

The Expression Analysis of KLF11, PCDH9 and TGF- β 2 Genes in Patients with Non-Small Cell Lung Cancer

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Abstract

Background: Lung cancer remains the leading cause of cancer mortality in men and women in the worldwide. Studies have confirmed the presence of multiple and effective genes such as KLF11, PCDH9 and TGF- β 2 can be involved in lung cancer. KLFs are transcriptional regulators that contribute to a wide range of cellular processes. PCDH9 gene belongs to the protocadherins family and its role is cell adhesion and TGF- β activates several proteins and signaling cascades including SMAD proteins and MAPK cascade. So, the aim of this study is to examine the expression of these genes.

Method: In this study, the expression of KLF11, PCDH9 and TGF- β 2 genes were checked by Quantitative real time PCR in 30 NSCLC tissue samples and adjacent normal tissue samples.

Results: The results of these genes indicated that the expression level in tumor samples is not significantly different with normal samples ($P > 0.05$). However, this study was designed as a pilot study, and further investigations are required to confirm our findings.

Keywords — Lung cancer; KLF11; PCDH9; TGF- β 2; Real Time-PCR; NSCLC.

I. INTRODUCTION

Lung cancer ranks as the top cause of cancer-related deaths in the world. Developing advanced therapies for lung cancer, including targeted molecular therapies, is a very important topic. For this purpose, it is necessary to evaluate the genes involved in lung cancer [1]. Lung cancer is divided into two categories: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Approximately 85% of lung cancer is related to NSCLC types, which are classified into adenocarcinoma (AdC), squamous cell carcinoma (SqCC), and large cell carcinoma [2, 3]. In 2016, around 224390 cases of lung cancer were diagnosed in the United States, of which 158080 died and in 2014, about 8251 people died of lung cancer in Australia [4, 5]. Studies have shown that genetic and epigenetic factors and environmental factors contribute to the development of lung

cancer [6]. The factors that cause lung cancer include such things as smoking, exposure to tobacco smoke, exposure to nickel, chromium, asbestos, and arsenic [7]. Lung cancer is often diagnosed in advanced stages. Individualized lung cancer therapy based on genetics has progressed in the past 10 years, especially in NSCLC [8, 9]. Therefore, the medical community needs to discover the diagnostic objectives and new molecular treatment for cancer patients [10]. Over the past decade, studies have confirmed the presence of multiple and effective genes in lung cancer samples, and some of them have been reported in the genes of KLF11, PCDH9 and TGF- β 2 in lung cancer [11, 12]. KLFs are transcriptional regulators that contribute to a wide range of cellular processes, including proliferation, differentiation, apoptosis and spread. KLFs can act as transcriptional activators or suppressors [13]. This gene produces a protein the same name as a zinc finger

transcription factor. The KLF11 protein binds to the SP1 sequences in the promoter of the epsilon and gamma globin genes and leads to inhibition of cell growth and induction of apoptosis [14]. The expression of KLF11 gene was first observed in osteoblast cells, and later it was expressed extensively in many tissues including the breast, lung, kidney, stomach and intestine, and its highest expression in the pancreas [11]. The PCDH9 gene belongs to the protocadherins family and its role is cell adhesion and is known as a tumor suppressor gene [15]. This gene has a high expression in various tissues of the body, including the breast, liver, prostate, brain, lung and pancreas. For this reason, many studies have been conducted in various researches and so far its role has been proved in a number of different diseases [12]. The PCDH9 gene also plays a role in the EMT signaling pathway [16]. TGF- β 2 gene belongs to the family of polypeptide growth factors. This gene performs various functions in the natural cells and acts in the proliferation, differentiation, apoptosis, and regeneration of the extracellular matrix. TGF- β activates several proteins and signaling cascades including SMAD proteins and

MAPK cascade and paths such as Erk, JNK and P38 [17]. The TGF- β 2 messenger path includes ligand binding to TGF β receptors and activation of two different signaling pathways: signaling path that dependent to SMAD and signaling path that un-dependent on SMAD [18]. In this study were investigated the expression of KLF11, PCDH9 and TGF- β 2 genes in NSCLC cancer by using Real-Time PCR method.

