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Yippee Like 1 Suppresses Glioma Progression and Serves as a Novel Prognostic Factor

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Glioma is the most common tumor of central nervous system in adults with poor prognosis. Yippee Like 1 (YPEL1) is a newly discovered protein that plays contradictory roles in pancreatic cancer and colon cancer. Here we initially explored the expression, clinical significance, and function of YPEL1 in glioma. The transcription level of *YPEL1* in glioma patients was extracted from TCGA datasets via GEPIA website. As a result, the mRNA level of *YPEL1* was significantly lower in glioma tissues than that in normal brain tissues. Immunohistochemistry staining was next conducted to test protein expression of YPEL1 in glioma tissues (n = 130). Consistently, lower protein expression of YPEL1 was observed in cases with larger tumor size and advanced WHO grades. Univariate and multivariate analyses identified YPEL1 as a novel independent prognostic factor of gliomas. Finally, overexpression of YPEL1 was performed in U87 and U373 cell lines to further validate its tumor-related functions, followed by proliferation, invasion, and subcutaneous mice xenografts assays. *In vitro* and *in vivo* data demonstrated that overexpressing YPEL1 can remarkably prevent glioma cell proliferation and invasion. Taken together, our data revealed that low YPEL1 expression was significantly correlated with poor overall survival of glioma patients and may play anti-tumor effects.

Keywords: glioma; prognosis; proliferation; Yippee Like 1
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Introduction

Glioma is originated from the central nervous system, representing the most common and aggressive type of brain tumor (Wen and Kesari 2008). Although with deep understanding of the molecular alterations of glioma, therapeutic options for glioma patients are still limited to surgery, chemotherapy and radiation therapy (Jiang et al. 2021). Moreover, most of glioma patients become therapeutic resistant and recurrent during the treatment (Osuka and Van Meir 2017). Therefore, it is important to search for novel molecular targets and prognostic makers to predict the therapeutic responses and clinical outcomes of glioma.

Yippee Like 1 (YPEL1) gene was initially cloned by Farlie et al. (2001) from embryonic mice, which was later mapped in human chromosome 22q11.2, a region associated with several congenital anomalies involving craniofacial malformation, including DiGeorge syndrome and velocardiofacial syndrome (Hosono et al. 2004). Dysregulated YPEL1 may also be correlated with development of oculo-auriculo-vertebral spectrum (hemifacial microsomia/OAVS) (Glaeser et al. 2020), a heterogenous and congenital condition caused by a morphogenesis defect of the first and second pharyngeal arches. YPEL1 protein contains a putative nuclear localization sequence, and located in the centrosome and nucleolus, thus may play a role in the regulation of cell division. Nevertheless, our knowledge about the physiological and pathological functions of YPEL1 is still limited.

It has been reported that transfection of YPEL1 into mouse fibroblasts resulted in confluent cultures with the cobblestone appearance characteristic of epithelial cells, including rearrangement of vimentin, increased circumferential F-actin, and increased expression of CD56. Therefore, YPEL1 may participate in modulating cellular morphology and behavior that is important for development of the craniofacial complex (Farlie et al. 2001). Indeed, a recent study reported that YPEL1 overexpression in early avian craniofacial mesenchyme causes mandibular dysmorphogenesis by up-regulating apoptosis (Tan et al. 2015).

Interestingly, YPEL1-mRNA level was reported to be downregulated in pancreatic ductal adenocarcinoma

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(PDAC) tissues and PDAC cell lines, comparing with the normal tissues and cells (Abiatari et al. 2009). Moreover, their data suggested that a reduced expression of YPEL1 in PDAC might be related to perineural invasion and prognosis, therefore implying its potential effects in malignant transformation. Consistently, YPEL1 was later reported to inhibit gastric cancer cell growth and invasion, highlighting its anti-tumor role in malignancies (Li et al. 2019).

In contrast, Penrose et al. (2017) developed a colonic ρ0 (rho0) colon cancer cells with reduced mitochondrial energy function and found that YPEL1 was upregulated in ρ0 cells and colon cancers, comparing with normal colon cells. Moreover, according to their data, high YPEL1 expression together with other altered genes can remarkably predict a poorer prognosis of colon cancer patients, thus implying its oncogenic role in colon cancer.

Therefore, YPEL1 may play oncogenic or anti-tumor functions in different tumor types. Here we investigate the mRNA and protein levels of YPEL1 in glioma tissues for the first time. By analyzing the clinicopathological characteristics and survival information, we found that lower YPEL1 was significantly correlated with advanced glioma stage and poorer prognosis. Finally, we conducted cellular and mice experiments to validate the anti-tumor effects of YPEL1 in glioma.

Methods

Online data mining

Gene Expression Profiling Interactive Analysis (GEPIA) microarray data mining platform (Tang et al. 2017) was used to collect and analyze *YPEL1* expression in glioma tissues and normal brain tissues based on TCGA and GTEx datasets. GEPIA platform also provided patient survival analysis based on *YPEL1* level.

Cohort enrollment

A total of 130 glioma patients who were treated in our hospital were selected as the research samples. All of them underwent surgery, including total resection, subtotal resection, or partial resection. All glioma diagnoses were based on pathological tests. None of the patients received anti-tumor treatment before surgery. This study was reviewed and approved by the Suining Central Hospital Ethic Committee. All patients agreed and signed an informed consent form. This research was in line with the Declaration of Helsinki.

