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Original Article

Increased expression of long non-coding RNA HOXC-AS1 associates with the malignant status and poor prognosis in glioma

Zhen-Hua Chen[1*], Hong-Kang Hu[2*], Cheng-Yin Lu[2*], Dong-Mei Wang[2], Yi Chen[2], Jun Qian[2], Wei Sun[2], Xiao-Jun Wu[2], Guo-Han Hu[2], Lei Jiang[2]

[1]Department of Neurosurgery, The Second Affiliated Hospital of Nantong University, Nantong, People's Republic of China; [2]Department of Neurosurgery, Changzheng Hospital, Second Military Medical University, Shanghai, People's Republic of China. [*]Equal contributors.

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Abstract: Long non-coding RNA (lncRNA), which is longer than 200 nucleotides, is a type of RNA without the function of encoding proteins. Growing evidence in recent years indicates that lncRNAs are novel regulators in cancerigenesis and progression. However, little is known about the therapeutic significance of lncRNAs in glioma. In this study, we focused on a typical lncRNA HOXC cluster antisense RNA 1 (HOXC-AS1) with unknown function and detected its expression in glioma and normal brain tissues, in an attempt to confirm the role of HOXC-AS1 in the pathogenesis of glioma and explore the relationship between HOXC-AS1 expression and the clinicopathological features of glioma patients. The results showed that the expression level of HOXC-AS1 was increased significantly in high-grade glioma tissues (WHO grade III-IV) compared with that in low-grade glioma tissues (WHO grade I-II) ($P<0.01$) and normal brain tissues ($P<0.05$). In addition, HOXC-AS1 expression was not significantly correlated with age (<50 vs. ≥50, $P=0.170$), gender (male vs. female, $P=0.467$), tumor size (<5 cm vs. ≥5 cm; $P=0.052$), and KPS (<70 vs. ≥70, $P=0.661$). The overall survival (OS) of glioma patients was significantly associated with the expression of HOXC-AS1 ($P<0.001$). Multivariate regression analysis showed that increased HOXC-AS1 expression was an independent risk factor for poor prognosis of glioma patients ($P=0.039$). Taken together, HOXC-AS1 may play an important role in the progression and prognosis of glioma, and may prove to be a latent biomarker and therapeutic target for glioma.

Keywords: Long noncoding RNA, HOXC-AS1, prognosis, glioma, tumor marker

Introduction

Glioma constitutes the most common and deadliest primary malignant brain tumor, accounting for 50-60% of all brain tumors [1]. According to the 2007 WHO classification of gliomas [2], they can be classified into grade I-IV according to their degree of malignancy. Although great advances have been made in the conventional treatments for glioma including surgery, radiotherapy and chemotherapy in recent years, patient outcomes remain unfavorable [3, 4]. The characteristics of progressive proliferation and diffuse invasion of glioma may directly result in the overall poor prognosis of patients with glioma. The currently available histopathological classification systems have undoubtedly provided a precious basis for defining the clinical assessment of groups of patients and predicting the clinical behavior of the corresponding cancer as the guideline for treatment [2]. However, studies in recent years suggest that these criteria alone may not be able to fully evaluate the prognosis of glioma patients [5]. Thus, there is an urgent need to identify new potential biomarkers to predict the prognosis of glioma patients more accurately, and find new targets for cancer therapy.

Long non-coding RNAs (lncRNAs), which were initially argued to be spurious transcriptional noise, are now recognized as a class of RNAs with transcripts longer than 200 nucleotides with no function of encoding proteins [6-8]. Recent studies have also found that lncRNAs play a critical regulatory role in many human diseases, including cancer [9, 10]. The first example is H19, which is identified as a RNA

that is associated with many tumors, including promoting glioma cell invasion by directly regulating miR-675 expression [11]. Some lncRNAs, such as HOTAIR (Hox transcript antisense intergenic RNA), have been considered to be signs of various cancers [12]. However, the relationship between most lncRNAs and cancerigenesis remains unclear.

We previously performed microarrays with the glioma specimens and found that HOXC-AS1 was aberrantly expressed in glioma [13]. HOXC-AS1 (ENST00000505700) is an lncRNA whose function has never been described. In the present study, we first detected the expression level of HOXC-AS1 in the glioma tissue and normal brain tissue to determine the role of HOXC-AS1 in the pathogenesis of gliomas, and then analyzed the relationship between HOXC-AS1 expression and clinicopathological features of glioma including the survival time of patients. It was found that the expression level of HOXC-AS1 in high-grade glioma tissues was significantly higher than that in low-grade glioma tissues and normal brain tissues. In addition, the relatively higher HOXC-AS1 expression was significantly related to the malignant status and poor prognosis of patients with glioma.

