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Expression of long non-coding RNA MAGI2-AS3 in human gliomas and its prognostic significance

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Abstract. – OBJECTIVE: It has been confirmed that the dysregulation of long noncoding RNAs (lncRNAs) is associated with various diseases, especially cancer. LncRNA MAGI2 antisense RNA 3 (MAGI2-AS3) has been reported to be involved in the progression of bladder cancer and breast cancer. In this study, we aimed to explore its expression and clinical significance in glioma.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) assays were performed to detect the expression levels of MAGI2-AS3 in fresh glioma and matched adjacent normal brain tissue specimens, which were collected from 178 patients. Then the association between MAGI2-AS3 expression and clinical-pathological parameters were further evaluated using the Chi-square test. The overall survival (OS) was analyzed by the log-rank test, and the survival curves were plotted according to Kaplan-Meier. Univariate and multivariate analyses were performed to analyze the prognostic significance of MAGI2-AS3 expression.

RESULTS: We found that the relative expression level of MAGI2-AS3 in glioma tissues was significantly lower than those in adjacent normal brain tissues ($p<0.01$). Lower MAGI2-AS3 expression was observed to be positively correlated with the World Health Organization (WHO) grade ($p=0.031$) and KPS score ($p=0.003$) in glioma patients. The Kaplan-Meier analysis indicated that patients with low MAGI2-AS3 expression levels tended to have worse overall survival than those with high levels of MAGI2-AS3 expression ($p=0.0042$). In the multivariate analysis, we further observed that MAGI2-AS3 expression in glioma tissues was an independent prognostic factor for overall survival (HR=3.098, 95% CI: 1.289-4.118, $p=0.014$).

CONCLUSIONS: MAGI2-AS3 expression represents a significant favorable prognostic factor for patients with glioma.

Key Words
LncRNA, MAGI2-AS3, Prognosis, Glioma.

Introduction

Gliomas, the most frequent type of primary brain cancer, account for about 80% of primary malignant neoplasms of the central nervous system in adults[1,2]. The grade III and IV (malignant) gliomas, also known as glioblastoma, are aggressive and lethal malignant neoplasms[3]. The patients diagnosed at an early stage have achieved a favorable result. However, high proliferation rate and invasiveness trait of glioblastoma make it poor prognosis in spite of efforts being made to improve therapeutic strategies[4,5]. Up to date, the overall survival rates vary between studies (22 to 60% at one year) and only 1.7-4.6% of glioblastoma patients are expected to survive longer than 24 months[6]. Although great advancements in genetic alterations have been made in understanding the progression of glioma in recent years, the complex molecular mechanisms involved in the metastasis of glioma remain largely unclear[7,8]. Therefore, it is essential to elucidate the roles of dysregulated genes in glioma progression to identify novel biomarkers for diagnosis and therapy in glioma patients.

The ENCODE (Encyclopedia of DNA elements) project showed that approximately 8% of the human genome is transcribed to RNA[9]. However, only about 2% of all RNAs can be used for protein coding. Long non-coding RNAs (lncRNAs) are a class of transcripts larger than 200 nt in length without protein-coding potential[10]. Although the potential function of lncRNAs remains largely unclear, it has been confirmed that lncRNAs play important roles in cell proliferation, apoptosis, angiogenesis, drug resistance, and tumorigenesis[11-13]. In glioma, several tumor-related lncRNAs have been identified to be

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involved in the glioma progression[14]. For instance, lncRNA LINC00958 promoted glioma cell proliferation and invasion via regulating miR-203/CDK2[15]. Besides, down-regulation of lncRNA LUCAT1 suppressed the proliferation and invasion of glioma cells via modulating miR-375[16]. These findings highlighted the potential of lncRNA as a new target for the metastasis-related glioma. In addition, the dysregulation of lncRNAs has also been reported to be associated with the clinical prognosis of glioma patients, suggesting that the detection of lncRNAs may be used as potential prognostic biomarkers for glioma patients[17-19]. LncRNA MAGI2 antisense RNA 3 (MAGI2-AS3), is a newly disease-related lncRNA whose length is 1726 nucleotides and locates in 7q21.11. Recently, scholars[20-22] had reported that MAGI2-AS3 was abnormally expressed in bladder cancer, breast cancer, and lung cancer, which suggested that MAGI2-AS3 may be involved in the development and progression of several tumors. Thus, we wondered whether MAGI2-AS3 may also display functional effects in glioma. In this study, we reported for the first time that MAGI2-AS3 expression was distinctly down-regulated in glioma tissues and associated with shorter overall survival of glioma patients. This new lncRNA may represent a novel biomarker for predicting prognosis of glioma patients.