Immunohistochemistry

Immunohistochemistry (IHC) was conducted to explore the YPEL1 protein expression level in clinical tissue samples. Briefly, the tissue sections were deparaffinized, rehydrated, and then incubated with 3% hydrogen peroxide. Antigen retrieval was achieved by using Tris (hydroxymethyl) aminomethane-ethylene-diamine-tetra acetic acid (Tris-EDTA) buffer (pH 9.0). The tissue sections were blocked with 5% bovine serum albumin (BSA) and then probed with anti-YPEL1 (1:300; LSBio, Seattle, WA, USA) at 4°C overnight. Secondary antibody was then added and incubated. The immunoreactivity was finally detected by using the diaminobenzidine (DAB) staining reagents according to the manufacturer's instructions (Liu et al. 2017).

The IHC results were next scored regarding both staining intensity and the percentage of positively stained cells. Staining intensity score was given as negative staining: 1; weak staining: 2; moderate staining: 3; and strong staining: 4. Percentage of positive cells was scored as 0-25%, 1; 26-50%, 2; 51-75%, 3; and > 75%, 4. The immunoreactivity score was obtained by multiplying the two scores above, ranging 0-16. Then patients were divided into negative-YPEL1 expression and positive-YPEL1 expression according to the IHC scores.

Cell culture and transfection

Glioma cell lines U87 and U373 were purchased from Shanghai Institute of Biochemistry and Cell Biology Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere with 5% CO_2 (Shi et al. 2020).

YPEL1 cDNA was cloned into pCDNA 3.0 vector, and then transfected into U87 and U373 cells through Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) using blank vector as control according to manufacturer's protocol. The over-expression of YPEL1 was validated using western blot.

Western blotting

Western blot was used to verify the transfection efficiency. The total protein of cells was extracted using radioimmunoprecipitation assay (RIPA) lysis buffer. The intact protein was separated by polyacrylamide gel electrophoresis (PAGE) and transferred onto a nitrocellulose membrane using a wet transfer method. The membrane was blocked with 5% BSA for 1 h and then probed at 4°C overnight with primary antibodies specific for YPEL1 (1:1,000) or GAPDH (1:1,000). After washing with TBST (Tris-buffered saline with 0.1% Tween® 20 Detergent) buffer, the membrane was incubated with horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG (1:10,000), washed with TBST, developed with an enhanced chemiluminescence (ECL) solution, and imaged for analyses (Chen et al. 2021).

Proliferation assay

The proliferation assay was conducted as we previously described (Luo et al. 2018). Transfected cells were seeded in the 96-well plate and cultured for 1, 2, 3, and 4 days. The CCK-8 (cell counting kit-8) assay was used to quantify cell proliferation. Briefly, 10 μL CCK-8 working solution was added to each well and incubation for 4 h. The absorption values at 450 nm of 96 well were detected.

Invasion assay

The invasive properties of the transfected cells were determined using a Matrigel-Transwell assay. The matrigel gel and serum-free RIPM-1640 medium were mixed with a ratio of 3:1, then each Boyden Chamber Transwell was added with 60 μL mixture. A total of 1×10^6 cells in 100 μL medium were seeded in the upper chamber while 600 μL complete medium was added into the lower chamber, followed by one-day incubation. The cells that migrate through the pores to the other side of the membrane were stained and counted.

Mice experiments

BALB/c nude mice (five weeks old) were bought from Shanghai Experimental Animal Center (Shanghai, China). The animal experiments were supervised by the Ethics Committee of Suining Central Hospital. Transfected U87 and U373 cells were subcutaneously injected into nude mice. The tumor volume was monitored and counted via the equation: volume = 0.5 × length × width × width. Thirty days later, all mice were euthanized, sacrificed, and tumors were isolated (Liu et al. 2021).

Statistics

As we previously described (Pan et al. 2020), overall survival of patients was compared by Kaplan-Meier curves and log-rank tests, using GraphPad Prism (version 7.0). Univariable and multivariable Cox regression analyses were performed using the SPSS (version 20.0) to compare the prognostic value of the risk model and clinicopathological characteristics. Student's t-test was used to compare the difference between groups in cellular and mice assays. The values of $P < 0.05$ in all tests were considered statistically significant.

Results

Patients' information

Among the 130 glioma cases, there were 48 females and 82 males. Seventy cases were diagnosed at older than 50 years old, while the other 60 cases were diagnosed at ≤ 50 years old. The tumor size was larger than 5.0 cm in 47 cases and ≤ 5.0 cm in the other 83 cases. The Karnofsky Performance Scale (KPS) score is a well-known evaluation to stratify patients' prognosis and determine appropriate management in gliomas (Chambless et al. 2015). Therefore, the Karnofsky score was also evaluated for each

Table 1. The correlations between clinical parameters and YPEL1 expression in gliomas.

Variables	Patients (n = 130)	YPEL1 expression		P value
		Negative (n = 63)	Positive (n = 67)	
Sex				
Female	48	21	27	0.411
Male	82	42	40	
Age (years)				
≤ 50	60	28	32	0.705
> 50	70	35	35	
Tumor size				
≤ 5 cm	83	33	50	0.008*
> 5 cm	47	30	17	
Karnofsky score				
≤ 90	88	45	43	0.377
> 90	42	18	24	
WHO grade				
II	22	5	17	0.001*
III	50	20	30	
IV	58	38	20	
Surgery				
Total resection	70	33	37	0.555
Subtotal resection	32	18	14	
Partial resection	28	12	16	
IDH1 status				
Wild type	66	34	32	0.317
Mutation	21	7	14	
Unknown	43	22	21	

*Statistically significant. *IDH1*, isocitrate dehydrogenase (NADP(+)) 1.

