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ORIGINAL ARTICLE

Predicting BRAF V600E mutation in glioblastoma: utility of radiographic features

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Abstract
Detection of BRAF V600E mutation in glioblastomas (GBMs) is important because of potential therapeutic implications. Still, the relative paucity of these mutations makes molecular detection in all GBMs controversial. In the present study, we analyzed clinical, radiographic and pathologic features of 12 BRAF V600E-mutant GBMs and 12 matched controls from 2 institutions. We found that a majority of BRAF V600E-mutant GBMs displayed a combination of well-circumscribed lesions, large cystic components with thin walls and solid cortical component on MRI, but with some overlap with matched *BRAF* wildtype controls ($p=0.069$). BRAF V600E-mutant GBMs were also apt to gross total resection (83% vs 17%, $p=0.016$) and morphologically displayed epithelioid features (83% vs 0%, $p<0.0001$). Identification of these clinical, radiographic, and pathologic characteristics should prompt testing for BRAF V600E in IDH-wildtype GBM.

Keywords BRAF V600E · Glioblastoma · Radigraphic features · Pathologic features

Introduction

Advances in our understanding of glioblastoma (GBM) genomics along with the increasing availability of molecular diagnostics in the clinic have fueled great interest in identifying targetable mutations for the treatment of GBM. One such targetable mutation is the BRAF V600E mutation, which is present in 3–15% of pediatric and adult GBMs, respectively [1–5]. The BRAF V600E mutation may be a positive prognostic indicator for adults with GBM. Studies have demonstrated that across multiple age groups, patients with BRAF V600E mutation exhibit better overall survival compared to patients with wildtype *BRAF* [6, 7]. Alternatively, epithelioid GBMs, which frequently harbor BRAF V600E mutations and can display leptomeningeal disease, are known to have dismal prognoses [8, 9]. Identification of the BRAF V600E mutation also has important therapeutic implications. BRAF or BRAF/MEK inhibitors effect a response in a significant subset of patients with BRAF V600E mutant recurrent GBM [10–12]. RAF-targeted therapy is currently being evaluated in clinical trials alone or in combination with radiation at diagnosis or recurrence in low and high-grade BRAF-mutant glioma. For these reasons, identifying BRAF mutations in patients with GBM is of great clinical value.

BRAF V600E alterations can be identified by immunostaining, Sanger sequencing or next-generation sequencing (NGS); however, availability of these modalities is not universal given the cost of NGS and the process of clinical laboratory improvement amendments (CLIA) certification [13, 14]. An alternative approach would be to utilize findings from routine clinical procedures, such as MRI and surgical pathology examination, to

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identify a subset of GBM patients for whom *BRAF* molecular testing may be warranted. Multiple recent small case series and case reports have identified unique radiographic features of BRAF V600E mutant GBM on pre-surgical MRI [15, 16]. In this manuscript, we sought to compare radiographic and histopathologic features of BRAF V600E mutant GBM and matched controls at presentation, to identify the features associated with BRAF V600E mutation status.

Methods

Case identification

This study was retrospectively conducted after approval of institutional review boards at Niigata University (IRB2020-0491) and Johns Hopkins University (IRB00243637). Cases with histologically confirmed GBM, known *BRAF* status through molecular testing, and available pre-operative MRIs, were identified using the pathology specimen database at Johns Hopkins Hospital and through the study investigators' personal clinical panel. Twelve cases with BRAF V600E mutant GBM and 12 matched controls with *BRAF* wildtype GBM were identified. Controls were matched for age, sex, tumor location, and IDH status. Clinical data including patient demographics, tumor location, tumor histopathology, molecular characteristics, extent of surgical resection, and overall survival, were extracted from the electronic medical record.

Imaging features

T2 weighted FLAIR and T1 post-gadolinium contrast enhanced MRIs were obtained for each patient from the pre-operative scan at time of diagnosis. Radiographic characteristics of the 24 GBM cases (12 BRAF V600E-mutant, 12 *BRAF*-wildtype cases) were scored by an experienced neuro-radiologist (K.O.). Well-circumscribed borders, presence of large cysts [17] with thin walls or necrosis, presence of solid portions, homogeneous or heterogeneous or slight enhancement, and cortical involvement were assessed on post-contrast MR images; peritumoral edema and infiltration were assessed on fluid attenuated inversion recovery (FLAIR) images, with infiltrative FLAIR-hyperintense disease extending to the brain parenchyma and ventricles or skip lesions were considered to be diffuse lesions. Large cysts were defined as cyst size $\geq 50\%$ of tumor [17]. Distinguishing between cysts and necrosis was often difficult; the presence of thin walls was diagnostic for cysts.

