Expression Analysis of *OIP5-AS1* in Non-Small Cell Lung Cancer

Expresní analýza *OIP5-AS1* u nemalobuněčného karcinomu plic

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Summary

Background: Lung cancer as the most fatal cancer of men has prompted researchers to find biomarkers for early detection and prognosis. Among the possible biomarkers are a group of non-coding transcripts with sizes more than 200 nucleotides called long non-coding RNAs (lncRNAs). ***Aims:*** In the present study, we evaluated the expression levels of the lncRNA OIP5 antisense RNA 1 (*OIP5-AS1*) in 32 non-small cell lung cancer (NSCLC) samples compared with their corresponding adjacent non-cancerous tissue (ANCTs) by means of real-time polymerase chain reaction. The samples were obtained from patients who were admitted at Labbafi-Nejad Hospital during 2015 and 2016. ***Results:*** OIP5-AS expression levels was significantly decreased in tumoral tissues compared with ANCTs in total samples and in male subgroup. However, no association was found between relative expression of *OIP5-AS1* and clinicopathological data of patients or history of smoking. Expression levels of this lncRNA were not correlated with patients' age. ***Conclusions:*** This lncRNA is possibly a novel biomarker of NSCLC in Iranian patients. Future studies are needed to confirm the results of our study in larger sample sizes. Moreover, based on the difference in lung cancer associated risk factors in different populations, population-based studies are needed to explore the role of this lncRNA in the pathogenesis of cancers in each region to design appropriate targeted therapies for each population.

Key words

lung cancer – *OIP5-AS* – lncRNA – long non-coding RNA

Souhrn

Úvod: Karcinom plic jako nejvíce fatální nádor mužů přiměl výzkumníky ke hledání biomarkerů pro včasnou detekci a prognózu. Mezi možné biomarkery patří skupina nekódujících transkriptů o velikosti více než 200 nukleotidů nazývaná dlouhé nekódující RNA (lncRNA). ***Cíle:*** V této studii jsme vyhodnotili hladiny exprese OIP5 antisense RNA 1 (*OIP5-AS1*) u 32 vzorků nemalobuněčného karcinomu plic (non-small cell lung cancer – NSCLC) ve srovnání s odpovídající sousedící nenádorovou tkání (adjacent non-cancerous tissue – ANCT) pomocí polymerázové řetězové reakce v reálném čase. Vzorky byly získány od pacientů, kteří byli přijati v nemocnici Labbafi-Nejad v letech 2015 a 2016. ***Výsledky:*** Úrovně exprese *OIP5-AS* byly významně sníženy v nádorových tkáních ve srovnání s ANCT v celkových vzorcích a v podskupině mužů. Nebyla však zjištěna žádná souvislost mezi relativní expresí *OIP5-AS1* a klinicko-patologickými daty pacientů nebo historií kouření. Expresní hladiny této lncRNA nebyly korelovány s věkem pacientů. ***Závěry:*** Tato lncRNA je možný nový biomarker NSCLC u iránských pacientů. K potvrzení výsledků naší studie jsou potřebné budoucí studie u větších počtů pacientů. Navíc na základě rozdílu v rizikových faktorech spojených s rakovinou plic v různých populacích jsou studie založené na populaci potřebné k prozkoumání role této lncRNA v patogenezi onkologických onemocnění v každé oblasti za účelem navržení vhodných cílených terapií pro každou populaci.

Klíčová slova

karcinom plic – *OIP5-AS* – lncRNA – dlouhé nekódující RNA

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Background

Lung cancer has been regarded as the most fatal cancer in men and women worldwide and in distinct regions, resp. [1]. Analysis of data provided by the Iranian National Pathology Based Cancer Registry (INPBCR) estimated the 5-year prevalence to be 4.21 (95% uncertainty level – 3.37–5.38) per 100,000 adults, with a male – female ratio of 2.01 [2]. The most common type of lung cancer is non-small cell lung cancer (NSCLC) which accounts for approximately 85% of all cases and is further categorized into three types – squamous-cell carcinoma, adenocarcinoma, and large-cell carcinoma [3]. The cure rate of patients is disappointedly low [4] possibly due to extensive disease burden, unsuitable performance condition, several comorbidities, deprived socio-economic status and malnutrition [5]. Identification of proper biomarkers for early detection of lung cancer is expected to enhance patients' outcomes. Among putative biomarkers for lung cancer are long non-coding RNAs (lncRNAs). These heterogeneous types of transcripts have various fundamental functions in gene regulation and are involved in the pathogenesis of human cancers [6–11]. A comprehensive study to find the lncRNA and mRNA signature in lung adenocarcinoma samples and normal tissues has identified more than two thousands lncRNAs with differential expression in these two sets of samples [12]. More recently, the lncRNA opa interacting protein 5-antisense 1 (*OIP5-AS1*) has been shown to be involved in the pathogenesis of lung adenocarcinoma through sponging miR-448 and indirectly altering the expression of *Bcl-2* [13]. The present study aimed to assess the expression level of *OIP5-AS1* in NSCLC samples compared with their corresponding adjacent non-cancerous tissues (ANCTs). We hypothesized that the expression level of this lncRNA is higher in cancerous tissues compared with ANCTs in association with patients' clinical data.

