The Immunoreactivity Patterns of BRAF VE1 and Its Diagnostic Value in Brain Tumors

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Keywords: brain tumors, BRAF-V600E, VE1, immunoreactivity patterns, diagnostic value

Posted Date: July 24th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-46822/v1>

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Abstract

Background: BRAF mutations have been detected in a high proportion of melanoma, papillary thyroid carcinoma, and various primary brain tumors. But the sensitivity and specificity of immunohistochemical detection of BRAF-V600E mutant protein were not evaluated in brain tumors. The aim of this study was to assess the utility of BRAF-V600E IHC compared to molecular biology on a large series of brain tumors, in order to provide a useful reference for the use of BRAF-V600E IHC in clinical practice.

Methods and results: We analyzed the BRAF-V600E immunoreactivity pattern and its expression profile by immunohistochemistry (IHC) in 122 patients diagnosed with different tumors of brain including gangliocytoma/ganglioma(GC/GG), pleomorphic xanthoastrocytomas(PXA), epithelioid glioblastoma(E-GBM), dysembryoplastic neuroepithelial tumour(DNT), pilocytic astrocytoma(PA), and papillary craniopharyngioma(p-CPG). VE1 immunostaining of 52 cases showed clear cytoplasmic diffuse positive pattern in majority tumor cells. 14 cases were presented with clear granular cytoplasmic positive pattern in single tumor cell or tumor cell cluster. 22 cases displayed equivocal positive with undefined location. 34 cases were negative. Including 81 immunopositive cases and 29 immunonegative cases were further confirmed by Real-time PCR. And 63 of 81 immunopositive cases were confirmed with BRAF-V600E mutation (77.8%), and all of 29 negative cases were confirmed to have wild-type BRAF (100%). Interestingly, only the cases showing clear immunoreactivity patterns (e.g cytoplasmic) with clean background had immunostaining results consistent with the molecular detection results, regardless of the number of positive cells (61/61,100%). However, samples with indeterminate immunoreactivity patterns were most likely to have false positive results (18/20, 90%).

Conclusions: VE1 immunostaining could replace molecular detection to some extent, on the premise of mastering the key points in the interpretation of BRAF VE1 immunostaining: 1) As long as the positive signal was accurately located in the cytoplasm of tumor cells, the sample was considered to have BRAF V600E mutation, disregarding the number of positive cells; 2) Tissue samples that had no signal of BRAF VE1 expression with clear background could be confirmed with wild-type BRAF-V600E; 3) Some equivocal positive with uniform “coating” or nucleus positive cases were often considered as false-positive and usually required further molecular detection.

Background

BRAF is an oncogene, which mutation has been detected in several types of cancer, such as malignant melanoma, papillary thyroid carcinoma, colorectal cancer, Langerhans cell histiocytosis and brain tumors, including gangliocytoma/ganglioglioma(GC/GG), pleomorphic xanthoastrocytoma(PXA), Dysembryoplastic Neuroepithelial tumor(DNT), pilocytic astrocytoma(PA), Epithelioid glioblastoma(E-GBM) and Papillary craniopharyngioma(P-CPG)^[1; 2; 3; 4; 5]. BRAF gene, which is located on chromosome 7q34, can activate the MAP kinase/ERK signaling pathway in response to cellular growth signals. B-Raf protein is a serine/threonine kinase and a component of the receptor tyrosine kinase (RTK) signaling pathway. The majority of mutations on BRAF affects a mutational hot spot at amino acid position 600 and is characterized by the exchange of valine by glutamate (referred to as BRAFV600E)^[6]. BRAF mutation is considered as an important biomarker with diagnostic, prognostic, and predictive potential in several clinical settings, especially in central nervous system tumors. Anti-BRAF V600E (clone VE1) antibody, a mouse monoclonal primary antibody, is used in the identification of the BRAF V600E mutant protein. Previous studies have verified the sensitivity and specificity of anti-BRAF V600E (clone VE1) antibody in melanoma^[7] and papillary thyroid carcinoma^[8]. However, few studies have assessed the reliability of anti-BRAF-V600E immunohistochemistry (IHC) in brain tumors. Breton has examined the expression of BRAF VE1 in a series of neuroepithelial neoplasias, but the sensitivity and specificity of immunohistochemical detection of BRAF-V600E mutant protein were not evaluated due to the fact that some of the tissue samples were not insufficient for further confirmation by molecular assay^[9]. The aim of this study was to assess the utility of BRAF-V600E IHC compared to molecular biology on a large series of CNS tumors, in order to provide a useful reference for the use of BRAF-V600E IHC in clinical practice.

