Exosomes in Cancer: Diagnostic and Therapeutic Applications

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Quratulain Maqsood[1], Aleena Sumrin[1], Yasar Saleem[2], Abdul Wajid[3] and Muhammada Mahnoor[4]

[1]Department of Centre for Applied Molecular Biology, University of the Punjab, Lahore, Pakistan. [2]Department of Food and Biotechnology Research Centre, Pakistan Council of Scientific and Industrial Research Laboratories Complex Lahore, Lahore, Pakistan. [3]Department of Biotechnology, Balochistan University of Information Technology, Engineering and Management Sciences, Quetta, Pakistan. [4]Department of Rehabilitation Science, The University of Lahore, Lahore, Pakistan.

ABSTRACT: Small extracellular vesicles called exosomes are produced by cells and contain a range of biomolecules, including proteins, lipids, and nucleic acids. Exosomes have been implicated in the development and spread of cancer, and recent studies have shown that their contents may be exploited as biomarkers for early detection and ongoing surveillance of the disease. In this review article, we summarize the current knowledge on exosomes as biomarkers of cancer. We discuss the various methods used for exosome isolation and characterization, as well as the different types of biomolecules found within exosomes that are relevant for cancer diagnosis and prognosis. We also highlight recent studies that have demonstrated the utility of exosomal biomarkers in different types of cancer, such as lung cancer, breast cancer, and pancreatic cancer. Overall, exosomes show great promise as noninvasive biomarkers for cancer detection and monitoring. Exosomes have the ability to transform cancer diagnostic and therapeutic paradigms, providing promise for more efficient and individualized. This review seeks to serve as an inspiration for new ideas and research in the never-ending fight against cancer. Moreover, further studies are needed to validate their clinical utility and establish standardized protocols for their isolation and analysis. With continued research and development, exosomal biomarkers have the potential to revolutionize cancer diagnosis and treatment.

KEYWORDS: Exosomes, cancer, diagnosis, therapeutics, biomarker

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CORRESPONDING AUTHOR: Quratulain Maqsood, Centre for Applied Molecular Biology, University of the Punjab, 87 west canal bank road thokar niaz baig, Lahore, Pakistan. Email: qmabc160403473@gmail.com

Introduction

Worldwide, cancer continues to be one of the primary causes of illness and death. Improving patient outcomes requires early identification. Cancer continues to demand innovative solutions for early identification and effective treatment, making it one of the most challenging issues in modern medicine. Cancer cells are characterized by their high rate of division, ability to self-renew, cancer stem cell (CSC) features, proclivity for metastasis, and ability to switch between different metabolic pathways to gain drug resistance.[1] Based on these features, novel therapeutics such as nucleic acid medicines and anticancer pharmaceuticals has been developed to target cancer cells and inhibit their spread. Furthermore, cutting-edge approaches, like the use of nanoparticles, have been employed to precisely deliver therapies to cancer cells.[2] Extracellular vesicles (EVs), a unique kind of structure, have lately emerged as having a role in cancer. Extracellular vesicles are micro- or nanovesicles that emerge from the cell membrane.[3] All prokaryotic and eukaryotic cells can produce these structures in a way that hasn't changed much throughout the course of evolution. Extracellular vesicles were once thought to be cellular byproducts or entities generated by cellular damage. However, further study of EVs has shown that they perform critical biological activities and serve as important cellular building blocks. Extracellular vesicles are classified into many categories according to their size, origin, and geographic location, and exosomes are one of the examples of EVs. Recently, exosomes have taken on a tiny but important function in the intricate realm of cancer research. Numerous physiological and pathological processes have been linked to these small vesicles, which are produced by several cell types.[4] Particularly, their significance in cancer biology has drawn a lot of interest. Exosomes, formerly thought to constitute cellular waste, are now understood to be essential mediators of intercellular communication that transport bioactive substances including proteins, nucleic acids, and lipids between cells.[5] A rapidly expanding body of research is being done in an effort to understand the different ways that exosomes play a role in the origin, development, and spread of cancer as a result of this increased understanding of their functional variety. Conventional cancer biomarkers have limits in sensitivity and specificity, and their application in clinical practice is restricted.[6] Examples include tumor antigens and circulating tumor cells (CTCs). In recent years, exosomes have become a potential new class of biomarkers for the identification and monitoring of cancer. Small EVs known as exosomes are produced by cells and contain a range of biomolecules, including proteins, lipids, and nucleic acids.[7,8] These vesicles have been shown to be essential for intercellular communication as well as the control of cancer development and metastasis.[5] Moreover, exosomes are a desirable noninvasive method for cancer detection and

monitoring since they can be extracted from a range of physiological fluids, such as blood, urine, and saliva. According to recent research, exosomes might be used to identify cancer patients and follow how they react to therapy.[5] Exosomes are EVs with a lipid bilayer membrane and a diameter of 40 to 150 nm. The lipid bilayer membrane keeps the proteins, microRNA (miRNA), mRNA, and DNA that are carried by exosomes stable as they move through the body.[5,9] Because exosomes are stable, they can be quickly separated from a number of fluids that are easy to get. As a result, they are attractive candidates for the development of innovative cancer detection systems.[10] The most often unregulated genes for a certain cancer type must be identified before exosomes and exosomal cargo may be used to diagnose cancer. This is because tumors are complicated and exhibit tumor heterogeneity.[11] Tumor heterogeneity refers to differences in genetic changes and cell behaviors exhibited in the same tumor type in different people, as well as differences between separate tumor forms and their metastases.[12] To optimize patient survival, the proper treatment must be selected, and tumor heterogeneity is critical in this process. As a result, a list of the most likely deregulated genes for a certain tumor type or a group of tumors must be established.[13] As a consequence, much research has focused on identifying deregulated genes specific to a certain tumor type using bioinformatics, sequencing, and machine learning. This review article aims to summarize the current knowledge on exosomes as biomarkers of cancer.[14] We will discuss the various methods used for exosome isolation and characterization, as well as the different types of biomolecules found within exosomes that are relevant for cancer diagnosis and prognosis.[15] We will also highlight recent studies that have demonstrated the utility of exosomal biomarkers in different types of cancer, including lung cancer, breast cancer, and pancreatic cancer.[16] This review article provides a thorough analysis of the several functions that exosomes perform in cancer biology. It explores both their potential as diagnostic indicators for the early diagnosis of cancer and the consequences for the creation of cutting-edge treatment approaches. We want to present a clear picture of the current state of knowledge and the immense potential that lies ahead by carefully reviewing new research and therapeutic developments.[17] Our examination of this complex web of exosome-mediated functions will emphasize their significance in cancer detection, opening the door to more effective and individualized treatment choices. We'll also examine exosome-based therapeutic strategies for effective drug delivery, immunotherapy, and tumor microenvironment (TME) control.[18]

