BRAFnon-V600E more frequently co-occurs with IDH1/2 mutation in adult patients with gliomas than patients harboring BRAFV600E, but without survival advantage

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Abstract

Background

The effect of $\textit{BRAF}^{\text{non-V600E}}$ and $\textit{BRAF}^{\text{V600E}}$ on the outcome and the molecular characteristics in adult glioma patients is unknown and needs to be explored, although $\textit{BRAF}^{\text{V600E}}$ 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 $\textit{BRAF}^{\text{V600E}}$ and $\textit{BRAF}^{\text{non-V600E}}$ mutations was also investigated.

Results

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

Conclusions

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

Background

$\textit{BRAF}$ (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 $\textit{ARAF}$ and $\textit{RAF1}$, $\textit{BRAF}$ kinase activity is prominent (4). A previous study showed that $\textit{RAF1}$ is activated by $\textit{BRAF}$ through direct interaction between proteins and phosphorylation (5). $\textit{BRAF}$ participates in the pathological mechanism of 7% human neoplasms, especially in patients with melanoma, colorectal, thyroid, and lung cancer (6, 7). The expression of $\textit{BRAF}$ is highly restrained (1, 8). The high expression of $\textit{BRAF}$ in neural cells indicates that it is a vital MEK kinase in neuronal tissues (9, 10). $\textit{BRAF}$ 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). $\textit{BRAF}^{\text{V600E}}$ mutation is scarce in adult LGGs and glioblastomas, which can be found in 1–5% of samples (12, 13). While $\textit{BRAF}$ activation contributes to tumor development and progression in the neural stem cells and progenitor cells of Homo sapiens, $\textit{BRAF}$ mutations are detected in adult diffuse gliomas, which are associated with poor outcomes (14).

Most studies have focused on the $\textit{BRAF}^{\text{V600E}}$ mutation, although more than 70 $\textit{BRAF}$ mutations have been reported till date. Mutations in $\textit{BRAF}$ 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 $\textit{BRAF}^{\text{V600E}}$ mutation is the most potent MAPK pathway activator, whereas $\textit{BRAF}^{\text{non-V600E}}$ mutations are low-active kinases, which slightly stimulate the MAPK pathway (16). However, these low-activity

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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 a 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 BRAFV600E 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 BRAFnon-V600E and BRAFV600E 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 BRAFV600E and BRAFnon-V600E was evaluated. We determined that BRAFnon-V600E exhibited a higher association with IDH1/2 mutation than BRAFV600E, 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 BRAFV600E and BRAFnon-V600E cohorts using univariate logistic regression analysis**

The enrolled populations were divided into BRAFV600E and BRAFnon-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 BRAFV600E and BRAFnon-V600E cohorts using univariate and multivariate logistic regression analyses**

The numbers and percentages of categorical variables were calculated in the BRAFV600E and BRAFnon-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 \textit{BRAF}\textsuperscript{V600E} and \textit{BRAF}\textsuperscript{non-V600E} cohorts using heatmap and univariate logistic regression analysis

