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Down-regulation of long noncoding RNA PGM5-AS1 correlates with tumor progression and predicts poor prognosis in clear cell renal cell carcinoma

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Abstract. – OBJECTIVE: Growing studies have shown that long non-coding RNAs (lncRNAs) have critical regulatory roles in tumorigenesis. Recently, a newly identified lncRNA, Homo sapiens PGM5 antisense RNA 1 (PGM5-AS1), was found to be dysregulated in several tumors. However, its roles in clear cell renal cell carcinoma (ccRCC) have not been investigated. The aim of the present study was to clarify the clinical significance of PGM5-AS1 in ccRCC patients.

PATIENTS AND METHODS: The PGM5-AS1 expression levels were evaluated in 182 primary ccRCC patients using quantitative real-time PCR assays. The associations between expression of PGM5-AS1, clinicopathological parameters, and prognosis of ccRCC were examined using Chi-square test, Kaplan-Meier assays, and multivariate assays.

RESULTS: The expressions of PGM5-AS1 in cancer specimens were lower than those in matched non-tumor specimens from the ccRCC patient (p<0.05). Downregulation of PGM5-AS1 was closely associated with more advanced clinical features, including lymph nodes metastasis (p=0.007) and distant metastasis (p=0.037). A clinical study revealed that ccRCC patients with lower PGM5-AS1 expressions had substantially shorter overall survival (OS) and disease-free survival (DFS) than patients with higher PGM5-AS1 expressions. Further multivariate assays demonstrated that PGM5-AS1 was identified as an independent prognostic factor for patients with ccRCC.

CONCLUSIONS: Down-regulation of PGM5-AS1 in ccRCC tissues had a strong association with unfavorable outcomes and PGM5-AS1 might be a potential tumor suppressor.

Key Words:
LncRNA PGM5-AS1, Clear cell renal cell carcinoma, Prognosis.

Introduction

Renal cell carcinoma (RCC) encompasses a heterogeneous group of tumors derived from epithelial cells of kidney and is the most lethal genitourinary cancer[1]. The global incidence of RCC is increasing annually due to some very complex and multifactorial causes[2]. The distinct advancements in histopathological and molecular characterization of RCC in recent years have resulted in improved revisions for the classification of this tumor[3,4]. The major subtype of RCC is clear cell RCC (ccRCC). For early-stage ccRCC, partial nephrectomy has been applied for the removal of localized ccRCC with a favorable clinical outcome[5]. However, the five-year survival rates of metastatic ccRCC are only 15%[6]. In addition, patients with advanced ccRCC typically respond poorly to adjuvant therapy. It is thus urgent to find novel molecular biomarkers for ccRCC.

Long noncoding RNAs (LncRNAs), a class of transcripts > 200 nucleotides without protein-coding abilities, have attracted growing attention recently due to their potential effects on the regulation of gene expression via various complex mechanisms[7]. The advancements of the human genome project have demonstrated that 98% of the genome is transcribed to various non-coding RNAs, and only 1.5% of the mammalian genome has the potential of encoding proteins[8]. Growing studies have indicated that a large number of lncRNAs are abnormally expressed in cells and are frequently involved in the progression of various diseases, including tumors[9,10]. In addition, various cellular researches provide evidence that some lncRNAs display functional effects on cell pro-

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liferation, apoptosis, cellular metastasis, and so on[11,12]. With the development of high throughput sequencing, the disposable detection of substantial lncRNAs expressions in tumor tissues becomes a reality, highlighting the great potential of lncRNAs used as a novel biomarker due to their critical effects on the regulation of tumor progression[13,14]. Although several lncRNAs have been functionally characterized, the clinical significance of many lncRNAs in ccRCC remained largely unexplored.

