

Up-regulation of long non-coding RNA BLACAT1 predicts aggressive clinicopathologic characteristics and poor prognosis of glioma

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Abstract

Bladder cancer-associated transcript 1 (BLACAT1) is one of the most common cancer-associated long non-coding RNAs (lncRNAs), which has been reported as a tumor promoter in several malignancies. Previously, BLACAT1 was found to be overexpressed in glioma tissues and cell lines. Functional assays determined that BLACAT1 promoted glioma cell proliferation, migration, invasion and epithelial-mesenchymal transition, suggesting that BLACAT1 might serve as an oncogene in glioma. In the present study, we aimed to investigate its clinical significance and prognostic value in glioma patients.

A total of 137 paired glioma tissue samples and adjacent normal brain tissue samples were collected from 137 glioma patients who underwent surgery from May 2014 to February 2019. The Student *t* test was applied to determine the statistical significance of the observed differences between 2 groups. Survival curves were constructed and differences among groups were calculated using the Kaplan–Meier method.

The relative expression of BLACAT1 in glioma samples was significantly higher than that of matched normal tissues ($P < .001$). The expression level of tissue BLACAT1 was statistically correlated with tumor size ($P = .04$), Karnofsky Performance Status (KPS) ($P = .006$), and WHO grade ($P = .017$). Kaplan–Meier analysis with the log-rank test revealed that BLACAT1 up-regulation was correlated with shorter overall survival time of patients with glioma (Log Rank test, $P = .012$). In multivariate Cox analysis, BLACAT1 expression was found to be an independent prognostic factor for overall survival in patients with glioma (HR = 2.739; 95% CI: 1.785–8.229; $P = .035$). Our study demonstrates that up-regulation of BLACAT1 is able to predict aggressive clinicopathologic characteristics and poor prognosis of glioma patients. These findings may have significant implications for potential treatment options and prognosis for patients with glioma.

Abbreviations: BLACAT1 = bladder cancer-associated transcript 1, KPS = Karnofsky Performance Status, lncRNAs = long non-coding RNAs.

Keywords: bladder cancer-associated transcript 1, expression, glioma, prognosis

1. Introduction

Glioma is the most common brain cancer and is the leading cause of brain tumor mortality worldwide.^[1] There are 4 different grades (2 classes) of glioma: grade I and II (low8 #1" wglioma) as

well as grade III and IV (high low8 glioma).^[2] Glioma genesis is a complex evolutionary process involving multiple molecular mechanisms that eventually leads to malignant lesions. Though some progresses in early diagnosis and treatment, majority of the patients with glioma are still detected at advanced stages, and clinical outcomes of them are still disappointing after treatment.^[3] Therefore, determining molecular targets for new therapies is imperative.

Long non-coding RNAs (lncRNAs) are RNA genomically transcribed non-coding transcripts longer than 200 nucleotides.^[4,5] Dysregulation of lncRNAs expression has been found in various types of human cancers, including glioma.^[6,7] Increasing studies have shown that the dysregulation of lncRNAs are closely related to the occurrence and development of glioma.^[7] This offers new insight in the diagnosis, targeted therapy and prognosis for glioma.

Bladder cancer-associated transcript 1 (BLACAT1) is one of the most common cancer-associated lncRNAs, which has been reported as tumor promoter in several malignancies, such as non-small-cell lung cancer (NSCLC), hepatocellular carcinoma (HCC), breast cancer, osteosarcoma, cervical cancer, gastric cancer, papillary thyroid cancer (PTC), and colorectal cancer.^[8–15] Previously, the role of BLACAT1 has also been investigated in glioma. Li et al found that BLACAT1 expression was significantly increased in glioma tissues compared to adjacent non-tumor tissues ($P < .05$). BLACAT1 expression was also markedly

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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increased in glioma tumors of high grade (III-IV) compared with low grade (I-II) tumors ($P < .05$). Functional assays determined that BLACAT1 promoted glioma cell proliferation, migration, invasion and epithelial-mesenchymal transition *in vitro*. In addition, it was demonstrated that BLACAT1 activated the Wnt/ β -catenin signaling pathway.^[16] However, its clinical significance and prognostic value in glioma patients have not been investigated.

2. Materials and methods

2.1. Patient information and tissue samples

A total of 137 paired glioma tissue samples and adjacent normal brain tissue samples were collected from 137 patients who underwent surgery at Department of Craniocerebral Surgery, People's Hospital of Lanling County from May 2014 to February 2019. All the glioma patients have not received preoperative chemotherapy or radiotherapy. The inclusion criteria were as follows:

1. a histological diagnosis of glioma,
2. patients with explicit clinical prognostic data.

