



***BRAF*^{AMP} Frequently Co-occurs With *IDH1/2*, *TP53*, and *ATRX* Mutations in Adult Patients With Gliomas and Is Associated With Poorer Survival Than That of Patients Harboring *BRAF*^{V600E}**

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Abnormal RAS/RAF signaling plays a critical role in glioma. Although it is known that the V600E mutation of v-raf murine viral oncogene homolog B1 (*BRAF*^{V600E}) and *BRAF* amplification (*BRAF*^{AMP}) both result in constitutive activation of the RAS/RAF pathway, whether *BRAF*^{V600E} and *BRAF*^{AMP} have different effects on the survival of glioma patients needs to be clarified. Using cBioPortal, we retrieved studies of both mutations and copy number variations of the *BRAF* gene in CNS/brain tumors and investigated data from 69 nonredundant glioma patients. The *BRAF* mutation group had significantly more male patients (64.00% vs. 36.84%; $P = 0.046$) and a higher occurrence of glioblastoma multiforme (66.00% vs. 31.58%; $P = 0.013$) compared to those in the other group. The *BRAF*^{AMP} group had significantly more patients with the mutant isocitrate dehydrogenase 1 and 2 (*IDH1/2*) (73.68% vs. 18.00%; $P = 0.000$), tumor protein p53 (*TP53*) (73.68% vs. 30.00%; $P = 0.002$), and alpha thalassemia/mental retardation syndrome X linked (*ATRX*) (63.16% vs. 18.00%; $P = 0.001$) than the mutation group. The *BRAF*^{AMP} and *IDH1/2*^{WT} cohort had lower overall survival compared with the *BRAF*^{AMP} and *IDH1/2*^{MT} groups ($P = 0.001$) and the *BRAF* mutation cohort ($P = 0.019$), including the *BRAF*^{V600E} ($P = 0.033$) and *BRAF*^{T10N-V600E} ($P = 0.029$) groups, using Kaplan–Meier survival curves and the log rank (Mantel–Cox) test. The *BRAF*^{AMP} and *IDH1/2*^{WT} genotype was found to be an independent predictive factor for glioma with *BRAF* mutation and *BRAF*^{AMP} using Cox proportional hazard regression analysis (HR = 0.138, $P = 0.018$). Our findings indicate that *BRAF*^{AMP} frequently occurs with *IDH1/2*, *TP53*, and *ATRX* mutations. Adult patients with glioma with *BRAF*^{AMP} and *IDH1/2*^{WT} had worse prognoses compared with those with *BRAF* mutation and *BRAF*^{AMP} and *IDH1/2*^{MT}. This suggests that the assessment of the status of *BRAF*^{AMP} and *IDH1/2* in adult glioma/glioblastoma patients has prognostic value as these patients have relatively short survival times and may benefit from personalized targeted therapy using *BRAF* and/or MEK inhibitors.

Keywords: *BRAF*, *IDH1/2*, *TP53*, *ATRX*, glioma, copy number amplification, mutation, overall survival

INTRODUCTION

Gliomas are the most frequent primary brain neoplasms occurring in both the pediatric and adult populations (1). The 2016 WHO Classification of Tumors of the Central Nervous System was the first to provide combined data regarding the genetic and histological characteristics of tumors and is, thus, considered a cornerstone for understanding and diagnosing tumors. When diagnosing the disease, mutation site genotypes of genes such as isocitrate dehydrogenase (*IDH*), tumor protein p53 (*TP53*), and alpha thalassemia/mental retardation syndrome X linked (*ATRX*) and 1p/19q codeletion should be evaluated. Hence, determining the status of *IDH* mutation and 1p/19q is essential for the 2016 classification of diffused gliomas, including astrocytoma, oligoastrocytoma, oligodendroglioma, and glioblastoma (2). The RAS/RAF/MEK/extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) pathway, which transduces mitogenic stimuli *via* the activation of growth factor receptors, is critical for cell proliferation, survival, and differentiation. Abnormal activation of RAS/RAF signaling plays a role in various tumors, and studies have revealed that the MAPK pathway is of great clinical significance in gliomas (3). Oncogenic mutations as well as the copy number amplification of RAS/RAF and/or abnormal activation of upstream growth factor receptors can cause hyperactivation of the RAS/RAF pathway (4), resulting in various neoplasms.

BRAF (v-raf murine viral oncogene homolog B1) participates in the pathological mechanism of 7% of human neoplasms, especially in melanoma, colorectal, thyroid, and lung cancers (5, 6). Because of the negative outcome of high-grade glioma, *BRAF* mutations have gained considerable interest in the possible benefit of the MAPK pathway inhibitors for glioma treatment. The *BRAF*^{V600E} mutation in which the thymine at nucleotide 1799 is substituted by adenine results in the substitution of valine with glutamic acid at amino acid 600; this is the most common *BRAF* mutation in glioma (6). It imitates the normal phosphorylation of T599 and S602, resulting in the overactivation of *BRAF* kinase and hyperactivation of the ERK signaling pathway (7). In addition, *BRAF* amplification (*BRAF*^{AMP}) can also cause hyperactivation of MAPK signaling, which plays essential roles in the acquired resistance to MAPK inhibitor therapy in cancers harboring *BRAF*^{V600E} (8). Moreover, *BRAF*^{AMP} is also found in primary pediatric low-grade gliomas (9).

Although both *BRAF*^{V600E} and *BRAF*^{AMP} can lead to the hyperactivation of MAPK signaling, the differences between the patterns of *BRAF*^{V600E} and *BRAF*^{AMP} signaling in glioma, their influence on the survival of glioma patients, and the involvement of other genes, remains unclear. In this study, based on cBioPortal data, we found that patients with glioma harboring *BRAF*^{AMP} had lower overall survival compared with those harboring *BRAF*^{V600E}. Furthermore, we found that *BRAF*^{AMP} frequently co-occurred with *IDH1/2*, *TP53*, and *ATRX* mutations.

