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Expression of long non-coding RNA CRNDE in glioma and its correlation with tumor progression and patient survival

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Abstract. – OBJECTIVE: Long non-coding RNAs (lncRNAs) CRNDE has been identified as a tumor oncogene in glioma. However, its clinical significance and prognostic value in glioma have not been investigated until now. The aim of this study was to explore CRNDE expression levels and evaluated its clinical significance in glioma patients.

PATIENTS AND METHODS: Expression levels of lncRNA CRNDE in 164 glioma specimens were determined by quantitative real-time PCR (qRT-PCR). The chi-square test was used to explore CRNDE expression with respect to clinicopathological parameters. The overall survival was analyzed by log-rank test, and survival curves were plotted according to Kaplan-Meier. Univariate and multivariate analyses were performed to analyze the prognostic significance of CRNDE expression.

RESULTS: Compared with nonneoplastic brain tissues, the expression level of CRNDE was significantly increased in glioma tissues ($p < 0.01$). CRNDE upregulation was correlated with larger tumor size ($p = 0.011$), higher WHO grade ($p = 0.001$), and recurrence ($p = 0.008$). Also, survival analysis proved that up-regulated CRNDE expression was associated with poor overall survival of glioma patients ($p < 0.001$). The multivariate Cox regression analysis indicated that CRNDE expression was an independent prognostic factor for overall survival.

CONCLUSIONS: These results indicated that lncRNA CRNDE was associated with tumor progression and could be an independent prognostic factor for glioma patients.

Key Words:
lncRNA CRNDE, Overall survival, Glioma, Prognosis.

Introduction

Gliomas are the most common and aggressive type of primary adult brain tumor and persist as serious clinical and scientific problems[1]. According to the World Health Organization (WHO) classification, gliomas are classified into four grades (I, II, III and IV), of which glioblastoma multiforme (GBM, WHO grade IV) is the most frequent and aggressive malignant type[2]. Despite aggressive surgery, radiation and chemotherapy, the median survival of the most frequent and most malignant type of glioma – the glioblastoma – is only 14.6 months[3]. Therefore, it is imperative to comprehend the pathogenic mechanism of GBM and identify new biomarkers and therapeutic targets for GBM patients.

Long noncoding RNAs (lncRNAs above 200 nucleotides) have been identified as multifunctional regulators of gene expression at different levels such as chromatin remodeling and post-transcriptional control[4,5]. Increasing evidence shows that lncRNAs could play an important role in cellular development, differentiation, and many other biological processes[6,7]. In this regard, highlighting the potentially widespread functional roles of lncRNAs in human cancer is important. Due to the advances in high-throughout technology, such as microarray and RNA-sequencing, numerous lncRNAs have been detected and profiled in glioma. For instance, Han et al[8] found that knockdown of MALAT1 in human glioma cell lines significantly promoted the invasion and proliferation of the glioma cells by in vitro assays. Zhen et al[9] showed that overexpression of Long noncoding RNA NEAT1 promotes glioma pathogenesis by regulating miR-449b-5p/c-Met axis. Zhang et al[10] reported that Long non-coding RNA HOTAIR Regulate glioma cell cycle progression through EZH2. However, these lncRNAs lack the capability to fully explain the mechanism of the recurrence and metastasis of glioma, and the overall pathophysiological function of lncRNAs on glioma remains largely unknown.

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Recently, CRNDE was treated as a long non-coding RNA (lncRNA), in the present study, we focused on the effect of CRNDE in glioma. We investigated the expression level of CRNDE in glioma tissues and adjacent non-tumor tissues. Then, the association of CRNDE with clinicopathological characteristics and outcome of the glioma patients was investigated.

Patients and Methods

Patients and tissue Samples

Patients with glioma who underwent initial surgery in The Second Hospital of Hebei Medical University from 2007 to 2013 were retrospectively selected for this study. All patients did not receive chemotherapy or radiotherapy before the surgery. All samples were confirmed by pathological examination and stored in liquid nitrogen for later total RNA extraction. The patients' characteristics are described in Table I. Tissue sample use was approved by the Ethics Committees of our Hospital and written informed consent was obtained from all study participants.

RNA Extraction and qRT-PCR Analyses

The total RNA was extracted from the tissues or cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was reverse transcribed to complementary DNA (cDNA) by using a PrimeScript RT reagent kit (Takara, Dalian, Liaoning, China). Its synthesis was conducted at 37 °C for 15 min, then 85 °C for 5 s according to the experimental protocols. Real-time PCR reactions were performed by the ABI7500 system (Applied Biosystems, Foster City, CA, USA). Real-time PCRs was performed in triplicate. The relative expression of CRNDE was calculated and normalized using the $2^{-\Delta\Delta Ct}$ method relative to GAPDH. The primers for CRNDE: 5′-ATATTCAGCCGTTGGTCTTTGA-3′, 5′-TCTGCGTGACAACTGAGGATTT-3′; and for GAPDH: 5′-GGTGAAGGTCGGAGTCAACG-3′, 5′-CCATGTAGT TGAGGTCAATGAAG-3′.