Materials And Methods

Patients

At Sanjiu Brain Hospital, 122 patients with brain tumors are registered in the brain tumor database of the Department of Pathology. Tumors were histologically diagnosed by two pathologists (one junior and one expert neuropathologist) and graded according to WHO Classification of Tumors of the Central Nervous System, published in 2016. The samples were obtained through biopsy or surgical excision between October 2016 and October 2018. The protocol and procedures employed were reviewed and approved by the appropriate institutional review committee of Sanjiu Brain Hospital.

Routine histology and immunohistochemical study of BRAF V600E mutation

Formalin fixed, paraffin-embedded (FFPE) tissue was sectioned at 4 μ m and mounted on precoated glass slides (Thermo scientificsuperfrost plus, USA). Immunostaining for BRAF V600E (clone VE1, Immunologic, Roche, USA) using the Opti ViewDAB kit (Immunologic, Roche, USA) was performed on a Ventana Bench Mark XT immunostainer (Ventana Medical Systems, Tucson, AZ, USA) as previously reported. BRAF V600E expression patterns were divided into five categories: 1) Diffuse strong granular cytoplasmic positive in tumor cells; 2) Credible granular cytoplasmic positive in single tumor cell or tumor cell cluster; 3) Cytoplasmic positive in tumor cells with different degrees of nucleus positive; 4) When it was not possible to distinguish a weak immunostaining from a background staining, the case was considered uniform "coating" positive; 5) No BRAF V600E mutant protein expression with clear background was considered as negative. Category 1 and 2 were defined as immunohistochemically reliable immunopositive and category 3 and 4 were classified as equivocally positive. Immunohistochemical staining of GFAP, Olig-2, IDH-1, Syn, CgA, CD34, EMA, P53, ATRX, β -catenin and Ki-67 were also performed in some cases.

Detection of BRAF V600E mutation by Real-time PCR

110 DNA samples were successfully obtained from the included 122 tissue samples. The other 12 tissue samples contained insufficient DNA for further analysis. PCR primers use in the present study were BRAFf 5'-TCATAATGCTTGCTCTGATAGGA-3' and BRAFr 5'-GGCCAAAATTTAATCAGTGGA-3'. DNA was extracted from FFPE tissue using the FFPE extraction kit (Takara Bio, Japan) according to manufacturer's instructions. PCR settings were modified as follows: activation at 94 °C for 10 mins, initial denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, extension at 72 °C 1min/kb for 35 cycles. DNA samples after purification were further quantified using Roche Cobas480 (Roche, USA).

Statistical analysis

Statistical analyses were performed with SPSS for Windows (SPSS 13, SPSS Inc, Chicago, IL, USA). Continuous variables were described with mean and ranges; categorical variables were described with proportions and percentages.

Results

Patient Cohort

Clinical data and demographic features are provided in Table 1. This series included 73 male and 49 female patients, with median age of 19 years (range, 1 to 63 years). The histopathological diagnosis for these 122 patients was as follows: gangliocytoma/ganglioma (GG/GA) (53 cases), dysembryoplastic neuroepithelial tumour (DNT) (18 cases), pilocytic astrocytoma (PA) (15 cases), pleomorphic xanthoastrocytomas (PXA) (9 cases), epithelioid glioblastoma (E-GBM) (13 cases) and papillary craniopharyngioma (P-CPG) (14 cases). In the GG/GA group, there were 30 males and 23 females with the median age of 21 years, ranging from 2 to 63 years. 39 cases had tumors located in temporal lobe (39/53, 73.6%), 6 cases had tumors located in frontal lobe (6/53, 11.3%), 2 cases had tumors located in occipital region (2/53, 3.8%), 2 cases had tumors located in parietal region (2/53, 3.8%), 2 cases had tumors located in sellar region (2/53, 3.8%) and 2 cases had