Recently, Zhihua et al[15] first identified a novel tumor-related lncRNA, *Homo sapiens* PGM5 antisense RNA 1 (PGM5-AS1), which was found to be downregulated in esophageal squamous cell carcinoma and exhibits tumor-promotive roles in the progression of this tumor. In addition, several other studies[16,17] also reported the dysregulation of PGM5-AS1 in several tumors, including colorectal cancer and gliomas. However, whether PGM5-AS1 acted as a regulator in ccRCC has not been identified. In this study, we showed that PGM5-AS1 levels were distinctly downregulated in ccRCC using an online statistical tool, Gene Expression Profiling Interactive Analysis (GEPIA). Then, in our cohort of 182 ccRCC patients, distinct downregulation of PGM5-AS1 was also observed in ccRCC specimens compared to matched normal tissues. In addition, our group performed a series of clinical assays, indicating that PGM5-AS1 might be a novel molecular biomarker for predicting the prognosis of patients.

Patients and Methods

Patients and Tissue Samples

Samples of 182 ccRCC tissues from patients, including 110 males and 72 females were obtained from patients who had undergone surgical resection at Jinan First People's Hospital. All tumor and normal tissues were immediately snap-frozen in liquid nitrogen soon after surgical operation. None of these patients underwent chemotherapy or radiotherapy before surgery, and all specimens were histopathologically confirmed by two pathologists. The clinicopathological data are shown in Table I. The protocol was approved by the Human Ethics Committee at the Jinan First People's Hospital. All participants provided informed consent prior to their inclusion in the study.

RNA Extraction and QRT-PCR

Total RNA from ccRCC samples and adjacent normal tissues was extracted using TRIzol Reagent (Invitrogen, Hangzhou, Zhejiang, China) according to the manufacturer's instructions. RNA concentration and quality were assessed by

Table I. Clinicopathological features and the expression of PGM5-AS1 in ccRCC patients.

Characteristics	No. of patients	PGM5-AS1 expression		*p*-value*
		Low	High	
Gender				0.274
Male	110	58	52	
Female	72	32	40	
Age (years)				0.543
< 55	99	51	48	
≥ 55	83	39	44	
Histological grade				0.184
I-II	106	48	58	
III-IV	76	42	34	
Tumor size (cm)				0.183
< 4 cm	110	50	60	
≥ 4 cm	72	40	32	
Tumor stage				0.380
T1-T2	111	52	59	
T3-T4	71	38	33	
Lymph nodes metastasis				0.007
Absence	130	56	74	
Presence	52	34	18	
Distant metastasis				0.037
Absence	132	59	73	
Presence	50	31	19	

the 260/280 nm ratio by the use of a Nanodrop Spectrophotometer (ND-200, Haidian, Beijing China). Real-time PCR was performed using an ABI 7500 Fast Real-time PCR System (#4351107, Biosystems, Pudong, Shanghai, China) with a SYBR Premix Ex Taq kit (TaKaRa, Dalian, Niaoning, China). The specific condition for this experiment was as follows: melting at 95°C for ten seconds, annealing at 95°C for five seconds, and extension at 60°C for twenty seconds, for a total of forty-five cycles. Quantitative assays of the changes in the expressions of PGM5-AS1 were assessed and normalized using the $2^{-\Delta\Delta Ct}$ method relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All experiments were conducted in duplicate and repeated twice. The primers used in this study were as follows: PGM5-AS1 forward, 5'-GCCCTACCAGGAGTGAATG-3', and reverse, 5'-TTCCTGGTTTTGGAGTTTG-3'; GAPDH forward, 5'-AGCCACATCGCTCAGACA-3', and reverse, 5'-GCCCAATACGACCAAATC-3';

Statistical Analysis

SPSS 17.0 (SPSS, Chicago, IL, USA) was used to perform statistical analyses. Comparisons between two groups were conducted using two-tail Student's *t*-test. Chi-square test was applied for the determination of the association of PGM5-AS1 levels with clinicopathological features. The overall survival (OS) and disease-free survival (DFS) were analyzed by log-rank test, and Kaplan-Meier assays were applied to plot survival curves. Univariate and multivariate assays were carried out on each clinical covariate to analyze its possible impact on ccRCC patient survival. $p < 0.05$ was considered to indicate a significant difference.

