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Review Article

Cerebrospinal fluid biomarkers for brain tumor detection: clinical roles and current progress

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Abstract: Brain tumors include those that originate within the brain (primary tumors) as well as those that arise from other cancers (metastatic tumors). The fragile nature of the brain poses a major challenge to access focal malignancies, which certainly limits both diagnostics and therapeutic approaches. This limitation has been alleviated with the advent of liquid biopsy technologies. Liquid biopsy represents a highly convenient, fast and non-invasive method, which allows multiple sampling and dynamic pathological detection. Biomarkers derived from liquid biopsies can promptly reflect changes on the gene expression profiling of tumors. Biomarkers derived from tumor cells contain abundant genetic information, which may provide a strong basis for the diagnosis and the individualized treatment of brain tumor patients. A series of body fluids can be assessed for liquid biopsy, including peripheral blood, cerebrospinal fluid (CSF), urine or saliva. Interestingly, the sensitivity and specificity of biomarkers from the CSF of patients with brain tumors is typically higher than those detected in the peripheral blood and other sources. Hence, here we describe and properly discuss the clinical roles of distinct classes of CSF biomarkers, isolated from patients with brain tumors, such as circulating tumor DNA (ctDNA), microRNA (miRNA), proteins, and extracellular vesicles (EVs).

Keywords: Liquid biopsy, cerebrospinal fluid, brain tumor, biomarkers

Introduction

CSF is an important source of potential molecular biomarkers, mostly collected by lumbar puncture or surgery around the brain area. For instance, CSF contains various biomarkers, such as ctDNA, miRNA, proteins, and EVs, which are typically derived from brain tumor cells [1]. Usually, tumor cells co-exist with their microenvironment. Therefore, tumor-related markers can be more prominent in fluids nearby the site of the disease. CSF is usually considered an extension of the extracellular compartment within the central nervous system (CNS) and, as such, a major route for brain tumors [2]. The biomarker content of patients with brain tumors is mostly low or even undetectable, since the blood-brain barrier has a major impact on the release of putative biomarkers into the systemic circulation. Nevertheless, CSF is a suitable repository of clinical biomarkers, and an increasing number of studies have reported that CSF-derived biomarkers are more abundant than those in the peripheral blood and other sources. For instance, ctDNAs derived from brain tumor cells are more abundantly present in the CSF than in the plasma [3]. In addition, CSF is a better source of circulating nucleic acids than the plasma from brain tumor patients [4]. One particular clinical study, composed by eight brain tumor patients, indicated that the detection of tumor-specific mutations in CSF ctDNA has higher sensitivity when comparing with plasma ctDNA (100% vs. 37.5%, respectively) [5]. Although plasma is still a more common source for the quantitative isolation and detection of nucleic acids (possibly due to the negative impact of the blood brain barrier), CSF has been a more qualitative source of nucleic acids [5, 6]. Hence, CSF may provide a less invasive diagnosis and treatment monitoring of brain tumors patients [7]. Currently, liquid biop-

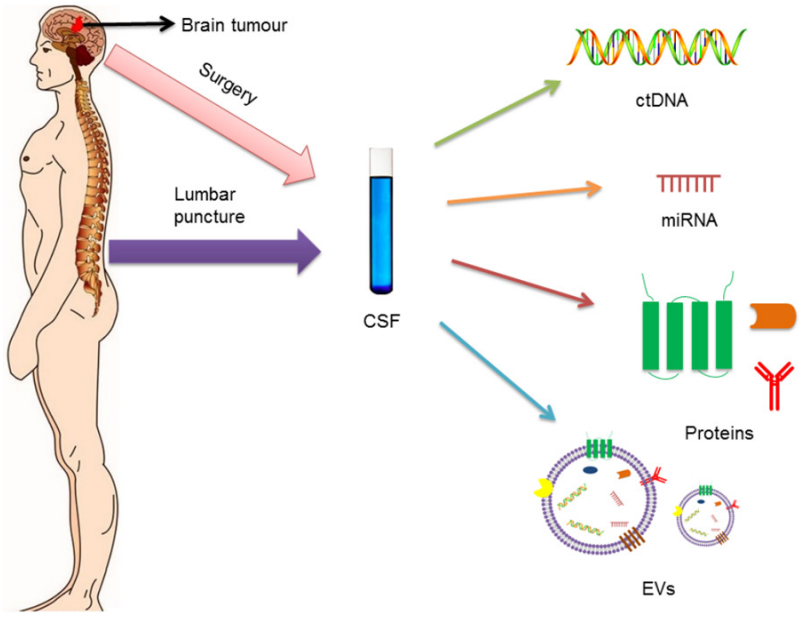

Figure 1. Molecular biomarkers, secreted by brain tumor cells, can enter into the cerebrospinal fluid (CSF). Therefore, CSF may contain increased levels of ctDNA, miRNA, proteins and EVs. Fortunately, CSF is easily obtained through lumbar puncture or surgery. Using liquid biopsy techniques, changes on the expression of ctDNA, miRNA, proteins and EVs from brain tumor cells can be examined in the CSF.

sy techniques including Enzyme-linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR), and Next-Generation Sequencing (NGS) have been standardized for the detection of potential CSF biomarkers. Based on these techniques, changes on the expression of ctDNA, miRNA, proteins, and EVs (**Figure 1**) from brain tumor cells can be examined in the CSF and, more precisely, translated into the diagnosis and treatment, as well as monitoring recurrence and treatment response of brain tumors.

