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Original Article

Overexpression of ZEB1 and YAP1 is related to poor prognosis in patients with gliomas with different IDH1 status

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Abstract: Objective: Whether there is a correlation between zinc-finger E-box-binding homolog 1 (ZEB1) and Yes-associated protein 1 (YAP1) with clinical outcome in gliomas remains unclear. Hence, this study aimed to investigate the effects of ZEB1 and YAP1 on the prognosis of human gliomas and its relationship with the isocitrate dehydrogenase 1 (IDH1) gene state. Methods: Immunohistochemical staining was used to record the expression levels of ZEB1, YAP1, and p-YAP1 in 122 cases of low-grade glioma (LGGs) and 69 cases of glioblastoma (GBMs). The correlations of ZEB1 and YAP1 with pathological data were determined by Pearson's Chi-square test. Spearman correlation analysis was then used for analyzing the relationship among YAP1, ZEB1, and IDH1 gene status. The effects of ZEB1 and YAP1 on prognosis were investigated through survival analysis. Results: We detected high ZEB1 expression levels in 29 LGGs (23.8%) and 39 GBMs (56.5%), and high YAP1 expression levels in 22 LGGs (18.0%) and 44 of GBM (63.8%). These results revealed that the protein expression levels of ZEB1 and YAP1 were higher in GBM ($P < 0.001$). There was a significantly positive correlation between ZEB1 and YAP1 ($P < 0.001$; $r = 0.533$). High ZEB1 expression was related to tumor grade ($P < 0.001$) and Ki-67 ($P = 0.0037$). YAP1 overexpression was correlated with Ki-67 ($P < 0.001$), P53 ($P = 0.009$), tumor grade ($P < 0.001$), and tumor location ($P = 0.018$). Patients with ZEB1 and YAP1 high expression had worse overall survival (OS) ($P < 0.001$). The multivariate analysis showed that YAP1 was an independent prognostic factor for OS. In the LGG group, worse OS were observed in glioma patients with elevated YAP1 expression level. Spearman correlation analysis revealed no association between ZEB1 expression and IDH1 state ($P = 0.360$; $r = -0.084$), and YAP1 expression had a negative correlation with IDH1 mutation ($P < 0.001$, $r = -0.364$). Conclusions: Our study showed that ZEB1 and YAP1 were significantly activated in GBM, and patients with high ZEB1 and YAP1 expression had worse OS. ZEB1 expression was significantly correlated with YAP1 in glioma. ZEB1 and YAP1 coexpression may serve as a useful prognostic biomarker for glioma, and aberrant YAP1 expression may be associated with IDH1 gene state.

Keywords: Glioma, ZEB1, YAP1, IDH1, pathological parameter

Introduction

Gliomas are the most common tumor of the central nervous system in adults [1]. According to the World Health Organization (WHO) criteria, gliomas are classified as grades I-IV, and WHO grade IV is referred to as glioblastoma (GBM). GBM is the most common type of glioma and is almost always fatal [2, 3]. The survival time of WHO grade II-III ranges from 1 year to 15 years [4, 5]. Owing to the highly aggressive nature of gliomas, complete neurosurgical resection is usually impossible. The presence of residual tumor leads to recurrence and malignant progression, and some gliomas progress to GBMs within months, whereas others are stable for several years [6]. Understanding the exact etiology and pathogenesis of the occurrence and

development of gliomas is of great significance for the personalized treatment of glioma. The WHO (2016) is the first to combine molecular markers with histological features to classify gliomas [7]. For example, isocitrate dehydrogenase (IDH) gene mutation is considered one of the important events in gliomas [8], and IDH1 mutations (IDH1mut) are present in 70%-80% of WHO type II-III gliomas [9, 10]. Low-grade gliomas (LGGs) with IDH1mut had favorable clinical outcome. However, the specific mechanisms by which IDH1mut drives gliomagenesis and improves prognosis have yet to be analyzed.

In our previous study, proteomics revealed that the Hippo/YAP signaling pathway is associated with the occurrence of gliomas [11], and further studies have shown that Yes-associated protein 1 (YAP1) may play an important role in the progression of gliomas [12]. Some studies have reported an association between Hippo/YAP signaling and zinc-finger E-box-binding homolog 1 (ZEB1), and YAP1 cooperates with ZEB1 in tumorigenesis [13]. ZEB1 is a member of the ZEB family of transcription factors involved in neoplastic transformation, tumor progression, and immunosuppression. ZEB1 also confers cells with an invasive and stem-like phenotype by driving epithelial-mesenchymal transition (EMT) [14, 15]. Elevated ZEB1 expression levels were observed in some tumor tissues, including colon cancer, lung cancer, and bladder cancer, and are associated with metastasis and poor prognosis [16]. Although ZEB1 overexpression is mainly associated with tumorigenicity and malignant progression, several studies differ from traditional reports. Thus, the contradictory functions of ZEB1 in various types of cancer highlight the complex role of ZEB1 in tumorigenesis. Similar ZEB1 duality conflicts can be found in gliomas, where ZEB1 may act as a transcriptional activator or blocker, depending on the cellular environment and different regulatory signals [17].