Pathologic features

All tumors were given the diagnosis of GBM at time of diagnosis by neuro-pathologists at each institution. Cases in which tissue blocks were available were re-reviewed to confirm diagnosis at Niigata University (R.G. and A.K.) and Johns Hopkins (F.R.). Histopathologic features and molecular features including epithelioid and PXA-like features, BRAF V600E mutation, and *IDH1/2* mutation, were recorded. *MGMT* promoter methylation status was also evaluated.

Statistical analysis

Differences between BRAF V600E-mutant and matched controls were assessed using paired *t*-test or one-way ANOVA as appropriate. Test for associations between different parameters were carried out by χ^2 test for 2×2 contingency tables using STATA/IC15 (StataCorp LP) and Prism 9 software (GraphPad Software). $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

BRAF V600E mutations were identified in 12 patients with newly diagnosed GBM. Twelve matched control (*BRAF* wildtype) patients were selected, and demographics were summarized in Table 1. Median age at diagnosis was 59 years (range 20–79) and 8 out of 12 (67%) were female

Table 1 Patient demographics of BRAF V600E-mutant and wildtype glioblastoma

Characteristic	BRAF V600E ($n=12$)	Control ($n=12$)	p value
Median age at diagnosis (yrs)	59 (20–79)	58.5 (26–73)	
Females	8 (67%)	6 (50%)	0.42
Overall survival (months)	33 ± 39	20 ± 16	0.31
Extent of resection			0.016*
Biopsy	1 (8%)	2 (17%)	
Subtotal resection	1 (8%)	8 (67%)	
Gross total resection	10 (83%)	2 (17%)	
Epithelioid features	10 (83%)	0 (0%)	< 0.0001*
Tumor grade			
IV	12 (100%)	12 (100%)	
BRAF mutation	12 (100%)	0 (0%)	
IDH status			0.83
Wildtype	11 (92%)	12 (100%)	
Mutant	0 (0%)	0 (0%)	
Unknown	1 (8%)	0 (0%)	
MGMT status			0.19
Methylated	2 (17%)	5 (42%)	
Unmethylated	10 (83%)	7 (58%)	

patients in the BRAF V600E-mutant group. Interestingly, gross total removal was achieved in a significantly higher percentage (83%) of patients compared to control (17%) ($p = 0.016$) and epithelioid features were observed in 10 out of 12 (83%) of cases, compared to 0% in the control group ($p < 0.0001$). Median overall survival was longer in the BRAF V600E group compared to control (33 ± 39 months vs 20 ± 16 months), although not significant ($p = 0.31$). IDH status ($p = 0.83$) and *MGMT* promoter methylation status ($p = 0.19$) were not significantly different between the 2 groups.

Radiographic features

Radiographic features thought to be associated with BRAF V600E mutation included well-circumscribed borders, presence of large cysts with thin walls, and cortical involvement (Fig. 1A, B) as opposed to classical GBM with less-circumscribed borders, presence of ring enhancement on post-contrast MR images reflecting necrosis, and predominantly white matter localization (Fig. 1C, D). We found that indeed BRAF V600E-mutant GBMs often had well-circumscribed borders (92%), presence of large cysts with thin walls (50%), and cortical involvement (75%), but we also found that matched controls lacking BRAF V600E mutation also frequently displayed well-circumscribed borders (67%), presence of large cysts (25%) and cortical involvement (50%), suggesting that GBMs in the younger age groups have considerable overlap in radiographic appearance, irrespective of BRAF V600E status. All 3 characteristics were simultaneously observed in 6 out of 12 cases (50%) in the BRAF V600E-mutant group, compared to only 1 out of 12 (8%) cases in the control group ($p = 0.069$, Table 2). Apparent dural invasion of tumor (dural tail) was observed in 2 out 12

