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Up-regulation of long non-coding RNA SNHG6 predicts poor prognosis in renal cell carcinoma

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Abstract. – OBJECTIVE: We aim to investigate the expression of long non-coding RNA SNHG6 and analyze its clinicopathological significance in renal cell carcinoma (RCC).

PATIENTS AND METHODS: A total of 81 cases of RCC tissues were collected and enrolled. Total RNA was extracted using the TRIzol method followed by qRT-PCR detection of the mRNA level of SNHG6. The x2-test was used to analyze the correlation between SNHG6 expression and clinicopathological variables, including age, gender, TNM stage, Fuhrman grade, tumor size, and overall prognosis. Kaplan-Meier survival curve was plotted to analyze the association between SNHG6 expression and overall survival. Univariate and multivariate analysis were carried out with the Cox proportional hazard analysis.

RESULTS: SNHG6 was shown to be markedly upregulated in RCC tissues as compared with normal controls. Elevated SNHG6 was found to significantly correlate with clinical stage, lymph node metastasis, Fuhrman grade, and tumor size ($p<0.05$). Kaplan-Meier survival analysis exhibited that elevated SNHG6 was remarkably associated with poor overall survival ($p<0.001$). Moreover, multivariate analysis revealed that SNHG6 expression was an independent prognostic factor in RCC.

CONCLUSIONS: We showed that up-regulated SNHG6 was significantly associated with tumor progression and was an independent prognostic factor in RCC, suggesting that SNHG6 can work as a promising prognostic predictor in RCC.

Key Words:
Long non-coding RNA SNHG6, Renal cell carcinoma, Prognosis, Predictor.

Introduction

As the most common neoplasm in the kidney, renal cell carcinoma (RCC) is the most common lethal of all urological malignancies, accounting for 2-3% of adult malignancies[1]. Clinically, surgical treatment can prolong life, but only for patients with early-stage tumors. However, it is difficult for early detection to distinguish between benign and malignant kidney tumors[2]. Therefore, potential biomarkers for earlier diagnosis and prognosis of RCC[3,4] are required. Long non-coding RNAs (lncRNAs) are a class of RNA polymerase II transcripts which are greater than 200 nucleotides in size[5]. Recently, accumulating evidence revealed that lncRNAs are implicated in the initiation and progression[6] of carcinoma, as well as development and spread of metastasis[7]. Researches[8-10] showed that lncRNAs can be used as potential prognostic markers of carcinoma. LncRNA SNHG6, a contraction of Small Nucleolar RNA Host Gene 6, has been reported to be elevated in several types of carcinoma, including colon adenocarcinoma[10], breast carcinoma[11], and colorectal carcinoma[12]. Despite these relevant earlier reports, it has been seldom described in RCC. Given this, to learn about the clinicopathological and prognostic significance of SNHG6 expression, herein we detected, by using quantitative Real-time-PCR (qRT-PCR), the expression of SNHG6 in RCC tissues, numbering 81 cases.

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Patients and Methods

RCC Patients and Tissue Specimens

A total of 81 cases of RCC tissues and its matched normal controls were collected from the Department of Urinary Surgery, Jining First People's Hospital from July 2007 to June 2018. No patient was given any radiotherapy or chemotherapy before undergoing nephrectomy. All cases were diagnosed histologically by two experienced pathologists independently and staged according to the TNM staging of the American Joint Committee on Cancer (AJCC 7th, 2017). All tissue samples were immediately frozen in liquid nitrogen after resection and stored at -80°C until use. This study was approved by the Committee for Ethical Review of Research involving human subjects of Jining First People's Hospital, and informed consent was obtained from each participant.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was isolated from fresh tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the instruction. Total RNA (1 μg) was reversely transcribed into cDNA with the Moloney Leukemia Virus Reverse Transcriptase kit (Promega, Madison, WI, USA). qRT-PCR was performed on ABI 7500 fast Real-time PCR platform (Applied Biosystems, Waltham, MA, UK) with SYBR Green Mix (Promega, Madison, WI, USA) to detect the expression of SNHG6. Results were normalized to the expression of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as an internal loading control. The primers involved were SNHG6 Forward 5'-TTAGTCATGCCGGTGTGGTG-3'; Reverse 5'-AATACATGCCGCGTGATCCT-3'; GAPDH Forward 5'-GGGAGCCAAAAGGGTCAT-3'; Reverse 5'-GAGTCCTTCCACGATACCAA-3'. The PCR program was denaturation at 95°C for 5 s, annealing at 60°C for 30 s, elongation at 68°C for 20 s for 40 cycles. Each sample was performed in triplicates and fold changes were calculated using the relative quantification $2^{-\Delta\Delta CT}$ method. The tissues were divided into two groups according to the expression of SNHG6 (a fold-change ≥ 1 stands for high expression of SNHG6, whereas a fold-change < 1 represents low expression), high expression group (n=46) and low expression group (n=35).