Cerebrospinal fluid biomarkers related to brain tumors

CtDNAs

CtDNA is referred to the DNA that comes from tumor cells and stably circulates in body fluids. Tumor-derived ctDNAs have been extracted from CSF samples of patients with brain tumors, and a series of genes mutations have been assessed [8]. Interestingly, one particular study has been demonstrated that CSF-derived ctDNAs can better reflect sequence mutations in driving genes when compared to plasma ctDNAs [9]. Two new ways have been utilized to detect genetic mutations: droplet-digital PCR (ddPCR) and NGS [10-12]. One particular study has performed ddPCR with targeted amplicon sequencing to search for mutations in CSF ctDNA of primary and metastatic brain tumor patients [13]. A number of tumor gene mutations were detected in CSF-derived ctDNAs from 7 patients with solid brain tumors, where 6 had detectable tumor mutations in at least one of the following genes: *NF2*, *AKT1*, *BRAF-V600*, *NRAS*, *KRAS*, *TP53*, and *EGFR* (**Table 1**) [14]. Interestingly, gene mutations in *RGS12*, *CASR*, *AQR*, *MTMR4*, and *KDM6A* were detected in CSF-derived ctDNA from medulloblastoma patients [8]. Moreover, CSF-derived ctDNAs were extracted from 53 patients to study alterations in 341 cancer-associated genes by NGS, and somatic alterations were detected in more than half of the patients with primary and metastatic brain tumors, but not detected in patients without brain tumors [11].

Gene mutations in *IDH1*, *TP53*, *EGFR*, *PTEN*, *FGFR2*, and *ERBB2* (**Table 1**) have been detected in CSF-derived ctDNA of patients with glioblastoma (GBM) [3]. The genetic alterations including amplification of *EGFR* and deletions of *CDKN2A/B* have been also observed in CSF-derived ctDNA from GBM patients by NGS (**Table 1**) [15]. Gene mutation analyses of other genes, such as *IDH1/2*, *TP53*, *ATRX*, *TERT*, *H3F3A*, and *HIST1H3B*, have been detected in CSF ctDNAs and equally contributed to the diagnosis and treatment of diffuse gliomas patients (**Table 1**) [6]. Similarly, the most frequently genes mutations, such as *H3F3A*, *HIST1H3B*, *TP53*, *ATRX*, *PDGFRA*, *FAT1*, *PPM1D*, *IDH1*, *NF1*, *PIK3CA*, and *ACVR1* (**Table 1**), have been detected in CSF ctDNA of brainstem glioma patients [5]. Of note, it is appropriate to point out that *H3F3A* and *HIST1H3B* mutations were also detected in CSF-derived ctDNAs of diffuse midline glioma patients (**Table 1**) [16].

Table 1. CtDNAs characterized in CSF samples derived from patients with brain tumors

Brain Tumor Types	Example	Findings	Clinical Roles	Reference
primary tumors				
Gliomas	*IDH1, TP53, EGFR, PTEN, FGFR2, ERBB2*	mutations	diagnosis/therapy	[3]
	EGFR	amplification	diagnosis/therapy	[15]
	CDKN2A/B	deletions	diagnosis/therapy	[15]
	IDH1/2, TP53, ATRX, TERT, H3F3A, HIST1H3B	mutations	diagnosis/therapy	[6]
	H3F3A, HIST1H3B, TP53, ATRX, PDGFRA, FAT1, PPM1D, IDH1, NF1, PIK3CA, ACVR1	mutations	diagnosis/therapy	[5]
	H3F3A, HIST1H3B	mutations	diagnosis/therapy	[16]
PCNSL	*MYD88*	mutations	diagnosis/therapy	[17-19]
Medulloblastoma	*RGS12*, *CASR*, *AQR*, *MTMR4*, *KDM6A*	mutations	diagnosis/therapy	[8]
VS	*NF2*	mutations	diagnosis/therapy	[14]
Meningioma	*AKT1*	mutations	diagnosis/therapy	[14]
Metastatic tumors				
SCNSL	*MYD88*	mutations	diagnosis/therapy	[20]
Melanoma	*BRAF-V600, NRAS*	mutations	diagnosis/therapy	[11, 14]
Lung cancer	*EGFR*	mutations	diagnosis/therapy	[11, 14, 26]
	KRAS	mutations	diagnosis/therapy	[11]
Breast cancer	*TP53, PIK3CA*	mutations	diagnosis/therapy	[27]
Colon cancer	*KRAS, TP53*	mutations	diagnosis/therapy	[14]
Bladder cancer	*TP53, AKT2*	mutations	diagnosis/therapy	[11]
Ovarian cancer	*BRCA1, CDKN2B*	mutations	diagnosis/therapy	[11]

Similarly, *MYD88* mutation (**Table 1**) has been detected in CSF extracted from patients with primary central nervous system lymphoma (PCNSL) [17-19]. Gene mutation in *MYD88* has also been detected in CSF-derived ctDNA of one patient with secondary central nervous system lymphoma (SCNSL) [20]. Another genes whose mutations have diagnostic potential, such as *CD79B*, were found in PCNSL patients [21]. However, no *CD79B* mutation has been detected in CSF so far. Interestingly, several studies have also shown that neither *CD79B* nor *MYD88* mutations have been found in glioma patients [22, 23]. Still, *CD79B* and *MYD88* mutations, which were detected in CSF-derived ctDNAs, may play an important role distinguishing PCNSL from other brain tumors [24]. Therefore, *MYD88* and *CD79B* could be potentially used as molecular signatures for lymphomas.

In patients with brain metastases derived from melanoma, *BRAF-V600* and *NRAS* mutations (**Table 1**) have been monitored in CSF-derived ctDNAs [11, 14]. Since ctDNA is not suitable to track tumor evolution in the brain, it is unable to monitor brain metastasis due to melanoma [25]. Similarly, we may also find other genetic mutations in CSF-derived ctDNAs from patients with other types of brain metastases. For example, *EGFR* [11, 26] and *KRAS* [11] mutations (**Table 1**) might be detected in CSF-derived ctDNAs in cases of brain metastasis due to primary lung cancer. *TP53* and *PIK3CA* mutations (**Table 1**) were also detected in CSF-derived ctDNAs from HER2-positive brain metastasis originated from breast cancer [27]. In patients with brain metastases due to primary bladder cancer, *TP53* and *AKT2* mutations have been detected in CSF-derived ctDNAs. Moreover, *BRCA1* and *CDKN2B* mutations (**Table 1**) were linked to brain metastases due to primary ovarian cancer [11].

