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Research Paper

AIB1 Genomic Amplification Predicts Poor Clinical Outcomes in Female Glioma Patients

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Abstract

Amplified in breast cancer 1 (*AIB1*) gene, a coactivator for steroid receptor, is frequently amplified in diverse cancers and is considered as an oncogene in tumorigenesis. However, the prognostic significance of *AIB1* amplification in gliomas remains totally unclear. In this study, 115 gliomas and 16 benign meningiomas as control subjects were enrolled, and the copy number of *AIB1* was analyzed in these samples. In addition, we explored potential correlation of *AIB1* amplification with clinicopathological characteristics and clinical outcomes of glioma patients. Our data showed that glioma samples exhibited a significantly higher *AIB1* copy number than control subjects as determined by quantitative polymerase chain reaction (qPCR) approach. Moreover, univariate analysis showed that *AIB1* amplification (≥3.5 copies) was strongly correlated with cancer-related death (P =0.03). Interestingly, our data revealed a significant association of *AIB1* amplification with WHO grade (P =0.03), tumor recurrence (P =0.03) and survival status (P =0.03) in female patients but not in male patients. Multivariate analysis further demonstrated that *AIB1* amplification was independent factor for cancer-related death in female patients. Importantly, *AIB1* amplification was closely relevant to worse survival in female patients (P =0.001), but not in male patients (P =1.00). In addition, the patients with *AIB1* amplification were resistant to radiotherapy. Altogether, our data demonstrate that *AIB1* amplification is a common genetic event in glioma tumorigenesis, and suggest that *AIB1* amplification is not only a prognostic factor for poor clinical outcomes in glioma patients, but also a predictor of radiotherapy resistance in gliomas.

Key words: Glioma, *AIB1* amplification, clinical outcomes, radiotherapy resistance

Introduction

Glioma is the most prevalent primary brain tumor in the central nervous system (CNS) and is characterized by high recurrence and mortality rates [1, 2]. Gliomas are generally categorized into four grades (I-IV) based on the World Health Organization (WHO) classification, including pilocytic astrocytoma (PA), diffuse astrocytoma (DA), anaplastic astrocytoma (AA) and glioblastoma (GBM) [3]. Despite considerable progresses in the application of comprehensive treatment strategies for glioma patients, the 5-year survival rate still remains poor [4, 5]. Therefore, valuable genomic biomarkers with increased sensitivity and reliability are critically required for predicting clinical outcomes and

establishing new therapeutic and preventive strategies for gliomas.

Gene amplification is considered as a pivotal molecular process of tumorigenesis through increasing gene copy number and subsequently activating the oncogenic potential of proto-oncogenes [6]. Similar to other tumors, glioma is characterized by changes in the expression of oncogenes and tumor suppressor genes due to numerical chromosomal abnormalities such as genomic gains and losses [7-10]. Molecular profiling of glioblastoma has recently demonstrated that expression of ~76% of genes with recurrent genomic copy number alterations (CNAs) is closely correlated with their copy number [7].

AIB1 (also known as steroid receptor coactivator-3, SRC-3) is a member of the p160 steroid receptor coactivator family. It is well-known that *AIB1* plays an oncogenic function in tumorigenesis by affecting several important carcinogenic signaling pathways [11, 12]. In recent years, *AIB1* has been reported to be frequently amplified in different types of cancer such as breast, ovarian, esophageal, colorectal, hepatocellular, gastric, pancreatic, bladder, nasopharyngeal and non-small-cell lung cancers (NSCLC) [12-20], and this genetic event is correlated with poor prognosis, aggressive tumor phenotype, progression and metastasis of tumors [21-23]. A recent study has revealed that AIB1 protein levels are much higher in high-grade astrocytomas than that in low-grade astrocytomas [24]. However, the prognostic significances of *AIB1* genomic amplification in gliomas remain totally unclear.

In this study, *AIB1* copy number was investigated in a cohort of gliomas and control subjects using qPCR approach. In addition, the correlation between *AIB1* amplification and clinical outcomes of glioma patients was also explored in this study.

Methods

Patients and Tissue Samples

A total of 115 glioma patients and 16 benign meningiomas as control subjects (10 females and 6 males, age 54.6 ± 7.1 years), who underwent surgery for brain tumors at the Department of Neurosurgery of First Affiliated Hospital of Xi'an Jiaotong University from 2006 to 2012, were randomly enrolled in this study. All patients did not receive radiotherapy or chemotherapy prior to surgery. Glioma patients received adjuvant radiotherapy and/or chemotherapy after surgery according to standard clinical protocols. All samples were histopathologically classified according to the WHO classification criteria. Overall survival was calculated as time duration starting from surgery until cancer-related death or last follow-up. Clinicopathological data of patients were presented in Table 1. All of the patients were enrolled after providing a written informed consent. This study was approved by the institutional review board.

Table 1. Clinicopathological characteristics of glioma patients.