The Hippo/YAP pathway regulates tissue growth by controlling cell proliferation, differentiation, metabolism, and apoptosis [18]. The regulation of these cellular processes is ultimately accomplished by the pathway-mediated localization of the downstream effectors YAP and transcriptional co-activator with PDZ-binding motif (TAZ). However, YAP phosphorylation prevents their nuclear localization, and thus YAP is negatively regulated by the Hippo signaling pathway. When the signaling pathway is inactive, nonphosphorylated YAP can enter the nucleus and bind to DNA by interacting with cofactors to induce the transcription of target genes [18]. However, persistent aberrant YAP activation enhances aberrant cell cycle progression and carcinogenesis. YAP has become a major determinant of malignancy in some tumors [19]. YAP includes YAP1 and YAP2. Although YAP1 plays an important role in the malignant progression of gliomas [20], the exact pathogenesis of glioma is not fully understood.

Recent reports have revealed a synergistic effect between Hippo/YAP signaling pathway and ZEB1 in tumor progression, but whether the association between ZEB1 and YAP1 has clinical significance to gliomas remains unclear. Furthermore, the IDH1 gene is involved in the development of LGG, but the relationships among YAP1, ZEB1, and IDH1 gene state have rarely been studied. In our study, the expression of ZEB1 and YAP1, p-YAP1 in 191 clinical glioma specimens were evaluated, and the clinicopathological data and survival analysis of glioma was analyzed. We found that the expression levels of ZEB1 and YAP1 were prominent in GBM compared to LGG, and patients with high ZEB1 and YAP1 expression had worse OS. Correlation analysis showed that ZEB1 is positively correlated with YAP1 expression, suggesting that the coexpression of ZEB1 and YAP1 may serve as a useful prognostic biomarker for glioma. Through further analysis, we found that the OS time of patients in the LGG group was short when YAP1 expression levels were high. YAP1 expression was negatively correlated with IDH1 mutation.

Materials and methods

Tissue specimens of surgical resections

The study was retrospective. A total of 191 formalin-fixed paraffin embedded (FFPE) blocks of gliomas were collected from the First Affiliated Hospital of Xinjiang Medical University from January 2010 to October 2014. According to the WHO (2016) classification of tumors of the central nervous system, all tissue sections were assessed by two pathologists. Grades II and III gliomas were classified as LGGs, and GBM was classified as high-grade gliomas (HGG) [20]. A total of 191 gliomas included 122 cases of LGG and 69 cases of GBM. All participants were informed of the purpose and con-

Table 1. Clinicopathologic characteristics of patients

Characteristic	LGG (II + III, n = 122)	HGG (GBM, n = 69)
Age		
< 50	83 (68.0%)	22 (31.9%)
≥ 50	39 (32.0%)	47 (68.1%)
Gender		
Male	50 (41.0%)	40 (58.0%)
Female	72 (59.0%)	29 (42.0%)
Ethnic		
Han	58 (47.5%)	40 (58.0%)
Other	64 (52.5%)	29 (42.0%)
Size of the main lesion		
< 3 cm	20 (16.4%)	12 (17.4%)
≥ 3 cm	102 (83.6%)	57 (82.6%)
Tumor location		
Frontal	69 (56.6%)	23 (33.3%)
Other	53 (43.4%)	46 (66.7%)
Selection of operation method		
Total resection	97 (79.5%)	25 (36.2%)
Partial resection	25 (20.5%)	44 (63.8%)
Postoperative		
Radiochemotherapy		
Yes	69 (56.6%)	37 (53.6%)
No	53 (43.4%)	32 (46.4%)
Ki-67		
< 30%	66 (54.1%)	15 (21.7%)
≥ 30%	56 (45.9%)	54 (78.3%)
P53		
< 5%	87 (71.3%)	36 (52.2%)
≥ 5%	35 (28.7%)	33 (47.8%)
IDH1 state		
IDH1mut	92 (75.4%)	-
IDH1wt	30 (24.6%)	-
Vital status		
Alive	72 (59.5%)	6 (9.4%)
Dead	49 (40.5%)	58 (90.6%)

tent of this study, and this study was also supported and approved by the hospital's ethics committee.

