European Review for Medical and Pharmacological Sciences 2018; 22: 5519-5524

The SNORA21 expression is upregulated and acts as a novel independent indicator in human gastric cancer prognosis

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Abstract. – OBJECTIVE: Emerging evidence indicates that small nucleolar RNAs (snoRNAs) act crucial roles in oncogenesis. Herein, the aim of this study is to investigate the clinical value of SNORA21 expression in gastric cancer (GC).

PATIENTS AND METHODS: The expression of SNORA21 was determined in 79 cases of GC tissues and adjacent normal tissues by quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) analysis. The association between SNORA21 expression and clinicopathological features was analyzed by the chi-square test. The survival curves were calculated by the Kaplan-Meier method and log-rank test. Univariate and multivariate analyses were used to assess the prognostic value of SNORA21 expression.

RESULTS: Our results first demonstrated that SNORA21 expression was significantly upregulated in human GC tissues and cells compared to their corresponding adjacent normal tissues and GES-1 cells, respectively ($p<0.05$). Furthermore, elevated SNORA21 expression was significantly associated with distant metastasis ($p<0.05$) and lymph node metastasis ($p<0.05$) in GC patients. Kaplan-Meier survival plots demonstrated that higher SNORA21 expression was associated with poor disease-free survival (DFS) and overall survival (OS) rate, respectively. Univariate analysis and multivariate regression analysis indicated that a higher SNORA21 was an independent risk factor for prognosis in GC patients.

CONCLUSIONS: The current results indicate that SNORA21 expression may be served as a predictor of GC prognosis.

Key Words

Small nucleolar RNAs, SNORA21, Gastric cancer, Prognosis.

Introduction

Gastric cancer (GC) is the third leading cause of cancer-related mortality worldwide, with more than 300,000 deaths every year[1]. China alone accounts for nearly 42% of all new gastric cancer cases worldwide, and unfortunately, about 80% of all Chinese patients are diagnosed at advanced stage[2]. The survival rate for gastric cancer at advanced stage remained about 25-30%[3,4]. Thus, to explore effectively diagnostic makers in order to obtain an excellent long-term survival rate for GC patients are important.

SnoRNAs are identified as some abundant non-coding RNAs (ncRNAs) in the nucleus[5,6]. Recent evidence[7,8] showed that snoRNAs exhibit a wide variety of functions including ranging from RNA silencing, pre-mRNA splicing to chromatin decondensation. For instance, snoRNA U50 plays a role in the development and progression of breast cancer and re-expression of snoRNA U50 resulted in the inhibition of colony formation in breast cancer cell lines[9]. In the previous study, elevated SNORA21 emerged as an independent factor for predicting poor survival. CRISPR/Cas9-mediated inhibition of SNORA21 expression resulted in decreased cell proliferation and invasion[10]. However, little research has been done for clinical significance of SNORA21 expression in GC.

In the present work, our results first demonstrated that SNORA21 expression was significantly upregulated in GC tissues and cells, respectively. Furthermore, a higher SNORA21 expression was associated with unfavorable disease-free survival (DFS) and overall survival (OS) in GC patients, respectively. Thus, our results proved that high SNORA21 expression could serve as a biomarker for predicting GC prognosis.

Patients and Methods

Patient Tissue Samples

A total of 79 samples of GC and matched adjacent normal tissues were collected from patients who received surgery at the Affiliated Hospital of

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Inner Mongolia Medical University. There were 49 males and 30 females, with an average age of 51.6 ±5.4 year-old. All the patients signed the written informed consent before surgery. None of the patients had received chemotherapy or radiotherapy before surgery. The tissue samples were frozen immediately after surgery and stored at -80°C until RNA analyses. All procedures were approved by the Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University. All the GC patients were followed up records from the date of surgery to death or the last follow-up date. The clinical data was shown in Table I.

Cell Lines Culture

Four human gastric cancer cell lines (BGC-823, MGC-803, MKN-45, and SGC-7901), one normal gastric epithelial cell line (GES-1) was obtained from the Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences (Shanghai, China). Cells were maintained in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone, GE Healthcare Life Sciences, Logan, UT, USA), 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen, Waltham, MA, USA). Cells were cultured at 37°C in a humidified air atmosphere containing 5% CO_2.