The History and Function of Exosomes

Exosomes were found in sheep reticulocyte culture supernatants in 1983. Due to a lack of knowledge about exosomes' functions, it was previously believed that cells produced nonfunctional microparticles.[19] Exosome research has gotten a lot of interest since then, as have other bioactive substances identified in exosomes. Extracellular vesicles with a membrane are exosomes, and their sizes and marker proteins can help to distinguish them from other EVs.[20] Exosomes are created when multivesicular bodies (MVBs) join the plasma membrane. Exosomes have the ability to travel through extracellular fluid and may be consumed by other cells. The bioactive compounds that exosomes carry affect a wide range of healthy and unhealthy processes in the cells they are sent to.[21] The lipid bilayer walls of exosomes offer effective resistance against the potential of the extracellular environment to destroy exosomal contents.[22] Exosomes are a special kind of intercellular communication since they act as carriers for the *in vivo* transfer of signal molecules between different cells. Exosome roles in pathophysiology have piqued the curiosity of many researchers throughout the past decade.[23] Exosomes regulate a variety of biological processes, such as apoptosis, differentiation, and proliferation. Exosomes contain tens of thousands of mRNAs, miRNAs, proteins, and long noncoding RNAs (lncRNAs).[24] Exosomal proteins and RNAs are variably expressed in a variety of disorders, involving cancer, cardiovascular disease, and diabetes mellitus, according to mounting evidence. Exosome research is becoming more popular as a useful indicator of disease, particularly cancer.[25] Exosomal miRNA-103, tripartite motif-containing 3 protein, glypican-1 protein, and hepatocyte growth factor-regulated tyrosine kinase substrate protein have all been connected to the detection of stomach, liver, pancreatic, and colon cancers. For many malignancies, including Colorectal cancer (CRC), exosomes may serve as a source of tumor markers.[26]

Formation, Release, and Uptake

Inward budding of the plasma membrane and the formation of early endosomes are the first steps in the creation of exosomes. Multivesicular bodies are formed throughout the early to late stages of endosome formation.[27] Multivesicular bodies may subsequently bind to the plasma membrane and be released into the extracellular environment. Exosomes are produced by a number of proteins, but the precise mechanisms by which these vesicles are secreted vary across cell types and remain unknown.[28] Exosome synthesis and release involve complex pathways, including both endosomal sorting complex required for transport (ESCRT)-dependent and ESCRT-independent mechanisms. ESCRT-Dependent Pathway: In the early endosome, specific proteins and other molecules destined for exosomes are sorted into intraluminal vesicles within the endosome.[29] The ESCRT machinery consists of multiple protein complexes that are recruited to the endosomal membrane as shown in Figure 1. ESCRT-0 recognizes and binds to ubiquitinated cargo proteins, while other ESCRT complexes assemble to form a bud on the endosomal membrane. ESCRT-3 drives membrane scission, pinching off the bud to form intraluminal vesicle ILVs containing cargo within the endosome.[30] The endosome, now filled with (ILVs), is transformed into a MVB. Multivesicular

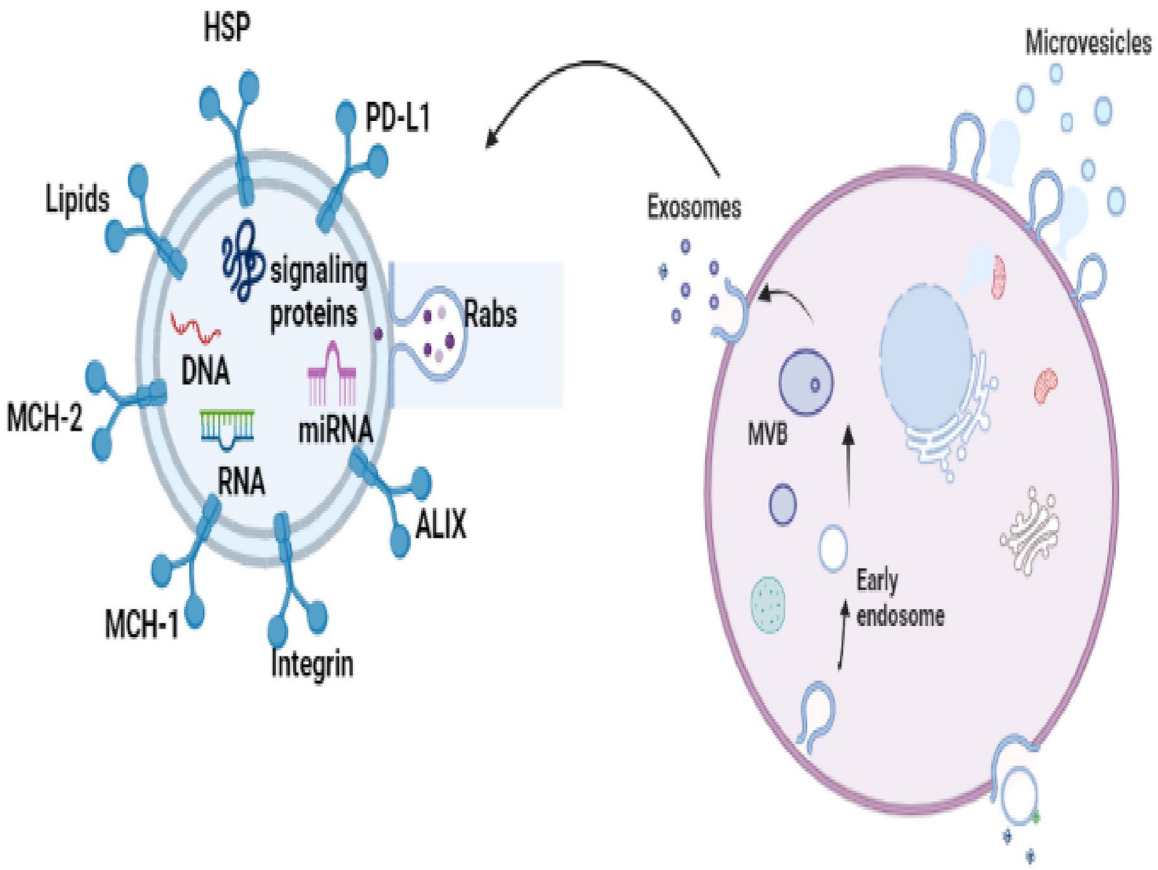

Figure 1. An example of biogenesis and the components of HNSCC. Early endosomes are generated by plasma membrane invagination, which is followed by the payload budding into the endosomal membrane to create multivesicular endosomes. Exosome release and plasma membrane fusion help to mature the late endosome. Some late endosomes collaborate with lysosomes to degrade lysosomes. HNSCC exosomes include a range of biological components, including proteins, lipids, and nucleic acids, all of which are required for signal transmission.
HNSCC indicates head and neck squamous cell carcinoma.

bodies may fuse with the cell's plasma membrane, releasing ILVs into the extracellular area as exosomes. ESCRT-Independent Pathway: Some cells use ceramides to induce membrane curvature and ILV formation without the ESCRT machinery. Tetraspanins and lipid rafts can also play a role in the ESCRT-independent formation of exosomes. Small GTPases such as Rab35 and ARF6 are involved in the regulation of exosome release through ESCRT-independent mechanisms.[31] Exosomes are released into the extracellular space following fusion of the MVB or endosomal membrane with the plasma membrane, whether generated by ESCRT-dependent or ESCRT-independent mechanisms. Exosomes secreted by donor cells may enter receiving cells through endocytosis or membrane fusion. They deliver their cargo, which may include miRNA s, proteins, and lipids, to modulate various cellular processes and participate in intercellular communication.[32] It's important to note that the mechanisms of exosome synthesis and release can vary depending on cell type and context. These pathways are the subject of ongoing research, and new details continue to emerge regarding their regulation and functions. In certain cancer cells, the Rab GTPase family of molecules, which includes membrane trafficking proteins Rab11, Rab27a, and Rab31, facilitates exosome secretion. The Rab family, namely Rab35, regulates exosome secretion by interacting with the TBC1D10Ac protein to activate GTPases.[33] Moreover, p53 activation increases exosome release considerably. This tumor suppressor enhances exosome synthesis by activating route 6 (TSAP6).[34] According to Lespagnol et al, TSAP6-null mice showed significantly lower exosome synthesis. Exosome uptake has been demonstrated to occur by phagocytosis as well as endocytosis via clathrin, lipid rafts, and heparin sulfate proteoglycans.[35] In addition to endocytosis, exosomes can also get into cells by fusing directly with the plasma membrane. Also, these EVs have molecules such as phosphatidylserine and lysophosphatidyl-choline on their surface that help them stick to cell receptors such as T-cell immunoglobulin mucin protein 4 (TIM4), T-cell immunoglobulin and mucin domain 1 (TIM1), and Lymphocyte function-associated antigen 1 (LFA1).[36]