The available copy number alterations of the \textit{BRAF}\textsuperscript{V600E} and \textit{BRAF}\textsuperscript{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 \textit{CDKN2A} HD and \textit{CDKN2B} HD. The ORs and 95% CIs were estimated. A \textit{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 \textit{the BRAF}\textsuperscript{V600E} and \textit{BRAF}\textsuperscript{non-V600E} cohorts were compared using Kaplan-Meier curves and the Log Rank (Mantel-Cox) test (23). To exclude the side effects of \textit{IDH1/2} on survival, the survival of the \textit{BRAF}\textsuperscript{V600E} and \textit{IDH1/2\textsuperscript{WT}} groups was compared with \textit{that of the BRAF}\textsuperscript{non-V600E} and \textit{IDH1/2\textsuperscript{WT}} groups. In addition, the \textit{BRAF}\textsuperscript{V600E} and \textit{IDH1/2\textsuperscript{WT}} groups were compared with the glycine-rich loop (G-loop) \textit{BRAF}\textsuperscript{non-V600E} and \textit{IDH1/2\textsuperscript{WT}} group and the activation segment \textit{BRAF}\textsuperscript{non-V600E} and \textit{IDH1/2\textsuperscript{WT}} groups, respectively. Furthermore, the G-loop \textit{BRAF}\textsuperscript{non-V600E} and \textit{IDH1/2\textsuperscript{WT}} groups were compared with the \textit{BRAF}\textsuperscript{non-V600E} and \textit{IDH1/2\textsuperscript{WT}} groups, except for the G-loop \textit{BRAF}\textsuperscript{non-V600E} and \textit{IDH1/2\textsuperscript{WT}} groups. A \textit{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 \textit{BRAF} mutations, including 53 samples (53 patients) with \textit{BRAF}\textsuperscript{V600E} and 37 samples (37 patients) with \textit{BRAF}\textsuperscript{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 \textit{BRAF}\textsuperscript{V600E} or \textit{BRAF}\textsuperscript{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 *BRAF*<sup>V600E</sup> | Samples with *BRAF*<sup>non-V600E</sup> | 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 Oligogliomocytoma (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                                                     |             |                            |                                     |                                        |            |
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

| Variables                  | BRAF<sup>V600E</sup> (n = 27) | BRAF<sup>non−V600E</sup> (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<sup>V600E</sup> and BRAF<sup>non−V600E</sup> cohorts using univariate and multivariate logistic regression analysis

Available co-occurring gene mutations of the BRAF<sup>V600E</sup> and BRAF<sup>non−V600E</sup> 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<sup>non−V600E</sup> 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<sup>V600E</sup> group. The variables with P < 0.10 were analyzed using multivariate logistic regression analysis, and the BRAF<sup>non−V600E</sup> group exhibited a significantly higher TP53 mutation frequency (56.00% vs. 7.41%; P = 0.031) than the BRAF<sup>V600E</sup> group (Table 3).
Table 3

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

| Gene          | $\textit{BRAF}^{\text{V600E}}$ (n = 27) | $\textit{BRAF}^{\text{non-V600E}}$ (n = 25) | Univariate analysis | Multivariate analysis |
|---------------|------------------------------------------|--------------------------------------------|---------------------|-----------------------|
|               | Number | % | Number | % | Odds Ratio | 95% Confidence Interval | P Value | Odds Ratio | 95% Confidence Interval | P Value |
| KRAS          | 0       | 0.00 | 1       | 4.00 | 1817409198 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 |
| HRAS          | 0       | 0.00 | 1       | 4.00 | 1817409198 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 |
| RAF1          | 0       | 0.00 | 2       | 8.00 | 1896426989 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 |
| MAP3K1        | 1       | 3.70 | 6       | 24.00 | 8.211 | 0.911-73.959 | 0.060 | 1.120 | 1.067-118.721 | 0.031 |
| MAP2K1        | 0       | 0.00 | 2       | 8.00 | 1896426989 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 |
| MAP2K2        | 0       | 0.00 | 4       | 16.00 | 2077039084 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 |
| MAP2K4        | 0       | 0.00 | 2       | 8.00 | 1896426989 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 |
| TP53          | 2       | 7.41 | 14      | 56.00 | 15.909 | 3.078-82.224 | 0.001 | 12.186 | 1.251-118.721 | 0.031 |
| MDM2          | 1       | 3.70 | 3       | 12.00 | 3.545 | 0.344-36.561 | 0.298 | 0.754 | 0.087-62.924 | 0.624 |
| MDM4          | 0       | 0.00 | 4       | 16.00 | 2077039084 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 |
| CDKN2A        | 0       | 0.00 | 3       | 12.00 | 1982628216 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 |
| CDKN2B        | 0       | 0.00 | 1       | 4.00 | 1817409198 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 | 0.000-1.000 |
| IDH1/2        | 1       | 3.70 | 9       | 36.00 | 14.625 | 1.690-126.537 | 0.015 | 5.498 | 0.512-59.020 | 0.159 |
| ATRX          | 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 $\textit{BRAF}^{\text{V600E}}$ and $\textit{BRAF}^{\text{non-V600E}}$ cohorts using heatmap and univariate logistic regression analyses

