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Prediction of relapse and prognosis by expression levels of long noncoding RNA PEG10 in glioma patients

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Abstract

Background: Long noncoding RNA paternally expressed 10 (lncRNA PEG10) is highly expressed in a variety of human cancers and related to the clinical prognosis of patients. However, to date there has been no previous study evaluating the prognostic significance of lncRNA PEG10 in gliomas. In the present study, we investigated the expression levels of lncRNA PEG10 to determine the prognostic value of this oncogene in human gliomas.

Methods: Expression levels of lncRNA PEG10 were detected by real-time polymerase chain reaction in a hospital-based study cohort of 147 glioma patients and 23 cases of patients with craniocerebral trauma tissues. Associations of lncRNA PEG10 expression with clinicopathological variables and clinical outcome of glioma patients were investigated.

Results: The results indicated that expression levels of lncRNA PEG10 were significantly increased in human gliomas compared to normal control brain tissues. In addition, lncRNA PEG10 expression was progressively increased from pathologic grade I to IV ($P = .009$) and correlated with the Karnofsky performance status ($P = .018$) in glioma patients. Furthermore, we also found that glioma patients with increased expression of lncRNA PEG10 had a higher risk to relapse and a statistically significant shorter overall survival (OS) than patients with reduced expression of lncRNA PEG10. In multivariate analysis, expression level of lncRNA PEG10 was found to be an independent prognostic factor for both progression-free survival and OS in glioma patients.

Conclusions: LncRNA PEG10 served as an oncogene and played crucial roles in the progression of glioma. Molecular therapy targeted on lncRNA PEG10 might bring significant benefits to the clinical outcome of malignant glioma.

Abbreviations: HGG = high-grade glioma, HR = hazard ratios, KPS = Karnofsky performance status, LGG = low-grade glioma, LncRNA = long noncoding RNAs, OS = overall survival, PEG10 = paternally expressed 10, PFS = progression-free survival, WHO = World Health Organization.

Keywords: glioma, long noncoding RNA, paternally expressed 10, prognosis, quantitative real-time PCR

1. Introduction

Glioma is the most frequent and lethal neoplasm of the central nervous system. This dismal tumor occurs in the glial cells and can be divided into grades I to IV with respect to malignancy according to the World Health Organization (WHO) classification.[1] Among all the subtypes of primary brain gliomas, glioblastoma (GBM, grade IV) constitutes the most malignant and carries the worst prognosis even after surgical resection combined with radiotherapy and/or chemotherapy.[2] Although accumulating studies have indicated that age, Karnofsky performance status (KPS) score, preoperative duration of symptoms, histologic grade, tumor necrosis, surgical resection extent, and multimodal therapeutic strategies including radiotherapy as well as chemotherapy can be identified as prognostic factors for gliomas, the clinical outcome of this terrible disease remains heterogeneous.[3] In addition, several new molecular prognostic factors have been proved to contribute to assessment of the survival probability, it is still not possible to accurately evaluate the relapse and clinical outcome for each patient, due to the remarkable molecular heterogeneity in each tumor tissue.[4,5] Therefore, finding a new specific tumor biomarker that is more suitable for the prognostic assessment of gliomas may provide important clinical insights into this disease.

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Long noncoding RNAs (lncRNAs, transcripts of >200 nucleotides) are defined as non–protein-coding RNAs.[6] Previous studies have shown that lncRNAs have critical functions in various cellular events, including cell proliferation, cell differentiation, nuclear architecture, immune surveillance, imprinting, epigenetic regulation, cellular trafficking, splicing, pluripotency of embryonic stem cells, and chromosome inactivation through regulating gene expression at transcriptional and post-transcriptional levels.[7–10] Furthermore, accumulating evidence has identified aberrant expression of lncRNAs in a variety of human tumors and highlighted its contribution to oncogenesis. Nowadays, it is well known that lncRNAs serve as oncogenes or tumor suppressors and implicated in carcinogenesis and cancer proliferation, invasion, and metastasis.[10] LncRNA paternally expressed 10 (PEG10; NONCODE Gene ID NONHSAG048235), which also functions as an oncogene in hepatocellular carcinoma, B-cell lymphocytic leukemia, lymphoma, and esophageal cancer, is closely related to the tumor malignancy in a variety of human cancers.[11–20] For example, knockdown of lncRNA PEG10 significantly inhibited the malignant behavior, such as growth, migration, and invasion in gastric and esophageal cancer.[20,21] However, overexpression of PEG10 could promote proliferation, invasion, and migration in breast, lung, and pancreatic cancer.[18,19,22] In our previous study, we found that PEG10 expression was significantly increased in hypopharyngeal squamous cell carcinoma and was associated with primary tumor size, lymph node status, and tumor node metastasis stage by real-time polymerase chain reaction (PCR). We also found that PEG10 played curial roles in cell proliferation, invasion, and metastasis.[23] Together these evidences suggest important functions for aberrant expression of PEG10 in tumor malignancy progression. However, to our knowledge, no correlations of PEG10 with relapse and prognosis have been addressed in gliomas yet.