Exosomal Markers in the Diagnosis and Monitoring of Cancer

Because they are so common in human fluids, exosomal markers are great targets for the tools used to find cancer.[37] Also, tumors and other types of diseases make more exosomes than healthy cells do, which may be a sign that a disease is present or that a tumor is heavy. Exosomes can be taken out of any bodily fluid, but plasma is the best way to find most solid tumors.[38]

Table 1. Some of the different types of exosomes that have been studied as potential biomarkers for cancer diagnosis.

EXOSOME TYPE	CANCER TYPE	BIOMARKER(S)	DETECTION METHOD
CD63-positive exosomes	Breast cancer	miR-21, miR-1246, HER2	enzyme-linked immunosorbent assay (ELISA), quantitative polymerase chain reaction (qPCR), western blot
CD81-positive exosomes	Lung cancer	EGFRvIII, EGFR L858R, EGFR T790M	ELISA, immunoblotting, flow cytometry
EpCAM-positive exosomes	Pancreatic cancer	KRAS mutation	Digital PCR
Hsp70-positive exosomes	Colorectal cancer	CD44v6, CD133, EpCAM	ELISA, flow cytometry, western blot
Annexin V-positive exosomes	Prostate cancer	PSA, PSMA	ELISA, flow cytometry, western blot
Glypican-1-positive exosomes	Melanoma	S100B, MIA	ELISA, flow cytometry, western blot

Abbreviations: PCR, polymerase chain reaction

Multiple studies on plasma exosomes have shown a link between the amount of exosomes and the presence of tumors, as well as a drop in the amount of exosomes after a tumor is treated or removed.[39] A pilot study, for example, looked at people with stage 4 oral squamous cell carcinoma (OSCC) who had exosomes that expressed CD63 and caveolin 1. After oral tumors were removed, the number of exosomes that expressed CAV1 went up. This goes against the theory that rising CAV1 expression is linked to tumor growth in oral malignancies.[40] Conversely, a week after resection, CD63-expressing exosomes started to decline. They noticed that longer-term survival was connected to decreased levels of exosomes that expressed CAV1 and CD63 in both situations. This study, therefore, supports the use of exosomal markers to monitor and predict the prognosis of therapy-treated individuals. Cancer research needs to learn more about how to use exosomes to diagnose cancer and keep track of patients.[40] In the United States, it is now possible to get regular tests for breast, cervical, colorectal, endometrial, lung, and prostate cancers. Nevertheless, innovative exosome-based screening strategies may be useful for validating tumor types for which established routine screenings already exist as well as tumors for which such processes are not yet customary or reliable.[41] While imaging scans cannot always distinguish between benign and malignant lesions, exosomal-based diagnostics might provide an additional tool to validate the presence of a worrisome tumor as shown in Table 1. Those with prostate cancer, for example, may have elevated blood levels of the prostate-specific antigen (PSA) even in the presence of benign illnesses. Yet, it was shown that the levels of exosomes expressing CD81 and PSA in blood plasma were exclusively higher in men with prostate cancer.[42] The plasma of 80 men with prostate cancer, 80 men with benign prostatic hyperplasia (BPH), and 80 healthy donors was examined in a second investigation. In the patient sample, exosomes expressing CD81 and PSA were discovered. Prostate cancer patients and healthy donors could be distinguished in this clinical research with 100% sensitivity and specificity.[43] Also, they noted that this method has a 98% specificity and an 80% sensitivity for differentiating between men with BPH and those with prostate cancer. In both situations, the diagnostic performance of blood PSA levels alone is superior to that of PSA with CD81-expressing exosomes.[44]

In another study, which was similar to the first, exosomes were looked at as a way to confirm ovarian cancer when imaging and CA-125 expression levels suggested it. They saw that people with ovarian cancer had a lot more exosomes that were positive for phosphatidylserine (PS) than people who didn't have cancer.[45] In a study that used PS-labeled exosomes to find pancreatic and breast cancers, an increase in the production of tumor exosomes was found in the early stages of the disease, even before there were any visible signs of the tumors. These findings hint at a bright future for exosome-based pan-cancer detection.[46] It's worth noting that research has revealed that the acidic tumor environment may help explain why more exosomes are produced, despite having a lower pH than exosomes from healthy cells.[47] Hence, based just on the pH of the exosomes, it may be possible to detect cancer in a patient. Our findings suggest that exosomes have clinical promise for tumor identification, imaging scan confirmation, and tumor burden monitoring in patients undergoing treatment.[48]

Techniques for Identifying and Purifying Exosomes in Cancer Diagnostics

The potential of exosomes as biomarkers is huge, and they have the ability to dramatically change how we diagnose and treat cancer. Vesicle purification and characterization might benefit from the use of a number of contemporary techniques, such as electron microscopy, Raman spectroscopy, and resistive pulse sensing.[49] These include optical methods including flow cytometry, dynamic light scattering (DLS), and nanoparticle tracking analysis. Vesicular morphology may be revealed using the teaching tool of electron microscopy. Nevertheless, this method is inappropriate for everyday usage since it cannot identify the quantity of soluble contaminants in the sample. Proteins that do not take part in vesicle formation, such as calnexin and gp96, have also been found using western blotting. While this method is helpful, choosing such proteins is challenging since it lacks quantitative data.[50] Nevertheless, existing particle tracking techniques cannot assess the whole size range of sample vesicles or distinguish between vesicular

Table 2. Some of the published clinical research on the use of exosomes as biomarkers for cancer diagnosis, prognosis, or prediction.

