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Review Article

Liquid biopsy in central nervous system tumors: the potential roles of circulating miRNA and exosomes

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Abstract: The Central nervous system (CNS） tumor still remains the most lethal cancer, and It is hard to diagnose at an earlier stage on most occasions. It is found that recurrent disease is finally observed in patients who occurred chemo-resistance after completely primary treatment. It is a challenge that monitoring treatment efficacy and tumor recurrence of CNS tumors are full of risks and difficulties by brain biopsies. However, the brain biopsies are considered as an invasive technique with low specificity and low sensitivity. In contrast, the liquid biopsy is based on blood and cerebrospinal fluid (CSF) test, which is going to acceptable among the patients through it's minimally invasive and serial bodily fluids. The advantages of liquid biopsy are to follow the development of tumors, provide new insights in real time, and accurate medical care. The major analytical constituents of liquid biopsy contain the Circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating cell-free microRNAs (cfmiRNAs), and circulating exosomes. Liquid biopsy has been widely utilized in CNS tumors in recent years, and the CTCs and ctDNA have become the hot topics for researching. In this review, we are going to explain the clinical potential of liquid biopsy biomarkers in CNS tumor by testing circulating miRNAs and exosomes to evaluate diagnose, prognosis, and response to treatment.

Keywords: Central nervous system (CNS), tumor, liquid biopsy, circulating miRNAs, exosomes, diagnosis, treatment respond

Introduction

Central nervous system (CNS) tumors are a kind of tumors with a large variety, high morbidity, and mortality. Such tumors usually originate from different cells, where these cells may appear from the CNS itself, or they may be caused by systemic tumors that metastasize to the CNS [1]. At present, the main treatment strategy is radio-chemotherapy which is according to the results of the pathological examination of surgical resection. Temozolomide (TMZ) is mainly used as postoperative adjuvant chemotherapy. The follow-up treatment is decided by the original tissues, as the tumor may change longitudinally over time, which makes it treatment resistance after initial treatment. Meanwhile, which makes it less effective is due to the therapeutic treatment is also affected by the difference of individual genotype, the degree of surgical edge resection, and the existence of the blood-brain barrier [2].

Currently, imaging examination and cytology are widely used in the diagnosis and prognosis of CNS tumors. Especially, as the early stage of drug intervention affects the imaging characteristics of the tumor, the therapeutic effect is hard to confirm, and the phenomenon is called "false progression". Secondly, there is hysteresis in imaging examination, which cannot synchronically reflect the exact changes of the tumor, makes it invariable in drug guidance, until there is the occurrence of visible changes in glioma patients' imaging exams [2, 3]. However, there are some defects in the traditional cytological examination. Firstly, the cytological examination is difficult to obtain materials, often only rely on intraoperative resection of tissue for examination, which can not be dynamically monitor tumor changes. Secondly, there are confounding factors in the positive rate of cytology, such as sampling site, sampling method and sample processing time. In addition, there are some risks as it is an inva-

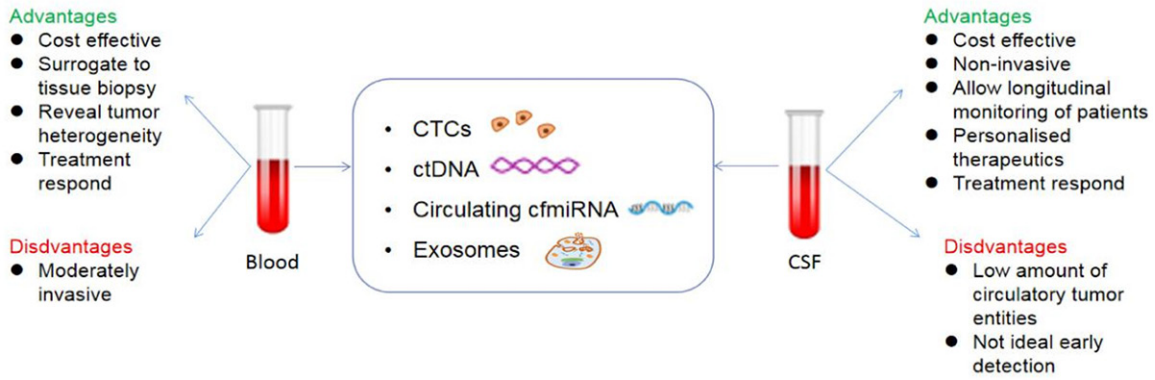

Figure 1. Comparision liquid biopsy of blood and CSF in central nervous system tumors.

sive operation, especially for the tumor patients in special parts, which makes the postoperative complications easily occur. Therefore, to find more sensitive, reliable tumor markers that can dynamically reflect tumor genetic information and treatment response has become a difficult problem for the diagnosis and treatment of CNS tumors [4].

Liquid biopsy is used to monitor the dynamic low-invasive monitoring of tumor by monitoring the CTCs, ctDNAs, circulating cfmiRNAs, and circulating tumor extracellular vesicles (EVs) of primary or metastatic tumor in urine, CSF and blood samples [5]. A large number of researches have proved that the gene information of tumor-specifies can be detected from the patients with malignant tumors in their blood. Since then, blood has become the most commonly used liquid biopsy specimen for various tumors with the advantages of convenient access, non-invasive, and dynamic reflection of tumor progress [6]. For the CNS, CSF can circulate continuously in the ventricles and cisterns of the spinal cord. It can fully contact the CNS and carry tumor metabolites and exfoliated tumor cells, which makes CSF as an ideal sample for CNS tumor fluid biopsy (**Figure 1**). Viral DNA was detected in the CSF of patients with herpes encephalitis in 1990. In 1994, Rhodes et al. detected tumor-specific p53 gene mutations in the CSF of patients with glioma [4, 6]. After that, the liquid biopsy of CSF has become a research hotspot. To compare with traditional biopsies, the liquid biopsies will not cause brain tissue damage and can be performed continuously at different stages of the disease, which is easier to be accepted by patients with few complications. in addition, it can dynamically monitor the disease. Beyond that, guiding to adjust the treatment plan according to the Liquid biopsy's detection results. The detection of tumor-specific targets is conducive to the development of targeted drugs. In the past 30 years, a large quantity of researches have shown the clinical significance of CTCs and ctDNA in the CNS [7].