Characteristics	No. of patients (%)
Gender	
Male	64 (55.7)
Female	51 (44.3)
Age, years	
Mean	45.3
Standard deviation	16.5
WHO grade	
I	16 (13.9)
II	57 (49.6)
III	28 (24.3)
IV	14 (12.2)
Recurrence	
Yes	86 (74.8)
No	29 (25.2)
Radiotherapy	
Yes	72 (62.6)
No	43 (37.4)
Chemotherapy	
Yes	48 (41.7)
No	67 (58.3)
KPS sore	
High	45 (39.1)
Low	70 (60.9)
Epilepsy	
Yes	54 (47.0)
No	61 (53.0)
Smoking	
Yes	33 (28.7)
No	82 (71.3)
Survival status	
Dead	66 (57.4)
Alive	49 (42.6)

Tissues and DNA Preparation

Formalin-fixed paraffin-embedded tissues were cut at 5 mm, and stained by hematoxylin and eosin (H&E). Tumor representative tissues were marked in each section by an expert cancer pathologist. Manual microdissection was carried out under an inverted light microscope by using the marked sections. DNA was extracted according to a previously described protocol [25]. In brief, the sections were first treated with xylene for 12h at room temperature for deparaffinization, followed by digestion with 1% sodium dodecylsulfate (SDS) and proteinase K at 48°C for 48h. Genomic DNA was then isolated from these tissues using a standard protocol.

Table 2. The primer and TaqMan probe sequences used in this study.

Gene	Forward primer sequence (5′→3′)	Probe sequence (5′→3′)	Reverse Primer sequence (5′→3′)
AIB1	CCTTACCAGGGTGAATTTTTTATTG	6FAM-ATCTGTGTGGCACGCCGCATTACTACA-TAMRA	GGGTTTGATGGAAATGTTCTTTCT
β-actin	TCACCCACACTGTGCCCATCTACGA	6FAM-ATGCCCTCCCCCATGCCATCC-TAMRA	TCGGTGAGGATCTTCATGAGGTA

Copy Number Analysis

Copy number of *AIB1* was analyzed in gliomas and control subjects by a well-established real-time quantitative PCR approach, which was previously validated by fluorescence *in situ* hybridization (FISH) [26, 27]. Primer Express 3.0 software (Applied Biosystems, Foster City, CA) was utilized to design specific PCR primers and TagMan probes for the amplification of *AIB1* gene and internal control *β-actin*. TaqMan probes were labeled at the 5' end with a fluorescent reporter 6-carboxyfluorescein (6FAM) and at the 3' end with a fluorescent quencher 6-carboxy-tetramethylrhodamine (TAMRA). The sequences were presented in Table 2. The PCR reaction was performed according to a previously described protocol [26]. Each sample was run in triplicate, and *β-actin* was performed in parallel to normalize the input DNA. Serially diluted leukocyte DNA was used to establish standard curves. DNA copy number was calculated as previously described [27, 28]. A copy number ≥3.5 was defined as gene amplification (or copy gain).

Statistical Analysis

Statistical analysis was performed using the SPSS 11.5 software (Chicago, IL, USA). *P* value <0.05 was considered statistically significant. Mann-Whitney *U*-test was applied to compare the copy number of *AIB1* between gliomas and control subjects. SPSS 11.5 software was used to univariately analyze the correlation of *AIB1* copy number and clinicopathological features. Multivariate analysis was performed to calculate multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for *AIB1* copy number, and other factors such as age, recurrence, radiotherapy and epilepsy. Cancer-related survival was calculated from the date of the operation to cancer-related death or last follow-up. Kaplan-Meier survival analysis was performed to evaluate the effect of *AIB1* amplification on patient survival. Log-rank test was used to analyze the differences between curves. The impact of *AIB1* amplification on the independent survival of age, radiotherapy and WHO grade was determined by multivariate Cox regression analysis.

Results

Frequent *AIB1* Amplification in Gliomas

The copy number of *AIB1*gene was examined in a cohort of gliomas and control subjects using qPCR assay. As shown in Figure 1, glioma patients exhibited significantly higher copy number of *AIB1* than the controls (meningioma patients) (Median, 2.78 copies *vs.* 1.99 copies; *P* =0.0003). When a copy number of ≥3.5was considered as gene amplification, we found *AIB1* amplification in 28/115 (24.3%) gliomas, whereas none in control subjects. To test the relationship between of *AIB1* copy number and its mRNA expression, we analyzed the corresponding data in a total of 435 low-grade gliomas using The Cancer Genome Atlas (TCGA) dataset from the Cancer Browser database (https://genome-cancer.soe.ucsc.edu). We divided all cases into low copy (L)-, median copy (M)- and high copy (H)-groups by use of two cutoff points (the 25 and 75 percentile of *AIB1* copy number). As shown in Figure 2A, mRNA expression of *AIB1* in H-group was significantly higher than that M- and L-groups. Given that chemotherapy and radiation therapy may affect mRNA expression of *AIB1*, we only analyzed their association in the cases who did not receive any therapy. Similar to the findings in Figure 2A, we still found a high mRNA expression of *AIB1* in H-group compared with M- and L-groups (Figure 2B).