MiRNAs

MiRNAs are small non-coding RNAs (~22 nucleotides in length) that can be released from brain tumor cells [28]. Free miRNAs possibly result from tumor cell death or secretion of tumor cells, leading to the release of nucleic acids in the extracellular matrix. The main function of miRNAs includes the modulation of gene expression by mRNA silencing and/or degradation. Interestingly, a single miRNA may be able to target several mRNAs simultaneously (pleiotropic effects) [28, 29]. The association between miRNAs and brain tumorigenesis was first introduced in 2005. Three years later, the presence of miRNAs in circulating body fluids from patients with brain tumor was finally detected [30]. In fact, miRNAs can be released into biological fluids such as plasma or CSF [31]. However, due to the existence of the blood-brain barrier, it has been hypothesized that miRNAs present in the CSF can better reflect the brain physiology and related pathologies more accurately than plasma miRNAs [32]. Several studies have demonstrated the causes and significance of extracting miRNAs from CSF [14, 32-34]. Still, due to the presence of RNA-degrading enzymes in the blood [35], the expression/secretion of miRNAs in the CSF appears to define, more accurately, the malignant process of brain tumors [36].

Differences in brain miRNA profiles may depend on the source of the brain region [37], suggesting that different types of brain tumors correspond to distinct types and levels of miRNAs [38, 39]. Extracellular vesicles (EVs) are nanometer size membrane-closed particles that can contain a variety of miRNAs [40, 41]. The incorporation of miRNAs into EVs results in protection from degradation in the biofluid environment [42]. EVs can be isolated from CSF [43, 44] and, apparently, this procedure can be more feasible that isolating and sequencing exosomal miRNAs from CSF [32, 33, 37]. Multiple CSF-related miRNAs have been found to be significantly associated with primary and metastatic brain tumors. Intriguingly, certain miRNAs may be upregulated in some brain tumors while downregulated in others, indicating that combinations of miRNA signatures can be useful to distinguish brain tumors. For instance, meningiomas and brain metastasis show elevated expression of miR-935, while miR-935 expression is absent in lymphomas and gliomas (**Table 2**) [45]. Similarly, miR-451 and miR-711 are upregulated in meningiomas, gliomas, and medulloblastoma while downregulated in lymphomas (**Table 2**) [45]. In particular, miR-125b and miR-223 are important diagnostic biomarkers for GBM, medulloblastoma, and brain metastasis (**Table 2**) [45]. Therefore, differential miRNA expression can be used as an unique approach for the minimally invasive diagnosis of GBM [33]. CSF-related miRNAs have also been extracted from 118 patients diagnosed with different types of brain tumors

Table 2. MiRNAs characterized in CSF samples derived from patients with brain tumors

Brain Tumor Types	Example	Findings	Clinical Roles	Reference
primary tumors				
Gliomas	miR-451, miR-711	upregulated	diagnosis/therapy response	[45]
	miR-125b, miR-223	upregulated	diagnosis/therapy response	[45]
	miR-10b	Upregulated	diagnosis/therapy response/tumor relapse	[32]
	miR-21	upregulated	diagnosis/therapy response/tumor relapse	[32, 44, 90, 91]
	miR-15b	upregulated	diagnosis/therapy response	[33]
	miR-21, miR-218, miR-193b, miR-331, miR-374a, miR-548c, miR-520f, miR-27b, miR-30b	upregulated	diagnosis/therapy response	[43]
	miR-151a	upregulated	therapy response	[104]
PCNSL	miR-21, miR-19b, miR-92	upregulated	diagnosis/therapy response	[38, 46]
	miR-451, miR-711	upregulated	diagnosis/therapy response	[45]
Medulloblastoma	miR-451, miR-711	upregulated	diagnosis/therapy response	[45]
	miR-125b, miR-223	upregulated	diagnosis/therapy response	[45]
Meningioma	miR-451, miR-711, miR-935	upregulated	diagnosis/therapy response	[45]
Metastatic tumors				
SCNSL	miR-30c	upregulated	diagnosis	[47]
Lung cancer	miR-10b, miR-21, miR-200	upregulated	diagnosis/therapy response/tumor relapse	[32]
	miR-125b, miR-223, miR-935	upregulated	diagnosis/therapy response	[45]
Breast cancer	miR-10b, miR-21, miR-200	upregulated	diagnosis/therapy response/tumor relapse	[32]
	miR-125b, miR-223, miR-935	upregulated	diagnosis/therapy response	[45]

and non-neoplastic neuropathologies [32]. As a result, miR-10b and miR-21 levels in the CSF were noticeably increased in GBM and brain metastasis patients when compared with tumors in remission and other non-neoplastic conditions (**Table 2**) [32]. In addition, miR-200 levels (**Table 2**) were solely elevated in brain metastases, but not under other pathological conditions, which allows the discrimination between GBM and metastatic brain tumors. Comparative analysis of these particular miRNAs allowed the distinction of GBM and metastatic brain tumors from healthy controls, with an accuracy of 91-99% [32]. The levels of miR-15b were also significantly elevated in gliomas, suggesting that the combined measurement of miR-15b and miR-21 levels could permit a more comprehensive diagnosis of gliomas than solely analyzing miR-21 yields (**Table 2**) [33]. In addition, a number of miRNAs, including miR-21, miR-218, miR-193b, miR-331, miR-374a, miR-548c, miR-520f, miR-27b, and miR-30b were also detected in the CSF and closely related to GBM differentiation (**Table 2**) [43].