II. 2. MATERIAL AND METHODS

2.1 Patient's characteristics

Human non-small cell lung cancer specimens (n = 30) and adjacent non-tumor tissues were obtained from patients at Masih daneshvari Hospital, Tehran, Iran, with informed consent from each patient. According to the information presented in Table 1, 8 patients were male and 22 of the patients were female, 17 patients had adenocarcinoma and 13 patients had squamous cell carcinoma, 22 patients were non-smokers and 8 patients were smokers. The proportion of patients with pathological stages I, II, III was 9 out of 30, 11 out of 30 and 10 out of 30, respectively.

Table 1. Clinicopathologic characteristics of thirty NSCLC patients.

Variable	Value (n = 30)
Gender	
Male	22 (73%)
Female	8 (27%)
Smoking history	
Never-smoker	22 (73%)
Current smoker	8 (27%)
Cell type	
Adenocarcinoma	17 (57%)
Squamous cell carcinoma	13 (43%)
Stage	
I	9 (30%)
II	11 (36.66%)
III	10 (33.34%)

2.2 RNA extraction and cDNA preparation

Total RNA was isolated from each tumor tissue and adjacent non-tumor tissue by using RiboEx (GeneAll, Korea) according to the manufacturer's specifications. The concentration of total RNA in the final eluate was determined by spectrophotometry and the absorbance 260/280 ratio was controlled between 1.8 and 2.0. The synthesis of cDNA (240 ng of total RNA per 20 μ L reaction mixture) was performed using the Prime Script RT reagent

kit (Perfect Real Time) RR037A (Takara, Japan) according to the manufacturer's specifications. The obtained cDNAs were stored in -80°C until use.

2.3 Real-time quantitative PCR

Real-time PCR was performed using an StepOnePlus™ Real-Time PCR Systems (ABI Applied Bio-systems, Thermo Fisher Scientific, USA) in a 15- μ L reaction containing 7.5- μ L of RealQ Plus 2x Master Mix Green High

ROX™ (Ampliqon, Denmark), 1-μl of cDNA, 5.5-μl of H₂O and 1-μl of mixed forward and reverse primers (3 Pmol/μl concentration). Real-time PCR amplifications were done as follows: for three selected genes, PCR amplification was set to an initial 95°C for 15 min and then for TGFβ2, PCDH9, and KLF11 genes, a total of 40 cycles, 95°C for 15

seconds and 58°C for 1 min (step and hold). All samples were analyzed in duplicate. GAPDH was used as an internal control. Gene expression was calculated using the comparative threshold cycle ($2^{-\Delta\Delta CT}$) method. The primers used for real-time PCR are listed in Table 2.

Table 2. Primer sequences of three selected genes and GAPDH.

Target genes		Sequences (5'→ 3')	Product length
TGF-β2	Forward	TGACCCACATCTCCTGCAAT	135bp
	Reverse	GTGGACGTAGGCAGCAATTATC	
PCDH9	Forward	AACAGATCCTGACACAGGCTT	75bp
	Reverse	TCCAGTCCAAAAACACTCTGC	
KLF11	Forward	GCATGACAGCGAAAGGTCTAC	128bp
	Reverse	GGGGTCTTATCCGCAACAGG	
GAPDH	Forward	CATCAAGAAGGTGGTGAAGCA	120bp
	Reverse	GCGTCAAAGGTGGAGGAGTG	

2.4 Statistical Analysis

Statistical analysis was performed using the GraphPad Prism v7.03 (GraphPad Software Inc., USA) and T-test. For all tests, a *P* value <0.05 was considered statistically significant.

