flow, can travel from their source to distant tissues where they localize to target cells by attaching their surface molecules to receptors on the surfaces of the target cells. Exosomes have attracted a lot of interest in the field of cancer treatment due to their advantageous biocompatibility and high stability as a potent anti-cancer drug delivery or gene delivery system. [38]

Exosome-based cancer therapies

Exosomes have generated a lot of interest in the field of cancer treatment because they can deliver bioactive cargo to cancer cells. Several methods for cancer therapy are currently being developed such as: [38]

1. Use of exosomes derived from immune cells to inhibit cancer cells;
2. Inhibition of the cancer-derived exosomes release;
3. Exosomes as a gene/ drug delivery system

1. Use of exosomes derived from immune cells to inhibit cancer cells

The cells of the immune system (DCs, T cells, B cells) release exosomes. [39] Dendritic cells (DCs) are involved in the first step of tumour cell growth inhibition in cancer immunology by capturing neoantigens and activating the tumor-specific cytotoxic lymphocyte response. [40] DC-derived exosomes (Dex) contain a variety of bioactive cargoes involved in antigen presentation, making them ideal for cancer treatment. [41] Zitvogel et al. discovered in 1998 that tumour peptide-pulsed Dex could activate antigen-specific cytotoxic T lymphocytes in vivo and eradicate or suppress the growth of established murine tumours in a T cell-dependent manner. [42] Furthermore, Munich et al. discovered that Dex can directly kill tumour cells as well as activate natural killer (NK) cells via TNF superfamily ligands. It has also been discovered that cancer cell-derived exosomes stimulate anti-tumor DCs. As a result, Dex is an important strategy for cancer therapy. [43]

Exosomes act to mediate immune modulation (immunosuppressive and immune-activating effects) by communicating within the immune system. Antigen presenting cells such as Dendritic Cells (DCs) transfer antigens between other DCs, activate T helper cells and initiate adaptive immune response. Exosomes released by Mature DCs contains MHC membrane molecules which directly bind to T cell receptors that lead to T cell activation. The T helper cells then trigger B cells resulted in increased production and releasing exosomes which stimulate CD4+ T cells thus play an important role in immune response modulation. [44] Exosomes derived from Immature DCs suppress adaptive immune response that resulted in inducing

T cell apoptosis and promote tolerogenic immune response. These exosomes play an important role of balancing pro-inflammatory as well as anti-inflammatory effector T cells by inducing and differentiating T helper cells into regulatory T cells. [45]

2. Inhibition of the cancer-derived exosomes release
Cancer cell-derived exosomes are thought to accelerate cancer pathogenesis by contributing to the development of the tumor growth, progression, pre-metastatic microenvironment, immune evasion, angiogenesis, drug-resistance and anti-apoptotic signaling. [46] As a result, inhibiting the synthesis, release, and uptake of cancer cell-derived exosomes may be an effective cancer therapy. [47] Bobrie et al. discovered, using a mouse model, that blocking Rab27a, a key mediator of exosomes secretion, resulted in decreased primary tumor growth and the dissemination of metastatic carcinoma (4T1) cells in the lungs. More particularly, inhibiting the function of Rab27a blocked exosomal transfer of miR-494 and resulted in inhibiting the growth and spread of melanoma. [48]

Exosomes as a gene/ drug delivery system
Exosomes have a great potential for cancer therapy as they are able to transfer their cargo to exosomes-recipient cells due to their active targeting and specificity. However, using natural exosomes is difficult and rarely has the desired therapeutic effect. Discovery of modified exosomes carrying particular proteins, RNAs, or drugs have a significant potential for use in the treatment of cancer. Due to their superior biocompatibility, excellent payload capacity, and decreased immunogenicity as compared to other polymeric-based carriers, exosomes offer a tremendous amount of potential as a drug delivery vehicle.

