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REVIEW PAPER

Application of Circulating Tumor DNA in the Auxiliary Diagnosis and Prognosis Prediction of Glioma

Ying Lu[1] · Zhouyu Wang[2] · Danmeng Zhang[1] · Ningning Luo[2] · Hui Yang[1] · Dongsheng Chen[2,3,4] · Haixin Huang[1]

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Abstract

Glioma is the most common primary malignant brain tumor. Despite significant advances in the past decade in understanding the molecular pathogenesis of this tumor and exploring therapeutic strategies, the prognosis of patients with glioma remains poor. Accurate diagnosis of glioma is very important for the treatment and prognosis. Although the gold-standard method for the diagnosis and prognosis prediction of patients with glioma is tissue biopsy, it still has many limitations. Liquid biopsy can provide information on the auxiliary diagnosis and prognosis of gliomas. In this review, we summarized the application of cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) in the auxiliary diagnosis and prognosis of glioma. The common methods used to detect ctDNA in gliomas using samples including blood and cerebrospinal fluid (CSF) and the detection techniques for ctDNA, including droplet digital PCR (ddPCR) and next-generation sequencing (NGS), were discussed. Detection of ctDNA from plasma of patients with brain tumors remains challenging because of the blood–brain barrier (BBB). CSF has been proposed as a medium for ctDNA analysis in brain tumors, and mutation detection using plasma ctDNA was less sensitive than CSF ctDNA sequencing. Moreover, ongoing relevant clinical studies were summarized. Finally, we discussed the challenges, and future directions for the studies on ctDNA in glioma.

Keywords Glioma · CtDNA · Auxiliary diagnosis · Prognosis · Next-generation sequencing

Introduction

Glioma is a tumor in central nervous system (CNS) originating from glial cells and is the most common primary malignant brain tumor (Yang et al. 2022; Otsuji et al. 2024). Treatment and management of glioma remain a challenge globally, and improving treatment strategies to reduce mortality and morbidity is a top priority in neuro-oncological research (Weiser et al. 2023). Patients with glioma usually experience symptoms such as headache, vomiting, and vision loss due to the tumor (Li et al. 2023). Glioma includes groups of heterogeneous tumors that differ in histology and molecular genetic features (Alpen et al. 2023). With the advances in the use of molecular biomarkers in the diagnosis of brain tumors, the fifth edition of the World Health Organization (WHO) classification of the CNS tumors (WHO CNS5) in 2021 has reported the division of glioma as follows: adult-type diffuse gliomas, pediatric-type diffuse low-grade gliomas, pediatric-type diffuse high-grade gliomas, and circumscribed astrocytic gliomas (Louis et al. 2021). The most commonly occurring histopathology of malignant glioma is glioblastoma (GBM) (Sabu et al. 2023).

Ying Lu and Zhouyu Wang are Co-first authors.

✉ Dongsheng Chen
dongsheng.chen@simceredx.com

✉ Haixin Huang
Huanghx_2024@163.com

Ying Lu
1786734840@qq.com

Zhouyu Wang
zhouyu.wang@simceredx.com

1 Department of Oncology, The Fourth Affiliated Hospital of Guangxi Medical University, Liuzhou 545000, China

2 The State Key Laboratory of Neurology and Oncology Drug Development, Jiangsu Simcere Diagnostics Co., Ltd, Nanjing Simcere Medical Laboratory Science Co., Ltd, Nanjing 210002, China

3 Cancer Center, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou 121001, China

4 Center of Translational Medicine, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou 121001, China

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GBM is characterized by aggressive biological behavior and a high degree of inter- and intratumor heterogeneity (Bv and Jolly 2024). Increasing understanding of the molecular and cellular heterogeneity of GBM will help to not only define specific subgroups more accurately for precise diagnosis but also set the foundation for successful implementation of targeted therapies (Lan et al. 2024; Skouras et al. 2023). The standard treatment for high-grade gliomas is a combination regimen; it consists of intensive multimodal treatment including maximally safe surgical resection followed by radiation therapy and temozolomide (TMZ) concurrent chemotherapy, after which adjuvant TMZ is administered (Luo et al. 2023; Fisher and Adamson 2021). Even with this regimen, recurrence is common and the prognosis is poor, particularly in GBM (with mean survival of 14–16 months) (Chai et al. 2022). Therefore, it is urgent to develop less invasive methods to identify and validate tumor biomarkers that provide real-time information to help in the auxiliary diagnosis and monitoring of gliomas.

At present, the diagnosis and prognosis of glioma mainly depends on imaging techniques and tissue biopsy (Cohen et al. 2018). However, the traditional detection technologies have several limitations (Chen and Zhao 2019; Perakis and Speicher 2017). For example, in the early-stage of treatment, tissue inflammation easily lead to false spread on magnetic resonance imaging (MRI), which leads to misdiagnosis and missed diagnosis (Hygino da Cruz et al. 2011). Current imaging methods are not sufficient to confirm the diagnosis of glioma, and tissue biopsy can provide more pathological information (Valerius et al. 2024). Although the gold-standard approach for the diagnosis and prognosis prediction in glioma is tissue biopsy, many recent studies have emphasized the limitations of this method (Marrugo-Ramírez et al. 2018; Cheng et al. 2016). First, tissue biopsy sampling is a highly invasive method and can be extremely difficult because of the poor anatomical location of the tumor (Hu et al. 2024). Second, tissue samples are not fully representative of tumor heterogeneity, limiting the accuracy of prediction results (Constâncio et al. 2019). In addition, it is difficult to achieve diagnosis and detect residual conditions and disease recurrence in this method (Poulet et al. 2019). Therefore, a noninvasive or minimally invasive method is urgently required for auxiliary diagnosis of cancer and ease of follow-up.

The application of liquid biopsy in glioma is significantly increasing in recent years. In this review, we reviewed the progress in the application of ctDNA in the auxiliary diagnosis and prognosis of gliomas (Fig. 1). The purpose of this review is to better understand the use and significance of ctDNA in the auxiliary diagnosis and prognosis of glioma and to suggest better strategy for the application of ctDNA in glioma in clinical practice.