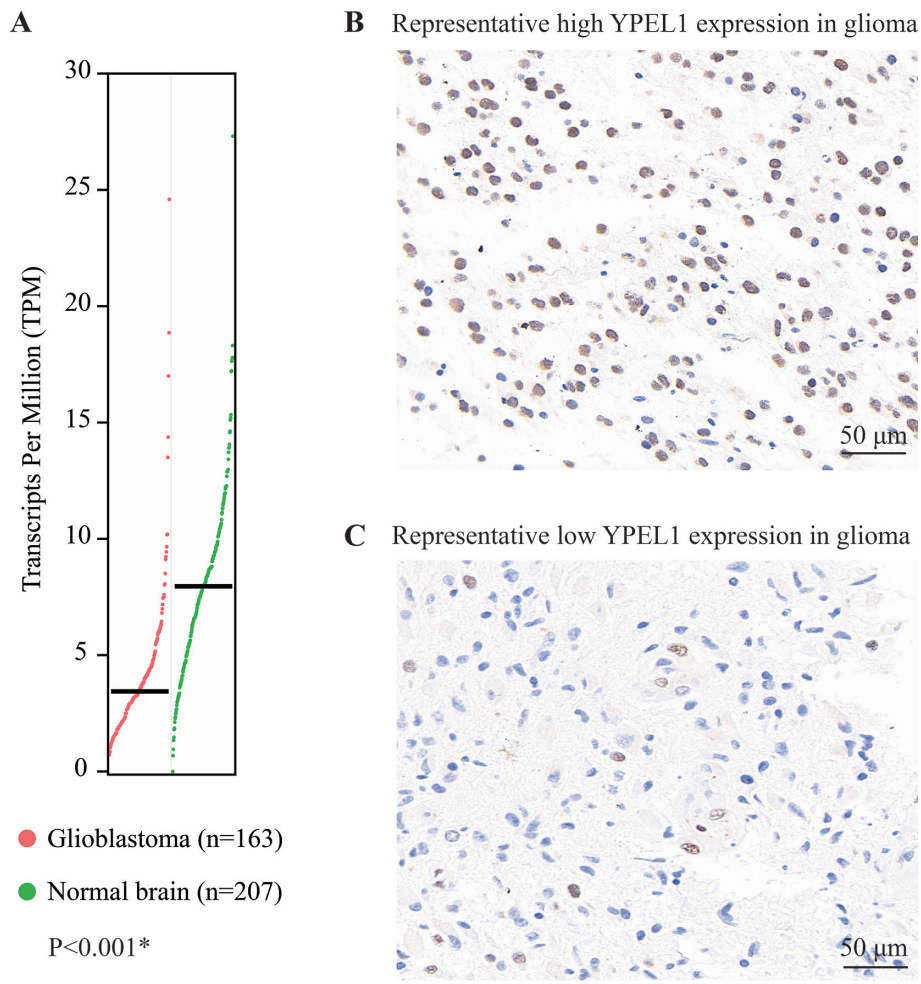

Fig. 1. Expression of YPEL1 in gliomas.
(A) mRNA levels of *YPEL1* in glioma tissues and normal brain samples were retrieved from TCGA and GTEx datasets, which were exhibited as transcripts per million (TPM). According to the Student's t-test, *YPEL1* exhibited significantly lower level in gliomas than that in normal brains. (B) Representative positive YPEL1 protein expression in glioma tissues as reflected by IHC experiments. Magnification: 400 ×. (C) Representative negative YPEL1 protein expression in glioma tissues. Magnification: 400 ×.

enrolled patient. Accordingly, 88 cases were scored as ≤ 90, while the other 42 cases were scored larger than 90. As for the WHO grade based on pathological test, there were 22 cases with grade II, 50 cases with grade III, and the other 58 cases with grade IV (glioblastoma). Total tumor resection was conducted for 70 patients, subtotal resection was conducted in 32 cases, while partial resection was performed in the other 28 cases. The *IDH1* [isocitrate dehydrogenase (NADP(+)) 1] mutation was also well-recognized as an important prognostic factor of gliomas (Yan et al. 2009). Our cohort contained 66 cases with wild type *IDH1*, 21 cases with mutated *IDH1*, while the other 43 cases were untested (Table 1).

YPEL1 expression in gliomas

We firstly extracted the mRNA expression information of *YPEL1* from TCGA and GTEx datasets. Accordingly, YPEL1 showed significantly lower mRNA level in glioma tissues than that in normal brain tissues ($P < 0.001$, Fig. 1A). Therefore, we next tested the protein expression level of YPEL1 in the glioma tissues collected from our hospital. Accordingly, YPEL1 mainly localized in the nucleus and showed positive expression in 67 cases (Fig. 1B), while YPEL1 expression was negative in the other 63 tissue samples (Fig. 1C).

Moreover, Chi-square test revealed that gliomas with larger tumor size were more prevalent to exhibit negative YPEL1 expression ($P = 0.008$, Table 1). Consistently, YPEL1 protein expression was negatively correlated with the WHO grade ($P = 0.001$, Table 1), indicating that low YPEL1 may promote glioma progression.

Prognostic analyses of enrolled glioma cohort

We next conducted univariate survival analyses by Kaplan-Meier method for each parameter (Table 2). Accordingly, patients in the negative-YPEL1 group showed significantly shorter survival time (23.6 ± 2.6 months) than those in the positive-YPEL1 group (58.2 ± 3.9 months),

Table 2. Univariate analyses of overall survival of glioma patients.