CANCER TYPE	EXOSOME SOURCE	BIOMARKER(S)	RESULTS	REFERENCE(S)
Prostate cancer	Serum	miR-107, miR-574-3p, miR-122-5p	Exosomal miRNA panel had AUC of 0.952 for discriminating prostate cancer from healthy controls.	Zou et al[52]
Lung cancer	Serum	Colorectal neoplasia differentially expressed - h (CRNDE-h)	Exosomal CRNDE-h levels were elevated and related to a worse prognosis in NSCLC patients compared with healthy controls.	Zhang et al[53]
Colorectal cancer	Plasma	miR-146a-5p, miR-106b-3p	Poor overall survival and disease-free survival were correlated with exosomal miR-146a-5p and miR-106b-3p levels.	Ghafouri-Fard et al[54]
Breast cancer	Plasma	Multiple proteins	Exosomal protein panel had AUC of 0.944 for discriminating breast cancer from healthy controls and was associated with survival outcomes.	van der Watt et al[55]
Pancreatic cancer	Serum	prostate cancer-associated transcript 1 (PCAT1)	High levels of exosomal PCAT1 were associated with poor prognosis and overall survival.	Li et al[56]
Melanoma	Plasma	Multiple miRNAs	Exosomal miRNA panel had AUC of 0.974 for discriminating early stage melanoma from healthy control.	Makino et al[57]

Abbreviations: AUC, area under the curve; miRNA, microRNA; NSCLC, non–small cell lung cancer.

and nonvesicular materials. Moreover, these techniques lack the sensitivity needed to validate the observed EV heterogeneity.[51]

Exosomes as a Promising Treatment Approach

Exosomes have properties that could make them a good choice for developing new ways to treat cancer, in addition to their use in diagnostic procedures as shown in Table 2. Earlier studies have shown that tumor cells produce more exosomes than normal cells.[58] As a result, lowering the abnormally high quantity of exosomes in the blood to a normal level may be a viable therapy method. There have been several research suggestions to restrict tumor derived exosomes (TDE) release by concentrating on their creation, secretion, or interactions with recipient cells.[59] Tetraspanins are 4-domain transmembrane proteins with a variety of physiological functions in cells. Moreover, by triggering certain signaling pathways, Ras homolog family member A or ADP ribosylation factors-6 (ARFs) may regulate exosome production.[60] By concentrating on these pathways, TDE generation is reduced. According to Bobrie et al, lowering Rab27a inhibited TDE-dependent and TDE-independent mechanisms that change the TME and presumably limit cancer progression.[61] Other members of the Rab family, such as Rab35 and Rab11, prevent TDEs from entering the plasma membrane, lowering exosome synthesis.[62] Moreover, Alonso et al demonstrated that downregulation of diacylglycerol kinase alpha (DGKA) may result in a reduction in the production of exosomes containing Fas ligand.[63] Later research demonstrated that increasing dimethyl amiloride, a voltage-gated calcium channel inhibitor, reduces exosome release.[64] The pH of the microenvironment may also impact exosome release. Therefore, changing proton pump inhibitors is another possibility. Exosomes are secreted by healthy cells to impact a range of physiological processes; suppressing exosome production may result in complications and injury.[65]

Another treatment strategy for cancer is to inhibit exosome uptake. Many endocytic mechanisms, including clathrin-dependent and autonomous endocytosis, allow exosomes to enter cells.[66] There is evidence that the exosome surface proteins may be necessary for exosome absorption. Phosphatidylserine, which is found on the surface of TDEs, is one of these molecules that aid in exosome absorption.[67] Diannexin, a homodimer of annexin A5, has a high affinity for this mediator and may bind to it to prevent absorption.[68] Heparan sulfate proteoglycan receptors play an important role in absorption. TDE-induced tumor cell movement was greatly inhibited by treating them with heparin. Moreover, heparin inhibits the transfer of oncogenic Epidermal growth factor receptor variant III (EGFRvIII) mRNA by interfering with exosome fusion with recipient cells.[69]

Exosomes as Biomarkers

As we learn more about the exosome biological basics and how they relate to cancer and treatment resistance, exosomes and TME are becoming more and more fascinating therapeutic targets.[70] Exosomes are sensitive, precise, and very encouraging indicators of illness, drug resistance, and therapeutic response. Exosomes may originate from any kind of tumor cell; therefore, they can provide a complete image of the whole tumor as shown in Figure 2.[71] Exosome profiling from a heterogeneous cell population, such as a tumor, may 1 day enable noninvasive deconvolution of cell types and status with subsequent high-throughput, high-content probing of pure exosomes.[72] More useful tactics are based on our present comprehension of exosomes and the TME. Further advanced tumors are more likely to develop exosomes. It has been hypothesized that the

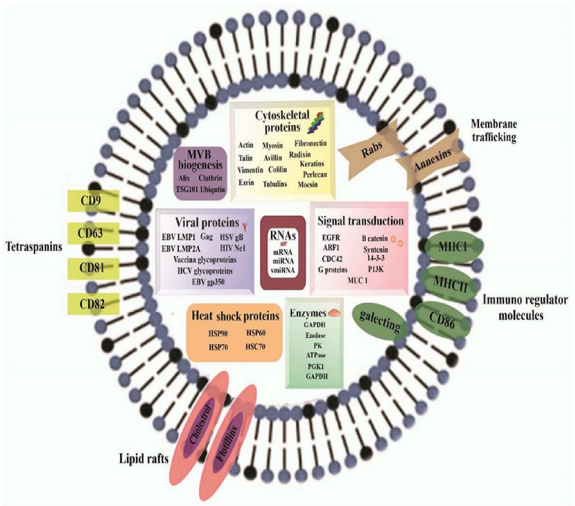

Figure 2. Exosome biomarkers.[11] License Number: 5510271467159. MVB indicates multivesicular body.

total exosome load in circulation may thus detect illness. Exosome composition may also point to a disease.[73] The membrane-bound protein GPC1, which has been shown to be a highly sensitive and specific diagnostic of early stage sickness, has been revealed to be present in exosomes from individuals with precancerous lesions or pancreatic cancer.[74] Contrary to breast cancers with hormone receptors, triple-negative breast cancer patients' serum samples contain a greater number of RNA types. As previously noted, exosomal programmed death ligand 1 (PD-L1) may serve as a regulator and biomarker of melanoma patients' responses to PD-1 inhibition. Exosomes generated by endothelial cells may act as short-term markers of cellular stress, cancer cell health, and the success of antiangiogenic therapy.[75] Exosomes may include nuclear DNA from cancer cells, and their mutational status may be detected. How much of the tumor DNA is present in exosomes or other EVs is unknown. It's also crucial to keep in mind that circulating tumor DNA may now be detected using extremely precise and sensitive technologies, so exosome separation may not be beneficial. It is hard to estimate the extra utility of exosomes as DNA biomarkers since it has not been properly described which cell-free compartments contain circulating tumor DNA.[76] Exosomes show enormous promise as cancer biomarkers; however, consistent isolation and characterization procedures are required before they can be used in clinical trials. The delivery of repeatable exosome yield and purity for therapeutic applications is anticipated to be improved by developments in acoustic and/or microfluidic techniques.

Exosomes in Breast Cancer

Breast cancer is a heterogeneous disease, and the development of effective biomarkers for its diagnosis and treatment is essential. Exosomes secreted by breast cancer cells contain unique biomolecules that can reflect the tumor's biological state, making them a favorable source of biomarkers for breast cancer diagnosis, prognosis, and treatment.[77] Studies have shown that the protein and nucleic acid content of exosomes can provide information on the breast cancer subtype, stage, and response to treatment. For example, the expression of certain proteins, such as HER2 and EGFR, in exosomes has been associated with the human epidermal growth factor receptor 2 (HER2)-positive subtype of breast cancer.[78] Exosomal miRNAs have also been shown to express themselves differently in breast cancer patients as compared with healthy people. Exosomes have several advantages over traditional biomarkers for breast cancer. For example, exosomes can provide a more accurate representation of the tumor's heterogeneity because they are released by various tumor cells within the TME.[79] In contrast, traditional biomarkers may only reflect the characteristics of a single tumor cell. Exosomes can be isolated from blood, serum, and other body fluids, making them a minimally invasive source of biomarkers for breast cancer.[80] Furthermore, exosomes can be isolated using a variety of techniques, including ultracentrifugation, size-exclusion chromatography, and immunoaffinity capture. Exosomes hold great promise as biomarkers for breast cancer prognosis, diagnosis, and treatment. Future research is needed to further characterize exosomes' molecular content and validate their clinical utility as biomarkers for breast cancer.[5]