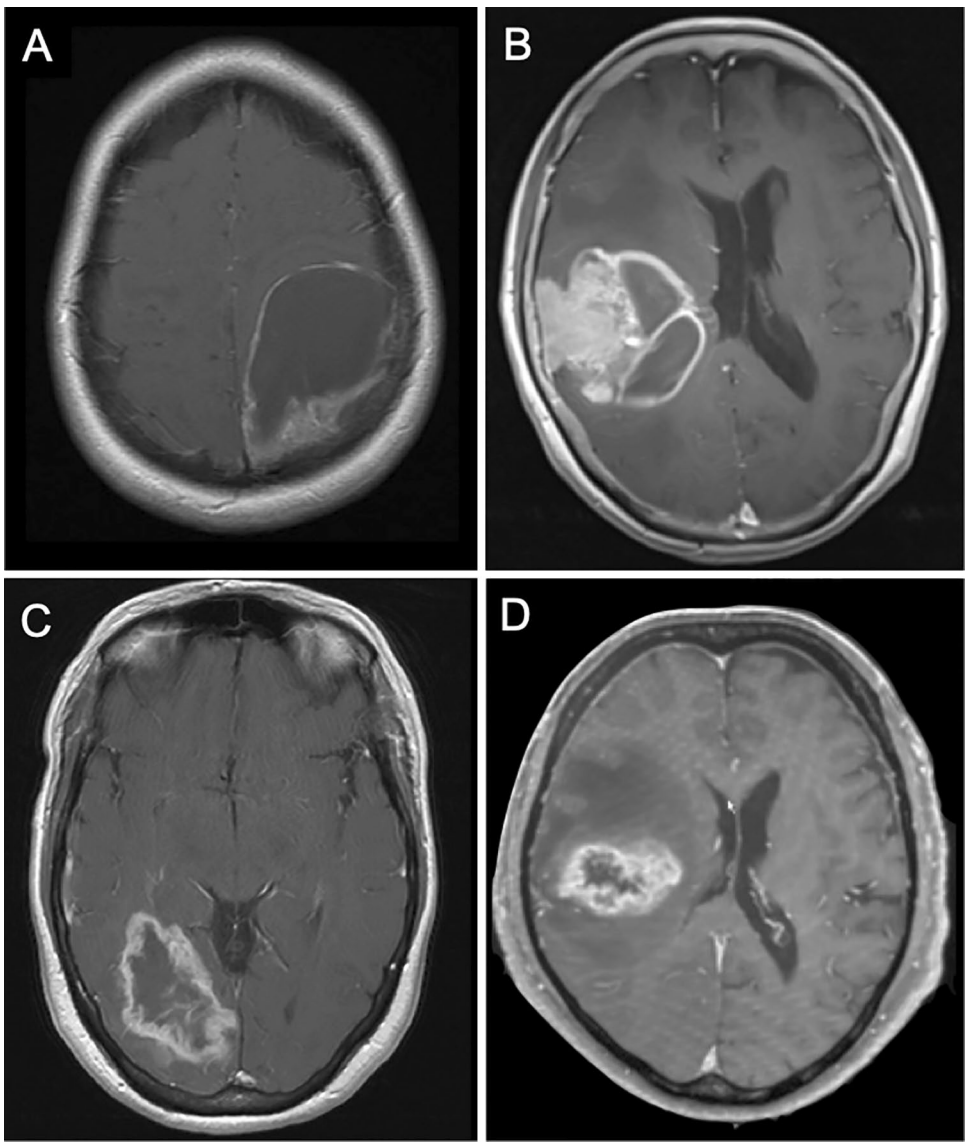

Fig. 1 Radiographic features of select cases. Post-contrast MR images of BRAF V600E-mutant glioblastoma from **A** 20-year-old and **B** 70-year-old female showing well-circumscribed borders, cortical involvement, and large, cystic components with thin walls. Post-contrast MR images of control *BRAF* wildtype glioblastoma from **C** 66-year-old female showing necrosis and **D** 70-year-old male showing indistinct borders

Table 2 Radiographic features of BRAF V600E-mutant and wildtype glioblastoma

	BRAF V600E ($n=12$)	Control ($n=12$)	p value
Borders			
Well circumscribed (A)	11 (92%)	8 (67%)	0.32
Cysts/Necrosis			
Large cysts (B)	6 (50%)	3 (25%)	0.40
Small cysts	1 (8%)	2 (17%)	>0.99
Necrosis	3 (25%)	6 (50%)	0.40
Solid	2 (17%)	1 (8%)	>0.99
Multi-cyst	2/7 (29%)	2/5 (40%)	>0.99
Single cyst	5/7 (71%)	3/5 (20%)	
Superficial location			
Cortical involvement (C)	9 (75%)	6 (50%)	0.40
Dural invasion	2 (17%)	0 (0%)	0.48
(A)+(B)+(C)	6 (50%)	1 (8%)	0.069
Enhancement			
Homogeneous	3 (25%)	1 (8%)	0.59
Heterogeneous	7 (58%)	10 (83%)	0.37
Slight	2 (17%)	1 (8%)	>0.99
Peritumoral edema			
Extensive	5 (42%)	4 (33%)	>0.99
Localized	7 (58%)	8 (67%)	
Infiltration			
Diffuse	3 (25%)	7 (58%)	0.21
Localized	9 (75%)	5 (42%)	
Tumor side			
Right	8 (67%)	4 (33%)	0.22
Left	4 (33%)	8 (67%)	
Tumor location			
Frontal	2 (17%)	2 (17%)	
Fronto-temporal	0 (0%)	1 (8%)	
Fronto-parietal	0 (0%)	1 (8%)	
Temporal	4 (33%)	5 (42%)	
Temporo-parietal	0 (0%)	1 (8%)	
Temporo-parieto-occipital	1 (8%)	1 (8%)	

