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Exosome-Mediated Brain Tumor Diagnostics from Peripheral Fluids: A Review of Clinical Data

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ABSTRACT

Definitive diagnoses in neuro-oncology often require invasive procedures, such as surgical biopsies to obtain tissue for histopathologic and molecular interrogation. Patients with small lesions that may respond to nonsurgical treatments, such as chemoradiation, may nevertheless undergo surgery with potential risks to obtain diagnostic tissue. A means for noninvasively obtaining diagnostic information from brain tumors may improve patient care by limiting the need for surgery. Molecular evaluation of exosomes may provide such a means. Exosomes are small vesicles excreted from tumor cells that contain molecular information. Isolation of these vesicles from peripheral fluids, such as blood and urine, may provide diagnostic information for rendering a definitive diagnosis. Here, we review current clinical data for exosome-mediated brain tumor diagnostics.

KEYWORDS: NeuroOncology, Neurosurgery, Glioma, Liquid biopsy, Exosome, mRNA

INTRODUCTION

Approximately 250,000 Americans are diagnosed with primary or metastatic brain tumors annually (44). Treatment for these tumors is guided by pathologic diagnosis from tissue biopsies. Biopsies can be obtained directly from a brain tumor, or peripherally from a suspicious lesion in the case of suspected metastasis. Recently, there have been efforts to develop non-invasive diagnostic techniques using molecules isolated from body fluids to make specific neuro-oncology diagnoses. One group of molecules in particular, known as exosomes, has become a point of interest; they are small extracellular vesicles currently being interrogated for diagnostic utility.

Exosomes begin intracellularly as intraluminal vesicles (ILVs) (13). When a multivesicular body (MVB), an intermediate endocytic compartment, fuses with the plasma membrane, ILVs are shed into the extracellular milieu and become exosomes (13) (Figures 1 and 2). Initially, exosomes were regarded as waste products, but studies have shown they are a unique class of vesicles involved in biological and pathological processes.

Exosomes have distinct biological purposes. They play a role in intercellular communication and are involved in molecular transmission. They transport a variety of cellular components: proteins, lipids, DNA, and mRNA which are specific to their cell of origin. Namely, exosomes have been linked with the ability to transport circulating microRNAs (46). Because exosomes are enriched with miRNAs and can be isolated through centrifugation techniques, detection of miRNA from exosomes has been investigated as a means for detecting primary brain tumors, such as gliomas. Furthermore, miRNAs have been

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found in the plasma and saliva of glioblastoma (GBM) patients (46). Specifically, elevated levels of miRNA-582-5p and miRNA-363 in the serum are correlated with GBM (5). Additionally, exosomes have the same MHC-peptide complexes that T-lymphocytes adhere to, activating the adaptive immune response. Studies show that exosomes are secreted from neoplastic tissue and may help prime sites for metastasis (9,20). Methods for isolating and identifying these exosomes have been explored for diagnostic utility as shown in Tables I and II.

Not to be confused with microvesicles or apoptotic bodies, exosomes are a separate class of extracellular vesicles measuring anywhere from 30 to 100 nm (49). Whereas microvesicles are vesicular structures ranging in size from 0.1-1.0 μm and shed by outward blebbing of the plasma membrane, exosomes are the smallest vesicles and are formed by fusion of multivesicular bodies containing intraluminal vesicles *with* the plasma membrane (43). Apoptotic bodies are the largest extracellular vesicles (1-5 μm) and are released during cell death. While all three are released under various cellular and physiologic circumstances, given how small exosomes are, it is important to separate them from other content and subsequently evaluate their usefulness. In order to segregate them, researchers commonly employ the use of liquid biopsies, which are samples of non-solid tissue, namely blood, taken non-invasively from cancer patients. Because the composition of blood is so diverse – consisting of cell-free DNA, proteins, lipids, extracellular DNA from apoptotic bodies, cellular debris, chromatin, circulating tumor cells, and other substances that can also be removed along with exosomes – further steps to isolate the exosomes through various centrifugation techniques are employed. Having biological markers that can be used to pinpoint these vesicles is also key, and while it is important to identify such markers, an exosome-specific marker has yet to be identified. Currently, a combination of biomarkers are being evaluated for differentiating exosomes from non-exosomal vesicles. Additionally, not all ILVs become exosomes. Therefore, specific criteria for identifying and characterizing exosomes are required (13).

