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ORIGINAL PAPER

Molecular profiling of tumors of the brainstem by sequencing of CSF-derived circulating tumor DNA

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Abstract

Brainstem gliomas are molecularly heterogeneous diseases, many of which are difficult to safely surgically resect and have limited treatment options due to their eloquent location. These constraints pose challenges to biopsy, which limits the use of routine molecular profiling and identification of personalized therapies. Here, we explored the potential of sequencing of circulating tumor DNA (ctDNA) isolated from the cerebrospinal fluid (CSF) of brainstem glioma patients as a less invasive approach for tumor molecular profiling. CSF was obtained from patients either intraoperatively (91.2%, 52/57), from ventricular-peritoneal shunt (3.5%, 2/57), or by lumbar puncture (5.3%, 3/57), all prior to surgical manipulation of the tumor. Deep sequencing of glioma-associated genes was performed on CSF-derived ctDNA and, where available, matched blood and tumor DNA from 57 patients, including nine medullary and 23 diffuse intrinsic pontine gliomas (DIPG). At least one tumor-specific mutation was detected in over 82.5% of CSF ctDNA samples (47/57). In cases with primary tumors harboring at least one mutation, alterations were identified in the CSF ctDNA of 97.3% of cases (36/37). In over 83% (31/37) of cases, all primary tumor alterations were detected in the CSF, and in 91.9% (34/37) of cases, at least half of the alterations were identified. Among ten patients found to have primary tumors negative for mutations, 30% (3/10) had detectable somatic alterations in the CSF. Finally, mutation detection using plasma ctDNA was less sensitive than sequencing the CSF ctDNA (38% vs. 100%, respectively). Our study indicates that deep sequencing of CSF ctDNA is a reliable technique for detecting tumor-specific alterations in brainstem tumors. This approach may offer an alternative approach to stereotactic biopsy for molecular profiling of brainstem tumors.

Keywords Liquid biopsy · ctDNA · DIPG · Brainstem · H3K27M mutation

Introduction

Brainstem gliomas are heterogeneous diseases, many of which are difficult to safely surgically resect due to their location in highly eloquent regions of the brain. In

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particular, those tumors affecting the pons, known as diffuse intrinsic pontine gliomas (DIPG), which are more frequent in children, have a dismal survival of less than 1 year [18]. Despite numerous clinical trials, chemotherapy has proven to be ineffective in prospective studies, while radiation therapy, the standard of care, only prolongs survival by a few months [11, 13, 24].

In an effort to better understand the pathogenesis of human brainstem gliomas and to identify new therapeutic targets, recent collaborative efforts have leveraged the latest next-generation sequencing technologies on tissues obtained by stereotactic biopsy or post-mortem for comprehensive molecular characterization. These studies have identified the major genetic alterations and potential therapeutic targets of brainstem gliomas in adult and pediatric populations, including frequent alterations in *H3F3A, HIST1H3B/C, ACVR1, PPM1D, IDH1, TP53,* and *ATRX* [2, 10, 23, 33, 34, 38–40].

Based on these findings, brainstem gliomas are made up of multiple genetic subtypes and are distinct from supratentorial tumors [2, 4, 10, 31, 33, 34, 39, 40]. These results highlight the need for molecularly guided treatment and rational clinical trial design to ensure personalized treatment for patients (Suppl. Table 1, Online Resource 1). Stereotactic biopsy is becoming increasingly accepted as a safe approach for tumor profiling. However, traditional biopsies still pose some risk to patients, in particular in eloquent regions of the brain, and these approaches do not provide opportunities for dynamic monitoring [20, 30]. Reports of intratumoral heterogeneity in brainstem tumors such as DIPG make adequate sampling for effective therapy selection challenging [3, 14, 26].

Liquid biopsy, or sampling of tumor-derived DNA released into the circulation, offers an opportunity to profile tumors less invasively, while also enabling dynamic disease monitoring and capturing disease heterogeneity [1, 7]. Although plasma is a common source for circulating tumor DNA (ctDNA), brain tumors often lack detectable plasma ctDNA due to the blood brain barrier. However, for brain tumors, sampling the cerebrospinal fluid (CSF) has proven to be an adequate source for ctDNA [6, 25, 29, 37]. For brainstem gliomas, due to their anatomic location, the potential risks of stereotactic biopsy, liquid biopsy approaches may offer an alternative for obtaining critical genetic information for these tumors and also may enable tracking of tumor dynamics using molecular endpoints throughout a patient's clinical course.

Here, we investigated the use of deep sequencing of ctDNA from the CSF of brainstem glioma patients to identify tumor-specific genetic alterations. Our results indicate that CSF ctDNA is a potent resource to genetically characterize and identify potential therapeutic targets for these heterogeneous and difficult to sample tumors.

Methods

Ethics statement

This research was approved by the Institutional Review Board (IRB) and Ethics Committee of Beijing Tiantan Hospital (Beijing, China), which has full accreditation of the Association for the Accreditation of Human Research Protection Program (AAHPP). All methods were carried out in accordance with the approved guidelines. Written informed consent was obtained from all patients.