Patients and Methods

Patients and Tissue Samples

178 paired human glioma tissues and the matched tumor-adjacent tissues were obtained from glioma patients and histopathologically diagnosed at the Sixth Affiliated Hospital of Wenzhou Medical University. All specimens were handled and made anonymous according to the ethical and legal standards. Collected tissues were immediately snap-frozen and stored at -80°C for RNA extraction and further RT-PC experiments. Pathological diagnoses were determined by two pathologists, according to the 2007 World Health Organization (WHO) classification of central nervous system tumors[23]. The clinicopathological data are shown in Table I. Informed consent was obtained by all patients before the surgery. This study was approved by the Ethics Committees of our institutes.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from glioma tissue samples and matched normal brain tissues using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) as per the manufacturer's instruction. The purity and concentration of RNA samples were determined by a spectrophotometer

Table I. Association between MAGI2-AS3 expression and different clinicopathological features of 178 human gliomas.

Parameter	No.	MAGI2-AS3 expression		*p*-value
		Low	High	
Age				0.366
≤ 50	87	40	47	
> 50	91	48	43	
Gender				0.915
Male	118	58	60	
Female	60	30	30	
WHO grade				0.031
I-II	117	51	66	
III-IV	61	37	24	
KPS score				0.003
≤ 80	64	41	23	
> 80	114	47	67	
Extent of resection				0.298
≤ 98%	108	50	58	
> 98%	70	38	32	
Tumor size				0.100
≤ 5 cm	102	45	57	
> 5 cm	76	43	33	

(Thermo Fisher Scientific, Waltham, MA, USA). The RNA samples were then reverse-transcribed into cDNA using the PrimeScript™ RT reagent kit (TaKaRa, Otsu, Shiga, Japan). The corresponding cDNA was used for quantitative real time-PCR using SYBR-Green Real-Time Master Mix (TaKaRa, Otsu, Shiga, Japan). The qRT-PCR was performed using the SYBR Select Master Mix (Applied Biosystems, Waltham, MA, USA) on ABI 7500 system. The levels of MAGI2-AS3 were calculated using the $2^{-\Delta\Delta CT}$ method and normalized to the level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All assays were performed in triplicate. The primer sequences were as follows: GADPH: 5'- TGTAGTTGAGGTCAATGAAGGG-3'(forward), 5'- ACATCGCTCAGACACCATG -3' (reverse); MAGI2-AS3: 5'-CACCTTGCTTGA CAACTTGA -3' (forward), 5'- CATTACAGCTCGGCTACTGC -3' (reverse).

Statistical Analysis

The statistical analysis was performed using the SPSS 17.0 software package (SPSS Inc., SPSS Statistics for Windows, Chicago, IL, USA). The distinct expression of MAGI2-AS3 between tumor tissues and matched normal tissues was examined by independent samples *t*-test. The Chi-square test was applied to the examination of the relationship between MAGI2-AS3 expression levels and clinicopathologic characteristics. The Kaplan-Meier curve was plotted for survival analysis, and the difference in survival was compared between the two groups using the log-rank test. The significance of survival variables was analyzed using the Cox multivariate proportional hazards model. A $p<0.05$ was considered statistically significant for all tests.