Variables	Patients (n = 130)	Overall survival time (months)		P value
		Mean ± SD	Median	
Sex				
Female	48	44.3 ± 4.1	46.0	0.365
Male	82	39.6 ± 3.7	21.0	
Age (years)				
≤ 50	60	52.1 ± 4.3	44.0	0.001*
> 50	70	31.8 ± 3.3	16.0	
Tumor size				
≤ 5 cm	83	47.9 ± 3.7	44.0	0.005*
> 5 cm	47	31.4 ± 4.4	16.0	
Karnofsky score				
≤ 90	88	41.0 ± 3.5	21.0	0.846
> 90	42	43.2 ± 5.1	42.0	
WHO grade				
II	22	66.6 ± 7.3	83.0	< 0.001*
III	50	42.0 ± 4.1	38.0	
IV	58	30.1 ± 3.2	19.0	
Surgery				
Total resection	70	42.6 ± 3.7	32.0	0.609
Subtotal resection	32	40.4 ± 5.3	20.1	
Partial resection	28	36.1 ± 5.8	18.0	
YPEL1 expression				
Negative	63	23.6 ± 2.6	15.0	< 0.001*
Positive	67	58.2 ± 3.9	70.0	
IDH1 status				
Wild type or unknown	109	35.9 ± 3.0	19.0	< 0.001*
Mutation	21	70.8 ± 5.7	72.0	

*Statistically significant. *IDH1*, isocitrate dehydrogenase (NADP(+)) 1.

indicating that YPEL1 negatively affected glioma prognosis (P < 0.001, Fig. 2A). Although patients' sex showed no statistically significant effect on the postoperative overall survival (P = 0.365, Fig. 2B), older patients exhibited poorer overall survival than that of younger ones (mean survival time 31.8 ± 3.3 vs. 52.1 ± 4.3 months, P = 0.001, Fig. 2C). As expected, the overall survival was poorer in patients with larger tumor size compared to those with smaller ones (P = 0.005, Fig. 2D). In contrast, the Karnofsky score showed no significant effect on patients' survival (P = 0.846, Fig. 2E) although it may affect patients' life quality. As a conventional prognostic factor with clinical application, WHO grade also help predict the overall survival of glioma patients (P < 0.001, Fig. 2F). However, survival curves showed that the surgery pattern has no statistically significant effect on the overall survival of our cohort (P = 0.609, Fig. 2G). Our previous meta-analysis indicated that total resection has better effect than subtotal resection (Tang et al. 2019), the insignificance in this study may be caused by the limited case numbers. As expected, patients with mutated *IHD1* exhibited significantly better overall survival than those with wild-type or unknown status of IDH1 (P < 0.001, Fig. 2H).

Moreover, by analyzing the prognostic significance of YPEL1 in gliomas with different WHO grades (Fig. 3A-C), we confirmed that YPEL1 expression would serve as a supplement to predict survival of glioma patients within the same WHO grade. To further identify independent prognostic factors, we subjected five variables into a Cox multivariate regression model (Table 3), including age, tumor size, WHO grade, YPEL1 expression, and *IDH1* mutation (all P < 0.05 by univariate tests). Accordingly, high YPEL1 was confirmed as a novel prognostic factor correlated with better survival [Hazard Ratio (HR) = 0.229, 95% Confidence Interval (CI) 0.131-0.400, P < 0.001]. Similarly, patients with mutated *IDH1* also exhibited better survival (HR = 0.328, 95% CI 0.158-0.679, P = 0.003). In addition, older age (HR = 3.001, 95% CI 1.846-4.880, P < 0.001), larger tumor size (HR = 2.514, 95% CI 1.538-4.109, P < 0.001), advanced WHO grade (HR = 1.945, 95% CI