Circulating miRNA is a subclass of non-coding RNA (ncRNA). It is composed of 18-22 nucleotides of single-stranded ncRNA fragments, which can be detected in almost all body fluids. Recent researches have demonstrated that circulating miRNAs play an important role in gene regulatory networks, which regulate many gene targets [8]. Circulating miRNAs are covered in intercellular communication, and their main function is to upregulate or down regulate the expression of their target genes. These pieces of evidence not only indicate that cfmiRNA is not only an important therapeutic target but also a potential tumor marker, because their expression reflects many gene information. At the same time, circulating exosomes are secreted by living cells, which is a special type of exosomes exist in the blood circulation or extracellular cavity. Exosomes can mediate intercellular communication and change the molecular activity of receptor cells by releasing biological factors [9]. Therefore, the exosomes released by tumor cells contain tumor-specific biomarkers and can detect the characteristics of primary tumors. At present, a large quantity of researches have proved that humoral circulating exosomes are rich in tumor gene information, which can accurately predict tumor progression and treatment response [10, 11].

In this paper, the latest research progress of CTCs and ctDNA in nervous system tumors is briefly introduced, and the research on circulating cfmiRNA and circulating exosomes is summarized in detail. At the same time, this paper we discusses the clinical potential of liquid biopsy in CNS tumors and tries to clarify its role in the early diagnosis, prognosis, and treatment response of CNS tumors.

Circulating tumor cells (CTCs)

CTCs are tumor cells that fall off from primary or metastatic tumors to the blood, CSF, or urine. Results of several reports have demonstrated that CTCs were an effective biomarker for predicting the prognosis of various cancers, such as lung cancer, melanoma, osteosarcoma, and pheochromocytoma [12]. It is worth noting that CTCs can be used as tumor substitutes and analyze tumor molecular biomarkers. The most commonly used CTCs monitoring method relies on monitoring epithelial cell adhesion molecules (EpCAM), which are expressed on the surface of most cancer cells but not glioma cells. Therefore, different CTCs enrichment and identification methods are currently used to monitor the CTCs in the CSF of glioma patients [13]. Several reports claimed that CTCs play an important clinical role in early diagnosis, drug resistance monitoring, and prognosis of CNS malignant tumors [14].

Studies have shown that CTCs in CSF of glioma patients have high mesenchyme and a small amount of nerve signature. As the copy number of epidermal growth factor receptor (epithelial growth factor receptor, EGFR) increases, the number of chromosomes also changes (Chromosome 3, 7, and 12 added, chromosome 10, 13, and 22 lost) which is synchronous with the primary tumor [16]. The use of next-generation sequencing (NGS) technology or more sensitive targeted detection methods to analyze the mutations of CTCs in the CSF of patients with CNS tumors may help screen patients with a higher risk of recurrence and direct dynamic intervention treatment strategies [15]. Therefore, the analysis of CTCs in CSF helps to more accurately distinguish the molecular subtypes, so it can be used as a biomarker to evaluate the prognosis and also measure the efficacy. In addition, the process of epithelial-mesenchymal transition in glioblastocyte regulates tumor progression and dissemination, and this process is also expressed in CTCs of GBM patients [17]. Therefore, the evaluation of CTCs may also indicated vital clues to the pathogenesis and pathophysiology of intracranial tumor physiology [18]. At the same time, some research results show that the dynamic monitoring of CTCs in patients with advanced CNS tumors can distinguish tumor recurrence and pseudoprogression. Moreover, the CTCs count reflects the state of the disease, which increases with the progression of the disease, and decreases after chemoradiotherapy. CTCs analysis may be used as a supplement to conventional MRI to more accurately monitor changes in the course of patients [19].

In previous studies, the significance of CTCs in the CNS tumors has been fully confirmed. CTCs provides good and low-invasive tumor detection samples. By detecting the genetic characteristics and specific genotypes of CTCs, it can reflect the progress of primary tumor and the change of specific genetic information in the process of recurrence. Characterizing a CTC fraction from patients with metastatic breast cancer confirmed this hypothesis and highlighted the Notch pathway and immunomodulatory (tumour necrosis factor (TNF), IL-1β and nuclear factor kB (NF-kB)), inflammatory (the chemokine CXCL8, the chemokine receptor CXCR4 and CD86) and mitogenic (platelet-derived growth factor BB (PDGF-BB))-activated pathways as a signature of CTCs associated with brain metastasis. This contributes to a more comprehensive understanding of the biological characteristics in the occurrence and development of tumors in the central nervous system, and is a reliable tumor biomarker.

Circulating tumor DNA (ctDNA)

The ctDNAs are released into blood, CSF, and urine through the decomposition of diseased tissues. The difficulty of analyzing ctDNA is that it requires high-sensitivity and specific technology to extract low-concentration ctDNA from the circulation, and it needs to be separated from the high-concentration circulating free DNA and RNA released by normal cells [20, 21]. In advanced solid tumors, such as advanced lung cancer patients, there are higher concentrations of ctDNAs in the blood, and it has been widely studied It has been widely studied and

proven to be an early predictor of systemic therapeutic response [22].