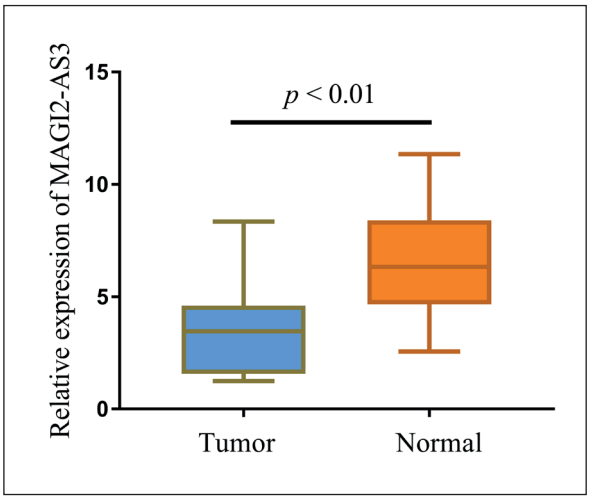

Figure 1. Comparison of MAGI2-AS3 expression levels between glioma tissues and adjacent normal tissues using RT-PCR.

Results

MAGI2-AS3 Expression Is Downregulated in Glioma Tissues

To explore the potential impact of MAGI2-AS3 in glioma development, we analyzed the expression pattern of MAGI2-AS3 in glioma tissues using RT-PCR. As shown in Figure 1, we found that MAGI2-AS3 was downregulated in glioma tissues compared with the adjacent non-cancerous tissues ($p<0.01$). Our results indicated that MAGI2-AS3 may play a functional role in the progression of glioma.

Expression Levels of MAGI2-AS3 and Clinicopathological Parameters in Glioma

In order to study the clinicopathological significance of MAGI2-AS3 in glioma, all patients were divided into two groups, high-MAGI2-AS3 expression group (n=90) and low-MAGI2-AS3 expression group (n=88), using the median value of MAGI2-AS3 in all glioma tissues as a cutoff value. As shown in Table I, the results showed that high-expression of MAGI2-AS3 was significantly associated with WHO grade ($p=0.031$) and KPS score ($p=0.003$). However, there was no association between MAGI2-AS3 expression and other clinical features, such as gender, age, the extent of resection, and tumor size ($p>0.05$).

MAGI2-AS3 Is Associated with Clinical Prognosis of Glioma Patients

To further examine whether MAGI2-AS3 expression has a prognostic value in glioma patients, we measured the association between the levels of MAGI2-AS3 and patients' overall survival using the Kaplan-Meier analysis. As presented in Figure 2, we found that the overall survival of patients with low MAGI2-AS3 expression was significantly shorter than those with high MAGI2-AS3 expression ($p=0.0024$). Then, we performed univariate analysis to determine potential prognostic factors and identified three prognostic factors: WHO grade (I-II, III-IV), KPS score (≤80, >80) and MAGI2-AS3 expression (High, Low) (All $p<0.05$, Table II). Age, gender, the ex-

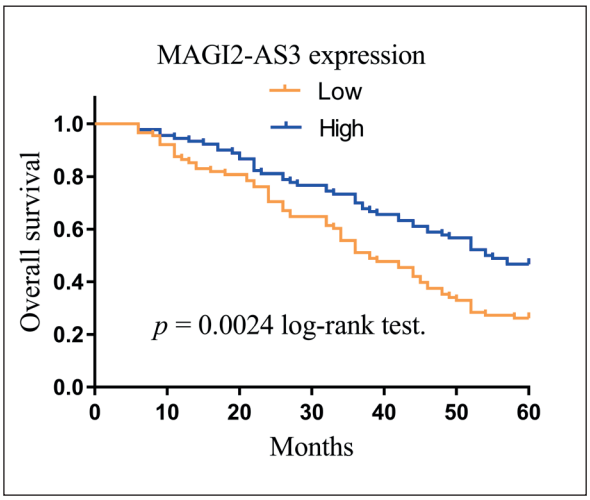

Figure 2. Kaplan-Meier survival curve for overall survival of glioma patients (n=178). Kaplan-Meier analysis was performed to evaluate the overall survival of glioma patients according to MAGI2-AS3 expression patterns.

tent of resection, and tumor size were not statistically significant prognosis factors (all $p>0.05$). In addition, the multivariate analysis showed that low MAGI2-AS3 expression was a significant independent predictor of poor survival of glioma (HR=3.098, 95% CI: 1.289-4.118, $p=0.014$), in addition to WHO grade and KPS score (Table II).

