P21-Associated ncRNA DNA Damage-Activated Expression in Bladder Cancer

Exprese ncRNA spojené s P21 aktivovaná poškozením DNA u karcinomu močového měchýře

Abdolmaleki F.[1], Ghafouri-Fard S.[1], Taheri M.[2], Omrani M. D.[3]

[1] Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Summary

Background: Long non-coding RNAs (lncRNA) have recently been the focus of attention of cancer researchers due to their diverse roles in the carcinogenesis process. These transcripts regulate critical steps in the normal cellular processes, so dysregulation of their expression participate in the pathogenesis of several cancers. P21-associated ncRNA DNA damage activated (*PANDA*) has a special situation in this regard due to its adjacency to the *CDKN1A* locus. It is involved in the regulation of DNA damage response as well as cell senescence and proliferation. ***Material and methods:*** In the current study, we assessed the expression of this lncRNA in bladder cancer tissue, adjacent non-cancerous tissues (ANCTs) and normal bladder samples by means of quantitative real time PCR method. ***Results:*** No significant difference has been detected in *PANDA* expression either between tumour tissue and ANCTs (expression ratio 1.75, P = 0.11) or between tumour tissue and normal tissues (expression ratio 2.72, P = 0.57). The expression level of this lncRNA was not associated with any of the demographic or clinical data of patients such as tumor grade or recurrence or cancer-associated risk factors such as cigarette smoking or opium addiction. ***Conclusion:*** Consequently, the current study implies that *PANDA* is not involved in the pathogenesis of bladder cancer. Assessment of expression of other lncRNAs would help in identification of biomarkers for this cancer.

Key words

P21-associated ncRNA – DNA damage-activated – *PANDA* – RNA – long non-coding – urinary bladder neoplasms

Souhrn

Úvod: Dlouhé nekódující ribonukleové kyseliny (long non-coding ribonucleic acids – lncRNA) jsou v poslední době vzhledem ke své úloze v procesu karcinogeneze předmětem zkoumání vědců zabývajících se nádory. Tyto transkripty regulují kritické kroky v normálních buněčných procesech, takže dysregulace jejich exprese se účastní patogeneze karcinomů. Z důvodu své blízkosti k lokusu *CDKN1A* má ncRNA spojená s P21 aktivovaná poškozením DNA (P21-associated ncRNA DNA damage activated – *PANDA*) v tomto ohledu zvláštní pozici. Podílí se na regulaci reakce na poškození DNA, stárnutí buněk a proliferace. ***Materiály a metody:*** V této studii jsme metodou kvantitativní polymerázové řetězové reakce hodnotili expresi této lncRNA ve tkáních karcinomu močového měchýře, sousedních nerakovinných tkání (adjacent non-cancerous tissues – ANCT) a v normálních vzorcích močového měchýře. ***Výsledky:*** Nebyl detekován žádný významný rozdíl v expresi *PANDA*, a to ani mezi nádorovými tkáněmi a ANCT (poměr exprese = 1,75; p = 0,11) nebo mezi nádorovými tkáněmi a normálními tkáněmi (poměr exprese = 2,72; p = 0,57). Úroveň exprese této lncRNA nebyla spojena s žádnými demografickými ani klinickými údaji o pacientech, jako je grade nádoru nebo recidiva, ani s rizikovými faktory souvisejícími s rakovinou, mezi něž patří např. kouření cigaret nebo závislost na opiu. ***Závěr:*** Tato studie tedy naznačuje, že *PANDA* není zapojena do patogeneze karcinomu močového měchýře. Hodnocení exprese jiných lncRNA by mohlo pomoci při identifikaci biomarkerů pro tyto karcinomy.

Klíčová slova

ncRNA spojená s P21 – aktivace poškozením DNA – *PANDA* – RNA – dlouhé nekódující – karcinomy močového měchýře

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Mir Davood Omrani, M.D.
Urology and Nephrology Research Center
Shahid Beheshti University of Medical Sciences
9th Boostan St, No. 103
Tehran, Iran
e-mail: davood_omrani@yahoo.co.uk