MATERIALS AND METHODS

Data Collection and Enrollment

We used cBioPortal (<https://www.cbioportal.org/>) (10, 11). The Cancer Genome Atlas Program (TCGA) data mining tool to

collect the necessary data. TCGA is a public database, and we strictly followed its publication guidelines (<https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/using-tcga/citing-tcga>) for collecting and generating data. Multiple patient cohorts, including all 19 available studies on central nervous system (CNS)/brain tumors (6122 samples) were queried. The data were filtered to include studies that listed both gene mutation and copy number data. In each study, mutations and putative copy number alterations (CNA) identified using the Genomic Identification of Significant Targets in Cancer (GISTIC) tool were selected to analyze the genomic profiles. We first selected tumor samples with mutations and CNA data for creating the patient/case set. Then, the gene names *BRAF*, *ATRX*, *TP53*, *IDH1*, and *IDH2* were entered, and the query was submitted. Among the retrieved data files, we selected samples harboring the *BRAF* mutation with AMP. The mutation data and CNA as well as the patient and sample data were retrieved. All data were recorded in a chart for further analysis.

Characteristics Associated With *BRAF*^{AMP} and *BRAF* Mutation in Glioma Using Univariate and Multivariate Logistic Regression Analysis

The study population was divided into the *BRAF*^{AMP} and *BRAF* mutation groups, and the numerical values of the categorical variables were calculated. The demographic characteristics of the patients, pathological classification, and molecular biomarkers in the two groups were analyzed using univariate logistic regression analysis. Then, the statistically significant variables ($P < 0.10$) were analyzed using multivariate logistic regression analysis. The odds ratios and 95% confidence intervals were estimated. P value < 0.05 was considered statistically significant. For greater precision of characteristic evaluation, we created a descriptive table and divided the *BRAF*^{AMP} group into two groups based on the non- and co-occurrence of the *IDH1/2* mutation, and the *BRAF* mutation group into *BRAF*^{V600E} and *BRAF*^{non-V600E} groups.

Cross-Over Analysis Using Kaplan–Meier Survival Curves and the Log Rank (Mantel–Cox) Test

The overall survival of the *BRAF*^{AMP} and *IDH1/2*^{MT}, *BRAF*^{AMP} and *IDH1/2*^{WT}, *BRAF*^{V600E}, and *BRAF*^{non-V600E} groups was determined by a crossover comparison using Kaplan–Meier survival curves and the log rank (Mantel–Cox) test (12). The survival of the *BRAF* mutation group was compared with that of the *BRAF*^{AMP} and *IDH1/2*^{MT} and *BRAF*^{AMP} and *IDH1/2*^{WT} groups, respectively. P value < 0.05 was considered statistically significant.

Multivariate Analysis of Overall Survival Using Cox Regression Analysis

The *BRAF*^{AMP} and *IDH1/2*^{WT}, *TP53*, and *ATRX* were analyzed using the Cox regression analysis in the 69 samples with *BRAF*^{AMP} or *BRAF* mutation. P value < 0.05 was considered statistically significant.

String Analysis of BRAF, IDH1, IDH2, TP53, and ATRX

Using STRING: functional protein association networks (<https://string-db.org/>) (13), the association among BRAF, IDH1, IDH2, TP53, and ATRX was investigated, and the combined scores among those four proteins were obtained.

RESULTS

Data Enrollment in the Study

Among the 19 CNS/brain studies available (6122 samples), 12 studies (5487 samples) matched the required inclusion criteria, containing both gene mutation and CNA data (Table 1). The cancer types in these 12 CNS/brain studies included diffuse glioma, glioblastoma, oligodendroglioma, and miscellaneous neuroepithelial tumors. A schematic representation of the flow of data screening and enrollment is shown in Figure 1. A total of 115 samples (109 patients) with BRAF mutation or BRAF^{AMP} were enrolled in this study, and data from 69 nonredundant patients were investigated. Integrated data of major patient characteristics, including sex, age, cancer type, BRAF mutation, BRAF CNA, and mutation of IDH1/2, TP53, and ATRX, were collected for further analysis (Supplementary Table S1).

Characteristics Associated With BRAF^{AMP} and BRAF Mutation of Glioma

The study population was divided into two groups, BRAF^{AMP} and BRAF mutation. The demographic characteristics and clinical data of the two groups are summarized in Table 2. The age of patients ranged from 20 to 85 years with an average of 45.46 years. Twenty-five patients harbored BRAF^{non-V600E} mutations; of these, two

patients harbored a D594G mutation; two patients, a G469A mutation; and the remaining patients, an A320T mutation combined with A171E, A404Cfs*9, E375*, G466E, G466V, G469R, G469V, G596D, G69S, L331F, L382V, L597R, M531, P708S, S394P, S614P, T121I, V504_R506dup, V504I, W476*, and X709_splice mutations. The BRAF mutation group had significantly more male patients (64.00% vs. 36.84%; $P = 0.046$) and a higher occurrence of glioblastoma multiforme (66.00% vs. 31.58%; $P = 0.013$). In contrast, the BRAF^{AMP} group had significantly more patients harboring IDH1/2 (73.68% vs. 18.00%; $P = 0.000$), TP53 (73.68% vs. 30.00%; $P = 0.002$), and ATRX (63.16% vs. 18.00%; $P = 0.001$) mutations. Variables with P value < 0.10 were analyzed using multivariate logistic regression analysis; the BRAF mutation group had more male patients (64.00% vs. 36.84%; $P = 0.027$), and the BRAF^{AMP} group had significantly more patients harboring IDH1/2 mutations (73.68% vs. 18.00%; $P = 0.029$) (Table 2). Further analysis indicated that the BRAF^{AMP} group had no simultaneously detected BRAF mutations, and that the BRAF mutation group had no simultaneously detected BRAF^{AMP}. The BRAF^{AMP} and IDH1/2^{MT} group had a significantly higher percentage of co-occurrence of TP53 (13/14, 92.86%) and ATRX (12/14, 85.71%) mutations (Table 3).