III. 3. RESULTS

3.1 Results obtained of KLF11 gene expression by used RT-PCR method

Investigating the expression of KLF11 gene in 30 NSCLC tissue samples and 30 adjacent normal tissue samples in this study showed that 11 out of 30 (36.6%) cases of tumor samples overexpressed in comparison with normal samples, while 19 out of 30 (63.4%) cases showed a decrease in expression levels. Therefore, there was no significant difference in expression level of this gene in the samples (*P*=0.15). (Fig. 1 and 4a show the results of the KLF11 gene).

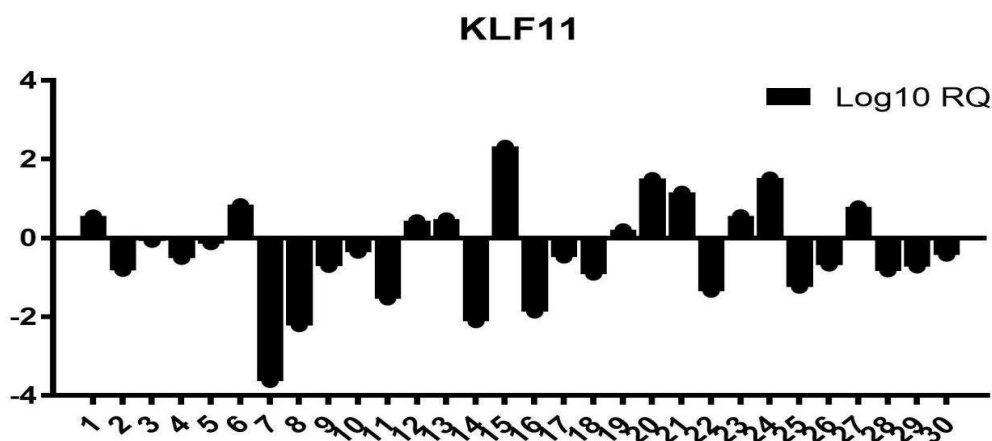


Fig. 1. The graph related to KLF11 gene expression

3.2 Results obtained of PCDH9 gene expression by used RT-PCR method

In this study, the expression of PCDH9 gene was investigated in 30 NSCLC tissue samples and 30 adjacent

normal tissue samples. The results of PCDH9 expression indicated that only 12 out of 30 (40%) cases of tumor samples overexpressed in comparison with normal samples and the rest of the samples (60%) were reduced. So analysis

on results did not show significant difference between NSCLC tissue samples and adjacent normal tissue samples

($P=0.44$). (Fig. 2 and 4b show the results of the PCDH9 gene).

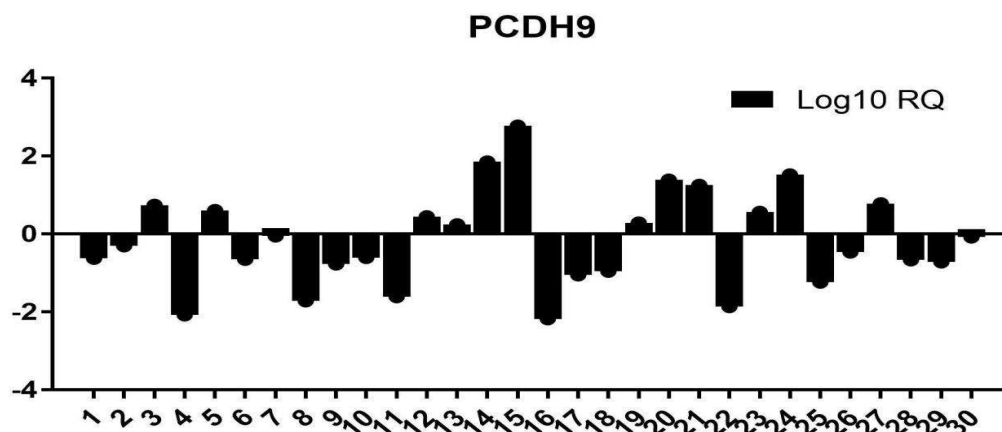


Fig. 2. The graph related to PCDH9 gene expression

3.3 Results obtained of TGF- β 2 gene expression by used RT-PCR method

The expression of TGF- β 2 gene was checked in 30 NSCLC tissue samples and 30 adjacent normal tissue samples. Consequence of expression this gene demonstrated

that 18 out of 30 (60%) cases of tumor samples were reduced compared to normal samples and 12 out of 30 (40%) other cases overexpressed. So that there were no significant difference expression levels of tumor samples comparison with normal samples ($P=0.78$). (Fig. 3 and 4c show the results of the TGF- β 2 gene).

