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Abstract

Noninvasive molecular profiling of tumors using plasma-based next-generation sequencing (NGS) is increasingly used to aid in diagnosis, treatment selection, and disease monitoring in oncology. In patients with glioma, however, the plasma cell-free DNA (cfDNA) tumor fraction, defined as the fractional proportion of circulating tumor-derived DNA (ctDNA) relative to total cfDNA, is especially low, in large part due to the blood-brain barrier. As a result, commercial plasma-based NGS assays, designed to screen for a small number of actionable genomic alterations, are not sensitive enough to guide the management of patients with glioma. As this has been long recognized in neuro-oncology, significant research efforts have been undertaken to improve the sensitivity of plasma ctDNA detection in patients with glioma and to understand the biology and clinical relevance of non-tumor-derived cfDNA, which makes up most of the total cfDNA pool. Here, we review key recent advances in the field of plasma cfDNA analysis in patients with glioma, including (1) the prognostic impact of pre-treatment and on-treatment total plasma cfDNA concentrations, (2) use of tumor-guided sequencing approaches to improve the sensitivity of ctDNA detection in the plasma, and (3) the emergence of plasma cfDNA methylomics for detection and discrimination of glioma from other primary intracranial tumors.

Keywords

biomarker | cfDNA | DNA methylation | glioblastoma | liquid biopsy

There is an urgent need for minimally invasive biomarkers to diagnose, molecularly characterize, and monitor glioma given the location of this tumor within the central nervous system and infeasibility of repeat tissue biopsies. Moreover, magnetic resonance imaging (MRI), the standard for assessing glioma response and progression, is notoriously limited in its ability to accurately measure the status of the tumor during and after treatment.¹ In an effort to address these issues, the field of liquid biopsy for patients with glioma has grown over the past decade.² Compared to other solid tumors, however, it remains in its infancy in terms of routine clinical applications.

Of the numerous analytes that have been explored for liquid biopsy across solid tumors, plasma cell-free DNA (cfDNA) has been the most widely studied and has achieved the greatest extent of clinical implementation, especially for detection of therapeutically targetable mutations and treatment selection.³ In patients with cancer, the total cfDNA pool in the plasma is composed primarily of cfDNA that is shed from cellular sources other than the tumor, while tumor-derived cfDNA (circulating tumor DNA or ctDNA) comprises only a small fraction of the overall plasma cfDNA burden.⁴ In patients with glioma, this “tumor fraction” of plasma cfDNA is even lower, in large part due to the blood-brain barrier.⁵,⁶ As a result, detection of ctDNA in the plasma of patients with glioma has been extremely challenging to date, rendering the use of commercially available, targeted next-generation sequencing (NGS) panels largely uninformative in the neuro-oncology clinic.⁵,⁶ While cerebrospinal fluid (CSF) represents a richer source of ctDNA in glioma,⁹ serial acquisition of CSF is invasive and not routinely performed in patients with this disease. The following sections will review recent advances in our understanding of plasma cfDNA and ctDNA in glioma, including novel research directions to improve the sensitivity and clinical utility of these analytes for patients with this disease. An overview of cfDNA biomarkers previously evaluated in glioma is provided in Figure 1.
Non-Tumor-Derived Plasma cfDNA

In all patients with cancer, and even more so in patients with glioma, the vast majority of the circulating cfDNA pool consists of non-tumor-derived cfDNA. Although the exact origins and molecular properties of this non-tumor-derived cfDNA remain unknown, most cfDNA is thought to originate from cellular events, including apoptosis, necrosis, and cell secretion. Which of these mechanisms of release is most relevant in a given patient likely depends on a range of biological and environmental factors, including age, gender, body mass index, organ health, and the presence of infections or systemic inflammatory conditions. The main non-tumor tissues of origin for cfDNA, as determined through deconvolution of plasma cfDNA methylation patterns, include white blood cells, which represent the predominant source, as well as erythrocyte progenitors, vascular endothelial cells, hepatocytes, and others. In patients with cancer, there may also be a significant contribution of cfDNA from cells of the tumor microenvironment. A recently emerging literature has suggested numerous functional roles for this cfDNA, including as an immune system regulator in healthy individuals, as well as a promoter of malignant transformation and tumor progression in the setting of cancer.