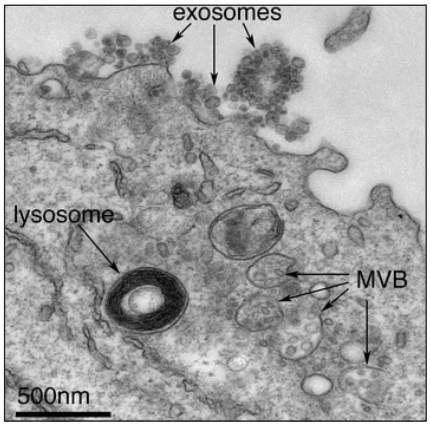

Figure 1: Transmission electron microscopy showing an expelled exosome from the plasma membrane. Multivesicular bodies (MVB) can be seen containing extracellular cargoare endosomes that contain intraluminal vesicles. Lysosomes are a potential destination for MVB cargo (13).

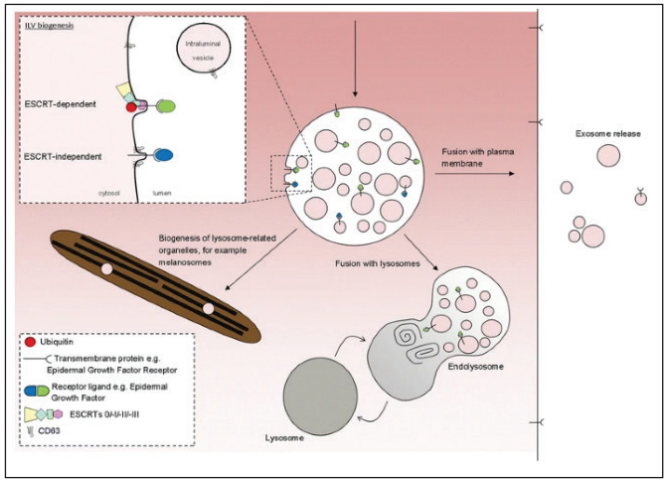

Figure 2: Intraluminal vesicles (ILV) are formed by invagination of the endosomal membrane through ESCRT-dependent or ESCRT-independent processes. ILVs accumulate in mature endosomes and result in three different outcomes. ILVs can transmit content used for the formation of lysosome-related organelles such as melanosomes, ILVs can fuse with lysosomes or ILVs can fuse with the cell membrane to form exosomes (13).

In this non-clinical review, we describe the role of exosomes as robust prognostic and diagnostic markers through careful scrutiny of current clinical studies exploring non-invasive exosome-based neuro-oncology diagnoses and how successfully they are isolated from body fluids and other molecules. Data are included from an extensive PubMed search of all English-language clinical studies evaluating the utility of exosome-mediated diagnoses for human CNS malignancies from 2008 through 2023. We discuss current technology implemented for establishing these diagnoses and the state of the field.

Methods for Article Review

Using the PubMed database for literature review, three separate advanced article searches were initially conducted: one on diagnostic potential, another on prognostic significance, and the last on isolation and detection methods. The various queries consisted of terms, such as “exosomes”, “diagnosis”, “diagnostic potential”, “prognosis”, “prognostic potential”, “isolation methods”, “liquid biopsy”, “brain tumors”, and “neuro-oncology” to optimally refine the searches. Combined, the queries came to a total of 580 publications. Two blinded reviewers went through all the articles, carefully selecting original scientific works and relevant literature reviews and meta-analyses. Any case reports, trials, animal studies, cadaver studies, and any articles non-clinical in nature and completely unrelated to neuro-oncology were excluded. Any duplicated articles were resolved. Ultimately, we narrowed our literature review to 42 relevant articles covering each area of interest in our paper.

