INTRODUCTION

Timely detection of BRAF V600E mutation and malignant cells by less-invasive methods such as cerebrospinal fluid (CSF) cytology and liquid biopsy can be beneficial in cases of epithelioid glioblastomas, which are characterized by frequent spinal dissemination, recurrent BRAF V600E mutations and are candidates for targeted therapy. Herein, we report a case of disseminating epithelioid glioblastoma harboring BRAF V600E, diagnosed by less-invasive analysis of CSF.

Epithelioid glioblastoma is a rare, aggressive variant of glioblastoma, characterized by frequent dissemination, poor prognosis, and recurrent BRAF V600E mutations. Dramatic response to BRAF and MEK inhibitor treatment, including the present case, has been reported, so screening for BRAF V600E in epithelioid glioblastoma is imperative. We have previously reported reliable detection of the driver mutation MYD88 P265L in circulating tumor DNA (ctDNA) extracted from the cerebrospinal fluid (CSF) of primary central nervous system lymphoma (PCNSL). In the present case, cytopathologic examination and liquid biopsy of CSF were diagnostic for BRAF V600E-mutant epithelioid glioblastoma.

CASE REPORT

A 57-year-old man presented with headaches and dysphasia. A left frontal tumor, relatively well circumscribed, showing subependymal enhancement of the left frontal horn,
was observed on MR images. Total removal of the parenchymal tumor was achieved. Hematoxylin and eosin staining of the tumor revealed the presence of discohesive, round tumor cells with abundant cytoplasm, and laterally positioned nuclei and focal necrosis in a mucinous background (Figure 1A). Thus, the pathological diagnosis was epithelioid glioblastoma. BRAF V600E mutation was detected by both droplet digital PCR (ddPCR) (Figure 1B) and the Sanger method (Figure 1C) in CSF. Variant allele frequency (VAF) determined by ddPCR was 52.1%. During radiation and concomitant temozolomide treatment, the patient became comatose and MR images subsequently taken showed hydrocephalus and diffuse leptomeningeal enhancement. An emergent lumbo-peritoneal shunt was placed, but obstruction of the lumbar side shunt tube was observed after only 3 days, so the shunt was removed, and an external ventricular drainage was placed. Hematoxylin and eosin staining of the obstructed shunt tube revealed aggregation of tumor cells (Figure 1D).

After completion of adjuvant treatment, a ventriculo-peritoneal shunt procedure was performed. However, immediately after shunting, the patient displayed symptoms of paraplegia. Spinal MR images showed thick spinal dissemination and diffuse syringomyelia. Cyto logical analysis of cerebrospinal fluid (CSF) by Papanicolaou staining revealed apparent epithelioid tumor cells with abundant cytoplasm, laterally displaced nuclei and lacking cellular processes (Figure 2A). One mL of CSF was immediately centrifuged at 500 g for 10 min, and the supernatant was stored at −80°C for ctDNA analysis. BRAF V600E mutation from circulating tumor DNA was detected by both ddPCR (Figure 2B) and Sanger sequencing (Figure 2C) and after approval from the institutional review board of Niigata University (#G2018-0008) and obtaining written consent. Detailed description of ddPCR methods has been previously described. VAF was comparable to that of the tumor at 47.5%. The disseminated lesions showed dramatic response to whole spine irradiation and combined BRAF and MEK inhibitor treatment, which has previously been reported in detail.

3 DISCUSSION

In the present case, tumor cells with epithelioid appearance were found by cytological analysis of CSF in an epithelioid glioblastoma patient with spinal dissemination. Leptomeningeal dissemination is observed in a third of these patients, and survival after dissemination is especially dismal. We speculate that epithelioid glioma can easily disseminate because of two reasons. First, these glioma cells are unique in that they lack cytoplasmic processes and are round shaped. This morphological characteristic may help these tumor cells readily spread through the neuraxis. Induction of BRAF V600E mutation in neuroprogenitor cells in Ink4a/Arf knockout mice produced well-demarcated gliomas with growth into subarachnoid and Virchow-Robin perivascular spaces. Secondly, these cells are naturally discohesive and may be able to stay alive and multiply even at the single cell state in CSF. We established the cell line NGT41 from tumor cells taken at autopsy of the present patient. These cells grew as neurospheres from single cells in serum free culture media and had high expression of CD133. Interestingly, epithelioid glioblastoma cells are morphologically similar to melanoma cells, and cultured melanoma cells, which frequently harbor BRAF V600E mutations, are known to have increased expression of stem cell markers including CD133, CD166, and nestin.

