***BRAF*^{non-V600E} more frequently co-occurs with *IDH1/2* mutation in adult patients with gliomas than patients harboring *BRAF*^{V600E}, but without survival advantage**

Rong Da

The First Affiliated Hospital of Xi'an Jiaotong University

Maode Wang

The First Affiliated Hospital of Xi'an Jiaotong University

Haitao Jiang

The First Affiliated Hospital of Xi'an Jiaotong University

Tuo Wang

The First Affiliated Hospital of Xi'an Jiaotong University

Wei Wang (✉ wei_wangdoc@xjtu.edu.cn)

The First Affiliated Hospital of Xi'an Jiaotong University <https://orcid.org/0000-0002-6954-9056>

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Abstract

Background

The effect of $BRAF^{\text{non-V600E}}$ and $BRAF^{\text{V600E}}$ on the outcome and the molecular characteristics in adult glioma patients is unknown and needs to be explored, although $BRAF^{\text{V600E}}$ has been extensively studied in pediatric glioma.

Methods

Co-occurring mutations and copy number alterations of associated genes in the MAPK and p53 pathways were retrieved and investigated using the cBioPortal. The prognosis of available adult glioma cohorts with $BRAF^{\text{V600E}}$ and $BRAF^{\text{non-V600E}}$ mutations was also investigated.

Results

Glioblastoma multiform was the most common cancer type with $BRAF^{\text{non-V600E}}$ and $BRAF^{\text{V600E}}$. $TP53$ (56.00% vs. 7.41%), $IDH1/2$ (36.00% vs. 3.70%), and $ATRX$ (32.00% vs. 7.41%) exhibited more mutations in $BRAF^{\text{non-V600E}}$ than in $BRAF^{\text{V600E}}$, and $TP53$ was the independent risk factor (56.00% vs. 7.41%). Both $BRAF^{\text{non-V600E}}$ and $BRAF^{\text{V600E}}$ frequently overlapped with $CDKN2A/2B$ homozygous deletions (HD), whereas there was no significant difference. Survival analysis showed no difference between $BRAF^{\text{non-V600E}}$ and $BRAF^{\text{V600E}}$ cohorts, even excluded the effects of $IDH1/2$ mutations, and concerned the $BRAF^{\text{non-V600E}}$ mutations in the glycine-rich loop (G-loop) and in the activation segment. The estimated mean survival of $BRAF^{\text{non-V600E}}$ and $IDH1/2^{\text{WT}}$ with mutations in the G-loop groups was the shortest.

Conclusions

$BRAF^{\text{non-V600E}}$ exhibited a higher association with $IDH1/2$ mutation than $BRAF^{\text{V600E}}$, but no survival advantage was found.

Background

$BRAF$ (v-raf murine sarcoma viral oncogene homolog B1) is a serine-threonine kinase in the Ras/Raf /mitogen-activated protein kinase (MAPK) pathway (1, 2), which transduces mitogenic stimuli by activating growth factor receptors in cell survival, proliferation, and differentiation (3). MAPK pathway activation is common in various neoplasms. Active RAS mutations have been detected in approximately 15% of malignant human tumors. Compared with $ARAF$ and $RAF1$, $BRAF$ kinase activity is prominent (4). A previous study showed that $RAF1$ is activated by $BRAF$ through direct interaction between proteins and phosphorylation (5). $BRAF$ participates in the pathological mechanism of 7% human neoplasms, especially in patients with melanoma, colorectal, thyroid, and lung cancer (6, 7). The expression of $BRAF$ is highly restrained (1, 8). The high expression of $BRAF$ in neural cells indicates that it is a vital MEK kinase in neuronal tissues (9, 10). $BRAF$ mutations are found in some central nervous system neoplasms. In pediatric low-grade gliomas (LGGs), these alterations correlate with oncogenic senescence, which may contribute to improved prognosis (11). $BRAF^{\text{V600E}}$ mutation is scarce in adult LGGs and glioblastomas, which can be found in 1–5% of samples (12, 13). While $BRAF$ activation contributes to tumor development and progression in the neural stem cells and progenitor cells of Homo sapiens, $BRAF$ mutations are detected in adult diffuse gliomas, which are associated with poor outcomes (14).