The levels of miR-21 are largely overexpressed in gliomas but, importantly, they can be also overexpressed in PCNSL. Except for miR-21, other miRNAs like miR-19b and miR-92a can also be helpful in the diagnosis and monitoring of PCNSL [38]. One clinical study, composed by thirty-nine PCNSL patients, indicated that ~97% sensitivity could be achieved in the diagnosis of PCNSL by combining with the CSF analyses of miR-21, miR-19b, and miR-92 levels (**Table 2**) [46]. Interestingly, one particular study has indicated that miR-30c levels in CSF noticeably increased in SCNSL patients when compared with PCNSL (**Table 2**) [47].

Melanoma has a strong tendency to metastasize to the brain. CSF cytology is often used to search for melanoma-derived brain metastases. However, this procedure is not sensitive enough to diagnose this metastatic subtype [30]. Fortunately, it has been observed that the presence of three mRNA markers in the CSF-MAGE-3, MART-1 and tyrosinase-may diagnose melanoma brain metastasis. The correlation between the detection of these melanoma-associated RNAs in the CSF and the development of CNS metastases, after 3 months, is significant [48]. Nevertheless, the clinical utility of miRNAs as CSF biomarkers has not been validated yet. Further research aiming the detection of miRNAs in the CSF of patients with melanoma-derived brain metastases is still warranted.

Proteins

Similar to nucleic acids detected in the CSF, certain protein biomarkers also appear to be more concentrated in this compartment than in the plasma. For instance, the levels of glial fibrillary acidic protein (GFAP) were quantitatively determined in the CSF of brain tumor patients. Hence, it was observed that GFAP levels from GBM patients surpassed those from other brain tumors and cerebral lesions of distinct etiology (**Table 3**) [49]. Other proteins detected in the CSF, including Tenascin, Osteopontin (OPN), and Matrix metalloproteinases (MMPs), have also been elevated in glioma patients. Extracellular matrix (ECM) is a significant component of this environment. Tenascin is present in the ECM and it is highly expressed during development in migratory cells. The levels of tenascin in CSF are reported to increase in astrocytic tumors when compared to non-astrocytic primary CNS tumors, metastases and non-malignant controls (**Table 3**) [50]. Tenascin levels appear to increase proportionally to the grade of astrocytic tumors [50]. OPN is a crucial chemokine for macrophages, mediates the interaction between GBM tumor cells and the innate immune system [51]. In the CSF, the levels of OPN and its derivatives are markedly increased in glioma patients when compared to healthy patients and control patients affected by other brain tumors (**Table 3**) [52]. MMPs exist in both membrane-bound and secreted forms. Analysis of MMP-2 and MMP-9 levels in the CSF may be conducive to diagnose gliomas and even estimate tumor recurrence (**Table 3**) [53]. Due to its role in the metastasis and invasion of brain tumors, extensive work has been pursued to develop MMP inhibitors for their treatment.

Growth factors and cytokines have also been identified as potential biomarkers present in the CSF of GBM patients. About 90% of patients with malignant gliomas present elevated vascular endothelial growth factor (VEGF) levels in the CSF (**Table 3**) [54]. Interestingly, related to CSF levels of cleaved OPN, VEGF and C-C motif chemokine ligand (CCL) 4 levels in the CSF were significantly increased in glioma patients when compared with non-tumors (**Table 3**) [55].

Table 3. Proteins characterized in CSF samples derived from patients with brain tumors

Brain Tumor Types	Example	Findings	Clinical Roles	Reference
primary tumors				
Gliomas	GFAP	high levels	diagnosis/therapy response	[49]
	Tenascin	high levels	diagnosis/therapy response	[50]
	OPN	high levels	diagnosis/therapy response/tumor relapse	[52]
	MMP-2, MMP-9	high levels	diagnosis/therapy/tumor relapse	[53]
	VEGF	high levels	diagnosis/therapy/therapy response	[54-56]
	CCL4	high levels	diagnosis/therapy	[55]
	FGF	high levels	diagnosis	[56]
	NGF	high levels	diagnosis	[57]
	IL-6	high levels	diagnosis/therapy/therapy response	[58]
	IL-8	high levels	diagnosis/therapy	[59]
PCNSL	CXCL13	high levels	diagnosis	[60]
	IL-10	high levels	diagnosis	[60, 61]
	IL-6, B2M	high levels	diagnosis	[61]
	sIL-2R	high levels	diagnosis	[61, 62]
	sCD27	high levels	diagnosis	[64]
	ATIII	high levels	diagnosis/therapy response	[65]
	OPN	high levels	diagnosis	[66]
	Neopterin	high levels	diagnosis	[67]
	sTACI, sBCMA	high levels	diagnosis/therapy response	[68]
	APRIL, BAFF	high levels	diagnosis/therapy response/tumor relapse	[69]
	BAFF, TACI	high levels	diagnosis/therapy	[70]
	haemopexin, apolipoprotein A1, transferrin	high levels	diagnosis	[71]
Medulloblastoma	PGD2S	low levels	diagnosis/therapy response/tumor relapse	[106]
VS	ABCA3, KLF11	high levels	tumor relapse	[105]
	BASP1, PRDX2	low levels	tumor relapse	[105]
Meningioma	EFEMP1	high levels	diagnosis	[74]
Metastatic tumors				
SCNSL	CXCL13	high levels	diagnosis	[60]
	IL-10	high levels	diagnosis	[60, 61]
	IL-6, B2M, sIL-2R	high levels	diagnosis	[61]
	OPN	high levels	diagnosis	[66]
	haemopexin, apolipoprotein A1, transferrin	high levels	diagnosis	[71]
Melanoma	CXCL10, IL-8	high levels	diagnosis/therapy	[72]
Lung cancer	CEA	high levels	diagnosis/therapy	[73]
Breast cancer	CEA	high levels	diagnosis/therapy	[73]

In addition, one particular study has shown that CSF fibroblast growth factor (FGF) and VEGF levels in patients with high-grade glioma were apparently higher than patients with low-grade glioma (**Table 3**) [56]. A further study has indicated that nerve growth factor (NGF) levels in the CSF elevate proportionally to the glioma grade (**Table 3**) [57]. The levels of 19 tumor-related CSF proteins have been examined, and results demonstrated that GBM patients have significant increases in interleukin (IL)-6 levels compared with patients with low grade gliomas and normal subjects (**Table 3**) [58]. Similarly, CSF IL-8 levels were also markedly increased in astrocytic tumors patients when compared with healthy patients (**Table 3**) [59]. Altogether, these studies suggest that CSF proteins have a potential use as glioma biomarkers.