Relevant research has shown that ctDNAs have been detected in some patients with primary CNS tumors, including astroglioma and oligodendroglioma. In a study, higher concentrations of ctDNAs can be detected in the serum of all oligodendrogliomas and 80.5% of astroglioma patients, and ctDNAs show tumor-related specific biomarkers (MGMT gene methylated/10q LOH/1p or 19q LOH) [24]. In another research, the methylation status of genes (MGMT, RASSF2B, CDKN2A) related with primary CNS tumors were found in the serum of 33 patients with CNS tumors (7 cases of primary GBM, 8 cases of astrocytoma, 2 cases of glioma, 6 cases of meningiomas, and 10 cases of metastatic tumor). The results showed that one gene promoter methylation at least was detected in the serum ctDNAs of 70% of patients in the astrocytoma group. Similarly, one gene promoter methylation at least was detected in 7 patients of the metastasis group and 3 patients of the meningioma group [25]. Lower concentrations of ctDNAs were detected in the serum of 5 GBM patients, and biomarkers related to the primary tumor were found. But it is different from other tumors that the concentration and positive rate of ctDNA in the serum of glioma patients are low. In 2014, Bettegowda et al. confirmed that CSF is better than serum as a sample for detecting ctDNAs derived from primary brain tumors [26]. Wang et al. sequenced the ctDNAs in the CSF of 35 patients with CNS tumors. The results indicated that 74% of samples contained primary tumor DNA, and the detection rate of tumor DNA was related to the anatomical location and grade of the tumor, but not to the size of the tumor [27]. Therefore, some clinical researches have shown that it is easier to obtain patients' CSF and blood during treatment. Detecting ctDNAs in body fluids makes up for those shortcomings [28, 29].

With the introduction of the concept of precision medicine and its application in the field of oncology, it has promoted the development of individualized treatment mode of tumor. A single tissue biopsy or intraoperative sampling in a central nervous system tumor may not be able to obtain tumor-specific mutant gene targets. The detection of ctDNA in body fluid makes up for these shortcomings [30]. On the one hand, ctDNA dynamically reflects the tumor progress, on the other hand, it provides the specific gene mutation and drug resistance mechanism of the primary tumor. ctDNA reflects the molecular composition of tumors in patients with central nervous system tumors, including information on targeted mutations and drug resistance mechanisms under selective therapy. Analysis of ctDNA can detect tumor progression and drug resistance mutations at an early stage (previously detected by imaging or obvious treatment resistance, but irreversible changes often occur at this time). This information may provide more effective information at an early stage and improve the effectiveness of treatment.

Circulating cell-free miRNAs and exosomes

MicroRNA (miRNA) is a type of highly conserved, endogenous non-coding small RNAs with a length of 18-22 bp, which completes the post-transcriptional level regulation of gene expression by degrading or inhibiting target mRNA [32]. In addition, MicroRNA plays a key role in the dynamic balance of normal with tumor tissues and cell-to-cell communication. In recent years, MicroRNA has been considered to be related to tumorigenesis and tumor suppression and can be used as an important biomarker for tumor diagnosis, detection, and prognosis. With more in-depth research on miRNA, it has been discovered that different types of miRNA are expressed in different types of CNS tumors. For example, miR-21 works by inhibiting caspase in glioblastoma cells [33]. Compared with normal brain cells, the expression level of miR-21 continues to be up-regulated in glioblastoma tissues, but not all miRNAs are up-regulated. MiR-21 in glioblastoma has been confirmed as a specific tumor marker for predicting overall survival and treatment response. Although some authors have got the same conclusion, their conclusions are still contradictory. In view of the value of a single miRNA in tumor samples, there is still further confirmation [34].

Exosomes are extracellular vesicles wrapped by membranes, generally 40-100 nm in diameter. Many cells actively secrete exosomes under normal and pathological conditions, it carries various cellular compositions such as proteins, nucleic acids, and lipids (mRNA, DNA, ncRNA) [35]. Exosomes can mediate cell-to-cell com-

munication and change the molecular activities of recipient cells by releasing biological factors [36]. Therefore, the tumor cells release the exosomes which contain tumor-specific biomarkers and can detect the characteristics of the primary tumor. The results of a study showed that in a mouse model transplanted with human cancer stem cells, extracellular vesicles associated with tumor cells were detected in the mouse CSF [37]. Another study showed that extracellular vesicles can be isolated in the CSF of gliomas patients with low-grade and high-grade gliomas, and reflect the degree of disease progression. These findings prove that exosomes can be used to detect as clinical prognostic biomarkers. For example, the detection of IDH1-R132H mutations in extracellular vesicles derived from CSF [38].

The role of circulating cell-free miRNA in CNS tumor

Diagnosis

In recent years, the rapid development of gene chip technology has provided great help for the screening of circulating miRNAs. A large number of researchers hope to understand the biological characteristics of tumors by screening specific circulating miRNA, which is helpful for early diagnosis and accurate treatment of tumors. Therefore, circulating miRNA has become the focus of tumor research. At present, some studies have confirmed that the abnormal expression of miRNA has guiding significance in some solid tumors. A study through meta-analysis found that miR-125b had high sensitivity (63%~89.4%) and specificity (66.40%~91%) in the diagnosis of breast cancer, thyroid cancer, non-small cell lung cancer, and bladder cancer [83]. Zhang et al. reported that overexpression of circulating miR-205, could be detected in the blood of patients with early ovarian cancer and ROX curve (AUS=0.831) was used to evaluate the value of miR-205 combined with CA-125 in the diagnosis of ovarian cancer [93]. Similarly, Wu and other studies found that the expression of miR-145 in serum of patients with early endometrial carcinoma was significantly lower than that of normal subjects, and the diagnostic value was evaluated by ROX curve (AUC=0.82) [94]. In addition, Conti et al. found that miR-221/222 family has diagnostic value in thyroid cancer, breast cancer and oral cancer [43].