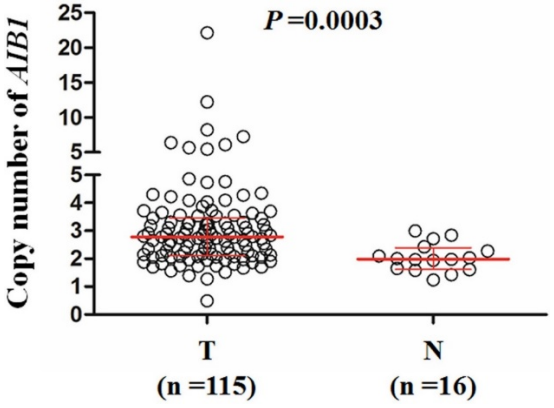

Figure 1. Copy number of *AIB1* in a cohort of gliomas and control subjects. *AIB1* copy number of each case was determined by a qPCR assay. Each circle represents the *AIB1* copy number of an individual case. Horizontal lines indicate median and inter-quartiles (25-75%). T: tumor tissues; N: control subjects.

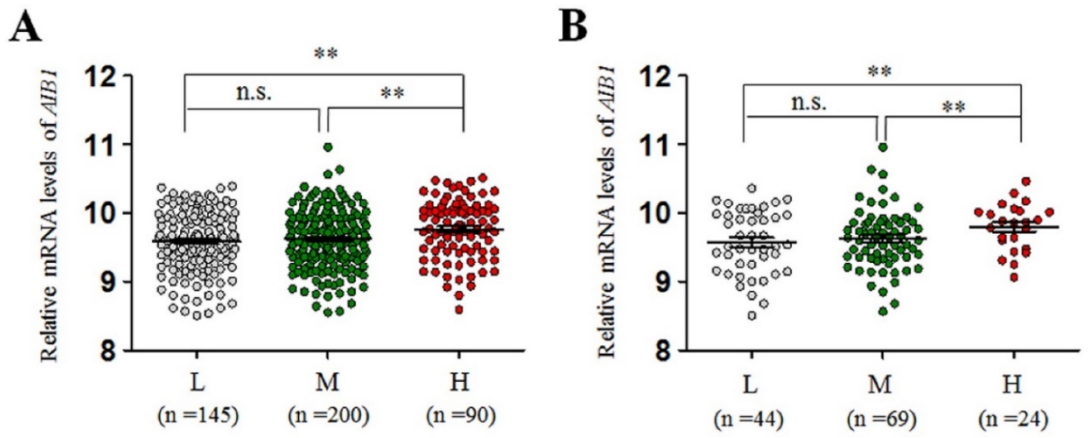

Figure 2. The relationship between copy number of *AIB1* and its mRNA expression in low-grade gliomas from The Cancer Genome Atlas (TCGA) dataset. (**A**) All glioma patients. (**B**) The patients who did not receive chemotherapy or radiation therapy. Horizontal lines indicate median and inter-quartiles (25-75%). L, low copy number of *AIB1*; M, medium copy number of *AIB1*; H, high copy number of *AIB1*; **, *P*<0.01.