Given the crucial role of lncRNA PEG10 in tumorigenesis, the present study mainly investigated expression levels of lncRNA PEG10 and further evaluated the correlation of PEG10 expression with clinical variables, relapse, and the prognostic significance in human gliomas.

2. Materials and methods

2.1. Patients and specimens

The current study was approved by the Second Hospital of Shandong University (Jinan, China) and Shandong Provincial Hospital affiliated to Shandong University. All patients involved provided full consent before participation in the study. Every eligible patient was randomly selected from patients consecutively diagnosed with glioma between May 2004 and December 2016 in Department of Neurosurgery, the Provincial Hospital Affiliated to Shandong University. All the primary tumor tissues were immediately collected following resection and frozen in liquid nitrogen for 10 minutes and stored at −80 °C for mRNA extraction. All the specimens had been histologically diagnosed (according to the WHO classifications in 2007) by Department of Pathology, the Provincial Hospital Affiliated to Shandong University, including 43 cases of low-grade glioma (LGG, 9 pilocytic astrocytomas, 5 myxopapillary ependymomas, 16 diffuse astrocytomas, 7 oligoastrocytomas, and 6 oligodendrogliomas) and 104 cases of high-grade glioma (HGG, 7 anaplastic astrocytomas, 5 anaplastic oligodendrogliomas, 8 anaplastic oligoastrocytomas, 84 GBMs). In addition, specimens from 23 cases of patients with craniocerebral trauma were used as normal control brain tissues.

2.2. Measurement of disease-free survival and overall survival

Patients were followed-up every 3 months by telephone or questionnaire letters for 5 years. Progression-free survival (PFS) was defined as the time from the date of the initial surgery to the first recurrence or death. Recurrence was confirmed by the imaging method such as computed tomography, magnetic resonance imaging, and position emission tomography. Overall survival (OS) was calculated from the date of the initial surgery until death or the last follow-up. Death of patients was ascertained by reporting from the family and verified by review of public records. Study physicians who collected all the following-up data of glioma patients were totally blind to the clinicopathological information and PEG10 expression status.

2.3. RNA extraction and reverse transcription quantitative polymerase chain reaction assay

Total RNA from all the 147 cases of glioma tissues and 23 cases of normal control brain tissues specimens was purified as recommended by the manufacturer using RecoverAll Total Nucleic Acid Isolation kit (Thermo Fisher Scientific, Inc., Waltham, MA). Synthesis of cDNA was performed using 2 μg of total RNA, lncRNA PEG10 reverse transcription primer (Invitrogen; Thermo Fisher Scientific, Inc.), 5X first-strand buffer (Thermo Fisher Scientific, Inc.), 0.1 mol/L DTT (Thermo Fisher Scientific, Inc.), a dNTP mixture (Takara, Bio, Inc., Otsu, Japan), Moloney Murine Leukemia Virus reverse transcriptase (Thermo Fisher Scientific, Inc.) and recombinant RNasin RNase inhibitor (Promega Corporation, Madison, WI). After first-strand synthesis, quantitative PCR (qPCR) was performed by a 7900 HT Fast RealTime PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. All primers were designed and synthesized by Invitrogen (Thermo Fisher Scientific, Inc.) as our previous study described.[23] Each sample was used in a single reaction that cycled at 95 °C for 10 minutes (denaturation), followed by 40 cycles of 95 °C for 15 seconds, and 60 °C for 1 minute on the 7900 HT Fast RealTime PCR system. The mRNA expression of PEG10 was analyzed using the $2^{-\Delta\Delta Cq}$ method and the results were replicated 3 times. Dissociation curve analysis was examined for biphasic melting curves, indicative of whether primer-dimers or other nonspecific products to assess the specificity of the amplified product. Sequencing of 4 μL randomly selected real-time PCR product by 1.5% nondenaturing agarose gel electrophoresis was utilized to ensure the quantity and specificity of real-time PCR.

2.4. Statistical analysis

All statistical analyses were carried out by the statistical package SPSS (SPSS Inc., Chicago, IL). Associations between PEG10 expression levels and clinical variables were analyzed by the Pearson χ^2 test or Fisher exact test as appropriate. Survival curves were estimated using the Kaplan–Meier method, and the prognostic differences in groups with different PEG10 expression were tested by log-rank analysis. Hazard ratios (HRs) of different

prognostic factors were evaluated by Cox proportional hazards modeling analysis to identify the independent prognostic factor on survival. Differences with a $P < .05$ were considered to be statistically significant and values are presented as the mean ± standard deviation.