Liquid Biopsies Based on Cell-Free DNA (cfDNA) or Circulating Tumor DNA (ctDNA)

Soluble molecules such as DNA, RNA and proteins released by tumor cells can spread to other sites through the blood circulation system to form so-called circulating tumor markers (Wang et al. 2024). Liquid biopsy has the advantages of less trauma, convenient sampling and real-time dynamic detection, which plays an important role in early screening, molecular typing, recurrence monitoring and prognosis evaluation of tumors (Trinidad et al. 2023; Ren et al. 2024). Liquid biopsy involves analysis of patient's blood, urine, cerebrospinal fluid (CSF), and other body fluid samples for the diagnosis of diseases (Anitha et al. 2024). Liquid biopsy

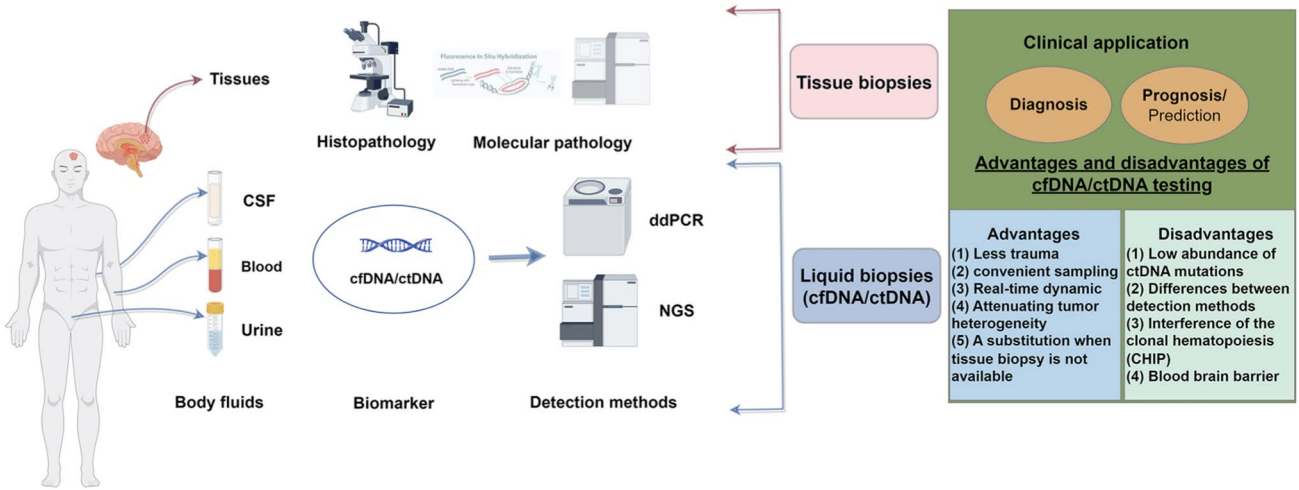

Fig. 1 Application of ctDNA in gliomas (By Figdraw). ctDNA, circulating tumor DNA

helps in understanding the molecular evolution and achieves the purpose of dynamic monitoring of the disease (Wu et al. 2023a, 2023b). In glioma, various molecules are released to regulate signal transduction and metabolic catalytic pathways between cells. These molecules can be isolated and detected in liquid biopsy as the markers for auxiliary diagnosis and prognosis of glioma (Müller Bark et al. 2020). cfDNA is an extracellular fragment of double-stranded DNA released into body fluids including blood, CSF, and urine as a result of natural body mechanisms such as apoptosis, necrosis, and active secretion (Thierry et al. 2016; Wan et al. 2017; Escudero et al. 2021). cfDNA exhibits short half-life, ranging from 4 min to 2 h, which is suitable for detection applications (Dao et al. 2023). Studies have found that the cfDNA concentration in healthy people ranges from 0 to 100 ng/ml of blood, with an average of 30 ng/ml, while the cfDNA concentration in the blood of cancer patients ranges from 0 to 1000 ng/ml, with an average of 180 ng/ml (Phallen et al. 2017). The vast majority of the circulating cfDNA pool in patients with all cancers, but particularly in those with gliomas, consists of cfDNA of nontumor origin. The exact origin and molecular properties of this nontumor-derived cfDNA remain unknown, but most cfDNA is thought to arise from cellular events, including apoptosis, necrosis, and cellular secretion (Bronkhorst et al. 2019). In patients with cancer, a fraction of cfDNA is derived from tumor cells and is termed ctDNA, which may contain tumor-corresponding specific variants, such as mutated tumor suppressor or oncogene, microsatellite instability (MSI), and DNA methylation (Yan et al. 2021). cfDNA fragments from tumor cells have a different size compared to cfDNA fragments from normal cells (Underhill et al. 2016; Mouliere et al. 2018a). ctDNA mainly comes from necrotic or apoptotic tumor cells and circulating tumor cells(Dai et al. 2023a). In cancer patients, the machinery to clear DNA fragments is overwhelmed by the DNA released by tumor cells. Thus, the proportion of ctDNA in circulating cfDNA varies from 0.01 to 90% (Wang and Bettegowda 2017). When surgery is not recommended or has not been informative, liquid biopsy, and in particular, detection of ctDNA in glioma may provide information on differential auxiliary diagnosis (Miller et al. 2019). In addition, ctDNA detection is more suitable for clinical follow-up, helping in understanding the molecular changes in brain tumor during therapeutic interventions (Diaz et al. 2024). Therefore, ctDNA plays a crucial role in the management of gliomas. However, the proportion of ctDNA in glioma patients is small compared with other tumors (Bettegowda et al. 2014). In addition, distinguishing true somatic mutations from sequencing artifacts and clonal hematopoiesis in patients with glioma has been challenging, and background cfDNA levels in cerebrospinal fluid are lower than those in blood (Okamura et al. 2021) (Okamura et al. 2021). Many recent studies have aimed to explore how to improve the detection rate of ctDNA in blood and cerebrospinal fluid (CSF).