Materials and methods

Clinical samples

This study was approved by the Specialty Committee on Ethics of Biomedicine Research of the Second Military Medical University (Shanghai, China). Informed consent was obtained from all patients concerned.

Forty-four glioma tissues were selected, and the pathological information was identified according to the WHO classification by experienced clinical pathologists. Six samples of normal brain tissues were obtained from six patients sustaining severe head trauma, for whom partial resection of the normal brain was required for decompression during surgery. None of the patients had received chemotherapy or radiotherapy before resection. All the samples were resected from primary surgery, and the specimens were put into liquid nitrogen for real-time polymerase chain reaction (PCR). The treatment was carried out according to the National Comprehensive Cancer Network (NCCN) guideline in all glioma patients included in this study. Clinical follow-up was available for all patients. Overall survival (OS) time of the patients was calculated from the date of initial surgery to the date of patient death.

RNA isolation and quantitative real-time PCR

Total RNA was extracted from the tissue with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After purification, complementary DNA (cDNA) was synthesized from 1 μg total RNA using the Prime Script RT Master Mix (Takara). The primers (Sangon) were designed as follows: for human HOXC-AS1, the forward primer was 5'-CCATCTCTGCGACACTTCC-3' and the reverse primer was 5'-AGCTACTTGCCCACGACC-3'. For human GAPDH, the forward primer was 5'-GGGAAACTGTGGCGTGAT-3' and the reverse primer was 5'-GAGTGGGTGTCGCTGTTGA-3. Real-time PCR was conducted by SYBR Premix Ex Taq™ II (Takara) on 7900HT (Applied Biosystems). Change in expression level was calculated by quantitative analysis in triplicate using the comparative cycle threshold method. The raw data of target lncRNA were normalized to GAPDH.

Statistical analysis

All data were analyzed using SPSS version 21.0 and GraphPad 5.0 software. Data are expressed as mean ± SD. One-way analysis of variance (ANOVA) was used to test for differences between the glioma and normal brain tissues in all groups, and a least significant difference post-hoc test was used to obtain individual *P* values followed by ANOVA. The chi-square test was used to examine the relationship between HOXC-AS1 expression level and the clinicopathologic features. Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. The Cox multivariate proportional hazards model was used to analyze the significance of survival variables. Differences were considered statistically significant when the p value was <0.05.

Results

HOXC-AS1 up-regulation in high-grade glioma tissues

To determine the role of HOXC-AS1 in glioma, HOXC-AS1 expression was detected in 44 glioma tissues and six normal brain tissues by

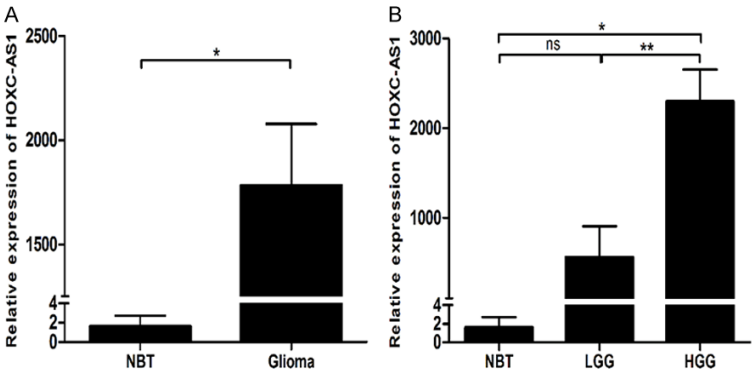

Figure 1. Quantitative real-time PCR analysis of HOXC-AS1. A. HOXC-AS1 expression was significantly higher in the glioma tissues compared with that in the normal brain tissues. B. HOXC-AS1 expression was significantly higher in the high-grade glioma tissues compared with that in the normal brain tissues and low-grade glioma tissues. NBT: normal brain tissue, LGG: low-grade glioma, HGG: high-grade glioma, ns; P>0.05, *P<0.05, **P<0.01.