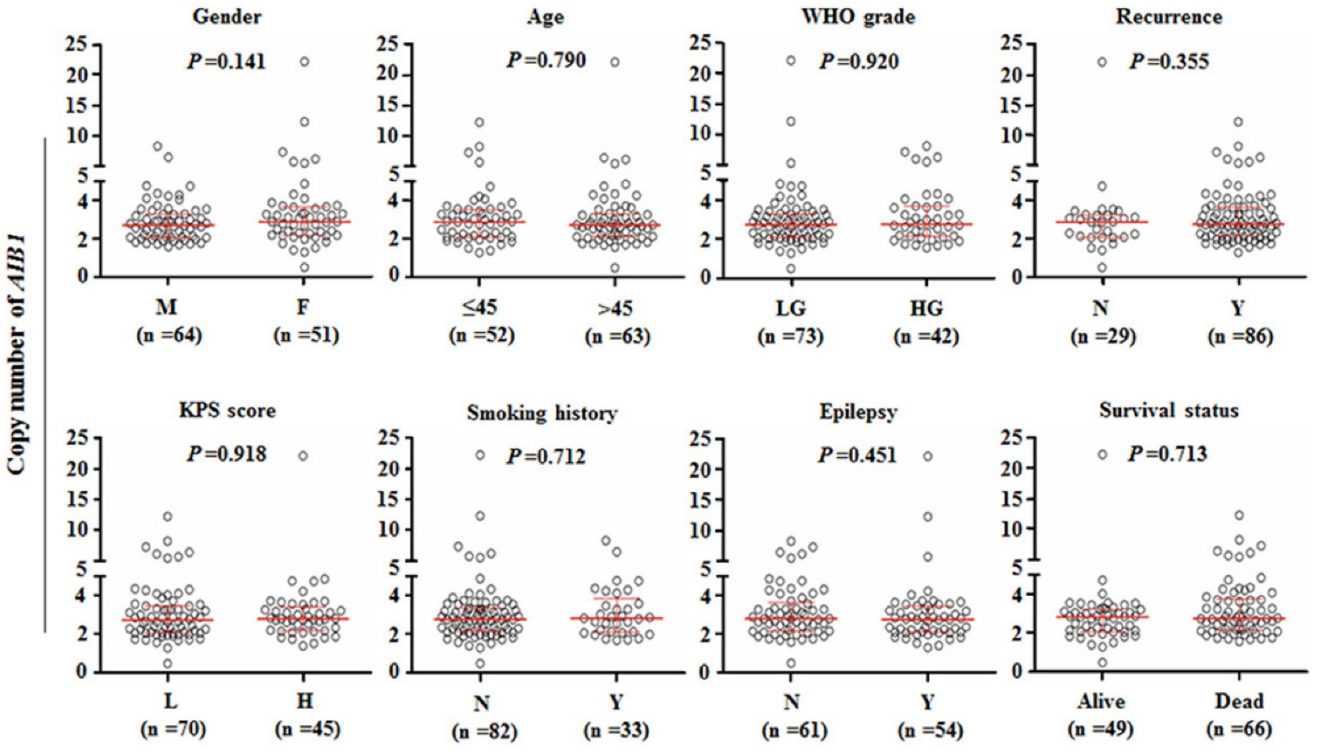

Figure 3. Relationship between *AIB1* copy number and clinicopathological features in glioma patients. Copy number of *AIB1* was evaluated by a qPCR method. Each circle represents the *AIB1* copy number of an individual case. Horizontal lines indicate median and inter-quartiles (25-75%). Mann-Whitney U test was used for the comparison of sample medians. M: male; F: female; LG: low-grade tumors; HG: high-grade tumors; L: low; H: high; N: No; Y: Yes.

Next, we analyzed the *AIB1* copy number grouped by the indicated clinicopathological features such as gender, age, WHO grade, tumor recurrence, Karnofsky performance status (KPS) score, smoking history, epilepsy and survival status. As shown in Figure 3, our data did not show significant relationships between *AIB1* copy number and clinicopathological features. However, we noted that copy number of *AIB1*was slightly higher in female patients than male patients (Median, 2.87 copies *vs.* 2.69 copies).

Association of *AIB1* Copy Number with Clinicopathological Features in Gliomas

Given frequent *AIB1* amplification in gliomas, the relationships between *AIB1* amplification and clinicopathological features were investigated in a cohort of gliomas. We defined a copy number of ≥3.5 as amplification. The glioma patients were subsequently categorized into *AIB1* amplification and non-amplification groups. As shown in Table 3, the risk of cancer-related death was significantly increased by the presence of *AIB1* amplification (OR

=2.80, 95% CI =1.08-7.26; *P* =0.03). However, there were no significant correlations between *AIB1* amplifications and other clinicopathological characteristics such as gender, age, WHO grade, recurrence, radiotherapy, chemotherapy, KPS score, epilepsy and smoking.

Table 3. *AIB1* amplification in gliomas: univariate associations with clinicopathological characteristics.

Characteristics	Copy number	
	OR* (95% CI)	*P*
Gender	0.61 (0.26-1.44)	0.26
Age[1]	0.78 (0.33-1.82)	0.56
WHO grade[2]	1.23 (0.75-2.00)	0.41
Recurrence	2.42 (0.76-7.69)	0.13
Radiotherapy	2.12 (0.81-5.51)	0.12
Chemotherapy	0.87 (0.37-2.09)	0.76
KPS score[3]	0.99 (0.41-2.37)	0.99
Epilepsy	0.80 (0.34-1.90)	0.62
Smoking	1.24 (0.50-3.13)	0.64
Survival status[4]	2.80 (1.08-7.26)	0.03

*OR: odds ratio with 95% confidence interval (CI); [1]Age (per 10 years); [2]WHO grade (I, II, III and IV); [3]KPS (>80; ≤80); [4]Survival status (alive *vs.* dead).

The patients were further categorized into two groups according to gender. Interestingly, we did not find that *AIB1* amplification was correlated with cancer-related death (OR =0.95, 95% CI =0.27-3.33; *P* =0.94) in male patients (Table 4). However, we found a significant association of *AIB1* amplification with cancer-related death (OR =11.50, 95% CI =2.24-59.01; *P* =0.03) in female patients (Table 4). Moreover, *AIB1* amplification was significantly associated with WHO grade (OR =4.00, 95% CI =1.11-14.43; *P* =0.03) and tumor recurrence (OR =11.20, 95% CI =1.33-94.49; *P* =0.03) in female patients (Table 4). Next, multiple multivariable logistic regressions were conducted to analyze the independent association of *AIB1* amplification with age, WHO grade, radiotherapy, epilepsy and cancer-related death. Similar to the findings from univariate analysis, *AIB1* amplification was still closely correlated with cancer-related death in glioma patients (OR =3.76, 95% CI =1.21-11.66; *P* =0.02), particularly in female patients (OR =10.60, 95% CI =1.56-72.14; *P* =0.02) (Table 5).