Table I: Literature Review of Exosome-Mediated Brain Tumor Diagnostics

Name of Study	Important Findings	Prognostic Significance	Diagnostic Significance
Systemic T Cells Immunosuppression of Glioma Stem Cell-Derived Exosomes Is Mediated by Monocytic Myeloid=Derived Suppressor Cells (11)	Monocytes, responding to GSC-derived exosomes, mediate the CD3+ T cell suppression through the de-differentiation into Monocyte-Myeloid-Derived Stem Cells (Mo-MDSC). The role of GSC-derived exosomes in glioma cell evasion from the immune surveillance is elucidated.	Enhanced immunosuppression secondary to de-differentiation of monocytes may lead to a poorer outcome for patients.	The presence of tumor exosomes worsens the immune response in glioma patients.
Comprehensive proteome profiling of glioblastoma-derived extracellular vesicles identifies markers for more aggressive disease (30)	More invasive GBM cells secrete more exosomes, a strategy that may allow tumors to manipulate their microenvironment and modulate anti-tumor immunity. Additionally, cavitron ultrasonic aspirator (CUSA) washings were identified as a novel source of brain tumor-derived EVs. The analysis of which could expedite the translation of clinically relevant blood-based biomarkers for GBM patient management.	Increased exosome release from tumors is related to a poorer prognosis.	CUSA washings facilitate identification of exosome biomarkers to manage GBM patients
Optimizing preservation of extracellular vesicular miRNAs derived from clinical cerebrospinal fluid (2)	EVs in CSF are stable at RT for at least seven days. Repeated cycles of freezing/thawing should be avoided to minimize experimental artifacts.	Presence of various miRNA within exosomes can be collected and analyzed to determine contribution(s) to poorer outcome.	CSF can be a source of exosomes for identifying tumor markers
Initial evidence that blood-borne microvesicles are biomarkers for recurrence and survival in newly diagnosed glioblastoma patients (15)	The slope and the trend (increasing vs. decreasing over time) in the number of Annexin V positive MV are prognostic of both GBM recurrence and survival.	Annexin V is a protein carried by exosomes that correlates with faster recurrence and shorter overall survival in GBM.	Exosomes released in the presence of GBMs carry certain biomarkers that reflect a GBM diagnosis.
Exosomal levels of miRNA-21 from cerebrospinal fluids associated with poor prognosis and tumor recurrence of glioma patients (40)	Exosomal miR-21 levels may be a promising indicator for glioma and metastasis diagnosis and prognosis, particularly with values to predict tumor recurrence.	miR21 levels in exosomes can determine tumor recurrence or metastasis.	Exosomes released in the presence of GBMs and metastasis carry certain biomarkers that reflect diagnosis.
Serum exosomes and cytokines promote a T-helper cell type 2 environment in the peripheral blood of glioblastoma patients (19)	Th2 bias in the periphery of GBM patients is likely as a result of products elaborated by the tumor. Consequentially, through immune modulation these brain tumors exert systemic effects beyond the confines of the CNS.	Exosomes allow tumors to exert immunosuppressive effects elsewhere in the body, contributing to lower survival and poor prognosis.	GBM releases exosomes with elements that lead to proliferation TH2 T cells and M2 monocytes that perpetuate tumor promotion.
miRNA contents of cerebrospinal fluid extracellular vesicles in glioblastoma patients (1)	Most EVs derived from clinical biofluids are devoid of miRNA content. The relative distribution of miRNA species in plasma exosomes or microvesicles is unpredictable. In contrast, CSF exosomes are the major EV compartment that harbor miRNAs.	The concentration of miRNA in CSF exosomes is much greater than that of peripheral exosomes, which could help determine patient prognosis.	Various miRNA may prove to be a useful biomarkers in the diagnosis of GBM.