Immunohistochemical staining and assessment

Tissue microarrays (TMA) were constructed using FFPE, and a 4 μm-thick TMA section was made. All experimental procedures were carried out on an automated immunostainer (Bench Mark Ultra), and positive and negative controls were conducted. The primary antibodies were anti-(p)YAP1 (Abcam, EP1675Y, 1:250), anti-YAP1 (Abcam, EP1674Y; 1:100), and anti-ZEB1 (Abcam, EPR17375, 1:250). p-YAP1 staining was dominated by cytoplasmic staining, YAP1 localized in the cytoplasm and nucleus, and ZEB1 localized in the nucleus. In addition, the following biomarkers were routinely used for diagnosis, such as P53 (Bioss, bs-4181R, 1:200), Ki-67 (Bioss, bs-2130R, 1:500), GFAP (Bioss, bsm-52254R, 1:150) and Olig2 (Bioss, bs-11194R, 1:350).

The immunohistochemical results were evaluated as described by Guichet [20]. The percentage of positive cells was assessed as follows: 0 (no positive staining for tumor cells), 1 (positive staining for tumor cells ≤ 10%), 2 (positive staining for tumor cells ≤ 50%), and 3 (positive staining for tumor cells > 50%). The intensity of staining was evaluated with the following criteria: 0 (no staining), 1 (canary yellow), 2 (brown), and 3 (dark brown). The two scores were multiplied to produce histoscores (0-9), and then the final staining scores were divided into a low-expression group (0-4) and a high-expression group (5-9).

Statistical analysis

SPSS version 23.0 (IBM) and Graphpad Prism 7 software were used. The correlations between protein expression and clinicopathologic characteristics were determined by Pearson's chi-square test. Spearman correlation was used for analyzing the relationships among YAP1, ZEB1, and IDH1 status. Progression-free survival (PFS) and overall survival (OS) were used to represent the survival time. Kaplan-Meier method and Cox hazard regression analysis were used in determining univariate and multivariate significance in the survival analysis. The follow-up time ranged from 1 month to 115 months. The medians of PFS and OS were 23 and 43 months, respectively.

Results

Clinicopathologic characteristics of patients

The characteristics of glioma patients are summarized in **Table 1**. The total of 191 gliomas

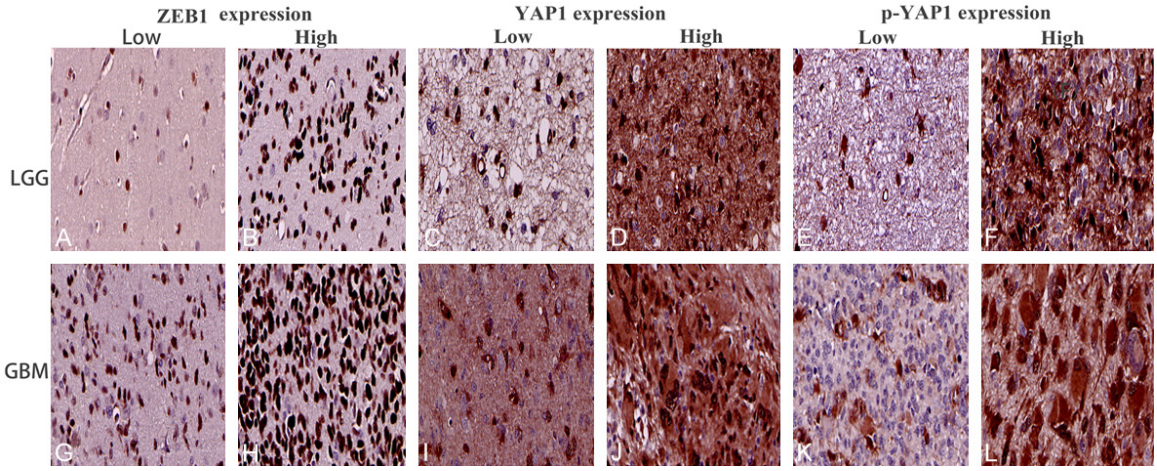

Figure 1. Expression of ZEB1, YAP1, and p-YAP1 in human glioma tissues (original magnification ×400). A-F. ZEB1, YAP1, and p-YAP1 expression in LGG. G-L. ZEB1, YAP1, and p-YAP1 expression in GBM.