The exclusion criteria were as follows:

1. patients have history of other tumors,
2. patients received radiotherapy, chemotherapy, targeted treatment and immunotherapy before surgery.

Tissue samples were snap-frozen in liquid nitrogen at the time of surgery and subsequently stored at -80°C . Final histological classifications and findings were made by 2 pathologists. The clinical information of the glioma patients were shown in Table 1. This study was approved by the Ethics Committee of People's Hospital of Lanling County. Informed written consents were obtained from all recruited patients.

2.2. RNA isolation and quantitative real-time PCR

Total RNA from glioma tissues and normal brain tissues was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was reverse transcribed to cDNA using a SYBR Green PCR kit (TaKaRa,

Otsu, Shiga, and Japan). Quantitative real-time polymerase chain reaction parameters were as follows: predegeneration for 15 minutes at 95°C , degeneration at 94°C for 15 second, annealing at 55°C for 30 second, and extension at 72°C for 30 second, for a total of 40 cycles. The BLACAT1 expression levels were normalized to GAPDH. The primers used were as followed: BLACAT1 forward, 5'-CAAGAGGAGCCGGCTTAG-CATCTA-3', reverse 5'-ACGGTTCAGTCCTCAGTCAG-3'; GAPDH, forward: 5'-CAAGAGGAGCCGGCTTAG-CATCTA-3', reverse 5'-ACGGTTCAGTCCTCAGTCA-3'.

2.3. Statistical analysis

The Student *t* test was applied to determine the statistical significance of the observed differences between groups. Chi-Squared test was used to compare the expression level of BLACAT1 and clinical variables. Survival curves were constructed and differences among groups were calculated using the Kaplan–Meier method. Significant variables in univariate analyses were used in multivariate analyses according to the Cox regression analyses. SPSS software, version 20.0 for Windows (SPSS Inc., Chicago, IL) was used for statistical analysis. $P < .05$ was considered statistically significant.

3. Results

3.1. The expression level of BLACAT1 in glioma tissues

To investigate the role of BLACAT1 in glioma development, we tested the expression of BLACAT1 in glioma tissues and adjacent normal brain tissues. The relative expression of BLACAT1 in glioma samples was significantly higher than that of matched normal brain tissues ($P < .001$, shown in Fig. 1). Hence, we considered the up-regulation of BLACAT1 may contribute to glioma tumorigenesis.

3.2. Association between BLACAT1 expression levels and clinicopathologic parameters in glioma patients

We evaluated the correlation of tissue BLACAT1 expression level with clinicopathological parameters of glioma patients. The expression level of tissue BLACAT1 was statistically correlated with tumor size ($P = .04$), KPS ($P = .006$), and

Table 1
Summary of clinicopathologic features of the glioma patients.

Variables	Cases (n)	BLACAT1 expression		P value
		High (n=67)	Low (n=70)	
Gender				
Male	97	51	46	.194
Female	40	16	24	
Age (yrs)				
<60	81	37	44	.389
≥ 60	56	30	26	
Tumor size (cm)				
<5	73	42	31	.040
≥ 5	64	25	39	
KPS				
<70	45	30	15	.006
≥ 70	92	37	55	
WHO grade				
I/II	68	26	42	.017
III/IV	69	41	28	

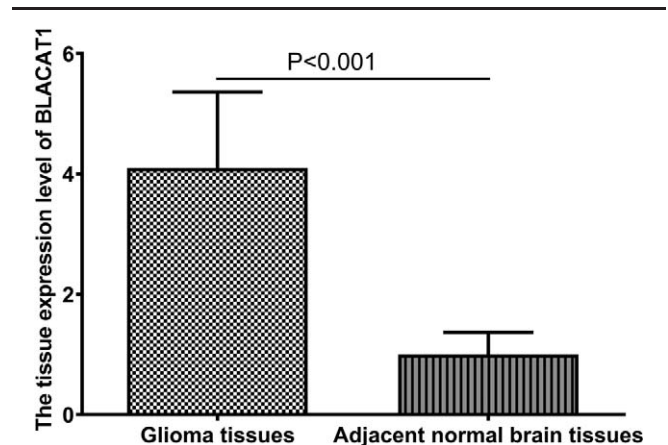


Figure 1. The expression level of BLACAT1 in glioma tissues and adjacent normal brain tissues.