Most studies have focused on the $BRAF^{\text{V600E}}$ mutation, although more than 70 $BRAF$ mutations have been reported till date. Mutations in $BRAF$ at V600 can activate ERK, which plays a critical role in the G1/S transition by adjusting the expression of cyclin D, cyclin E, and p21Cip1 (15). The $BRAF^{\text{V600E}}$ mutation is the most potent MAPK pathway activator, whereas $BRAF^{\text{non-V600E}}$ mutations are low-active kinases, which slightly stimulate the MAPK pathway (16). However, these low-activity

BRAF mutants could activate MAPK signaling in COS-1 cells at a high level by activating *RAF1* (16). Isocitrate dehydrogenase (IDH) is a frequent mutation and associated with survival benefit in glioma patients, which has been defined as an molecular parameter to define the category of brain tumor in the updated 2016 edition of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS)(17). *IDH1* and *BRAF*^{V600E} mutations are associated with infiltrative gliomas or circumscribed gliomas and glioneuronal tumors respectively (18, 19), and they are exclusive in most cases (20). The exact effect of *BRAF*^{non-V600E} and *BRAF*^{V600E} on the prognosis of glioma patients and whether there are unique molecular characteristics in the MAPK and p53 pathways remain largely unknown.

In this study, co-occurring mutations and copy number alterations of 35 associated genes in the MAPK and p53 pathways were retrieved and investigated, and the prognosis of available adult glioma cohorts with *BRAF*^{V600E} and *BRAF*^{non-V600E} was evaluated. We determined that *BRAF*^{non-V600E} exhibited a higher association with *IDH1/2* mutation than *BRAF*^{V600E}, but no survival advantage was found.

Methods

Data collection and enrollment

All data were collected and generated from The Cancer Genome Atlas (TCGA) public database using the TCGA data mining tool cBioPortal (<https://www.cbioportal.org/>) (21, 22). We strictly followed the TCGA publication guidelines (<https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/using-tcga/citing-tcga>). In multiple patient cohorts of all twenty available CNS/Brain studies (6164 samples), the available data were queried, including gene mutation, copy number alteration, mRNA expression, and protein expression data of patients with *BRAF* gene mutations. In each study, mutations were selected for genomic profiles. Samples with mutation data were selected for the patient/case set and entered into three groups: (1) General: *Ras-Raf-MEK-Erk/JNK* signaling (26 genes), including *KRAS*, *HRAS*, *BRAF*, *RAF1*, *MAP3K1*, *MAP3K2*, *MAP3K3*, *MAP3K4*, *MAP3K5*, *MAP2K1*, *MAP2K2*, *MAP2K3*, *MAP2K4*, *MAP2K5*, *MAPK1*, *MAPK3*, *MAPK4*, *MAPK6*, *MAPK7*, *MAPK8*, *MAPK9*, *MAPK12*, *MAPK14*, *DAB2*, *RASSF1*, and *RAB25*; (2) General: p53 signaling (6 genes), including *TP53*, *MDM2*, *MDM4*, *CDKN2A*, *CDKN2B*, *TP53BP1*; (3) Other frequently mutated genes, including *IDH1*, *IDH2*, and *ATRX*, were then submitted for query. Among downloadable data files, the available data regarding mutations, copy number alterations, mRNA expression, and protein expression were downloaded. In the type of genetic alterations across all samples, samples harboring the *BRAF* mutation were chosen. Data regarding mutations and copy number alterations on the summary page, and patient and sample data on the clinical data page were downloaded. All the data were recorded in a chart for further analysis.

Major characteristics of the *BRAF*^{V600E} and *BRAF*^{non-V600E} cohorts using univariate logistic regression analysis

The enrolled populations were divided into *BRAF*^{V600E} and *BRAF*^{non-V600E} groups. The numbers and percentages of categorical variables were calculated. The demographic characteristics, including sex, diagnosis age, cancer type, and overall survival status, in the two groups were analyzed using univariate logistic regression analysis. The odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. A *P* value < 0.05 was considered statistically significant.

Co-occurring mutations of the *BRAF*^{V600E} and *BRAF*^{non-V600E} cohorts using univariate and multivariate logistic regression analyses

The numbers and percentages of categorical variables were calculated in the *BRAF*^{V600E} and *BRAF*^{non-V600E} groups. The available data of co-occurring mutation genes in these two groups were analyzed using univariate logistic regression analysis. Thereafter, significant variables (*P* < 0.10) were analyzed using multivariate logistic regression analysis. The ORs and 95% CIs were estimated. A *P* value < 0.05 was considered statistically significant.