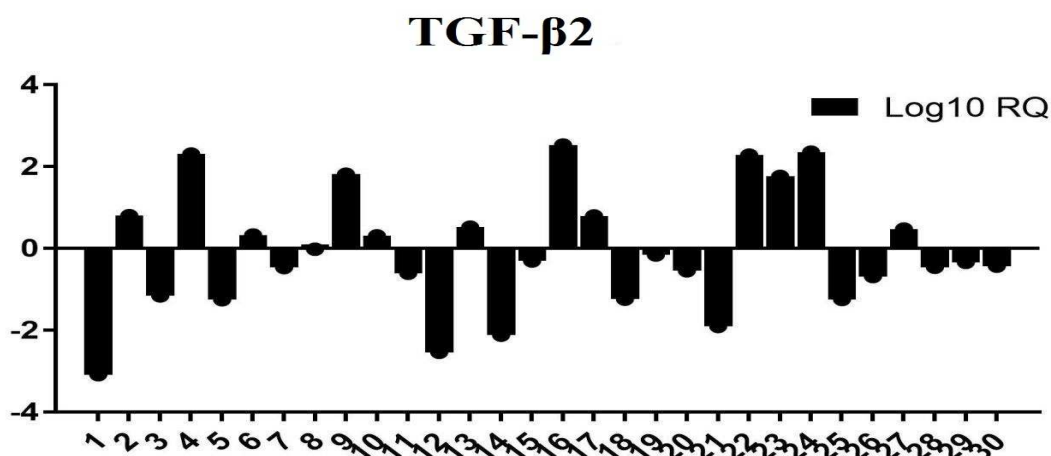


Fig. 3. The graph related to TGF- β 2 gene expression

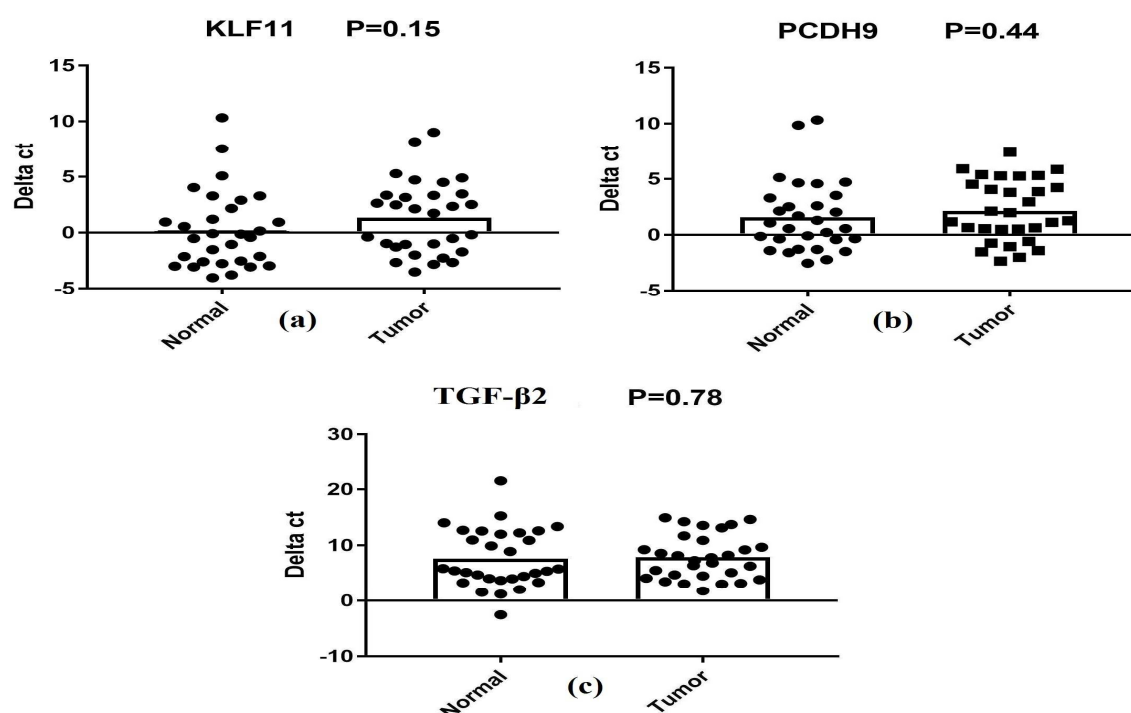


Fig. 4. The graph (a) related to Comparison of KLF11 gene expression between tumor samples and normal samples (b) related to Comparison of PCDH9 gene expression between tumor samples and normal samples and (c) related to Comparison of TGF- β 2 gene expression between tumor samples and normal samples.

IV. DISCUSSION

Better recognition of gene expression patterns and molecular mechanisms which involved in non-small cell lung cancer, can lead to early detection of cancer and increase the 5-years survival rate for patients. For this purpose, we have analyzed the expression level of TGF- β 2, KLF11 and PCDH9 genes in thirty NSCLC patients by real-time quantitative PCR.

Earlier research on these genes determines different results in various cancers. Hachim et al, by studying on the expression of three TGF- β isoforms in breast cancer tissues demonstrated that TGF- β 1 and TGF- β 3, but not TGF- β 2, expression levels were increased in breast tumors compared to normal breast tissues [19]. In another study, it was found that the expression of this gene was significantly increased in the serum of patients with gastric cancer [20]. As mentioned, TGF- β 2 gene performs various activities in natural tissues such as proliferation, differentiation, apoptosis, and regeneration of the extracellular matrix [17]. According to the roles that TGF- β 2 gene plays in many processes, it's clear that dysregulation of this gene occurs in NSCLC tumors, but in this study, only 40% of samples were

overexpressed for TGF- β 2 gene, and expression in 60% of samples have been reduced.

