Cytogenetic diagnosis of disseminated epithelioid glioblastoma harboring BRAF V600E mutation

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INTRODUCTION

Epithelioid glioblastoma is a rare, aggressive variant of glioblastoma, characterized by frequent dissemination, poor prognosis and recurrent*BRAF* V600E mutations.[1] Dramatic response to BRAF and MEK inhibitor treatment, including the present case,[2] 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).[3, 4] In the present case, cytopathologic examination and liquid biopsy of CSF was 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.[2]Total removal of the parenchymal tumor was achieved. Hematoxylin 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). 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 three 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.[2]Cytological 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). *BRAF* V600E mutation from circulating tumor DNA was detected by both Sanger sequencing (Figure 2B) and ddPCR (Figure 2C) after approval from the institutional review board of Niigata University (#G2018-0008) and obtaining written consent. 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.[2]

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,[1] 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.[5] 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.[2]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,[1] 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.[6]

Liquid biopsy, usually by detection of circulating tumor cells or circulating tumor DNA (ctDNA), has revolutionized the diagnosis, treatment and monitoring of cancer.[7] 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, Leiden, the Netherlands) is feasible.[3, 4] 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.[4] However, in cases such as the present one, in which diffuse spinal dissemination is observed, mutations may be detected by Sanger sequencing alone.

Though detection of *BRAF* V600E is not diagnostic for epithelioid glioblastoma, as it is also found in brain tumors such as pleomorphic xanthoastrocytoma, ganglioglioma and pediatric low-grade gliomas, it can serve as a rationale for targeted treatment. Next generation sequencing panels for liquid biopsy such as Guardant360^{R} and FoundationOne^{R} Liquid CDx are available for use in solid tumor patients, albeit from blood. At least one genetic alteration was found from plasma in 55% of glioblastoma patients by Guardant360^{R},[8] but concentrations of ctDNA is 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,[9] is awaited.

Because of the high risk of dissemination in epithelioid glioblastoma, CSF cytology and post-contrast whole

spine MRI should be periodically repeated. For patients with evidence of dissemination at diagnosis, we propose that craniospinal irradiation should be performed. In a different epithelioid glioblastoma patient showing disseminating disease of the cervical spine at presentation, CSI was performed upfront and lead to long-term control of disseminating disease for more than 2.5 years (Figure S1). Other brain tumors showing leptomeningeal dissemination include glioblastoma, PCNSL, metastatic brain tumors such as metastasis of breast cancer and *EGFR* -mutant non-small cell lung cancer (NCSLC), 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[10] are just some of the possible examples of diagnostic markers for the various brain tumors.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

1. Louis DN, Ohgaki H, Wiestler OD, et al. *WHO classification of tumours of the central nervous system* . IARC; 2016.

2. Kanemaru Y, Natsumeda M, Okada M, et al. Dramatic response of BRAF V600E-mutant epithelioid glioblastoma to combination therapy with BRAF and MEK inhibitor: establishment and xenograft of a cell line to predict clinical efficacy. *Acta Neuropathol Commun* . Jul 25 2019;7(1):119.

3. Watanabe J, Natsumeda M, Kanemaru Y, et al. Comparison of circulating tumor DNA between body fluids in patients with primary central nervous system lymphoma. *Leuk Lymphoma* . Dec 2019;60(14):3587-3589.

4. Watanabe J, Natsumeda M, Okada M, et al. High detection rate of MYD88 mutations in cerebrospinal fluid from patients with central nervous system lymphomas. *JCO Precis Oncol* . 2019;3:1-13.

5. Robinson JP, VanBrocklin MW, Guilbeault AR, Signorelli DL, Brandner S, Holmen SL. Activated BRAF induces gliomas in mice when combined with Ink4a/Arf loss or Akt activation. *Oncogene* . Jan 21 2010;29(3):335-44.

6. Klein WM, Wu BP, Zhao S, Wu H, Klein-Szanto AJ, Tahan SR. Increased expression of stem cell markers in malignant melanoma. *Mod Pathol* . Jan 2007;20(1):102-7.

7. Ignatiadis M, Lee M, Jeffrey SS. Circulating Tumor Cells and Circulating Tumor DNA: Challenges and Opportunities on the Path to Clinical Utility. *Clin Cancer Res* . Nov 1 2015;21(21):4786-800.

8. Piccioni DE, Achrol AS, Kierdrowski LA, et al. Analysis of cell-free circulating tumor DNA in 419 patients with glioblastoma and other primary brain tumors. *CNS Oncol* . 2019;8(CNS34)

9. Miller AM, Shah RH, Pentsova EI, et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. *Nature* . Jan 2019;565(7741):654-658.

10. Shingyoji M, Kageyama H, Sakaida T, et al. Detection of epithelial growth factor receptor mutations in cerebrospinal fluid from patients with lung adenocarcinoma suspected of neoplastic meningitis. *J Thorac Oncol* . 2011;6(7):1215-1220.