Table I: Cont.

Name of Study	Important Findings	Prognostic Significance	Diagnostic Significance
Inflammatory cytokines, interleukin-1 beta and tumor necrosis factor-alpha, upregulated in glioblastoma multiforme, raise the levels of CRYAB in exosomes secreted by U373 glioma cells (25)	U373 cells produce and secrete CRYAB via exosomes and that stimulation with IL-1β and TNF-α significantly increase the levels of CRYAB in not only the cells but also in the secreted exosomes. In addition, cytokine stimulation of U373 cells brings about changes in the secreted exosomal proteome, many of which are involved in cancer progression.	The secretion of CRYAB from GBM exosomes may lead to tumor progression through cytokine stimulation.	CRYAB is an exosome biomarker that may be useful in GBM diagnosis.
MicroRNA and protein profiling of brain metastasis competent cell-derived exosomes (8)	Identification of dysregulated miRNAs and proteins in BM versus non-BM cell-derived exosomes and an increase in adhesion and invasion properties in non-BM cells when they are incubated with BM cell-derived exosomes.	Exosomes derived from metastatic brain tumor cells indicate a worse prognosis.	One up-regulated (miR-210) and two down-regulated miRNAs (miR-19a and miR-29c) in brain metastasis are markers that contribute to the promotion of GBM.
Medulloblastoma exosome proteomics yield functional roles for extracellular vesicles (14)	Identification of a potentially novel tumor suppressor in medulloblastomas based on the presence of HNF4A in D283MED exosomes.	HNF4A acts as a tumor suppressor that points to a better prognosis in medulloblastoma.	HNF4A may prove to be a useful marker against medulloblastoma.
RNA expression patterns in serum microvesicles from patients with glioblastoma multiforme and controls (33)	Overall yields of exoRNA from GBM patient serum microvesicles was higher than yields from normal controls	Certain RNA in exosomes makes for a worse prognosis in GBM versus others.	Exosomes carrying specific RNA proteins point to a diagnosis of GBM.
Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers (42)	Bioanalysis of RNA from microvesicles and their donor cells revealed that microvesicles contain a broad range of RNA sizes consistent with a variety of mRNAs and miRNAs, but lack the ribosomal RNA peaks characteristic of cellular RNA	GBM microvesicles promote tumor progression, making for a poor prognosis.	There are a subset of 11 miRNA that are involved in GBM tumors (miR-11b, -16, -12b, -21, -26a, -27a, etc.).
The roles of exosomes as future therapeutic agents and diagnostic tools for glioma (49)	This review communicates the current knowledge of exosomes' roles that make them crucial future therapeutic agents and diagnostic tools for gliomas. Exosomes secreted by tumor cells carry tumor-specific antigens that enable and suppress the immune system and promote the proliferation, invasiveness, and chemoresistance of glioma.	This review describes exosomes as suitable prognostic and diagnostic markers in GBM patients.	

Table II: Exosome Isolation

Liquid Biopsy/Isolation Technique	Advantages	Disadvantages
ExoQuick Exosome Precipitation Solution	High protein purity.	Low yield and recovery.
Mass Spectrometry	Fast, simple, low-cost, and separated exosomes have complete structure and uniform size.	Reduced purity due to difficulty separating particles of similar size.
Differential Centrifugation	useful for separating heterogeneous solutions into independent components	Damage to exosomes during homogenization. Contamination with other cellular components.
Centrifugation	Highly effective at removing low-molecular weight molecules. Centrifugation tubes are readily decontaminated and sterilized.	Limited sample capacity.
Ultracentrifugation	No need to mark the outer cut body to avoid cross contaminations.	High cost, time-consuming, structural failure, aggregation, and lipoprotein separation is not conducted to downstream analysis.
Sequential Centrifugation	Higher yield of desired product.	Time-consuming. Possible contamination with small, soluble proteins.
Density Gradient Centrifugation	Improves purity of exosomes.	High viscosity of sucrose solution will reduce the settling velocity of exosomes and lead to more time consumed.
Immunoaffinity Chromatography	Small sample size needed. Exosomes can be detected both qualitatively and quantitatively. Strong specificity, high sensitivity, high purity, and high yied.	Harsh conditions for exosome preservation. Not suitable for large-scale separation of exosomes.
Phlebotomy*	Minimally invasive. Fast procedure. Can be repeated and followed up on.	Difficult to do on some patients. More complications may arise.
Cerebrospinal Fluid (Lumbar Puncture)*	Direct contact with CNS. Less background proteins compared to blood draws.	Invasive. Easy to introduce infection directly into the CNS.