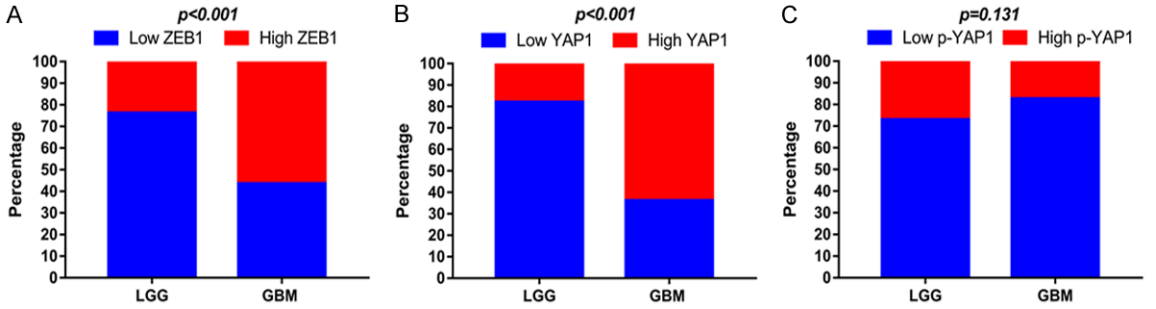

Figure 2. Proportion of ZEB1, YAP1, and P-YAP1 expression in LGG and GBM. A. ZEB1 high expression in 29 LGG (23.8%) and 39 GBM (56.5%). B. YAP1 high expression in 22 LGG (18.0%) and 44 GBM (63.8%). C. p-YAP1 high expression in 33 LGG (27.0%) and 12 GBM (17.4%).

included LGGs (122, 63.9%) and GBMs (69, 36.1%). 90 males and 101 females were included, with a mean age of 49 years (6-76 years). Other features included ethnicity (Han; 98, 51.3%), tumor size (< 3 cm; 32, 16.8%), tumor location in the frontal lobe (92, 48.2%), partial resection (69, 36.1%), postoperative radiochemotherapy (106, 55.5%), and live status (84, 43.9%). IDH1mut (92/122, 75.4%), IDH1 wild type (IDH1wt; 30/122, 24.6%), ki-67 expression of < 30% (81, 42.4%), and P53 expression of < 5% (123, 64.4%) were obtained.

Relationship between protein expression levels (ZEB1, YAP1, p-YAP1) and clinicopathologic features

We conducted immunostaining with ZEB1, YAP1, and YAP1 on 191 clinical glioma samples (**Figure 1**). The results showed that ZEB1 showed nuclear staining, and YAP1 was located in the cytoplasm and nucleus. The staining for p-YAP1 was mainly found in the cytoplasm. The ZEB1 protein was highly expressed in 29 LGGs (23.8%) and 39 GBMs (56.5%). ZEB1 expression was more pronounced in GBMs than in LGGs ($P < 0.001$; **Figure 2A**). YAP1 was highly expressed in 22 LGGs (18.0%) and 44 GBMs (63.8%), indicating that YAP1 expression was more obvious in GBM ($P < 0.001$; **Figure 2B**). p-YAP1 expression was not different in LGG and GBM ($P = 0.131$, **Figure 2C**). We assessed the relationship between the protein expression level and clinicopathologic characteristics (**Table 2**). Statistical differences in ZEB1 expression were found between tumor grade ($P < 0.001$) and Ki-67 ($P = 0.037$), and statistical differences in YAP1 expression were found among Ki-67 ($P < 0.001$), P53 ($P = 0.009$), tumor grade ($P < 0.001$), and tumor location ($P = 0.018$). No correlation linked p-YAP1 expression with clinicopathologic characteris-

Table 2. Relationship between protein expression levels (ZEB1, YAP1, p-YAP1) and clinicopathologic features