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Introduction

Long non-coding ribonucleic acids (lncRNAs) with sizes of more than 200 nucleotides constitute the main part of the human transcriptome. Although they do not encode proteins, they participate in multiple biological activities [1,2]. Dysregulation of their expression contributes to conferring malignant phenotypes in diverse tissues [3]. The lncRNA P21 associated ncRNA deoxyribonucleic acid damage activated (*PANDA*) gene location is near the cyclin-dependent kinase inhibitor 1A (CDKN1A) gene and is transcribed antisense to CDKN1A [4]. The expression of this lncRNA is induced in a p53-dependent fashion following deoxyribonucleic acid (DNA) damage. Its interaction with the transcription factor NF-YA leads to suppression of the expression of pro-apoptotic genes [4]. Furthermore, this lncRNA interacts with scaffold attachment factor A to recruit polycomb repressive complexes and inhibit the expression of senescence-enhancing genes [5]. Peng et al. have reported a lower expression of *PANDA* in hepatocellular carcinoma samples compared with peri-tumour tissues [6]. However, forced overexpression of this lncRNA has enhanced cell proliferation and tumourigenesis potential both *in vitro* and *in vivo* [6]. In the osteosarcoma cell line, *PANDA* stimulates G1-S transition and increases cell proliferation by suppressing p18 transcription [7]. Zhan et al. have demonstrated up-regulation of this lncRNA in bladder cancer tissue compared with the corresponding adjacent non-cancerous tissues (ANCTs) [8]. In addition, they reported positive correlations between *PANDA* over-expression and higher histological and advanced tumour, node, metastasis (TNM) stage [8]. Based on the inconsistency of data regarding the expression pattern of *PANDA* in diverse cancer types, we designed the current study to evaluate its expression in bladder cancer tissues, ANCTs and normal bladder tissues.

Materials and Methods

Study participants

In the current study, we recruited 50 patients with histopathologically-defined bladder cancer. Both tumour tissue and ANCTs were excised during bladder surgery. The patients received no prior chemo/radiotherapy. Furthermore, 30 samples were excised from the bladder tissue of corpses to be used as controls. The individuals recruited as controls had no history of urogenital disease or cancer. Permission to use these tissues was obtained from the guardians of the deceased. The study protocol was approved by the ethical committee of the Shahid Beheshti University of Medical Sciences. All the patients signed written informed consent forms.

Assessment of *PANDA* expression

Total RNA was extracted from the tissue samples using TRIzol™ Reagent (Invitrogen, Carlsbad, California, USA). The quality of the RNA was assessed using a Thermo Scientific NanoDrop Spectrophotometer. The RNA purity was assessed by measuring the ratio of absorbance at 260 and 280 nm. Samples with ratios around 1.9 were regarded as acceptable. About 500 ng of RNA was converted to complementary DNA (cDNA) using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) according to the manufacturer's instructions. The expression levels of *PANDA* were compared between tumour tissues, ANCTs and control samples in a Rotor Gene 6000 Real-Time PCR Machine (Corbett, Australia) using a TaqMan® Universal PCR Master Mix (Applied Biosystems, USA). The *HPRT1* gene was used as the endogenous control. The PCR programme included a preliminary step at 94 °C for 10 min, forty cycles of 94 °C for 20 sec and 60 °C for 40 sec and a final extension step at 72 °C for 5 min. The sequences of the primers and probes are shown in Tab. 1.

Statistical analysis

The transcript levels of *PANDA* in tumour tissues were compared with ANCTs/controls using REST 2009 software. The significance of the difference in the expression of *PANDA* between the tumour tissues and the ANCTs/controls was evaluated using a t-test. The association between the clinical data and relative expression of *PANDA* was evaluated using a Chi-square test. P values of less than 0.05 were considered as significant.

Results

General information on the recruited persons

General information on the study participants has been summarised in Tab. 2.

Relative expression of *PANDA* in bladder cancer tissues, ANCTs and normal tissues

No significant difference has been detected in *PANDA* expression either between tumour tissues and ANCTs (expression ratio 1.75, P = 0.11) or between

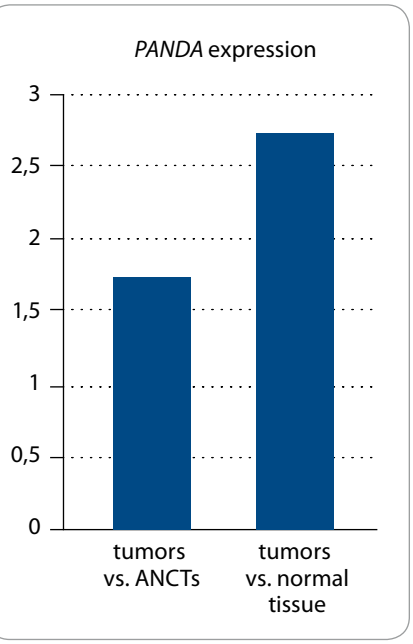
Tab. 1. The nucleotide sequence of primers and probes.

Gene name	Primer and probe sequences	Primer and probe length	Product length
HPRT1	F: AGCCTAAGATGAGAGTTC	18	88
	R: CACAGAACTAGAACATTGATA	21	
	FAM -CATCTGGAGTCCTATTGACATCGC- TAMRA	24	
PANDA	F: GTTTTCCTGTTCGTCGATTCTGG	24	81
	R: GGAAAGCTGAGAGAGACTTTGAAC	23	
	FAM- CTGGACCACCTCTGAAGGCAGGCA - TAMRA	24	

Tab. 2. General information of study participants.