(17%) cases in the BRAF V600E-mutant group as opposed to 0 (0%) in the control ($p=0.48$). In terms of location, only 2 of 12 (17%) BRAF V600E-mutant GBMs involved the frontal lobe while 5 of 12 (42%) involving the temporal lobe.

Pathologic analysis

In 2 (17%) cases in the BRAF V600E group, areas resembling pleomorphic xanthoastrocytoma (PXA)-like pathology (Fig. 2A) were observed. We also found presence of epithelioid components in 10 out of 12 cases (83%) in the BRAF V600E group (Fig. 2B–F), but none in the control group ($p<0.0001$). Epithelioid pathology was apparent in 7 cases, leading to the initial pathologic diagnosis of epithelioid GBM or glioblastoma with epithelioid features in these cases. Reevaluation of cases revealed 3 additional cases with focal areas of epithelioid cells.

Discussion

BRAF V600E mutations are relatively frequent in brain tumors such as PXAs (60–70%), gangliogliomas (50%), and papillary craniopharyngiomas (95%), as well as pediatric (20–35%) and adult low-grade gliomas (5–15%), and pediatric high-grade gliomas (10–20%) [1, 3, 4, 18]. However, BRAF V600E mutation is found in only ~3% of all GBMs [1, 2, 4, 5, 19], and appropriate selection of cases for BRAF alteration testing in a cost-effective fashion is not well understood. We [9, 10] and others [20–22] have reported dramatic responses of BRAF V600E-mutant malignant gliomas to BRAF inhibitor and/or combination BRAF inhibitor and MEK inhibitor treatment. Therefore, understanding the characteristics of GBMs harboring BRAF V600E mutations is vital.

In this case–control cohort, we identified unique radiographic and pathologic features of BRAF-mutant GBM. Specifically, we observed the frequent co-occurrence of solid, enhancing areas involving the cortex and large cysts with thin walls in BRAF V600E-mutant GBMs in 6 out of 12 (50%) BRAF V600E-mutant GBMs, compared to 1 out of 12 (8%) matched controls ($p=0.069$). These observations were in agreement with past case series on BRAF V600E mutant GBMs [15] and epithelioid GBMs [23]. However, somewhat surprisingly, we found that age- and location-matched *BRAF*-wildtype cases also featured frequent overlap of these radiographic characteristics (well circumscribed 92% vs. 67%, cortical involvement 75% vs 50%, and presence of large cysts 50% vs. 25%), suggesting that the specificity of these individual findings is not very high for the BRAF V600E mutation. This observation underscores the importance of matched controls, though our cohort is small and at risk for selection bias. Also of note, our cohort was relatively older than some other reports of BRAF V600E mutations in adult GBM (median 59 years, range 20–79), highlighting the importance of considering BRAF-mutations even in older adults. We further found that BRAF V600E-mutant GBMs were more amenable to gross total resection compared to their wildtype counterparts (83% vs 17%, $p=0.016$) and BRAF V600E-mutant GBMs tended to involve the temporal lobe (42%) and be less localized to the frontal lobe (17%), in agreement with radiomic analyses showing frontal lobe preference for *IDH1*-mutant gliomas and temporal lobe for glioblastoma, *IDH1*-wildtype [24, 25] and past case series of epithelioid GBMs [23, 26].

