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Expression analysis of OIP5-AS1 in bladder tissues

Feraydoon Abdolmaleki[1], Soudeh Ghafoui-Fard[1], Mohammad Taheri[2], Alireza Mordadi[3], Mandana Afasharpad[4], Abolghasem Nikfallah[5], Bashir Nazparvar[6], Vahid Kholghi Oskooei[1], Mir Davood Omrani[7*]

[1]Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3]Department of Microbiology, Hamadan University of Medical Science, Hamadan, Iran

[4]Cancer Control Research Center, Cancer Control Foundation, Iran University of Medical Sciences, Tehran, Iran

[5]Urology Division, Brain and Spinal Injury Research Center, Tehran University of Medical Sciences, Tehran, Iran

[6]Department of Anatomy, Legal Medicine Research Center, Tehran, Iran

[7]Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding author: Mir Davood Omrani

E-mail: davood_omrani@yahoo.co.uk

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ABSTRACT. Long non-coding RNAs (lncRNAs) have crucial roles in regulation of many aspects of cell functions which are related with carcinogenesis process. The lncRNA Opa Interacting Protein 5-antisense 1 (OIP5-AS1) is among lncRNAs whose roles in some human malignancies have been elucidated. In the current study, we aimed at identification of the role of this lncRNA in bladder cancer. Expression level of OIP5-AS1 was assessed in 50 bladder cancer tissues, the corresponding adjacent non-cancerous tissues (ANCTs) and 30 normal bladder tissues using quantitative real time PCR. OIP5-AS1 was significantly down-regulated in tumor tissues

compared with normal bladder tissues (expression ratio=0.14, p=0.008). However, there was no significant difference in its expression between tumor tissues and ANCTs (expression ratio=1.79, p=0.23). No significant association was detected between relative expression of this gene and clinicopathological data of patients. Based on the area under curve (AUC) value, the diagnostic power of this gene was estimated to be 0.67. OIP5-AS1 expression levels might differentiate bladder tumor tissues from normal bladder samples but not from ANCTs.

Keywords: OIP5-AS1; Bladder cancer; lncRNA

INTRODUCTION

Long non-coding RNAs (lncRNAs) participate in the regulation of expression of several genes which are implicated in the carcinogenesis process in numerous tissues including bladder tissue. Both *in vitro* and clinical studies have shown that lncRNAs might exert oncogenic or tumor suppressor roles in this tissue (Taheri M, Omrani MD, Ghafouri-Fard S, 2018). The distinct pattern of their expressions in malignant tissues vs. normal tissues and their fundamental roles in the determination of cell fate have implied that they can be used as diagnostic or prognostic biomarkers in the bladder cancer (Taheri M, Omrani MD, Ghafouri-Fard S, 2018). More importantly, their transcript levels in urinary exosomes could also be used in this regard (Yazarlou F, hossein Modarressi M, Oskooei VK, 2018). Among the lncRNAs with putative roles in the tumorigenesis process is Opa Interacting Protein 5-antisense 1 (OIP5-AS1). Expression of this lncRNA in morula stage of the mouse embryo has been associated with preservation of stem cell self-renewal (Smith KN et al., 2017). In human, OIP5-AS1 is transcribed from the antisense strand of OIP5 gene at chromosome 15q15.1. OIP5-AS1 has been demonstrated to participate in the evolution of lung cancer through sponging miR-448 and indirectly changing the expression of Bcl-2 (Deng J et al., 2018). In undifferentiated oral tumors, expression of this lncRNA has been associated with higher degree of cancer stemness, and therefore, poor patients' outcome (Arun Kumar G et al. (2018). We have recently assessed expression of OIP5-AS1 in lung cancer tumors and found significant down-regulation of this lncRNA in tumor tissues compared with adjacent non-cancerous tissues (ANCTs) (Esfandi F, Kholghi OV, Taheri F, Kiani A, 2018). In the present study, we aimed at identification of the role of this lncRNA in the pathogenesis of bladder cancer through evaluation of its expression in bladder cancer samples, ANCTs and normal bladder tissues.

MATERIAL AND METHODS

Study participants

The present research was conducted on samples obtained from 50 patients with definite diagnosis of bladder cancer. Both tumoral and adjacent non-cancerous tissues (ANCTs) were obtained during bladder surgery before any chemo/radiotherapy. The inclusion criteria for patients were the presence of primary bladder tumor with definite pathological diagnosis and availability of clinical data and patients' willingness for cooperation. The exclusion criteria were lack of proper tissue for evaluation and presence of concomitant urinary disorder or cancer in other organs. All tissue samples were examined by pathologists to appraise the existence of tumoral cells. Moreover, 30 samples excised from bladder tissues of dead bodied were used as controls. Control subjects had no history of urogenital diseases or cancers. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences. All patients have signed written informed consent forms.

Expression study

Total RNA was extracted from all samples using TRIzol™ Reagent (Invitrogen, Carlsbad, CA, USA) according to company instructions. cDNA was produced from approximately 50 ng of RNA samples using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). Relative expression of OIP5-AS1 was compared between tumor tissues, ANCTs and control samples in the Rotor Gene 6000 Real-Time PCR Machine (Corbett, Australia) using TaqMan® Universal PCR Master Mix (Applied Biosystems, USA). Expression levels were normalized to HPRT1 transcripts. The sequences of primers and probes are shown in Table 1.