Lung Cancer

Lung cancer is the primary reason for cancer-related fatalities worldwide. The 3 main types of lung cancer are small-cell lung cancer (SCLC), non–small cell lung cancer (NSCLC), and lung carcinoid. Non–small cell lung cancer (80%-85%) and SCLC (10%-15%) are the 2 most prevalent forms, nonetheless.[78] Non–small cell lung cancer is further split into subtypes that originate from various lung regions; all of these subtypes are treated the same way and have comparable prognoses. The 3 most common subtypes of lung cancer are adenocarcinoma, which accounts for 40% of all diagnosed cases; squamous cell carcinomas, which account for 25% to 30% of lung tumors; and large cell carcinomas, which are often undifferentiated tumors and account for 10% to 15% of lung cancers. Less than 5% of lung tumors are lung carcinoid tumors, which are connected to neuroendocrine cells.[78] Even though modern lung cancer screening systems involve imaging scans, a diagnosis cannot be made without examining lung cells for the existence of a tumor. Collecting a patient's sputum is the least invasive method of searching for lung cancer. This method is effective for malignancies that arise from the airways, like squamous cell carcinoma. Pleural effusion collection is a somewhat more invasive procedure; however, it may not be enough to determine the tumor kind. A more invasive approach, like a needle biopsy of the tumor, might cause the lung to collapse. As a result, less invasive procedures for lung cancer diagnostics are necessary. Exosomes isolated from serum or blood may be a less invasive and potentially dangerous alternative. In pleural effusion, exosomal RNA expression levels may be used to distinguish

between people who have lung inflammation and those who have lung adenocarcinomas. A group of miRs, including miR-200b, miR-200c, miR-141, and miR-375, showed much higher expression levels in lung cancer than in lung inflammation that was not harmful.[81] Moreover, when assessed using receiver operating characteristic (ROC) curves, these miRs had values greater than 0.95. With an area under the curve (AUC) of 0.99, lipocalin-2 (LCN2) has the best diagnostic potential of any exosomal transcript. Even though this is a hopeful diagnosis, collecting pleural effusions is a bit more invasive than collecting blood. It would be intriguing to find out whether the same results could be obtained using blood from individuals with lung inflammation as opposed to lung cancer. In a clinical experiment, the ability of exosomes to monitor patient response to lung cancer treatment is being tested. In the clinical study NCT02869685, the value of PD-L1 mRNA expression in plasma exosomes as a measure of how well radiation works is being looked into. Programmed death ligand 1 is a crucial receptor for the activation of naive T cells, which is critical for the elimination of tumor cells.[82]

Exosomes in Endometrial Cancer

Endometrial carcinoma is the fourth most common type of cancer in the genital tracts of women around the world.[78] Recently, endometrial cancer has become more prevalent, especially in Europe. The endometrium is where the tumor first develops because of aberrant cell proliferation that has the potential to spread to other bodily organs.[83] The main cause of early detection in most endometrial cancer patients is symptomatic postmenopausal hemorrhagia; nevertheless, 20% of cases develop into high-stage malignancies.[84] That is crucial since the survival rate for these people is less than 15%. Even if surgery is recommended as the primary treatment, patients may have adjuvant radiation and chemotherapy. Identifying new targets and indications to employ as valuable tools for endometrial cancer control is so critical. It is becoming more common to look for circulating exosomes in a range of bodily fluids from patients with different malignancies.[85] Moreover, it is anticipated that cancer cells will create more exosomes than normal cells. Exosome significance in the emergence of endometrial cancer is gaining prominence as a research topic. It is believed that exosomes harboring various regulatory RNAs allow endometrial fibroblasts and endometrial cancer cells to communicate with one another.[85] Exosomes produced by cancer-associated fibroblasts (CAFs) were discovered to hasten the development of endometrial cancer, in part because the exosomes lost miR-148b.[86] A tumor suppressor called miR-148b works to stop the progression of endometrial cancer by specifically targeting DNA (cytosine-5) methyltransferase 1. By accelerating the epithelial-mesenchymal transition, DNA methyltransferase 1 enhances metastasis. Moreover, another study discovered that the expression of exosomal miR-320a, which is produced by CAFs, was lower in tissues and cells carrying endometrial cancer.[87] They observed that miR-320a inhibits cell proliferation by targeting HIF1, which lowers vascular endothelial growth factor production. According to recent research, endometrial cancer cells migrated exosomal miRNA-21 when oxygen levels were low.[88] This helped turn monocyte taxotere, herceptin, and pertuzumab-1 cells into macrophages with an M2-like polarization.[89] Also, as was already said, it was found that exosomes taken from the blood of polycystic ovary syndrome (PCOS) patients caused endometrial cancer cell lines to move and spread. Surprisingly, these exosomes have the highest amount of miR-27a-5p targeting sma of caenorhabditis elegans and mad of drosophila melanogaster4.[89] Exosomal hsa-miR-200c-3p was found to be the most important biological miRNA in the urine of endometrial cancer patients. It is a biomarker that doesn't need to be taken out of the body.[90] Another study found that EVs taken from the serum of stage 3 endometrial cancer patients had 209 circular RNA (circRNA)s that were up-regulated and 66 that were down-regulated.[91] The sequestering of miRNAs linked to cancer was the primary mechanism of action of these circRNAs. Most notably, real-time quantitative PCR revealed that hsa circ 0109046 and hsa circ 0002577 could generate a fold change greater than 2. In uterine and peripheral blood samples from endometrial cancer patients, increased concentrations of endothelial (CD144+), total (TF+), and monocytic (CD14+) microparticles were found as potential indicators.[92] The histologic grade and clinical cancer stage agreed with these findings.

Exosomes in Cervical Cancer

Squamocolumnar junction cells are the source of cervical cancer, which is the second most common type of cancer in young women. The most prevalent sexually transmitted infection, the human papillomavirus (HPV), is virtually always to blame.[93] Early detection and disease progression prevention may arise from successful early screening technology. Hence, research into cervical cancer biomarkers is critical for early identification and therapy.[94] The finding of extracellular survival protein inside exosomes in cervical cancer HeLa cells in 2009 resulted in the first study showing the involvement of exosomes in HPV pathogenesis. These cells have pro-proliferative and antiapoptotic properties. In HeLa exosomes, there were 52 miRNAs with various levels of expression, and the silencing of E6 and E7 had an effect on 23 of them.[95] MicroRNA that were up-regulated showed antiapoptotic and pro-proliferative effects, while miRNAs that were down-regulated had the opposite effect. Exosomal miRNAs may have a significant role in the development of cervical cancer, according to multiple studies. Previous studies revealed that cervical cancer patients had higher levels of the miRNAs let-7d-3p and miR-30d-5p in their plasma samples, higher levels of the miRNAs miR-21 and miR-146a in their cervicovaginal lavage specimens, lower levels of the miRNA miR-125a-5p in their cervical cancer cell lines, and higher levels of the miRNAs miR-221 and miR-222

in their cervical cancer cell lines.[96,97] miR-221-3p has an important role in controlling Epithelial–mesenchymal transition (EMT) in cancer cells. It is crucial for keeping local angiogenesis under control. In addition, miR-221-3p may directly target the thrombospondin-2 gene, according to bioinformatics studies. A gene needed for angiogenesis is Thrombospondin-2. It's noteworthy that releasing exosomes rich in miR-22 has been suggested as a potential radiation delivery strategy for cervical cancer. Human telomerase reverse transcriptase and c-Myc binding protein (MYCBP) levels have been shown to be decreased by miR-22.[98] Several studies have also shown that exosomes from different experimental forms of cervical cancer have other chemicals in them. So, the Hedgehog signaling system is important for cervical cancer to start, grow, spread, and become resistant to treatment. Moreover, rat sarcoma (RAS) and activating transcription factor 1 (ATF1) levels were elevated in mice tumors with cervical cancer. Cellular growth, survival, and other processes all depend on ATF1.[99] Moreover, RAS proteins are tiny GTPases that are necessary for differentiation and proliferation processes as well as activities involving growth factor receptors.[100] More research needs to be done to figure out what role exosome cargo plays in cervical cancer and to come up with new ways to use exosomes for therapeutic and diagnostic purposes. This is because exosomes have a wide range of contents and effects.