Aberrant expression of the KLF11 gene has been reported in several human cancers. Wang et al. reported that the expression of KLF11 was significantly reduced in the tumor tissues compared to the normal ovarian tissues [11]. This gene was also inactivated in myelodysplastic syndrome [21]. KLF11 along with TGF- β 2 involved in TGF- β signaling pathway and They accomplish a variety of activities including proliferation, differentiation, and apoptosis [22]. We expected that these genes dysregulated in NSCLC tumors and disturb the TGF-beta signaling pathway. However, in this research, reduce expression of KLF11 gene was observed only in 63.33% of NSCLC patients.

Another gene that was investigated in this study was PCDH9, and it has already been studied in various cancers. The expression of PCDH9 decreases in Hepatocellular carcinoma and primary gliomas [12, 23]. Loss of PCDH9 gene is associated with the differentiation of tumor cells, metastasis and predicts poor survival in gastric cancer [24]. In this study, we didn't see any significant difference in

expression level of PCDH9 genes ($p=0.44$). PCDH9 is thought to function in cell adhesion and the reduced expression of this gene causes metastasis and this process occurs in the advanced stages of the disease, but samples in this research are often at the early stages of NSCLC. Moreover, Nooshin et al. showed the methylation level of the PCDH9 gene didn't change in early stages of NSCLC tumors [25].

In summary, we didn't see any significant difference in expression level between tumor and adjacent normal lung tissues for these three selected genes. However, this study was designed as a pilot study, and further investigations are required to confirm our findings.

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