Characteristic	ZEB1			YAP1			p-YAP1		
	Low	High	*P*-value	Low	High	*P*-value	Low	High	*P*-value
Tumor grade									
LGG	93 (76.2%)	29 (23.8%)	P < 0.001*	100 (82.0%)	22 (18.0%)	P < 0.001*	89 (73.0%)	33 (27.0%)	P = 0.131
GBM	30 (43.5%)	39 (56.5%)		25 (36.2%)	44 (63.8%)		57 (82.6%)	12 (17.4%)	
Age									
< 50	70 (66.7%)	35 (33.3%)	P = 0.469	71 (67.6%)	34 (32.4%)	P = 0.485	80 (76.2%)	25 (23.8%)	P = 0.929
≥ 50	53 (61.6%)	33 (38.4%)		54 (62.8%)	32 (37.2%)		66 (76.7%)	20 (23.3%)	
Gender									
Male	52 (57.8%)	38 (42.2%)	P = 0.071	55 (61.1%)	35 (38.9%)	P = 0.234	69 (76.7%)	21 (23.3%)	P = 0.944
Female	71 (70.3%)	30 (29.7%)		70 (69.3%)	31 (30.7%)		77 (76.2%)	24 (23.8%)	
Ethnic									
Han	59 (60.2%)	39 (39.8%)	P = 0.214	64 (65.3%)	34 (34.7%)	P = 0.967	80 (81.6%)	18 (18.4%)	P = 0.083
Other	64 (68.8%)	29 (31.2%)		61 (65.6%)	32 (34.4%)		66 (71.0%)	27 (29.0%)	
Tumor size									
< 3 cm	21 (65.6%)	11 (34.4%)	P = 0.874	19 (59.4%)	13 (40.6%)	P = 0.429	24 (75.0%)	8 (25.0%)	P = 0.833
≥ 3 cm	102 (64.2%)	57 (35.8%)		106 (66.7%)	53 (33.3%)		122 (76.7%)	37 (23.3%)	
Tumor location									
Frontal	64 (69.6%)	28 (30.4%)	P = 0.151	68 (73.9%)	24 (26.1%)	P = 0.018*	70 (76.1%)	22 (23.9%)	P = 0.912
Other	59 (59.6%)	40 (40.4%)		57 (57.6%)	42 (42.4%)		76 (76.8%)	23 (23.2%)	
Ki-67									
< 30%	59 (72.8%)	22 (27.2%)	P = 0.037*	67 (82.7%)	14 (17.3%)	P < 0.001*	59 (72.8%)	22 (27.2%)	P = 0.314
≥ 30%	64 (58.2%)	46 (41.8%)		58 (52.7%)	52 (47.3%)		87 (79.1%)	23 (20.9%)	
P53									
< 5%	84 (67.2%)	41 (32.8%)	P = 0.266	90 (72.0%)	35 (28.0%)	P = 0.009*	96 (76.8%)	29 (23.2%)	P = 0.872
≥ 5%	39 (59.1%)	27 (40.9%)		35 (53.0%)	31 (47.0%)		50 (75.8%)	16 (24.2%)	

**P*-value < 0.05 indicates statistical significance.

Table 3. Relationship between the protein expression levels (ZEB1, YAP1) and IDH1 gene state

Protein	ZEB1 expression	r	*P*-value
YAP1 expression		0.533	< 0.001*
Protein	IDH1 mutation	r	*P*-valve
ZEB1 expression		-0.084	0.360
YAP1 expression		-0.364	< 0.001*

**P*-value < 0.05 indicates statistical signifificance.

tics. Given the strong staining of YAP1 and ZEB1 in GBM, we conducted a correlation analysis to reveal the relationship between YAP1 and ZEB1. Pearson's correlation analysis showed that YAP1 was positively correlated with ZEB1 expression ($P < 0.001$; r = 0.533; **Table 3**).

Survival analysis

Survival analysis was performed for all patients (**Table 4**). Of the 191 cases, 12 were missing visits. By univariate survival analysis, the following prognostic factors were associated with OS: tumor grade, age, selection of operation method, Ki-67 index, ZEB1 expression, YAP1 expression, and coexpression of ZEB1 and YAP1 (**Figure 3**). Further analysis of the study showed that tumor grade, age, and YAP1 were independent prognostic factors for OS, and tumor grade and age were independent prognostic factors for PFS. The survival analysis of LGG and GBM was further evaluated based on the expression of ZEB1 and YAP1 (**Table 5**). In the LGG group, the study showed that patients with high YAP1 expression had poor OS (**Figure 4B**). The level of ZEB1 expression had no statistical significance for OS (P = 0.105, **Figure 4A**). Patients with IDH1mut have a better prognosis than patients with IDH1wt (P < 0.001, **Figure 4C**). We further studied the relationship between ZEB1, YAP1 and IDH1, we found that ZEB1 expression had no statistical significance in IDH1mut compared to IDH1wt (P = 0.360, **Figure 4D**), and the expression of YAP1 was significantly decreased in IDH1mut compared to IDH1wt (P < 0.001, **Figure 4E**). In the GBM group, the expression levels of ZEB1 and YAP1 had no effect on OS and PFS.

Table 4. Overall survival and progression-free survival in all glioma patients

Clinicopathologic variable	Overall survival			Progression-free survival		
	Univariate	Multivariate	HR (95% CI)	Univariate	Multivariate	HR (95% CI)
	P-value	*P*-value		*P*-value	*P*-value	
Grade						
LGG vs. GBM	0.001*	0.001*	0.207 (0.120-0.358)	0.001*	0.001*	0.223 (0.135-0.368)
Age						
< 50 vs. ≥ 50	0.001*	0.040*	0.646 (0.427-0.979)	0.001*	0.028*	0.651 (0.443-0.955)
Gender						
Male vs. female	0.138			0.703		
Ethnic						
Han vs. other	0.620			0.760		
Tumor location						
Frontal vs. other	0.065			0.005*	0.335	
Tumor size						
< 3 cm vs. ≥ 3 cm	0.934			0.679		
Selection of operation method						
Total resection vs. partial resection	0.001*	0.808		0.001*	0.512	
Postoperative radiochemotherapy						
Yes vs. no	0.905			0.747		
Ki-67						
< 30% vs. ≥ 30%	0.001*	0.083		0.001*	0.142	
P53						
< 5% vs. ≥ 5%	0.365			0.404		
YAP1 expression						
Low expression vs. high expression	0.001*	0.039*	0.585 (0.351-0.974)	0.001*	0.171	
p-YAP1 expression						
Low expression vs. high expression	0.599			0.557		
ZEB1 expression						
Low expression vs. High expression	0.001*	0.708		0.001*	0.166	
YAP1 and ZEB1 co-expression						
$ZEB1^{low}YAP1^{low}$ vs. $ZEB1^{low}YAP1^{high}$ vs. $ZEB1^{high}YAP1^{low}$ vs. $ZEB1^{high}YAP1^{high}$	0.001*			0.025*		