Statistical Analysis

The comparison of the expression level of CRNDE between glioma tissue and adjacent normal tissue was performed using the two-sample Student's *t*-test. The chi-square test and Fisher's exact test were used to examine the associations between CRNDE expression and the clinicopathological characters. In addition, survival curves were constructed using the Kaplan-Meier method and analyzed using log-rank test. Cox

Table I. Correlation between the expression level of CRNDE with clinicopathological features.

Characteristic	Case number	CRNDE expression		*p*-value
		Low (n = 81)	High (n = 83)	
Age (years)				0.591
≤ 45	58	27	31	
> 45	106	54	52	
Gender				0.353
Male	103	48	55	
Female	61	33	28	
Onset				0.387
Epilepsia	20	8	12	
Intracranial hypertension	79	42	37	
Others	65	31	34	
Tumor size (cm)				0.011
≤ 3	59	37	22	
> 3	105	44	61	
Necrosis				0.490
Yes	34	15	19	
No	130	66	64	
WHO grade				0.001
I-II	46	34	12	
III-IV	118	47	71	
Recurrence				0.008
Yes	59	21	38	
No	105	60	45	

proportional-hazards regression analysis was applied to estimate univariate and multivariate hazard ratios for OS. A value of $p < 0.05$ was regarded as significantly different. SPSS software 16.0 (SPSS Inc., Chicago, IL, USA) was applied in the current work.

Results

CRNDE is Upregulated in Glioma Tissues

The qRT-PCR was used to measure CRNDE expression levels in a total of 164 patients with glioma. The results showed that CRNDE had higher expression in glioma tissues than that in matched adjacent normal tissues ($p < 0.01$, shown in Figure 1A). Moreover, CRNDE expression levels were further analyzed in glioma tissue samples with different WHO grade. The expression of CRNDE was obviously increased in patients with high WHO grade (WHO grade III and IV, $p < 0.01$, Figure 1B).

Correlation Between CRNDE Expression and Clinicopathological Factors of Glioma patients

Then, we analyzed the correlation between CRNDE expression and clinicopathological factors of glioma patients. The median expression level of CRNDE was used as a cut-off point to divide all 164 patients into two groups (high and low). Table I summarizes the association between CRNDE expression and clinicopathological parameters in glioma. The results showed that high expression of CRNDE was significantly associated with larger tumor size ($p = 0.011$), higher WHO grade ($p = 0.001$) and recurrence ($p = 0.008$). However, no significant differences about other characteristics of patients were found.

Upregulation of CRNDE Confers Poor Prognosis in Patients with Glioma

Using Kaplan-Meier method and log-rank test, the overall survival durations was significantly shorter in patients with high CRNDE expression compared to those with low CRNDE expression ($p < 0.001$, Figure 2). Univariate and multivariate analyses were utilized to evaluate whether the CRNDE expression level and various clinicopathological features were independent prognostic parameters of glioma patient outcomes. The detailed data has ben shown in Table II. Univariate analysis indicated that the overall survival of patients with CRNDE was associated with recurrence, WHO grade, CRNDE expression. Moreover, multivariate confirmed that CRNDE expression was an independent prognostic factor for overall survival of patients with glioma ($p = 0.002$).

Discussion

Glioma is one of the most common malignant brain tumors. Prognostic factor detection in glioma is essential to predict patients' survival and determine optimal therapeutic strategies. So