The above studies promote the study of ctRNAs in CNS tumors. Lawrie et al. found that the overexpression of plasma miR-21 and miR-210 in patients with primary diffuse large B lymphoma of the central nervous system compared with the normal group (P=0.009). This result describes for the first time the potential of miRNA as a diagnostic marker of tumors in the central nervous system. Since then, the significance of miRNA in central nervous system tumors has attracted the attention of more researchers [82]. In 2012, Ilhan-Mutlu et al. conducted a small sample study to confirm the expression levels of plasma S100B, secretagogues (SCGN), neuropeptide-Y (NPY) and miR-21 in 10 patients with glioblastoma and 10 healthy controls. The results showed that the expression level of miR-21 was 4 times higher than that of the control group (P=0.02), while there was no significant difference in other proteins between the control group and the control group (P=0.06), indicating that the overexpression of miR-21 may be related to glioblastoma [40]. Roth et al. analyzed the distribution of miRNAs in the blood of 20 patients with glioblastoma and 20 healthy controls. 52 miRNAs were screened from 1158 miRNAs by gene chip. After many adjustments and corrections, it was still observed that compared with the control group, the expression of miR-128 was significantly down-regulated and the expression of miR-317 was significantly up-regulated. The results show that the down-regulation of miR-128 and the up-regulation of miR-317 have higher specificity (75%-83%) and sensitivity (71%-85%) for GBM diagnosis [51]. Dong et al. screened more abnormal expressions of circulating cfmiRNAs using Keegan microarray. Among the 752 miRNAs, 115 miRNAs were up-regulated and 24 down-regulated in the blood of patients with glioblastoma (multiple change $\geq$ 2.0). After progressive verification, it was found that the expressions of miR-124, miR-27a, miR-29, miR-210, miR-122, miR-182 and miR-223 were significantly up-regulated, while the expressions of miR-137, miR-203, miR-485 and miR-16-5p were significantly down-regulated ($P < 0.01$). It has potential diagnostic value in the diagnosis of glioma patients [52] (**Table 1**).

Wang et al. [53] analyzed gliomas (WHO II~IV, 30 cases), pituitary adenomas (10 cases), meningiomas (10 cases), patients with glioblastoma 2 weeks after surgery (10 cases), com-

Table 1. Clinical application of circulating miRNA in primary and metastatic CNS tumors

miRNA	Expression	Source	Diagnosis	Prognosis	Response to treatment	Reference
miR-21	Up-regulation	CSF/Plasma	Yes	Yes	Yes	[39-41]
miR-15a/b	Up-regulation	CSF/Plasma	Yes	Yes		[42]
miR-221	Up-regulation	Plasma	Yes	Yes		[43, 57]
miR-182	Up-regulation	Plasma		Yes		[44, 56, 70]
miR-128	Down-regulation	CSF/Plasma	Yes		Yes	[45]
miR-20a	Up-regulation	Plasma	Yes	Yes		[46]
miR-125b	Down-regulation	CSF/Plasma	Yes		Yes	[47]
miR-106a	Up-regulation	CSF/Plasma		Yes	Yes	[48, 49]
miR-19	Up-regulation	CSF	Yes			[50]
miR-27a/b	Up-regulation	CSF	Yes		Yes	[52]
miR-92a	Up-regulation	CSF	Yes			[50]
miR-137	Down-regulation	Plasma		Yes		[51, 52]
miR-203	Down-regulation	Plasma		Yes		[52]
miR-485	Down-regulation	Plasma	Yes	Yes		[52, 53]
miR-205	Down-regulation	Plasma	Yes	Yes		[52]
miR-210	Up-regulation	Serum		Yes	Yes	[54]
miR-100	Down-regulation	Serum		Yes		[55]
miR-222	Up-regulation	Plasma	Yes	Yes		[56, 57]
miR-26a	Down-regulation	Plasma		Yes		[58]
miR-122	Down-regulation	Plasma	Yes	Yes		[52]
miR-376a/b/c	Down-regulation	Plasma	Yes	Yes		[59]
miR-200a/b/c	Down-regulation	CSF	Yes			[60]
miR-124	Down-regulation	Plasma		Yes		[52]
miR-29b	Down-regulation	Plasma	Yes		Yes	[52]
miR-223	Up-regulation	CSF/Plasma	Yes	Yes		[51, 53]
miR-185	Up-regulation	Serum		Yes		[62]
miR-29	Up-regulation	Serum	Yes	Yes		[63]
miR-497	Down-regulation	Plasma	Yes			[64, 65]
miR-181d	Down-regulation	Plasma			Yes	[42]
miR-16	Down-regulation	CSF/Plasma		Yes	Yes	[52]
miR-520h	Up-regulation	Plasma	Yes	Yes		[42]

bined The expression level of miRNAs in the blood of glioblastoma patients (10 cases) who received chemotherapy and radiotherapy for 1 month further confirmed that the expression of miR-21 was up-regulated in the plasma of patients with glioma and that miR-128 and miR-342-3p were significantly down-regulated, and The three expressions are normal in other brain tumors, indicating the specificity of plasma miR-21, miR-128, and miR-342-3p in the diagnosis of glioma. Tang et al. [61] measured the expression of miR-185 in the blood of 66 patients with glioma, 11 patients with pituitary adenoma, 32 patients with meningioma, and 14 patients with acoustic neuroma, and found that plasma miR-185 expression in patients with glioma was obviously increased, while the other benign ones There was no obvious change in brain tumors; among them, plasma miR-185 expression levels in patients with glioblastoma were almost restored to normal levels after surgery and chemotherapy. Therefore, it can be considered that the expression of miR-185 is related to the progression of gliomas and may be a potential biomarker for the diagnosis of gliomas. Studies such as D'urso have proved that plasma miR-15b and miR-21 can also be used as a combination to distinguish gliomas from other types of brain tumors or diseases, including gliomas (30 cases), large B-cell lymphoma of the CNS (PCNSL, 36 cases), secondary brain metastases (16 cases) and

various neurological diseases (30 cases). Studies have shown that the expression of serum miR-15b and miR-21 was significantly up-regulated in patients with gliomas (P=0.02). However, the abnormal expression was not obvious in other tumors (P=0.09). At the same time, the sensitivity and specificity of the diagnosis of glioma can reach 90% and 100% [85]. It is worth thinking about whether the combined diagnostic significance of circulating miRNAs in CNS tumors is greater than that of a single circulating miRNA?