Effect of *AIB1* Amplification on Poor Survival of Glioma Patients

We next conducted the univariate survival analysis to determine the potential relationship between *AIB1* amplification and poor patient survival. As shown in Table 6, *AIB1* amplification was notably correlated with poor survival of patients (HR =1.77, 95% CI =1.05-2.98; *P* =0.03). Further analysis showed a significant relationship between *AIB1* amplification and poor survival in female patients (HR =3.41, 95% CI =1.57-7.43; *P* =0.002), but not in male patients (HR =1.00, 95% CI =0.46-2.18; *P* =0.99). In order to clarify clinical significance of *AIB1* amplification in prognosing patient survival, Cox multivariate regression analysis was conducted in the present study. Also shown in Table 6, *AIB1* amplification was identified as an independent variable for predicting the poor survival in glioma patients (HR =1.78, 95% CI =1.00-3.13; *P* =0.048).

Table 4. *AIB1* amplification in female and male glioma patients: univariate associations with clinicopathological characteristics.

Characteristics	Female patients		Male patients	
	OR* (95% CI)	P	OR* (95% CI)	P
Age[1]	1.02 (0.31-3.42)	0.97	0.60 (0.18-2.04)	0.41
WHO grade[2]	4.00 (1.11-14.43)	0.03	0.82 (0.43-1.57)	0.55
Recurrence	11.20 (1.33-94.49)	0.03	0.71 (0.16-3.13)	0.66
Radiotherapy	3.67 (0.72-18.88)	0.12	1.31 (0.38-4.57)	0.67
Chemotherapy	1.55 (0.46-5.25)	0.48	0.50 (0.14-1.83)	0.30
KPS score[3]	1.50 (0.44-5.09)	0.52	0.73 (0.21-2.60)	0.63
Epilepsy	0.78 (0.23-2.62)	0.69	0.76 (0.22-2.65)	0.67
Survival status[4]	11.50 (2.24-59.01)	0.03	0.95 (0.27-3.33)	0.94

*OR: odds ratio with 95% confidence interval (CI); [1]Age (per 10 years); [2]WHO grade (I, II, III and IV); [3]KPS (>80; ≤80); [4]Survival status (alive *vs.* dead).

Table 5. *AIB1* amplification in all and female glioma patients: multivariable models assessing age, WHO grade, radiotherapy, epilepsy and survival status.

Characteristics	All patients		Female patients		Male patients	
	OR* (95% CI)	P	OR* (95% CI)	P	OR* (95% CI)	P
Age[1]	0.65 (0.25-1.68)	0.38	1.20 (0.24-5.97)	0.82	0.58 (0.15-2.22)	0.42
WHO grade[2]	0.98 (0.55-1.74)	0.95	1.06 (0.38-3.00)	0.91	0.78 (0.36-1.67)	0.51
Radiotherapy	2.59 (0.94-7.15)	0.07	3.64 (0.51-25.86)	0.20	1.22 (0.30-5.00)	0.78
Epilepsy	0.93 (0.36-2.40)	0.87	1.09 (0.23-5.14)	0.91	0.62 (0.15-2.54)	0.50
Survival status[3]	3.76 (1.21-11.66)	0.02	10.60 (1.56-72.14)	0.02	1.35 (0.26-6.98)	0.72

*OR: odds ratio with 95% confidence interval (CI); [1]Age (per 10 years); [2]WHO grade (I, II, III and IV); [3]Survival status (alive *vs.* dead).

Table 6. Prognostic value of clinicopathological factors and *AIB1* amplification using univariate and multivariate Cox regression analysis (n =115).

Variables	Univariate analysis		Multivariate analysis	
	Hazard Ratio (95% CI)	*P*	Hazard Ratio (95% CI)	*P*
Copy number				
<3.50	1.00 (reference)		1.00 (reference)	
≥3.50	1.77 (1.05-2.98)	0.03	1.78 (1.00-3.13)	0.048
Age				
≤45	1.00 (reference)		1.00 (reference)	
>45	2.26 (1.36-3.76)	0.002	2.05 (1.21-3.46)	0.008
Radiotherapy				
No	1.00 (reference)		1.00 (reference)	
Yes	0.51 (0.31-0.82)	0.006	0.51 (0.30-0.85)	0.01
WHO grade				
I	1.00 (reference)		1.00 (reference)	
II	2.43 (0.83-7.13)	0.11	2.51 (0.84-7.49)	0.10
III	9.93 (3.34-29.52)	<0.001	7.66 (2.52-23.31)	<0.001
IV	10.10 (3.17-32.25)	<0.001	10.12 (3.10-33.04)	<0.001