tumors located in thalamus (2/53, 3.8%). In the DNT group, there were 11 males and 7 females with median age of 22 years, ranging from 3 to 61 years. Tumor mainly located in the temporal lobe (10/18, 55.6%), frontal lobe (5/18, 27.8%), parietal region (2/18, 11.1%) and occipital region (1/18, 5.5%). In the PA group, there were 10 males and 5 females with median age of 13 years, ranging from 1 to 60 years. Patients in these group had tumors mainly located in cerebellum (11/15, 73.3%), sellar region (2/15, 13.3%), medulla oblongata (1/15, 6.7%) and frontal lobe (1/15, 6.7%). In the PXA group, there were 4 males and 5 females with median age of 28 years, ranging from 5 to 63 years. Patients in these group had tumor primarily located in temporal lobe (5/9, 55.6%), frontal lobe (2/9, 22.2%), occipital (1/9, 11.1%) and parietal region (1/9, 11.1%). In the E-GBM group, there were 6 males and 7 females with median age of 28 years, ranging from 5 to 51 years. Patients in these group had lesions mainly located in temporal lobe (7/13, 53.8%), frontal lobe (3/13, 23.1%), occipital region (1/13, 7.7%), parietal region (1/13, 7.7%) and sellar region (1/13, 7.7%). In the P-CPG group, there were 12 males and 2 females with median age of 45 years, ranging from 29 to 61 years. Patients in these group had lesions primarily located in sellar region (8/14, 57.1%) and suprasellar region (6/14, 42.9%).

Histopathological features and immunoreactivity pattern of BRAF V600E mutation

Routine Hematoxylin-Eosin (HE) staining and BRAF-V600E (VE1) immunohistochemical staining was performed on all paraffin-embedded tumor specimens, and then GFAP, Olig-2, IDH-1, Syn, CgA, CD34, EMA, P53, ATRX, β -catenin and Ki-67 were also performed in some cases. BRAF-V600E (VE1) immunohistochemical staining characteristics of various tumors were listed in supplementary table 1.

The GC/GG group included 3 cases of gangliocytoma (WHO grade I), 49 cases of ganglioglioma (WHO grade II), and 1 case of anaplastic ganglioglioma (WHO grade III). Representative HE staining image showed that clusters of immature neurons were embedded in mild hyperplastic glial components and low to medium cellularity glial component of a ganglioglioma. The scattering distribution of neoplastic ganglionic cells were also observed (Figure 1a, 1b and 1c). Immunostaining of BRAF V600E (VE1) showed that tumors with prominent glial components had most intense level of BRAF VE1 expression, which was predominantly located in the cytoplasm of single ganglion cell-like tumor cell, but were absent in the glial cell-like tumor cells. (Figure 1d, 1e and 1f). Out of the 53 cases, 47 cases were further tested by Real-time PCR. And 37 of the 47 cases (74.5%) were confirmed with BRAF-V600E mutation. The immunoreactivity pattern of the GC/GG group were as follows: 27 cases were diffuse strong positive, 10 cases were positive in single tumor cells or in tumor cell cluster, 5 cases were uniform "coating" (equivocally positive), and 11 cases were negative. 35 of 37 positive cases were further assessed by Real-time PCR, all of which had BRAF-V600E mutation (35/35, 100%). 4 of 5 equivocal cases were also assessed by Real-time PCR, all of which were wild-type BRAF. The sensitivity of BRAF VE1 staining was 89.7% (35/39). 8 of the 11 negative cases were further verified by Real-time PCR, all of which were wild-type BRAF. The specificity of BRAF VE1 staining was 100% (8/8). All cases showed diffusely immunoreactive for GFAP and Olig2. It is noteworthy that no neoplastic glial cell components was observed in the HE staining of GG, but rich glial fibrillary was observed in the GFAP staining. Immunohistochemistry staining were positive for Syn, CgA and CD34, while it was negative for IDH1. Ki-67 proliferation index of GG ranged from 1% to 5%, with increasing in anaplastic gangliogliomas.