Results

Decreased Expressions of PGM5-AS1 in Human CcRCC Samples

To screen potential ccRCC-related lncRNA, we searched an online tool, "GEPIA" which has a basic function in screening dysregulated lncRNAs by analyzing microarray data from TCGA datasets. As shown in Figure 1A, PGM5-AS1 was observed to be distinctly down-regulated in ccRCC specimens. Besides, in our cohort of 182 ccRCC patients, the results of RT-PCR also revealed that PGM5-AS1 was markedly down-regulated in ccRCC specimens compared with adjacent non-neoplasm tissues ($p < 0.01$, Figure 1B). Thus, the finding from our experiments, together with online data suggested that PGM5-AS1 may be a potential oncogenic lncRNA in ccRCC progression.

Associations Between PGM5-AS1 Expression and Clinicopathologic Factors

To explore whether PGM5-AS1 had a functional effect on the clinical progression of ccRCC, using the ratio of their normal/tumor specimens mean expression levels of PGM5-AS1 from qRT-PCR assays, 182 patients were divided into high and low expressing group. Then, Chi-square test was

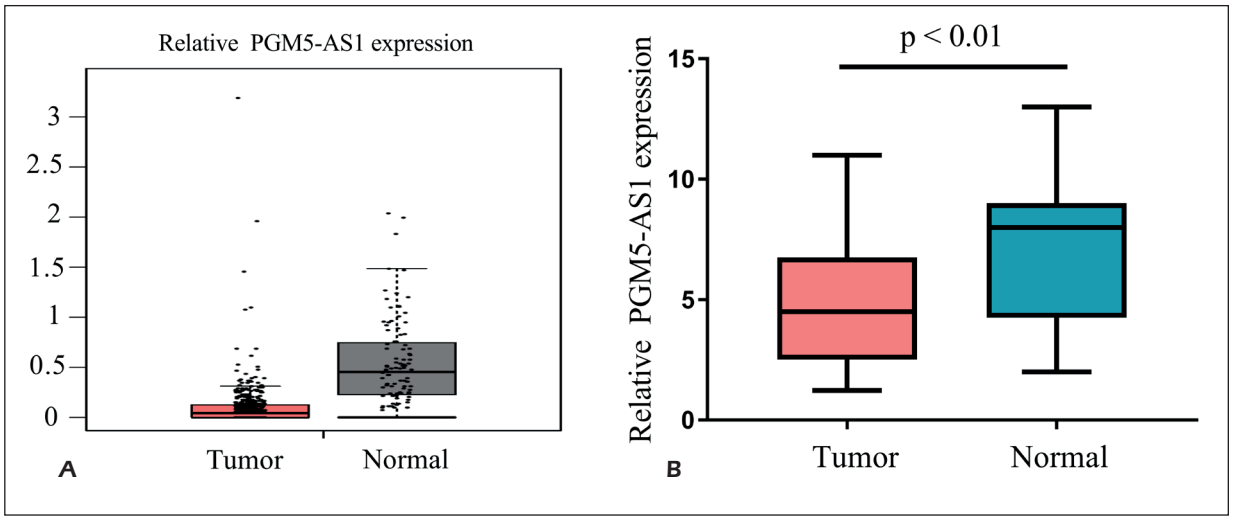

Figure 1. Analysis of PGM5-AS1 expression in ccRCC patients by using qRT-PCR. **A**, "GEPIA" was used to screen the dysregulated lncRNA in ccRCC tissues. **B**, RT-qPCR analysis was performed to examine the expression of PGM5-AS1 in the ccRCC specimens and adjacent non-tumor tissues.