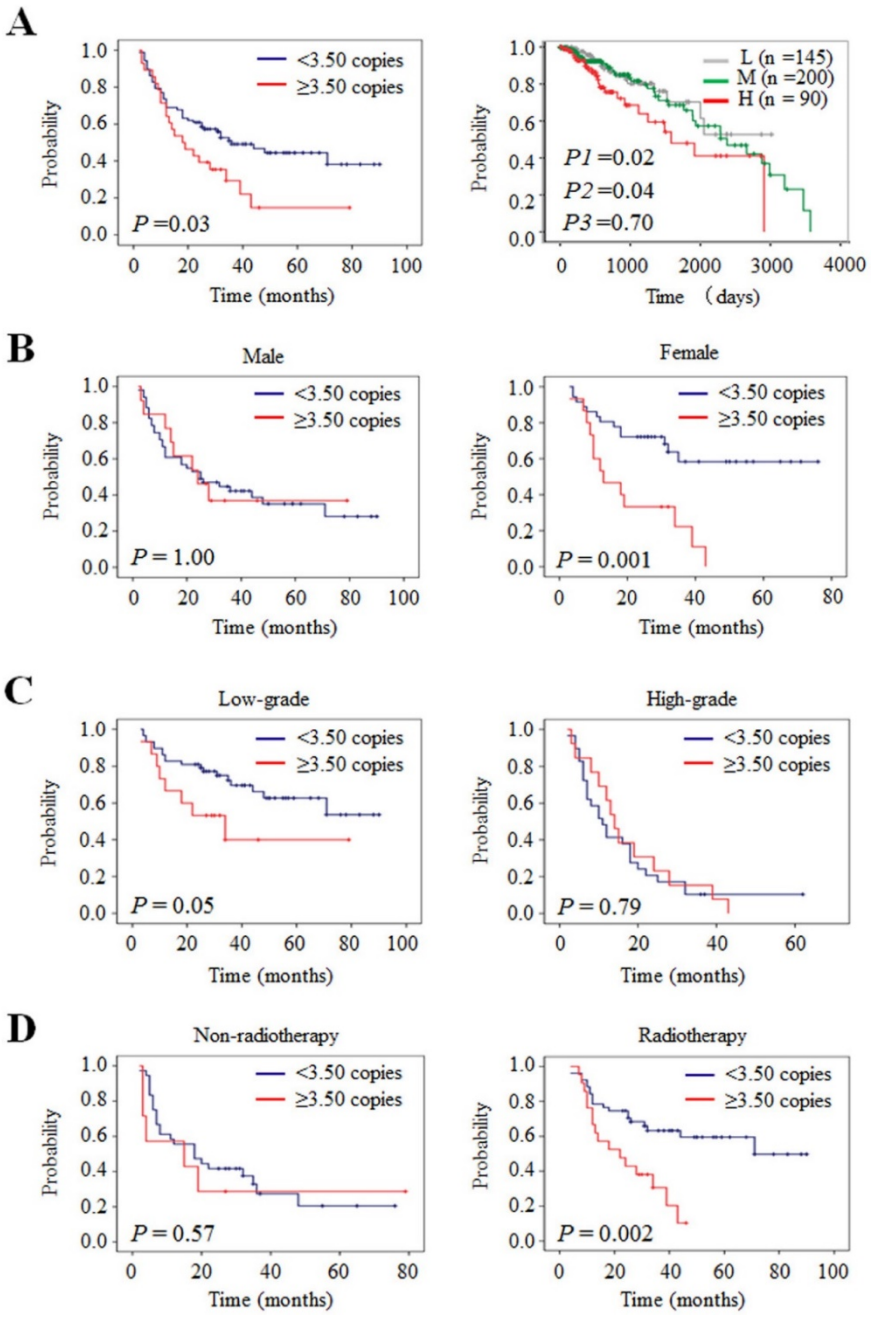

Figure 4. The impact of *AIB1* amplification on the survival of glioma patients. **(A)** Kaplan-Meier survival curves were grouped based on the status of *AIB1* amplification in gliomas from our cohort (left panel) and TCGA cohort (right panel). The presence of *AIB1* amplification caused a poorer overall survival than the absence of *AIB1* amplification in female patients (**B**), the patients with low-grade tumors (**C**), and the patients receiving radiotherapy (**D**), but not in male patients, the patients with high-grade tumors and the patients who did not receive radiotherapy. L, low copy number of *AIB1* (L-group); M, medium copy number of *AIB1* (M-group); H, high copy number of *AIB1* (H-group); *P1* for H-group *vs.* L-group; *P2* for H-group *vs.* M-group; *P3* for M-group *vs.* L-group.