The histopathological features of the P-CPG (WHO grade II) group were as follows: 1) Few squamous cystic epithelial cells were observed in the sellar region, and the cells were mildly atypia; 2) It was difficult to differentiate craniopharyngioma from Rathke cleft cysts. Among the 14 P-CPG cases, 12 cases showed diffuse positive in BRAF VE1 immunohistochemistry staining, and two cases showed granular positive in clustered tumor cells (Figure 1g, 1h and 1i). These 12 positive cases were further assessed by Real-time PCR, total of which were confirmed with BRAF-V600E mutation, and β -catenin immunohistochemistry showed localizes to the cell membrane whereas the nuclear accumulation. The sensitivity of BRAF VE1 staining was 100% (12/12) for the P-CPG group.

The PXA group included 7 cases of PXA (WHO grade II), 2 cases of anaplastic PXA (WHO grade III). Out of all the PXA cases tested for BRAF V600E expression, 5 cases had diffuse granular cytoplasmic staining in tumor cells, 1 case showed both cytoplasmic and nucleus staining, 2 cases had coating-like fuzzy uniform background positive (equivocally positive), 1

case was negative (Figure 2). 8 of all 9 cases were further verified by Real-time PCR, and 62.5% (5/8) were confirmed with BRAF-V600E mutation. Moreover, 4 of 5 positive cases were confirmed with BRAF-V600E mutation (4/4,100%). Among the 3 equivocal cases, one of them had BRAF-V60E mutation, and the rest 2 cases did not have BRAF V600E mutation. BRAF V600E mutation was not found in the negative case either. The sensitivity of BRAF VE1 detection was 71.4% (5/7), and the specificity was 100% (1/1) for the PXA group.

The 13 E-GBM cases shared similar histopathological features with anaplastic PXA: 1) epithelioid cells and rhabdoid cells of E-GBM showed diffuse growth; 2) Eosinophilic cytoplasm, a laterally positioned nucleus, focal discohesion, spindled tumor cells intermingled, giant cells and lipidization were observed in both E-GBM and PXA cells. Out of all the E-GBM cases tested for BRAF V600E expression, 5 cases had diffuse granular cytoplasmic staining in tumor cells, 2 cases showed granular cytoplasmic positive in clustered tumor cells, 1 case showed both cytoplasmic and nucleus staining, 3 cases of coating-like fuzzy uniform background positive (equivocally positive), 2 case was negative (Figure 2). 11 of all 13 cases were further verified by Real-time PCR, 54.5% (6/11) were confirmed with BRAF-V600E mutation. Moreover, 5 of 7 positive cases were confirmed with BRAF-V600E mutation (100%, 5/5). Among the 4 equivocal cases, one of them had BRAF-V600E mutation, 3 of them did not have BRAF V600E mutation. The sensitivity of VE1 detection was 77.8% (7/9), and the specificity was 100% (2/2) for the E-GBM group.

Out of all the DNT cases tested for BRAF V600E expression, 2 cases had diffuse granular cytoplasmic staining in tumor cells, 1 case showed both cytoplasmic and nucleus staining, 4 cases had of coating-like fuzzy uniform background positive, and 11 cases were negative. The 17 of all 18 cases were further verified by Real-time PCR, and 11.7% (2/17) were confirmed with BRAF-V600E mutation. All of 2 positive cases were confirmed with BRAF-V600E mutation. All of 5 equivocal cases were verified to have wild-type BRAF. 10 of 11 negative cases tested by Real-time PCR were also confirmed with wild-type BRAF. The sensitivity of VE1 detection was 33.3% (2/6), and the specificity was 100% (10/10) in the DNT group.

Among the 15 cases in the PA group, 1 case had diffuse granular cytoplasmic staining in tumor cells, 1 case showed both cytoplasmic and nucleus staining, 4 cases had equivocal positive, and 9 cases were negative. 13 of all 15 cases were further tested by Real-time PCR, and 7.8% (1/13) were confirmed with BRAF-V600E mutation. The positive case was confirmed with BRAF-V600E mutation. 4 of 5 equivocal positive cases and 8 of 9 negative cases were verified to have wild-type BRAF. The sensitivity of VE1 detection was 25% (1/4), and the specificity was 100% (8/8) in the PA group.