Statistical Analysis

All statistical analyses were conducted using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) or GraphPad Prism 5.0 version (GraphPad Prism, La Jolla, CA, USA). Each experiment was performed independently in triplicate, and data are presented as mean ± standard deviation (SD) of three independent experiments. The Student's *t*-test was used to compare the means of two groups. The correlation between SNHG6 expression and clinicopathological variables was calculated by x^2-test. The Kaplan-Meier survival curve and Log-Rank test were used to plot the survival curve and compare differences between survival curves respectively. *p*-values less than 0.05 were considered statistically significant.

Results

SNHG6 Was Elevated in RCC Tissues

To understand the expression level of SNHG6, qRT-PCR was conducted in 81 RCC tissues and its paired normal control tissues. It was found that SNHG6 was markedly over-expressed in RCC tissues as compared with its paired normal control tissues (Figure 1), hinting that SNHG6 could be oncogenic in RCC.

Elevated SNHG6 Significantly Correlated with Tumor Progression and Lymph Node Metastasis in RCC

Subsequently, to learn about the clinicopathological significance of SNHG6 expression in RCC, detailed statistical analysis was performed with the clinicopathological variables available, including gender, age, T classification, Clinical stage, Lymph

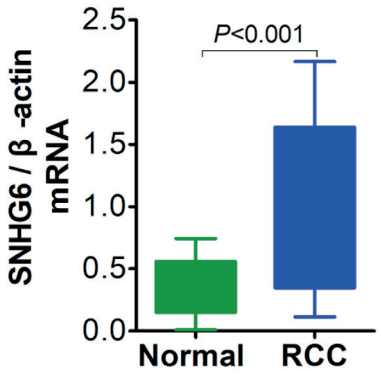

Figure 1. Evaluation of SNHG6 expression by qRT-PCR in RCC and paired normal control tissues, with a total of 81 cases, respectively. Normal, a contraction of matched normal control tissues; RCC, renal cell carcinoma. Independent sample *t*-test was used to analyze the significant difference. $p<0.001$ compared with control group.

node metastasis, Fuhrman Grade, tumor size and pathological subtypes. It was shown that up-regulated SNHG6 significantly correlated with T classification (p =0.033), clinical stage (p =0.023), Fuhrman grade (p =0.009), lymph node metastasis (p =0.001) and tumor size (p =0.012). No significant association was observed between up-regulation of SNHG6 and gender, age and pathological subtypes (Table I), indicating that SNHG6 was associated with tumor progression and lymph node metastasis in RCC.

Elevated SNHG6 was an Independent Prognostic Factor in RCC

After observing that up-regulated SNHG6 correlated with tumor progression and lymph node metastasis, we investigated whether or not there was an association between overall prognoses and SNHG6 expression in RCC. Kaplan-Meier survival curve was plotted on the basis of survival data. Analysis of Kaplan-Meier survival curve exhibited that there was an extremely significant difference of overall survival between patients with high expression of SNHG6 and patients with low expression of SNHG6 (p<0.001, Figure 2), indicating that SNHG6 expression was associated with overall prognosis in RCC. Given that SNHG6 significantly correlated with T classification, clinical stage, Fuhrman grade, lymph node metastasis, and tumor size, we next performed the multivariate Cox regression analysis that is more stringent than univariate analysis. Results of multivariate analysis showed that among all the significant parameters obtained by univariate analysis, only SNHG6 expression was still significant (Table II), demonstrating that SNHG6 expression was an independent prognostic factor in RCC. It is noteworthy that there are strong tendencies toward the statistical significance of clinical stage (p =0.067) and Fuhrman Grade (p =0.051), although no significant correlations can be observed (Table II).

Discussion

In the present study, LncRNA SNHG6 was shown to be remarkably up-regulated in RCC

Table I. The clinicopathological significance of SNHG6 expression in RCC.

Clinicopathological parameters	Total	SNHG6 expression		χ^2	p
		High (-, +)	Low (++)		
Renal carcinoma	81	46	35	32.044	0.000
Paired normal control	81	13	68		
Gender					
Male	41	26	15	1.485	0.266
Female	40	20	20		
Age (years)					
≤ 60	36	19	17	0.425	0.652
> 60	45	27	18		
T classification					
T_{1-2}	28	11	17	5.343	0.033
T_{3-4}	53	35	18		
Clinical stage					
Stage I-II	32	13	19	5.633	0.023
Stage III-IV	49	33	16		
Lymph node metastases					
N0	38	14	24	12.662	0.001
N1-2	43	32	11		
Fuhrman grade					
G1-2	31	10	21	7.491	0.009
G3-4	50	36	14		
Tumor diameter (cm)					
≤ 3	33	13	20	6.868	0.012
> 3	48	33	15		
Pathological subtypes					
Clear cell RCC	29	18	11	0.647	0.886
Papillary RCC	26	14	12		
Chromophobe RCC	12	6	6		

Table II. Univariate and multivariate Cox regression analysis of SNHG6 and survival in RCC.