Co-occurring copy number alteration in the $BRAF^{V600E}$ and $BRAF^{non-V600E}$ cohorts using heatmap and univariate logistic regression analysis

The available copy number alterations of the $BRAF^{V600E}$ and $BRAF^{non-V600E}$ cohorts were retrieved and displayed using a heatmap by Morpheus (<https://software.broadinstitute.org/morpheus>). Putative copy-number alterations are as follows: -2 = homozygous deletion; -1 = hemizygous deletion; 0 = neutral/no change; 1 = gain; 2 = high-level amplification. Univariate logistic regression analysis was used to calculate the numbers and percentages of *CDKN2A* HD and *CDKN2B* HD. The ORs and 95% CIs were estimated. A *P* value < 0.05 was considered statistically significant.

Cross-over analysis with Kaplan–Meier survival curves and the log rank (Mantel-Cox) test

The overall survival rates of the $BRAF^{V600E}$ and $BRAF^{non-V600E}$ cohorts were compared using Kaplan-Meier curves and the Log Rank (Mantel-Cox) test (23). To exclude the side effects of *IDH1/2* on survival, the survival of the $BRAF^{V600E}$ and $IDH1/2^{WT}$ groups was compared with that of the $BRAF^{non-V600E}$ and $IDH1/2^{WT}$ groups. In addition, the $BRAF^{V600E}$ and $IDH1/2^{WT}$ groups were compared with the glycine-rich loop (G-loop) $BRAF^{non-V600E}$ and $IDH1/2^{WT}$ group and the activation segment $BRAF^{non-V600E}$ and $IDH1/2^{WT}$ groups, respectively. Furthermore, the G-loop $BRAF^{non-V600E}$ and $IDH1/2^{WT}$ groups were compared with the $BRAF^{non-V600E}$ and $IDH1/2^{WT}$ groups, except for the G-loop $BRAF^{non-V600E}$ and $IDH1/2^{WT}$ groups. A *P* value < 0.05 was considered statistically significant.

Results

Data enrollment in the study

In all the 20 CNS/Brain studies (6164 samples), 4674 samples with mutation data were queried; 90 samples (90 patients) with *BRAF* mutations, including 53 samples (53 patients) with $BRAF^{V600E}$ and 37 samples (37 patients) with $BRAF^{non-V600E}$, are shown in Table 1. The cancer types of 20 CNS/Brain studies included diffuse glioma, glioblastoma, oligodendroglioma, embryonal tumor, encapsulated glioma, and miscellaneous neuroepithelial tumor. The scheme for the final enrolled and investigated data is shown in Fig. 1. Ninety patients with $BRAF^{V600E}$ or $BRAF^{non-V600E}$ were enrolled in this study, and data from 52 non-redundant patients were investigated. The integrated data of major patient characteristics, including sex, age, diagnosis age, cancer type, data of co-occurring mutations, copy number alterations, and overall survival time and status, were collected for further analysis.

Table 1
The CNS/Brain projects of TCGA database enrolled in the study retrieved by cBioPortal

Project	All Samples	Samples with mutation data	Samples with <i>BRAF</i> ^{V600E}	Samples with <i>BRAF</i> ^{non-V600E}	References
Diffuse Glioma					
Brain Lower Grade Glioma (TCGA, Firehose Legacy)	530	286	1	1	https://www.cancer.gov
Brain Lower Grade Glioma (TCGA, PanCancer Atlas)	514	512	1	2	(42–47)
Glioma (MSK, Nature 2019)	91	91	2	1	https://www.cancer.gov
Glioma (MSKCC, Clin Cancer Res 2019)	1004	1004	22	19	(48)
Low-Grade Gliomas (UCSF, Science 2014)	61	61	2	0	(49)
Merged Cohort of LGG and GBM (TCGA, Cell 2016)	1102	812	5	2	(50)
GLIOBLASTOMA					
Brain Tumor PDXs (Mayo Clinic, 2019)	95	83	2	1	https://www.cbioportal.org
Glioblastoma (Columbia, Nat Med. 2019)	42	32	1	1	(51)
Glioblastoma (TCGA, Cell 2013)	543	257	3	0	(52)
Glioblastoma (TCGA, Nature 2008)	206	91	0	0	(53)
Glioblastoma Multiforme (TCGA, Firehose Legacy)	604	290	5	1	https://www.cancer.gov
Glioblastoma Multiforme (TCGA, PanCancer Atlas)	592	397	5	3	(42–47, 54)
OLIGODENDROGLIOMA					
Anaplastic Oligodendroglioma and Anaplastic Oligogastrocytoma (MSKCC, Neuro Oncol 2017)	22	22	0	0	(55)
Embryonal Tumor					
MEDULLOBLASTOMA					
Medulloblastoma (Broad, Nature 2012)	92	92	0	0	(56)
Medulloblastoma (ICGC, Nature 2012)	125	125	0	0	(57)
Medulloblastoma (PCGP, Nature 2012)	37	37	0	0	(58)
Medulloblastoma (Sickkids, Nature 2016)	46	46	0	1	(59)
Encapsulated Glioma					