Table 1. HOXC-AS1 expression and clinicopathological features of human gliomas

Characteristics	Patients, n	HOXC-AS1 expression, n		*P* value
		High	Low	
Age (years)				
<50	19	7	12	0.170
≥50	25	14	11	
Gender				
Male	28	14	14	0.467
Female	16	7	9	
WHO grade				
I-II	13	2	11	0.006
III-IV	31	19	12	
Tumor size (cm)				
<5	17	5	12	0.052
≥5	27	16	11	
KPS				
<70	4	2	2	0.661
≥70	40	19	21	

KPS: Karnofsky performance score.

quantitative real-time PCR. It was found that HOXC-AS1 expression was significantly higher in the glioma tissues compared with that in the normal brain tissues (**Figure 1A**). HOXC-AS1 expression was significantly up-regulated in the high-grade glioma tissues compared with that in the normal brain tissues (P<0.05) and low-grade glioma tissues (P<0.01) (**Figure 1B**), while there was no significant difference in HOXC-AS1 expression between the low-grade glioma and normal brain tissues.

Correlation between HOXC-AS1 expression and clinicopathological features in patients with glioma

We next identified the correlation between HOXC-AS1 expression and the clinicopathological features of glioma. The glioma tissues were divided as high-expression group (n=21) and low-expression group (n=23), based on the median expression level of all gliomas (mean expression value 1568.208). As summarized in **Table 1**, HOXC-AS1 was significantly associated with WHO grade (I-II vs. III-IV, P=0.006). However, no significant association was observed between HOXC-AS1 expression and other clinicopathological parameters, including age (<50 vs. ≥50, P=0.170), gender (male vs. female, P=0.467), tumor size (<5 cm vs. ≥5 cm, P=0.052), and karnofsky performance score (KPS) (<70 vs. ≥70, P=0.661).

High HOXC-AS1 expression indicates poor prognosis

To evaluate the prognostic value of HOXC-AS1 expression in patients with glioma, we used Kaplan-Meier analysis with the log-rank test to confirm the correlation between the HOXC-AS1 expression level and OS of glioma patients. It was found that OS was significantly shorter in glioma patients with high HOXC-AS1 expression levels than that in those with low HOXC-AS1 expression levels (P<0.001) (**Figure 2A**), suggesting that HOXC-AS1 up-regulation might play a critical role in the development and progression of glioma. Additionally, WHO grade and age were also significantly correlated with the patient outcomes (**Figure 2B** and **2C**). Univariate analysis identified three prognostic factors: age (<50 or ≥50), WHO grade (I-II or III-IV), and HOXC-AS1 expression. The other clinicopathological characteristics (gender, tumor size and KPS) were not statistically significant prognostic factors. Multivariate analysis of the prognostic factors confirmed that high HOXC-AS1 expression was an independent predictor of poor survival in glioma patients (P=0.039), in

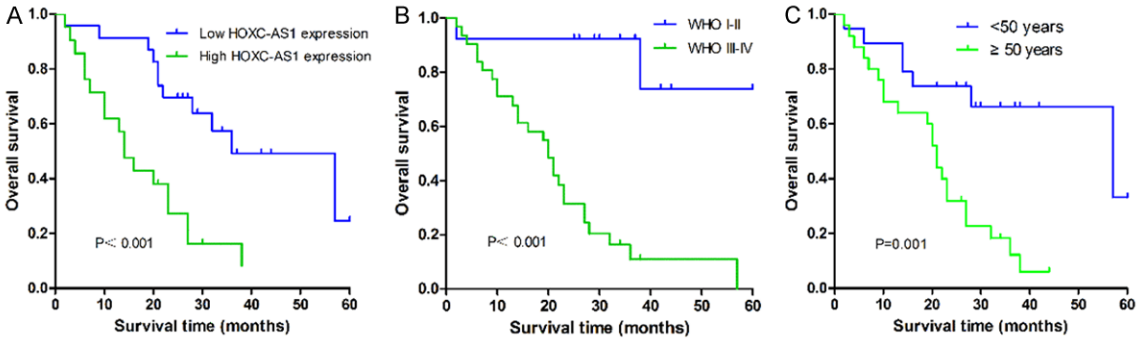

Figure 2. Kaplan-Meier curves for overall survival by HOXC-AS1 expression (A), WHO grade (B), age (C).