Table 1. Nucleotide sequences of primers and probes.

Gene name	Primer and probe sequence	Primer and probe length	Product length
HPRT1	F: AGCCTAAGATGAGAGTTC	18	88
	R: CACAGAACTAGAACATTGATA	21	
	FAM -CATCTGGAGTCCTATTGACATCGC- TAMRA	24	
OIP5-AS1	F: TCAGCCTCCCAAGTAGCTAGG	20	77
	R: GTCCCAGCCTTTTCAGCCTAG	21	
	FAM- CGCACCACCACGCTCAGCCTGATT- TAMRA	24	

Statistical analysis

Relative expression levels of genes in tumor tissues vs. ANCTs/ controls were assessed using REST 2009 software. The significance of difference in expression of OIP5-AS1 between tumor tissues and ANCTs/ controls was evaluated using t-test. The association between clinical data and relative expression of OIP5-AS1 was judged using Chi-square test. p-values less than 0.05 were considered as significant. The diagnostic power of OIP5-AS1 in bladder cancer was measured by scheming the receiver operating characteristic (ROC) curve. The area under curve (AUC) value was calculated.

RESULTS

General data of study participants

General information of study participants are shown in Table 2.

Table 2. General information of study participants.

Study Groups	Total Numbers	Variables	Values	
Patients	50	Age (mean±SD)	61.78 ± 18.29 (9-88)	
		Gender	Male	47 (94%)
			Female	3 (6%)
		Smoking	Negative	14 (28%)
			Positive	36 (72%)
		Opium	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 (59-84)	
		Gender	Male	28 (93.3%)
			Female	2 (6.7%)

Relative expression of OIP5-AS1 in tumor tissues compared to normal tissues and ANCTs

OIP5-AS1 was significantly down-regulated in tumor tissues compared with normal bladder tissues (expression ratio=0.14, P=0.008). However, there was no significant difference in its expression between tumor tissues and ANCTs (expression ratio=1.79, P=0.23). Figure 1 shows relative expression of this lncRNA in these three sets of samples.

Associations between relative expression of OIP5-AS1 in tumor tissues and patients' clinical data

Based on the relative expression of OIP5-AS1 in tumor tissues compared with the paired ANCT, we divided patients to up-regulated and down-regulated groups. Subsequently, we assessed associations between relative expression of OIP5-AS1 and tumor features (Table 3). No significant association was detected between relative expression of this gene and clinicopathological data of patients.

Table 3. Association analysis between relative expression of *OIP5-AS1* and tumor

	***OIP5-AS1* up-regulation**	***OIP5-AS1* down-regulation**	**P value**
Age			0.79
<60 years	8 (57.1%)	6 (42.9%)	
≥ 60 years	22 (61.1%)	14 (38.9%)	
Smoking			0.79
Yes	22 (61.1%)	14 (38.9%)	
No	8 (57.1%)	6 (42.9%)	
Opium addiction			0.56
Yes	14 (56%)	11 (44%)	
No	16 (64%)	9 (36%)	
Recurrence			0.63
Positive	10 (55.6%)	8 (44.4%)	
Negative	20 (62.5%)	12 (37.5%)	
Hematuria			0.48
Positive	8 (72.7%)	3 (27.3%)	
Negative	22 (56.4%)	17 (43.6%)	
Cytology			0.47
Positive	18 (56.3%)	14 (43.8%)	
Inconclusive	12 (66.7%)	6 (33.3%)	
Grade			0.63
High grade	10 (55.6%)	8 (44.4%)	
Low grade	20 (62.5%)	12 (37.5%)	

ROC curve analysis

We assessed the diagnostic power of OIP5-AS1 in differentiation of tumor tissues from normal tissues (Figure 2). Based on the AUC value, the diagnostic power of this gene was estimated to be 0.67. Table 4 shows the detailed parameters calculated for ROC curve analysis.

Table 4. The results of ROC curve analysis (a: Youden index, b: Significance level P (Area=0.5), Estimate criterion: optimal cut-off point for gene expression).

	Estimate criterion	**AUC**	**J[a]**	**Sensitivity**	**Specificity**	**P-value[b]**
OIPS5-AS1	≤ 0.23	0.67	0.4	60	80	0.003

DISCUSSION

In the current study, we evaluated expression of OIP5-AS1 in bladder cancer tissues, ANCTs and normal bladder tissues. Although OIP5-AS1 was significantly down-regulated in tumor tissues compared with normal bladder tissues, there was no significant difference in its expression between tumor tissues and ANCTs. This lncRNA has been previously shown to be associated with stemness properties in both normal and cancer cells. However, data regarding its role in the tumorigenesis process are inconsistent. While Deng et al. have demonstrated an oncogenic role for this lncRNA in lung adenocarcinoma (Deng J et al. (2018), we detected its down-regulation in lung cancer tissues compared with ANCTs. Arunkumar et al. reported elevated levels of OIP5-AS1 in undifferentiated oral tumors. However, *in vitro* studies have shown that this lncRNA suppresses HeLa cell proliferation probably through interaction with the RBP HuR and reducing its function in targeting cyclins A and D1 transcripts. Such inconsistencies might be partly explained by distinct roles of this lncRNA in each cancer type or the intrinsic dissimilarity in the pathogenesis of certain type of cancer due to geographic-related distinctions or risk exposures. The presence of certain genomic variants within this lncRNA might affect its interactions with its putative targets in distinctive malignancies and influence its role in the pathogenesis of cancer.