**P*-value < 0.05 indicates statistical significance.

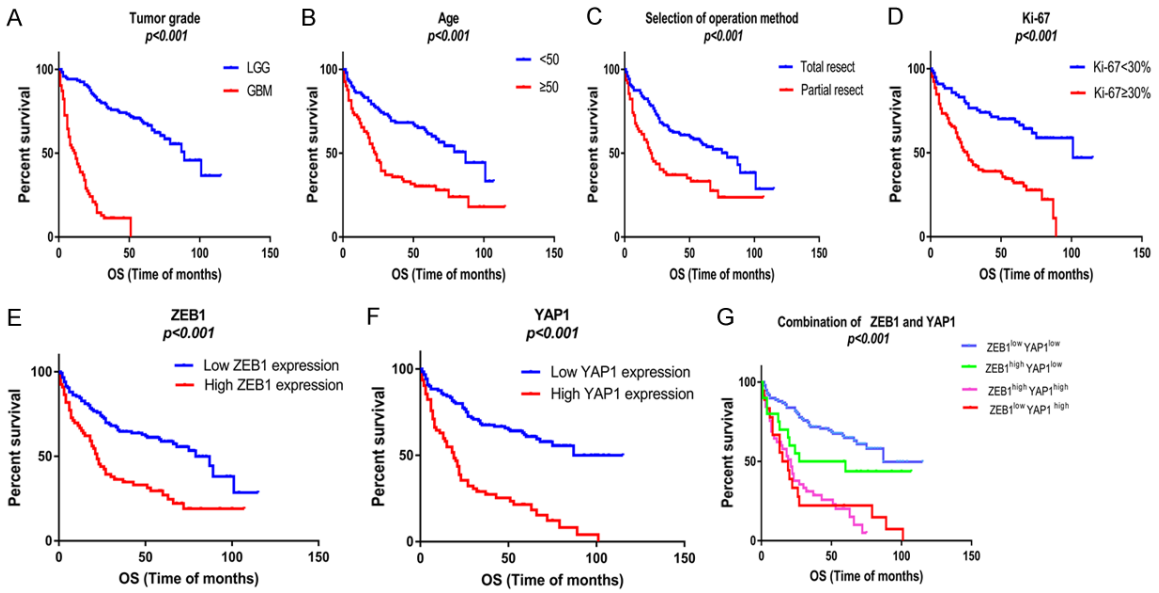

Figure 3. Overall survival (OS) in all glioma patients. A. Patients with LGG had a better OS ($P < 0.001$). B. Patients with age < 50 had a longer OS time ($P < 0.001$). C. Patients with partial resection had a poorer OS ($P < 0.001$). D. Patients with Ki-67 < 30% had a better OS ($P < 0.001$). E. Patients with ZEB1 high expression showed worse OS ($P < 0.001$). F. Patients with low expression of YAP1 showed better OS ($P < 0.001$). G. Patients with $ZEB1^{low}YAP1^{low}$ had the best OS ($P < 0.001$).

Table 5. Survival analysis of LGG and GBM patients according to the expression of ZEB1 and YAP1

Protein expression	Overall survival		Progression-free survival	
	LGG	GBM	LGG	GBM
	P-value	*P*-value	*P*-value	*P*-value
ZEB1 expression				
Low expression vs. high expression	0.105	0.402	0.209	0.054
YAP1 expression				
Low expression vs. high expression	0.009*	0.240	0.023*	0.248
YAP1 and ZEB1 co-expression				
$ZEB1^{low}YAP1^{low}$ vs. $ZEB1^{high}YAP1^{low}$ vs. $ZEB1^{low}YAP1^{high}$ vs. $ZEB1^{high}YAP1^{high}$	0.001*	0.077	0.025*	0.075

*P-value < 0.05 indicates statistical signifificance.