Project	All Samples	Samples with mutation data	Samples with <i>BRAF</i> ^{V600E}	Samples with <i>BRAF</i> ^{non-V600E}	References
PILOCYTIC ASTROCYTOMA					
Pilocytic Astrocytoma (ICGC, Nature Genetics 2013)	96	96	4	3	(60)
Miscellaneous Neuroepithelial Tumor					
Pheochromocytoma and Paraganglioma (TCGA, Cell 2017)	178	178	0	1	(61)
Pheochromocytoma and Paraganglioma (TCGA, Firehose Legacy)	184	162	0	1	https://www.cancer.gov

Major characteristics of cohorts with *BRAF*^{V600E} and *BRAF*^{non-V600E}

The study populations were divided into two groups, *BRAF*^{V600E} and *BRAF*^{non-V600E}. The major demographic characteristics and clinical data of the two groups are summarized in Table 2. The patients' ages ranged from 20 to 85 years and were divided into early adulthood, midlife, mature adulthood, and late adulthood (aged 20–35, 35–50, 50–80, and > 80 years, respectively). The two groups had comparable male patients, diagnosis age, cancer type, and overall survival status. Glioblastoma multiform was the most common cancer type in both cohorts (74.07% vs. 56.00%; *P* = 0.175; Table 2).

Table 2
The major characteristics of cohorts including *BRAF*^{V600E} and *BRAF*^{non-V600E}

Variables	<i>BRAF</i> ^{V600E} (n = 27)		<i>BRAF</i> ^{non-V600E} (n = 25)		Univariate analysis		
	Number	%	Number	%	Odds Ratio	95% Confidence Interval	P Value
Male	16	59.26	18	72.00	0.566	0.177–1.809	0.337
Diagnosis Age							
Ages 20–35	9	33.33	6	24.00	1.583	0.469–5.350	0.459
Ages 36–50	9	33.33	8	32.00	1.062	0.333–3.390	0.918
Ages 51–80	7	25.93	11	44.00	0.445	0.139–1.433	0.175
Age 80+	2	7.41	0	0.00	1615474843	0.000-	0.999
Cancer type detailed							
Glioblastoma Multiform	20	74.07	14	56.00	2.245	0.698–7.219	0.175
Astrocytoma	3	11.11	6	24.00	0.396	0.087–1.794	0.229
Oligoastrocytoma	1	3.70	0	0.00	1553341195	0.000-	1.000
Oligodendroglioma	0	0.00	3	12.00	0.000	0.000-	0.999
Gliosarcoma	0	0.00	2	8.00	0.000	0.000-	0.999
Other glioma	3	11.11	0	0.00	1682786295	0.000-	0.999
Overall survival status							
Deceased	14	51.85	11	44.00	1.371	0.460–4.087	0.572

Co-occurring mutations of the *BRAF*^{V600E} and *BRAF*^{non-V600E} cohorts using univariate and multivariate logistic regression analysis

Available co-occurring gene mutations of the *BRAF*^{V600E} and *BRAF*^{non-V600E} cohorts were retrieved, and differences between the two groups were compared; the results are summarized in Table 3. The mutation frequencies of *KRAS*, *HRAS*, *RAF1*, *MAP3K1*, *MAP2K1*, *MAP2K2*, *MAP2K4*, *MDM2*, *MDM4*, *CDKN2A*, and *CDKN2B* were comparable between the two groups. In contrast, the *BRAF*^{non-V600E} group exhibited a significantly higher mutation frequency of *TP53* (56.00% vs. 7.41%; *P* = 0.001), *IDH1/2* (36.00% vs. 3.70%; *P* = 0.015), and *ATRX* (32.00% vs. 7.41%; *P* = 0.037) than the *BRAF*^{V600E} group. The variables with *P* < 0.10 were analyzed using multivariate logistic regression analysis, and the *BRAF*^{non-V600E} group exhibited a significantly higher *TP53* mutation frequency (56.00% vs. 7.41%; *P* = 0.031) than the *BRAF*^{V600E} group (Table 3).