Next, we used the Kaplan-Meier survival analysis to confirm the impact of *AIB1* amplification on patient survival. As expected, our data showed a significantly poorer survival in the patients with *AIB1* amplification than those without amplification (18.0 months *vs.* 36.0 months; P =0.03) (Table 7 and Figure 4A, left panel). This was supported by the TCGA dataset that high copy number of *AIB1* was closely related with worse survival as compared with medium (P =0.04) and low copy number (P =0.02), respectively (Figure 4A, right panel). In addition, to exclude the effect of chemotherapy and radiotherapy on patient survival, we only evaluated the prognostic value of *AIB1* amplification in the patients who did not receive any therapy. As expected, we still found that *AIB1* amplification was significantly related to poor survival of these patients (Supplementary Figure 1). Further analysis revealed that *AIB1* amplification

almost did not influence the survival of male patients (Figure 4B, left panel). However, it significantly shortened the survival times in female patients (Figure 4B, right panel). Accordingly, 1-, 2- and 3-year overall survival rates were much worse in female patients than male patients (Table 7). When the data were stratified according to WHO grade, we found that *AIB1* amplification was related to poor survival in patients with low-grade gliomas, but not in high-grade gliomas, although no statistical significance was obtained (Table 7 and Figure 4C).

Effect of *AIB1* Amplification on Radiotherapy Outcome of Glioma Patients

Given that radiotherapy is an important adjuvant therapy after surgical resection for glioma patients, we thus tested the effect of *AIB1* amplification on radiotherapy outcome in a cohort of gliomas. As shown in Figure 4D, *AIB1* amplification significantly shortened median survival times in the patients receiving radiotherapy (22.0 months *vs.* 71.0 months; P =0.002), but not in the patients who did not receive radiotherapy (15.0 months *vs.* 18.0 months; P =0.57). Accordingly, *AIB1* amplification was markedly associated with worse overall survival rates in the former, but not in the latter (Table 7). In addition, we did not find significant effect of *AIB1* amplification on chemotherapy outcome in glioma patients (Supplementary Figure 2). Collectively, our data indicate that *AIB1*amplification may be considered as a predictor of radiotherapy resistance in gliomas.

Discussion

Malignant glioma, a common primary tumor of central nervous system, is characterized by complex molecular heterogeneity and associated with poor clinical outcomes of patients [2, 28]. Therefore, understanding the underlying molecular disease mechanisms may lead to better management and appropriate therapeutic strategies to improve clinical outcomes of glioma. Gene amplification is a well-known prevalent mechanism of oncogene overexpression in human cancers [6]. Recent genomic studies in gliomas have shown frequent changes in DNA copy number, resulting in high levels of chromosomal instability [7, 29]. As a critical oncogene, *AIB1* amplification has been widely found in different types of cancer [30]. However, the association of *AIB1* amplification with therapeutic outcomes of glioma patients, and prognostic value of *AIB1* amplification in gliomas remains totally unknown. In the present study, we compared the copy number of *AIB1*gene between glioma patients and control subjects, and determined the prognostic significance of *AIB1*amplification in gliomas. Our data showed that *AIB1* was frequently amplified in gliomas, but not in control subjects. Moreover, our data showed that female glioma patients had higher *AIB1* copy number as compared to male patients. This was supported by a previous study that female patients had higher *AIB1* expression than male patients in NSCLC [31]. Another study demonstrated that there was a significantly higher *AIB1* expression in high-grade astrocytomas than that in low-grade astrocytomas, and high *AIB1* expression was associated with poor prognosis [24]. These results suggest that *AIB1* amplification is likely involved in glioma tumorigenesis.

Table 7. Overall survival by grouping with *AIB1* amplification.

Characteristics	n	Overall survival rate (%)			Overall survival time (months)	
		1 year (95% CI)	2 years (95% CI)	3 years (95% CI)	Median	95% CI
Total patients						
<3.50 copies	87	69.0 (59.2-78.8)	60.9 (50.7-71.1)	49.2 (38.0-60.4)	36.0	19.3-52.7
≥ 3.50 copies	28	64.3 (46.5-82.1)	39.3 (21.3-57.3)	29.5 (11.3-47.7)	18.0	7.6-28.4
Male						
<3.50 copies	51	60.8 (47.5-74.1)	52.9 (39.2-66.6)	42.1 (28.2-56.0)	25.0	9.3-40.7
≥ 3.50 copies	13	76.9 (54.0-99.8)	46.2 (19.2-73.2)	36.9 (9.9-63.9)	24.0	9.7-38.3
Female						
<3.50 copies	36	80.6 (67.7-93.5)	72.2 (57.5-86.9)	58.4 (39.8-77.0)	Not reached	--
≥ 3.50 copies	15	53.3 (28.0-78.6)	33.3 (9.4-57.2)	22.2 (-1.7-46.1)	13.0	2.9-23.1
Low-grade						
<3.50 copies	58	82.8 (73.0-92.6)	81.0 (71.0-91.0)	69.7 (57.0-82.4)	Not reached	--
≥ 3.50 copies	15	66.7 (42.8-90.6)	53.3 (28.0-78.6)	40.0 (10.4-69.6)	34.0	10.4-57.6
High-grade						
<3.50 copies	29	41.4 (23.6-59.2)	20.7 (6.0-35.4)	10.3 (-0.9-21.5)	11.0	6.8-15.2
≥ 3.50 copies	13	61.5 (35.0-88.0)	23.1 (0.2-46.0)	15.4 (-4.2-35)	14.0	10.5-17.5
Non-radiotherapy						
<3.50 copies	36	55.6 (39.3-71.9)	41.7 (25.6-57.8)	27.3 (10.2-44.4)	18.0	6.3-29.7
≥ 3.50 copies	7	57.1 (20.4-93.8)	38.6 (6.3-62.1)	28.6 (-4.9-62.1)	15.0	0.0-43.2
Radiotherapy						
<3.50 copies	51	78.4 (67.0-89.8)	74.5 (62.5-86.5)	63.2 (49.5-76.9)	71.0	--
≥ 3.50 copies	21	66.7 (46.5-86.9)	42.9 (21.7-64.1)	30.5 (9.1-51.9)	22.0	7.0-37.0