3. Results

3.1. Characteristics of glioma patients and clinical outcome

The characteristics of the 147 cases of newly diagnosed glioma patients involved in the study cohort were shown in Table 1. The mean age was 54.5 years, with a range of 21 to 79. There were 79 (53.7%) males and 68 (46.3%) females. The KPS scores of 93 (63.3%) glioma patients were <80 and that of 54 (36.7%) patients was >80 (including 80). According to the WHO classifications in 2007, 14 (9.5%), 29 (19.7%), 20 (13.6%), and 84 (57.1%) of 147 glioma patients were classified as WHO grade I, II, III, and IV, respectively. Eight-eight (59.9%) gliomas were located in supratentorial and 59 (40.1%) gliomas were located infratentorial. The tumor size of 74 (50.3%) gliomas was <3 cm and that of 73 (49.7%) patients was >3 cm (including 3 cm). Eight-four patients (57.1%) underwent total surgery resection and 63 (42.9%) patients underwent subtotal surgery resection, which was evaluated by the postoperative magnetic resonance imaging (MRI) within 72 hours after operation.

The survival curves of the PFS and OS were shown in Figure 1. The mean PFS and OS at the time of analysis was 17.96 ± 15.50 and 23.03 ± 16.41 months, respectively. The mean PFS in LGG and HGG were 34.60 ± 17.97 and 11.08 ± 6.72, respectively, whereas the mean OS in LGG and HGG were 42.12 ± 15.60 and 15.13 ± 8.23, respectively. At the end of follow-up, 114 (77.6%) patients died of the disease and 18 (12.2%) cases remained free of disease progression.

3.2. Association between lncRNA PEG10 expression and clinicopathological variables of gliomas

The expression of lncRNA PEG10, which was presented as 2-ΔΔCq values, was detectable in all analyzed clinical specimens and normal control brain tissues. The relative mean value of lncRNA PEG10 expression in 23 cases of normal control brain tissues was 1.023 ± 0.362. In addition, glioma patients were stratified as reduced (1.983 ± 1.752) or increased (4.006 ± 1.383) expression of lncRNA PEG10 according to the mean values of lncRNA PEG10 (2.566 ± 2.147). The difference between glioma patients and normal control brain tissues was statistically significant ($P < .001$), which indicated that expression of lncRNA PEG10 in glioma was increased and was in accordance with our previous finding.[23] Furthermore, 76 (51.7%) patients exhibited reduced PEG10 expression, whereas 71 (48.3%) cases exhibited increased PEG10 expression. The correlation of lncRNA PEG10 expression levels with different clinicopathological variables are shown in Table 1. LncRNA PEG10 expression increased with the progression of pathologic grades ($P = .009$). In addition, Dec1 expression was also significantly correlated with the KPS scores ($P = .018$). However, no statistically significant correlations were observed between lncRNA PEG10 expression levels and age, sex, tumor location, tumor size, and surgery resection.

Table 1

Correlations of lncRNA PEG10 expression with clinical variables in glioma patients.

Variable	Description	N	Expression of lncRNA PEG10		P
			Reduced	Increased	
Age, y	<50	70	33	37	.295[b]
	≥50	77	43	34	
Sex	Male	79	46	33	.089[b]
	Female	68	30	38	
KPS	<80	93	55	38	.018[b]
	≥80	54	21	33	
WHO Grade	I	14	12	2	.009[c]
	II	29	15	14	
	III	20	13	7	
	IV	84	36	48	
Tumor location	Supratentorial	88	46	42	.867[b]
	Infratentorial	59	30	29	
Tumor size	<3 cm	74	40	34	.568[b]
	≥3 cm	73	36	37	
Surgery resection[a]	Total	84	45	39	.603[b]
	Subtotal	63	31	32	

Surgery resecton[a]: Surgery resection was evaluated by the postoperative magnetic resonance imaging (MRI) within 72 hours after operation. Complete resolution on the postoperative MRI (T1 low-intensity lesion for grade I, II and III; T1 gadolinium -enhanced lesion for grade IV) was considered as total resection. *P* values were evaluated using Pearson [b]χ^2 test or [c]Fisher exact test.

