Long noncoding RNA SPRY4-IT1 is a prognostic factor for poor overall survival and has an oncogenic role in glioma

Y. ZHOU1, D.-L. WANG2, Q. PANG3

1Shandong University, Jinan, Shandong, P.R. China
2Department of Neurosurgery, Shandong Provincial Traditional Chinese Medical Hospital, Jinan, Shandong, P.R. China
3Department of Neurosurgery, Shandong Provincial Hospital, P.R. China

Abstract. – OBJECTIVE: Long non-coding RNAs (lncRNAs) are emerging as biomarkers and as important regulators of the biological processes and tumorigenesis in cancer. The purpose of this study is to investigate the clinical significance of lncRNA SPRY4-IT1 in glioma.

PATIENTS AND METHODS: The expression level of SPRY4-IT1 was examined by the quantitative Real-time PCR (qRT-PCR) in glioma tissues and control tissues and its association with overall survival of patients was analyzed by statistical analysis. Survival curves were made using the Kaplan-Meier method, and the log-rank test was used to analyze the differences between clinicopathological characteristics and survival in glioma patients.

RESULTS: The expression level of SPRY4-IT1 was significantly higher in glioma in comparison to normal matched tissue (p < 0.01). Furthermore, lncRNA SPRY4-IT1 was associated significantly with WHO grade (p = 0.009) and tumor size (p = 0.003).

A significant difference was found that glioma patients with high SPRY4-IT1 expression level had distinctly shorter OS than patients with low SPRY4-IT1 expression level. Furthermore, multivariate analysis indicated SPRY4-IT1 as an independent prognostic indicator for glioma patients (p = 0.003).

CONCLUSIONS: The lncRNA SPRY4-IT1 may be a potential prognostic biomarker of glioma.

Key Words
lncRNAs, SPRY4-IT1, Prognosis, Glioma.

Introduction

Glioma is the most common and aggressive primary tumor in the nervous system, and accounts for about 80% of primary malignant brain tumors12. Despite recent advances in the application of multidisciplinary (surgery followed by chemotherapy and radiotherapy) treatment13. The 5-year overall survival rates are still unsatisfactory3. Thus, it is urgent to develop effective screening strategies for detecting glioma at an early, treatable stage.

Long non-coding RNAs (lncRNAs, >200 nt in length), previously disregarded as transcriptional noise, are emerging as new regulators in the cancer paradigm56. Emerging evidence indicates that lncRNAs may play complex and extensive roles in promoting the development and progression of cancer78. For example, Yang et al9 found that high MALAT1 expression is associated with poor prognosis and promotes cancer cell growth and invasion in cervical cancer; Sun et al10 revealed that lncRNA GAS5 expression was markedly down-regulated in gastric cancer and associated with tumor size and pathologic stage; Wang et al11 showed that lncRNA promotes the glioma cell growth and invasion through mTOR signaling. However, to our knowledge the clinical significance and biological function of lncRNA SPRY4-IT1 in glioma remains unclear.

In the present study, we examined the expression level of SPRY4-IT1 in glioma tissues. Furthermore, we analyzed the association between SPRY4-IT1 and glioma patients’ prognosis. Our results indicated that SPRY4-IT1 could be an independent marker for predicting the clinical outcome of glioma patients.

Patients and Methods

Patients and tissue samples

The 163 cases of clinical specimens were collected from surgical resections of brain glioma.
in the Department of Neurology, Shandong Provincial Hospital, Shandong Province, China. The histopathological diagnosis of all samples was respectively diagnosed by two pathologists. Overall survival (OS) was defined as the interval between the dates of surgery and death. The clinical and pathological characteristics of each patient were also collected. All patients gave written informed consent for the collection of biomaterials. Approval for this study was received from the Ethics Committee of the host institution.

**RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)**

Total RNA was extracted from tissues or cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. qRT-PCR assays were performed to detect SPRY4-IT1 expression using the Prime Script RT reagent Kit and SYBR Premix ExTaq. The TaqMan microRNA assay and TaqMan universal PCR master mix were used to detect the expression of SPRY4-IT1, and the GAPDH gene was used as an internal control. All experiments were performed using the 2^(-ΔΔCt) method. Each experiment was performed in triplicate.

**Statistical Analysis**

Statistical analyses were performed using SPSS Statistics 13.0 (IBM, Chicago, IL, USA). The gene expression levels in glioma were compared with those in adjacent non-tumor tissues with the use of Wilcoxon test. Comparisons of continuous data among multiple groups were calculated using the one-way analysis of variance. The effects of SPRY4-IT1 expression on the overall survival were evaluated by the Kaplan-Meier curves. Survival data were evaluated using univariate and multivariate Cox proportional hazards models. Statistical significance was set at \( p < 0.05 \).

**Results**

**The expression level of SPRY4-IT1 in glioma**

Quantitative reverse transcription-polymerase chain reaction was used to examine the expression level of SPRY4-IT1 in glioma tissues and adjacent non-tumorous tissues. As shown in Figure 1, the relative expression of SPRY4-IT1 in glioma tissues was significantly higher than that in matched noncancerous tissues \( (p < 0.01) \).
LncRNA SPRY4-IT1 expression in glioma

Table 1. Relationship between SPRY4-IT1 expression and clinicopathological

<table>
<thead>
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<th>Characteristics</th>
<th>n</th>
<th>High expression</th>
<th>Low expression</th>
<th>p</th>
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<tr>
<td>≥45</td>
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<tr>
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<td>122</td>
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<tr>
<td>I–II</td>
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Discussion

Glioma is one of the most common types of primary brain tumors in adults\(^1,^2\). The reliable identification of glioma progression-specific targets has huge implications for its prevention and treatment. Several authors\(^13,^14\) have also demonstrated that lncRNAs play important roles in carcinogenesis and cancer metastasis. It is necessary to identify novel therapeutic targets from lncRNA to improve the clinical outcome of HCC patients.

Recently, many studies reported that lncRNA may serve as novel biomarker to predict prognosis for glioma patients. For instance, He et al\(^15\) found that NEAT1 expression was up-regulated in glioma tissues and associated with poor prognosis in glioma patients. Zhu et al\(^16\) showed that HULC silencing suppressed angiogenesis by targeting ESM-1. Furthermore, they identified HULC as a novel predictive marker for the prognosis of glioma. Moreover, Zhang et al\(^17\) reported that lncRNA HOTAIR was positively associated with histological grade, unfavorable prognosis of glioma. SPRY4-IT1 was a newly found lncRNA. Khaitan et al\(^18\) reported that SPRY4-IT1 promoted proliferation and invasion of melanoma cells. Liu et al\(^19\) showed that knockdown of long noncoding RNA SPRY4-IT1 suppressed glioma cell proliferation, metastasis. These results indicated that SPRY4-IT1 may function as a tumor promoter in cancers.

In the present study, to our best knowledge, this is the first time to investigate the clinical significance of SPRY4-IT1 in glioma patients. Our data showed that SPRY4-IT1 expression in glioma tissues was significantly lower than that in matched normal adjacent tissues. In addition, lncRNA SPRY4-IT1 overexpression was proved to be correlated positively with WHO grade and tumor size. Kaplan-Meier analyses show that glioma tissues with high SPRY4-IT1 expression levels had poorer overall survival. Multivari-
ate analyses confirmed that SPRY4-IT1 expression level was independent prognostic factors for overall survival.

Conclusions

SPRY4-IT1 may be further developed as a novel noninvasive biomarker for glioma prognosis. A more in-depth and larger scale study remains to identify the role of SPRY4-IT1 in glioma.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References