In all of 122 cases, the immunoreactivity patterns of BRAF V600E mutant protein were as follows: 52 cases showed diffuse strong positive, 14 cases had positive staining in single tumor cell or tumor cell clusters, 4 cases showed both cytoplasmic and nucleus staining, 18 cases were uniform "coating", and 34 cases were negative. In 61 of 66 positive cases were further verified by Real-time PCR, all of which were confirmed with BRAF-V600E mutation (Figure 3). 20 of the 22 equivocally positive cases were tested by Real-time PCR, and 18 of the 20 cases were confirmed as false positive (Figure 3), and the other two actually had BRAF-V600E mutation. 29 of 34 negative cases were also verified Real-time PCR, which were further confirmed with wild-type BRAF. The sensitivity of BRAF V600E detection was 75.3% (61/81) and specificity was 100% (29/29) for all the enrolled samples. Therefore, our results suggested that, by using the same anti-BRAF V600E (VE1) antibodies, samples with indeterminate immunoreactivity patterns were most likely to have false positive results (90%,18/20), while samples with clear immunoreactivity patterns were almost certainly to had positive results (100%,61/61) for BRAF V600E mutation, regardless of the number and range of tumor cells.

Table 1
Clinical data and BRAFV600E molecular findings in 122 patients with nervous system tumors

Tumor entity (N)	Age median (range)	Gender (N)	Localization	BRAF V600E(VE1)			BRAF V600E		
				Reliable positive (N)	Equivocal positive (N)	Negative (N)	PCR (N)	Mutation (N)	Wild type (N)
GC/GG (53)	21(2–63)	M (30)	Temporal, Frontal, Occipital, Parietal, Sellar region, Thalamus,	37(69.8%)	5	11	47	35(74.5%)	12(25.5%)
		F (23)							
DNT (18)	22(3–61)	M (11)	Temporal, Frontal, Occipital, Parietal,	2 (11.1%)	5	11	17	2 (11.8%)	15(88.2%)
		F (7)							
PXA (9)	28(5–63)	M (4)	Temporal, Frontal, Occipital, Parietal	5 (55.6%)	3	1	8	5 (62.5%)	3(37.5%)
		F (5)							
E-GBM(13)	28(5–51)	M (6)	Temporal, Frontal, Parietal, Occipital, Sellar region	7 (53.8%)	4	2	11	6 (54.5%)	5(45.5%)
		F (7)							
PA (15)	13(1–60)	M (10)	Cerebellum, Pineal gland, Frontal, Medulla oblongata,	1 (6.7%)	5	9	13	1 (7.8%)	12(92.2%)
		F (5)							
P-CPG(14)	45(29–61)	M (12)	Sellar region, Suprasellar region	14 (100%)	0	0	14	14(100%)	0

Discussion

BRAF, a serine/threonine protein kinase belonging to the RAF family, is a key intermediary in the RAS-RAF-MEK-ERK-MAP kinase signaling pathway. It is also a key regulator of cellular functions, including cell proliferation, cell-cycle arrest, terminal differentiation, and apoptosis^[10]. BRAF V600E mutation have been detected in several types of cancer, including a variety of tumors in central nervous system. The detection of BRAF V600E is essential in the diagnosis of brain tumors for the following reasons: 1) Some circumscribed gliomas (GGs and PXAs) are difficult to be differentiated from diffuse gliomas via histopathology alone, especially on biopsy specimens as they have different molecular characteristics. Therefore, BRAF-V600E detection has important differential diagnostic value^[5]; 2) Detection of BRAF-V600E has a certain stratification effect on the prognosis of adult invasive glioma^[11]; 3) With the clinical development of BRAF-targeted therapeutics, the detection of BRAF-V600E has important diagnostic and prognostic value^[12]. However, the current molecular biology techniques are expensive and not yet widely available. If the BRAF-V600E expression status can be clarified through immunohistochemistry, it will be more efficient in the diagnosis and treatment evaluation for brain tumors. In melanoma,

papillary cholangiocarcinoma, thyroid cancer, and colorectal cancer, VE1 detection has the specificity and sensitivity of more than 95%^[2; 7; 8], but few study has evaluated the sensitivity and specificity of BRAF V600E immunochemical staining in central nervous system tumors. This study aimed to elucidate the current bias in the histopathological interpretation of BRAF VE1 staining, and provide the clinical insight into the potential future use of BRAF V600E immunochemical staining in the diagnosis of brain tumor by analyzing its sensitivity and specificity.