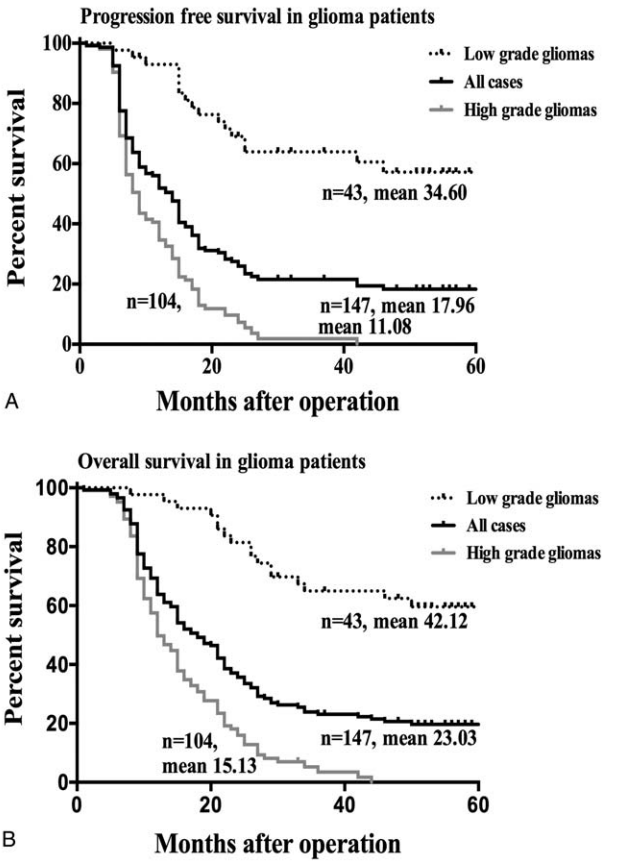

Figure 1. Survival curves of different groups of glioma patients: progression-free survival (A) and overall survival (B).

3.3. Association between lncRNA PEG10 expression and PFS of glioma patients

The postoperative mean PFS time of patients with reduced expression of lncRNA PEG10 was 22.36 ± 17.52 months, whereas that of patients with increased expression of lncRNA PEG10 was 13.25 ± 11.34 months. The Kaplan–Meier analysis was used to evaluate the PFS of glioma patients and lncRNA PEG10 expression levels. Results indicated that patients with reduced expression of lncRNA PEG10 had better PFS than those with increased expression of lncRNA PEG10 (Fig. 2A, $P < .001$). Glioma patients with increased expression of lncRNA PEG10 had a higher risk to relapse than in those with reduced expression of lncRNA PEG10. Moreover, patients with age >50, KPS of less 80 and higher WHO grade also had shorter PFS and higher risk to relapse than those without (Fig. 2B–D). However, sex, tumor location, tumor size, or surgery resection had no prognostic value on PFS in the current study cohort (data not shown).

The Kaplan–Meier analysis was also performed with stratification according to the malignant degree. Because high grade of glioma was considered a marker of high risk, we classified patients into subgroups of LGG and HGG, respectively. The postoperative mean PFS in LGG and HGG were 34.60 ± 17.97 and 11.08 ± 6.72 months, respectively. The postoperative mean PFS of patients with reduced and increased lncRNA PEG10 expression in LGG were 39.44 ± 17.42 and 26.44 ± 16.28 months, respectively, whereas those of patients with reduced and increased lncRNA PEG10 expression in HGG were 12.94 ± 7.75 and 9.42 ± 5.16 months, respectively. Expression levels of lncRNA PEG10 were correlated with PFS both in LGG and HGG. PFS was significantly longer in patients with reduced lncRNA PEG10 expression versus increased expression both in LGG and HGG (Fig. 2 E and F, $P = .0112$ and .0097, respectively). In addition, age and KPS had prognostic value for PFS both in LGG and HGG, whereas see, tumor location, tumor size, or surgery resection did not contribute to long-term benefits in the current cohort (data not shown).

A Cox proportional hazards model adjusted for age, sex, KPS, WHO grade, tumor location, tumor size, and surgery resection was performed to verify the independent prognostic value of lncRNA PEG10 expression levels. As a result, reduced lncRNA PEG10 expression was proved to be a favorable independent prognostic factor for PFS after controlling for all the clinicopathological variables ($P = 0.009$; HR, 1.738). Similarly, age and KPS were also shown to be independent prognostic factors for PFS. However, no statistically significance was found among other variables, including gender, tumor location, tumor size and surgery resection (Table 2).

3.4. Association between lncRNA PEG10 expression and OS of glioma patients

A statistically significant association between OS and expression levels of lncRNA PEG10 was found in glioma patients. The postoperative mean OS time of patients with reduced expression of lncRNA PEG10 was 27.54 ± 17.33 months, whereas that of patients with increased expression of lncRNA PEG10 was 18.20 ± 13.95 months. The results of Kaplan–Meier analysis indicated that patients with reduced expression of lncRNA PEG10 had better OS than those with increased expression of lncRNA PEG10 (Fig. 3A, $P < .001$). Similar to results of PFS, age of >50, KPS <80, and higher WHO grade proved to be poor prognostic factors for OS (Fig. 3B-D) and sex, tumor location, tumor size or surgery resection had no prognostic value for OS in glioma patients (data not shown).