Methods

Patients' samples

A total of 32 patients with definite diagnosis of NSCLC who were admitted at Labbafi-Nejad Hospital during 2015 and 2016 entered the study. Lung cancer tissues and the corresponding ANCTs were removed during surgery and snap-frozen in liquid nitrogen instantly. All patients signed the informed consent forms. The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1395.525).

Sampling and RNA extraction

Total RNA was isolated from cancerous tissues and ANCTs using the TRIzol™ Reagent (Invitrogen, Carlsbad, CA, USA) based on the protocol provided by the company except for inclusion of a DNase I treatment step. RNA concentration was assessed by 260/280 nM absorbance using Nanodrop equipment (Thermo Scientific).

cDNA synthesis and quantitative real-time polymerase chain reaction (RT-PCR)

Applied Biosystems High-Capacity cDNA Reverse Transcription Kits was used for cDNA synthesis based on the manufacturer's instructions. Afterwards, synthesized cDNAs were stocked at –20 °C until PCR was performed. Primers and probes used for PCR were designed using the allele ID 7 for x64 Windows software (Premier Biosoft, Palo Alto, USA). *HPRT1* was chosen as the reference gene. The primers and probes sequences and PCR product length are shown in Tab. 1. All experiments were performed on the rotor gene 6000 corbett RT-PCR System. Applied Biosystems TaqMan® Universal PCR Master Mix was used for quantification of relative levels of transcripts. PCR program included a denaturation step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 sec and 60 °C for 60 sec. The final extension step was performed at 72 °C for 5 min.

Statistical analysis

SPSS version 18 (Chicago, IL, USA) was used for statistical analysis. Relative expression of *OIP5-AS1* in tumoral tissues compared with ANCTs was assessed using Ln [Efficiency^ΔCT] values. The association between *OIP5-AS1* relative expressions and clinicopathologic data of patients was estimated using Chi-square test. Spearman correlation coefficient was calculated to assess the correlation between *OIP5-AS1* transcript levels and patient's age. To test the significance of difference in means of transcript levels between tumor and ANCT groups we used Kruschke's Bayesian estimation to fit two-sample Bayesian paired t-test. P values less than 0.05 were considered significant.

Tab. 1. The primers and probes sequences and PCR product length.

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	

PCR – polymerase chain reaction

Results

Relative expression of *OIP5-AS* in lung cancer tissues compared with ANCTs

OIP5-AS1 expression levels were significantly decreased in tumor tissues compared with ANCTs in total samples and in male subgroup (Tab. 2).

Tab. 2. Relative expression of *OIP5-AS1* in lung cancer samples compared with ANCTs in age- and sex-based subgroups of patients (HDI – 95% credible interval based on Bayesian approach).

***OIPS-AS* expression**		**Sample number**	**Relative expression**	**SD**	**Effect size**	**p**	**95% HDI**
total		32	–2.815	0.73	–0.721	< 0.0001	–4.28, –1.37
male		24	–2.976	0.9191	–0.718	0.016	–4.75, –1.11
female		8	–2.385	1.6	–0.667	0.101	–5.52, 0.78
< 60	male	15	–4.201	1.462	–0.947	0.006	–7.06, –1.24
	female	5	–1.668	5.15	–0.385	0.406	–10.15, 7.24
≥ 60	male	9	–1.774	1.288	–0.456	0.12	–4.35, 0.75
	female	3	–2.975	5.533	–0.703	0.223	–11.89, 6.55

ANCT – adjacent non–cancerous tissue, HDI – human development index, SD – stadard deviation

Association study of *OIP5-AS1* expression and clinicopathological data of patients

No association was found between relative expression of *OIP5-AS1* and clinicopathological data of patients (Tab. 3).