Exosomes in Ovarian Cancer

Ovarian cancer, which is the most frequent kind of malignant tumor in a woman's reproductive system, is the leading cause of mortality from gynecologic cancer across the globe. Most ovarian cancer patients who are sent to clinics have the illness in late stages. Ovarian cancer is estimated to cause 230 000 new cases each year and kill 150 000 individuals.[78] It's interesting to note that fewer than 50% of patients survive for 5 years. The lack of early diagnostic techniques contributes to the patients' poor quality of life and low survival rates.[101] As a result, it is critical to develop more helpful applications for sickness diagnosis and treatment to halt the rise in disease occurrence.

Ovarian cancer cells may produce exosomes that can be ingested by other cancer cells or healthy cells.[102] This improves communication between cells, which is needed for tumors to grow, spread, and invade. Exosomes from ovarian cancer may possibly serve as new biomarkers and therapeutic targets. Exosomes from ovarian cancer have been demonstrated to contain or include a variety of proteins.[103] Some of these proteins contribute to the aggressiveness of the tumor. Exosomes do, in fact, communicate with other cells and function as carriers for numerous proteins. Proteins may change the TME or impact cell signaling in this case, promoting metastasis and tumor formation. Tetraspanins such as CD24, CD9, CD63, and CD44, as well as exosome-transferred membrane proteins including Alix and tumor susceptibility gene 101 (TSG 101), have been connected to the emergence of ovarian cancer.[104] Exosomal Hsp70 and Hsp90 are also thought to be involved in the illness's genesis. It's worth noting that one study discovered that ovarian cancer patients' exosomes had higher levels of Hsp27 expression.[105]

Important exosomal proteins in ovarian cancer have been found to be enzymes and antigens such as phosphate isomerase, aldehyde reductase, peroxiredoxin, and fatty acid synthase. These factors either influence tumor metastasis or tumor development. Exosomal proteins may possibly play a role in drug resistance. For instance, increased production of the exosomal protein annexin A3 by cisplatin-resistant cells has been associated with platinum resistance in cancer cells. Exosomes have been proven in recent studies to affect recipient cells' chemo-susceptibility via influencing a number of biological processes such as cell cycle and death. miR-130a, miR-106a, miR-221, miR-222, miR-591, and miR-433 have been identified as modulators of therapy resistance in ovarian cancer.[106] Moreover, a recent study discovered that miR-223 is transported to epithelial ovarian cancer cells via macrophage-derived exosomes, increasing treatment resistance through the PI3K/serine/threonine AKT kinase signaling pathway. Since those with epithelial ovarian cancer had greater levels of miR-200f in their blood, earlier research suggested that miR-200f may be employed as a diagnostic marker.[107] Moreover, recent research has shown that exosomal miRNAs generated by epithelial ovarian cancer, such as miR-205 and miR-141-3p, promote endothelial cell vascularization.[108]

Clinical Applications of Exosomes

Because of their flexibility, exosomes are an intriguing prospect for therapeutic usage as personalized nanocarriers for biological substances and markers of patient illness. The efficacy and efficiency of exosome-derived therapies will advance as we understand more about the molecular make-up and physiological function of exosomes. Given the significance of exosome signaling in the TME, focusing clinical therapeutics on exosomes may be a potential technique for reducing the development of cancer therapeutic resistance.

Exosomes in Immunotherapy

It has been shown that antibodies can break down immune checkpoints like cytotoxic T-Lymphocyte associated protein 4 and the PD-L1/Programmed cell death protein 1 (PD-1) signaling axis, which has dramatic and long-lasting effects on a number of cancers. On the contrary, most patients don't get better from these treatments alone because they have natural and learned ways to resist them.[109] To treat solid tumors, there is a lot of interest in combining immunotherapies with conventional or targeted cytotoxic medications.[110] Exosome-based response indicators and the development of rational combinations of treatments may both benefit from a better understanding of how exosomes in the TME suppress or stimulate the immune system.[111] Recent research has shown

that tumor exosomes contain functional PD-L1 and suppress immunologic responses. Exosomal PD-L1 levels were also associated with tumor size and treatment effectiveness in melanoma patients receiving PD-1 inhibition.[112] Exosomal PD-L1 state may be beneficial as a biomarker for PD-1 inhibition prediction, even if it is uncertain whether it directly correlates with immunologic or neoplastic PD-L1 status. Exosomes that carry PD-L1 may serve as indicators of treatment resistance and regulators, much as exosomes do in conventional therapy.[75]

Cancer-Derived Exosomes in the Formation of Biomarkers

The utilization of certain exosomal cargo as a cancer biomarker in different malignancies and the diagnostic effectiveness of such biomarkers in a particular clinical setting are the subjects of several prospective observational studies. Due to rapidly developing nanotechnologies that are producing a new type of cell-free nanomedicine, exosomes may now be used and produced for therapeutic applications.[113] In the development of cancer treatments, it may be tempting to pharmacologically decrease exosome production to stop cancer progression at certain phases of the illness. The use of responsive exosome nanobioconjugates for cancer treatment has recently been shown. These nanobioconjugates may actively target tumors by accurately locating on the surface of tumor cells, blocking signaling, and enhancing macrophage phagocytosis.[114] Further research is being done on synthetic exosomes as possible therapeutic agents or active drug delivery systems. By using the exosomal organotropic properties, it may be possible to specifically target a recipient cell for gene therapy using exosomes that deliver therapeutic substances.