These research results answer this question. Some scholars have suggested that a single plasma miRNA as a biomarker for the diagnosis of low-grade glioma lacks sensitivity and specificity, but it has certain significance in the diagnosis of high-grade glioma [67]. A study of 83 patients with different grades of gliomas showed that the expression of plasma miR-29 family was significantly lower than that of healthy controls, showing high diagnostic value (AUC=0.91) for high-grade gliomas, but lower sensitivity and specificity for the diagnosis of low-grade gliomas. Therefore, the researchers believe that circulating miR-29 has a certain significance in the diagnosis of high-grade gliomas [62]. Liu et al. studied the expression and significance of serum miR-29 in 120 patients with glioma and 120 healthy patients. The age and sex of the two groups were matched. It was found that the serum miR-29b of glioma patients was down-regulated, while the expression of VEGFA was up-regulated. In addition, the researchers used ROX curves to evaluate the diagnostic value of miR-29b and VEGFA in patients with gliomas, with AUC of 0.913 and 0.752 [63]. In addition, a study of 112 patients with gliomas (including 69 patients with WHO I and II, 43 patients with WHO III and IV) and 54 healthy controls showed that there was no difference in serum miR-182 between low-grade gliomas and healthy patients (P > 0.05), but the level of serum miR-182 expression in patients with high-grade gliomas was significantly higher than that in low-grade gliomas and healthy controls (P < 0.01) [64]. The results show that serum miR-182 has a certain diagnostic significance in high-grade gliomas.

Santangelo et al. studied the serum samples of 30 healthy controls and 44 patients with GBM. The expressions of miR-21, miR-222 and miR-124-3p in serum were analyzed by RT-qPCR. The sensitivity and specificity of diagnosis of circulating miRNA were discussed by ROC curve. The results showed that there was no significant difference in the expression of miR-21, miR-222 and miR-124-3p between the low-grade glioma group and the healthy group, but the diagnostic accuracy was significantly improved by combined detection (AUC=0.87, P < 0.001) [74]. In high-grade glioma patients, the levels of serum miR-21, miR-222 and miR-124-3p were significantly higher than those in the healthy group. There was no significant difference between using miR-21 alone (AUC= 0.839, P < 0.001) and combined detection (AUC=0.81, P < 0.003) [74]. Combined with previous studies, it can be inferred that the diagnostic significance of combined detection of serum miRNA in low-grade glioma patients is significantly higher than that of circulating miRNA alone and can be used as a potential biomarker for early diagnosis of low-grade glioma patients. There was no significant difference between the diagnostic significance of specific serum miRNA and combined detection in patients with high-grade gliomas.

The above study liquid biopsy samples are mainly serum or plasma samples of CNS tumors patients. With the deepening of understanding of CSF, it is more and more widely used in CNS tumors, and it can be used as a good liquid biopsy sample. Baraniskin et al. reported that tumor-related miRNAs were detected in the CSF of patients with CNS tumors and that CSF miRNAs gradually attracted attention in the diagnosis of CNS tumors. They found that primary CNS lymphoma (primary CNS lymphoma, in the CSF of patients with PCNSL), the combined monitoring of miR-21, miR-19, and miR-92a can distinguish PCNSL from CNS infectious diseases with a specificity of 96.7% and a sensitivity of 95.7% Open [50]. Based on previous work, Baraniskin et al. found that the combined detection of miR-15b and miR-21 in the CSF of patients with glioma can distinguish glioma from the control group and primary CNS lymphoma [50]. Similarly, Teplyuk et al. also found that miR-10b and miR-21 can distinguish malignant glioma from other diseases, while the MiR-200 family can help distinguish brain metastases from malignant glioma. They also established an independent variable composed of 7 miRNAs (miR-10b, miR-21, miR-125br-141, miR-200a, miR-200b, and miR-200c) to distinguish them

with an accuracy of nearly 90% The non-tumor normal control/malignant glioma/solid tumor brain metastasis [60]. Compared with serum miRNA, CSF can fully contact the focus and contain tumor cell-related miRNA with high stability, high specificity and repeatability. However, the source of CSF samples is more difficult and the risk is relatively higher than that of serum samples.

Numerous studies have documented the clinical relevance of cfmiRNAs as diagnostic markers for a CNS tumors, which used mainly blood plasma, serum or CSF for their analyses. In these studies, tumour-associated cfmiRNA expression profiles from blood and CSF were detected, and those cfmiRNA signatures were related to CNS tumour development, disease progression and metastases. Therefore, in order to better explain the diagnostic significance of different types of circulating miRNA in CNS tumors, we summarize the relevant studies on circulating miRNA. The investigation may not be comprehensive, but based on the author's research concept, we provide substantial clinical insights into the diagnosis of CNS tumors (**Table 1**).

Prognosis

Circulating miRNA shows high diagnostic value in patients with CNS tumors. At the same time, it also shows certain advantages in predicting tumor progression and predicting prognosis. In the early stage of the study, Lai et al. compared the plasma miR-210 expression level of 136 patients with different grades of glioma and 50 controls, and confirmed that the plasma miR-210 expression level of glioma patients increased by about 7 times compared with the control group ($P < 0.01$), and found that the increased expression of miR-210 in glioma patients was related to tumor grade and prognosis ($P < 0.001$) [54]. Shao et al. analyzed the blood miR-20a expression level of 70 glioma patients and 70 healthy controls and found that the miR-20a expression level of glioma patients was significantly higher than that of healthy controls, while the miR-20a expression level of glioma patients was significantly higher than that of healthy controls. Plasma miR-20a expression was significantly down-regulated compared with preoperatively ($P < 0.01$). After follow-up, it was found that the increase in blood miR-20a expression was related to the shorter survival time of patients. Later more studies confirmed that the abnormal expression of certain plasma miRNAs in patients with glioma is related to overall survival [72]. At the same time, Li et al. compared 64 GBM patients with different grades and 64 healthy people, and detected the expression levels of serum miR-137 in the 2 groups by RT-PCR, and found that the expression of miR-137 in the serum of the GBM group was significantly decreased. And the serum of high-grade GBM patients was significantly lower than that of low-grade glioma patients ($P=0.003$), and the low expression of miR-137 in serum was associated with poor clinical prognosis [86].