2.1 Exosomes as miRNA transport in cancer therapy
Endogenous miRNAs are small, non-coding RNAs that can control gene expression by binding to specific target mRNAs. As a result, miRNAs may be an effective tool for cancer therapy. Exosomes are stable small vesicles that can transport functional bioactive molecules over long distances with high target specificity. [49] They have been proposed as a potential miRNA carrier in the treatment of cancer. Many researchers have been concentrating on exosomal-based delivery of miRNAs and miRNA inhibitors for cancer treatment during the past few years. Katakowski et al. reported in 2013 that exosomes enriched with the anti-glioma miRNA (miRNA-146b) can suppress glioma growth in vitro and significantly reduce glioma xenograft growth in rats. Similarly, exosomes enriched in miR-101 can inhibit osteosarcoma cell invasion/migration in vitro and metastasis in vivo. [50]

Wang et al. discovered that exosomes containing miR-335-5p can inhibit cancer growth and invasion in vitro and in vivo. Interestingly, O'Brien et al. treated breast cancer in vivo by using modified mesenchymal stem cells to release exosomes enriched with miR-379. They also discovered that systemic delivery of exosomes enriched in miR-379 can significantly lower tumour activity. Another miRNA, miR-145-5p, was similarly shown to promote apoptosis and reduce pancreatic ductal adenocarcinoma cell growth. [51]

Exosomes as protein carrier for cancer therapy
Many scientists have recently started working on developing an exosomal-based cancer vaccine [52] TNF-alpha-related apoptosis-inducing-ligand (TRAIL), for example, is a cytokine that acts as a ligand to induce cell apoptosis. [53] According to Rivoltini et al., TRAIL-armed exosomes have the ability to inhibit the growth of tumours in vivo and promote apoptosis in cancer cells. [54] Additionally, IL-18 enriched exosomes increase Th1 cytokine release and proliferation of peripheral blood mononuclear cells, implying that IL-18 enriched exosomes are more capable of inducing specific anti-tumor immunity because they elicit a larger immune response. Yang et al. discovered that IL-2 enriched exosomes more efficiently induce the antigen-specific Th1-polarized immune response and the cytotoxic T lymphocyte response, resulting in a significant inhibition of tumor growth in mice. [55]

Table 2: Exosomal protein/miRNAs biomarker for cancer therapy

Disease/ Mechanism	Associated Proteins/ miRNAs	Role in Dieases	Reference
Oesophageal Squamous Cell Carcinoma	miR326, PCAT1	promotes cell proliferation by sponging miR-326	[32]
Prostate cancer	miR-125b, miR-130, miR-155, as well as HRas and Kras mRNAs	participate in neoplastic reprogramming through transfer of their miRNA cargo	[35]
Breast cancer	miR-122/ miR-379	promotes metastasis by suppressing pyruvate kinase and subsequent glucose uptake in the lungs & exosomes enriched with miR-379 use in vivo therapy and significantly lower tumour activity	[36]
Gastric cancer	miR-501	miR-501 targeted the BH3-like motif protein in gastric cancer, promoting carcinogenesis and chemoresistance	[32]
Cancer	Dex	Involved in antigen presentation, kill tumour cells, activate natural killer (NK) cells via TNF superfamily ligands & stimulate anti-tumor DCs, ideal for cancer treatment.	[41]
Lung Metastatic Carcinoma	Rab27a, miR494	Blocking Rab27a inhibited exosomal transfer of miR-494 and resulted in decreased primary tumor growth & preventing the growth and spread of melanoma.	[48]
Glioma	miRNA-146b	Suppress glioma growth in vitro & significantly reduce glioma xenograft growth in rats	[50]
Osteosarcoma	miR-101	Inhibition of osteosarcoma cell invasion/migration in vitro and metastasis in vivo	[50]

Pancreatic ductal adenocarcinoma	MiR-145-5P	Promoting apoptosis and reducing pancreatic ductal adenocarcinoma cell growth	[51]
Cell apoptosis	TRAIL(TNF-related apoptosis-inducing ligand)	TRAIL-armed exosomes could promote apoptosis in cancer cells and control tumor progression in vivo.	[54]
Anti tumor immunity	IL18	exosomes enriched with IL-18 enhance Th1 cytokine release, proliferation of peripheral blood mononuclear cells & inducing specific anti-tumor immunity	[55]

CONCLUSION

Over the last decade, research on the biology, purpose, and prospective applications of exosomes has expanded. Nano-sized exosomes are essential as cell communication in cancer-related processes, such as cancer progression, metastasis, and medication resistance. Recent technical advances in exosomes isolation not only facilitated exosomes research but also made exosomal diagnostics more cost-efficient. RNAs and exosomal proteins have both been demonstrated to have diagnostic potential. Exosomes are emerging as prospective indicators for cancer detection and prognosis since they are present in the majority of biofluids. Exosomal biomarkers are generally still in the research and development stages, and their potential use in clinical diagnostics and therapy has not yet been adequately investigated. Further studies are needed to explore the role of exosomes in diagnosis and therapy.

Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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