Expression of ZEB1 and YAP1 in LGG according to different IDH1 status

According to the WHO (2016) classification of tumors of the central nervous system, the IDH1 mutation status of LGG was examined. We found that ZEB1 expression had no statistical significance in IDH1mut compared with IDH1wt ($P = 0.360$), and no correlation was found between ZEB1 expression and IDH1 state ($P = 0.360$; r = -0.084; **Figure 4**; **Tables 2**, **6**). For YAP1, high YAP1 expression in 8.7% (8/92) IDH1mut and 40.0% (12/30) IDH1wt was found, showing that YAP1 expression was more pronounced in IDH1wt ($P < 0.001$). Correlation analysis showed that YAP1 expression was negatively correlated with IDH1 mutation ($P < 0.001$, r = -0.364).

Discussion

Given the conflicting data of ZEB1 in gliomas, more studies were needed to further elucidate the role of ZEB1. In this study, we analyzed the clinical significance of ZEB1 expression in 191 human gliomas. The results showed that ZEB1 expression was associated with tumor grade and Ki-67. The ZEB1 expression was more pro-

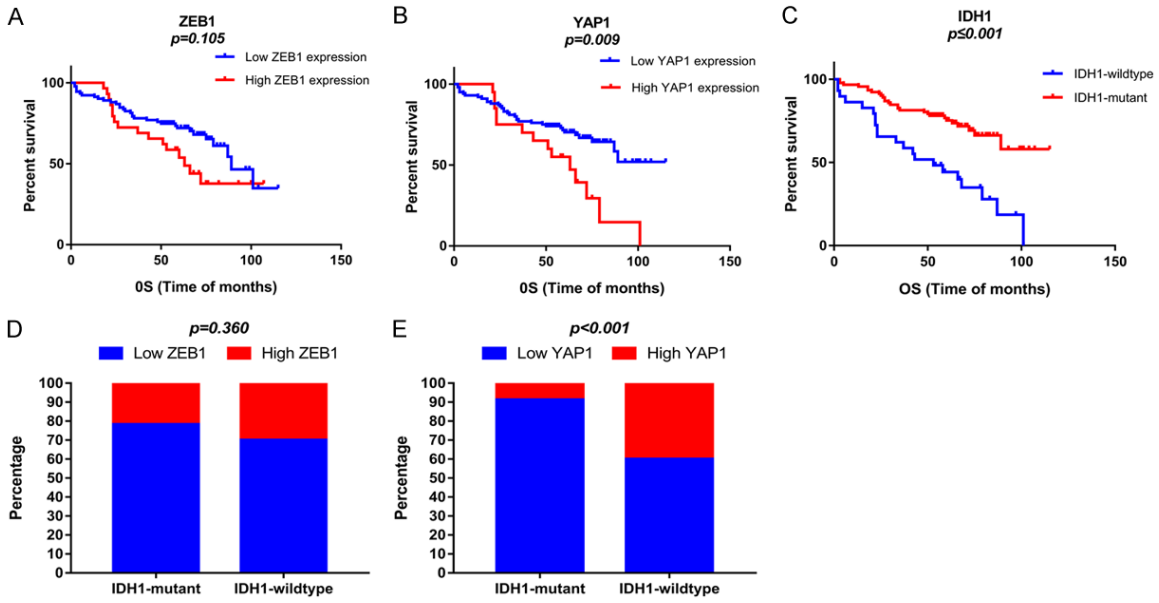

Figure 4. Expression of ZEB1 and YAP1 in LGG and their relationships to prognosis. A. The level of ZEB1 expression had no statistical significance for OS (P = 0.105). B. Patients with high YAP1 expression had poor OS (P = 0.009). C. Patients with IDH1mut have a better prognosis than patients with IDH1wt (P < 0.001). D. ZEB1 expression had no statistical significance in IDH1mut compared to IDH1wt (P = 0.360). E. The expression of YAP1 was significantly decreased in IDH1mut compared to IDH1wt (P < 0.001).

Table 6. Expression of ZEB1 and YAP1 in LGG with different IDH1 gene states

Protein	IDH1mut (92)	IDH1wt (30)	*P*-value
ZEB1			
Low expression	72 (78.3%)	21 (70.0%)	0.360
High expression	20 (21.7%)	9 (30.0%)	
YAP1			
Low expression	84 (91.3%)	18 (60.0%)	< 0.001*
High expression	8 (8.7%)	12 (40.0%)	

*P-value < 0.05 indicates statistical signifificance.