Crossover Analysis Using Kaplan–Meier Survival Curves and Log Rank (Mantel–Cox) Test

The crossover Kaplan–Meier survival curves and log rank (Mantel–Cox) test were performed to explore the influence of BRAF gene alteration on the overall survival of glioma patients. The estimated mean survival time was 67.026 months for patients harboring BRAF^{AMP} and IDH1/2^{MT}, 9.750 months for patients harboring BRAF^{AMP} and IDH1/2^{WT}, 41.573 months for patients

TABLE 1 | The CNS/Brain projects of TCGA data enrolled in the study retrieved using cBioPortal.

Project	All Samples	Samples with mutation and CNA data	Samples of BRAF ^{AMP}	Samples of BRAF ^{V600E}	Samples of BRAF ^{non-V600E}	References
Diffuse Glioma						
Brain Lower Grade Glioma (TCGA, Firehose Legacy)	530	283	10	1	1	https://www.cancer.gov
Brain Lower Grade Glioma (TCGA, PanCancer Atlas)	514	507	7	1	2	(14–19)
Glioma (MSK, 2018)	91	91	1	2	1	https://www.cancer.gov
Glioma (MSKCC, Clin Cancer Res 2019)	1004	1004	3	22	26	(20)
Merged Cohort of LGG and GBM (TCGA, Cell 2016)	1102	794	9	5	2	(21)
Glioblastoma						
Brain Tumor PDXs (Mayo Clinic, 2019)	95	83	0	2	1	https://www.cbioportal.org
Glioblastoma (TCGA, Cell 2013)	543	248	2	3	0	(22)
Glioblastoma (TCGA, Nature 2008)	206	91	0	0	0	(23)
Glioblastoma Multiforme (TCGA, Firehose Legacy)	604	273	1	5	1	https://www.cancer.gov
Glioblastoma Multiforme (TCGA, PanCancer Atlas)	592	378	4	5	4	(14–19, 24)
Oligodendroglioma						
Anaplastic Oligodendroglioma and Anaplastic Oligogastrocytoma (MSKCC, Neuro Oncol 2017)	22	22	0	0	0	(25)
Miscellaneous Neuroepithelial Tumor						
Pheochromocytoma and Paraganglioma (TCGA, Firehose Legacy)	184	162	1	0	1	https://www.cancer.gov

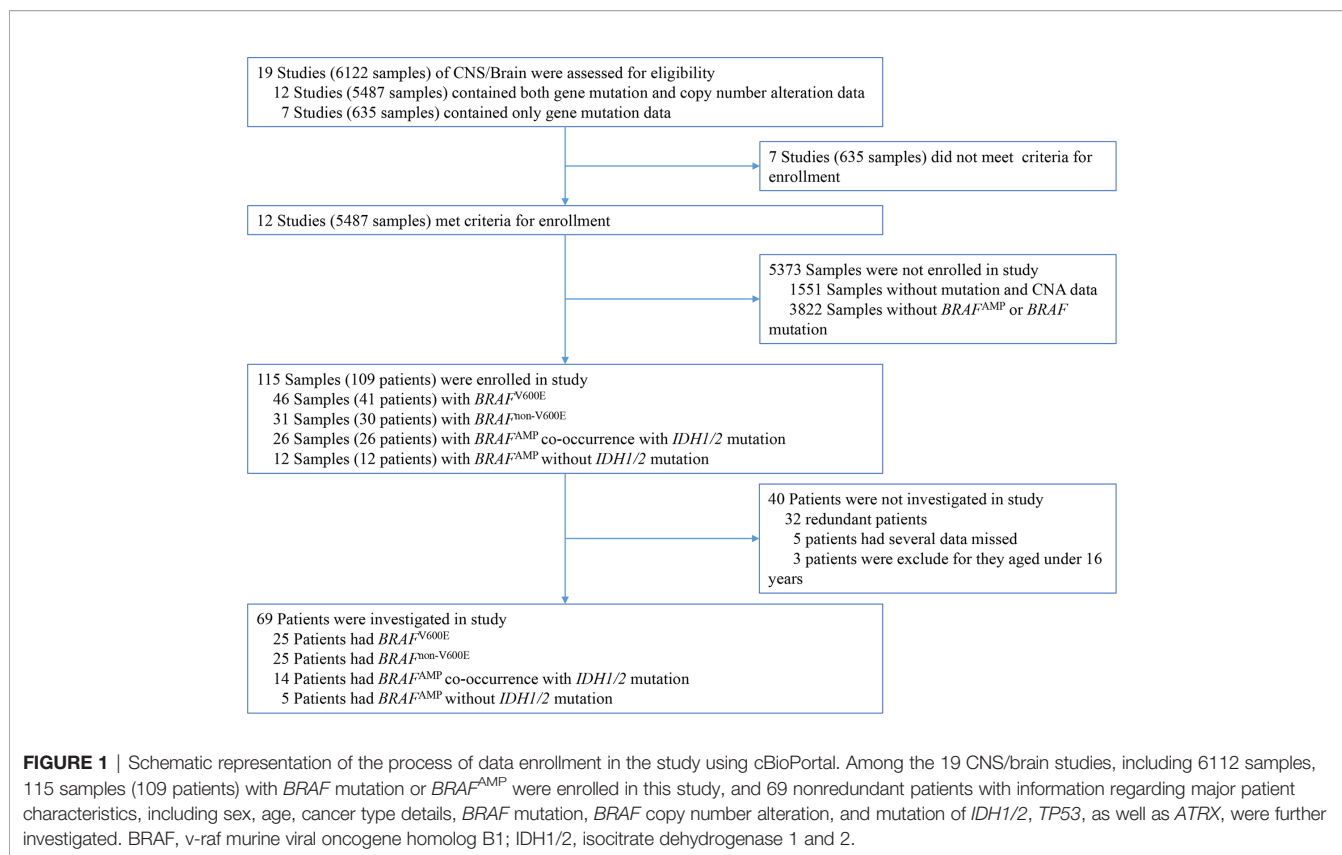


TABLE 2 | Univariate and multivariate analysis: characteristics associated with BRAF^{AMP} and BRAF mutation in gliomas.