Sample Types for ctDNA Detection in Gliomas

At present, the sample types used for ctDNA detection in glioma are mainly blood and CSF (Krynina et al. 2024). ctDNA detection based on blood and CSF samples have their own capabilities and shortcomings (Fig. 2). Studies have

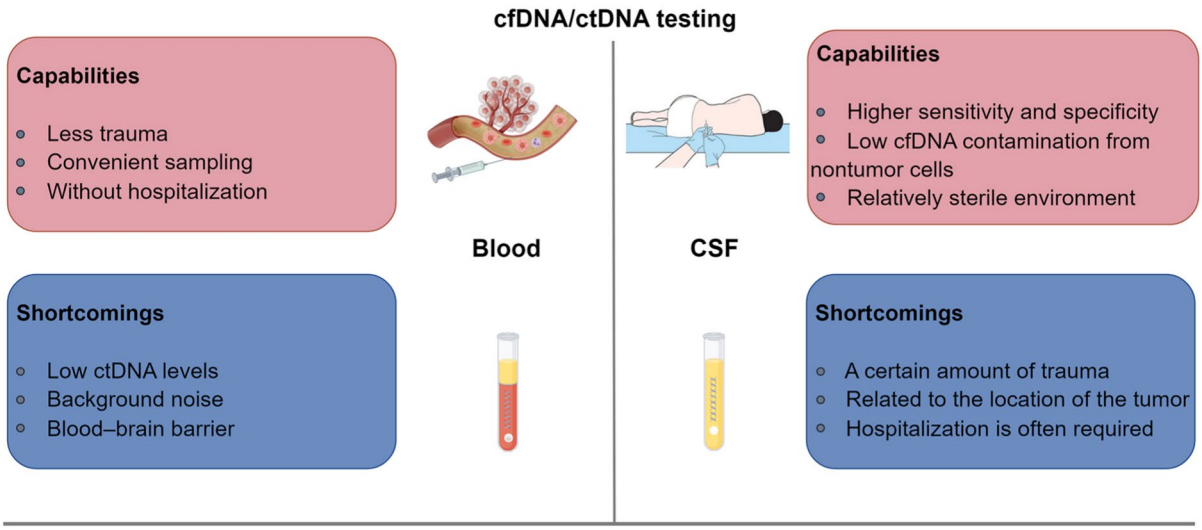

Fig. 2 Capabilities and shortcomings of cfDNA/ctDNA detection based on blood and CSF samples (By Figdraw). cfDNA, cell-free DNA. ctDNA, circulating tumor DNA. CSF, cerebrospinal fluid

reported that ctDNA level is significantly reduced in glioma compared with other malignancies and speculated that the blood–brain barrier (BBB) may be a major obstacle to detect ctDNA in plasma (Bettegowda et al. 2014; Estival et al. 2019). The known rate of clonal hematopoiesis may lead to the accumulation of nontumor-derived somatic mutations in hematopoietic cells, resulting in significant background noise in plasma, particularly after radiotherapy or chemotherapy (Okamura et al. 2021). The relatively low amount of ctDNA in glioma in plasma, in contrast to the significantly increased amount of nontumor-derived cfDNA, limits its utility in the diagnosis and monitoring of tumor evolution (McMahon et al. 2022). The detection of ctDNA in blood in patients with primary brain tumors remains challenging. BBB protects nerve tissue from toxins in the circulation, but it also hinders the release of brain tumor-derived molecular biomarkers into the blood, resulting in extremely low concentrations of circulating biomarkers (Connolly et al. 2016). Using advanced biomarker detection techniques, ctDNA can be detected in more than 75% of patients with advanced pancreatic, ovarian, colorectal bladder, melanoma, and head and neck cancers, but in less than 10% of glioma patients (Bettegowda et al. 2014). Current strategies focus on developing advanced, highly sensitive biomarker detection techniques to analyze collected blood samples, such as ddPCR, NGS, and advanced spectroscopy (Pacia et al. 2022). Local disruption of the BBB may contribute to the release of tumor-derived DNA into the blood circulation, thereby improving the detection rate of glioma ctDNA. Pacia et al. developed the "sonobiopsy" technique using focused ultrasound (FUS) in combination with microbubbles to disrupt the BBB (Pacia et al. 2022). This technique can greatly improve the detection sensitivity of EGFRvIII in mouse and porcine GBM models. Besides, cfDNA sequencing has much room for improvement in reducing ccfDNA isolation time, size selection, library preparation, and deep sequencing of targeted panels (Szadkowska et al. 2022).

Tumor-specific DNA alterations are more readily detected in CSF than in plasma. Massively parallel sequencing of ctDNA from CSF enables a more comprehensive characterization of genomic alterations in brain tumors compared with that of ctDNA from plasma, enabling the identification of actionable somatic mutations in brain tumor (De Mattos-Arruda et al. 2015). Notably, sequencing of ctDNA from CSF may provide an alternative approach with lower morbidity and cost. In cases where tumor cannot be approached surgically, lumbar puncture can be used to obtain a molecular signature and potentially definitive diagnosis (On et al. 2021). However, shedding of ctDNA from CSF does not appear to be a universal characteristic of diffuse glioma, even in previously treated patients (Miller et al. 2019). For CNS tumors, such as medulloblastoma characterized by invasive growth patterns and diffuse endogenous pontine gliomas, molecular analysis of cfDNA from CSF exhibits superior performance than that of cfDNA from plasma. This advantage is mainly attributed to the existence of the BBB (De Mattos-Arruda et al. 2015; Arvanitis et al. 2020). Although collection of CSF occasionally requires hospitalization, the use of cfDNA from CSF instead of that from plasma in patients with brain tumors has attracted increasing attention. Analysis with CSF exhibits advantages such as more cell-free, sterile environment in which ctDNA is enriched, and sequencing depth is no longer a technical limitation (Pan et al. 2015). Studies have reported that in patients with high tumor burden, lesions adjacent to the CSF space, and leptomeningeal disease, analysis of ctDNA from CSF is more beneficial (Miller et al. 2019, 2022). In a study, 38 patients had *TERT*p-mutant/*IDH* wild-type glioblastomas. The matched *TERT*p mutation in the ctDNA from CSF was successfully detected with 100% specificity and 92.1% sensitivity. In contrast, the sensitivity in the ctDNA from plasma was far lower (Juratli et al. 2018).