Patients and sample collection

Patients with primary tumor in the brainstem or fourth ventricle ($n = 57$) were enrolled in this retrospective study between August 2015 and September 2017 in the Department of Neurosurgery at Beijing Tiantan Hospital. The median age at diagnosis was 12.0 *y* (range 3.7 *y* to 56.0 *y*). All patients underwent MRI scans. CSF (5–10 ml) was collected at the time of surgery or ventricular-peritoneal shunt prior to any surgical manipulation of the tumor in 54 patients and from preoperative lumbar puncture in 3 patients (Fig. 1a–d). Fresh tumor tissue was obtained from surgical resection or open biopsy for 47 patients in the study and confirmed by a neuropathologist to contain greater than 70% tumor cells by H&E staining. Additionally, blood samples for germline DNA controls from each patient were obtained.

Tumor volume calculation on MRI scans

A conventional MRI scan was performed on all patients preoperatively. Tumor volumes were determined through manual segmentation of the corresponding MRI scans using a 3D slicer (version 4.8.0) [8]. Contrast-enhanced T1WI and T2WI/T2Flair images were used as the primary reference for determining tumor boundaries. When patchy enhancement was observed, the hyperintensity on the T2WI/T2Flair images was used to delineate the tumor boundaries. This process was performed by a single neurosurgeon. The senior author then independently verified all results.

Extraction of genomic DNA and cell-free DNA

EDTA tubes containing whole blood (5 ml) samples or CSF (5–10 ml) were centrifuged for 10 min at 1900 g, and the supernatants from these samples were further centrifuged for 10 min at 16,000 g. Samples were then collected and stored at − 80 °C until extraction. Genomic DNA was extracted from fresh tumor tissue using the QIAamp DNA Tissue & Blood Kit (Qiagen; Germantown, MD, USA) [12, 19]. Cell-free

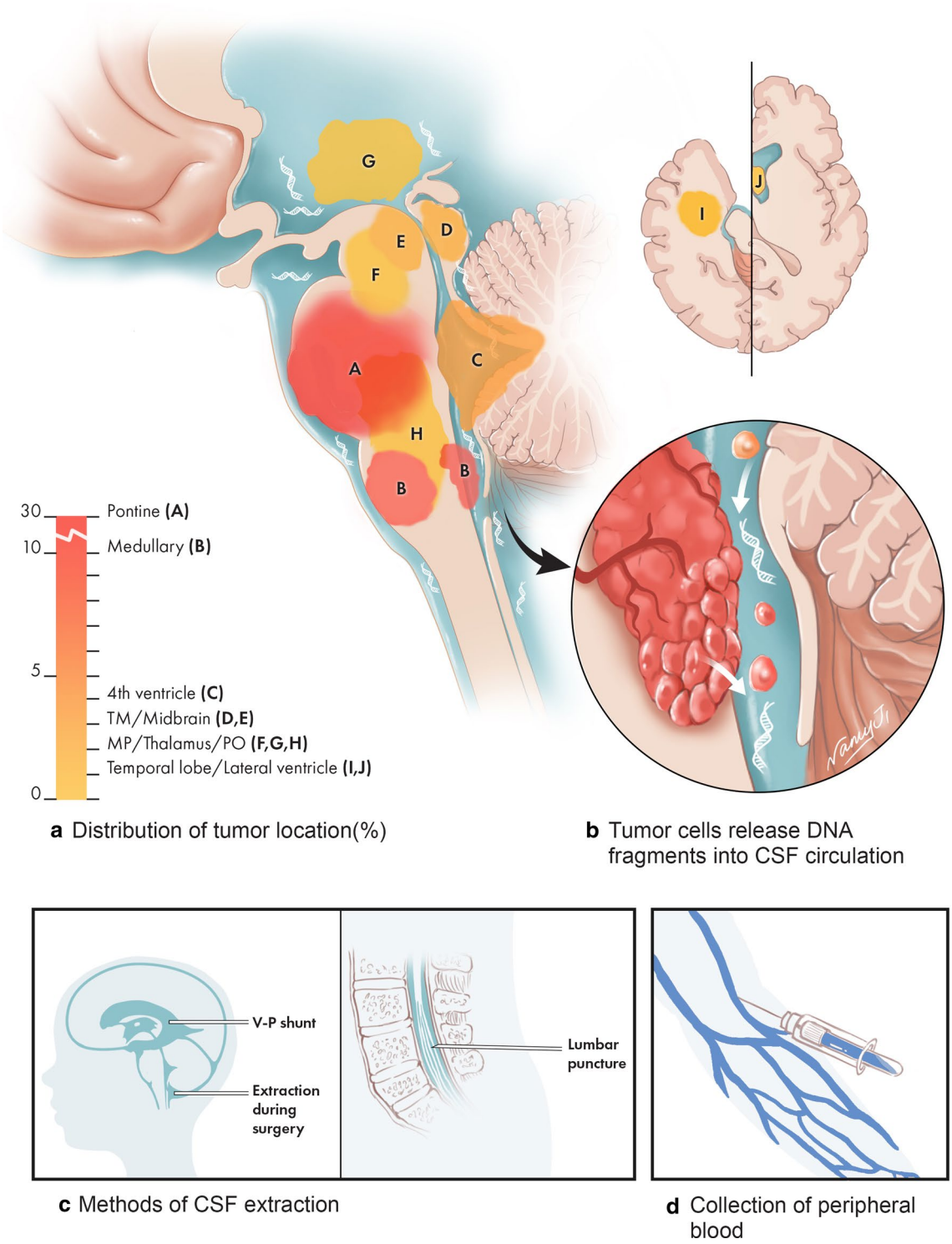

Fig. 1 The anatomic distribution of brainstem tumors that underwent CSF-derived ctDNA sequencing and methods used to collect samples. **a** The majority of patient tumors in this study are diffuse intrinsic pontine glioma (DIPG), or medullary tumors, while the remaining are other brainstem tumors also located in eloquent regions of the brain. Due to their anatomic locations, procedures such as surgical resection and stereotactic biopsy are challenging and may harbor risks for patients. **b** Circulating tumor DNA is shed into the CSF. **c** CSF-derived circulating tumor DNA was isolated in our study by either VP shunt, during surgery, or by lumbar puncture. **d** For select patients, plasma-derived circulating tumor DNA was isolated by peripheral blood draw