Both in LGG and HGG, significant association of OS was found in patients with reduced lncRNA PEG10 expression versus those with increased lncRNA PEG10 expression. The postoperative mean OS of patients with reduced and increased lncRNA PEG10 expression in LGG were 45.74 ± 13.43 and 36.00 ± 17.45

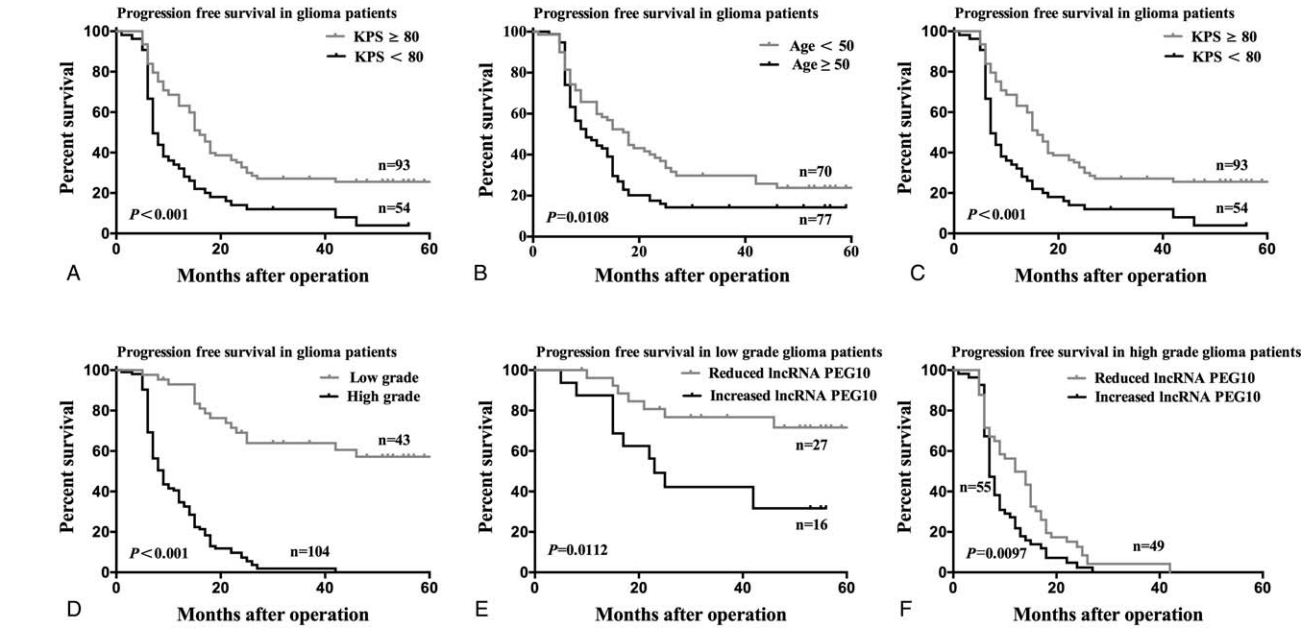

Figure 2. Correlation of clinicopathological variables with progression-free survival: lncRNA PEG10 expression (A), age (B), KPS (C), and degree of malignancy (D) in all cases of gliomas; lncRNA PEG10 expression in low-grade of gliomas (E) and high-grade gliomas (F). KPS = Karnofsky performance status.

Table 2

Univariate and multivariate analyses of progression-free survival in glioma patients.

Clinical variables	Unadjusted HR[a] (95% CI)	*P*	Adjusted HR[b] (95% CI)	*P*
Age, y				
<50/≥50	1.588 (1.091–2.310)	0.016	1.712 (1.135–2.580)	.010
Sex				
Male/female	1.121 (0.776–1.620)	0.542	0.993 (0.067–1.463)	.971
KPS				
≥80/<80	2.509 (1.411–3.005)	0.000	2.551 (1.660–3.918)	.000
WHO grade				
I+II/III+IV	7.595 (4.229–13.42)	0.000	9.550 (5.255–17.35)	.000
PEG10 expression				
Reduced/increased	1.994 (1.370–2.930)	0.000	1.738 (1.145–2.640)	.009
Tumor location				
Sup./Inf.	1.006 (0.691–1.466)	0.973	1.085 (0.738–1.596)	.677
Tumor size				
≥3 cm/<3 cm	0.776 (0.534–1.125)	0.181	1.043 (0.694–1.567)	.839
Surgery resection				
Total/subtotal	1.262 (0.872–1.827)	0.218	1.020 (0.694–1.499)	.919

Hazard ratios in [a]univariate models or [b]multivariable models.