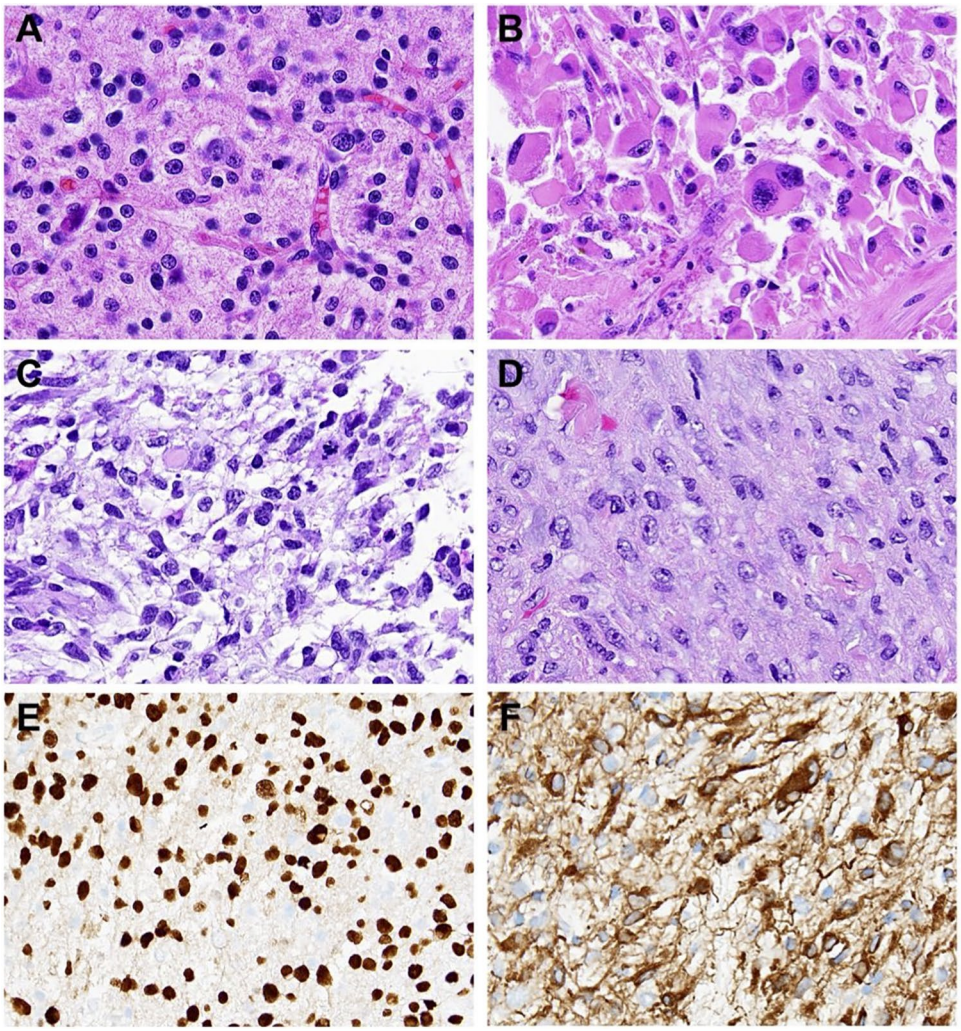

Fig. 2 Pathologic features of select cases. Hematoxylin and eosin stain from representative sections in BRAF V600E-mutant glioblastoma from **A** a 54-year-old female demonstrating high-grade astrocytoma with some areas reminiscent of PXA but not diagnostic. **B** Epithelioid glioblastoma from a 79-year-old female with **C** frequent mitotic figures. **D** BRAF V600E-mutant glioblastoma from a 60-year-old female displays focally epithelioid features on hematoxylin and eosin stain. **E** Olig2 and **F** GFAP stain from the same patient

Close pathologic examination of the BRAF V600E-mutant GBM cases revealed 10 out of 12 (83%) cases had epithelioid features, and 2 (17%) cases had localized areas of distinct, PXA-like features. Though neither epithelioid nor PXA-like features were detected in 2 out of 12 (17%) cases, identifying these features in GBM, IDH-wildtype cases is important for selecting possible BRAF V600E-mutant cases, as epithelioid GBM are known to be enriched for BRAF V600E mutations (50–93%) [23, 27].

In summary, we found that the radiographic features of well-circumscribed borders, presence of large cystic components, and a solid component involving the cortex were frequently observed in BRAF V600E-mutant GBM, but with significant overlap amongst matched BRAF-wildtype controls. On the other hand, the simultaneous presence of the above characteristics, the ability to undergo a gross total resection, and epithelioid features were more-specifically associated with the BRAF V600E mutation. Identification of these clinical, radiographic, and pathologic characteristics should prompt testing for BRAF V600E in IDH-wildtype GBM, even among older adults.

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Declarations

Ethics standards This retrospective study was approved by the institutional review boards at Niigata University (IRB2020-0491) and Johns Hopkins School of Medicine (IRB00243637) in accordance with the Declaration of Helsinki. The authors have no conflicts of interest to disclose.

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