DNA was extracted from plasma (3 ml) and CSF using the MagMAX™ CellFree DNA Isolation Kit (ThermoFisher Scientific; Waltham, MA, USA). Finally, all isolated DNAs were quantified with the Qubit 2.0 Fluorometer using the Qubit dsDNA HS Assay kit (Life Technologies; Carlsbad, CA, USA) according to the recommended protocol.

NGS library preparation and sequencing data analysis

Genomic DNA was sheared into 150–200 bp fragments with the Covaris M220 Focused-ultrasonicator™ Instrument (Covaris; Woburn, MA, USA). Fragmented DNA and ctDNA libraries were constructed with the KAPA HTP Library Preparation Kit (Illumina platforms; KAPA Biosystems; Wilmington, MA, USA) following the manufacturer's instructions [5, 17]. DNA libraries were captured with a designed brain tumor panel of 68 genes (GenetronHealth; Beijing, China) that included major brain tumor related genes. The captured samples were subjected to Illumina HiSeq X-Ten for paired end sequencing.

Sequencing reads from the HiSeq X-Ten platform were demultiplexed allowing zero mismatches in barcodes, and the read quality statistics were calculated by FastQC. Sequence adapters and low-quality regions were removed with Trimmomatic (v0.36), and then mapped to the hg19 reference genome with BWA (v0.7.10). PCR duplicates were marked using Picard. Local realignment was run using GATK. Pileup files that were converted from bam files were generated for the genomic regions targeted by exome enrichment. Using the pileup file as input, SNV or indel was called with SAMtools (v0.1.1722) and Pindel; structural variation was detected with Crest. The criteria we adopted for retaining a mutation in CSF and plasma ctDNA were that it had an allele fraction of $\geq 0.1\%$ and a total of ≥ 4 reads. Additionally, known recurrent loci were manually reviewed using Integrative Genomics Viewer (IGV v2.3.34) in the target tissue as compared to the normal blood DNA. All mutations were annotated for genes and function as well as repeated genomic regions using ANNOVAR, Oncotator and Vep. The dbNSFP and the Exome Aggregation Consortium (ExAC) database were used to filter out either the benign mutations with pp2_hdiv score < 0.452 or polymorphic nonsynonymous mutations sites. Finally, all mutations were annotated for genes using ANNOVAR, Oncotator and Vep.

Results

Clinical and pathological features affecting the detection of CSF ctDNA

We isolated CSF ctDNA from 57 patients diagnosed with primary CNS tumors of the brainstem (Suppl. Table 2, Online Resource 2). The median age was 12.0 years old (range 3.7–56 years old) and tumors were classified as WHO grades I–IV. The majority of tumors were located in the pons ($n = 30$) and medulla ($n = 9$), while the remaining tumors were located in the fourth ventricle ($n = 4$), the thalamus ($n = 2$), or had intermediate locations between two regions of the brainstem (Fig. 1). The median concentration of cell-free DNA fragments from the 57 patients was 3.2 ng/mL for CSF, which was lower than the median concentration of 8.4 ng/mL for plasma. All patients in the study had detectable cell-free DNA fragments in their CSF.

We investigated the relationship between clinical features and the detectability of tumor-specific genetic alterations in CSF ctDNA, (Suppl. Table 3, Online Resource 3; Suppl. Figure 1, Online Resource 4), which were defined as alterations identified both in tumor sample and CSF or new compared to the germline DNA controls, in particular for cases without available tumor tissue. The results demonstrated that there were significant differences in tumor grade ($p = 0.001$), location abutting the CSF space ($p = 0.028$), and symptom duration ($p = 0.05$) between samples with detectable tumor-specific alterations in the CSF versus those with undetected alterations. Additionally, samples with detectable tumor-specific alterations generally also had increased amounts of cell-free DNA fragments in the CSF ($p = 0.022$). Other features such as tumor location in the brainstem, sex and age did not significantly vary between both groups, while CSF-detectable alterations trended towards being present in larger tumors ($p = 0.097$), although tumor alterations were still successfully identified in the CSF in patients with tumor as small as 0.95 cm^3. Although tumor location was not a significant variable affecting the detectability of tumor-specific alterations in CSF ctDNA, the two tumors (one pilocytic astrocytoma and one glioblastoma) located in regions away from CSF reservoirs had undetectable tumor-specific alterations in the cell-free DNA fragments from the CSF. On univariate logistic regression analysis, tumor grade, in particular lower grades (I, $p = 0.001$; I and II, $p = 0.016$) vs. higher grades had significant effects on the detectability of tumor-specific alterations in the CSF (Suppl. Table 4, Online Resource 5).