Quantitative Real-Time Polymerase Chain Reaction (QRT-PCR)

Total RNA was extracted from tissue samples and cell lines using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cDNA was reversed using RNA via the Prime Script RT kit (TaKaRa Biotechnology Co., Ltd., Dalian, China) following the manufacturer's protocol. The SYBR Premix Ex Taq II (TaKaRa Biotechnology Co., Ltd., Dalian, China) was used to detect the mRNA expression on ABI7500HT system (Applied Biosystems, Foster City, CA, USA). The qRT-PCR thermocycling conditions were as follows: 30 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 30 sec. The mRNA expression was normalized to U6 snRNA expression. The relative gene expression was calculated using $2^{-\Delta\Delta Ct}$ methods. The primers were purchased from RiboBio Co., Ltd. (Guangzhou, China). The primer sequences were as follow: SNORA21 forward primer: CCTGTGGCTAATGACCTATT, SNORA21 reverse primer: CTTGTCACACCACCGATT.

Table I. Correlation between SNORA21 expression and clinicopathological variables of GC cases.

Variable	Number (n=79)	Low (n=42)	High (n=37)	*p*-value
Age (years)				0.176
≤60	55	32	23	
>60	24	10	14	
Sex				0.659
Male	49	27	22	
Female	30	15	15	
Tumor size				0.148
≥3 cm	38	17	21	
<3 cm	41	25	16	
Invasion depth				0.905
T1/T2	39	21	18	
T3/T4	40	21	19	
Lymph node metastasis				0.022*
Negative	32	22	10	
Positive	47	20	27	
Distant metastasis				0.034*
Negative	58	35	23	
Positive	21	7	14	
TNM stage				0.087
I-II	38	24	14	
III-IV	41	18	23	

*$p<0.05$.

Statistical Analysis

Data were analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The relevant results were shown as mean ± standard error. The differences between two groups were analyzed by the Student's *t*-test. The association between SNORA21 expression and clinicopathological features was tested using the Chi-square test. Kaplan-Meier and the log-rank test were used to calculate the survival curves. The prognostic factors were evaluated using univariate and multivariate Cox proportional hazards models. The $p<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of SNORA21 is Upregulated in GC Tissues and Cells

First, the relative expression of SNORA21 in human GC tissues and adjacent normal tissues was examined using qRT-PCR analysis. As presented in Figure 1A, the relative expression of SNORA21 in GC tissues was significantly increased (3.6-fold) compared with their adjacent normal counterparts ($p<0.001$). Moreover, we examined the relative expression of SNORA21 in four human GC cell lines (MGC-803, SGC-7901, BGC-823, and MKN-45) compared to GES-1 cells. The results showed that expression of SNORA21 was also significantly increased in four GC cells compared to GES-1 cells (Figure 1B, $p<0.05$). Altogether, these results indicated that expression of SNORA21 was higher in GC tissues and cells.

Association Between SNORA21 Expression and Clinical Pathological Features

To assess the potential association between SNORA21 expression and clinicopathologic features, we classified the patients into high (above the median, n=37) and low (below the median, n=42) SNORA21 expression groups according to the median expression of SNORA21 in GC tissues. The association between SNORA21 expression and clinicopathological features was tested using the Chi-square test. Our results showed that high SNORA21 expression levels were significantly associated with distant metastasis ($p=0.034$, Table I) and lymph node metastasis ($p=0.022$, Table I) in GC patients. However, there was no significant association of SNORA21expression with other clinical features including age, sex, tumor size, and so on (all $p > 0.05$, shown in Table I).

Association Between SNORA21 Expression and Prognosis of GC Patients

Next, we assessed the prognostic value of SNORA21 expression in GC patients. Kaplan-Meier and the log-rank test were used to calculate the survival curves. The results showed that high SNORA21 expression group in GC patients was related to worse disease-free survival (DFS) (Figure 2A, log-rank test=14.223, $p<0.05$) and overall survival (OS) (Figure 2B, log-rank test=15.055, $p<0.05$) of GC compared to lower SNORA21 expression group. Moreover, we performed univariate and multivariate Cox proportional hazards models