Liquid biopsy, usually by detection of circulating tumor cells or circulating tumor DNA (ctDNA), has revolutionized the diagnosis, treatment, and monitoring of cancer. Both methods are promising, but presently, methods to detect ctDNA are more sensitive. We have previously reported reliable detection MYD88 L265P mutation in ctDNA extracted from CSF in primary central nervous system lymphomas using the Maxwell RSC ccfDNA Plasma Kit (RSC; Promega) is feasible. Using the same methods, we were able to detect BRAF V600E in CSF by both Sanger sequencing and ddPCR. ddPCR is 100 times more sensitive than Sanger sequencing, and we found that of 10 (40%) lymphoma cases which were thought to be MYD88 P265L wildtype by Sanger sequencing, 4 (40%) were in fact P265L mutant by ddPCR. However, in cases such as the present one, in which diffuse spinal dissemination is observed, mutations may be detected by Sanger sequencing alone. We have successfully separated ctDNA from cellular DNA by centrifugation at 500 g for 10 min. However, we do not know if the same conditions are sufficient to separate ctDNA from cellular DNA in CSF with gross disseminated of tumor cells. Interestingly, a study detected higher variant allele frequency for BRAF V600E in CSF ctDNA compared to CSF pellet DNA.

Though detection of BRAF V600E is not diagnostic for epithelioid glioblastoma, as it is also found in brain tumors, such as pleomorphic xanthoastrocytoma, gangliogioma, and pediatric low-grade gliomas, it can serve as a rationale for targeted treatment. Some recent papers detect BRAF V600E mutations in CSF of brain tumor patients harboring metastatic melanoma, and medulloblastomas. Li et al. and Ballester et al. serially collect ctDNA in CSF in melanoma patients with brain metastases and leptomeningeal disease; Li et al. show a decrease in mutant ctDNA fraction...
of BRAF V600E during treatment with BRAF inhibitors. Pentsova et al.\(^8\) reported detection of BRAF V600E mutations, as well as other alterations such as NRAS G12R, PTEN del in CSF ctDNA of metastatic melanoma patients using the MSK-IMPACT next-generation sequencing genome panel. Furthermore, Garcia-Romero et al.\(^11\) show a discrepancy in BRAF V600E mutation status between tumor, CSF, plasma, and serum ctDNA in pediatric brain tumors. The present report is the first to successfully detect BRAF V600E from ctDNA in CSF of an epithelioid glioblastoma patient.

Next generation sequencing panels for liquid biopsy such as Guardant360\(^\circledR\) and FoundationOne\(^\circledR\) Liquid CDx are available for use in solid tumor patients, albeit in blood. At least one genetic alteration was found from plasma in 55% of glioblastoma patients by Guardant360\(^\circledR\),\(^12\) but concentrations of ctDNA are known to be in higher in CSF of brain tumor patients compared to plasma.\(^3\) Clinical application of a liquid NGS panel analyzing ctDNA extracted from CSF in brain tumor patients\(^13\) is awaited.

Because of the high risk of and dismal prognosis of dissemination in epithelioid glioblastoma,\(^1,2,14-21\) CSF cytology and post-contrast whole spine MRI should be periodically repeated. Other brain tumors showing leptomeningeal dissemination include glioblastoma, PCNSL, metastatic brain tumors, medulloblastoma, atypical teratoid rhabdoid tumor, and malignant germ cell tumors. Screening for ctDNA can be sequentially performed to monitor for disseminating disease or CNS relapse in addition to CSF cytology and/or tumor markers such as AFP and β-HCG in germ cell tumors. Hotspot (C228T, C250T) TERT promoter mutations for glioblastoma and oligodendroglioma, IDH1 R132H for IDH1-mutant gliomas, MYD88 L265P for PCNSL,\(^3\) EGFR mutations for metastatic NCSLC\(^22\) are just some of the possible examples of diagnostic markers for the various brain tumors.

**FIGURE 2** (A) Cytological examination revealed the presence of tumor cells (Papanicolaou ×400) with apparent epithelioid features (inset). BRAF V600E mutation was detected in circulating tumor (ctDNA) extracted from cerebrospinal fluid (CSF) by Sanger sequencing (B) and droplet digital PCR (ddPCR) (C) with comparable variant allele frequency (VAF) to that of the tumor.
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CONFLICT OF INTEREST
None declared.

AUTHOR CONTRIBUTIONS
MN managed the patient, interpreted the findings, reviewed the literature, and drafted the manuscript. YK managed the patient, collected data, and critically reviewed the manuscript. YF collected data. HU and AK pathologically reviewed the cytology and brain tumor samples, respectively. YF supervised the study. All authors have read and approved the final manuscript.

ETHICAL APPROVAL
This study was conducted after approval from the institutional review board of Niigata University (#G2018-0008) and obtaining written consent.

DATA AVAILABILITY STATEMENT
The data presented in this study are available upon reasonable request to the corresponding author.

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