Variables	BRAF ^{AMP} (n = 19)		BRAF mutation (n = 50)		Univariate analysis			Multivariate analysis		
	Number	%	Number	%	Odds Ratio	95% Confidence Interval	P Value	Odds Ratio	95% Confidence Interval	P Value
Male	7	36.84	32	64.00	0.328	0.110–0.982	0.046	0.181	0.040–0.824	0.027
Diagnosis Age										
20–40 years	9	47.37	21	42.00	1.243	0.430–2.592	0.688			
41–60 years	7	36.84	18	36.00	1.037	0.346–3.105	0.948			
> 61 years	3	15.79	11	22.00	0.665	0.163–2.704	0.568			
Cancer type detailed										
Glioblastoma multiform	6	31.58	33	66.00	0.238	0.077–0.736	0.013	0.590	0.120–2.893	0.515
Astrocytoma	5	26.32	9	18.00	1.627	0.466–5.680	0.445			
Oligoastrocytoma	4	21.05	0	0.00	5384916143	0.000–	0.999			
Oligodendroglioma	4	21.05	3	6.00	4.178	0.839–20.814	0.081	0.807	0.098–6.633	0.842
Gliosarcoma	0	0.00	2	4.00	0.000	0.000–	0.999			
Other glioma	0	0.00	3	6.00	0.000	0.000–	0.999			
Mutation										
BRAF ^{V600E}	0	0.00	25	50.00	1227760777	0.000–	0.998			
BRAF ^{non-V600E}	0	0.00	25	50.00	0.000	0.000–	0.998			
IDH1/2	14	73.68	9	18.00	12.756	3.653–44.534	0.000	8.805	1.242–62.406	0.029
TP53	14	73.68	15	30.00	6.533	1.994–21.407	0.002	1.463	0.165–13.000	0.733
ATRX	12	63.16	9	18.00	7.810	2.403–25.383	0.001	1.832	0.273–12.310	0.534
Copy number variation										
BRAF ^{AMP}	19	100.00	0	0.00	–	–	–			
Overall survival status										
Deceased	7	36.84	24	48.00	0.632	0.214–1.870	0.407			

TABLE 3 | Characteristics associated with BRAF^{AMP} and BRAF mutation in gliomas.

Variables	BRAF ^{AMP} & IDH1/2 ^{MT} (n = 14)		BRAF ^{AMP} & IDH1/2 ^{WT} (n = 5)		BRAF ^{V600E} (n = 25)		BRAF ^{non-V600E} (n = 25)	
	Number	%	Number	%	Number	%	Number	%
Male	6	42.86	1	20.00	14	56.00	18	72.00
Diagnosis Age								
20–40 years	7	50.00	2	40.00	12	48.00	9	36.00
41–60 years	6	42.86	1	20.00	7	28.00	11	44.00
> 61 years	1	7.14	2	40.00	6	24.00	5	20.00
Cancer type detailed								
Glioblastoma multiform	2	14.29	4	80.00	19	76.00	14	56.00
Astrocytoma	4	28.57	1	20.00	3	12.00	6	24.00
Oligoastrocytoma	4	28.57	0	0.00	0	0.00	0	0.00
Oligodendroglioma	4	28.57	0	0.00	0	0.00	3	12.00
Gliosarcoma	0	0.00	0	0.00	0	0.00	2	8.00
Other glioma	0	0.00	0	0.00	3	12.00	0	0.00
Mutation								
BRAF ^{V600E}	0	0.00	0	0.00	25	100.00	0	0.00
BRAF ^{non-V600E}	0	0.00	0	0.00	0	0.00	25	100.00
IDH1/2	14	100.00	0	0.00	0	0.00	9	36.00
TP53	13	92.86	1	20.00	1	4.00	14	56.00
ATRX	12	85.71	0	0.00	1	4.00	8	32.00
Copy number variation								
BRAF ^{AMP}	14	100.00	5	100.00	0	0.00	0	0.00
Overall survival status								
Deceased	5	35.71	2	40.00	13	52.00	11	44.00

harboring BRAF^{V600E}, and 89.958 months for patients harboring BRAF^{non-V600E}. The estimated survival time of the BRAF^{AMP} and IDH1/2^{WT} cohort was the shortest and was significantly lower compared with that of the BRAF^{AMP} and IDH1/2^{MT} (9.750 vs. 67.026, chi-square 10.526, $P = 0.001$), the BRAF^{V600E} (9.750 vs. 41.573, chi-square 4.536, $P = 0.033$), and the BRAF^{non-V600E} (9.750 vs. 89.958, chi-square 4.747, $P = 0.029$) cohorts. The estimated mean survival time of the BRAF mutation cohort was significantly greater than that of the BRAF^{AMP} and IDH1/2^{WT} cohort (71.698 vs. 9.750, chi-square 5.469, $P = 0.019$). The estimated mean survival times of the three cohorts were significantly greater than that of the BRAF^{AMP} and IDH1/2^{WT} cohort (74.401 vs. 9.750, chi-square 7.639, $P = 0.006$) (Figure 2). When analyzed using Kaplan–Meier survival curves and the log rank (Mantel–Cox) test, there was no significance between the following groups: BRAF^{AMP} cohort vs. BRAF mutation cohort (58.835 vs. 71.698, chi-square 0.020, $P = 0.886$), BRAF^{V600E} cohort vs. BRAF^{non-V600E} cohort (41.573 vs. 89.958, chi-square 1.999, $P = 0.157$), BRAF^{AMP} and IDH1/2^{MT} cohort vs. BRAF^{V600E} cohort (67.026 vs. 41.573, chi-square 1.031, $P = 0.310$), BRAF^{AMP} and IDH1/2^{MT} cohort vs. BRAF^{non-V600E} cohort (67.026 vs. 89.958, chi-square 0.025, $P = 0.875$), BRAF^{AMP} and IDH1/2^{MT} cohort vs. BRAF mutation cohort (67.026 vs. 71.698, chi-square 0.513, $P = 0.474$) (Supplementary Figure S1). The estimated survival time of the BRAF^{V600E} cohort above 30 years of age was 40.135 months, whereas that of the BRAF^{AMP} and IDH1/2^{WT} cohort was significantly lower (9.750 vs. 40.135, chi-square 5.575, $P = 0.018$) (Supplementary Figure S2).