Tumor-specific alterations detected in CSF-derived ctDNA

We used a panel that captures the coding regions of the 68 most frequently mutated genes in brain tumors (Suppl. Table 5, Online Resource 6) and performed next generation sequencing on CSF cell-free circulating DNA isolated from 57 patients (Suppl. Table 6, Online Resource 7). We sequenced this CSF cell-free circulating DNA to an average depth of 1581× (range 102–1864×). The mutant allele fraction (MAF) of variants detected was 12.1% (range 0.15–100%). At least

one tumor-specific mutation among 68 brain tumor-associated genes was detected in over 82.5% of patients (47/57) (Fig. 2). The average number of different mutations identified in the CSF ctDNA was 2.07 (range 0–5). The most frequently altered genes were *H3F3A* (47.7%), *TP53* (43.86%), *ATRX* (12.28%), *PDGFRA* (10.53%), *FAT1* (8.77%), *HIST1H3B* (8.77%), *PPM1D* (8.77%), *IDH1* (7.02%), *NF1* (7.02%), *PIK3CA* (7.02%) and *ACVR1* (7.02%). The average MAF for the most frequently mutated genes in the CSF were the following: *H3F3A* (26.18%), *TP53* (20.81%), *ATRX* (27.68%),

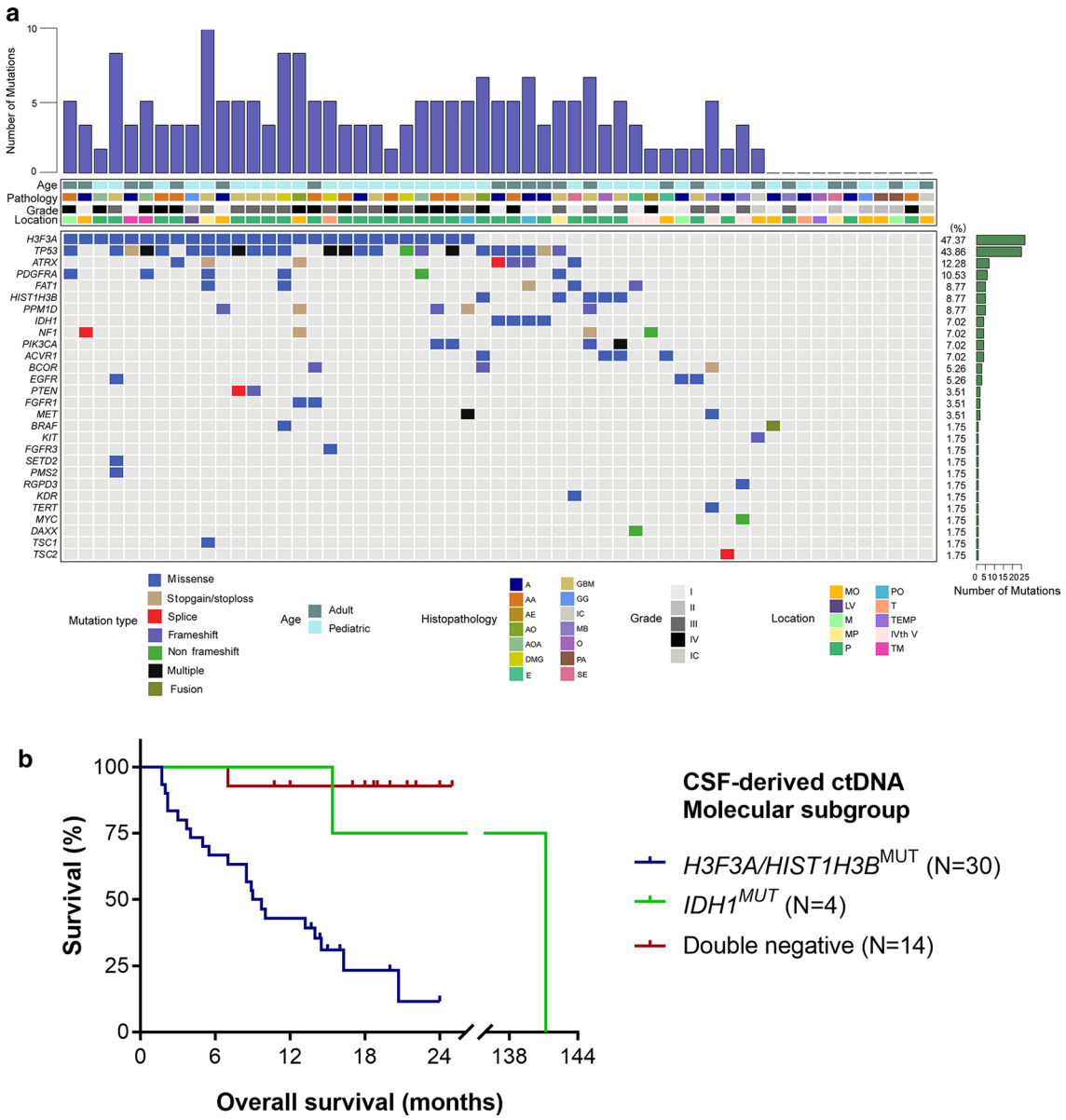

Fig. 2 Mutational characteristics for alterations detected by deep sequencing of the cerebrospinal fluid-derived cell-free DNA. **a** CSF ctDNA was successfully isolated from patients with brainstem tumors ($N=57$). We used a panel-based next generation sequencing approach to identify recurrent tumor-specific alterations in the majority of CSF ctDNA samples. The most frequently mutated genes included *H3F3A, TP53, ATRX* and *PDGFRA*. Tumors were located in the following locations: *MO* medullary oblongata, *P* pontine, *TM* thalamus and midbrain, *M* midbrain, *MP* midbrain and pontine, *PO* pontine and medullary oblongata, *TEMP* temporal lobe, *LV* lateral ventricle, *T* thalamus, *IVth V* the fourth ventricle, *IC* inconclusive. The following histologies were included in our study: *A* astrocytoma, *AA* anaplastic astrocytoma, *DMG* diffuse midline glioma, *GBM* glioblastoma, *AOA* anaplastic oligoastrocytoma, *PA* pilocytic astrocytoma, *GG* ganglioglioma, *E* ependymoma, *AO* anaplastic oligodendroglioma, *SE* subependymoma, *O* oligodendroglioma, *AE* anaplastic ependymoma, *MB* medulloblastoma. **b** Kaplan–Meier estimates of overall survival in brainstem and thalamic tumors based on molecular subgroups from CSF-derived ctDNA. The *H3F3A*/*HIST1H3B* mutant subgroup has a significantly worse survival of 9.35 months as compared to the *IDH1* mutant group (141.2 months, $p=0.0318$) and the double negative (*H3F3A/HIST1H3B* and *IDH1* wildtype) group (only one death in this subgroup, undefined median OS, $p<0.0001$)

PDGFRA (20.76%), *FAT1* (19.69%), *HIST1H3B* (12.54%), *PPM1D* (22.01%), *IDH1* (1.67%), *NF1* (3.50%), *PIK3CA* (22.50%) and *ACVR1* (13.25%).