Assessment of correlation between *OIP5-AS1* expression and patient's age

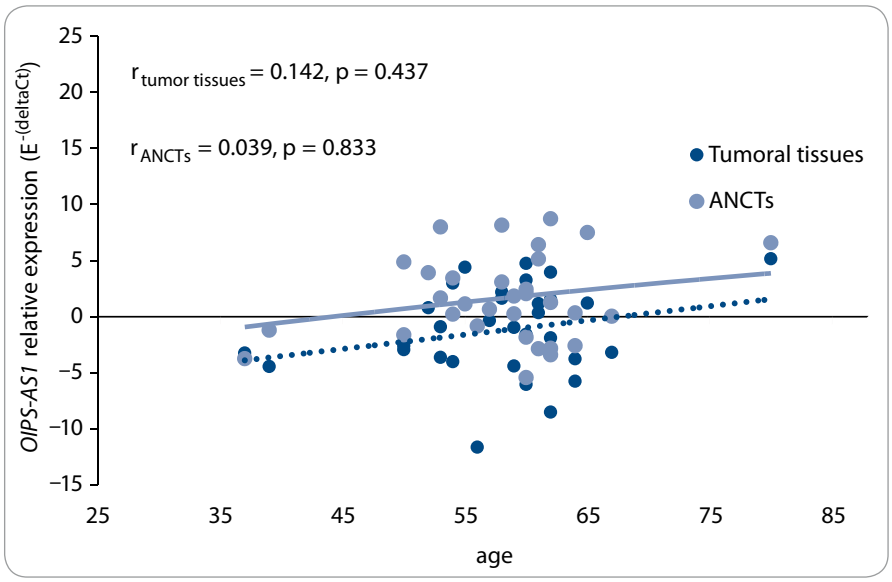
There was no correlation between patient's age and relative expression of *OIP5-AS1* either in tumor tissues or in ANCTs (Graph 1).

Tab. 3. Association study of *OIP5-AS1* expression and clinicopathological data of patients.

	***OIP5-AS1* up-regulation**	***OIP5-AS1* down-regulation**	**Total number**	**p**
Age				
< 60 years	7 (43.8%)	9 (56.3%)	16	0.723
≥ 60 years	8 (50%)	8 (50%)	16	
Gender				
male	12 (50%)	12 (50%)	24	0.691
female	3 (37.5%)	5 (62.5%)	8	
Smoking				
yes	12 (48%)	13 (52%)	25	1
no	3 (42.9%)	4 (57.1%)	7	
Stage				
I	6 (85.7%)	1 (14.3%)	7	0.068
II	3 (27.3%)	8 (72.7%)	11	
III	6 (42.9%)	8 (57.1%)	14	
Subtype				
adenocarcinoma	7 (38.9%)	11 (61.1%)	18	0.305
squamous cell carcinoma	8 (57.1%)	6 (42.9%)	14	

Discussion

The critical roles of lncRNAs in regulation of genes involved in DNA repair [14], induction of epithelial to mesenchymal transition (EMT) [15,16], inhibition of tumor suppressors [17,18], control of apoptosis and cellular metabolism [19] and regulation of nuclear receptors function [20] potentiate them as molecular markers of cancer. Expression levels of several lncRNAs have been assessed in lung cancer specimens and cell lines. For instance, the metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) has been recognized as a predictive marker of metastatic capacity of lung cancer cells which acts through modulation of expression of several metastasis-associated genes [21]. The maternally expressed gene 3 (*MEG3*) has also been involved in the EMT in lung cancer cells [22]. The oncogenic effects of *OIP5-AS1* have also been clarified in lung adenocarcinoma recently [13]. This lncRNA is transcribed in the antisense direction from the *OIP5* gene, an oncogene overexpressed in numerous malignancies [23]. While *OIP5-AS1* plays oncogenic roles in lung cancer [13], in HeLa cells, it inhibits cell proliferation possibly through interaction with the RNA-binding protein of human antigen (RBP HuR) and decreasing its readiness to target mRNAs of cyclins A and D1 (*CCNA2* and *CCND1*) and *SIRT1* [24]. In an effort to validate the results of the previous study on the role of *OIP5-AS1*



Graph. 1. Correlation between patient´s age and *OIP5-AS1* relative expression in tumoral tissues as well as ANCTs (E – efficiency, delta Ct = $Ct_{OIP5\text{-}AS}$–Ct_{HPRT}).
ANCT – adjacent non-cancerous tissue

in lung cancer [13] and assess its potential as a biomarker in these patients, we evaluated the expression levels of this lncRNA in NSCLC samples from Iranian patients compared with the corresponding ANCTs. However, contrary to Deng et al. we detected down-regulation of its expression levels in tumor tissues compared with ANCTs. Nevertheless, our study is in line with Deng et al., study regarding the lack of association between *OIP5-AS1* expression levels and patient's age. We also assessed the association between its expression levels and history of smoking in patients as well as disease stage and found no associations. The latter parameters were not evaluated in Deng et al. study. The discrepancy between the results of our current study and those of Deng et al. [13] can be explained by the heterogeneity of samples in our study (comprising both adenocarcinoma and squamous cell carcinoma) or the inherent difference in the pathogenesis of lung cancer due to geographic-related dissimilarities or hazards exposures. A previous study revealed geography-related patterns in the methylation profiles of NSCLC tumors [25]. Moreover, exposure to inorganic dusts, heavy metals and chemical compounds has been associated with lung cancer risk in Iranian patients [26]. Thus, the possibility of specific geographic-based hazards and their possible effects on gene expression signature cannot be ignored. Consequently, population-based studies are needed to explore the role of lncRNAs in the pathogenesis of cancers in each region to design appropriate targeted therapies for each population.

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