Table 3

The co-occurred mutations of *BRAF*^{V600E} and *BRAF*^{non-V600E} cohort using univariate and multivariate logistics regression analysis

Gene	<i>BRAF</i> ^{V600E} (n = 27)		<i>BRAF</i> ^{non-V600E} (n = 25)		Univariate analysis			Multivariate analysis		
	Number	%	Number	%	Odds Ratio	95% Confidence Interval	P Value	Odds Ratio	95% Confidence Interval	P Value
<i>KRAS</i>	0	0.00	1	4.00	1817409198	0.000-	1.000			
<i>HRAS</i>	0	0.00	1	4.00	1817409198	0.000-	1.000			
<i>RAF1</i>	0	0.00	2	8.00	1896426989	0.000-	0.999			
<i>MAP3K1</i>	1	3.70	6	24.00	8.211	0.911–73.959	0.060			
<i>MAP2K1</i>	0	0.00	2	8.00	1896426989	0.000-	0.999			
<i>MAP2K2</i>	0	0.00	4	16.00	2077039084	0.000-	0.999			
<i>MAP2K4</i>	0	0.00	2	8.00	1896426989	0.000-	0.999			
<i>TP53</i>	2	7.41	14	56.00	15.909	3.078–82.224	0.001	12.186	1.251–118.721	0.031
<i>MDM2</i>	1	3.70	3	12.00	3.545	0.344–36.561	0.298			
<i>MDM4</i>	0	0.00	4	16.00	2077039084	0.000-	0.999			
<i>CDKN2A</i>	0	0.00	3	12.00	1982628216	0.000-	0.999			
<i>CDKN2B</i>	0	0.00	1	4.00	1817409198	0.000-	1.000			
<i>IDH1/2</i>	1	3.70	9	36.00	14.625	1.690–126.537	0.015	5.498	0.512–59.020	0.159
<i>ATRX</i>	2	7.41	8	32.00	5.882	1.110–31.170	0.037	0.665	0.048–9.188	0.761

Co-occurring copy number alteration in the *BRAF*^{V600E} and *BRAF*^{non-V600E} cohorts using heatmap and univariate logistic regression analyses

There were no available copy number data for five patients with *BRAF*^{V600E} and five patients with *BRAF*^{non-V600E}. The copy number alterations of the available co-occurring genes included *BRAF*, *RAF1*, *MAP3K1*, *MAP2K1*, *MAP2K2*, *MAP2K4*, *MAPK1*, *MAPK3*, *TP53*, *MDM2*, *MDM4*, *TP53BP1*, *IDH1*, *IDH2*, *ATRX*, *CDKN2A*, and *CDKN2B*. The homozygous deletion (HD) copy number was frequently retrieved in these two genes, including *CDKN2A* and *CDKN2B* (Fig. 2), and the HD of both *CDKN2A* (77.27.00% vs. 60.00%; *P* = 0.032) and *CDKN2B* (77.27.00% vs. 60.00%; *P* = 0.032) was more frequent in the *BRAF*^{V600E} cohort than in the *BRAF*^{non-V600E} cohort (Table 4).

Table 4
The HD of *CDKN2A/2B* of *BRAF*^{V600E} and *BRAF*^{non-V600E} cohort using univariate logistics regression analysis

Variables	<i>BRAF</i> ^{V600E} (n = 22)		<i>BRAF</i> ^{non-V600E} (n = 20)		Univariate analysis		
	Number	%	Number	%	Odds Ratio	95% Confidence Interval	P Value
<i>CDKN2A</i>	17	77.27	12	60.00	0.193	0.043–0.867	0.032
<i>CDKN2B</i>	17	77.27	12	60.00	0.193	0.043–0.867	0.032

Cross over analysis using Kaplan–Meier survival curves and the log rank (Mantel-Cox) test