For patients with tumors of the brainstem and thalamus, we examined the survival trends in overall survival based on the most frequent genomic alterations detected in the CSF ctDNA, namely those in the histone H3.3 and H3.1 genes (*H3F3A* or *HIST1H3B*), *IDH1* mutation, and tumors wildtype for both markers ("double negative"). The patients with detectable CSF-derived ctDNA alterations in H3.3 and H3.1 exhibited significantly worse survival of 9.35 months as compared to the *IDH1*-mutant group (141.2 months, $p = 0.0318$) and the double negative group (only one death in this subgroup, undefined median OS, $p < 0.0001$, Fig. 2b). The two-year survival of the H3-mutant group was only 11.6%, as compared to the *IDH1*-mutant (75%) and double negative (92.9%) groups.

The sensitivity and specificity of CSF liquid biopsy

Matched DNA from the primary tumor biopsy and germline DNA from the patient's blood (reference DNA) were available for 47 cases and were sequenced to determine the specificity and sensitivity of sequencing CSF ctDNA (Fig. 3a). In 97.3% of cases (36/37) with detectable mutations in the primary tumor, matched tumor-specific alterations were identified in the CSF ctDNA. In 83.8% (31/37) of cases, all primary tumor alterations were detected in the CSF, and in 91.9% (34/37) of cases, at least half of the alterations were identified in the CSF (Suppl. Table 2, Online Resource 2; Fig. 3b, c).

We also found that among cases in which alterations were undetected in the primary tumor DNA, mutations were readily detected in the CSF-derived ctDNA in 30% (3/10) of these cases (6.4% of entire cohort, Suppl. Table 2, Online Resource 2; Fig. 3d). The alterations exclusively detected in the CSF ctDNA were mutations in *NF1* (p.Ala2035_Ala2037del), *KIT* (p.Gln775ArgfsTer39), and *EGFR* (p.Ser768Ile). We also found that among primary tumors sequenced that had detectable mutations, 10.6% of these tumors had additional somatic alterations detected exclusively in the CSF-derived ctDNA, not originally identified in the primary tumor (Suppl. Table 2, Online Resource 2; Fig. 3d). These additional alterations were *PDGFRA, BRAF, FAT1* in case RD993, *EGFR* in case RE964, *HIST1H3B, NF1* in case RF007, and *TP53* (p.Arg273Cys) in case RF182.

CSF ctDNA offers improved detection of brain tumor mutations compared to plasma

We detected tumor-specific alterations in the CSF ctDNA in the majority of brainstem glioma patients using next-generation sequencing, indicating that CSF was a reliable source for liquid biopsy. However, we sought to examine if the plasma could also be used as a source of ctDNA for less invasive profiling of brainstem tumors. We therefore isolated ctDNA from the plasma of eight patients with matching CSF ctDNA, primary tumor DNA and germline DNA, and performed targeted capture sequencing on these samples. The genetic profiles generated from CSF ctDNA, primary tumor tissue, and plasma were compared (Fig. 4a–c). Tumor-specific mutations were detected in the CSF ctDNA in 100% (8/8) of cases, but in the plasma ctDNA in only 37.5% (3/8) of cases (Fig. 4c). Furthermore, for the four mutations detected in both CSF and plasma, the median MAF was higher in all CSF ctDNA than in plasma ctDNA (Fig. 4d, e).