Study Groups	Total numbers	Variables	Values	
Patients	50	age (mean ± SD)	61.78 ± 18.29	
		age range	29–88	
		gender	male	47 (94%)
			female	3 (6%)
		smoking	negative	14 (28%)
			positive	36 (72%)
		opium addiction	negative	25 (50%)
			positive	25 (50%)
		recurrence	negative	32 (64%)
			positive	18 (36%)
		hematuria	negative	39 (78%)
			positive	11 (22%)
		cytology	inconclusive	18 (36%)
			positive	32 (64%)
		grade	high-grade	32 (64%)
			low-grade	18 (36%)
Normal individuals	30	age (mean ± SD)	71.33 ± 6.97	
		age range	59–84	
		gender	male	28 (93.3%)
			female	2 (6.7%)

SD – standard deviation



Graph 1. Relative expression of *PANDA* in bladder cancer tissues, ANCTs compared with normal tissues.
ANCT – adjacent non-cancerous tissues

tumour tissues and normal tissues (expression ratio 2.72, P = 0.57) (Graph 1).

Association between relative expression of *PANDA* in bladder cancer tissues and tumour features

Based on the relative expression of *PANDA* in each tumour tissue compared with the corresponding ANCT, the patients were categorised into two groups (up-regulated vs. down-regulated). Subsequently, the associations between *PANDA* expression and clinicopathological features were assessed. The expression level of this lncRNA was not associated with any of the demographic or clinical data of patients or cancer-associated risk factors such as cigarette smoking or opium addiction (Tab. 3).

Discussion

Bladder cancer is one of the most frequently occurring cancers worldwide [9]. Based on the lack of specific symptoms in the initial phases of bladder cancer evolution, diagnosis of this malignancy is delayed and subsequently the therapeutic options are less effective [10]. The need for identification of diagnostic biomarkers has prompted researchers to evaluate the expression of several genes in the tissues or body fluids of the patients [11,12]. LncRNAs are among the putative biomarkers and therapeutic targets for this kind of human malignancy [1].

In the present study, we assessed the expression levels of *PANDA* in three types of bladder tissues including normal, ANCT and tumour tissues and found no significant difference in its expression between these three sets of samples. This lncRNA has been suggested to be involved in a variety of human disorders including neuroinflammatory and malignant conditions [6,13]. Moreover, it participates in several cancer-related processes such as DNA damage response, cell proliferation and cell senescence [4,5]. Previous studies in bladder cancer cells have revealed that *PANDA* knock-down suppresses proliferation/migration and stimulates cell apoptosis. Based on these observations, the authors proposed *PANDA* as a potent tumour biomarker and a therapeutic target in bladder cancer [8]. However, we could not find any difference in the expression of this lncRNA between normal, peri-tumoural and tumour tissues. Moreover, we could not detect any association between the expression levels of this gene and any of the clinical data of the patients. This lack of association further disproves the theory that lncRNA is a tumour biomarker. The inconsistency between our results and those of Zhan et al. [8] might be attributed to ethnic-based factors or differences in environmental risk factors.

We also detected a high prevalence of opium addiction in the patients. Based on the small sample size of the study, we cannot suggest opium addiction as a risk factor for bladder cancer. Previous studies have shown similar roles for both

Tab. 3. Association between relative expression of *PANDA* in bladder cancer tissues and tumor features.

	***PANDA* up-regulation**	***PANDA* down-regulation**	**P-value**
Age			0.27
< 60 years	7 (50%)	7 (50%)	
≥ 60 years	24 (66.7%)	12 (33.3%)	
Smoking			0.13
yes	20) 55.6%)	16 (44.4%)	
no	11 (78.6%)	3 (21.4%)	
Opium addiction			0.38
yes	14 (56%)	11 (44%)	
no	17 (68%)	8 (32%)	
Recurrence			0.92
positive	11 (61.1%)	7 (38.9%)	
negative	20 (62.5%)	12 (37.5%)	
Hematuria			1
positive	7 (63.6%)	4 (36.4%)	
negative	24 (61.5%)	15 (38.5%)	
Cytology			0.92
positive	20 (62.5%)	12 (37.5%)	
inconclusive	11 (61.1%)	7 (38.9%)	
Grade			0.13
high-grade	14 (77.8%)	4 (22.2%)	
low-grade	17 (53.1%)	15 (46.9%)	

cigarette smoking and opium addiction in the development of bladder cancer [14]. However, we could not find any association between the expression of *PANDA* and these two risk factors.

The main advantage of the current study was the incorporation of two sets of control samples including normal tissue and ANCT. The former tissue was used to control the intervening effects of tumour cells or tumour microenvironment on the expression of genes in the peri-tumour tissues, while the latter control was applied to adjust the effects of personal risk factors or environmental hazards. However, our study had some limitations regarding sample size and lack of mechanistical studies. Consequently, we propose large-scale studies in different populations to assess the diagnostic power of this lncRNA as a putative biomarker for cancer.

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