**These are the two liquid biopsy techniques used in the context of CNS tumors. Every other method is an isolation technique.*

Materials and Methods Used in Isolation Techniques

Exosome isolation is a topic in both clinical and basic science research. While there are multiple methods of isolation as shown in Table II, the most common technique to isolate exosomes is the differential ultracentrifugation based technique (26). This approach is often perceived as easy to use, requiring little technical expertise, affordability over time (i.e. one ultracentrifuge machine for long term use), and only moderately time-consuming with little or no sample pretreatments. For these reasons, ultracentrifugation-based techniques have become a rather popular option among exosome researchers. Ultracentrifugation uses centrifugation forces up to 1,000,000 x g, and accounts for 56% of exosome isolation techniques used in the research setting, it is considered the gold standard (26).

Differential ultracentrifugation consists of a series of centrifugation cycles at ranges of force between 100,000 and 120,000 x g, and facilitates the removal of cells, vesicles, and debris to purify and isolate exosomes (26,50). Before exosome isolation, protease inhibitors are used to prevent degradation and a cleaning step is used to remove bioparticles (26). Between centrifugation cycles, the supernatant is removed and the pellet is re-suspended with phosphate buffered saline.

There are several methods for ultracentrifugation. Some of these include simple ultracentrifugation (pelleting method), ultracentrifugation with an iodixanol cushion (cushion method), and ultracentrifugation on an iodixanol density gradient (gradient method) (51).

The different isolation methods all result in similar numbers of exosome particles isolated, but exosomes isolated from the iodixanol density gradient technique are more dispersed. Furthermore, when exosomes are filtered, the recovery rate is higher through the gradient method than both the pelleting and cushion method (51). As a result, the dispersibility of exosomes and the recovery rate of exosomes after filtration may be affected after exosome isolation.

Another technique of exosomal isolation is ultrafiltration. This method uses membranes with holes in order to separate products based on size. The larger particles are removed initially by using filters ranging from 0.45 to 0.8 micrometers (41). Membranes with holes smaller than the targeted exosomes are then utilized to remove small particles from the filtrate (41). Ultrafiltration can be used alone or with other isolation methods such as ultracentrifugation. When compared to ultracentrifugation, ultrafiltration has a decreased exosomal recovery, increased contaminants, and lower quality RNA (3).

Poly-ethylene glycol (PEG) based precipitation is another method that can be utilized to isolate exosomes. A water soluble PEG mixture is used to surround exosomes (48). Exosomes can then precipitate out of solution with low velocity centrifugation (24). Other soluble proteins can precipitate out of solution with exosomes such as immunoglobulins and viral particles (41). Therefore the final product can have contaminants, resulting in a high out but low quality isolation. The immunoaffinity capture isolation method enables exosomes to be separated due to their surface markers. Specifically, antibodies targeting the surface molecules on exosomes including CD9 or CD81 can be used (41). This technique involves incubation of the solution with magnetic beads that have antibodies against the exosome markers attached to them (23). Immunoaffinity is frequently combined with other exosome separation methods especially ultracentrifugation in order to improve exosome purity (41).