Discussion

Recent genetic profiling studies of brainstem tumors, in particular DIPG, have identified the most frequent molecular alterations in these tumors and highlighted the existence of multiple distinct molecular subtypes of these tumors. Considering the limited treatment options available, these molecular markers will be critical for personalized therapy selection and patient stratification. However, the significant risks associated with biopsy of these tumors pose challenges to truly harnessing these molecular markers for treating patients.

Here, we have shown, through comparison of mutational analyses from deep sequencing of ctDNA isolated from the CSF with the matched primary tumor samples, that the CSF represents an attractive source for liquid biopsy of tumors located in the eloquent brain areas, such as brainstem and thalamus. The major genetic alterations associated with brainstem tumors were readily detected in the CSF-derived ctDNA and these mutation patterns reflected distinct survival trends consistent with more aggressive *H3F3A/HIST1H3B*-mutant tumors and less aggressive tumors with *IDH1* mutation or the double-negative tumors lacking mutations in these genes. Sampling of the CSF for this analysis may assist in determining the expected clinical course for patients and need for immediate therapeutic intervention. *H3F3A/HIST1H3B*-mutated tumors may be candidates for early intervention with radiotherapy and targeted therapies including epigenetic modifiers, such as HDAC and BET inhibitors, which are currently in clinical trials for DIPG patients [22]. Similar to previous studies, the *IDH1*-mutated brainstem gliomas had co-occurring mutations in *TP53* and *ATRX* and were present in adults with a more favorable prognosis (median OS = 141.2 months) [40]. These tumors resemble their cerebral counterparts in terms of their genetic signature and less aggressive clinical course. *IDH1*-mutated

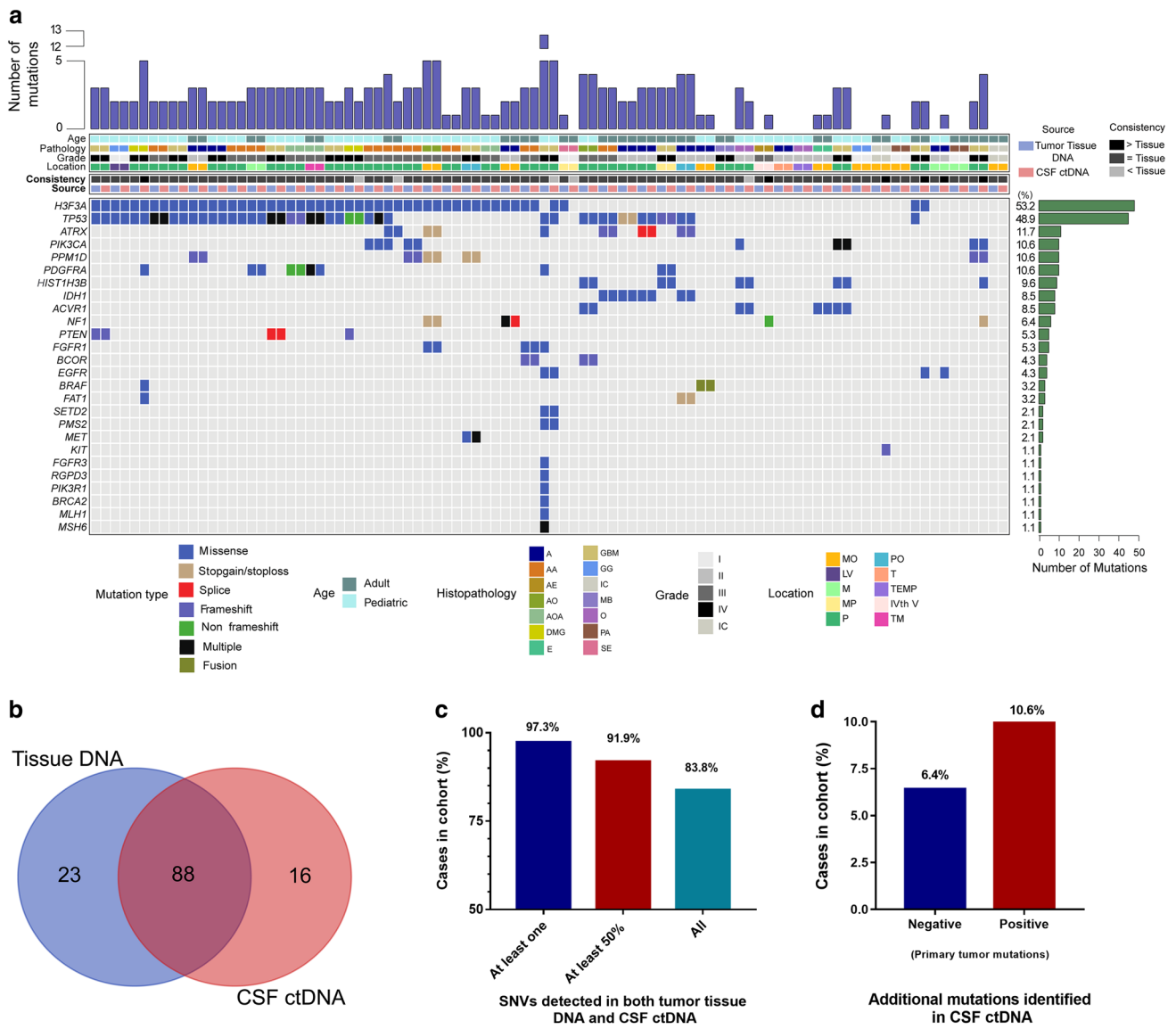

Fig. 3 Sequencing of CSF-derived ctDNA accurately detects tumor-specific alterations found in the primary tumor tissue ($N=47$). **a** Using parallel sequencing of the primary tumor DNA as well as CSF-derived ctDNA, tumor-specific alterations were identified and compared to one another. **b**, **c** The majority of cases show a high level of consistency between the tissue DNA detected variants and those identified in the CSF ctDNA. **d** In 17.0% of cases overall, mutations were detected in the CSF ctDNA that were undetectable in the primary tumor. In half of these cases, the primary tumor had undetectable mutations from the tissue specimen