Zhi et al. have shown that the detection of circulating miRNAs combination is an independent and important index to evaluate the prognosis in patients with CNS tumor. it also showed that the expression levels of 9 kinds of miRNAs, contain miR-15b, miR-21, miR-19a, miR-19b, miR-20a, miR-106a, miR-182, miR-182 and miR-520h in the blood of patients with astrocytoma were obviously higher than those of the control group, and the expression levels of these 9 miRNAs were obviously higher after surgery reduce. In subsequent classification analysis, the authors found that the up-regulation of miR-20a, miR-106a, and miR-181b is related to the advanced clinical stage of astrocytoma. Kaplan-Meier survival analysis indicated that the high expression of miR-19a, miR-106a, and miR-181b was significantly correlated with the survival rate of patients [42]. Zhao and other recent studies have found that the up-regulation of blood miR-20a and miR-106a expression in glioma patients is related to the poor survival rate, and provides new evidence for the conclusions of Zhi et al. It is also found that the combination of up-regulation of blood miR-222 and miR-17 expression and down-regulation of miR-145 expression is related to the prognosis of glioblastoma [87].

There have been a lot of studies shows that over expression or under expression specific circulating cfmiRNAs is significantly associated with shorter overall survival (OS) and/or progression-free survival (PFS), suggesting that it may have prognostic value in patients with CNS tumors. More specifically, the over-expression of circulating miR-21, miR-15b, miR-221, miR-182, miR-20a, miR-106a, miR-210, miR-222, miR-223, miR-520h, and under-expression of

circulating miR-137, miR-203, miR-485, miR-205, miR-122, miR-124, and miR-16 were obviously related with shorter OS (**Table 1**).

Respond to treatment

The evaluation of treatment effects in CNS tumors patients has always been the most concerned issue for clinicians. Therefore, finding a biomarker that can accurately reflect the efficacy has become a research focus. For example, in the treatment of glioma, chemotherapy resistance may be a key factor affecting the prognosis of patients. The ability to predict treatment response by the expression level of circulating miRNA has been proved by more and more studies that it can improve the prognosis by choosing the correct treatment process as soon as possible after diagnosis, which makes the treatment quickly adaptable to the acquisition of chemotherapy and radiation resistance. The rationality of using circulating cfmiRNA as a therapeutic target is based on inhibiting or knocking down the expression of miRNA, which can inhibit tumor growth, invasion, and metastasis. A synthetic miRNA using herpes simplex virus (HSV) as carrier successfully silenced specific target genes in phase I and phase II clinical trials, which may open up a new way for circulating cfmiRNA to treat gliomas [88]. Similar studies have also been conducted in phase I clinical trials on the enhancement of tumor specificity of GBM by miRNA with MV virus as a vector [89].

MiR-21 affects the signal pathways of glioma growth, differentiation, apoptosis, invasion, metastasis, and drug resistance. The study of single or combined dynamic detection of circulating cfmiRNA in the treatment of gliomas has been widely carried out. In glioma cells, inhibition of miR-21 expression can reduce tumor invasiveness, arrest cells in G or S phase and promote apoptosis, increase the sensitivity of glioma cells to paclitaxel, and may control the growth of GBM through EGFR/STAT-3 signal pathway [90]. In addition, the combination of miR-21 inhibitor and 5-FU can promote the apoptosis of glioma cells and reduce the metastasis of glioma cells. The expression of circulating cfmiR-21 in U87-MG cells decreased after being treated with temozolomide (TMZ) [18, 90]. Subsequently, Ilhan-Mutlu et al. carried out a further study and observed that the plasma miR-21 level of 9 patients decreased significantly during the first-line treatment, and the plasma miR-21 level of the other 1 patient increased during the treatment. After that, the tumor recurrence was found in this patient. The above results suggest that miR-21 may be a clinical biomarker of glioblastoma patients and may be related to the evaluation of the prognosis of the patients [40].

The presence of chromatin-modifying drugs, such as 4-phenyl butyric acid and 5-aza-20-deoxycytidine, was also detected in glioma cells, which further suggests that miRNAs have the potential to inhibit tumor growth. Researches have indicated that the expression level of miR-153 in patients with glioma is lower than that of the normal control group, and miR-153 can reduce the expression of Its-2, Bcl-2, and Akt Ser473. Therefore, increasing its level may be harmful Glioma has a therapeutic effect. Experiments have shown that simultaneous regulation of 4-phenyl butyric acid and 5-aza-20-deoxynucleoside in GBM cell lines can induce apoptosis and reach a peak at 72 h, It is suggested that the mechanism of action of these drugs may be that miR-153 acts through hs-2 [91]. Ujifuku et al. studied the serum or CSF miRNA expression of U87MG cells before and after radiotherapy and found that the expression of 5 miRNAs was up-regulated, while the expression of 6 miRNAs was down-regulated [64], specifically miR-21, miR-106a, miR-27a, MiR-210, miR-200a expression increased ($P < 0.01$), miR-128, miR-125b, miR-16, miR-181d, miR-497, miR-29b expression was down-regulated ($P < 0.05$) (**Table 1**).