In patients with glioblastoma, the most common and lethal form of adult glioma, we have previously demonstrated a role for total plasma cfDNA concentration as a prognostic biomarker. When quantified by qPCR for a 115 bp amplicon of the human ALU repeat element, high total plasma cfDNA concentration (defined as greater than the median value of the cohort, 25.2 ng/mL) prior to initial surgical resection of newly diagnosed glioblastoma was strongly and independently associated with inferior progression-free survival (PFS) and overall survival (OS). In addition, patients with increasing cfDNA concentration from baseline to 1-month post-chemoradiotherapy experienced substantially worse PFS and OS compared to patients with decreasing cfDNA concentration between these timepoints. Similar results were described by Fontanilles et al, who showed that an increase in cfDNA concentration from pre-chemoradiotherapy to time of tumor progression was observed in patients with glioblastoma who experienced tumor progression following chemoradiotherapy, while no differences over time were observed for non-progressive patients. Taken together, these results suggest that plasma cfDNA, the bulk of which is non-tumor-derived, has significant pre-treatment and on-treatment prognostic significance in patients with glioblastoma. Additional research is ongoing to determine (a) the predominant cellular origins of plasma cfDNA in glioblastoma, and (b) whether this cfDNA is itself playing a deleterious, pro-tumor functional role or is a proxy for some other pro-tumor process. Regardless of the answers to these questions, future studies are warranted to determine whether plasma cfDNA concentration should be used routinely in practice and clinical trials for enhanced patient risk stratification.

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**Figure 1.** Summary diagram of previously published biomarkers utilizing cell-free circulating DNA (cfDNA) in patients with glioma, including potential clinical applications. cfDNA is released from healthy, inflamed, or cancerous tissue from cells undergoing apoptosis or necrosis. Of the cfDNA that can be extracted from bodily fluid samples, a small proportion is derived from tumor cells, allowing for detection and quantification of tumor genetic aberrations. Analysis of non-tumor-derived cfDNA may also yield biologically relevant information, depending on the clinical setting. Abbreviations: CSF, cerebrospinal fluid; ddPCR, droplet digital polymerase chain reaction; NGS, next-generation sequencing.
Tumor-Derived Plasma cfDNA

Due to the extremely small fraction of cfDNA that is tumor cell-derived (ctDNA) in patients with glioma, currently available plasma-based targeted NGS panels have had low sensitivity and specificity for detecting tumor somatic mutations compared to matched tumor tissue sequencing.5,7 Moreover, distinguishing true somatic mutations from sequencing artifacts and clonal hematopoiesis in patients with glioma has been challenging.8,22 Targeted sequencing of cfDNA from CSF, on the other hand, has demonstrated significantly better performance for detection of potentially therapeutically relevant tumor mutations9 and is covered in detail in a separate article in this journal supplement. Thus, plasma-based detection and sequencing of tumor-derived cfDNA (ctDNA) has so far demonstrated little clinical utility as a noninvasive alternative to tissue acquisition for the identification of tumor somatic mutations in patients with glioma. Approaches for increasing the yield of ctDNA through transient opening of the blood-brain barrier, such as focused ultrasound,23 are currently being evaluated but remain in proof-of-concept stages.