Exosomes Function in the Development and Metastasis of Cancer

Some things are true of all malignant tumors, such as hypoxia, not getting enough nutrients, and extracellular acidity. Exosomes, which are EVs, have recently been found to be a possible way for cells in the TME to talk to each other and spread cancer.[115] When compared with a physiological pH, the amount of exosomes made at a pH of 6.5 was found to be higher. No matter what kind of cancer the cell lines came from (prostate, melanoma, osteosarcoma, breast, or colorectal), they all made more exosomes when they were grown in acidic conditions.[116] Also, exosomes generated at low pH consistently had fewer size differences than exosomes produced at pH 7.4, which exhibited more size variability.[117] Increased exosome release *in vitro* under acidic conditions was the same as the higher levels of plasmatic exosomes found in prostate cancer patients compared with healthy people or people with benign prostatic enlargement, which were used as controls.[65] On the contrary, more and more evidence suggest that the acidity of the TME may have a big effect on how much exosomes are released when there is cancer.[118] Exosomes play a critical role in both the encouragement of malignant transformation in localized mesenchymal stem cells and the establishment of tumor niches in the organs that serve as the hosts as shown in Table 3. All of these results show that exosomes are important in both the growth of primary tumors and their spread to other parts of the body (metastasis).[127] Some evidence, however, suggests that exosomes play a big role in a recent Darwinian-like theory about how tumors form. This theory says that as a tumor grows, it picks out cells that are very good at adapting to their environment. This lets tumors live and grow in an environment that is very bad for them.[128] Also, the results of the experiments show that the acidic environment of the tumor plays a key role in making cancer cells release more exosomes. So, it's possible that the dangerous microenvironment, which probably chooses cells that release vesicles outside the cell to get rid of toxic substances and stop them from building up inside the cell, is to blame for tumor cell exosome hyperproduction.[47] Exosomes produced in an acidic environment exhibit ion transporter like carbonic anhydrase IX (CA IX), which show complete enzymatic activity on exosomes, according to recent research. The hypothesis that CA-IX expression and activity in plasmatic exosome production could 1 day serve as a crucial new cancer diagnostic has been raised by several investigations that demonstrated higher CA-related enzymatic action in plasmatic exosomes from cancer patients.[129] Extracellular vesicles are a high-potential source of tumor biomarkers such as proteins, lipids, and a variety of nucleic acids because of their broad payload. On the plus side, this enables EVs to perform a variety of functions within the TME.[130] The overexpression of established tumor markers like PSA and proteins involved in ion and proton transport, as well as the harsh microenvironmental circumstances of hypoxia, acidity, and a lack of nutritional availability, have increased the release of exosomes by tumor cells.

The rise of circulating exosomes, which have been linked *in vitro* to an acidic pH but have also been found in the plasma of people with cancer, is a very interesting topic. Based on clinical data gathered using an immunocapture-based technique, this was assumed to be the case, and it was validated in patients with malignancies of all sorts in 2017.[38] Also, preclinical *in vivo* research found a link between the amount of plasmatic exosomes and the size of the tumor. This was proven by clinical trials that showed a big drop in the amount of plasmatic exosomes after the primary tumor was removed by surgery.[131] This suggests that counting the number of exosomes in the blood could be a useful way to track the results of both surgical and medical treatments and to keep up with patients. Still, given how important exosomes are to the spread of tumors, it is possible that this huge number of circulating tumor exosomes could pose a big risk to cancer patients. This finding also means that in the future, antitumor drugs may try to reduce the number of exosomes that cancers make.

Table 3. Plasmatic exosomes have been shown to contain protein tumor markers.

PROTEIN MARKER	CANCER TYPE	ROLE IN CANCER	REFERENCE(S)
EpCAM	Multiple types	Tumor cell adhesion, migration, invasion	Wortzel et al[119]
HER2/neu	Breast cancer	Cell proliferation, survival	Ayala-Breton et al[120]
EGFR	Lung cancer	Cell proliferation, angiogenesis, metastasis	Zheng et al[121]
CA125	Ovarian cancer	Cell adhesion, immune evasion	Iliescu et al[122]
MUC1	Multiple types	Cell adhesion, proliferation, immune evasion	Madhavan et al[123]
PSA	Prostate cancer	Cell growth, differentiation	McKiernan et al[124]
CD44v6	Multiple types	Cell adhesion, migration, invasion	Wrana et al[125]
L1CAM	Multiple types	Cell adhesion, invasion, angiogenesis	Wang et al[126]

Abbreviation: PSA, prostate-specific antigen.

Exosomes Clinical Significance as Cancer Biomarkers

Even though there is more research on exosomes as a source of tumor markers for a number of types of cancer, there are only a few diagnostic tests based on exosomes that can be used in clinical settings right now. Immunocapture-based ELISA (IC-ELISA) was used in the early 1990s to measure and describe plasmatic exosomes. Using this method, it was found that melanoma patients had a lot more plasma exosomes that were CD63+ and CAV1+ than healthy donors. Based on the early test results, the IC-ELISA was later made better and put up against other modern technologies such as nanoparticle tracking analysis (NTA) and nanofibrillated cellulose (NFC).[131] The combination of these 3 approaches demonstrated that cancer cells generated more exosomes in acidic environments, a feature common to nearly all malignancies. Immunocapture-based ELISA and NFC were used in the same study to demonstrate that when the environment was acidic, human prostate cancer cells produced more exosomes expressing PSA.[65] The results revealed that prostate cancer patients had much higher levels of PSA-expressing plasmatic exosomes, which had significantly higher sensitivity and specificity than traditional blood PSA in the same people. While serum PSA and exosome PSA levels are different measurements, the investigation results show that blood PSA levels and exosome PSA levels (as determined by either IC-ELISA or NFC) are significantly related, demonstrating the same biological process.[65] Although plasmatic levels of the exosomes that produce PSA could clearly identify cancer patients from both healthy people and those with nontumor illnesses like BPH, blood PSA was unable to discriminate between Pca and BPH patients.[43] This is a significant result since serum PSA testing has a high risk of false positives and false negatives, which may have a range of detrimental effects. Despite its widespread use today for Pca early diagnosis and clinical follow-up, study results provide strong support for the use of IC-based technologies for defining and monitoring circulating exosomes, potentially exposing new sources of clinical biomarkers for cancer patients and maybe others.[43] In particular, IC-ELISA deserves attention because (1) it is noninvasive; (2) it is quick, inexpensive, precise, quantitative, and versatile (easily extendable to other markers or conditions); (3) it requires little sample and has many readouts; (4) it allows for both screenings and follow-up applications; and (5) it is affordable enough to be used in all research and clinical laboratories around the world. The investigation of the expression of known tumor biomarkers, substitute tumor biomarkers, and even new tumor biomarkers on exosomes in a single plasma sample is made possible by the use of IC-ELISA, which specifically permits the evaluation of many markers in a single sample.

Exosomal miRNAs as Cancer Biomarkers

Along with the protein payload, exosomes from different body fluids have a lot of DNA, miRNA, mRNA, and lncRNAs. Since Valadi found exosomal mRNAs and miRNAs, which are the most common types of RNA in exosomes and EVs, there has been a lot of interest in exosomal miRNAs as shown in Table 4.[20] The content and amount of exosomal miRNAs have been demonstrated to differ considerably between healthy individuals and cancer patients, suggesting that they might be used as noninvasive clinical markers.[137] Nevertheless, technological and analytical biases that affect exosomal miRNA synthesis, purity, and integrity have hitherto prohibited its application in clinical cancer. The miRNAs in physiological fluids are packed in vesicles, attached to RNA-binding proteins or related to lipoprotein complexes.[138] RNA that isn't from exosomes gets mixed in with exosomal preparations during ultracentrifugation to get rid of exosomes.[139] This means that RNA gets into EV preparations. Exosome/EV immunocapture may be used as a feasible alternative before miRNA detection is continued. The related exosome or EV population may then be analyzed for miRNAs using this method. When EVs are isolated from patient samples, there seems to be a technological method that will permit more accurate RNA characterization.[140]

Table 4. A some of the RNA types that have been investigated as biomarkers in various cancer types.