CI = confidence interval, HR = hazard ratio, Inf. = infratentorial, KPS = Karnofsky performance status, Sup. = supratentorial.

months, respectively, whereas those of patients with reduced and increased lncRNA PEG10 expression in HGG were 17.51 ± 9.07 and 13.02 ± 6.80 months, respectively. Kaplan–Meier analysis indicated that patients with reduced lncRNA PEG10 expression had longer OS than patients with increased expression both in LGG and HGG (Fig. 3E and F, $P = .0122$ and .0040). In accordance with results in PFS, age and KPS had prognostic value for OS both in LGG and HGG, whereas sex, tumor location, tumor size, or surgery resection was not found to be correlated with patient clinical outcome (data not shown).

Results of multivariate analysis indicated that lncRNA PEG10 expression levels could be a prognostic factor for OS of patients with glioma independent of age, sex, KPS, WHO grade, tumor location, tumor size, and surgery resection ($P = .006$; HR, 1.763). In addition, age and KPS were also shown to be independent prognostic factors for OS after controlling for all other clinicopathological variables and other variables, including sex, tumor location, tumor size, and surgery resection, were not found to be significant independent predictors of OS (Table 3).

4. Discussion

Due to the indefinite proliferative potential, antiapoptotic nature, and the tendency to metastasize of gliomas, total surgical resection is nearly impossible for this malignant brain tumor. Therefore, the expression levels of crucial factors that are

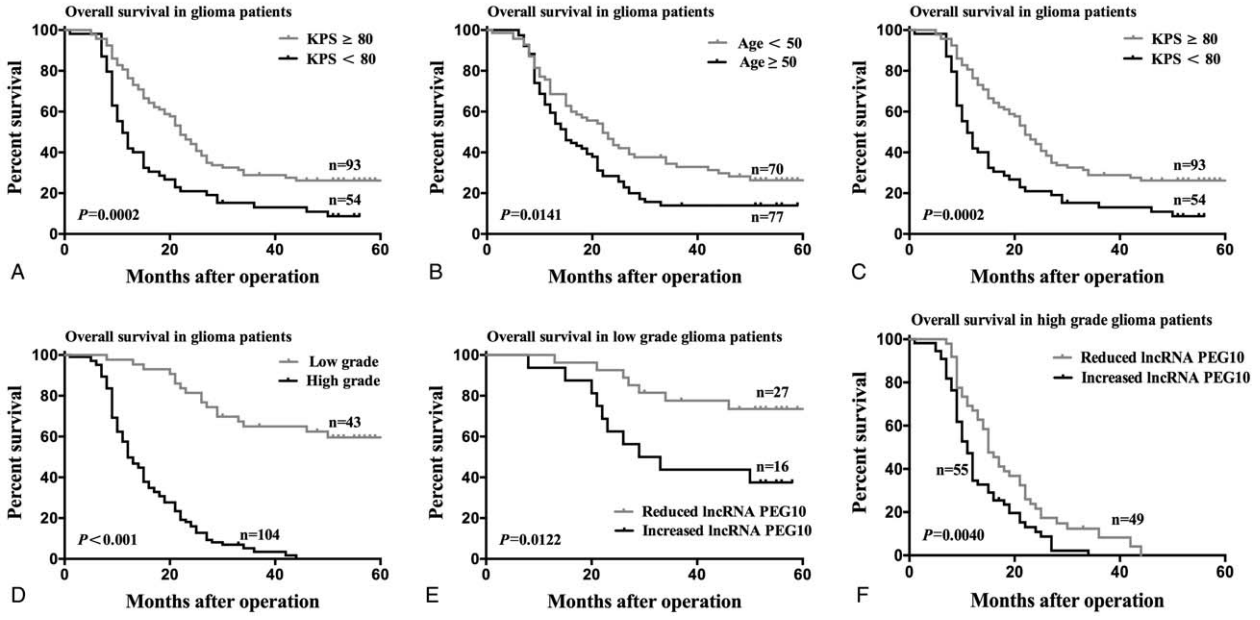

Figure 3. Correlation of clinicopathological variables with overall survival: lncRNA PEG10 expression (A), age (B), KPS (C) and degree of malignancy (D) in all cases of gliomas; lncRNA PEG10 expression in low-grade of gliomas (E) and high-grade gliomas (F). KPS = Karnofsky performance status.