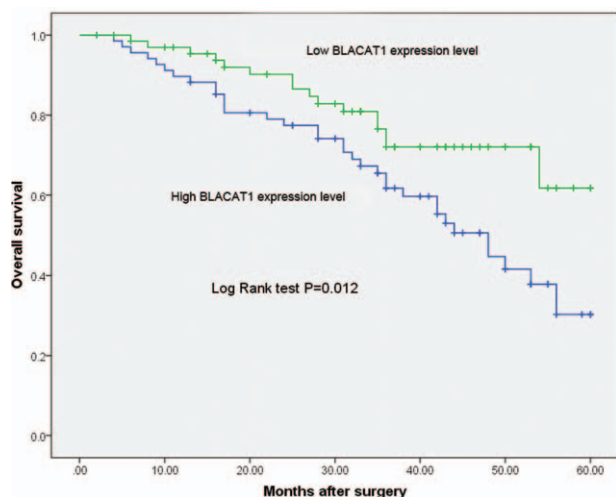


Figure 2. Kaplan–Meier overall survival curve for high and low BLACAT1 expression.

WHO grade ($P=.017$); whereas no significant correlation between tissue BLACAT1 expression level and gender, and age (all $P > .05$). The results suggest that tissue BLACAT1 expression level may be a potential marker for the reflection of disease status. These data are summarized in Table 1.

3.3. Correlation between tissue BLACAT1 expression level and prognosis of glioma patients

To further evaluate the prognostic value of tissue BLACAT1 expression level for glioma patients, we also analyzed the association between BLACAT1 expression and survival duration using Kaplan–Meier analysis with the log-rank test. The results revealed that BLACAT1 up-regulation was correlated with shorter overall survival time of patients with glioma (Log Rank test, $P=.012$, shown in Fig. 2). In addition, univariate and multivariate Cox analysis were used to examine whether tissue BLACAT1 expression was an independent risk factor for prognosis in glioma patients. The parameters that significantly correlated with survival in the univariate analysis were further assessed by multivariate analysis. In multivariate analysis, BLACAT1 expression was found to be an independent prognostic factor for overall survival in patients with glioma (HR=2.739; 95% CI: 1.785–8.229; $P=.035$, shown in Table 2).

4. Discussion

Glioma is one of the most aggressive and common primary malignant brain tumors, and it accounts for approximately 80% of all primary malignant brain tumor cases.^[2] In spite of the progress that researchers and medical workers made in the treatment of patients with glioma over the last decades, the prognosis and survival rate are still unpleasant.^[17–19] Therefore, it is critical to develop accurate risk evaluation methods with the aim of preventing the recurrence of glioma.

lncRNAs play important roles in some crucial biological processes, such as cellular proliferation, differentiation, apoptosis, and tumorigenesis.^[4,20,21] Recently, accumulating evidence indicates that lncRNAs expression level in tissues or serum can be served as novel diagnostic or prognostic biomarkers in many types of cancers.^[22,23] BLACAT1 is one of the most common cancer-associated lncRNAs, which has been reported as tumor promotor in several malignancies, such as hepatocellular carcinoma, colorectal cancer, osteosarcoma, esophageal cancer, gallbladder carcinoma, endometrial cancer, and bladder cancer. There are a lot of studies investigating its mechanism and clinical significance. For example, Xu et al found that non-small-cell lung cancer (NSCLC) patients with high BLACAT1 expression had shorter overall survival and progression-free survival than those with low BLACAT1 expression. Multivariate analyses showed that BLACAT1 was an independent prognostic factor of survival in NSCLC patients. In vitro assays showed that the downregulation of BLACAT1 significantly suppressed cell progression, migration, and invasion. The epithelial-mesenchymal transition was also inhibited when BLACAT1 was silenced, indicated by an increase in E-cadherin expression and a decrease in vimentin expression by mediating Wnt/ β -catenin signaling pathway.^[8] Peng et al found that BLACAT1 was aberrantly upregulated in HCC and its inhibition had tumor suppressing effects in human HCC, possibly through endogenously competing against has-miR-485-5p. The BLACAT1/ has-miR-485-5p regulatory axis may be a molecular target for future HCC therapy.^[9] Hu et al found that BLACAT1 took part in breast cancer cell aggressive phenotype. The real-time PCR result showed that BLACAT1 was up-regulated in tumor tissues compared to adjacent normal tissues. The molecular mechanism experiments demonstrated that BLACAT1 down-regulation suppressed the proliferation and metastasis of human breast cancer cells by regulating miR-150-5p targeting CCR2. The clinical studies indicated that lack of BLACAT1 was related to tumor size, and metastasis.^[10] Dong et al found that BLACAT1 was upregulated in osteosarcoma tissues and cells. Upregulation of BLACAT1 predicted unfavorable prognosis for patients with osteosarco-

Table 2
Univariate and multivariate analysis of overall survival in 137 patients with glioma.