Size-exclusion chromatography (SEC) can separate exosomes with the biofluid as the mobile phase and the gel polymer as the stationary phase (7). The stationary phase has pores which enable larger particles to elute followed by the smaller particles. This is accomplished because the larger particles have less holes to travel through and will therefore have a shorter avenue to get to the end of the column allowing quicker elution when compared to smaller particles. Although Lindqvist and Sotgards developed the mechanism behind SEC in 1955 in order to separate proteins, it was not until 2014 when SEC was used to isolate exosomes from biofluid (7,27). SEC is efficient with only an average processing time of 20 minutes and is able to isolate pure exosome samples (45). However, it provides a low total yield of exosomes (41,45).

Improved sensitivity at detecting brain tumor exosomes are thought to enhance the treatment outcomes of patients. Detection methods including surface-enhanced Raman scattering (SERS), localized surface plasmon resonance (LSPR), and atomic force microscopy can identify these markers. Raman spectroscopy is a technique that can detect the vibration of a sample by calculating the scattering effect by the laser (18). SERS further improves the Raman signal by absorbing the particles on nanometals (38). SERS is able to differentiate various exosomes from eachother. LSPR is able to detect exosomes in real time and can adjust sensor size to complement the specific exosome needed (35). As a result, LSPR is especially useful for high sensitivity detection of single exosomes (22). AFM is a scanning probe microscope that can be used to detect exosomes in brain tumors (22). AFM can detect the intermolecular forces between the probe and the biofluid allowing measurement of single molecular interactions (22).

Diagnostic Potential of Exosomes

Exosomes are involved in tumor growth and proliferation as they transfer oncogenic proteins and various macromolecules between cells and can alter the phenotype of recipient cells (32). Their microparticle transmission has also been implicated in the priming of metastatic microenvironments (37). Drug resistance can be heightened via compensatory mechanisms mediated by these very same components (28). Because exosomes are involved in tumor development and reflect the overall tumor habitat, after isolation and identification via liquid biopsy, they have the capacity to be used as diagnostic markers as shown in Table I.

Glioblastoma is an aggressive, primary brain tumor with a low survival rate even with treatment. According to the most up-to-date World Health Organization (WHO) guidelines, the diagnosis is restricted to isocitrate dehydrogenase (IDH) wild-type tumors, whereas IDH-mutated tumors have been re-classified as astrocytomas. To expedite its diagnosis, Shankar et al. introduced the concept of liquid biopsy: examination of blood samples to determine the presence of circulating cancer cells and/or fragments of DNA from tumors. This technique may be useful in facilitating early cancer diagnosis and may help establish precise patient treatments. Collection and analysis of liquid biopsies may allow physicians to monitor tumor changes at the molecular level, and help accurately diagnose a tumor (39). Unlike traditional brain biopsies which may pose surgical risks, liquid biopsies are easier to obtain and less invasive. Exosomes isolated from the serum of brain tumor patients have been shown to carry altered epidermal growth factor receptor (EGFR) genes and tumor-specific RNA expression patterns (39). Moreover, exosomes have detectable surface proteins that may facilitate diagnoses (39). Because exosomes are present in nearly all human body fluids, there may be few obstacles to their collection (34). Additionally, exosome biomarkers may be useful clinically to allow early detection of tumors or when biopsies are ambiguous. One such biomarker may be micro RNA, or miRNA, various forms of which have been detected as exosomes have been shown to carry significant amounts of this protein in their cargo. For instance, elevated miR-221 is a biomarker for glioma. One study analyzed the distribution of miRNA within a subpopulation of extracellular vesicles (EVs), which were isolated from glioblastoma cell lines, plasma, and CSF from patients. The study illustrated that glioma-specific miRNA could be identified in the CSF exosome fraction (34).