Several CSF-related protein biomarkers, such as CXCL13, IL-10, IL-6, B2M, sIL-2R, sCD27, ATIII, OPN, Neopterin, sTACI, sBCMA, APRIL, and BAFF, have a putative diagnostic value in lymphomas. The elevated CXC chemokine ligand (CXCL) 13 plus IL-10 was 99.3% specific for PCNSL and SCNSL, with a sensitivity significantly greater than standard CSF tests (**Table 3**) [60]. IL-10, IL-6, beta-2 microglobulin (B2M), and soluble IL-2 receptor (sIL-2R) levels in CSF from patients with CNS lymphoma were apparently higher than non-lymphoma patients (**Table 3**) [61]. A specificity of 90% was also

found for increased sIL-2R in the CSF, which correlated with the proper diagnosis of patients initially suspected of having PCNSL (**Table 3**) [62]. CD27 is a receptor molecule that integrates the tumor necrosis factor receptor (TNFR) superfamily and, as such, it may regulate the activation of B cell and synthesis of immunoglobulin [63]. A total of 42 CSF samples were collected from various types of brain tumor patients, and results indicated that the levels of Soluble CD27 (sCD27) were significantly higher in the PCNSL group when compared to controls with unrelated brain tumors (**Table 3**) [64]. Antithrombin III (ATIII) has been reported at significantly different levels in patients with CNS lymphoma and non-neoplastic patients. Indeed, ATIII concentrations in patients with CNS lymphoma were significantly higher than in control patients, and the elevation of ATIII was 75% sensitive and 98% specific along this population study (**Table 3**) [65]. Osteopontin (OPN), a pro-inflammatory cytokine, is frequently associated with the progression, metastatic spread and poor prognosis of various malignancies. One study has shown that the sensitivity and specificity of CSF OPN in a combined patient group of PCNSL and SCNSL was 87% and 86% higher than the control group, respectively (**Table 3**) [66]. Neopterin is part of the pteridin class of molecules, and it is considered a marker for the activation of the immune system. CSF neopterin levels have been significantly higher in the patients with PCNSL than in those with other brain tumors. In this context, the sensitivity of this approach was 96% and the specificity was 93% for the diagnosis of PCNSL (**Table 3**) [67]. CSF soluble transmembrane activator and CAML-interactor (sTACI) and soluble B-cell maturation antigen (sBCMA) levels were significantly increased in PCNSL patients when compared with control groups (**Table 3**) [68]. Similarly, a proliferation-inducing ligand (APRIL) and B cell activating factor (BAFF) levels in CSF from patients with PCNSL were also apparently higher than other brain tumors (**Table 3**) [69]. In addition, one particularly study has shown that the sensitivity and specificity of CSF-derived BAFF and TACI as diagnostic markers for PCNSL were 100% (**Table 3**) [70]. Another three proteins - haemopexin, apolipoprotein A1, and transferrin - were also detected at significantly increased levels in the CSF of CNS lymphoma patients (**Table 3**) [71].

CXCL10 and IL-8 chemokines are usually increased in the CSF of patients with melanoma-related brain metastasis (**Table 3**) [72]. Likely, carcino-embryonic antigen (CEA) has been measured in the CSF of patients with primary and metastatic brain tumors, suggesting that CEA levels in metastatic brain tumors are apparently higher than those in primary brain tumors (**Table 3**) [73]. EGF containing fibulin-like extracellular matrix protein 1 (EFEMP1) was also identified in the CSF of patients with meningioma and some healthy controls. Surprisingly, EFEMP1 levels were significantly higher in the CSF samples of meningioma patients when compared to the controls (**Table 3**) [74]. Prospective studies, including larger patient cohorts, will be further necessary to validate the diagnostic value of some attractive CSF protein biomarkers, for different types of brain tumors.

Extracellular vesicles

Extracellular vesicles (EVs) are membrane-bound nanoparticles, released by various types of cells [75-77]. Most of them range in size from 30 nm to 1000 nm [78, 79]. Based on the size, biogenesis, and biophysical characteristics, EVs can be classified as exosomes, microvesicles and apoptotic bodies [80, 81]. These vesicles are seminal to multiple biologic processes and, at the same time, capable of promoting tumor progression [82-84]. EVs, sometimes referred to as 'exosomes', carry an abundant array of lipids, DNAs, miRNAs, and proteins (**Figure 2A**), which can reflect their identity for analysis in liquid biopsy for brain tumors [85-88]. As shown in **Figure 2B**, the secretion of extracellular vesicles from brain tumor cell is quite complicated. A number of studies have shown that EVs can be found and isolated from the CSF of brain tumor patients [43, 44, 75, 89]. CSF-derived EVs provide a platform for detection of tumor specific biomarkers in the brain. For instance, the analysis of mutated IDH1 in CSF-derived EVs of patients with glioma may play a new role for the diagnosis [89]. Moreover, the levels of miR-21 in CSF-derived EVs of GBM patients were, in average, 10-fold higher than the levels in control subjects, and miR-21 in CSF-derived EVs yielded a diagnostic sensitivity and specificity of 87% and 93% for GBM, respectively [44]. Another study also indicated that the miR-21 signature from CSF-