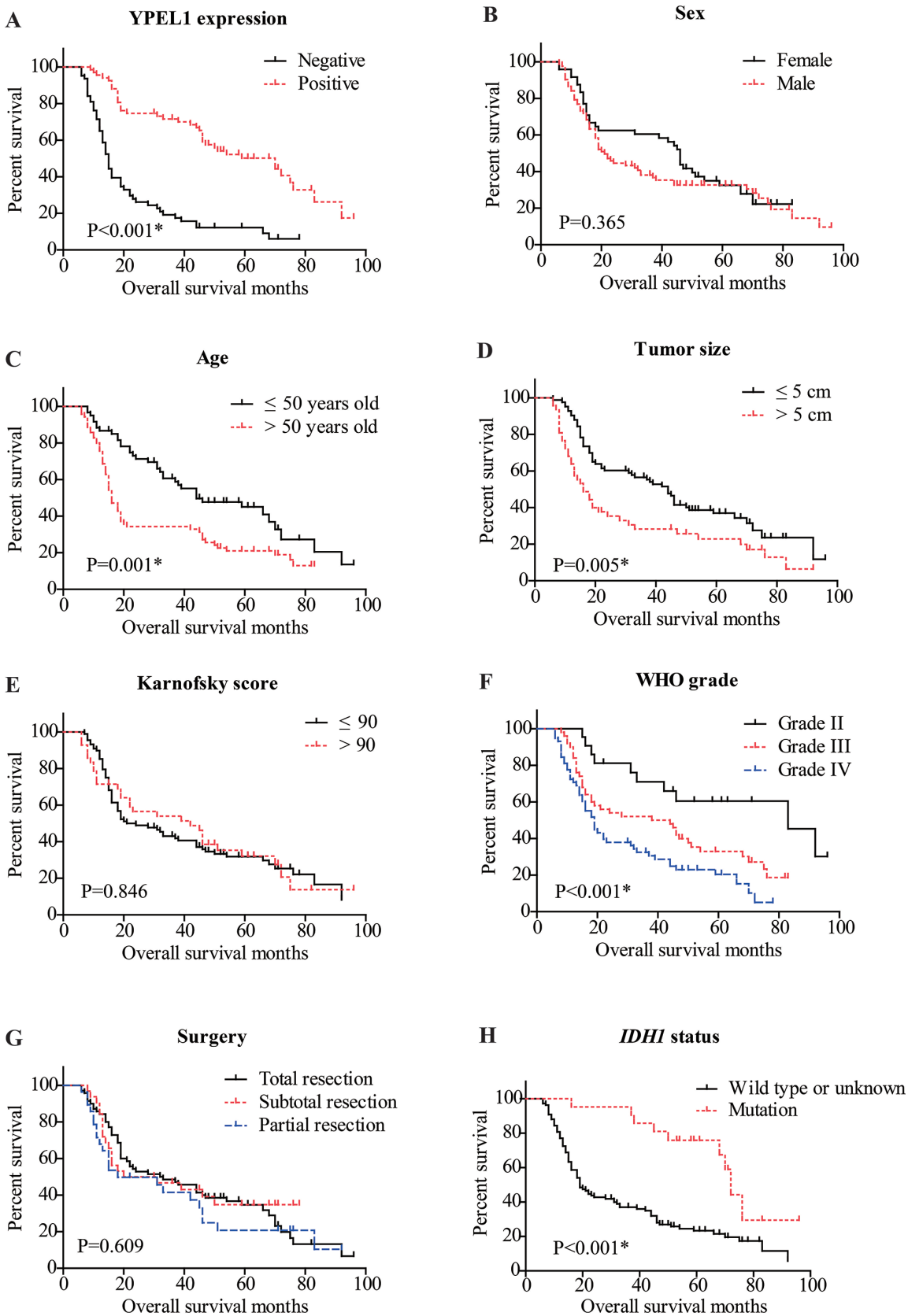

Fig. 2. Overall survival analyses of enrolled glioma cohort.
The overall survival curves were plotted by Kaplan-Meier method based on YPEL1 expression level (A), sex (B), age (C), tumor size (D), Karnofsky score (E), WHO grade (F), surgery (G), and *IDH1* mutation (H), respectively. Data was compared by log-rank test and *indicates $P < 0.05$.