At present, the gold standard for the treatment of glioblastoma is surgical resection added TMZ. Unfortunately, a small number of patients are resistant to TMZ treatment because patients with functional MGMT DNA repair protein reversed the guanine methylation caused by TMZ, leading to chemotherapy resistance. Serum miR-181d can be used as a biomarker to dynamically reflect the treatment response after receiving TMZ. MGMT is a candidate target of miR-181d. The high expression of miR-181d in serum and the low expression of MGMT are closely related to the enhanced response of glioma patients to TMZ [64, 65]. After first-line treatment, there will be corresponding changes in the tumor microenvironment, which promotes the development of chemotherapy resistance in patients with special

genes. With the application of circulating miRNA in patients with CNS tumors, it seems to have found a solution, because circulating miRNA carries genetic information contained in primary tumors and actively reflects the response of tumor cells to treatment. However, there is still a lack of more accurate and reliable circulating miRNA, which needs to be further explored.

Circulating exosomal miRNAs

Previous studies have indicated that malignant tumor cells will increase the discharge of exosomes, and miRNA encapsulated in exosomes are released into body fluids to participate in the regulation of tumor biological behavior. Researches have indicated that the exosomes released by tumor cells of nervous system have similar constituent with tumor tissues. The contents released may affect the host's immune response, just like resisting chemotherapy drugs, enhancing tumor proliferation and promoting tumor invasion [34, 92]. Consequently, the miRNA expression profile of exosomes is a potential biomarker, which can provide information about tumor grading and prognosis, so that a better tumor-specific treatment can be designed [92].

Manterola et al. found that one small nuclear RNA (rnu6-1) and two miRNAs (miR-320, miR-574-3p) were overexpressed in exosomes isolated from the plasma of glioblastoma patients. Further verification showed that the combination of rnu6 - or miR-320/miR-574-3p/rnu6-1 to identify glioblastoma patients from healthy controls is a biological marker for the diagnosis of glioblastoma [76]. This study opens up a new way that exosomal miRNA can be used as a biomarker for the diagnosis of glioblastoma.

Chen et al. Applied magnetic beads, emulsion, amplification, beads (emulsion, amplification, magnetics, BEAMing PCR) and digital PCR to detect IDH1 RNA mutation (G595A) in the extracellular vesicles of CSF of patients with glioma, and the mutation could only be detected in CSF, but not detected in EVs derived from blood [37]. This finding was confirmed by the study of Akers et al. At the same time Akers et al. also identified three miRNAs in exosomes by TaqMan open array openarray human miRNA gene chip (The results showed that miR-21, mir-218, mir-193b) were positive correlation with tumor size, and the expression level of these molecular markers was significantly higher than that of normal control group [66].

Currently, high levels of miR-451 and miR-21 were found in GBM-EVs isolated from human glioblastoma cells. miR-451 plays a key role in the proliferation and migration of glioma cells. miR-451 plays a key role in the proliferation and migration of glioma cells. The selective output of miRNAs can indicate whether a miRNA is tumor specific or not. Generally, tumor cell originated miR-451 has not been found in non-tumoral exosomes. miR-451 has been shown to inhibit the adenosine monophosphate activated protein kinase (AMPK) signaling pathway, which is normally regulated to meet metabolic requirements. Therefore, miR-451 in exosomes released by GBM can dynamically reflect tumor progression and may be used as a biomarker for diagnosis and prediction of tumor progression. Many studies have proved that miR-21 is over expressed in glioma patients body fluids, while the expression level of circulating exosomal miR-21 is 10 times higher than that of normal controls ($P < 0.001$), which is higher than that of circulating miR-21 in brain tissue, blood and CSF [77]. However, miR-21 is also sensitive to most cancers, so exosome miR-21 cannot be used as a specific marker for gliomas. MiR-10b was expressed in tumor cells of central nervous system, but not in normal brain tissues [78]. When analyzing different circulating miRNA in CSF of glioma patients, it was found that circulating exosomal miR-10b was positively correlated with tumor malignant degree, so miR-10b may be used as a remarkable tumor marker for judging prognosis [79].

Temozolomide as a first-line chemotherapeutic drug for glioma. Chemotherapy resistance is the main reason for treatment failure. In recent years, some studies have shown that exosomes can regulate signaling pathways by mediating miRNAs and other substances, and thus become one of the mechanisms of chemotherapy resistance in glioma cells. Yang et al. established cell models in vitro. Flow cytometry was used to detect the apoptosis rate of different cell model groups after treatment with TMZ for 48 h. It was found that the apoptotic ability of the cell model group co-incubated with exosomes was the weakest, which suggested that the exosomes in glioma cells might participate in the mechanism of enhancing the chemore-

Table 2. Clinical application of circulating exosomal miRNA in primary and metastatic CNS tumors

miRNA	Expression	Source	Diagnosis	Prognosis	Response to treatment	Reference
Exosomal miR-21	Up-regulation	CSF/Plasma	Yes		Yes	[37, 66, 69]
Exosomal miR-301a	Up-regulation	Serum	Yes		Yes	[67, 74, 81]
Exosomal miR-34s	Up-regulation	Serum	Yes	Yes		[71]
Exosomal miR-218	Up-regulation	CSF	Yes			[66]
Exosomal miR-454-3p	Down-regulation	Plasma	Yes		Yes	[72]
Exosomal miR-210	Up-regulation	Plasma	Yes	Yes		[73]
Exosomal miR-193b	Up-regulation	CSF	Yes			[66]
Exosomal miR-222	Up-regulation	Plasma		Yes		[74]
Exosomal miR-124-3p	Up-regulation	Plasma	Yes	Yes		[74]
Exosomal miR-320	Up-regulation	Serum	Yes			[75]
Exosomal miR-574-3p	Up-regulation	Plasma		Yes		[76]
Exosomal miR-451	Up-regulation	Plasma	Yes	Yes		[78]
Exosomal miR-1238	Up-regulation	Plasma			Yes	[80]
Exosomal miR-221	Up-regulation	Plasma			Yes	[81]
Exosomal miR-10b	Up-regulation	CSF		Yes		[77]