Other sample types, such as tumor in situ fluid (TISF), have also been explored in glioma. TISF is the fluid in the local surgical cavity. Sheng et al. used cfDNA sequencing to compare genomic changes between TISF and tumor tissue. The results showed that cfDNA fragment concentrations in TISF patients ranged from 7.2 to 1397 ng/ml. At least one tumor-specific mutation identified in all 10 patients (100%) (Sheng et al. 2021). Two years later, they analyzed the value of TISF ctDNA analysis for tracking tumor evolution during glioma treatment (Sheng et al. 2023). At least one tumor mutation was detected in 179 of 205 (87.3%) ctDNA samples from TISF. Moreover, analysis of ctDNA from TISF detects mutations that are not present in tumor tissue. ctDNA parameters during treatment can predict recurrence, and continuous ctDNA monitoring has diagnostic value for early detection of recurrence. ctDNA from TISF may be more sensitive than ctDNA from CSF to describe the genetic characteristics in the evolution of gliomas after surgery(Sheng et al. 2021; Liu et al. 2023). One study identified the value of cfDNA in brain tumor cystic fluid for mutation detection in brain tumors (On et al. 2024). They detected all variants in brain tumor tissue in cyst fluid cfDNA based on ddPCR, with variant allele frequencies (VAF) in cyst fluid cfDNA comparable to those in tumor DNA.

Detection Platforms for ctDNA Detection in Gliomas

ctDNA-based liquid biopsy has been extensively studied in many solid tumors and can be used for determining tumor burdens and mutations (Henriksen et al. 2023; Tran et al. 2023; Coakley et al. 2023). Various approaches are applied in recent studies to detect single nucleotide variants

(SNVs) and copy number variations (CNVs) in the ctDNA of patients with cancer, including ddPCR, whole-exome sequencing (WES), low-pass whole-genome sequencing (lpWGS), and cancer-type-specific high-throughput DNA sequencing panels (Chicard et al. 2018; Cimmino et al. 2020; Klega et al. 2018). Detection and quantification of ctDNA in glioma require the use of highly sensitive assays such as ddPCR or NGS that can detect and quantify mutated genes in the context of normal cfDNA (Table 1). Isocitrate dehydrogenase-1 (*IDH1*) mutation is considered to be one of the earliest events in IDH1 mutant glioma (astrocytoma or oligodendroglioma) tumorigenesis. Tuna et al. used ddPCR to identify *IDH1* mutations in CSF and plasma of 49 glioma patients and found that *IDH1* mutations were detected in CSF and plasma ctDNA in 63.2% and 25.0%, respectively (Tuna et al. 2022). Detection of low-level ctDNA is highly dependent on platform sensitivity and sample type. A study optimized the ddPCR method to detect common somatic mutations in pediatric high-grade gliomas (HGG) and diffuse midline glioma, H3 K27-altered, and tumor-specific DNA alterations were more readily detected in CSF compared to plasma (Izquierdo et al. 2021). Another study optimized two ddPCR platforms (RainDance and BioRad) and validated methods for detecting the H3F3A c.83A > T (H3.3K27M) mutation in diffuse midline glioma, H3 K27-altered CSF, plasma, and primary diffuse midline glioma, H3 K27-altered specimens. This study found 100% sensitivity and specificity for mutation detection in matched diffuse midline glioma, H3 K27-altered tissue and CSF across different assays, platforms, and institutions.

The detection limit of PCR-based methods is 0.001–0.01%, and the sensitivity of NGS technology is 0.1–1%(Bohers et al. 2021). Despite their high sensitivity, ddPCR can only detect known variants using specific probes. Although ddPCR can quantitatively assess mutation frequencies in cfDNA, it is limited by the number of fluorescent probes (up to 5) that can be used in a single assay (Butler et al. 2017). Detection of ctDNA form plasma is generally more sensitive via PCR-based assays than via NGS-based methods for the detection of specific SNVs (Postel et al. 2018). Multiplex ligation-dependent probe amplification (MLPA) is a relatively simple semi-quantitative PCR assay that can detect DNA copy number changes at up to 50 loci. Otsuji et al. extracted cfDNA from CSF of patients with diffuse glioma and successfully performed copy number analysis using MLPA (Otsuji et al. 2023). The limitations of this study are the small sample size and the small number of tumor cases with CNA and the fact that lower grade gliomas were not included. PCR-based methods often fail to reflect the broader genomic changes associated with glioma progression (Kurtz et al. 2021). The detection based on NGS method is more and more widely used in gliomas.

NGS-based methods are targeted to genes of interest and can detect not only known variants but also novel variants (Table 1). Existing approaches to improve ctDNA analysis have primarily focused on test sensitivity, with only little

Table 1 Selected studies using ddPCR and NGS to detect ctDNA in brain gliomas

Tumor	Sample type	Patients(n)	Detection methods	Detection index	References
Gliomas	CSF, urine, and plasma	49	ddPCR	*IDH1*	(Tuna et al. 2022)
Pediatric high-grade and diffuse midline glioma, H3K27-altered	Plasma, serum, CSF, cystic fluid, and tumor samples	32	ddPCR	*H3F3A*_K27M; *BRAF*-V600E	(Izquierdo et al. 2021)
Gliomas	CSF	13	sWGS	DNA fragmentation and copy number alterations	(Mouliere et al. 2018b)
GBM and other primary brain tumors	Blood samples	419	NGS	Targeted NGS sequencing	(Piccioni et al. 2019)
Brainstem gliomas	CSF, plasma, and tumor samples	57	NGS	68 genes	(Pan et al. 2019)
Pediatric solid tumors	Blood samples	39	NGS and sWGS	67 genes	(Stankunaite et al. 2022)
Gliomas	CSF and tumor samples	26	NGS	520 genes	(Guo et al. 2022)
GBM	CSF and tumor samples	10	WES	ctDNA mutations	(Duan et al. 2020)
Gliomas	TISF and tissue samples	10	NGS	68 genes	(Sheng et al. 2021)
Gliomas	TISF and tumor samples	107	NGS	68 genes	(Sheng et al. 2023)
Diffuse midline glioma, H3 K27-altered	CSF, plasma, tumor samples, and human primary pediatric glioma cells	CSF ($n=6$), plasma ($n=4$)	ddPCR	H3F3A c.83A > T	(Li et al. 2021)
Pan-cancers	Blood and tumor samples	200	sWGS	Fragment sizes	(Mouliere et al. 2018a)

ddPCR Droplet digital PCR; *NGS* next-generation sequencing; *sWGS* shallow whole-genome sequencing; *WES* whole-exome sequencing; *ctDNA* circulating tumor DNA; *GBM* glioblastoma; *CSF* cerebrospinal fluid