The cross over Kaplan–Meier survival curves and the log rank (Mantel-Cox) test were performed to explore the difference between the overall survival of glioma patients with *BRAF*^{V600E} and *BRAF*^{non-V600E}. The estimated mean survival time was 51.394 months for patients with *BRAF*^{V600E}, 89.958 months for patients with *BRAF*^{non-V600E}, 44.550 months for patients with *BRAF*^{V600E} and *IDH1/2*^{WT}, and 93.821 months for patients with *BRAF*^{non-V600E} and *IDH1/2*^{WT}. There was no difference between the survival of *BRAF*^{V600E} and *BRAF*^{non-V600E} (51.394 vs. 89.958, chi-square 1.130, *P* = 0.288). In addition, there was no difference between the survival of *BRAF*^{V600E} and *IDH1/2*^{WT} and *BRAF*^{non-V600E} and *IDH1/2*^{WT} (44.550 vs. 93.821, chi-square 0.007, *P* = 0.935), which excluded the side effect of *IDH1/2*. We also evaluated the survival of *BRAF*^{non-V600E} and *IDH1/2*^{WT} with mutations in the G-loop and activation segment. The estimated survival time of these two subgroups was 12.250 months for patients with *BRAF*^{non-V600E} and *IDH1/2*^{WT} with mutations in the G-loop, and 34.800 months for patients with *BRAF*^{non-V600E} and *IDH1/2*^{WT} with mutations in the activation segment. In addition, there was no difference between the *BRAF*^{V600E} and *IDH1/2*^{WT} cohorts and those of the *BRAF*^{non-V600E} and *IDH1/2*^{WT} cohorts. As shown below, *BRAF*^{V600E} and *IDH1/2*^{WT} vs. *BRAF*^{non-V600E} and *IDH1/2*^{WT} with mutations in the G-loop (44.550 vs. 12.250, chi-square 0.122, *P* = 0.727), and *BRAF*^{V600E} and *IDH1/2*^{WT} vs. *BRAF*^{non-V600E} and *IDH1/2*^{WT} with mutations in the activation segment (44.550 vs. 34.800, chi-square 0.145, *P* = 0.703). Since the estimated mean survival of *BRAF*^{non-V600E} and *IDH1/2*^{WT} with mutations in the G-loop was the shortest, we compared the *BRAF*^{non-V600E} and *IDH1/2*^{WT} with mutations in the G-loop with the remaining *BRAF*^{non-V600E} and *IDH1/2*^{WT} patients. There was no difference between them (12.250 vs. 95.100, chi-square 0.008, *P* = 0.927) (Fig. 3).

Discussion

BRAF mutations critically affect cancer growth and progression and are supposed to be a founder event for mutations occurring early in the initiation process of cancer. However, *BRAF* mutations must cooperate with other mechanisms for a fully cancerous state, as they are insufficient to induce cancer alone (5). *BRAF*^{V600E} has been the focused mutation in previous studies on glioma, especially in pediatric glioma patients, for the available molecule-targeted drugs. However, various *BRAF*^{non-V600E} cells exert different activation effects on the MAPK pathway. The exact impact on the clinical prognosis and possible molecular mechanism of associated co-occurring genes with mutations or copy number alterations co-occurring with *BRAF* mutations remains unclear in adult glioma patients. In this study, the available data of patients with *BRAF*^{non-V600E} and *BRAF*^{V600E} in the TCGA CNS/Brain database were investigated to determine the possible mechanisms of *BRAF* gene mutations in adult glioma patients.

Our data indicated that in adult glioma patients with *BRAF* mutations, including both *BRAF*^{non-V600E} and *BRAF*^{V600E} cohorts, glioblastoma multiform was the most common cancer type. The available co-occurring mutation genes in the MAPK and p53 pathways showed that mutation genes frequently co-occurred in the *BRAF*^{non-V600E} cohort, and there were more *TP53*, *IDH1/2*, and *ATRX* mutations in *BRAF*^{non-V600E} than in *BRAF*^{V600E} (20). Lai *et al.* (24) found that a *TP53* point mutation at position 273 (Arg to Cys) was more common than *IDH1* mutations at position 132 (Arg to His). They hypothesized that the *TP53* mutation (C→T) occurred in the non-transcribed strand, while *IDH1* mutation existed in the transcribed strand, which is a strand