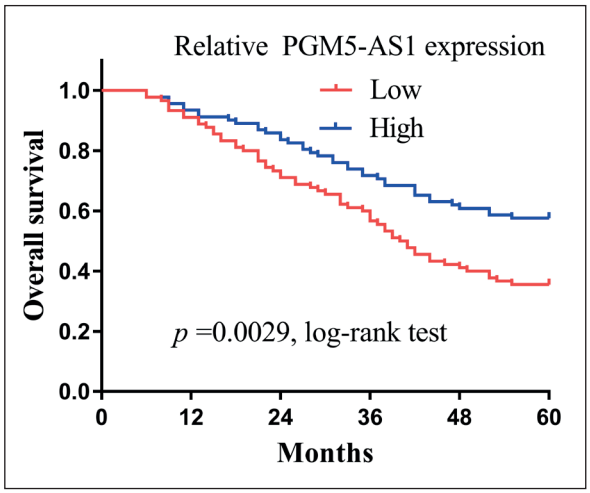

Figure 2. Overall survival curves for two groups defined by low and high expression of PGM5-AS1 in ccRCC patients.

performed to calculate the clinical data, and our results were presented in Table I, which showed that the expressions of PGM5-AS1 were distinctly correlated with distant metastasis (p = 0.037) and lymph nodes metastasis (p = 0.007). However, no distinct difference was observed between PGM5-AS1 levels and other clinical factors.

Associations Between PGM5-AS1 Expression and Prognosis of CcRCC Patients

Given the positive association between low levels of PGM5-AS1 and clinical metastasis in ccRCC patients, we wondered whether PGM5-AS1 may influence the clinical outcome of tumor patients. The five-year survival data were collected from the Follow-up Office with five years follow-up. Then, we performed Kaplan-Meier analysis, finding that patients with low expressions of PGM5-AS1 had shorter OS ($p < 0.0029$, Figure 2) and DFS (p= 0.003, Figure 3) as compared with the PGM5-AS1-high group. In addition, we performed univariate and multivariate assays for further exploration of the clinical values of PGM5-AS1 used as a biomarker. As shown in Table II, PGM5-AS1 expression was demonstrated to be an independent poor prognostic factor for both OS (HR= 2.897, 95% CI: 1.275-4.387, p = 0.015) and DFS (HR=2.875, 95% CI: 1.185-4.462, p = 0.017) in ccRCC patients.

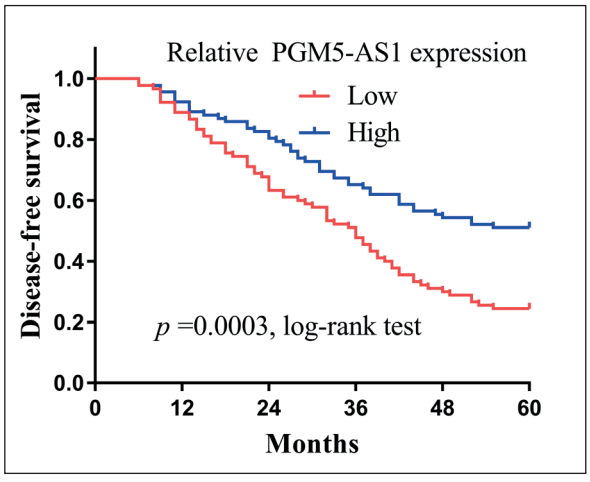

Figure 3. Disease-free survival curves for two groups defined by low and high expression of PGM5-AS1 in ccRCC patients.

Discussion

CcRCC, an aggressive tumor with 15-25% of patients presenting with metastasis at diagnosis, is one of the top 8 tumors in China[18]. Up to date, imaging remains the primary tool for ccRCC detection and screening[19]. In addition, the past ten years have seen the approvals of several molecular targeted therapies and one immunotherapy agent for the clinical management of ccRCC with metastasis[20]. Unfortunately, a satisfactory clinical prognosis has not been achieved. The identification of novel biomarkers guiding treatment to further improve long-term survival on the basis of individual tumor characteristics (so-called individualized treatment) has become an emerging opportunity[21]. In recent years, increasing attention focused on the possible clinical application of lncRNAs used as novel cancer markers or adjunct factors improving the specificity and sensitivity of existing markers due to high specificity and easy detections in the human specimens and plasma.