Although currently available plasma-based, multi-gene NGS panels lack proven clinical utility in patients with glioma, there have been numerous recent studies to suggest that serial collection and analysis of ctDNA using more sensitive methods may be useful for monitoring response and progression of glioma over time.24,25 This concept, referred to as “tumor-guided” or “tumor-informed” sequencing,26 assumes that prior knowledge of a patient’s tumor-specific mutations, obtained through previous acquisition and sequencing of the patient’s tumor tissue, may allow increased sensitivity for ctDNA detection in the plasma. While such an approach cannot yield new information about a given tumor’s molecular profile, it could potentially be used as a complement to MRI for monitoring glioma tumor burden during or after treatment. One example of this approach was described by Muralidharan et al, who developed a novel digital droplet PCR (ddPCR) assay for 2 common TERT promoter mutations in patients with glioma (C228T and C250T).24 In five patients known to harbor one of these mutations in their tumors, plasma TERT-mutant allele frequency reflected the clinical course of the disease, with levels decreasing after surgical intervention and therapy and increasing with tumor progression. Similar results have been observed using ddPCR to detect the H3K27M mutation in the plasma of patients with diffuse midline glioma,22,28 although rates of detection have generally been higher in CSF.29 These results are particularly intriguing given the inherent difficulty of obtaining tissue for midline tumors, such as those emerging from the brainstem. Moulere et al also used tumor-guided sequencing in matched tissue and liquid biopsy, including CSF, plasma, and urine, for highly sensitive detection of ctDNA.25 First, whole-exome sequencing was performed on multiple regions of a given patient’s tumor. The mutations detected were then used to generate targeted panels for high-depth sequencing of the liquid biopsy samples. Through this approach, which integrated mutation signal across hundreds of mutations, tumor-derived signal was detected in the majority of CSF (7/8), plasma (10/12), and urine samples (10/16). Although the sample size was small and serial longitudinal analysis was not performed, this study provides proof-of-principle that tumor-informed sequencing can provide high sensitivity for the detection of ctDNA in the plasma and urine of patients with glioma, thus opening the door to future studies of this technology for glioma disease monitoring. Other tumor-guided sequencing approaches are also being evaluated for use in plasma in patients with glioma, including whole-genome sequencing.30

Finally, another promising area of investigation for plasma cfDNA-based liquid biopsy in glioma has been the interrogation of epigenetic signatures. This topic is covered in greater detail in a separate article in this supplement but warrants brief discussion here. Based on previous studies demonstrating that detection of DNA methylation alterations in plasma can reliably identify extracranial cancers with distinct tissues of origin despite low ctDNA abundance, Nassiri et al recently published a ctDNA detection for brain tumors approach called cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq).31 Profiling 447 plasma samples across 9 tumors types, including 60 samples from patient with glioma, the authors trained binomial random forest classifiers using the top 300 differentially methylated regions for gliomas vs. each other tumor class. This technology was able to detect highly specific DNA methylation signatures from patient plasma that could accurately discriminate between common primary intracranial tumors, including IDH-mutant glioma vs. others (AUC 0.82), low-grade glial-neuronal tumors vs. others (AUC 0.93), and IDH wild-type glioma vs others (AUC 0.71). Similar results were obtained by Sabeldo et al using serum-based, genome-wide cfDNA methylation profiling.32 In this study, the authors identified a cfDNA-derived methylation signature that is associated with the presence of glioma and related immune features. The signature was tested in independent discovery and validation cohorts, leading to a score metric (the “glioma-epigenetic liquid biopsy score” or GeLB) that exhibited high sensitivity and specificity for distinguishing patients with or without glioma. In addition, serum cfDNA methylomes could be used to distinguish IDH-mutant from IDH wild-type gliomas.

Summary

Over the past decade, plasma cfDNA-based liquid biopsy has had a profound impact on the clinical management of patients with cancer. Although the benefits of a sensitive, noninvasive assay for detection of tumor genetic material in patients with glioma are obvious, the blood-brain barrier and other factors have severely limited the sensitivity of currently available targeted NGS panels for detection of tumor somatic mutations in glioma patient plasma. The use of CSF as an alternative to plasma for liquid biopsy in patients with glioma has gained traction, as CSF represents a much richer source of ctDNA compared to plasma in this population. However, lumbar puncture is invasive and not typically performed in the routine management of most patients with glioma, rendering serial CSF collection logistically difficult. Thus, we and others have concentrated
on developing novel approaches to plasma-based liquid biopsy for patients with glioma, including (1) focusing on the role of non-tumor-derived plasma cfDNA, which represents the vast majority of cfDNA in patients with glioma and has significant prognostic impact, (2) using tumor-guided sequencing approaches to increase the sensitivity of ctDNA detection, and (3) harnessing the power of methylyomics to distinguish epigenetic signatures of the tumor from those of other tissues. Ultimately, larger, prospective, multicenter studies are needed to truly understand the clinical utility of these and other approaches for improving the management and outcomes of patients with glioma.

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