asymmetry pattern (25). The study indicated that *IDH1/2* mutations represent early events in brain tumor formation (26). Liu *et al.* (27) found that *ATRX* alterations correlated with mutations in *IDH1/2* and *TP53* in glioma of all grades. It has been reported that *ATRX* deletions/mutations are correlated with *TP53* and *IDH1* mutations (28, 29). Somatic *TP53*, *ATRX*, and *IDH1/2* mutations have been found in adult LGGs (30). *ATRX* mutations are detected in adult diffuse gliomas and astrocytomas, harboring both *TP53* and *IDH1/2*. The co-occurrence of these three mutated genes, including *TP53*, *IDH1/2*, and *ATRX*, facilitates the growth of an adult diffuse astrocytoma subgroup (27). All the studies above indicate that *ATRX* mutations frequently overlap with *IDH1/2* and *TP53* mutations. In the present study, we also found the co-occurrence of these three mutations, which were frequently detected in the *BRAF*^{non-V600E} cohort, but not in the *BRAF*^{V600E} cohort. Our findings indicated that in adult glioma patients, a possible correlation between *BRAF*^{non-V600E} and those three common mutations simultaneously occurred in glioma. Multivariate logistic regression revealed that *TP53* was an independent risk factor in the *BRAF*^{non-V600E} cohort vs. the *BRAF*^{V600E} group. Our data proved the correlation between *BRAF*^{non-V600E} and *TP53* mutations in adult glioma patients. Previous findings have shown that active *Ras* can induce heterodimerization of *BRAF* and *RAF1* (31), and that this event may be critical for *RAF1* activation (32). *RAF1* directly regulates cell apoptosis, which does not depend on MAPK signaling (33, 34), but through direct interaction with *Bcl-2* (33). *TP53* can regulate *Bcl-2* by suppressing *Bcl-2* transcription (35). We proposed that *BRAF*^{non-V600E} mutation might activate the *BRAF-RAF1* heterodimer, which shows anti-apoptotic properties via the activation of *Bcl-2* through *RAF1* phosphorylation. Mutant *TP53*, which is frequently accompanied by *IDH1/2* mutation by a strand asymmetry mechanism, would fail to regulate *Bcl-2*. Therefore, with both activated *RAF1* and mutated *TP53*, an enhanced anti-apoptosis effect, which promotes cancer growth, might be predicted. Further studies using appropriate clinical tissue samples or animal models are needed, which might provide evidence for this proposal.

Our data showed that *CDKN2A* and *CDKN2B* HDs were more frequent in the *BRAF*^{V600E} cohort than in the *BRAF*^{non-V600E} cohort. Concomitant *CDKN2A* and *CDKN2B* HDs could be detected in patients with glioblastoma multiform, astrocytoma, and gliosarcoma. A previous report indicated that five of seven pediatric grade II–IV astrocytomas with *BRAF*^{V600E} had concomitant *CDKN2A* HD (36), and *CDKN2A* deletions combined with *BRAF*^{V600E} alterations, constituting a subgroup of secondary high-grade gliomas (37). We found that in adult glioma patients, *BRAF*^{V600E} and *BRAF*^{non-V600E} frequently co-occurred with *CDKN2A* HDs combined with *CDKN2B* HDs, especially in patients with *BRAF*^{V600E}. Except for astrocytoma, glioblastoma multiform was the most common cancer type with these combined alterations. Robinson *et al.* (38) indicated that activated Akt or Ink4a/ARF deletions are necessary for high-grade brain neoplasms with *BRAF* mutation in a Cre/lox animal model. Our results showed the possible synergy of *CDKN2A* and *CDKN2B* HDs with *BRAF* mutations, especially in adult glioma patients with *BRAF*^{V600E} and *BRAF*^{non-V600E}.

BRAF^{V600E} reportedly improves *BRAF* kinase activity 500-fold (39). According to kinase viability, *BRAF*^{non-V600E} mutations were classified into three groups: high activity (130–700 times), intermediate activity (1.3–64 times), and impaired activity (30%–80%) (16). Theoretically, the higher the *BRAF* kinase activity, the worse the prognosis. To clarify whether there is a difference between *BRAF*^{V600E} and *BRAF*^{non-V600E}, we compared the overall survival of these two cohorts, and no statistical significance was found. Besides, the status of *IDH* mutations in glioblastomas definitely influences the prognosis of patients with glioblastomas; therefore, *IDH*-wildtype glioblastomas are defined as primary tumors, while *IDH*-mutant ones are classified as secondary tumors (40). To exclude the side effects of *IDH* mutations on survival, we compared the *BRAF*^{V600E} and *IDH1/2*^{WT} and *BRAF*^{non-V600E} and *IDH1/2*^{WT} cohorts, and no difference was detected. Most *BRAF*^{non-V600E} mutations exist in the activation segment and the G-loop (16, 41); therefore, we selected the two cohorts as *BRAF*^{non-V600E} and *IDH1/2*^{WT} with mutations in the G-loop and activation segment. We compared them with *BRAF*^{V600E} and *IDH1/2*^{WT}, and no difference was found between the *BRAF*^{V600E} and *IDH1/2*^{WT} cohorts and those of the *BRAF*^{non-V600E} and *IDH1/2*^{WT} cohorts. Furthermore, we compared the *BRAF*^{non-V600E} and *IDH1/2*^{WT} with mutations in the G-loop with the remaining *BRAF*^{non-V600E} and *IDH1/2*^{WT} patients and found no difference between them. Although there was no statistical significance, the estimated mean survival of *BRAF*^{non-V600E} and *IDH1/2*^{WT} with mutations in the G-loop was the shortest in all cohorts. We propose that a large sample will benefit further confirmation. Our data indicated that *BRAF*^{non-V600E} cohort have no survival advantage from the co-occurrence with *IDH* mutation compared with that of *BRAF*^{non-V600E} cohort of adult patients with glioma.