Next, we investigated the clinical significance and prognostic value of *AIB1* amplification in a cohort of gliomas. The results demonstrated that *AIB1* amplification was significantly correlated with WHO grade and tumor recurrence, and was an independent risk factor for cancer-related death in female glioma patients. Given the association of high *AIB1* expression with poor prognosis of glioma patients [24], we investigated the impact of *AIB1* amplification on patient survival. As expected, *AIB1* amplification was closely related to poor patient survival, particularly in female patients. Multivariate analysis demonstrated that *AIB1* amplification was an independent risk factor for poor patient survival. These findings suggest that this molecular event may contribute to clinical outcomes, and may thus serve as a potential therapeutic target in glioma patients, particularly in female patients.

*AIB1*as a steroid receptor coactivator of estrogen and progesterone receptors can enhance the transcription of target genes through being recruited to their promoters or enhancers via nuclear receptors [12, 32, 33]. In addition, there is evidence showing that estradiol can enhance the transcriptional activity of *AIB1* through increasing its phosphorylation and decreasing its sumoylation [34]. Estradiol also promotes the proliferation of astrocytoma cells through estrogen receptor-α (ERα) and its interaction with *AIB1* [35]. It is the fact that *AIB1* interacts with ERα, and subsequently binds to ERα-binding site on the promoter of *SNAI1*, a transcriptional repressor for E-cadherin, to promote the transcription of *SNAI1*and repress *E-cadherin* expression, ultimately leading to the initiation and progression of epithelial mesenchymal transition (EMT) [36]. Thus, we speculate that specific role and prognostic value of *AIB1* amplification in female glioma patients may be related to sex hormones and nuclear receptor levels.

Further analysis revealed that *AIB1* amplification was only related to poor patient survival in low-grade tumors, but not in high-grade tumors. Moreover, *AIB1* copy number did not show a significant difference between them. These findings indicate that *AIB1* amplification may be an early molecular event, and may affect the prognosis in early stage of glioma tumorigenesis. Interestingly, we observed that *AIB1* amplification significantly impacted radiotherapy outcome in glioma patients. Similar to these findings, a previous study showed that *AIB1* was related to chemo-radiotherapy (CRT) response in esophageal squamous cell carcinoma (ESCC). Compared to the CRT-effective group, *AIB1* overexpression was more frequently found in the CRT-resistant group [37]. However, the underlying molecular mechanism is still unknown. The PI3K/AKT/mTOR signal pathway has been identified as the predominant downstream pathway of *AIB1* [38, 39], contributing to radioresistance in different types of cancer including glioma [40-47]. Thus, we speculate that the activation of PI3K/AKT pathway may be one of molecular mechanisms underlying *AIB1*-induced radiotherapy resistance.

In summary, we found frequent *AIB1* amplification in gliomas, and demonstrated that this genetic event was closely related to poor prognosis in glioma patients, particularly in female patients. To our knowledge, our data for the first time reveals that *AIB1* amplification affects radiotherapy outcome in glioma patients. Collectively, these observations raise the possibility that *AIB1* amplification may be one of major driving forces in glioma tumorigenesis, and may be potentially prognostic marker for glioma patients particularly female patients.

Abbreviations

AA: anaplastic astrocytoma; *AIB1*: Amplified in breast cancer 1; CI: confidence interval; CNAs: copy number alterations; CNS: central nervous system; CRT: chemo-radiotherapy; DA: diffuse astrocytoma; DNA: deoxyribonucleic acid; EMT: epithelial mesenchymal transition; ERα: estrogen receptor alpha; ESCC: esophageal squamous cell carcinoma; FISH: fluorescence *in situ* hybridization; GBM: glioblastoma; H&E: hematoxylin and eosin; HR: hazard ratio; KPS: Karnofsky performance status; mRNA: messenger ribonucleic acid; NSCLC: non small cell lung cancer; OR: odds ratio; PA: pilocytic astrocytoma; PCR: polymerase chain reaction; SRC-3: steroid receptor coactivator-3; SDS: sodium dodecylsulfate; TCGA: The Cancer Genome Atlas; WHO: World Health Organization.

Supplementary Material

Supplementary figures.
http://www.jcancer.org/v07p2052s1.pdf

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Competing Interests

The authors have declared that no competing interest exists.

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