Increasing evidence has demonstrated that lncRNAs are emerging and imperative modulators of a series of biological processes, acting as tumor suppressors or promoters in tumorigenesis[22]. For instance, lncRNA MRCCAT1, a highly expressed lncRNA in ccRCC, which was firstly reported by Li et al[23], was shown to be positively associated with the metastatic potential of ccRCC and poor prognosis, and promote ccRCC cell proliferation and invasion via regulating the p38-MAPK pathway. LncRNA H19 was reported to be highly expressed in ccRCC and promoted ccRCC progression by increasing E2F1 expressions via competitively sponging miRNA-29a-3p[24]. Deng et al[25] showed that LINC00511 levels were distinctly upregulated in ccRCC and were associated with pos-

Table II. Univariate and multivariate analyses of prognostic variables of DFS and OS in glioma patients.

Parameters	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Disease free survival				
Gender	0.984 (0.472-1.884)	0.218	–	–
Age	1.362 (0.683-2.189)	0.159	–	–
Histological grade	1.895 (0.875-2.472)	0.216	–	–
Tumor size	1.784 (1.029-2.331)	0.384	–	–
Tumor stage	1.482 (0.672-2.179)	0.191	–	–
Lymph nodes metastasis	2.985 (1.372-4.672)	0.009	2.784 (1.132-4.218)	0.014
Distant metastasis	3.058 (1.387-4.382)	0.015	2.775 (1.258-3.989)	0.022
PGM5-AS1 expression	3.175 (1.479-5.271)	0.006	2.875 (1.185-4.462)	0.017
Overall survival				
Gender	0.875 (0.582-1.954)	0.168	–	–
Age	1.174 (0.785-2.385)	0.199	–	–
Histological grade	1.785 (0.911-2.462)	0.317	–	–
Tumor size	1.654 (0.767-2.317)	0.218	–	–
Tumor stage	1.564 (1.128-1.896)	0.199	–	–
Lymph nodes metastasis	3.126 (1.358-4.552)	0.011	2.986 (1.276-4.276)	0.020
Distant metastasis	3.019 (1.218-4.762)	0.008	2.786 (1.195-4.362)	0.014
PGM5-AS1 expression	3.118 (1.185-4.657)	0.009	2.897 (1.275-4.387)	0.015

itive lymph node metastasis and short survivals among ccRCC patients. Functionally, LINC00511 was observed to exhibit tumor-promotive roles in regulating the abilities of the proliferation and metastasis in ccRCC cells via sponging miR-625. In addition, several lncRNAs had been reported to have clinical potential to be used as novel diagnostic and prognostic biomarkers for ccRCC patients, such as lncRNA LINC-PINT and lncRNA OTUD6B-AS1[26,27]. These findings highlighted the great application of lncRNAs used as biomarkers for ccRCC patients. However, only a few lncRNAs were clinically identified in ccRCC.

In the present study, we identified a novel ccRCC-associated lncRNA, PGM5-AS1. For the first time, we provided evidence that PGM5-AS1 expression was distinctly down-regulated in ccRCC specimens. Then, we analyzed its clinical significance using Chi-square test, finding that low PGM5-AS1 expressions were distinctly correlated with lymph nodes metastasis and distant metastasis, which suggested that PGM5-AS1 may play a negative role in the clinical progress of ccRCC. Moreover, the results from Kaplan-Meier assays confirmed that patients with a lower expression of PGM5-AS1 tended to have a shorter five-year survival. Of note, multivariate assays demonstrated that PGM5-AS1 expression was independently associated with the OS and DFS of ccRCC patients, suggesting that lower PGM5-AS1 level was a potential biomarker of poor prognosis for ccRCC patients.

Conclusions

We first identified PGM5-AS1 as a novel ccRCC-related lncRNA. PGM5-AS1 might be a potential clinical marker for ccRCC patients. Further studies are warranted to explore the possible tumor-related functions of PGM5-AS1 in ccRCC tumorigenesis and its potential as a novel therapeutic target for the prevention of ccRCC-based metastasis.

Conflict of Interests

The Authors declare that they have no conflicts of interests.

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