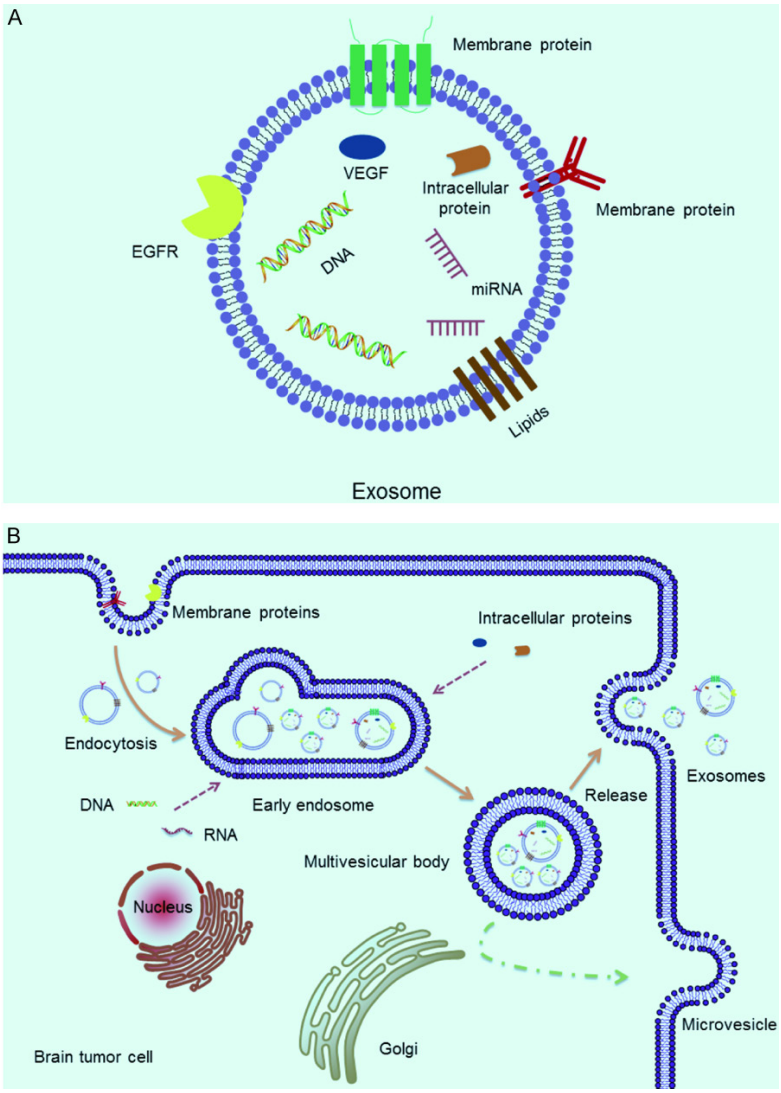

Figure 2. Extracellular vesicles (EVs) are small membrane-bound nanoparticles, released by all human cell types and tissues. A. Exosomes (nano-sized EVs) carry an abundant array of lipids, DNA, miRNA and proteins which reflect their identity for further analysis after liquid biopsy. B. Illustrative mechanism of EV secretion from brain tumor cells.

derived EVs have a diagnostic significance for GBM patients [90]. Indeed, it plays an important role not only in diagnosis but also in the prognosis and over the course of metastasis. The levels of exosomal miR-21 in the CSF from glioma patients were found significantly higher than in the controls. Furthermore, the levels of miR-21 may affect the expression of target genes. Therefore, miR-21 levels may have a predictive value in glioma recurrence and/or metastasis [91]. Epidermal growth factor receptor variant III (EGFRvIII) was also detected in CSF-derived EVs from EGFRvIII tissue-positive and tissue-negative GBM patients. In this case, EGFRvIII in CSF-derived EVs yielded a diagnostic sensitivity and specificity of 61% and 98% for EGFRvIII-positive GBM. Therefore, it looks feasible to direct mutation-specific therapies for GBM [92].

Despite recent advances, in-depth validation of CSF-derived EVs as biomarkers is still expected. For this, acquiring CSF-derived EV samples from brain tumor patients, in a larger large-scale, is warranted but will certainly require a coordinated multi-institutional effort.

Clinical roles of CSF biomarkers in brain tumors

Diagnosis

Some patients affected by brain tumors are eventually diagnosed at advanced stages and, therefore, lack the best timing for a more effective treatment. Therefore, early diagnosis of brain tumors and a timely treatment are of great importance to improve the survival rate of the patients. Although brain biopsy is still the gold standard for diagnosing brain tumors, biomarkers obtained from the CSF of brain tumor patients have unique advantages including easy access and less trauma. CSF-derived biomarkers, such as ctDNA, miRNA, proteins, and EVs, could be used for early diagnosis of brain tumors and, importantly, they could also indicate the type of brain tumor involved as well as the severity of the disease.

CSF-derived ctDNA has been a suitable tool for identifying genomic alterations of patients with primary and metastatic brain tumors. Many researchers have analyzed single and multiple genes present in CSF ctDNA, and they have discovered that most of identified genetic altera-

tions included point mutations, amplifications, and small deletions (**Table 1**) [3, 5, 6, 8, 11, 14-20, 26, 27, 93-96]. More than 50%-75% of the brain tumor patients had somatic alterations in the CSF ctDNA [3, 8, 11, 14]. Interestingly, brain tumors may be diagnosed by studying mutations in CSF ctDNA gene and, in addition, the respective tumor size can be correlated with level of mutation involved. In fact, one particular study has shown that the mutation levels detected by ddPCR analysis, can be closely associated with the tumor size [3]. In conclusion, the study of gene mutations (and their levels) in CSF ctDNA from patients with brain tumors have a great significance for the diagnosis. Multiple miRNAs identified in CSF have also been found to be significantly associated with primary and metastatic brain tumors. Similarly, several studies have also suggested that the miRNA yields in the CSF could help distinguish different types of brain tumors and other CNS-related diseases (**Table 2**) [30, 32, 33, 38, 43-47]. Protein markers can also play an important role in the diagnosis of brain tumors. A representative number of studies have demonstrated that the levels of CSF proteins have a diagnostic value for brain tumor patients (**Table 3**) [49, 50, 52-62, 64-74]. In this context, EVs might also play an indirect but supportive diagnostic role, due to its abundance of DNA, miRNA, and proteins.