RNA TYPE	CANCER TYPE	ROLE IN CANCER	REFERENCE(S)
miRNA	Multiple types	Gene regulation, tumor suppression, oncogenesis	Kikkawa et al[132]
lncRNA	Multiple types	Gene regulation, epigenetic modification, cell differentiation	Statello et al[133]
circRNA	Multiple types	Gene regulation, protein translation, cellular signaling	Qu et al[134]
tRNA	Multiple types	Protein synthesis, regulation of gene expression, apoptosis	Goodarzi et al[135]
rRNA	Multiple types	Protein synthesis, cell growth and division, tumor progression	Zander et al[136]

Abbreviations: circRNA, circular RNA; lncRNA, long noncoding RNA; miRNA, microRNA; rRNA, ribosomal RNA; tRNA, transfer RNA.

Examining the Exosomal Cargo: Limitations and Significance in Cancer Biomarker Development

Exosomes may record aberrant cellular function, making them potentially useful as biomarkers for cancer. Changes in a variety of biological components support this. Yet, this is a significant obstacle to using exosomes as a source of biomarkers. Research is being done on exosome enrichment, and it may be possible to customize each step for a particular cargo, such as protein, DNA, RNA, or another sort. Larger sample quantities or more sensitive methods for detection and quantification would be necessary for various biological preparations to evaluate each biological component in exosomes.[141] Exosome enrichment is required to boost the exosome signal in bodily fluids above nonexosomal noise using a certain volume of liquid biopsy. Yet, each exosome enrichment method is likely to have a specific bias based on the subtypes of exosomes recovered and potential contaminants copurified with the exosomes. Biologics that are co-enriched with exosomes but are not exosomal cargo in and of themselves are contaminants. They are valuable as cancer biomarkers and therapeutically important. The combination of numerous exosome cancer signals has so far largely concentrated on different miRNA sequences, a broad range of DNA alterations, or multiple proteins rather than evoking diverse nucleic acid combinations or nucleic acids in conjunction with proteins. The size of the sample and the availability of data for specialist biological material processing are the key causes of this. When CTCs are insufficient or undetectable, exosomes may provide additional or alternate sources of study materials. While CTCs in the blood may provide information about the prognosis of breast cancer, their use in liquid biopsies of cancer poses a number of practical difficulties. Exosomes are being studied as potential cancer biomarkers in addition to cancer cells, unlike CTCs, as was previously mentioned. As a consequence, we believe they might be helpful for monitoring cancer development and treatment response, as shown by stromal reactions.[142] Exosome biomarker research enables nucleic acid and other cargo analyses, which are presently difficult to investigate with CTCs, in contrast to CTC detection. Other previously mentioned exosome biologics, such as lipids and metabolites, have not yet been studied in large-scale samples for similar reasons. The lack of mass spectrometers, which are necessary for sensitive, high-throughput lipid, and metabolite analysis, makes these problems worse. Future studies combining different biologics for a particular target, such as DNA, RNA, and protein for mutant Kras in pancreatic cancer, may aid in predicting the course of the illness, with each biological material perhaps boosting the sensitivity and specificity of the exosome biomarker.[143] The "combined exosome cancer biomarker" would probably be more accurate if more biologics were included, with a relative score being assigned to the positive or negative detection of various proteins and miRNAs, for instance. Exosomes have the ability to record cellular activity and live cell activities, which means that they may provide information on not only the existence of a malignant lesion but also the state of reoccurring illness and possible recurrence.[144] Exosomes are constantly lost, so it is exciting and encouraging to create designs that incorporate exosome markers into microfluidic devices or other analytical platforms for in-depth studies of biological fluids. The exosome protein evaluation is constrained (when using immunocapture) in favor of a more complete analysis of a number of biological markers, which is one of these approaches' primary limitations. A multistep process that comprises enrichment and identification of a pool of exosomes based on protein presentation, followed by secondary analysis, may help to increase the signal-to-noise ratio for a cancer-specific biomarker.[145] With the development of new techniques based on exosome size, charge, immunoaffinity, or a combination of these, microfluidic devices may be used in exosome biomarker research more often. A multistep approach would be used to distribute liquid biopsy samples for research on diverse biologics, but it would introduce more errors than single-step techniques and be more difficult to implement for high-throughput clinical needs.[146] Even when the illness is asymptomatic, exosomes may be employed as cancer biomarkers, albeit it is still unclear whether their presence should signal invasive therapeutic action or an expensive work-up. Exosomes are heterogeneous in nature, both in their cargo and cellular origins. This heterogeneity can make it challenging to identify consistent and specific biomarkers for different cancer types. Isolating and purifying exosomes from biological fluids can be technically demanding. Existing isolation methods may

not yield pure exosome populations, potentially leading to contamination and inaccurate results. The sensitivity and specificity of exosomal biomarkers may not always meet clinical standards. False positives or false negatives can have significant implications for patient care and treatment decisions. In some cases, obtaining sufficient and high-quality samples for exosome analysis, especially in early stage cancer, can be challenging. This limitation can hinder the development and validation of exosomal biomarkers. Exosomes are sensitive to freeze-thaw cycles and long-term storage, which can affect the stability of biomarkers. Proper handling and storage conditions are critical. There are also an ethical and social implications of exosome use in cancer: The collection and analysis of biological samples for exosome research raise concerns about patient privacy and the need for informed consent, especially if genetic or sensitive information is revealed. The sensitivity of exosomal biomarkers may lead to the identification of cancers that might never cause symptoms or harm, potentially resulting in overdiagnosis and overtreatment. The psychological and emotional impact of receiving a cancer diagnosis based on exosomal biomarkers should be considered, as it may differ from traditional diagnostic methods. In summary, while exosomal biomarkers hold immense promise in cancer diagnosis and treatment, addressing the associated limitations and ethical and social implications is essential to harness their potential effectively and responsibly in the clinical setting. Balancing innovation with ethical considerations and equitable access is a complex challenge that requires collaboration among researchers, clinicians, policymakers, and ethicists.

Conclusions

In conclusion, exosomes represent a promising avenue for cancer biomarker discovery and detection. These small EVs contain a wealth of information about cancer cells, including proteins, lipids, and nucleic acids, that can be used to diagnose and monitor cancer progression. In addition, exosomes offer several advantages over traditional cancer biomarkers, including their noninvasive nature, stability in bodily fluids, and ability to provide real-time information about cancer cells. Exosomes also have the potential to revolutionize medicine. They may be used as natural nanocarriers for the delivery of pharmaceuticals, enabling the delivery of customized medicines with fewer adverse effects. Exosomes may be used in immunotherapy to change how the immune system responds to cancer cells. Exosomes' ability to modify the TME also creates new opportunities for therapeutic approaches that can slow the spread of cancer. It is abundantly evident that exosomes' huge significance transcends even their minute size as we get closer to wrapping up our inquiry into exosomes and cancer. These amazing EVs have risen from relative obscurity to play pivotal roles in the development of cancer medicines and diagnostics. The amazing advances gained in understanding their biology and function are shown by their potential to revolutionize therapeutic practice and enhance the lives of cancer patients. Further studies are needed to validate the clinical utility of exosomal biomarkers and to establish guidelines for their routine use in the clinic. With ongoing research and technological advancements, exosomal biomarkers are poised to make a significant impact on the field of cancer biology and clinical practice.

Author Contributions

QM, AS, YS, AW, and MM equally involved in concept and design of study drafting, data analysis, critically revisiting, and article formatting. All authors have made a significant contribution to this study and have approved the final article.

ORCID iD

Quratulain Maqsood https://orcid.org/0000-0001-7342-9797

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