Multivariate Analysis of Overall Survival Using the Cox Regression Analysis

The IDH1/2 mutation in 13 of the 14 BRAF^{AMP} patients was R132H, and one patient harbored the R132G mutation. The

IDH1/2 mutation in eight BRAF^{non-V600E} patients was R132H with the exception of one sample (R132S). The TP53 and ATRX mutations were highly diverse in all patients (Supplementary Table S1). The Cox regression analysis introduced three factors, including BRAF^{AMP} and IDH1/2^{WT}, TP53 mutation, and ATRX mutation in all BRAF^{AMP} and BRAF mutation patients and determined the BRAF^{AMP} and IDH1/2^{WT} genotype as an independent predictive factor for overall survival (HR = 0.138, $P = 0.018$) (Figure 3).

Associations Between BRAF, IDH1, IDH2, TP53, and ATRX Using String Analysis

The networks showed that there were functional links between BRAF, IDH1, IDH2, TP53, and ATRX except for BRAF and ATRX and BRAF and IDH2. BRAF was directly associated with TP53 and indirectly interacted with ATRX through TP53. BRAF was directly associated with IDH1 and indirectly interacted with IDH2 through IDH1. There were direct interactions among TP53, ATRX, IDH1, and IDH2 (Figure 4A). The combined score of the association showed that the highest score was that between IDH1 and IDH2 (0.976), followed by TP53 and ATRX (0.793), TP53 and IDH1 (0.770), IDH1 and ATRX (0.731), IDH2 and TP53 (0.700), ATRX and IDH2 (0.669), BRAF and TP53 (0.561), and BRAF and IDH1 (0.409) (Figure 4B).

DISCUSSION

Glioma is the most common primary brain malignancy and is characterized by high heterogeneity and extensive mutations (26). The roles of RAF serine/threonine protein kinases in various cancers have been investigated in the last two decades. BRAF regulates normal cell growth, differentiation, and survival via the