Treatment

After diagnosing a brain tumor type, a treatment plan should be followed according to the tumor characteristics. Currently, the strategies used for brain tumor therapy include neurosurgery, radiation and/or chemotherapy after surgery. In the current era of molecular classification of brain tumors, it sounds feasible that a preoperative knowledge of biomarkers with prognostic significance could help the surgical planning, intraoperative decision-making and dosage regimen.

CSF ctDNA provides a minimally invasive method to assess the genomic alterations of a brain tumor, in such a way that personalized treatment(s) might be established according to its molecular characteristics (**Table 1**) [3, 5, 6, 8, 11, 14-20, 26, 27, 97, 98]. Analyses of CSF-derived ctDNA from some lymphoma patients have indicated that the detection of tumor-specific mutations is conducive to the adoption of a targeted therapy [99]. The use of CSF miRNAs as biomarkers can also be extremely helpful to determine whether surgery should be performed in patients with brain tumor [32]. In fact, even after a complete surgical resection under optimal conditions, a number of patients can still relapse and be refractory to re-treatment. The relapsed tumor tends to evolve under treatment and may present different genetic changes from the original primary tumor [100]. Unfortunately, there is no standard treatment for the recurrent brain tumors. Still, some of treatment options may include temozolomide (TMZ) rechallenge, lomustine, and antiangiogenic therapy, with the addition of re-irradiation and/or re-resection depending on the tumor location and general condition of the patient [101]. The prognosis of brain metastases is poor, and the development of effective treatment plans is constantly challenging. Still, CSF ctDNA has the potential to identify specific genomic alterations during brain metastasis that might facilitate the design of personalized treatments to target this advanced condition. For example, brain metastases originated from lung adenocarcinoma, containing EGFR mutations detectable in the CSF, have responded well to treatment with EGFR-tyrosine kinase inhibitors [102]. Similarly, many researchers elaborated that CSF-derived proteins may be helpful in the management of brain tumors (**Table 3**) [53-55, 58, 59, 70, 72, 73]. Since CSF-derived EVs contain a large amount of genetic information related to brain tumor cells, they also have a potential value in targeted therapy [79].

Taken together, it has been assumed that the content of tumor biomarkers (i.e., ctDNA, miRNA, and proteins) in the CSF might correlate with brain tumor burden. Hence, after surgery or chemoradiotherapy, we could anticipate whether a selected regimen is correct or should be updated according to the biomarker profiling.

Monitoring recurrence and treatment response

Post-treatment monitoring of patients with primary or metastatic brain tumors is a standard medical procedure. Shortly after chemo and/or radiotherapy, the appearance of an enlarged or newly enhanced lesion, called pseudoprogression, is frequently found [103]. Indeed, the tumor itself does not necessarily grow, and

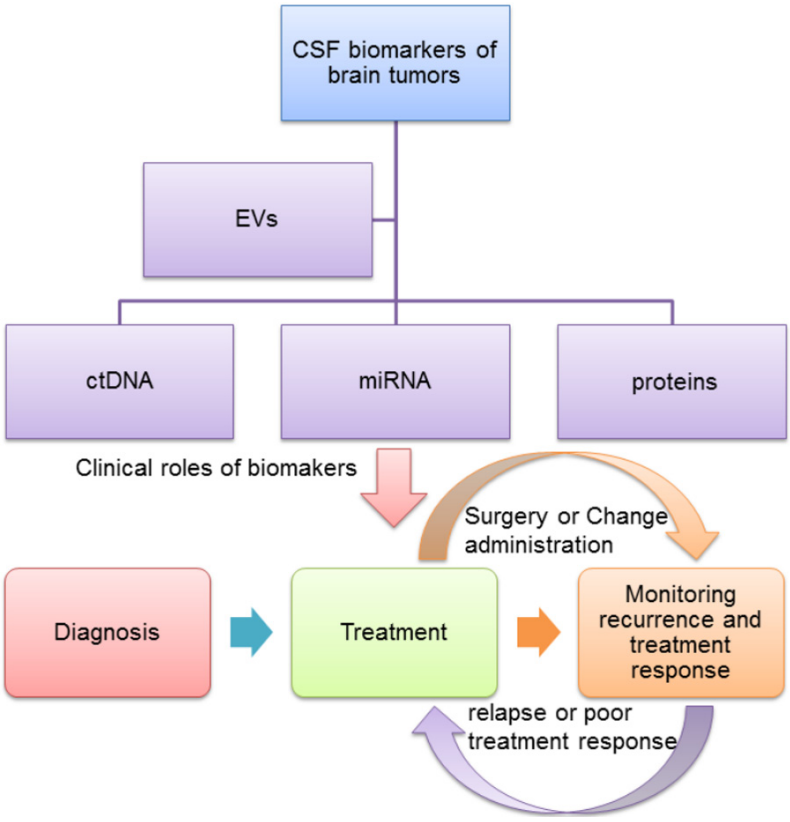

Figure 3. Changes on the expression of CSF-derived molecular biomarkers (i.e., ctDNA, miRNA, proteins and EVs) can be translated into the diagnosis, treatment, monitoring recurrence and treatment response of brain tumors. EVs play indirect clinical roles, due to its abundance of DNA, miRNA, and proteins. After diagnosing a brain tumor type, a treatment plan should be followed up according to the tumor characteristics. If the tumor recurs or responds poorly, we can re-operate on the patients or change the medication regimen.