sistance of TMZ [69]. Similarly, Yin et al. [80] detected the plasma and tissue samples of glioblastoma patients, and found that the circulating exosomes in tumor patients had higher expression levels of miR-1238 than healthy adults. In addition, further studies showed that miR-1238, the exosome transferred to the receptor cells, could induce the up regulation of miR-1238 level in TMZ sensitive cells, and then significantly decreased the expression level of cavein (cav1) in the receptor cells, which was activated by egfr-pi3k-akt-mtor pathway, which eventually led to TMZ sensitive cells obtaining drug resistance. There are similar studies in radiotherapy. Yue et al. found that glioblastoma-derived exosome miR-301a, can specifically inhibit the expression of TCEAL7 in recipient cells in hypoxic tumor microenvironment, and in this process, TCEAL7 can also block the transport of β-catenin from cytoplasm to nucleus, and then negatively regulate Wnt/β-catenin signal pathway, also promote the formation of radiation resistance of glioblastoma cells [81].

In general, emerging studies have revealed that exosome derived miRNAs may be involved in the molecular mechanism of chemoradiotherapy resistance of glioma. With the rapid development of gene chip and high-throughput sequencing technology, we may find the key exosome miRNAs or their related pathways that regulate the mechanism of chemoradiotherapy of glioma cells in the future, through the rational use of relevant inhibitors or block a key molecular pathway. Then the radiosensitivity of glioma cells was enhanced by chemoradiotherapy. We collected and summarized the studies on the relationship between exosomal miRNA and CNS tumors in order to provide new ideas for the diagnosis, prognosis and treatment efficacy of CNS tumors (**Table 2**).

Conclusions and future perspectives

A large number of studies have shown that liquid biopsies of CSF and plasma in patients with CNS tumors can be used as potential substitutes for tissue biopsies in order to tumor diagnosis and prognostic biomarker analysis [94-96]. Considering the relative difficulty and high risk of traditional biopsy, the development of biomarkers related to tumor development is essential in the trend of individualized treatment of CNS tumors. Liquid biopsy provides a safer and more convenient technology to obtain tumor-related molecular information, which can make low-invasive and dynamic diagnosis of diseases, adjust treatment plans and contribute to the development of targeted drugs. However, the scale of these studies is small and there is no strong statistical conclusion to prove the correlation between the detection of tumor biomarkers and disease parameters. Moreover, liquid biopsy needs to rely on some cutting-edge technologies, such as next-generation sequencing, digital PCR and so on, which has high technical cost and high requirements for testing personnel, which limits its clinical

application. Therefore, a large number of studies are needed to verify the effectiveness and reliability of liquid biopsy in patients with CNS tumors, and we need to constantly develop simpler and more accurate methods for biomarker monitoring and efficacy evaluation.

Circulating miRNA and exosomes will change and supplement the traditional understanding of tumorigenesis and development, these will contribute to widely understanding of tumor-related molecular mechanisms. miRNA is extensively existed and determines the regulation of gene expression and protein translation, and plays an indispensable role in the occurrence and development of CNS tumors. Exosomes represent a new way of intercellular communication, which is rich in miRNA. A large number of studies have shown that exosomal miRNA participates in intercellular communication in tumor microenvironment and regulates many characteristics of CNS tumors, which includes proliferation, invasion, angiogenesis, immune escape and therapeutic resistance by selective packaging, secretion and metastasis. In addition, circulating miRNA and exosomes carry biological information related to primary tumors, which can easily cross the biological barrier (for example, blood-brain barrier) and exist in a large number of body fluids from different sources, so it brings great hope for the diagnosis and treatment of CNS tumors.

Although more and more studies have proved that circulating miRNA and exosomes are promising biomarkers in CNS tumors. Nevertheless, its application in clinical practice is still controversial. In fact, the vast majority of cfmiRNA in blood comes from white blood cells, red blood cells and endothelial cells, as well as from some high-flow organs (liver, lung and kidney). Therefore, the isolation and accurate detection of tumor-derived circulating miRNA and exosomes rely on cutting-edge technology and high requirements for testing personnel, so its wide clinical application has been questioned and limited.

In short, the analysis of circulating miRNA and exosomes in body fluid of patients with CNS tumors has become a hot research field of tumor biomarkers. We mainly summarized the related studies of different circulating miRNA and exosomal miRNA, analyzing and discussing their values in patients with CNS tumors. We consider that circulating miRNAs and exosomes are the main components of liquid biopsy analysis, which may contribute to the development and establishment of personalized medication regimens for patients with CNS tumors. With the standardization of the detection methods of circulating miRNA and exosomes, we have a better understanding of the formation mechanism, biological function and relationship with CNS tumors of circulating miRNA and exosomes. We believe that circulating miRNA and exosomes will show a broad application prospect in the diagnosis, prognosis and treatment response of patients with CNS tumors in the future.

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Disclosure of conflict of interest

None.

Abbreviations

CNS, Central nervous system; CSF, cerebrospinal fluid; CTC, Circulating tumor cells; ctDNA, circulating tumor DNA; cfmiRNA, cell-free microRNA; TMZ, Temozolomide; EVs, extracellular vesicles; ncRNA, non-coding RNA; EpCAM, epithelial cell adhesion molecules; EGFR, epithelial growth factor receptor; NGS, next-generation sequencing; GBM, glioblastoma multiforme; MGMT, O6-methylguanine DNA methyltransfersse; mRNA, messenger RNA; IDH, Isocitrate dehydrogenase; SCGN, secretagogues; NPY, neuropeptide-Y; VEGFA, vascular endothelial growth factor A; OS, overall survival; PFS, progression-free survival; HSV, herpes simplex virus; MV, measlesvirus; 5-FU, 5-Fluo-rouracil; rnu6-1, small nuclear RNA; PCR, Polymerase Chain Reaction.

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