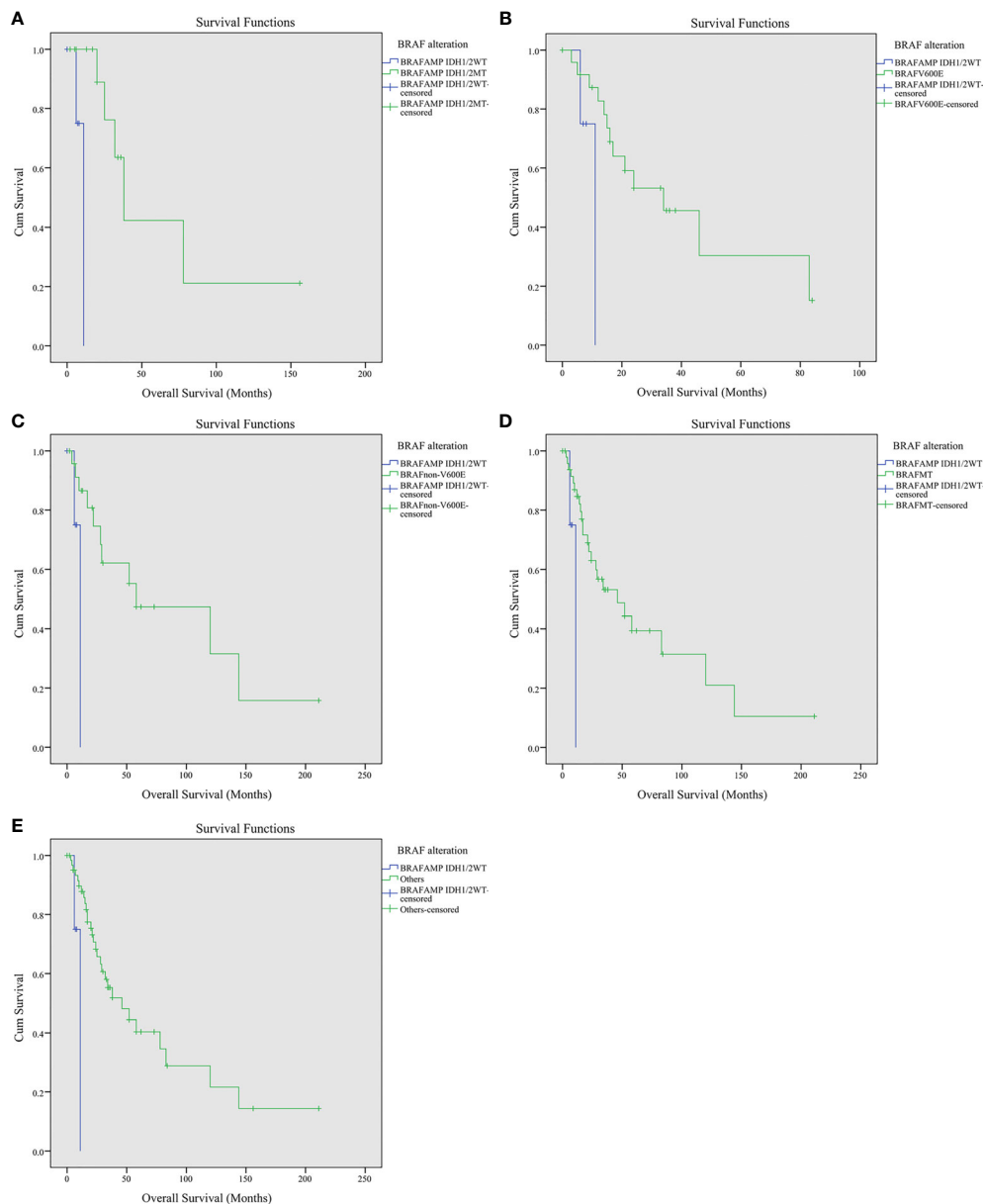


FIGURE 2 | Kaplan-Meier survival curves of patients with gliomas harboring *BRAF*^{AMP} and *BRAF* mutation. **(A)** *BRAF*^{AMP} and *IDH1/2*^{WT} cohort vs. *BRAF*^{AMP} and *IDH1/2*^{MT} cohort (9.750 vs. 67.026, chi-square 10.526, $P = 0.001$). **(B)** *BRAF*^{AMP} and *IDH1/2*^{WT} cohort vs. *BRAF*^{V600E} cohort (9.750 vs. 41.573, chi-square 4.536, $P = 0.033$). **(C)** *BRAF*^{AMP} and *IDH1/2*^{WT} cohort vs. *BRAF*^{non-V600E} cohort (9.750 vs. 89.958, chi-square 4.747, $P = 0.029$). **(D)** *BRAF*^{AMP} and *IDH1/2*^{WT} cohort vs. *BRAF* mutation cohort (9.750 vs. 71.698, chi-square 5.469, $P = 0.019$). **(E)** *BRAF*^{AMP} and *IDH1/2*^{WT} cohort vs. other three *BRAF* alteration cohorts, including the *BRAF*^{AMP} and *IDH1/2*^{MT}, *BRAF*^{V600E}, and *BRAF*^{non-V600E} cohorts (9.750 vs. 74.401, chi-square 5.469, $P = 0.019$). *BRAF*, v-ras murine viral oncogene homolog B1; *IDH1/2*, isocitrate dehydrogenase 1 and 2.

MAPK/ERK pathway (27, 28). *BRAF* mutations and copy number variation have been widely investigated in melanoma, thyroid carcinoma, and lung and colon cancers (6, 29). Although *BRAF*^{V600E} is rarely found in adult gliomas, it occurs predominately in pediatric gliomas, accounting for 68%–80% of pleomorphic xanthoastrocytoma (PXA), 20%–70% of ganglioglioma, 9%–10% of pilocytic astrocytoma (PA), 5%–15% of low-grade glioma (LGG), 20% of pediatric glioblastoma

(pGBM), and 3% of adult glioblastoma multiforme (GBM) cases (30–32). Because genetic alterations are important in tumor development and progression (33, 34) and both *BRAF*^{V600E} and *BRAF*^{AMP} can activate the MAPK pathway, we investigated the different effects of these two *BRAF* alterations and the mutations associated on the survival of glioma patients.

In this study, among the various *BRAF* mutations that were identified using next-generation sequencing, the most frequent

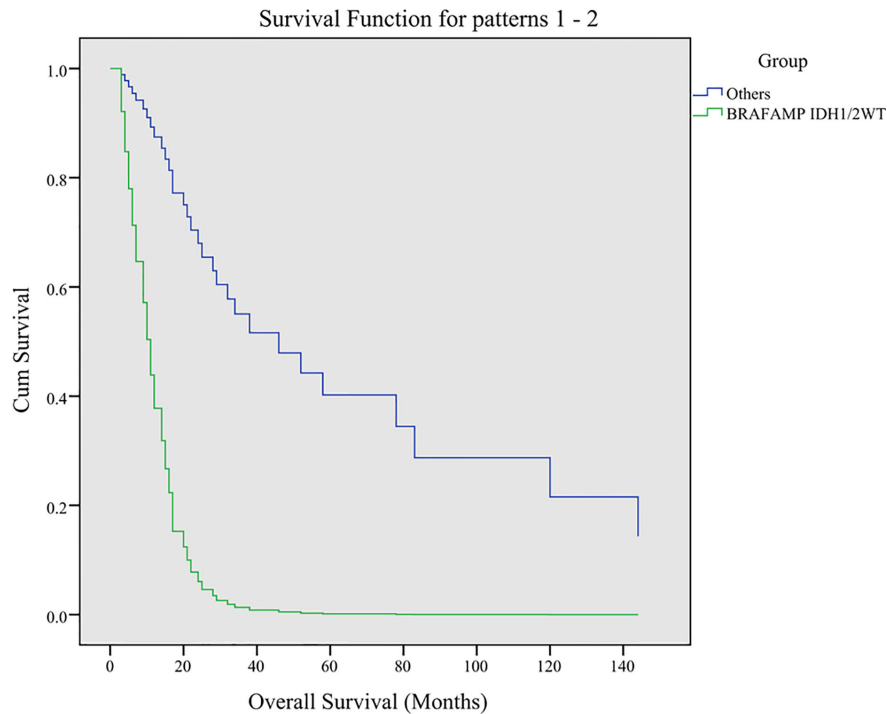


FIGURE 3 | Multivariate analysis of overall survival using Cox regression analysis. Three factors including BRAF^{AMP} and IDH1/2^{WT}, TP53, and ATRX were analyzed. The BRAF^{AMP} and IDH1/2^{WT} genotype was determined as an independent predictive factor for overall survival (HR = 0.138, P = 0.018). BRAF, v-raf murine viral oncogene homolog B1; IDH1/2, isocitrate dehydrogenase 1 and 2.