While progress has been made, attempting to find exosomal biomarkers that demonstrate high sensitivity and specificity for early diagnosis of GBM has proved difficult (6). Cancer patients' bodily fluids have a disproportionately higher number of exosomes than that of their healthy counterparts, and those exosomes demonstrate significant changes in protein expression (6). Because exosomes carry a heterogeneous combination of biological markers, a single biomarker that differentiates exosomes from other extracellular vesicles has not been found. The biomarker diversity, however, may be beneficial as these biomarkers can be combined to give an overall indication of tumor composition and progression (4). In order

for this technique to be effective, purification of exosomes with exclusion of other microvesicles must be perfected in the laboratory (4). At present, imaging flow cytometry (IMFC) is the preferred method for detection of multiple parameters on exosomes, which dually helps to avoid detection of non-exosome events (36).

Studies have demonstrated strong sensitivity and specificity for glioblastoma diagnosis using miRNA based exosomes extracted from patient serum. Exosomes from GBM can cross the blood brain barrier and enter systemic circulation with their cargo, including miRNA (12). Ebrahimkhani et al. reported in a 22-patient study that serum exosome miRNA was accurately diagnostic of GBM preoperatively. Twenty-six different miRNAs were expressed in GBM patients when compared to healthy controls. Furthermore, the 7 most stable miRNAs for classifying GBM were determined to be: miR-182-5p, miR-328-3p, miR-339-5p, miR-340-5p, miR-485-3p, miR-486-5p, and miR-543, which had a 91.7% GBM diagnostic predictive accuracy, and many of which have not been previously identified in free circulating studies (12). Additionally, miR-182-5p, miR-328-3p miR-485-3p, miR-486-5p isolated from circulating exosomes had a 100% accuracy at distinguishing GBM patients from the healthy control population (12). miR-182-5p, specifically, is a marker of angiogenesis and promotes tumor progression (6).

Different tumor types have distinct miRNA profiles that can distinguish specific clinical pathways (29). Ma et al. conducted a 16-study meta-analysis to analyze miRNA's potential as a biomarker to distinguish glioma from healthy controls. Studies in this meta-analysis utilized qRT-PCR to detect miRNA, a technique sensitive for detecting low serum levels of miRNA. Particularly, one study found that that the pooled sensitivity and specificity of miRNA as a diagnostic biomarker for gliomas was 0.87 and 0.86 (29). In addition, Manterola et al. conducted a study of 75 patients with newly diagnosed and untreated GBM in order to determine if a diagnostic exosome miRNA biomarker could be identified. His team found that RNU6-1, a small noncoding RNA, miR-320, and miR-574-3p were overexpressed in GBM patients (31). RNU6-1 had the best diagnostic performance with a sensitivity of 73% and specificity of 70% (31). Additionally, miR-320 had a sensitivity and specificity of 65% and miR-574-3p had a sensitivity and specificity of 59% (31). As a result, RNU6-1 on its own, or a combined signature of RNU6-1, miR-320, and miR-574-3p may have diagnostic utility for GBM.

In another study, Ivo D'Urso et al. incorporated 112 plasma samples from 53 male and 59 female patients. miR-15b and miR-21 were found to be increased in glioma patients, while miR-16 differentiated glioblastoma from other grades of glioma (21). Alternatively, miR-24 levels were low in both healthy and glioma patients, and miR-16 levels were decreased in patients with glioma (21).

For metastatic brain tumor cells, miRNA and protein profiling also provide insight into the molecular content of exosomes (8). Camacho et al. found that while key mitotic cell cycle components, like fibronectin and cyclin D1, were highly expressed in exosomes, tumor suppressors, such as caveolin-1 and neurofibromin-2, were not significant cargo in exosomes. Another molecule, microRNA-210, was also significantly elevated in these extracellular vesicles (8). About 90% of GBM tumors have also been shown to express cytomegalovirus (CMV) proteins, such as pp65, which has been found in exosomes. Aside from acting as a biomarker, this may be a viable target for vaccines against GBM as well to stimulate immunity against the tumor (22). By pinpointing the content and markers carried by exosomes, physicians may be able to better diagnose metastases in cancer patients.