brainstem gliomas may therefore benefit from treatment such as concomitant radiotherapy (RT) and chemotherapy followed by adjuvant therapy [9, 27]. Patients with double negative tumors (*H3F3A*/*HIST1H3B* and *IDH1* wildtype) demonstrated even better prognosis with only one death during follow-up and unreached median OS. These tumors were largely Grade I and II tumors and in our clinical experience, these tumors are often optimal candidates for surgical resection. For those undergoing total or near-total resection, they may be able to expect long-term tumor free of stable disease on serial observation, with RT and/or chemotherapy reserved for tumor recurrence [21, 28]. Other alterations were identified by sequencing of the CSF-derived ctDNA for which targeted therapies are in development or currently available, including mutations in *PDGFRA, EGFR, IDH1, BRAF, MET* and *PPM1D*. Based on the location of these tumors, the challenges of surgical resection and biopsy, and limited treatment options currently available, molecular profiling from the CSF may offer an alternative approach to identify patients that are candidates for new targeted therapies. Here, we show for the first time that a panel-based approach can accurately be used to sensitively detect multiple tumor-specific mutations from brainstem tumors by sampling the CSF, and that this approach is superior to plasma

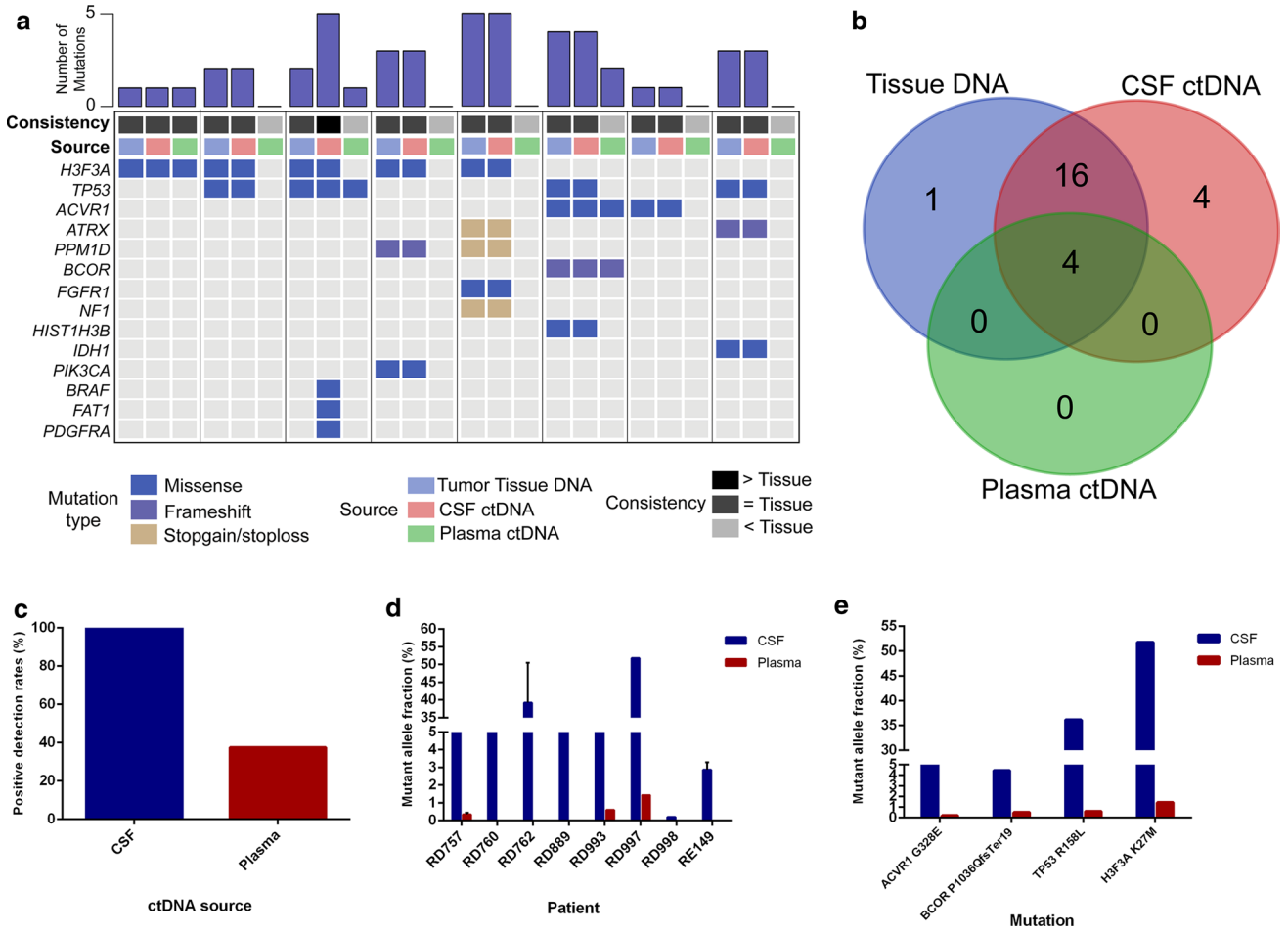

Fig. 4 CSF-ctDNA provides robust detection of tumor-specific alterations for brainstem tumors as compared to plasma ctDNA. **a** For eight cases with available primary tumor tissue DNA, both the CSF ctDNA and plasma ctDNA were isolated and sequenced by NGS for genes frequently mutated in brain tumors. **b** CSF identified the majority of alterations in the primary tumor, but also identified additional alterations undetected in the tumor. **c** Positive detection rates were higher for CSF ctDNA as compared to plasma ctDNA, as were the **d**, **e** mutant allele fractions of the variants identified

ctDNA in these tumor types. In addition, we show that this approach can be accurately used in a variety brainstem tumor types (including DIPG) of both adult and pediatric populations. Prior studies have focused on detection of single variants (such as H3 variants) in DIPG [15].

In several cases, the primary tumor was sequenced with no mutations being identified, but alterations were detectable in the CSF ctDNA. These results may be due to the sampling bias inherent in traditional biopsy or due to tumor heterogeneity known to be present in brainstem tumors such as DIPG, highlighting an additional potential benefit of CSF ctDNA over primary tumor DNA. However, this analysis is difficult to accurately assess in our study as the primary tumor samples were sequenced to lower depth than the CSF and plasma (either WGS to ~ 60× or 68-gene panel sequencing to 500×).

Recent studies of supratentorial brain tumors have also shown a similar pattern in which CSF ctDNA was unable to consistently detect alterations in tumors unless they abutted the CSF space. In our study, both tumors with undetectable CSF ctDNA alterations were non-brainstem tumors that were not directly adjacent to CSF reservoir (one oligodendroglioma of the temporal lobe and one pilocytic astrocytoma of the thalamus) [37]. For this reason, brainstem tumors are being bathed by the CSF and therefore may be ideal targets for CSF ctDNA sequencing. Patients with supratentorial gliomas often exhibit increased intracranial pressure (ICP), which is a strong contraindication for CSF sampling by lumbar puncture. However, patients with brainstem gliomas less commonly demonstrate symptoms of hydrocephalus or elevated intracranial pressure, making them much better candidates for safe use of lumbar puncture for CSF sampling.