Table 3

Univariate and multivariate analyses of overall survival in glioma patients.

Clinical variables	Unadjusted HR[a] (95% CI)	*P*	Adjusted HR[b] (95% CI)	*P*
Age				
<50/50	1.573 (1.082–2.287)	0.018	1.669 (1.112–2.506)	.013
Sex				
Male/female	1.151 (0.797–1.663)	0.454	1.103 (0.745–1.633)	.626
KPS				
≥80/<80	1.992 (1.369–2.899)	0.000	2.261 (1.738–4.076)	.000
WHO grade				
I+II/III+IV	8.574 (4.790–15.35)	0.000	11.368 (6.150–21.0)	.000
PEG10 expression				
Reduced/increased	2.039 (1.404–2.961)	0.000	1.763 (1.175–2.644)	.006
Tumor location				
Sup./Inf.	1.071 (0.735–1.599)	0.721	1.329 (0.892–1.980)	.163
Tumor size				
≥3 cm/<3 cm	0.766 (0.528–1.113)	0.162	1.067 (0.715–1.593)	.749
Surgery resection				
Total/subtotal	1.210 (0.836–1.750)	0.313	0.905 (0.617–1.329)	.611

Hazard ratios in [a]univariate models or [b]multivariable models.

CI = confidence interval, HR = hazard ratio, Inf. = infratentorial, KPS = Karnofsky performance status, Sup. = supratentorial.

involved in the malignant processes may dramatically impact the development and the clinical outcome of glioma patients. Recent study has implicated lncRNAs in a variety of human carcinomas and expression signature of these molecules can provide insight into the diagnosis, prognosis, and therapeutic strategies in human cancers.[24] Our previous study indicated that lncRNA PEG10 was overexpressed in hypopharyngeal squamous cell carcinoma and promotes proliferation, invasion, and metastasis both in vitro and in vivo.[23] However, to date there has been no previous study of the prognostic significance of lncRNA PEG10 in gliomas. In the present study, we mainly investigated the expression levels and prognostic value of lncRNA PEG10 in human gliomas. The results indicated that expression of lncRNA PEG10 was significantly increased in human gliomas compared to non-neoplastic brain tissues. In addition, lncRNA PEG10 expression also proved to be progressively increased from WHO grade I to IV. These results further supported the notion that lncRNA PEG10 served as an oncogene and played crucial roles in the development of glioma. Furthermore, we also found that glioma patients with increased expression of lncRNA PEG10 had a higher risk to relapse and a worse clinical outcome than in those with reduced expression. Thus, molecular targeted therapy on lncRNA PEG10 might bring significant benefits to the clinical outcome of malignant glioma. To our knowledge, this is the first investigation evaluating the expression pattern and clinical significance of lncRNA PEG10 in glioma patients.

As a less-known lncRNA, expression of PEG10 has been identified to be upregulated in a variety of cancers.[13,16–19,21–23] In the present study, expression of lncRNA PEG10 was significantly increased in human gliomas comparing with the normal control brain tissues. This result was consistent with our previous study and the findings mentioned above, which indicated that lncRNA PEG10 served as an oncogene and played crucial roles in oncogenesis. Uncontrolled proliferation is the most important characteristic in tumor progression. Recent studies have provided evidences for the oncogenic functions of lncRNA PEG10 by promoting cancer proliferation. First of all, the expression pattern of lncRNA PEG10 was cell cycle-dependent manner, which drives cell cycle progression from G0/G1 to S phase and promoted cancer proliferation.[22] Ishii et al identified that inhibiting expression of PEG10 in MKN7 cells reduced anchorage-independent colony formation.[25] Additionally, PEG10 was reported to be abundantly expressed in HCC and decreasing endogenous expression of PEG10 induced inhibition of proliferation.[26] As a target gene of c-Myc, PEG10 promoted the proliferation of several tumor cells by activating the transcription of c-Myc.[27] Together, these evidences suggest the oncogenic roles for lncRNA PEG10 in cancer proliferation and further indicated that lncRNA PEG10 could be used as a potential target for antiproliferation therapy in the molecular pathways determining the behavior of glioma.

On the contrary, a recent meta-analysis has identified the clinical significance of PEG10 expression in a set of human solid tumors. They found that PEG10 was overexpressed in a set of human tumors and its overexpression was significantly correlated with the tumor oncogenesis, lower degree of differentiation, increased lymph node metastasis, and advanced tumor node metastasis stage.[28] High malignancy and low differentiation are the main differences between low and high pathological grades of gliomas. HGGs were either poorly differentiated or undifferentiated, and consequently carried a dismal prognosis.[29] The current study observed that expression of LncRNA PEG10 was significantly correlated with the pathologic grades and KPS in glioma patients ($P = .009$ and .018, respectively, Table 1). This evidence was consistent with the previous study and its capacity to antagonize differentiation in various types of human tumor, which proved that expression of lncRNA PEG10 was strongly correlated with degree of malignancy and differentiation in human gliomas.