Table 2. Univariate and multivariate Cox regression analyses of overall survival

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Gender						
Female	1					
Male	1.467	0.677-3.182	0.332			
Tumor size (cm)						
≥5	1					
<5	0.988	0.459-2.126	0.975			
KPS						
≥70	1					
<70	1.520	0.446-5.182	0.504			
Age (years)						
≥50	1			1		
<50	0.281	0.132-0.599	0.001	0.310	0.119-0.806	0.016
WHO grade						
III-IV	1			1		
I-II	0.208	0.097-0.448	<0.001	0.117	0.026-0.523	0.005
HOXC-AS1						
High	1			1		
Low	0.253	0.114-0.562	<0.001	0.427	0.191-0.958	0.039

HR: Hazard ratio, 95% CI: 95% confidence interval, KPS: Karnofsky performance score.

addition to age (P=0.016) and WHO grade (P=0.005) (**Table 2**).

Discussion

It is widely accepted that genetic information is expressed as a protein. However, almost 98% human genomes do not code for proteins [14], and their function remains largely unknown. Research in recent years has focused more attention on noncoding genes, especially lncRNAs. More evidence has revealed that lncRNAs are generally transcribed in eukaryotic cells, and some studies have provided insights into the molecular mechanism by which lncRNAs function in tumorigenesis [9, 15]. They are believed to be involved in tumorigenesis, development, invasion, metastasis, and prognosis [16-18]. Differential expression of lncRNA profiles can be applied to find new potential biomarkers for cancer diagnosis and treatment. Using this method, some studies [19] found that HOTAIR (HOX transcript antisense intergenic RNA) was over-expressed in breast cancer and participated in the chromatin remodeling process. Yang et al [20] found that lncRNA-HEIH could promote tumor progression in hepatocellular carcinoma (HCC). Additionally, the down-regulation of lncRNA-LET was found to be related to hypoxia-induced cancer cell invasion [21]. These examples confirmed that lncRNAs could be used as candidate targets for cancer therapy.

LncRNAs are of great importance to gliomas [22, 23]. LncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has been reported to be significantly increased in glioma tissues compared with paired adjacent normal brain tissues. Additionally, lncRNA

MALAT1 over-expression was markedly associated with poor prognosis of glioma patients [24]. Yao et al [25] reported that lncRNA X-inactive specific transcript (XIST) expression was up-regulated in glioma tissues and glioblastoma stem cells, and knockdown of XIST expression exerted a tumor-suppressive function by reducing cell proliferation, invasion and migration as well as inducing apoptosis. The mechanism study showed that miR-152 mediated the tumor-suppressive effect that XIST knockdown produced. Guo et al [26] found that long intergenic noncoding RNA POU3F3 (linc-POU3F3) might affect the development and progression of glioma by altering the expression of POU3F3. However, our understanding about the concrete mechanism of lncRNAs in the pathogenesis of glioma is far behind other solid tumors, and more lncRNAs associated with glioma need to be found, especially as glioma specific biomarkers.

In this study, we found that HOXC-AS1 was over-expressed in high-grade glioma tissues compared with that in low-grade glioma and normal brain tissues, suggesting that it might play an important role in the development of glioma. Besides, we showed that HOXC-AS1 expression in glioma was negatively correlated with OS of glioma patients, and that OS in glioma patients with high HOXC-AS1 expression was relatively short. In addition, multivariate Cox regression analysis showed that HOXC-AS1 over-expression was an independent indicator of poor prognosis in glioma patients. Some previous studies reported that age was another important factor affecting the survival time of glioma patients [27, 28], and our results may provide more favorable evidence for them. Yet, the accuracy and reliability of this study may be affected by the small sample size and the lack of the normal brain tissue around the glioma.

In conclusion, our data offer convincing evidence that the increased expression of HOXC-AS1 may predict unfavorable prognosis in glioma patients, indicating that HOXC-AS1 may play a crucial role in promoting the progression of glioma and be a potential target for the treatment of this disease. However, the particular mechanism by which HOXC-AS1 is up-regulated in glioma is not clear. More studies are needed to verify the role of HOXC-AS1 as a reliable clinical predictor of the outcome for glioma patients in the future.

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Disclosure of conflict of interest

None.

Address correspondence to: Guo-Han Hu and Lei Jiang, Department of Neurosurgery, Changzheng Hospital, Second Military Medical University, No. 415, Fengyang Road, Shanghai 200003, People's Republic of China. Tel: +86-21-81885673; E-mail: huguohan@hotmail.com (GHH); Tel: +86-21-81885-680; E-mail: Jl13jan@hotmail.com (LJ)

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