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P value	Hazard ratio	95% CI	P value
Gender	0.692	0.478–2.738	.736			
Age	1.784	0.815–3.664	.163			
Tumor size	2.183	0.892–6.284	.075			
KPS	4.281	2.284–12.283	.009	3.928	2.775–11.923	.007
WHO grade	3.957	1.559–10.034	0.013	4.012	2.192–10.273	.009
BLACAT1 expression	2.885	1.628–8.285	0.047	2.739	1.785–8.229	.035

ma. Downregulation of BLACAT1 inhibited osteosarcoma cell proliferation and invasion, whereas upregulation of BLACAT1 accelerated cell proliferation and invasion. More importantly, BLACAT1 could interact with STAT3 and regulate the phosphorylation of STAT3.^[11] Su et al found that increased BLACAT1 was an independent unfavorable prognostic indicator for colorectal cancer, and revealed that BLACAT1 knockdown significantly repressed proliferation, both in vitro and in vivo. Mechanistic investigations demonstrated that BLACAT1 had a key role in G1/G0 arrest, and showed that BLACAT1 can repress p15 expression by binding to EZH2, thus contributing to the regulation of colorectal cancer cell cycle and proliferation. These results suggest that BLACAT1, as a cell cycle regulator, may serve as a potential target for cancer prevention and treatment in human colorectal cancer.^[15]

Previously, the role of BLACAT1 has also been investigated in glioma. Li et al found that BLACAT1 expression was significantly increased in glioma tissues compared to adjacent non-tumor tissues ($P < .05$). BLACAT1 expression was also markedly increased in glioma tumors of high grade (III-IV) compared with low grade (I-II) tumors ($P < .05$). Functional assays determined that BLACAT1 promoted glioma cell proliferation, migration, invasion and epithelial-mesenchymal transition in vitro. In addition, it was demonstrated that BLACAT1 activated the Wnt/ β -catenin signaling pathway.^[16] However, its clinical significance and prognostic value in glioma patients have not been investigated. In the present study, to investigate the role of BLACAT1 in glioma development, we tested the expression of BLACAT1 in glioma tissues and adjacent normal brain tissues. The relative expression of BLACAT1 in glioma samples was significantly higher than that of matched normal brain tissues. Hence, we considered the up-regulation of BLACAT1 may contribute to glioma tumorigenesis. We then evaluated the correlation of tissue BLACAT1 expression level with clinicopathological parameters of glioma patients. The expression level of tissue BLACAT1 was statistically correlated with tumor size, KPS, and WHO grade; whereas no significant correlation between tissue BLACAT1 expression level and gender, and age. The results suggest that tissue BLACAT1 expression level may be a potential marker for the reflection of disease status. To further evaluate the prognostic value of tissue BLACAT1 expression level for glioma patients, we also analyzed the association between BLACAT1 expression and survival duration using Kaplan-Meier analysis with the log-rank test. The results revealed that BLACAT1 up-regulation was correlated with shorter overall survival time of patients with glioma. In addition, univariate and multivariate Cox analysis were used to examine whether tissue BLACAT1 expression was an independent risk factor for prognosis in glioma patients. The parameters that significantly correlated with survival in the univariate analysis were further assessed by multivariate analysis. In multivariate analysis, BLACAT1 expression was found to be an independent prognostic factor for overall survival in patients with glioma. In the present study, we have not investigated the expression level of BLACAT1 in serum. This is the limitation of our study, and we will assess the BLACAT1 level in paired serum samples from glioma patients and healthy controls, and also investigate the prognostic value of serum BLACAT1 level in the future.

In conclusion, up-regulation of BLACAT1 is associated with aggressive clinicopathologic characteristics and poor prognosis in glioma patients. These findings may have significant

implications for potential treatment options and prognosis for patients with glioma. Further study with larger sample size is warranted to confirm our results.

Author contributions

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