There were no available copy number data for five patients with $\textit{BRAF}^{\text{V600E}}$ and five patients with $\textit{BRAF}^{\text{non-V600E}}$. The copy number alterations of the available co-occurring genes included $\textit{BRAF}$, $\textit{RAF1}$, $\textit{MAP3K1}$, $\textit{MAP2K1}$, $\textit{MAP2K2}$, $\textit{MAP2K4}$, $\textit{MAPK1}$, $\textit{MAPK3}$, $\textit{TP53}$, $\textit{MDM2}$, $\textit{MDM4}$, $\textit{TP53BP1}$, $\textit{IDH1}$, $\textit{IDH2}$, $\textit{ATRX}$, $\textit{CDKN2A}$, and $\textit{CDKN2B}$. The homozygous deletion (HD) copy number was frequently retrieved in these two genes, including $\textit{CDKN2A}$ and $\textit{CDKN2B}$ (Fig. 2), and the HD of both $\textit{CDKN2A}$ (77.27.00% vs. 60.000%; $P = 0.032$) and $\textit{CDKN2B}$ (77.27.00% vs. 60.000%; $P = 0.032$) was more frequent in the $\textit{BRAF}^{\text{V600E}}$ cohort than in the $\textit{BRAF}^{\text{non-V600E}}$ cohort (Table 4).
Table 4
The HD of CDKN2A/2B of BRAF<sup>V600E</sup> and BRAF<sup>non−V600E</sup> cohort using univariate logistics regression analysis

| Variables | BRAF<sup>V600E</sup> (n = 22) | BRAF<sup>non−V600E</sup> (n = 20) | Univariate analysis |
|-----------|-------------------------------|---------------------------------|---------------------|
|           | Number %                       | Number %                        | Odds Ratio | 95% Confidence Interval | P Value |
| CDKN2A    | 17 77.27                       | 12 60.00                        | 0.193      | 0.043–0.867              | 0.032   |
| CDKN2B    | 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<sup>V600E</sup> and BRAF<sup>non−V600E</sup>. The estimated mean survival time was 51.394 months for patients with BRAF<sup>V600E</sup>, 89.958 months for patients with BRAF<sup>non−V600E</sup>, 44.550 months for patients with BRAF<sup>V600E</sup> and IDH1/2WT, and 93.821 months for patients with BRAF<sup>non−V600E</sup> and IDH1/2WT. There was no difference between the survival of BRAF<sup>V600E</sup> and BRAF<sup>non−V600E</sup> (51.394 vs. 89.958, chi-square 1.130, P = 0.288). In addition, there was no difference between the survival of BRAF<sup>V600E</sup> and IDH1/2WT and BRAF<sup>non−V600E</sup> and IDH1/2WT (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<sup>non−V600E</sup> and IDH1/2WT 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<sup>non−V600E</sup> and IDH1/2WT with mutations in the G-loop, and 34.800 months for patients with BRAF<sup>non−V600E</sup> and IDH1/2WT with mutations in the activation segment. In addition, there was no difference between the BRAF<sup>V600E</sup> and IDH1/2WT cohorts and those of the BRAF<sup>non−V600E</sup> and IDH1/2WT cohorts. As shown below, BRAF<sup>V600E</sup> and IDH1/2WT vs. BRAF<sup>non−V600E</sup> and IDH1/2WT with mutations in the G-loop (44.550 vs. 12.250, chi-square 0.122, P = 0.727), and BRAF<sup>V600E</sup> and IDH1/2WT vs. BRAF<sup>non−V600E</sup> and IDH1/2WT 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<sup>non−V600E</sup> and IDH1/2WT with mutations in the G-loop was the shortest, we compared the BRAF<sup>non−V600E</sup> and IDH1/2WT with mutations in the G-loop with the remaining BRAF<sup>non−V600E</sup> and IDH1/2WT 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<sup>V600E</sup> has been the focused mutation in previous studies on glioma, especially in pediatric glioma patients, for the available molecule-targeted drugs. However, various BRAF<sup>non−V600E</sup> 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<sup>non−V600E</sup> and BRAF<sup>V600E</sup> 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<sup>non−V600E</sup> and BRAF<sup>V600E</sup> 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<sup>non−V600E</sup> cohort, and there were more TP53, IDH1/2, and ATRX mutations in BRAF<sup>non−V600E</sup> than in BRAF<sup>V600E</sup> (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 mutations in 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 BRAFnon-V600E cohort, but not in the BRAFV600E cohort. Our findings indicated that in adult glioma patients, a possible correlation between BRAFnon-V600E and those three common mutations simultaneously occurred in glioma. Multivariate logistic regression revealed that TP53 was an independent risk factor in the BRAFnon-V600E cohort vs. the BRAFV600E group. Our data proved the correlation between BRAFnon-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 BRAFnon-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 BRAFV600E cohort than in the BRAFnon-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 BRAFV600E had concomitant CDKN2A HD (36), and CDKN2A deletions combined with BRAFV600E alterations, constituting a subgroup of secondary high-grade gliomas (37). We found that in adult glioma patients, BRAFV600E and BRAFnon-V600E frequently co-occurred with CDKN2A HDs combined with CDKN2B HDs, especially in patients with BRAFV600E. 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 BRAFnon-V600E and BRAFV600E.