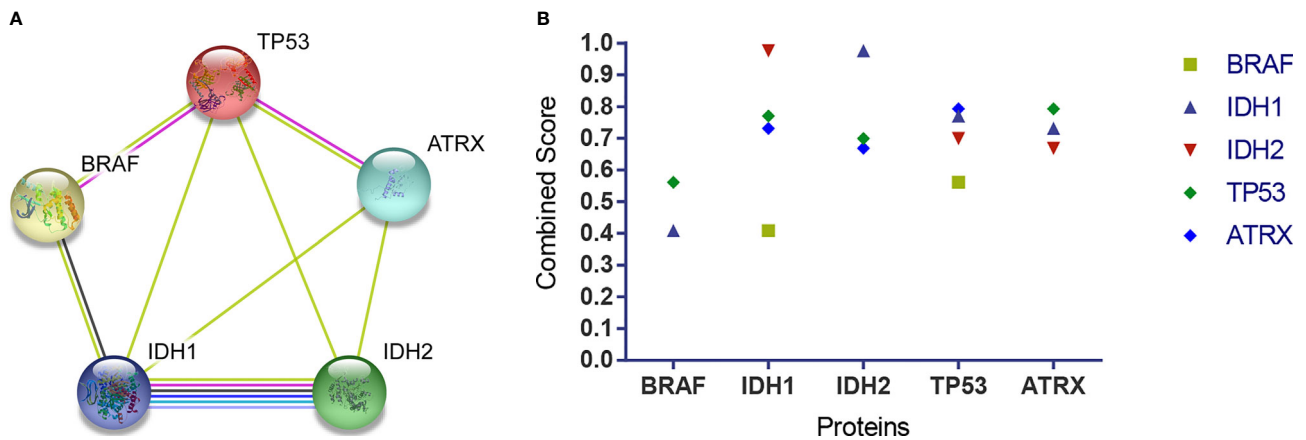


FIGURE 4 | The association among BRAF, IDH1, IDH2, TP53, and ATRX proteins. **(A)** The networks showed functional links among these four proteins, except for BRAF and ATRX and BRAF and IDH2. TP53 is associated with BRAF and ATRX via a known interaction (experimentally determined, the pink edge) and another interaction (text mining, the lime green edge), respectively. BRAF is associated with IDH1 via other interactions (coexpression, the black edge; text mining, the lime green edge). IDH1 is associated with IDH2 via known interactions (from curated databases, the jungle green edge; experimentally determined, the pink edge), predicted interaction (gene co-occurrence, the blue edge), and other interactions (text mining, the lime green edge; coexpression, the black edge; protein homology, the violet edge). TP53 is associated with IDH1 and IDH2, and ATRX is associated with IDH1 and IDH2 via another interaction (text mining, the lime green edge) respectively. **(B)** The combined score showed that the highest score was that between IDH1 and IDH2 (0.976), followed by TP53 and ATRX (0.793), TP53 and IDH1 (0.770), IDH1 and ATRX (0.731), IDH2 and TP53 (0.700), ATRX and IDH2 (0.669), BRAF and TP53 (0.561), and BRAF and IDH1 (0.409). BRAF, v-raf murine viral oncogene homolog B1; IDH1/2, isocitrate dehydrogenase 1 and 2; TP53, tumor protein p53; ATRX, alpha thalassemia/mental retardation syndrome X linked.

mutation was BRAF^{V600E}. Although some BRAF mutations are in the functional domains, other BRAF mutations with unknown functions occur across the gene (35). Patients with IDH1^{WT} glioma have a poor prognosis; however, patients with BRAF^{V600E} and IDH1^{WT} experience favorable outcomes. Andrew S. Chi et al. report that five patients with grade II glioma harboring BRAF^{V600E} without IDH1 mutation who had undergone gross total resection without treatment were progression-free for 14–35 months; two patients with glioblastoma harboring BRAF^{V600E} and IDH1^{WT} had a progression-free survival of 36 and 19 months, respectively (36). In addition, a study reported a glioma patient with BRAF^{V600E} without the IDH1 mutation who experienced 2 years of overall survival (37). Hiromichi Suzuki's study shows that IDH^{WT} in grade II and III gliomas (type III) is associated with a poorer overall survival rate compared with that of glioblastoma. In contrast, the grade II subtype (type IIIa) was associated with more BRAF mutations and better overall survival than the grade III subtype (type IIIb) (26). Patients with glioma harboring BRAF^{V600E} might benefit from MAPK pathway inhibitor target therapy, a rescue treatment that includes the use of RAF inhibitors and MEK inhibitors alone or in combination (38–41), and the results were encouraging (42). Our data show that the survival of the BRAF^{non-V600E} cohort was comparable to that of the BRAF^{V600E} cohort.

We also find that the gross survival of the BRAF^{AMP} cohort was comparable to that of both the BRAF^{V600E} and BRAF^{non-V600E} cohorts. Because the IDH1/2 mutation was frequently present in the BRAF^{AMP} cohort, we divided this cohort into two groups based on the absence/presence of the IDH1/2 mutation in order to elucidate the exact survival of patients with BRAF^{AMP} alone and without the interference of the IDH1/2 mutation. We found that the BRAF^{AMP} and IDH1/2^{WT} cohort had reduced overall survival compared with that of the BRAF mutation cohort (BRAF^{V600E} and BRAF^{non-V600E}) and the BRAF^{AMP} and IDH1/2^{MT} groups. We propose two possible reasons for this. First, the mRNA and protein expression levels of BRAF^{AMP} may be higher than those of BRAF^{V600E}, resulting in higher activation of the MAPK/ERK pathway and subsequent proliferation of cancer cells. Second, the survival of patients with BRAF^{AMP} and IDH1/2^{MT} was comparable to that of patients with BRAF mutations and greater than that of patients with BRAF^{AMP} and IDH1/2^{WT}, probably because the IDH1/2 mutation and 2-HG can induce oxidative stress, autophagy, and apoptosis in cancer cells. We believe that these two reasons may explain the poor survival of the BRAF^{AMP} and IDH1/2^{WT} cohorts. Young adult patients are enriched with BRAF^{V600E} mutations and have better survival than older patients; we reveal that the survival of patients above 30 years of age in the BRAF^{AMP} and IDH1/2^{WT} cohort was also significantly reduced compared with that of the BRAF^{V600E} cohort above 30 years ($P = 0.018$).

The results of Cox proportional hazard regression analysis show that BRAF^{AMP} and IDH1/2^{WT} genotype was an independent predictive factor for glioma with BRAF mutation and BRAF^{AMP}. IDH1/2 mutations exist in greater than 70% of lower-grade gliomas (grades II and III) and in some glioblastomas (43, 44). It is known that the IDH1/2 mutation leads to hypermethylation, which is the molecular basis of the

CpG island methylator phenotype in gliomas (45). We found that BRAF^{AMP} cohorts have lower survival compared with BRAF mutation cohorts, including BRAF^{V600E} and BRAF^{non-V600E}. However, the survival of patients with BRAF^{AMP} and IDH1/2^{MT} was better than that of patients with BRAF^{AMP} and IDH1/2^{WT} and comparable to that of the BRAF^{non-V600E} cohort. A previous study indicated that IDH1/2 mutation status alone was a predictive factor for longer overall survival and progression-free survival for the entire group of nonenhancing hemispheric grade II–III gliomas (46). Therefore, we propose that IDH1/2 mutations can improve the survival of cohorts with BRAF^{AMP}. Because the mutant IDH1 and 2-HG can induce oxidative stress, autophagy, and apoptosis (47), we propose that this is the mechanism underlying the improvement in survival conferred by the IDH1/2 mutation.