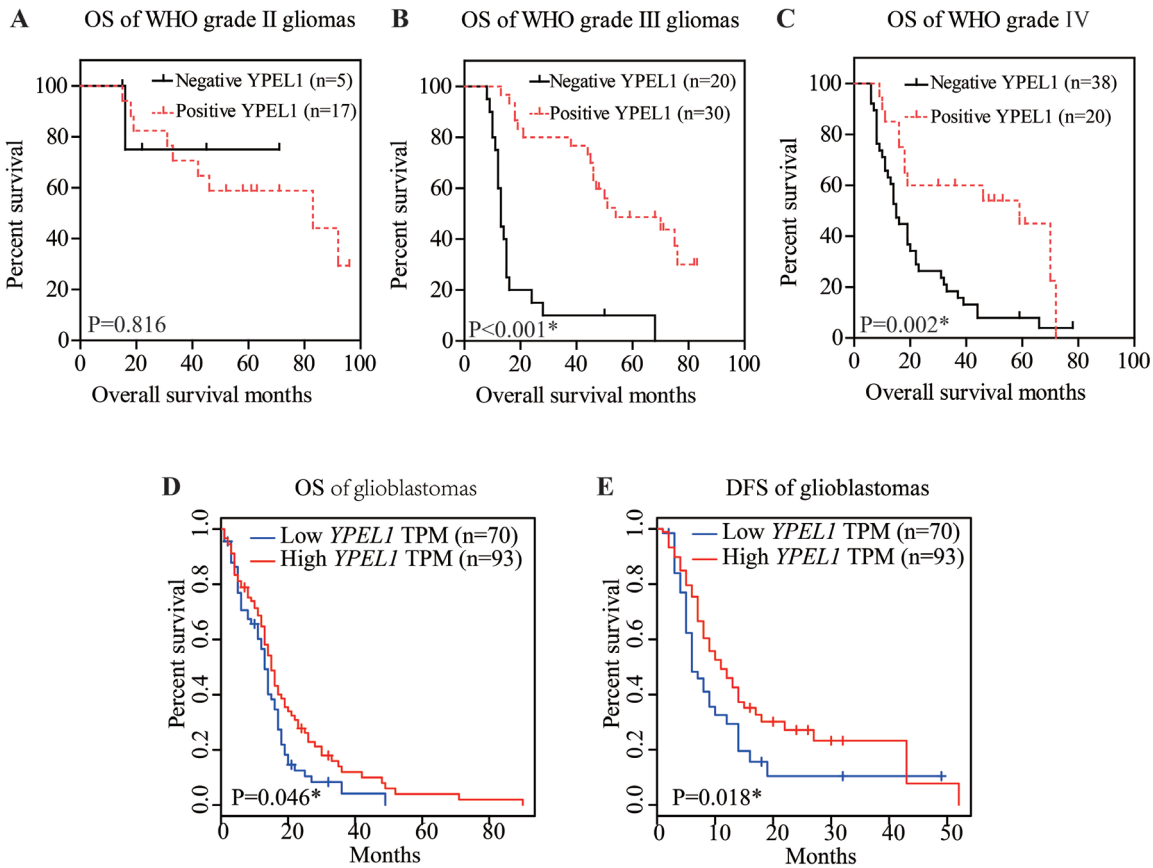

Fig. 3. Prognoses of glioma patients with different WHO grades.
Based on the protein expression level of YPEL1, Kaplan-Meier analyses were conducted for glioma patients with WHO grade II (A) (P = 0.816), WHO grade III (B) (P < 0.001), or WHO grade IV (C) (P = 0.002), respectively. In addition, survival analyses for the cases from TCGA cohort indicated that glioblastoma patients with lower *YPEL1* level exhibited poorer overall survival (D) (P = 0.046) and poorer disease-free survival (E) (P = 0.018). OS, overall survival; DFS, disease free survival.

Table 3. Multivariate analysis of the independent prognostic factors of glioma patients.

Variables	HR	95% CI	P value
Age	3.001	1.846-4.880	< 0.001*
Size	2.514	1.538-4.109	< 0.001*
Grade	1.945	1.255-3.014	0.003*
YPEL1 expression	0.229	0.131-0.400	< 0.001*
IHD1 mutation	0.328	0.158-0.679	0.003*

HR, hazard ratio; CI, confidence interval; *IDH1*, isocitrate dehydrogenase (NADP(+)) 1.
*Statistically significant.

1.255-3.014, P = 0.003) can all independently contribute to a poorer overall survival of glioma patients.

Considering that our cohort contains patients from a single medical center and may lead to biased conclusions, we next conducted survival analyses by using GEPIA website according to the mRNA level of *YPEL1* to validate our findings. As a result, lower *YPEL1*-mRNA level was significantly correlated with poorer overall survival (P = 0.046, Fig. 3D) and disease-free survival (P = 0.018, Fig. 3E) of glioblastoma patients in TCGA cohort. Therefore, we came to the conclusion that YPEL1 was negatively correlated with glioma prognosis.

YPEL1 inhibits glioma progression

Clinical findings implied that YPEL1 may play an anti-tumor role in glioma, which engaged us to perform further cellular experiments. Overexpression of YPEL1 was conducted in U87 and U373 cells (Fig. 4A), which achieved significance increase in the protein expression level of YPEL1. By subjecting the transfected cells into CCK-8 assays, we found that cells with YPEL1-overexpression showed significantly decreased proliferation capacities in both cell lines (Fig. 4B). Similarly, overexpressing YPEL1 resulted in remarkably impaired invasion process based on the Matrigel-Transwell data (Fig. 4C).

Finally, we performed subcutaneous xenografts in nude mice to validate the anti-tumor effects of YPEL1. By monitoring the growth curves of xenografts generated by different cells, we observed that overexpressing YPEL1 significantly attenuated the tumor growth in vivo (Fig. 4D), highlighting its potential as an anti-tumor protein.