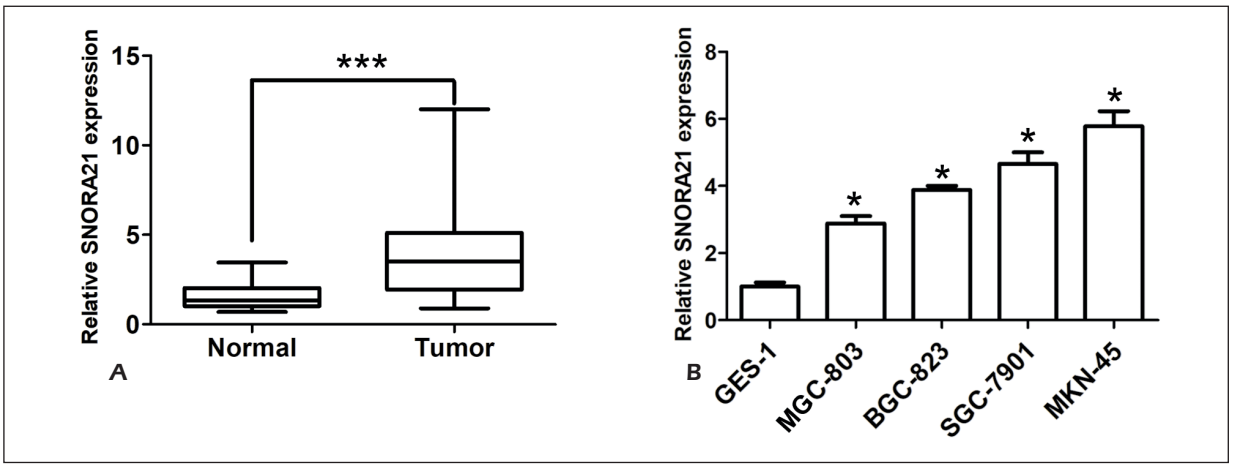

Figure 1. The expression of SNORA21 is higher in GC tissues and cells. **A**, The relative expression of SNORA21 was examined in 79 cases of GC tissues and adjacent normal tissues using qRT-PCR analysis. **B**, The relative expression of SNORA21 was examined in four GC cells and GES-1 cells using qRT-PCR analysis, ***$p<0.001$, *$p<0.05$.

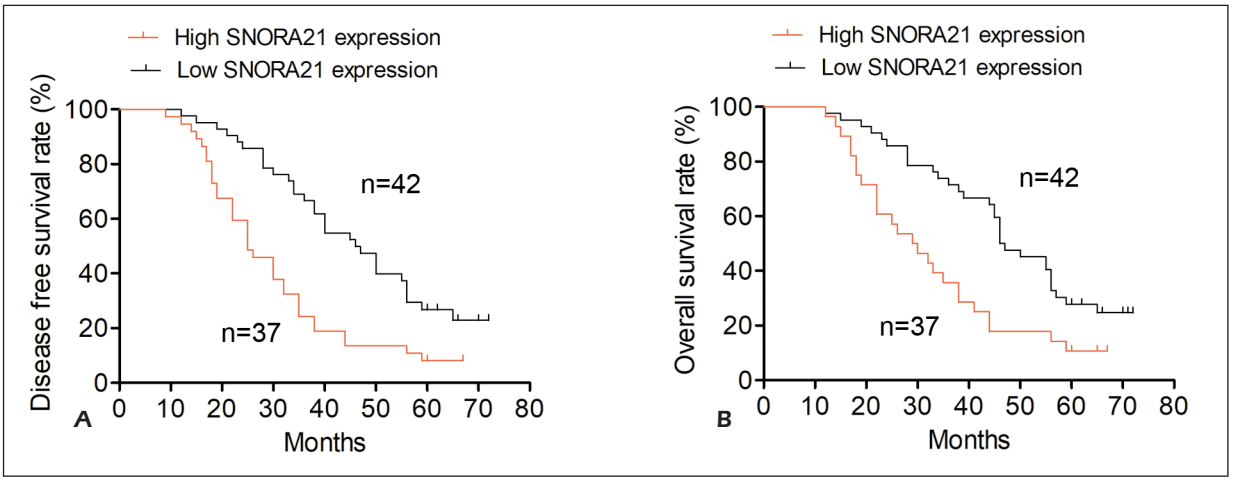

Figure 2. The association between SNORA21 expression and prognosis. **A**, Kaplan-Meier and the log-rank test were used to calculate the disease-free survival (DFS) curves between high SNORA21 expression group and low SNORA21 expression group. **B**, Kaplan-Meier and the log-rank test were used to calculate the overall survival (OS) curves between high SNORA21 expression group and low SNORA21 expression group.

to assess the prognostic factors of GC patients. Univariate and multivariate Cox proportional analysis showed that higher SNORA21 expression was a significant prognostic factor for DFS ($p<0.05$, Table III). Furthermore, the SNORA21 expression was also significant prognostic factor for OS ($p<0.05$, Table III). Thus, these results indicated that SNORA21 expression may be a prognostic biomarker of GC.