attention given to the biological properties of cfDNA from plasma. Fragment size analysis based on low-pass whole-genome sequencing (lpWGS) can facilitate ctDNA detection and can complement or provide an alternative to more in-depth cfDNA sequencing (Pesta et al. 2022). Another study performed shallow whole-genome sequencing (sWGS, $< 0.4\times$ coverage) of cfDNA in CSF of 13 patients with primary glioma, identified specific somatic copy number alterations and DNA fragmentation patterns, and found that cfDNA in CSF had different fragmentation patterns than that in plasma (Mouliere et al. 2018b). Piccioni et al. used a targeted NGS panel to detect ctDNA in blood samples from 419 primary brain tumors, and ≥ 1 somatic mutation was detected in 50% of patients, with meningiomas (59%) and GBM (55%) having the highest detection rates (Piccioni et al. 2019). However, this study did not use matched germline samples and variant calling/filtering was not well described, which led to the possible detection of SNVS unrelated to tumors**.** CSF ctDNA deep sequencing is a reliable technique to detect tumor-specific mutations in brainstem tumors. In a study, deep sequencing of glioma-related genes was performed on ctDNA derived from CSF of patients with brainstem glioma, and it was found that at least one tumor-specific mutation was detected in more than 82.5% of CSF ctDNA samples (47/57). Also, mutation detection using plasma ctDNA was less sensitive than CSF ctDNA sequencing (38 and 100%, respectively) (Pan et al. 2019). It should be noted that only three CSF samples were collected by conventional lumbar puncture in this study, and most of the samples were taken intraoperatively. Therefore, larger studies of these promising findings should be conducted to ensure the utility and sensitivity of CSF ctDNA testing based on lumbar puncture for the analysis of primary brainstem tumors. Stankunaite et al. developed a clinically relevant (67 genes) NGS capture panel and corresponding workflow that enables sensitive and reliable detection of low-frequency genetic variants in cfDNA from blood of patients with pediatric solid tumors (Stankunaite et al. 2022). To analyze the mutation profile and tumor mutational burden (TMB) of CSF ctDNA and compare them with corresponding tumor DNA samples, Guo et al. sequenced the samples using a 520-gene panel (Guo et al. 2022). It has been found that ctDNA mutations have a high concordance with tumor DNA mutations. ctDNA TMB in CSF also showed a strong correlation with TMB level in tumor tissues ($R^2 = 0.879$, $P < 0.001$), especially in GBM. WES assessment of ctDNA from CSF is a feasible method to detect genomic alterations in GBM and can provide useful information for deciding the treatment strategies. Duan et al. extracted ctDNA from CSF and genomic DNA from resected tumors of 10 GBM patients and performed WES (Duan et al. 2020). Their results showed that the mean mutation frequency was similar in CSF and tumor tissue samples ($74.1 \pm 6.0\%$ vs. $73.8 \pm 6.0\%$, $P = 0.924$). In conclusion, NGS-based ctDNA detection of gliomas, especially based on CSF samples, has a certain consistency with the mutation of tumor tissue DNA.

Application of ctDNA in the Auxiliary Diagnosis of Gliomas

Based on blood, CSF, or TISF sample types and ddPCR or NGS platforms, many studies have investigated the application of ctDNA in auxiliary diagnosis of glioma. Gliomas are often not identified until the patient presents with severe neurological symptoms, such as seizures (Löding et al. 2023). As treatment options are limited, definitive diagnosis can improve the prognosis and survival (Saito et al. 2023). Several studies have explored the use of ctDNA detection based on tumor tissue or blood, CSF, and urine samples in the auxiliary diagnosis of gliomas.

Auxiliary Diagnosis of Gliomas Based on Blood Samples

The detection of ctDNA based on blood samples can provide valuable information for the auxiliary diagnosis of glioma (Table 2). Detection of *TERT* promoter mutations (C228T, C250T) in cfDNA has been successfully used in some systemic cancers but has not been demonstrated in gliomas. Muralidharan et al. have developed a novel ddPCR assay that combines various features to increase sensitivity and allows simultaneous detection and longitudinal monitoring of two *TERT* promoter mutations (C228T and C250T) in cfDNA from the plasma of patients with glioma (Muralidharan et al. 2021). Compared with the gold-standard of tumor tissue-based *TERT* mutation detection, blood-based testing had an overall sensitivity of 62.5% (95% CI 52–73) and a specificity of 90% (95% CI 80–96). Current methods for detecting circulating tumor DNA include testing for somatic mutations with the use of cfDNA, but these methods may be less sensitive in patients with early-stage cancer, given the limited number of recurrent mutations (Phallen et al. 2017). In contrast, large-scale epigenetic variation (tissue- and cancer-type-specific) would not be similarly constrained (Hoadley et al. 2014). Despite the low abundance of ctDNA, DNA methylation alterations can reliably detect extracranial cancers with different cellular origins in plasma. Shen et al. analyzed the detection probability of different numbers of differentially methylated regions (DMRs), coverage, and ctDNA abundance (Shen et al. 2018). Sensitivity was found to improve with increasing number of DMRs even at lower sequencing depth and ctDNA abundance, suggesting that recovering cancer-specific DNA methylation

Table 2 Application of ctDNA in the auxiliary diagnosis of gliomas

Tumor	Sample types	Sample size	Detection methods	Detection index	References
Gliomas	Plasma	157	ddPCR	*TERT* promoter mutations	(Muralidharan et al. 2021)
Gliomas	Plasma samples	60	cfMeDIP-seq	DNA methylation	(Nassiri et al. 2020)
Gliomas	Serum	149	Genome-wide DNA methylation	cfDNA-derived methylation signature	(Sabedot et al. 2021)
Gliomas	frozen tumors and corresponding blood samples	80	NGS	Circulating cell-free DNA	(Szadkowska et al. 2022)
Gliomas	Tumor tissues and blood sample	21	NGS	1p/19q and *MDM2* followed by frequencies of *ERBB2, IDH1, CDKN2A, CDK4, PDGFRA, CCNE1, MET*	(Liang et al. 2020)
Diffuse gliomas	Tumor specimens and CSF	20	NGS and PCR	*IDH1, IDH2, TP53, TERT, ATRX, H3F3A, HIST1H3B*	(Martínez-Ricarte et al. 2018)
Diffuse gliomas	CSF	34	ddPCR	*IDH1 R132H*, *TERT* promoter (C228T and C250T), and *H3F3A* (K27M) mutations	(Fujioka et al. 2021)
Gliomas	CSF	48	ddPCR, liquid chromatography-mass spectrometry	*IDH1* p.R132H mutation and 2-hydroxyglutarate (2HG)	(Fujita et al. 2022)
Diffuse midline glioma, H3 K27-altered	CSF	6	Targeted Sanger sequencing	H3K27M mutation	(Huang et al. 2017)
Gliomas	Urine samples	35	WES	Whole exomes	(Mouliere et al. 2021)