BRAFV600E reportedly improves BRAF kinase activity 500-fold (39). According to kinase viability, BRAFnon-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 BRAFV600E and BRAFnon-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 BRAFV600E and IDH1/2WT and BRAFnon-V600E and IDH1/2WT cohorts, and no difference was detected. Most BRAFnon-V600E mutations exist in the activation segment and the G-loop (16, 41); therefore, we selected the two cohorts as BRAFnon-V600E and IDH1/2WT with mutations in the G-loop and activation segment. We compared them with BRAFV600E and IDH1/2WT, and no difference was found between the BRAFV600E and IDH1/2WT cohorts and those of the BRAFnon-V600E and IDH1/2WT cohorts. Furthermore, we compared the BRAFnon-V600E and IDH1/2WT with mutations in the G-loop with the remaining BRAFnon-V600E and IDH1/2WT patients and found no difference between them. Although there was no statistical significance, the estimated mean survival of BRAFnon-V600E and IDH1/2WT 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 BRAFnon-V600E cohort have no survival advantage from the co-occurrence with IDH mutation compared with that of BRAFnon-V600E cohort of adult patients with glioma.
Conclusions

In conclusion, we found that in adult patients with gliomas, $\textit{BRAF}^{\text{non-V600E}}$, rather than $\textit{BRAF}^{\text{V600E}}$, frequently co-occurs with TP53, IDH1/2, and ATRX mutations. Both $\textit{BRAF}^{\text{non-V600E}}$ and $\textit{BRAF}^{\text{V600E}}$ 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 $\textit{BRAF}^{\text{non-V600E}}$ and $\textit{BRAF}^{\text{V600E}}$ with and without IDH1/2 favorable effects on survival. We also found that the estimated mean survival of $\textit{BRAF}^{\text{non-V600E}}$ and IDH1/2$^{\text{WT}}$ 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 $\textit{BRAF}^{\text{non-V600E}}$ associated glioma in the future.

Abbreviations

HD, homozygous deletion; $\textit{BRAF}$, 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
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