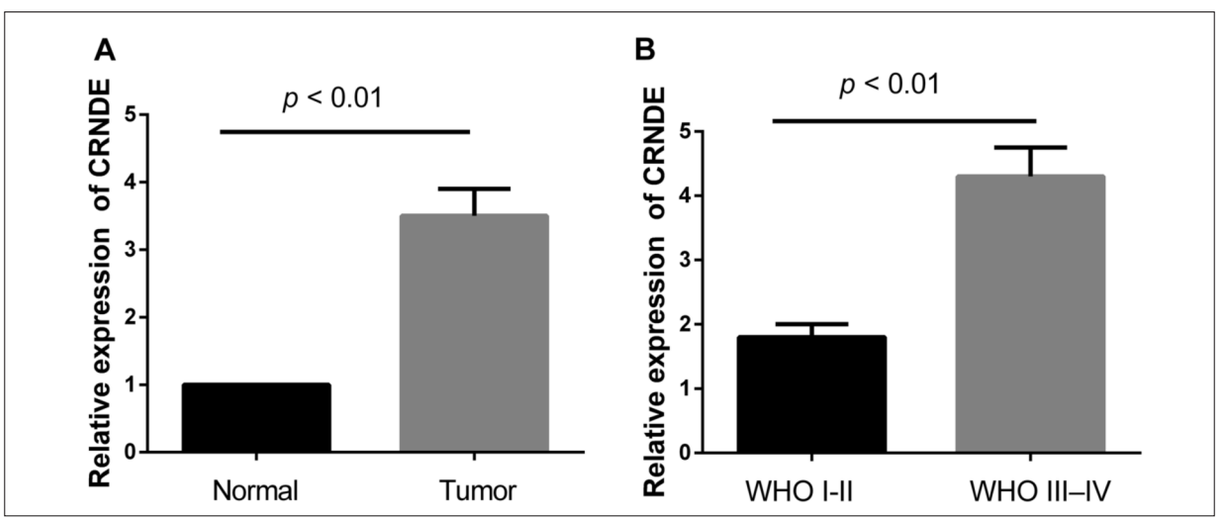

Figure 1. Expression levels of CRNDE in glioma tissues and adjacent nontumor tissues. ***A,*** CRNDE levels in glioma tissues were significantly higher than those in the noncancerous tissues ($p < 0.01$). ***B,*** CRNDE levels in glioma WHO III–IV tissues were significantly higher than those in glioma WHO I-II tissues ($p < 0.01$).

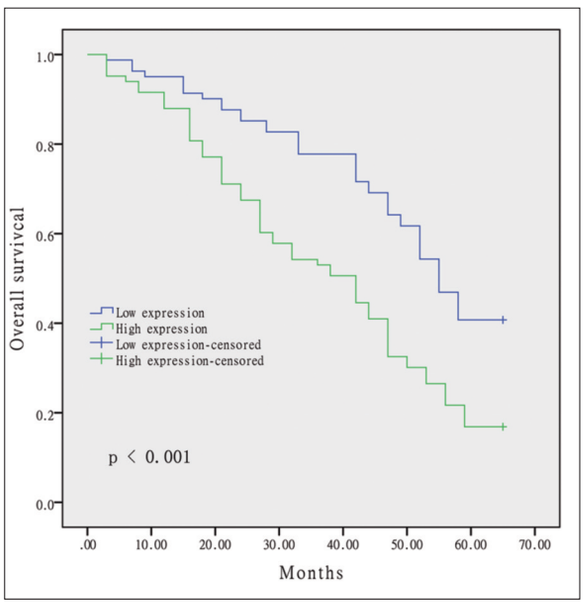

Figure 2. Kaplan-Meier overall survival curves of patients with glioma according to relative expression of CRNDE. Patients with higher expression of CRNDE showed worse overall survival compared with patients with lower expression of CRNDE ($p < 0.001$).

far, many different biological markers were reported[11,12]. As a novel biomarker, more and more studies focus on LncRNAs. Furthermore, application of lncRNAs as cancer diagnostic or prognostic biomarkers has been reported in several investigations[13,14]. However, the prognostic value of CRNDE in glioma has not been reported.

CRNDE was firstly found in colorectal cancer and located on chromosome 16, exerting oncogenic functions in diverse cancers[15]. Recently, Szafron et al[16] found that increased expression of CRNDE is associated with local invasion, lymph node metastasis and poor prognosis in glioma. Wang et al[17] reported that CRNDE was greatly upregulated both in glioma cell lines and in primary glioma samples. Furthermore, they found that the overexpression of CRNDE promoted cell growth and migration *in vitro* through mTOR signaling. Zheng et al[18] showed that overexpression of CRNDE could promote the cellular proliferation, migration, invasion and inhibit the apoptosis by negatively regulating miR-186. Above results informed that CRNDE might be an important modulator involved in glioma development and could serve as a tumor promoter.

In the present paper, we observed significantly higher CRNDE expression in glioma tissues. Besides, higher CRNDE expression was seen more frequently in advanced glioma. Increased expression of CRNDE was significantly correlated with larger tumor size, higher WHO grade, and recurrence. Moreover, Kaplan-Meier method and log-rank test were used to evaluate the differences of overall survival between the low-expression group and high-expression group. The findings showed that glioma patients with high CRNDE expression tend to have shorter overall survival. Moreover, multivariate analysis revealed that CRNDE expression was independently associated with the OS, indicating that higher CRNDE level was a marker of poor prognosis for patients with glioma.

Conclusions

We demonstrated that CRNDE was up-regulated in human glioma and could be considered an independent prognostic factor in patients with glioma. These results suggested that CRNDE could be an effective therapeutic target for glioma. In the future, the molecular mechanism of CRNDE involved in glioma development should be further studied.

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

Parameter	Univariate analysis			Multivariate analysis		
	HR	95 % CI	*p*	HR	95 % CI	*p*
Age (≤ 45/> 45)	1.371	1.067-2.662	0.351			
Gender (male/female)	1.563	0.832-3.372	0.542			
Tumor size (≤ 3 cm/> 3 cm)	2.369	1.541-4.427	0.137			
Necrosis (yes/no)	1.845	1.137-3.338	0.336			
Recurrence (yes/no)	2.235	1.269-7.745	0.017	3.328	1.554-8.849	0.008
WHO grade (I-II/III-IV)	3.871	1.893-6.654	0.012	4.321	2.136-8.822	0.005
CRNDE (high/low)	2.236	1.452-7.125	0.006	1.589	1.034-6.873	0.002

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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