Most of the studies of BRAF^{V600E} in gliomas focus on pediatric neoplasms, especially in gangliogliomas and PXA (48–50). As all the patients enrolled in this study were adults, our findings provide insight into the effects of BRAF alterations in adult glioma. In addition to their diagnostic role, BRAF mutations may also have a prognostic value (51). Our data show that males accounted for the majority of patients in the BRAF mutation cohort, compared with the BRAF^{AMP} cohort. The occurrence of GBM was higher in the BRAF mutation cohort than in the BRAF^{AMP} cohort, whereas the BRAF^{AMP} group had significantly more patients with the IDH1/2, TP53, and ATRX mutations. ATRX deletions/mutations are associated with several conventional molecular events, including IDH1 and TP53 mutations (52, 53). Somatic mutations in TP53, ATRX, and IDH1/2 have been identified in adult low-grade gliomas (54). Although IDH1/2 mutations are scarce in primary GBM, they are common in diffuse/anaplastic gliomas and secondary GBM (43, 44). ATRX mutations are detected in adult diffuse gliomas and astrocytomas harboring both TP53 and IDH1/2. The co-occurrence of TP53, IDH1/2, and ATRX mutations facilitates the growth of a subgroup of adult diffuse astrocytomas (55). All of the above studies indicate that ATRX mutations frequently overlap with IDH1 and TP53 mutations. Additionally, our string analysis reveals close connections between BRAF, IDH1, IDH2, TP53, and ATRX proteins, similar to previous studies (55). Moreover, our results show that BRAF has direct reactions with TP53 and IDH1 but not with ATRX.

Active Ras can induce the hetero-dimerization of BRAF and CRAF (56), and BRAF can phosphorylate CRAF through direct protein–protein interactions (57, 58). CRAF exerts anti-apoptotic effects, which are mediated by an independent MAPK pathway (59, 60) through direct binding to Bcl-2 (59). TP53 can regulate Bcl-2 by suppressing Bcl-2 transcription (61). Liu et al. (55) find that ATRX alterations are correlated with mutations in IDH1/2 and TP53 in glioma of all grades. Lai et al. (62) find that the rate of Arg-to-Cys substitutions at position 273 in TP53 is higher than that of Arg-to-His substitutions at position 132 in IDH1. They propose that this event is caused by a strand asymmetry mechanism (63) in which C to T mutations occur in the nontranscribed DNA strand in TP53 and IDH1 mutations occur in the transcribed strand in IDH. The

study indicates that *IDH1/2* mutations represent early events in brain tumor formation (64). We propose that an increase in BRAF activates Bcl-2 by phosphorylating CRAF, and mutated *TP53* fails to regulate Bcl-2 but frequently accompanies *IDH1/2* mutation *via* a strand asymmetry mechanism. Further work using appropriate clinical tissue samples or animal models is required to provide some evidence for this proposal.

In conclusion, our study shows that BRAF^{AMP} and *IDH1/2*^{WT} is related to the reduced survival in adult patients with glioma compared with BRAF^{V600E} and that BRAF^{AMP} is associated with mutations in *IDH1*, *TP53*, and *ATRX*. This suggests that assessment for BRAF^{AMP} and *IDH1/2*^{WT} alterations is of prognostic value in adult glioma/glioblastoma patients because patients with this gene alteration pattern have relatively shorter survival times and may benefit from personalized, targeted therapy using BRAF and/or MEK inhibitors. As noted above, a concentrated effort is required to prospectively evaluate these findings in adult glioma patients.

DATA AVAILABILITY STATEMENT

The results published or shown here are in whole or part based upon data generated by the TCGA Research Network: <https://www.cancer.gov/tcga>.

ETHICS STATEMENT

All data collected and generated from TCGA, which is a public database, and we strictly followed TCGA publication guidelines (<https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/using-tcga/citing-tcga>).

AUTHOR CONTRIBUTIONS

RD, WW, MW, and HJ conceived and designed the work. RD and WW performed data analysis. RD, WW, and TW wrote the

manuscript. MW and HJ revised the paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fonc.2020.531968/full#supplementary-material>

SUPPLEMENTARY FIGURE 1 | Kaplan-Meier Survival curves of patients with gliomas harboring BRAF^{AMP} and BRAF mutation. A: BRAF^{AMP} cohort vs. BRAF mutation cohort (58.835 vs. 71.698, Chi-Square 0.020, P = 0.886); B: BRAF^{V600E} cohort vs. BRAF^{non-V600E} cohort (41.573 vs. 89.958, Chi-Square 1.999, P = 0.157); C: BRAF^{AMP} & *IDH1/2*^{MT} cohort vs. BRAF^{V600E} cohort (67.026 vs. 41.573, Chi-Square 1.031, P = 0.310); D: BRAF^{AMP} & *IDH1/2*^{MT} cohort vs. BRAF^{non-V600E} cohort (67.026 vs. 89.958, Chi-Square 0.025, P = 0.875); E: BRAF^{AMP} & *IDH1/2*^{MT} cohort vs. BRAF mutation cohort (67.026 vs. 71.698, Chi-Square 0.513, P = 0.474). BRAF, v-raf murine viral oncogene homolog B1; *IDH1/2*, isocitrate dehydrogenase 1 and 2.

SUPPLEMENTARY FIGURE 2 | Kaplan-Meier Survival curves of patients above 30 years of age with gliomas harboring BRAF^{AMP} & *IDH1/2*^{WT} and BRAF^{V600E}. BRAF^{AMP} & *IDH1/2*^{WT} cohort vs. BRAF^{V600E} cohort (9.750 vs. 40.135, chi-square 5.575, P = 0.018). BRAF, v-raf murine viral oncogene homolog B1; *IDH1/2*, isocitrate dehydrogenase 1 and 2.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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