ddPCR droplet digital PCR; *NGS* next-generation sequencing; *WES* whole-exome sequencing; *cfDNA* cell-free DNA; *ctDNA* circulating tumor DNA; *CSF* cerebrospinal fluid; *cfMeDIP-seq* cell-free methylated DNA immunoprecipitation and sequencing

changes could enable highly sensitive and low-cost cancer detection, classification, and monitoring. Two years later, Nassiri et al. found that the tumor-specific plasma methylation model (cell-free methylated DNA immunoprecipitation and high-throughput sequencing, cfMeDIP-seq) could distinguish gliomas from extracranial tumors and healthy controls (AUC = 0.99, 95% CI 0.96–1.00), with similar performance in low-grade and high-grade gliomas (Nassiri et al. 2020). Sabedot et al. developed a noninvasive method to analyze the DNA methylation status in the serum of patients with glioma and identified cfDNA-derived methylation signatures associated with the presence of glioma and associated immunological features (the "glioma-epigenetic liquid biopsy score" or GeLB) (Sabedot et al. 2021). The model could best distinguish patients with or without glioma (sensitivity: 100%, specificity: 97.78%). However, these results have not been replicated, and the complexity of their analyses may affect the validation of the method in external and independent datasets. Another study reported that cfDNA sequencing was less efficient as a diagnostic tool in patients with glioma. After improvements in quality control and library preparation, ctDNA was detectable in 8 of 84 patients with brain tumors, including 5 of 80 patients with glioma (Szadkowska et al. 2022). In 32 of 84 patients, potentially pathogenic genetic alterations that were not detected in the tumor DNA were identified in cfDNA. However, the results of this study showed that plasma cfDNA sequencing is inefficient as a diagnostic tool for patients with glioma. Another study evaluated the differences in gene mutations between gliomas ($n = 21$) and metastatic brain tumors ($n = 7$) (Liang et al. 2020). This study suggested that the mutated genes are different between gliomas and metastatic brain tumors. ctDNA mutated genes in metastatic brain tumors included ALK and *MDM2*, and glioma-associated ctDNA mutated genes included 1p/19q and MDM2, followed by *ERBB2, IDH1, CDKN2A, CDK4, PDGFRA, CCNE1, and MET*. The sample size of this study is limited, and larger samples are needed to confirm these findings. In conclusion, ctDNA mutation detection based on blood samples can provide useful information for the auxiliary diagnosis of gliomas, while cfDNA methylation analysis has greater reference value.

Auxiliary Diagnosis of Glioma Based on CSF Samples

The high consistency of mutations between CSF and tumor tissues suggests the practicability of NGS-based CSF mutations detection in assisting the comprehensive diagnosis of gliomas (Wang et al. 2023). The genomic features of diffuse glioma contribute to its molecular diagnosis (Table 2). The anatomical localization of diffuse glioma complicates the acquisition of tumor specimens for the diagnosis and in some cases leads to high-risk surgical procedures and stereotactic biopsies. Martinez-Ricarte et al. reported that genomic analysis of *IDH1*, *IDH2*, *TP53*, *ATRX*, *TERT*, *H3F3A*, and *HIST1H3B* mutations in ctDNA from CSF can help in the timely diagnosis of diffuse glioma, facilitating surgical and clinical management of these patients (Martínez-Ricarte et al. 2018). The gene mutations identified in the tumor samples were found in 85% of CSF ctDNA. Fujioka et al. used a chip-based digital PCR system to achieve accurate molecular diagnosis of 20 diffuse gliomas (71%) using intracranial CSF. Lumbar CSF obtained from 6 of 7 patients (87%) with tumors harboring any mutation was used to accurately diagnose WHO grade 3 or 4 diffuse gliomas (Fujioka et al. 2021). The reliability of this method depends on the extent of CSF involvement in the tumor distribution and may be most appropriate for the diagnosis of advanced high-grade gliomas involving the CSF. Fujita et al. reported that detection of *IDH1* p. R132h mutation and D-2-hydroxyglutarate (D-2HG) level in CSF using ddPCR and liquid chromatography–mass spectrometry, respectively, may help to identify glioma with IDH mutation(Fujita et al. 2022). ctDNA mutations were detected in 7 of 9 IDH-mutant glioma samples. However, further prospective studies with larger cohorts are needed to validate these findings.

Diffuse midline glioma, H3 K27-altered are a group of aggressive CNS tumors that predominantly affect children and young adults. These tumors cannot be surgically resected due to their anatomical location, which limits the tissue available for diagnostic and molecular studies (Al Sharie et al. 2023). H3K27M mutations are observed in up to 80% of diffuse midline glioma, H3 K27-altered cases, and H3G34V/R mutations occur in up to 30% of pediatric hemisphere gliomas (Mueller et al. 2023; Lulla et al. 2016). CSF from patients with diffuse midline glioma, H3 K27-altered can be a reasonable alternative to detect these mutations without performing tissue biopsy. In a study on 6 children with diffuse midline glioma, ctDNA was isolated from CSF in sufficient quantity and quality for analysis from 5 samples (83%), and H3.3K27M was detected in 4 samples (66.7%) (Huang et al. 2017). Histone H3 gene mutations can be detected in ctDNA from CSF in children with brain tumors, including diffuse midline glioma, H3 K27-altered, which indicates the feasibility of using liquid biopsy to supplement tissue biopsy.