Discussion

Glioma is the first tumor classified based on both of the molecular markers and histology. High-grade gliomas occur approximately four times more frequently than low-grade gliomas, extremely poor clinical outcome[24,25]. The reasons causing the poor prognosis are very complex. In recent years, although more and more emerging targeted therapies for glioma were identified, drug resistance frequently occurred and contributed to poor clinical outcomes[4,26]. On the other hand, the development of individualized treatment was limited because the identification of sensitive cancer biomarker is very difficult[27]. Recently, growing evidence indicated that tumor development and progression can be mediated via multiple mechanisms involving lncRNAs, which suggested that lncRNAs may become new cancer diagnostic and prognostic biomarkers. However, the expression and clinical significance of a large number of lncRNAs remain unknown. With the development of high-resolution microarrays, lncRNAs have gained extensive attention to date and have been frequently reported to abnormally expressed in various tumors and associated clinical prognosis of tumor patients, including glioma[28,29]. In recent years, more than 100 lncRNAs were identified, and among them, some have been well studied, such as lncRNA MALAT1[30], lncRNA DANCR[31], and lncRNA HOTTIP[32]. Recently, a newly identified lncRNA, MAGI2-AS3, attracted our attention. Yang et al[22] firstly reported that MAGI2-AS3 was highly expressed in breast cancer and its overexpression suppressed tumor cells proliferation and promoted apoptosis via targeting the Fas/FasL pathway. In addition, Wang et al[21] showed that MAGI2-AS3 expression was significantly down-regulated and indicated poor prognosis in bladder cancer patients. By using *in vitro* assays and *in vivo* assays, they further confirmed that MAGI2-AS3 acted as a tumor suppressor in progression of bladder cancer because its forced expression can suppress the proliferation, migration, and invasion by sponging miR-15b-5p. Together with those cells experiments, the roles of MAGI2-AS3 acting as a tumor suppressor were confirmed. However, the expression and clinical significance of MAGI2-AS3 in glioma remain largely unclear. In this study, we firstly detected the expression levels of MAGI2-AS3 in glioma pa-

Table II. Univariate and multivariate analysis of overall survival in BC patients.

Variable	Univariate			Multivariate		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Gender	1.673	0.774-2.542	0.231	–	–	–
Age	1.532	0.554-1.978	0.129	–	–	–
WHO grade	3.241	1.342-4.782	0.013	2.886	1.185-4.157	0.021
KPS score	3.542	1.549-5.332	0.005	2.963	1.268-4.773	0.009
Extent of resection	1.442	0.567-1.942	0.452	1.215	0.628-1.752	0.672
Tumor size	1.389	0.742-2.031	0.325	1.266	0.842-1.885	0.289
MAGI2-AS3 expression	3.476	1.478-4.472	0.009	3.098	1.289-4.118	0.014

tients, finding that MAGI2-AS3 expression levels were significantly downregulated in glioma tissues compared with the corresponding noncancerous tissues, which also revealed the tumor-suppressive roles of MAGI2-AS3 in glioma. Then, we further explored its clinical significance, and the results showed that lower expression of MAGI2-AS3 was associated with advanced WHO grade and KPS score, which suggested that MAGI2-AS3 may contribute to a malignant progress. Next, we used Kaplan-Meier method to determine the prognostic value of MAGI2-AS3 in five-year overall survival of glioma patients, finding that the glioma patients with low MAGI2-AS3 expression level had poor overall survival than those with high MAGI2-AS3 expression level. More importantly, we performed the univariate and multivariate survival analyses to explore whether MAGI2-AS3 could be used as a new prognostic biomarker, finding that the down-regulation of MAGI2-AS3 was an independent factor for predicting overall survival in glioma patients, demonstrating that MAGI2-AS3 can be used as a potential prognostic cancer biomarker for glioma patients. However, the potential function and the molecular mechanism of MAGI2-AS3 in glioma progression remain unknown. In the future, additional investigations are needed to study the biological function of MAGI2-AS3, and the potential mechanism involved in the dysregulation of MAGI2-AS3.

Conclusions

We have demonstrated that MAGI2-AS3 is downregulated in patients with glioma, and correlated with tumor progression. Our findings further suggested that MAGI2-AS3 may be a potential prognostic biomarker for glioma.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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