nounced in GBM than in LGG. After survival analysis of all gliomas, glioma patients with high ZEB1 expression had poorer OS and PFS. Suzuki [21] reported that ZEB1 was highly expressed in high-grade gliomas, and ZEB1-positive cells were more abundant in specimens from patients with recurrent glioma. These results indicated that ZEB1 level was positively correlated with histopathologic grade and invasiveness. Kahlert [22] reported that targeting ZEB1 blocked glioblastoma cell invasion in hypoxia environments, suggesting that ZEB1 plays a critical role in promoting the invasion of the tumor core. On the contrary, Edwards [23] found that ZEB1 deletions occurred in more than 50% of GBMs and 15% of LGGs and further determined that heterozygous deletions in GBMs and LGGs were important causes of ZEB1 loss. ZEB1 deletion leads to low ZEB1 protein expression and poor prognosis, suggesting that ZEB1 is a positive predictor of survival [24, 25]. A study showed that ZEB1 mRNA expression was increased in LGG accompanied by IDH1mut, and ZEB1 protein was more prominently expressed in these tumors [26]. However, our study did not find a correlation between ZEB1 protein expression and IDH1 gene status.

Increasing evidence suggests that improper YAP activation can not only promote tumor formation and growth but also promote tumor progression and metastasis. Thus, YAP is an attractive target for cancer therapy [27]. Although YAP promotes the development of gliomas, the specific pathogenesis remains unclear. YAP is an important transcriptional coactivator that is negatively regulated by the Hippo signaling pathway. Genetic alterations in the Hippo pathway are present in some human malignancies. However, these genetic alterations are not prevalent enough to fully account for aberrant YAP expression, and other pathways can contribute to YAP overexpression.

In the present study, YAP1 immunohistochemical staining was performed in clinical glioma specimens. The results showed that YAP1 expression was correlated with Ki-67, P53, tumor grade and tumor location. Further analysis showed that YAP1 was more pronounced in GBM, consistent with Liu's study [28]. High YAP1 expression was accompanied by a high Ki-67 index, suggesting that YAP1 is involved in cell proliferation. Orr [29] found that YAP1 is frequently expressed in aggressive gliomas, including oligodendrogliomas, anaplastic astrocytomas, and GBMs but rarely in pilocytic astrocytomas. Moreover, survival analysis showed poor OS in patients with high YAP1 expression. Further analysis revealed that YAP1 overexpression was associated with poor OS and PFS in the LGG group. Interestingly, we found that YAP1 expression was significantly lower in IDH1mut than in IDH1wt, and correlation analysis revealed a negative correlation between YAP1 expression and IDH1 mutation. Other studies have obtained inconsistent results regarding the relationship between IDH1 mutations and YAP1 expression. For instance, Liu [28] found no correlation linking IDH1 mutation with YAP1 expression. Guichet found that YAP1 expression had a significant association with IDH1 mutation in gliomas [20]. Wei [30] proposed a mechanism underlying the relationship between IDH1 and YAP1 by efficiently introducing heterozygous IDH1 R132H mutation (IDH1R132H/WT) in human astroglial cells; their studies showed that some cell proliferation signaling pathways, including Wnt, MAPK, and Notch, were repressed and the transcriptional YAP level was downregulated to 50% by IDH1R132H/WT. Overall, our findings suggested that YAP1 was associated with the malignant progression of glioma, and YAP1 may be a therapeutic target for glioma. Further studies are needed to investigate the relationship between YAP1 and IDH1 to decipher the role of IDH1 mutation in the occurrence and development of glioma.

YAP1 lacks a DNA binding domain and must bind to other transcription factors to drive transcription. ZEB1 is an important member of the ZEB family of transcription factors, and some studies have reported the correlation between YAP1 and ZEB1. For instance, Qiu [31] demonstrated that YAP1 is a downstream effector of ZEB1, which can participate in EMT of hepatocellular carcinoma by binding to the promoter 5'UTR region of YAP gene; however, other studies have shown that YAP1 is implicated in EMT by regulating the downstream effector ZEB1 [32]. In the present study, the expression levels of ZEB1 and YAP1 were increased in the GBMs, and correlation analysis revealed that ZEB1 expression was significantly correlated with YAP1 in glioma tissues, suggesting that YAP1 and ZEB1 may play a synergistic role in the malignant progression of glioma.

In conclusion, our results showed that ZEB1 and YAP1 levels were higher in GBMs compared to LGGs, and patients with high ZEB1 and YAP1 expression levels had poor prognoses. Correlation analysis showed that ZEB1 and YAP1 expression were positively correlated, suggesting that the inhibition of ZEB1 and YAP1 coexpression may improve the prognoses of gliomas. Aberrant YAP1 expression may be associated with the IDH1 gene state. However, this study is limited to the analysis of protein expression in tissue samples, and the exact mechanism of glioma tumorigenicity is still poorly understood. Further studies are needed to elucidate the roles of ZEB1 and YAP1 in glioma cells and animal models.

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Disclosure of conflict of interest

None.

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