Conclusions

In conclusion, we found that in adult patients with gliomas, *BRAF*^{non-V600E}, rather than *BRAF*^{V600E}, frequently co-occurs with *TP53*, *IDH1/2*, and *ATRX* mutations. Both *BRAF*^{non-V600E} and *BRAF*^{V600E} frequently overlapped with *CDKN2A/2B* HDs, whereas there were no significant differences between the two cohorts. Although there were significant differences in co-occurring gene mutations and copy number alterations, no difference was found in survival between cohorts of *BRAF*^{non-V600E} and *BRAF*^{V600E} with and without *IDH1/2* favorable effects on survival. We also found that the estimated mean survival of *BRAF*^{non-V600E} and *IDH1/2*^{WT} with mutations in the G-loop was the shortest; however, no difference was observed between those cohorts and other cohorts. For the poor available mRNA and protein data in TCGA database we retrieved in the study, no expression data were evaluated. More clinical data or models are anticipated to be studied to elucidate the mechanism involved in the *BRAF*^{non-V600E} associated glioma in the future.

Abbreviations

HD, homozygous deletion; *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; LGGs, low-grade gliomas; TCGA, The Cancer Genome Atlas; ORs, odds ratios; Cis, confidence intervals; G-loop, glycine-rich loop; MAPK, mitogen-activated protein kinase.

Declarations

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Authors' contributions

Rong Da: Conceptualization, Methodology Maode Wang: Writing- Reviewing & Editing Haitao Jiang: Investigation Tuo Wang: Data curation Wei Wang: Formal analysis, Writing- Original draft preparation. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ Department of Clinical Laboratory, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

² Department of Neurosurgery, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

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Figures

Figure 1

The scheme of the enrollment and investigation of data. In all 20 CNS/Brain studies, including 6164 samples, 90 patients with *BRAF*^{V600E} or *BRAF*^{non-V600E} were enrolled; 52 non-redundant patients displayed major patient characteristics, including sex, age, cancer type detailed, co-occurring mutations, and copy number alteration genes, and were enrolled for further analysis.

Figure 2

The co-occurring copy number alteration of the BRAFV600E cohort and BRAFnon-V600E cohort using a heatmap. The cohorts of BRAFV600E (red) or BRAFnon-V600E (green) are shown, and putative copy-number alterations change from light to dark with value enhancement.

Figure 3

Cross-over analysis with Kaplan–Meier Survival Curves and the Log Rank (Mantel-Cox) test. (a) BRAFV600E vs. BRAFnon-V600E (51.394 vs. 89.958, Chi-Square 1.130, $P = 0.288$); (b) BRAFV600E & IDH1/2WT vs. BRAFnon-V600E & IDH1/2WT (44.550 vs. 93.821, Chi-Square 0.007, $P = 0.935$); (c) BRAFV600E & IDH1/2WT vs. BRAFnon-V600E & IDH1/2WT with mutations in G-loop (44.550 vs. 12.250, Chi-Square 0.122, $P = 0.727$); (d) BRAFV600E & IDH1/2WT vs. BRAFnon-V600E & IDH1/2WT with mutations in activation segment (44.550 vs. 34.800, Chi-Square 0.145, $P = 0.703$); (e) BRAFnon-V600E & IDH1/2WT with mutations in G-loop vs. the rest BRAFnon-V600E & IDH1/2WT patients (12.250 vs. 95.100, Chi-Square 0.008, $P = 0.927$).

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