The similar expression levels of this lncRNA in tumor tissues and ANCTs might reflect the effect of tumor microenvironment on its expression. A previous study has demonstrated similarities in gene expression pattern between tumor tissues and histologically-normal cancer-adjacent tissues. The authors suggested that this similar pattern of expression might be due to the presence of discrete immune populations in the microenvironment. A comprehensive study in diverse cancer types has shown that ANCT has a discrete expression pattern from both healthy and tumor tissues. Taken together, our study has the advantage of comparison of expression level of OIP5-AS1 in tumor tissues with its expression in both ANCTs and normal tissues.

We could not find any association between transcript levels of this gene and tumor characteristics. Such lack of association might be due to the small sample size of the current study. However, based on the similar expression pattern of this lncRNA between tumor tissues and ANCTs, the failure to show any association between expression levels and clinical data might imply lack of fundamental role of this lncRNA in the pathogenic process of bladder cancer. Future functional studies are needed to explore the effects of OIP5-AS1 up-/down-regulation in modulation of malignant phenotype in bladder cancer cells.

CONCLUSION

As we detected difference in the expression levels of this lncRNA between tumor and normal tissues, we assessed the diagnostic power of this lncRNA in differentiation of disease status between these two sets of samples. Although transcript levels of this lncRNA had 60% sensitivity and 80% specificity in this regard, AUC value indicates that this lncRNA has not the appropriate diagnostic power. However, incorporation of this lncRNA in a putative panel of biomarkers might increase the diagnostic power. Taken together, we suggest assessment of expression level of this lncRNA in larger samples sizes from bladder cancer patients with diverse ethnicities to unravel its participation in the pathogenesis of this cancer.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

REFERENCES

Taheri M, Omrani MD, Ghafouri-Fard S (2018) Long non-coding RNA expression in bladder cancer. Biophysical reviews 10: 1205-1213. https://doi.org/10.1007/s12551-017-0379-y

Yazarlou F, hossein Modarressi M, Oskooei VK (2018) Urinary exosomal expression of long non-coding Rnas as diagnostic marker in bladder cancer. Cancer Management and Research 10: 6357-6365. https://doi.org/10.2147/cmar.s186108

Smith KN, Starmer J, Miller SC, Sethupathy P, et al. (2017) Long Noncoding RNA Moderates MicroRNA Activity to Maintain Self-Renewal in Embryonic Stem Cells. Stem cell reports 9: 108-121. https://doi.org/10.1016/j.stemcr.2017.05.005

Deng J, Deng H, Liu C, Liang Y, et al. (2018) Long non-coding RNA OIP5-AS1 functions as an oncogene in lung adenocarcinoma through targeting miR-448/Bcl-2. Biomedicine & pharmacotherapy 98: 102-110. https://doi.org/10.1016/j.biopha.2017.12.031

Arunkumar G, Anand S, Raksha P, Dhamodharan S, et al. (2018) LncRNA OIP5-AS1 is overexpressed in undifferentiated oral tumors and integrated analysis identifies as a downstream effector of stemness-associated transcription factors. Sci Rep-Uk 8. https://doi.org/10.1038/s41598-018-25451-3

Esfandi F, Kholghi OV, Taheri F, Kiani A (2018) Expression analysis of OIP5-AS1 in non-small cell lung cancer. Klinicka Onkologie 31: 313-318. https://doi.org/10.14735/amko2018260

Deng J, Deng H, Liu CF, Liang YJ, et al. (2018) Long non-coding RNA OIP5-AS1 functions as an oncogene in lung adenocarcinoma through targeting miR-448/Bcl-2. Biomed Pharmacother 98: 102-110. https://doi.org/10.1016/j.biopha.2017.12.031

Kim J, Abdelmohsen K, Yang XL, De S, et al. (2016) LncRNA OIP5-AS1/cyrano sponges RNA-binding protein HuR. Nucleic Acids Res 44: 2378-2392. https://doi.org/10.1093/nar/gkw017

Casbas-Hernandez P, Sun XZ, Roman-Perez E, D'Arcy M, et al. (2015) Tumor Intrinsic Subtype Is Reflected in Cancer-Adjacent Tissue. Cancer Epidem Biomar 24: 406-414. https://doi.org/10.1158/1055-9965.epi-14-0934

Aran D, Camarda R, Odegaard J, Paik Het al. (2017) Comprehensive analysis of normal adjacent to tumor transcriptomes. Nat Commun 8. https://doi.org/10.1038/s41467-017-01027-z