Prognostic Potential of Exosomes

Exosomes may have a role in evaluating tumor progression and severity. One study found high serum levels of exosome-reactive antibodies from GBM patients, who also had high numbers of exosomes. Harshyne et al. demonstrated that a significant number of patients with GBM had tumor exosome-reactive immunoglobulins IgG2 and IgG4 antibody isotypes consistent with Th2 immunity (19). In turn, the immunomodulation exerted by a Th2-biased system allows for tumors to extend beyond the boundaries of the central nervous system (19).

Many clinical trials have corroborated the impact of exosomes on tumor growth, namely in anti-tumor immunity and immunosuppression. The immunomodulatory role exosomes have to facilitate tumor progression is quite extensive. They also contain mutated genes that, when expressed, expedite poorer prognoses in patients. In a new study by Ding et al, four glioma gene data sets were utilized to stratify the prognostic outcome of high- and low-risk patients, differentiated by the degree of infiltration by immune cells, by genes that were altered in the setting of GBM and carried by exosomes (10). It was found that while TP53 and EGFR were more commonly mutated in the low-risk group, these mutations were different from those in the high-risk group (10). The strongest concurrent pairs of gene alterations in the high-risk group were CARD6-TP53, PIK3-F5, DNAH-PIK3CG, whereas those in the low-risk group were VWF-SPTA1 and ATP2B3-PIK3CA; additionally, BCL11A, HMCN1, and TP53 were frequently altered between patients in both groups (10). All of these different combinations are in some way involved in cell growth, proliferation, cell cycle progression, or a role in immunologic function. These findings prove valuable in that they set the stage for more targeted therapy aimed at slowing disease advancement and tumor burden, and therefore a better prognosis.

Exosomes may also be used to arbitrate the recurrence and survival in newly diagnosed GBM patients (15). According to Evans et al., an increased number of exosomes carrying phosphatidylserine – a marker of apoptosis – on their external surfaces are associated with earlier recurrence and shorter survival in GBM patients receiving chemoradiation therapy (15). Those exosomes that also carry NANOG DNA play a key part in tumor progression and intercellular communication, promoting the spread of cancer (47). NANOG DNA encodes for the Homeobox protein NANOG which is a transcription factor that maintains the pluripotency in embryonic stem cells (17). However, the NANOG DNA that is associated with exosomes is not full length, and instead, comes in vari-

ations and consists of mixed populations of sequences (47). This poses a challenge because there is no single generic gene sequence that can be consistently used to classify such exosomes. These GBM-derived exosomes carry significantly more NANOG DNA than their normal cell counterparts and are also suggested to correlate to patient survival (47). The sooner such exosomes are identified, the faster physicians may work to identify patients at the greatest risk for recurrence and disease progression (15).

CONCLUSION

In the United States, brain tumors have a yearly incidence of 15 to 20 cases per 100,000 people and are a top 10 cause of cancer-related deaths (16). An early and accurate diagnosis is needed to effectively manage these cancer patients. Exosome-based diagnostics are growing rapidly and may provide a minimally invasive screening technique for brain tumors. Exosomes may compliment tumor biopsies by offering a quick, non-invasive method for capturing tumor-specific molecular information and their use may be extrapolated in the setting of chronic inflammation, metabolic diseases, and cardiorenal pathologies as well.

Declarations

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Availability of data and materials: The datasets generated and/or analyzed during the current study are available from the corresponding author by reasonable request.

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AUTHORSHIP CONTRIBUTION

Study conception and design: MB, JG
Data collection: MB, JG
Analysis and interpretation of results: MB, JG
Draft manuscript preparation: MB, JG
Critical revision of the article: JG, RM, CB, AV, SY
All authors (MB, JG, RM, CB, AV, JG, SY) reviewed the results and approved the final version of the manuscript.

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