	Univariate analysis			Multivariate analysis		
Clinical variables	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Gender (Male *vs*. Female)	0.776	0.500-1.203	0.257			
Age (≥ 60 y *vs*. < 60 y)	0.978	0.639-1.496	0.918			
Pathological subtype (Clear cell vs. the remainder 3)	0.784	0.512-1.199	0.261			
Tumor diameter (≥ 5 cm vs. < 5 cm)	1.413	0.913-2.185	0.121			
Clinical stage	1.855	0.960-3.587	0.046			
T stage (T1-2 vs. T3-4)	4.126	1.512-11.259	0.006			
Lymph node metastasis (N1-N2 vs. N0)	2.117	1.304-3.436	0.002			
Clinical stage (III-IV vs. II-I)	2.493	1.352-4.595	0.003	2.210	1.010-4.835	0.067
Fuhrman Grade (G1-2 vs. G3-4)	1.855	1.024-3.360	0.022	0.722	0.468-1.114	0.051
SNHG6 expression (high vs. low)	0.381	0.244-0.597	< 0.001	0.517	0.328-0.815	0.005

Abbreviation: HR, hazard ratio; CI, confidence interval.

tissues relative to normal controls, and elevated SNHG6 was displayed to be markedly associated with inferior overall survival. Elevated SNHG6 was also found to significantly correlate with T classification, clinical stage, Fuhrman grade, lymph node metastasis, and tumor size after detailed clinicopathological analysis. Moreover, the multivariate analysis showed that up-regulated SNHG6 was an independent prognostic factor in RCC. Recently, several excellent review articles[13-15] have been available regarding the involvement of lncRNAs in RCC. Therefore, we focus on lncRNA SNHG6 as an example for its potential use in RCC. Factually, the first study on SNHG6 in carcinoma came from hepatocellular carcinoma[8,16], and it was then extended to other different types of carcinoma, including colon adenocarcinoma[10], gastric carcinoma[9,17], glioma[18,19], breast carcinoma[11], esophageal squamous cell carcinoma[20], colorectal carcinoma[12,21,22], lung adenocarcinoma[23], and osteosarcoma[24]. This strongly suggested that SNHG6 was oncogenic whose expression was independent of tissue or cancer type. Despite this, it has been little described in RCC and the expression and its clinicopathological significance remain to be investigated. Given this, our study was important since we firstly provided direct evidence regarding SNHG6 in RCC that elevated SNHG6 was an independent prognostic factor in RCC. Several lines of evidence revealed that SNHG6 works as an oncogene in the initiation, development, and progression of carcinomas. Chang et al[25] suggested that SNHG6 was

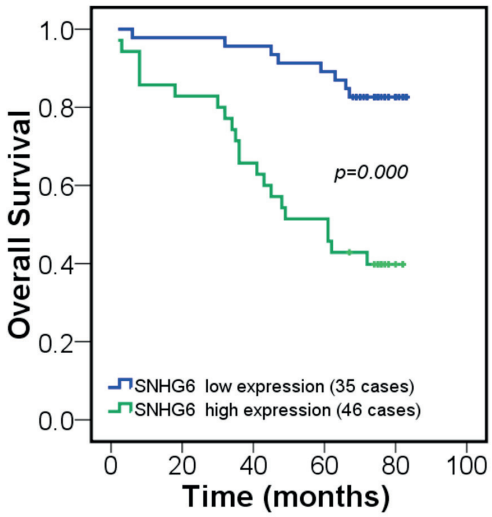

Figure 2. Kaplan-Meier survival analysis of SNHG6 expression. Of these 81 cases of RCC, the patients with high SNHG6 expression were 46, whereas the patients with low SNHG6 were 35 cases. Log-rank test was employed to analyze the significant difference.

up-regulated in hepatocellular carcinoma and up-regulation of SNHG6 was closely associated with histological grade, which partly supports our findings that elevated SNHG6 was shown to be significantly correlated with T classification, clinical stage, and Fuhrman grade in RCC. Concordantly, Cao et al[8] also reported that high expression of SNHG6 closely correlated with tumor progression and shorter survival, which was

totally in line with our data obtained on prognostic analysis of SNHG6 expression in RCC. Moreover, Yan et al[9] demonstrated in gastric carcinoma that over-expressed SNHG6 was significantly associated with invasion depth, lymph node metastasis, distant metastasis, TNM stage and poor prognosis, which was in agreement with our study in the case of the clinicopathological significance of over-expression of SNHG6. Nevertheless, Yan et al[9] failed to analyze using multivariate Cox regression whether or not over-expressed SNHG6 could be used as an independent prognostic predictor. By contrast, in our investigation, using multivariate Cox regression, we did a more stringent analysis of some significant correlations preliminarily obtained by univariate analysis, exhibiting that SNHG6 expression can independently predict the prognosis in RCC. In spite of our first description of SNHG6 in RCC, there were still some limitations that deserve to be acknowledged from the outset. Firstly, further study with a larger sample size needs to be warranted; secondly, the possible working mechanism by which SNHG6 works in RCC should have been explored.

Conclusions

We showed that lncRNA SNHG6 was pronouncedly up-regulated in RCC tissues as compared with paired normal controls and that up-regulated SNHG6 significantly correlated with T classification, clinical stage, Fuhrman grade, lymph node metastasis and tumor size of RCC. Moreover, SNHG6 expression was an independent prognostic factor of RCC, fully supporting that lncRNA SNHG6 was a generic oncogenic lncRNA in carcinomas, regardless of types.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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