might subside or stabilize without a modified treatment [103]. Due to the potential ocurrence of pseudoprogression, we cannot solely rely on imaging data to confirm or not tumor recurrence [38]. It is reasonable to consider that tumor recurrence may lead to an increase in the levels of tumor-derived biomarkers in the CSF, which could also distinguish recurrence from pseudoprogression. Afterwards, we may reduce unnecessary surgical procedures with imaging alterations according to the treatment outcome. MiRNA profiling could be used for monitoring recurrence and/or examining the efficacy of treatment (**Table 2**) [32, 38, 40, 43-46, 90, 91, 104]. Upon tumor removal, miRNA levels decrease below the levels as previously established but, if tumor recurrence appears, miRNA levels may re-adjust. As an example, levels of exosome-derived miR-21 significantly correlate with the recurrence or metastasis of gliomas [91]. By testing whether CSF levels of specific miRNAs can reflect disease activity and/or treatment response, Teplyuk and colleagues figured out that both miR-10b and miR-200 levels in CSF increase during relapse and, contrarily, they can be reduced by improving erlotinib dosage [32]. Similarly, the levels of CSF-derived exosomal miR-151a might predict the response to TMZ treatment in GBM patients [104]. A number of studies have shown that changes in levels of CSF-derived proteins may also reflect whether the tumor has recurred and/or treatment is effective (**Table 3**) [49, 50, 52-55, 58, 65, 68, 69]. Remarkably, increased ATP-binding cassette subfamily A member 3 (ABCA3) and Krueppel-like factor 11 (KLF11) levels and decreased brain acid soluble protein 1 (BASP1) and peroxiredoxin-2 (PRDX2) levels in CSF acquired during vestibular schwannoma (VS) surgery typically correlate with vestibular schwannoma growth at early phases or upon recurrence (**Table 3**) [105]. Furthermore, levels of CSF prostaglandin D2 synthase (PGD2S) were obviously decreased in medulloblastoma patients and, therefore, could also be used to monitor response to treatment and tumor recurrence (**Table 3**) [106].

In summary, tumor biomarkers in the CSF can be used to distinguish pseudoprogression from recurrence. In that way, some patients without true tumor recurrence will refrain from unnecessary surgical procedures, such as re-resections or brain biopsies to confirm recurrence. Meanwhile, the levels of tumor biomarkers in CSF can vary according to the dosage and the type of drug applied, so that a better drug regimen can be established according to the yields of distinct tumor markers (**Figure 3**).

Conclusion

Since brain tumors cannot be readily accessed by biopsy for frequent analysis, liquid biopsy techniques have been increasingly considered as a safer and more accessible approach to monitor disease progression. As sequencing-based technologies improve and related costs tend to be more manageable, the identification of "actionable" mutations have become even more important and accessible for medical assessment and therapeutics.

Biomarkers in the CSF of brain tumor patients can provide information on diagnosis, treatment, monitoring recurrence and treatment response. Distinguishing different types of brain tumors as well as accessing the severity of these tumors are major milestones that the analysis of tumor biomarkers in CSF have been provided. As the need for tailored therapies increase, biomarker analyses are required to predict response to specific treatments and, therefore, support the development of more individualized therapies. In fact, patient-specific tumor biomarkers are the basis of individualized treatment. Monitoring recurrence and treatment response is another important potential of the tumor biomarkers. The use of the tumor biomarkers in the CSF to distinguish pseudoprogression from recurrence will avoid unnecessary reoperations and biopsies. Moreover, changes on the levels of certain brain tumor biomarkers can reflect the efficacy of a certain treatment, whether drug resistance reaction occurs and/or when to stop treatment, especially when side effects outweigh therapeutic benefits.

Nevertheless, the acquisition of tumor biomarkers in CSF still has some shortcomings. The detection of tumor biomarkers in the CSF of brain tumor patients might be still challenging, possibly due limited concentration, sample size, sensitivity of gene mutation detection, improper extraction methods, lack of proper procedures for handling and storing samples, or even lack of standardized isolation procedures. In fact, one particular study has shown that CSF ctDNA cannot be detected in every patient with brain tumor, and ctDNA was only detected in 74% of the CSF samples [8]. Due to technological difficulties in their detection and lack of standardization, CSF miRNAs are still challenging to precisely measure [107].

We can certainly identify that the biological value of CSF-derived miRNAs, proteins, and EVs from certain conditions, including vestibular schwannoma and metastatic brain tumors, still require further validation. But, most importantly, we believe that continuous efforts to explore and test relevant biomarkers in the CSF of brain tumors patients will be translated, in the near future, into clinically relevant tools to support the diagnosis, treatment, and monitoring recurrence and treatment response of brain tumor and other CNS-related diseases.

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Disclosure of conflict of interest

None.

Abbreviations

CSF, cerebrospinal fluid; ctDNA, circulating tumor DNA; miRNA, microRNA; EVs, extracellular vesicles; CNS, central nervous system; ELISA, Enzyme-linked Immunosorbent Assay; PCR, Polymerase Chain Reaction; NGS, Next-Generation Sequencing; ddPCR, droplet-digital PCR; GBM, glioblastoma; PCNSL, primary central nervous system lymphoma; SCNSL, secondary central nervous system lymphoma; GFAP, glial fibrillary acidic protein; OPN, osteopontin; MMP, matrix metalloproteinase; ECM, extracellular matrix; VEGF, vascular endothelial growth factor; CCL, C-C motif chemokine ligand; FGF, fibroblast growth factor; NGF, nerve growth factor; IL, interleukin; CXCL, CXC chemokine ligand; B2M, beta-2 microglobulin; sIL-2R, soluble IL-2 receptor; TNFR, tumor necrosis factor receptor; sCD27, soluble CD27; ATIII, Antithrombin III; Staci, soluble transmembrane activator and CAML-interactor; sBCMA, soluble B-cell maturation antigen; APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor; CEA, carcino-embryonic antigen; EFEMP1, EGF containing fibulin-like extracellular matrix protein 1; EGFRvIII, epidermal growth factor receptor variant III; TMZ, temozolomide; ABCA3, ATP-binding cassette subfamily A member 3; KLF11, krueppel-like factor 11; BASP1, brain acid soluble

protein 1; PRDX2, peroxiredoxin-2; VS, vestibular schwannoma; PGD2S, prostaglandin D2 synthase.

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