The gold standard for BRAF mutation analysis is direct sequencing of tumor DNA, but Real-time PCR tests were considered more effective and widely accepted in recent year^[13]. In this study, the reliability of BRAF VE1 immunohistochemical staining was verified by Real-time PCR on the routinely processed formalin-fixed, and paraffin-embedded (FFPE) tumor tissue. Our results suggested that all of the BRAF VE1-positive cases with DNA available for Real-time PCR detection were further confirmed to had BRAF V600E mutation (61/61,100%). None of the VE1-negative cases carried a BRAF V600E mutation (29/29,100%). However, it should be noted that when the positive BRAF V600E immunostaining was located in the cell components other than cytoplasm or non-granular positive signal were presented, the possibility of false positive immunostaining should be taken into account. In this study, about 90% of equivocally positive cases (18/20) had wild-type BRAF when verified by Real-time PCR. Hence, our investigation revealed two key points in the interpretation of BRAF V600E immunostaining patterns: 1) Tissue displaying solely granular cytoplasmic positive or negative immunostaining could be considered to have BRAF-V600E mutation or wild-type BRAF respectively. The coincidence rate of BRAF V600E immunostaining and the Real-time PCR test results was 100%; 2) Tissue showing BRAF V600E immunopositive in the components other than cytoplasm required further verification by molecular detection.

The positive rates of BRAFV600E mutations determined by molecular detection in different brain tumor were not always consistent with those determined using immunohistochemistry. Our results showed that, brain tumor with high BRAF V600E positive rate such as GC/GG(35/47, 74.5%)and P-CPG 14/14, 100%) had high specificity and sensitivity in immunohistochemical detection when further verified by Real-time PCR (49/49, 100%). BRAF V600E positive rate of GC/GG was 74.5%, which was similar to that reported by Schindler^[9]. Among the 35 cases with BRAF V600E mutation, 10 cases showed cytoplasmic positive in only a small number of ganglion cell-like tumor cells (10/35, 28.6%), and there was no case showing positive staining of BRAF V600E only in glial cell-like tumor cell. This is similar to the results reported in the relevant research^[14]. Selected tumors in the GC/GG group were also screened for the expression of precursor cell marker CD34, and the results (38/53, 71.7%) were consistent with those reported in previous studies^[15]. Interestingly, among the 38 cases that were CD34-positive, 36 of them were also positive for BRAF V600E, and the CD34-negative cases were also negative for BRAF V600E. In agreement with previous studies, the presence of BRAF V600E mutation was significantly associated with the expression of CD34^[16; 17]. However, since histological features of tumor subtypes were overlapping, the differentiation between the diffusely infiltrating astrocytomas and the non-specific forms of dysembryoplastic neuroepithelial tumours with gangliogliomas could be very challenging, especially for biopsy samples, which required additional tests for accurate clinical diagnosis. Hence, the combination of immunohistochemical staining of VE1 and CD34 would be helpful for differential diagnosis. For single tumor cell-positive GC/GG samples, immunostaining using OptiView DAB kit was more reliable, as it was more sensitive for color development.