In the sight of the crucial roles in oncogenesis, several studies investigated the prognostic significance of PEG10 in human cancers.[16,18,19,30–33] For example, Ge et al[28] found that PEG10 could be served as an independent prognostic factor in a set of human solid tumors and its high-expression was significantly correlated with the poor clinical outcome. In addition, PEG10 was also identified as a biomarker for predicting early recurrence and patients with high expression of PEG10 have shorter recurrence-free survival in HCC.[13] However, little is known

about the prognostic value of lncRNA PEG10 in human gliomas. In the present study, the Kaplan–Meier analysis showed that glioma patients with increased expression of lncRNA PEG10 had worse PFS than glioma patients with reduced lncRNA PEG10 expression (Fig. 2). Multivariate analysis found that patients with increased expression of lncRNA PEG10 had higher risk of relapse, with adjusted HR of 1.738 (Table 2, 95% confidence interval [CI]: 1.145–2.640, $P = .009$). Moreover, glioma patients with increased expression of lncRNA PEG10 had worse OS than those with reduced expression of lncRNA PEG10 (Fig. 3). Multivariate analysis indicated that lncRNA PEG10 could be used as an independent prognostic indicator to evaluate the outcome of patients with glioma (Table 3, 95% CI: 1.175–2.644, $P = .006$, adjusted HR: 1.763). These evidences were consistent with the previous studies and further indicated that increased expression of lncRNA PEG10 could help to predict which patients with an intermediate risk of relapse would develop recurrent disease. Moreover, lncRNA PEG10 might be served as an important candidate therapeutic biomarker due to its prognostic value for the OS in gliomas.

Several previous studies might explain the prognostic value of lncRNA PEG10 in glioma patients. To date, the most effective used strategy for malignant glioma was surgical resection combined with radiotherapy and/or chemotherapy. However, these strategies are never curative due to the antiapoptotic nature of glioma cells.[29,34] Therefore, the proliferative ability and the antiapoptotic potential of the residual cancer cells will dramatically influence the clinical outcome of glioma patients. The function of promoting proliferation of lncRNA PEG10 has been discussed above. As an oncogene, recent studies have been identified the antiapoptotic role of PEG10 in a variety of human cancers.[15,35–38] In hepatocellular carcinoma, PEG10 could increase expression of the apoptosis promoting factor Bcl-2 and decrease expression of the apoptosis-inhibiting factor Bax, which induced the attenuation of apoptosis by doxorubicin.[39] Additionally, Yoshibayashi et al[37] found that PEG10 was overexpressed mediating by SIAH1 and played crucial roles in decreasing the cell death in hepatocellular carcinoma. Hu et al indicated that upregulated expression of PEG10 contributes to resistance to apoptosis by activation of caspase-3 and caspase-8 in CD19+CD34+ B cells from patients with B cell lineage acute and chronic lymphocytic leukemia.[15] Furthermore, our previous study revealed that lncRNA PEG10 was upregulated in hypopharyngeal squamous cell carcinoma cells and promoted an increase in the tumorigenic activities of proliferation, invasion and migration.[23] On the other hand, several recent investigations have also indicated that cancers with overexpression of PEG10 are more vulnerable to metastasis or invasion.[18–20,22,40,41] For example, through upregulating expression levels of MMP-1, -2 and -9 and downregulating expression levels of TIMP-1 and -2, PEG10 promoted the migration and invasion of breast cancer cell line MDA-MB-231.[17] In addition, overexpression of PEG10 also promoted cell proliferation, migration, and invasion via ERK/MMP7 pathway in pancreatic cancer.[22] Together, all these evidences indicated that expression levels of lncRNA PEG10 could clearly reflect the malignant characteristics of proliferative potential, antiapoptotic nature, degree of invasiveness, and metastasis in gliomas. Therefore, we considered that the higher expression of lncRNA PEG10 has the worse prognosis in patients with glioma.

In conclusion, our study provided the first evidence that expression level of lncRNA PEG10 was significantly correlated with the advanced clinicopathological features and poor outcome in patients with gliomas. LncRNA PEG10 can be identified as an oncogenic biomarker for the relapse and clinical outcome in glioma patients. Although further underlying mechanisms will be needed to determine the underlying mechanism of this observation, our findings support the notion that more effective therapeutic strategies targeting the lncRNA PEG10 may have important clinical implications for human gliomas.

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