Auxiliary Diagnosis of Gliomas Based on Urine Samples

The cfDNA signatures in the urine samples of glioma patients can be used to detect tumors. Mouliere et al. explored the potential of urine for cfDNA testing in patients with glioma. They reported that the cfDNA in the urine of patients with glioma was significantly more fragmented than that in the urine of patients with nonmalignant brain disease and healthy individuals. Fragment-length-integrated machine learning models could distinguish patients with glioma using urine samples (AUC = 0.80–0.91) (Mouliere et al. 2021). However, the study analyzed urine samples from only 35 patients and exhibited the limitation of the use of double-stranded DNA from urine samples; therefore, the results may be affected by potential biases introduced by the DNA extraction and sequencing methods used.

Application of ctDNA in Efficacy Prediction and Prognosis of Gliomas

Except providing information on auxiliary diagnosis, some relevant studies are available on the potential application of ctDNA-based liquid biopsies in efficacy assessment and prognosis stratification in primary and recurrent gliomas (Table 3). In a study, the genome in the CSF of 85 patients with glioma was evaluated who underwent lumbar puncture (Miller et al. 2019). This study included 54, 31, and 15% cases of WHO grade 4 GBM, WHO grade 3 gliomas, and WHO grade 2 gliomas, respectively. ctDNA from CSF was detected in 49.4% patients and was associated with adverse outcome and disease burden. This study suggested that the presence of ctDNA in CSF may be an early indicator of glioma progression. A study on 30 patients with grade 2–4 glioma reported that tumor DNA from TISF can reveal the mutational landscape of minimal residual disease after surgery and the risk of early recurrence and genomic changes in recurrent tumors, providing valuable information for prognosis and contributing to the clinical management glioma patients (Yu et al. 2021). In addition, serial monitoring of ctDNA from TISF had diagnostic value for detecting early recurrence and revealed that the pre-existing mismatch repair-deficiency is one of the mechanisms of TMZ resistance (Sheng et al. 2023).

Research on the application of ctDNA in prognosis is mostly conducted in GBM. A pilot prospective study highlighted the value of accurate and reliable detection

Table 3 The application of the ctDNA in prognosis of glioma

Tumor	Sample type	Sample size	Detection methods	Detection index	References
Gliomas	Tumor specimens and CSF	85	NGS	410 genes	(Miller et al. 2019)
GBM	CSF and Plasma	60	ddPCR	*TERT*p	(Juratli et al. 2018)
GBM	Plasma	42	NGS	Plasma cfDNA	(Bagley et al. 2020)
GBM	Plasma	62	qPCR	cfDNA concentration	(Bagley et al. 2021)
GBM	Plasma	49	ddPCR	cfDNA concentration	(Fontanilles et al. 2020)
GBM	CSF	1	Genome-wide analysis	GZMB, KRT80, CD5, CCNJL, ACTB, LCN2, CALN1, and DUSP27	(Dai et al. 2023a)
GBM	Tumor specimens and CSF	4	NGS	Methylation profiles of CSF ctDNA and Transcriptional profiles of tumor tissues	(Dai et al. 2023b)
Oligodendrogliomas	Tumor specimens, matched TISF samples, and blood samples	11	WES	68 genes	(Liu et al. 2023)
Diffuse midline glioma H3 K27-altered	Biopsy tissues	28	WES and RNAseq	TP53 mutation status, genome instability	(Kline et al. 2022)

ddPCR droplet digital PCR; *NGS* next-generation sequencing; *WES* whole-exome sequencing; *cfDNA* cell-free DNA; *ctDNA* circulating tumor DNA; *CSF* cerebrospinal fluid; *TISF* tumor in situ fluid; *GBM* glioblastoma

of *TERT*p mutations using ctDNA from CSF and further suggested that high VAF levels of *TERT*p mutation in ctDNA from CSF may predict poor survival in glioblastoma (Juratli et al. 2018). In addition, cfDNA concentration can also indicate the prognosis of glioma patients. In a prospective cohort study, cfDNA from plasma was collected at baseline prior to initial resection and longitudinally during chemoradiotherapy from patients with newly diagnosed glioblastoma (Bagley et al. 2020). The results indicated that the detection of somatic alterations is feasible in the plasma samples obtained prior to initial surgical resection. The concentration of cfDNA from plasma was related to radiographic tumor burden on first post-radiation MRI scan and tended to increase prior to or concurrently with radiographic tumor progression. This indicated that cfDNA from plasma may be a surrogate of tumor burden. Preoperative concentration of cfDNA from plasma above the mean value was associated with inferior progression-fee survival (PFS) and further indicated that cfDNA from plasma may be an effective prognostic tool in newly diagnosed glioblastoma. Another prospective cohort study indicated that high preoperative cfDNA concentration and increasing cfDNA concentration post-chemoradiotherapy were both correlated with worse PFS and overall survival (OS). cfDNA from plasma can be obtained noninvasively, and cfDNA concentration is a promising prognostic biomarker for the effective stratification of patients with GBM (Bagley et al. 2021). Fontanilles et al. reported that in newly diagnosed GBM, median cfDNA concentration was significantly decreased from baseline to pre-RT-TMZ and was significantly increased from pre-RT-TMZ to time of progressive disease (PD); the cfDNA concentration may be a biomarker of PD during TMZ phase (Fontanilles et al. 2020). Some studies reported the construction of diagnostic and prognostic models in GBM. In a clinical trial on oncolytic virus against GBM, genome-wide analysis of ctDNA methylation profiles in CSF were conducted, and 8 key genes were selected for constructing diagnostic and prognostic models (Dai et al. 2023a). In recurrent GBM, a study identified 8 hub genes for constructing diagnostic and prognostic models, providing new biomarkers for the clinical study on recurrent GBM (Dai et al. 2023b).

A study on oligodendrogliomas using ctDNA profiling from TISF samples for the assessment of dynamic tumor evolution demonstrated that great heterogeneity of oligodendroglioma during gene evolution leads to the rapid progression of tumor (Liu et al. 2023). This study indicated that the Sonic hedgehog (SHH) and PI3K/AKT signaling pathways may play an important role in promoting drug resistance and tumor distant relapse during progression**.** Most studies on the application of ctDNA in prognosis were on adult-type gliomas. In pediatric-type diffuse intrinsic pontine glioma, H3K27M mutation in ctDNA from plasma was longitudinally measured, and in 60% of cases, H3K27M mutation in ctDNA from plasma was detected at baseline and was associated with response to radiotherapy and survival. In diffuse midline glioma H3 K27-altered, TP53 mutations were associated with worse OS, genome instability, and radiotherapy resistance (Kline et al. 2022).