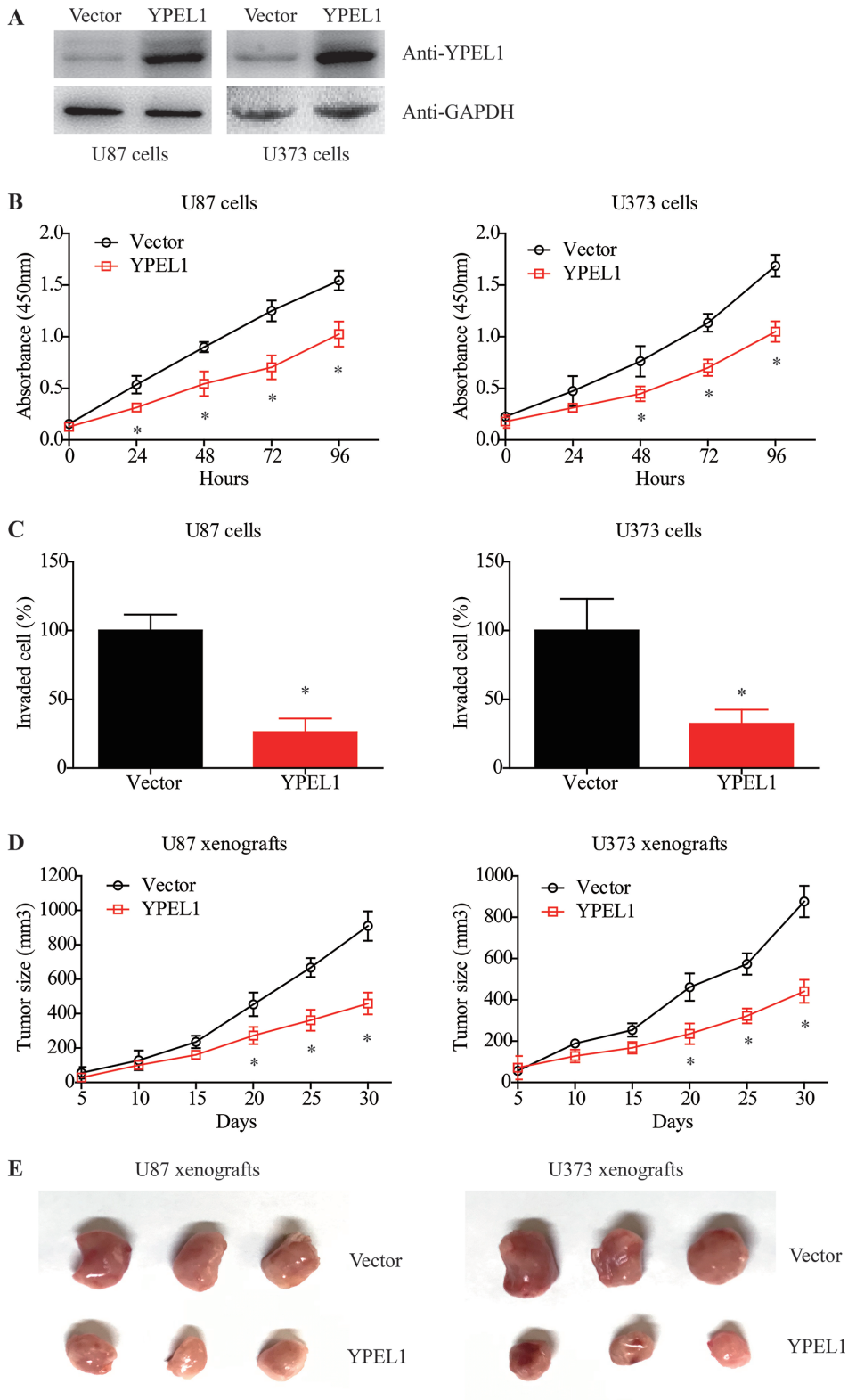

Fig. 4. YPEL1 inhibits glioma progression both *in vitro* and *in vivo*.
(A) Western blotting was used to test the transfection efficiencies of pcDNA-vector (Vector) and pcDNA-YPEL1 (YPEL1) in U87 and U373 cells. (B) CCK-8 assays were conducted to evaluate viabilities of transfected cells. (C) Matrigel-Transwell method was used to compare the difference on invasion capacities of different cell lines. (D) After subcutaneously injecting different cell lines in nude mice, the growth curves of xenografts were plotted to assess tumor growth capacities. (E) The isolated xenografts were pictured, which showed that overexpressing YPEL1 resulted in decreased tumor growth. Data were presented as mean ± SEM from three independent repeats and compared by Student's t-test. *P < 0.05.

Consistently, the isolated tumor size (Fig. 4E) also demonstrated that YPEL1 can restrain glioma growth.

Discussion

Glioma is a highly heterogeneous disease and many prognostic factors have been well recognized (Weller et al. 2015). Here in our study, using TCGA dataset, we confirmed the prognostic significance of *YPEL1*-mRNA expression. Moreover, we validated the data by enrolling an independent cohort in our hospital which included 130 glioma patients. Accordingly, YPEL1 was negatively associated with histological grades and tumor size.

Although a prediction model containing higher YPEL1 expression and other biomarkers can predict a poorer prognosis of colon cancer patients (Penrose et al. 2017), our data showed that lower expression level of YPEL1 can independently contribute to unfavorable outcomes of glioma patients. In addition, by using two glioma cell lines, we found that overexpressing YPEL1 resulted in attenuated cell proliferation and invasion. Our findings were consistent with a recent study which reported a similar role of YPEL1 in gastric cancer cells (Li et al. 2019). Therefore, YPEL1 may play anti-tumor roles in glioma progression. Furthermore, we conducted subcutaneous xenograft experiments in nude mice, which showed that xenografts with overexpressed YPEL1 possessed slower growth rate, thus validated our clinical and cellular findings.

Our study has several limitations. Firstly, all the clinical data were collected from a single medical center with limited cases, and thus may induce regional bias. We tried to solve this problem by analyzing the TCGA dataset to validate our major findings. Secondly, we did not fully dig into the detailed signaling mechanisms downstream of YPEL1, although our data suggested its role in suppressing glioma growth and invasion. In fact, few study reported the pathways YPEL1 may participate because it is a newly identified protein. It has been reported that YPEL1-related abnormal mandibular morphogenesis was associated with increased apoptosis and involvement of the BMP/MSX pathway (Tan et al. 2015), which can serve as an inspiration for validating its mechanisms in malignancies in the near future. Nevertheless, our paper represents the first evidence on confirming YPEL1's role in glioma both *in vitro* and *in vivo*, highlighting its potential as a novel therapeutic target.

Interestingly, it has been recently reported that YPEL1 can be upregulated by erlotinib stimulation in non-small cell lung cancer (Wu 2018). However, the detailed effect or mechanism of this alteration remains further investigation. Future studies focusing on the crosstalk between YPEL1 and chemotherapies would be invaluable for glioma treatment considering its high chemo-resistance rate.

In conclusion, low YPEL1 expression is significantly correlated with poor overall survival of glioma patients. YPEL1 plays anti-tumor effects by suppressing glioma proliferation and invasion both *in vitro* and *in vivo*.

Conflict of Interest

The authors declare no conflict of interest.

References

Abiatari, I., Kiladze, M., Kerkadze, V., Friess, H. & Kleeff, J. (2009) Expression of YPEL1 in pancreatic cancer cell lines and tissues. *Georgian Med. News*, 60-62.

Chambless, L.B., Kistka, H.M., Parker, S.L., Hassam-Malani, L., McGirt, M.J. & Thompson, R.C. (2015) The relative value of postoperative versus preoperative Karnofsky Performance Scale scores as a predictor of survival after surgical resection of glioblastoma multiforme. *J. Neurooncol.*, **121**, 359-364.