Craniopharyngioma could be divided into two subtypes, adamantinomatous craniopharyngioma and papillary craniopharyngioma according to their clinicopathological features. The WNT signaling pathway was strongly implicated in the pathogenesis of adamantinomatous craniopharyngioma. Aberrant nuclear accumulation of beta-catenin could be detected by immunohistochemistry^[18], but papillary craniopharyngioma harboured the BRAF V600E mutation in nearly all cases^[2]. In this study, a total of 14 P-CPG cases were found to carry the BRAF V600E mutation (14/14, 100%) by immunohistochemistry using the BRAF V600E mutation-specific antibody VE1 and then confirmed by Real-time PCR. When the craniopharyngioma is associated with cystic degeneration due to the the cyst wall epithelium atrophies caused by the pressure of the cyst, it is difficult to differentiate from Rathke cleft cysts in sellar region with epithelial squamous metaplasia, which can be resolved by conducting immunohistochemistry. Unlike craniopharyngioma, Rathke cleft cysts are

known for the lack of BRAF V600E mutations and cell membrane accumulation of beta-catenin localizes^[19]. In addition, BRAF-V600E status can provide an important evidence for the targeted therapies of recurrent and refractory craniopharyngioma^[20].

PXA and E-GBM are the other two types of central nervous system tumors with high positive rate of BRAF-V600E mutation. In agreement with previous studies^[5; 21], for the 8 PXA cases in the present study, the BRAF V600E positive rate was 62.5% (5/8). The BRAF-V600E positive rate in anaplastic PXA was not evaluated due to the small sample size. According to other literatures, the BRAF V600E positive rate in anaplastic PXA is 47.4%^[22], closer to that of E-GBM (50%)^[23]. Moreover, GG and PXA showed overlapping histological features. PXA had spindled cells arranged in inplexiform or storiform, large pleomorphic cells, lipidized cells, ganglioncell like cells and eosinophilic granular bodies. Sometimes differential diagnosis of PXA and GG was difficult, not only due to their similar histological features, but also to the fact that the immunopositive rates of both VE1 and CD34 were indifferent. Hence, accurate diagnosis would be based on more pathological tests, clinical features and imaging findings. Interestingly, in our study, 80% of the PXA located temporal lobe were associated with BRAF-V600E mutation, which positive rate was significantly higher than in other sites. This founding was similar to the previously reported results^[24]. E-GBM and anaplastic PXA also showed overlapping histological and molecular features, such as epithelioid pattern and high positive rate of BRAF V600E mutation. Hence, it may also be difficult to distinguish anaplastic PXA from E-GBM, and accurate diagnosis of these rare tumors is challenging. Previous studies suggested that PXA and E-GBM belonged to the same category, and occurred in primary and/or secondary fashion by acquiring genetic abnormalities, such as p53 and TERT-p mutations besides BRAF mutation^[25; 26; 27]. Immunohistochemical detection of BRAF V600E in PXA and E-GBM not only has an important differential diagnostic value, but also the therapeutic significance, due to the only available targeted therapy with vemurafenib^[28; 29]. What is also noteworthy is that equivocal positive of VE1 staining were commonly observed in PXA and E-GBM, which was further confirmed as false-positive by Real-time PCR. It was consistent with the results of some previous reports^[30; 31]. However, cases with clear cytoplasmic BRAF VE1 staining were confirmed with BRAF V600 mutation by molecular detection.

Most studies suggested that only small number of PA and DNT had BRAF-V600E mutation. It was identified in 3.3% of DNT^[32]. For DNT-like tumors with BRAF mutations, we should be wary of whether they were indeed GG or DNT-GG mixed tumors due to the challenges in the histopathological classification of GG and DNT^[33]. Frequently observed genetic change in Pilocytic astrocytomas was BRAF mutation, particularly, *KIAA1549-BRAF* fusion that was identified in > 70% of all PAs, while BRAF V600E mutation only existed in 5% of the supratentorial PA^[34]. The VE1 staining pattern in DNT and PA were mostly equivocal, which also tended to have false-positive results.

Conclusions

In summary, we found the key points in the interpretation of BRAF VE1 immunostaining through analyzing the correlation between the BRAF V600E immunoreactivity patterns and the BRAF V600E status determined by Real-time PCR detection: 1) As long as the positive signal was accurately located in the cytoplasm of tumor cells, the sample was considered to have BRAF V600E mutation, disregarding the number of positive cells. It should be noted that single ganglion cell-like tumor cells with reliable positive in GG were easy to be ignored; 2) Tissue samples that had no signal of BRAF VE1 expression with clear background could be confirmed with wild-type BRAF-V600E; 3) Some equivocal positive with uniform “coating” or nucleus positive cases, which were commonly observed in PXA and E-GBM, were often considered as false-positive and usually required further molecular detection. Therefore, due to the high concordance between BRAF V600E IHC and molecular detection of a BRAF V600E mutation, IHC could be used up-front to detect BRAF V600E in brain tumors, especially in P-CPG and GC/GG.