Discussion

The roles of small nucleolar RNAs (snoRNAs) in various disease processes and oncogenesis have been previously reported[11]. SNORD126 was upregulated in HCC compared with non-tumor tissues and correlated with Barcelona Clinic Liver Cancer (BCLC) stage in HCC patients[12]. The expression of snoRA15, snoRA41 displayed increased, whereas snoRD33 was downregulated in colorectal cancer compared with matched non-cancerous tissues, that were involve in the progress from chronic intestinal inflammation to malignant tumor[13]. SNORA55 expression is significantly associated with growth factor signaling and pro-inflammatory cytokine expression in prostate cancer[14]. Enforced SNORD76 expression in orthotopic tumors resulted in the decreased tumor growth and the reduction of tumor volume[15]. Small nucleolar RNA 113-1 (SNORD113-1) suppressed cancer cell growth of hepatocellular carcinoma (HCC)[16]. C/D-box snoRNA-derived RNA production is associated with malignant transformation and metastatic progression in prostate cancer[17]. These above results revealed that snoRNAs exhibited important regulators in tumor progression.

Table II. Univariate and multivariate analysis of DFS in GC patients.

Variable	Univariate Cox analysis		Multivariate Cox analysis	
	HR (95% CI)	p	HR (95% CI)	p
Age (years)	0.678 (0.345-1.245)	0.783	0.788 (0.455-1.344)	0.723
Sex	0.912 (0.554-1.654)	0.544	0.895 (0.655-1.344)	0.589
Tumor size (≥ 3cm)	1.128 (0.776-1.966)	0.446	1.178 (0.886-1.866)	0.422
Invasion depth	0.881 (0.544-1.466)	0.569	0.765 (0.344-1.568)	0.689
Lymph node metastasis	2.214 (1.088-3.887)	0.001*	2.088 (0.989-3.699)	0.001*
Distant metastasis	2.088 (0.880-3.766)	0.001*	1.906 (0.788-3.799)	0.001*
TNM stage	1.088 (0.455-1.866)	0.482	0.810 (0.402-1.955)	0.625
SNORA21 expression	2.588 (1.097-4.899)	0.001*	2.122 (0.886-3.778)	0.001*

*$p<0.05$.

Table III. Univariate and multivariate analysis of OS in GC patients.

Variable	Univariate Cox analysis		Multivariate Cox analysis	
	HR (95% CI)	p	HR (95% CI)	p
Age (years)	0.789 (0.466-1.388)	0.722	0.688 (0.344-1.244)	0.899
Sex	0.877 (0.488-1.788)	0.596	0.815 (0.604-1.411)	0.614
Tumor size (≥3 cm)	1.088 (0.665-1.877)	0.482	0.966 (0.667-1.755)	0.495
Invasion depth	1.187 (0.723-1.796)	0.410	0.899 (0.454-1.528)	0.502
Lymph node metastasis	2.344 (1.276-3.799)	0.001*	2.008 (0.811-3.441)	0.001*
Distant metastasis	2.155 (0.760-3.811)	0.001*	1.899 (0.668-3.739)	0.004*
TNM stage	1.211 (0.855-1.964)	0.213	1.094 (0.655-1.845)	0.466
SNORA21 expression	2.588 (1.097-4.899)	0.001*	2.188 (0.916-3.889)	0.001*

*$p<0.05$.

In the present study, we demostrated that the relative expression of SNORA21 in GC tissues was significantly increased compared to adjacent normal counterparts. Elevated SNORA21 expression levels were significantly associated with distant metastasis and lymph node metastasis in GC patients. Kaplan-Meier and the log-rank test showed that higher SNORA21 expression group in GC patients was related to worse disease-free survival (DFS) and overall survival (OS) of GC compared to lower SNORA21 expression group. In addition, univariate and multivariate Cox proportional analysis showed that high SNORA21 expression were significant prognostic factors for DFS and OS in GC patients. Thus, these results showed that SNORA21 expression could be served as prognostic biomarker. Of course, we only observed that important clinical value of SNORA21 in GC. In the future, the potential functional effects of SNORA21 in GC cells may be well investigated.

Conclusions

We first showed that SNORA21 expression was elevated in GC tissues and cells. Furthermore, we showed that higher SNORA21 expression predicted a prognostic outcome of GC. These findings indicated that SNORA21 expression could be served as prognostic biomarker of GC.

Conflict of Interest

The authors declare that they have no conflict of interests.

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