Chen, T., Liu, H., Liu, Z., Li, K., Qin, R., Wang, Y., Liu, J., Li, Z., Gao, Q., Pan, C., Yang, F., Zhao, W., Zhang, Z. & Xu, Y. (2021) FGF19 and FGFR4 promotes the progression of gallbladder carcinoma in an autocrine pathway dependent on GPBAR1-cAMP-EGR1 axis. *Oncogene*, **40**, 4941-4953.

Farlie, P., Reid, C., Wilcox, S., Peeters, J., Reed, G. & Newgreen, D. (2001) Ypel1: a novel nuclear protein that induces an epithelial-like morphology in fibroblasts. *Genes Cells*, **6**, 619-629.

Glaeser, A.B., Santos, A.S., Diniz, B.L., Deconte, D., Rosa, R.F.M. & Zen, P.R.G. (2020) Candidate genes of oculo-auriculovertebral spectrum in 22q region: a systematic review. *Am. J. Med. Genet. A*, **182**, 2624-2631.

Hosono, K., Sasaki, T., Minoshima, S. & Shimizu, N. (2004) Identification and characterization of a novel gene family YPEL in a wide spectrum of eukaryotic species. *Gene*, **340**, 31-43.

Jiang, T., Nam, D.H., Ram, Z., Poon, W.S., Wang, J., Boldbaatar, D., Mao, Y., Ma, W., Mao, Q., You, Y., Jiang, C., Yang, X., Kang, C., Qiu, X., Li, W., et al. (2021) Clinical practice guidelines for the management of adult diffuse gliomas. *Cancer Lett.*, **499**, 60-72.

Li, S., Sun, M.Y. & Su, X. (2019) MiR-885-5p promotes gastric cancer proliferation and invasion through regulating YPEL1. *Eur. Rev. Med. Pharmacol. Sci.*, **23**, 7913-7919.

Liu, H., Gong, Z., Li, K., Zhang, Q., Xu, Z. & Xu, Y. (2021) SRPK1/2 and PP1alpha exert opposite functions by modulating SRSF1-guided MKNK2 alternative splicing in colon adenocarcinoma. *J. Exp. Clin. Cancer Res.*, **40**, 75.

Liu, H., Xu, Y., Zhang, Q., Yang, H., Shi, W., Liu, Z., Li, K., Gong, Z., Ning, S., Li, S. & Chen, Y. (2017) Prognostic significance of TBL1XR1 in predicting liver metastasis for early stage colorectal cancer. *Surg. Oncol.*, **26**, 13-20.

Luo, K., Huang, W. & Tang, S. (2018) Sirt3 enhances glioma cell viability by stabilizing Ku70-BAX interaction. *Onco Targets Ther.*, **11**, 7559-7567.

Osuka, S. & Van Meir, E.G. (2017) Overcoming therapeutic resistance in glioblastoma: the way forward. *J. Clin. Invest.*, **127**, 415-426.

Pan, D., Xia, X.X., Zhou, H., Jin, S.Q., Lu, Y.Y., Liu, H., Gao, M.L. & Jin, Z.B. (2020) COCO enhances the efficiency of photoreceptor precursor differentiation in early human embryonic stem cell-derived retinal organoids. *Stem Cell. Res. Ther.*, **11**, 366.

Penrose, H.M., Heller, S., Cable, C., Nakhoul, H., Ungerleider, N., Baddoo, M., Pursell, Z.F., Flemington, E.K., Crawford, S.E. & Savkovic, S.D. (2017) In colonic rho(0) (rho0) cells reduced mitochondrial function mediates transcriptomic alterations associated with cancer. *Oncoscience*, **4**, 189-198.

Shi, H., Luo, K. & Huang, W. (2020) Bone marrow stromal antigen 2 is a potential unfavorable prognostic factor for high-grade glioma. *Onco Targets Ther.*, **13**, 8723-8734.

Tan, T.Y., Gordon, C.T., Miller, K.A., Amor, D.J. & Farlie, P.G. (2015) YPEL1 overexpression in early avian craniofacial mesenchyme causes mandibular dysmorphogenesis by

up-regulating apoptosis. *Dev. Dyn.*, **244**, 1022-1030.
Tang, S., Liao, J. & Long, Y. (2019) Comparative assessment of the efficacy of gross total versus subtotal total resection in patients with glioma: a meta-analysis. *Int. J. Surg.*, **63**, 90-97.
Tang, Z., Li, C., Kang, B., Gao, G., Li, C. & Zhang, Z. (2017) GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.*, **45**, W98-W102.
Weller, M., Wick, W., Aldape, K., Brada, M., Berger, M., Pfister, S.M., Nishikawa, R., Rosenthal, M., Wen, P.Y., Stupp, R. & Reifenberger, G. (2015) Glioma. *Nat. Rev. Dis. Primers*, **1**, 15017.
Wen, P.Y. & Kesari, S. (2008) Malignant gliomas in adults. *N. Engl. J. Med.*, **359**, 492-507.
Wu, X. (2018) Up-regulation of YPEL1 and YPEL5 and down-regulation of ITGA2 in erlotinib-treated EGFR-mutant non-small cell lung cancer: a bioinformatic analysis. *Gene*, **643**, 74-82.
Yan, H., Parsons, D.W., Jin, G., McLendon, R., Rasheed, B.A., Yuan, W., Kos, I., Batinic-Haberle, I., Jones, S., Riggins, G.J., Friedman, H., Friedman, A., Reardon, D., Herndon, J., Kinzler, K.W., et al. (2009) IDH1 and IDH2 mutations in gliomas. *N. Engl. J. Med.*, **360**, 765-773.