Abbreviations

IHC: immunohistochemistry; CNS: central nervous system; GC/GG: gangliocytoma/ganglioma; PXA: pleomorphic xanthoastrocytomas; E-GBM: epithelioid glioblastoma; DNT: dysembryoplastic neuroepithelial tumour; PA: pilocytic astrocytoma; p-CPG: papillary craniopharyngioma; PCR: Polymerase Chain Reaction.

Declarations

Acknowledgement

Not applicable

Authors' contributions

HNL and CZF made contributions to acquisition of clinical data, and analysis of the histological features. They are joint first co-authors and made an equal contribution to this work. MTL, XX and ZBZ carried out the H&E staining and immunohistochemical staining. LW carried out the Real-time PCR. WDC and GYJ participated in the revision of the manuscript. ZL revised manuscript critically for important intellectual content and had given final approval of the version to be published. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Funding

Guangdong Natural Science Foundation [NO.2017A030313779]

Guangdong Medical Science and Technology Research Foundation [NO.A2019315]

Program of Ministry of Science and Technology [NO.2016YFC1000307]

Acknowledgements

Not applicable

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Figures

Figure 1

H&E staining and VE1 immunohistochemical expression in GC/GG and P-PCG. 1a, Clusters of immature neurons were embedded in mild hyperplastic glial components. 1b, Low to medium cellularity glial component of a ganglioglioma. 1c, The scattering distribution of neoplastic ganglionic cells. 1d, VE1 immunohistochemical staining showing cytoplasmic granular positive in neoplastic ganglionic cells. 1e, VE immunohistochemical staining showing diffuse cytoplasmic positive in neoplastic glial elements. 1f, VE1 immunohistochemical staining showing granular cytoplasmic positive in single neoplastic ganglionic cells, which further confirmed with BRAF-V600E mutation by Real-time PCR. 1g, Squamous epithelium showing papillary hyperplasia with mildly atypia, and VE1 immunohistochemical staining showing cytoplasmic positive in tumor cells. 1h, A few squamous epithelial cells were observed in the cystic at the sellar region, and the cells were mildly atypia, 1i, VE1 immunohistochemical staining showing cytoplasmic positive in squamous epithelial cells. 1a, 1b, 1c, 1d, and 1f magnification $\times 200$, 1g, 1h, and 1i magnification $\times 100$.

Figure 2

Histopathological and VE1 immunohistochemical staining in E-GBM and PXA. 2a and 2b, Epithelioid cells and rhabdoid cells showing diffuse growth, focal decohesion, a laterally positioned nucleus, and eosinophilic cytoplasm. 2c, Spindled tumor cells intermingled, giant cells and lipidation were observed. However, mitotic activity was not easy to observe. 2d, VE1 immunohistochemical staining showing diffuse cytoplasmic positive. 2e, VE1 immunohistochemical staining showing clear cytoplasmic positive in tumor cell cluster. 2f, VE1 immunohistochemical staining showing clear cytoplasmic diffuse positive in partial tumor cells. 2g, H&E staining showing diffuse tumor cells, eosinophilic cytoplasm, a laterally positioned nucleus and decohesion. 2h, The location of positive signal was equivocal and uniform "coating" positive. 2i, VE1 immunohistochemical staining showing weak cytoplasmic and nucleus positive in glial cells. The BRAF V600E immunopositive presented in 2h and 2i were further verified as false positive by Real-time PCR. 2a, 2b, 2d, 2e, 2f, 2h, and 2i magnification $\times 200$, 2c and 2g magnification $\times 100$.

Figure 3

BRAF V600E status by Real-time PCR. 3a, BRAF-V600E mutations. 3b, BRAF-V600E wild-type.