Clinical trials of the ctDNA Application in Auxiliary Diagnosis and Prognosis of Gliomas

Several glioma-related clinical trials are registered involving the use of ctDNA (https://www.clinicaltrials.gov/) (Table 4). A multicenter study on children and young adults with primary brain tumors attempted to integrate liquid biopsy for cfDNA analysis from CSF into the clinical care of patients with entire pediatric brain tumors to facilitate tumor diagnosis or molecular subclassification and guide treatment decisions (NCT05934630). The primary objective was to investigate the concordance between alterations in cfDNA from CSF and tumor DNA in matched pairs to: (1) the concordance between matched samples and (2) genomic evolution across tumor types over time. Enrollment is ongoing, and the study will eventually enroll 300 patients. Another study will enroll 220 patients with glioma to validate a newly developed RT-PCR assay for the analysis of tissue, plasma, and CFS samples (NCT04539431). The qualitative and quantitative value of the results obtained using the new method will be then compared with the results already obtained with the standard method for the routine diagnosis using surgical tissue samples from the same patients. In addition, studies are ongoing that will confirm the presence of glioma-type tumors and provide real-time information to classify astrocytomas or oligodendrogliomas by analyzing specific mutations in ctDNA in the blood of patients with glioma using ddPCR (NCT05964153). Interestingly, a study was conducted to evaluate the relationship between multiparametric imaging biomarkers (CT, PET/CT, MRI) and genetic analysis (tumor tissues and ctDNA in tumor in situ fluid) in patients with glioblastoma (NCT05541042). Future research should be devoted to exploring how to improve the detection rate of ctDNA in gliomas.

Registered clinical studies aiming to determine the prognostic value of ctDNA in patients with glioma were reviewed. An ongoing phase 2, open-label, single-center, nonrandomized study involving 60 patients with GBM will explore the efficacy and safety of sintilimab (an anti-PD-1 antibody) plus low-dose bevacizumab in clinically recurrent GBM or ctDNA-level recurrence of GBM (NCT05502991). Another study will determine the utility of cfDNA from CSF as a prognostic biomarker for GBM (NCT05927610). Some studies reported the application of liquid biopsy to assess tumor response to standard radiation therapy and TMZ in patients with newly diagnosed GBM. Such studies will better define longitudinal genomic alterations in patients with GBM and determine whether ctDNA or cfDNA from plasma is associated with disease recurrence, survival, tumor characteristics, and prognosis (NCT05695976). Some studies are ongoing that will assess the value of ctDNA as a marker of tumor evolution in patients with high-grade gliomas and meningiomas. A total of 90 patients will be enrolled in the study, and plasma samples will be collected at four time points, including before surgery (T0), 1 month after surgery (T1), 1 month after the end of radiotherapy (T2), and at the progression of radiotherapy (T3) (NCT05630664).

Challenges and Limitations

In recent years, increasing interest in the application of liquid biopsy in primary and secondary brain tumors has led to several studies investigating ctDNA in blood and CSF. DNA analysis in liquid biopsy can complement current imaging-based surveillance techniques, which have limited effectiveness, and can provide genomic information for precision medicine while reducing complications from repeated biopsies (Nikanjam et al. 2022). In gliomas, targeted sequencing of ctDNA from CSF may help in auxiliary diagnosis when surgery is not possible, and ctDNA can overcome the

Table 4 Clinical trials on the application of ctDNA in the auxiliary diagnosis and prognosis prediction of glioma

Clinical trail no	Tumor	Diagnosis/Prognosis	Sample type	Sample size	Detection methods	Detection index
NCT05934630	Brain tumors	Auxiliary diagnosis	CSF samples	300	Genetic tests	Clonal mutations
NCT04539431	Gliomas	Auxiliary diagnosis	Blood and CSF	220	Real-time PCR	Mutations in ctDNA
NCT05964153	Gliomas	Auxiliary diagnosis	Blood	10	ddPCR	ctDNA mutations
NCT05541042	GBM	Auxiliary diagnosis	Tumor in situ fluid	50	/	ctDNA mutations
NCT05502991	GBM	Prognosis	Liquid within the surgical cavity	60	/	ctDNA mutations
NCT05927610	GBM	Auxiliary diagnosis	CSF	180	/	cfDNA concentration
NCT05695976	GBM	Prognosis	Serum specimens	100	/	cf/ctDNA concentration
NCT05630664	High-grade gliomas	Prognosis	Plasma	90	/	cfDNA concentration

cfDNA cell-free DNA; *ctDNA* circulating tumor DNA; *CSF* cerebrospinal fluid; *GBM* glioblastoma

spatial heterogeneity of gliomas to a certain extent. CSF has been proposed as an alternative medium for ctDNA analysis in case of brain tumors; however, the detection sensitivity remains poor. In addition, CSF sampling via lumbar puncture is an invasive and painful procedure for patients and requires skilled medical personnel, which severely limits its application in research, diagnosis, and repeated sampling. Given that obtaining CSF requires an invasive lumbar puncture, the analysis of ctDNA from plasma is a better option during follow-up. However, detection of ctDNA from plasma of patients with brain tumors remains challenging because of its low concentration, mostly because of the BBB (Carpenter and Bagley 2022). The detection rate of ctDNA in the plasma of glioma patients is usually approximately 10% (Bettegowda et al. 2014). The high frequency of alterations due to clonal hematopoiesis may confound these results. Alternatively, urine may be used for the auxiliary detection of ctDNA in case of brain tumor; however, studies on this are limited. NGS is becoming more and more popular as a technique for detecting glioma ctDNA, but technical improvements are still needed to improve the detection rate of ctDNA in blood CSF. The combination of multiple body fluid samples may elevate the sensitivity of the detection of ctDNA mutation in gliomas. Strategies to improve ctDNA detection in blood and CSF samples of brain tumor patients may be the direction of future research. Besides, the sample size of the previous studies is limited, and large-scale prospective studies should be conducted in the future.

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Declarations

Conflict of Interest The authors declare no competing interests.

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