We found that ctDNA isolated from the plasma was not a reliable DNA source for the detection of tumor-specific mutations, with the majority of patients having

undetectable mutations in the plasma ctDNA. Additionally, plasma ctDNA-detected mutations exhibited lower overall MAF than from the CSF ctDNA. Thus, direct CSF sampling might be superior for sensitive detection of tumor-specific alterations in brainstem glioma.

Despite our encouraging findings of detectable and consistent mutation profiles in the ctDNA of brainstem glioma patients compared to tumor tissue, there were several cases in our study that lacked any detectable alterations by both approaches. For more comprehensive annotation of these cases, the 68 gene panel could be expanded to encompass additional alterations. Several of these cases with undetectable mutations were Grade I tumors, indicating this liquid biopsy approach may be more appropriate for aggressive tumors. In addition, in our study, the majority of CSF samples was obtained during open biopsy or V-P shunt. Although tumor-specific mutations were detected by sequencing the ctDNA from all the three CSF samples collected by traditional lumbar puncture, the majority of samples in this study were obtained from intraoperative sampling prior to tumor manipulation. Larger studies should build off these promising findings to ensure the utility and sensitivity of lumbar puncture-based CSF ctDNA detection for primary tumor profiling. The incidence of hydrocephalus in pediatric DIPG varies from < 10% to approximately 25% [16, 32, 35, 36]. We believe lumbar puncture as an approach to CSF sampling for ctDNA should be avoided in patients with signs of hydrocephalus. Finally, our results could be expanded to determine if serial sampling of the CSF could be used to monitor tumor dynamics, including tracking minimal residual disease, recurrence, and response to therapy.

In conclusion, our study shows that deep sequencing of CSF-derived ctDNA from patients with tumors of the brainstem can accurately detect tumor-specific mutations. The molecular signature of tumors is becoming increasingly important for designing personalized therapy for cancer patients, ensuring homogeneous treatment populations and advancing clinical management of devastating cancers such as DIPG. This approach can potentially facilitate these goals in brainstem glioma patients and ultimately help to make significant advances in prolonging the lives of affected patients.

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Author contributions LZ, HY, and BHD designed the study. CP, XC, YW, XX, YG, PZ, WW, and YW collected the samples, CP, and LJ performed the experiments and collected the data. CP, LJ, YJ, and LZ analyzed the results. CP, LJ, BHD, HY, and LZ wrote the manuscript. CX, YS, ZW, JZ, and YJ, gave conceptual advice.

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Compliance with ethical standards

Conflict of interest HY is a founder of Genetron Health and receives royalties from Personal Genome Diagnostics (PGDX) and Agios. YJ is a co-founder of and Scientific Advisor for Genetron Health. BHD serves as a scientific consultant for Genetron Health.

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