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Study of SOX2, OCT4 and NANOG Genes Expression in Peripheral Blood of Patients with Non-small Cell Lung Cancer (NSCLC)

Niloofar Mojtahedifard[1], Shohre Zare Karizi[2*] , Morteza Karimi pour[2]

[1]Islamic Azad University Varamin Pishva Branch, Tehran, Iran
[2]Department of medical genetics, Islamic Azad University Varamin Pishva Branch, Varamin, Tehran, Iran

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ABSTRACT

Introduction: lung cancer is a disease that is characterized by an uncontrolled growth of the cell in the lung tissue. This cancer is the most common cause of cancer death worldwide. The SOX2, OCT4 and NANOG transcription factor are the main regulators of maintaining vitality state and self-renewal in embryonic stem cells. The aim of this study was to evaluate the genes expression of SOX2 in peripheral blood of patients with non-small cell lung cancer (NSCLC). Material and Methods: in this research, the expression of SOX2, OCT4 and NANOG transcription factors were investigated in peripheral blood of 30 patients with NSCLC. For this purpose, after RNA extraction from patients' blood by TRIzol then cDNA synthesis, the expression of the genes was analyzed by Real Time PCR technique and using specific primers for the genes. Results: after reviewing the results by Graph Pad software and t-test, SOX2, OCT4 and NANOG expression in patient samples was significantly higher than normal samples. Discussion: the expression of the genes studied in this research in the patients with lung cancer was significantly higher than normal. Regarding the gene expression profile, it seems that the gene is a potential biomarker for the diagnosis of lung cancer.

Keywords: NANOG, OCT4, SOX2, NSCLC, Non-small Cell Lung Cancer

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Corresponding author: Shohre Zare Karizi
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INTRODUCTION

There are many types of cancers in the human body. Lung cancer is a type of cancer that is created the tumor in the lung tissue. Most cancers that start with the lungs are carcinomas that originate from the lung lining tissue. In 2012, around 1.6 million people died from the cancer in worldwide. Most mortality from this cancer occurs in undeveloped countries [1]. Lung carcinomas are classified on basis of the size and characteristics of the malignant cells. In general, lung cancer is divided into two groups of small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [2]. NSCLC includes approximately 80% of lung cancers [3].

Several new reports have revealed the association of powerful factors with a variety of malignancies, including lung, breast, intestinal and esophageal cancer [4]. The main genes in preserving the ulcerative state are the genes of the transcriptional factors that control the expression of other genes. SOX2, OCT4 and NANOG are the most important transcription factors in sustaining powerful mode [5, 6]. A member of the family is the SOX transcription factor. The coding gene of this factor has no intron and is located in cytogenetic position 3q26.33 [7]. For the first time, the NANOG gene was identified as a gene that was expressed in previous embryonic implantation stage, also in pluripotent cells in the culture medium [8]. The NANOG gene is located in the cytogenetic position of 12p13.31 [9]. OCT4 is a major member of the family of Pou transcription factors, which is essential for the survival and self-

renewal of embryonic stem cells. The transcription factors of the Pou family cause to activate the expression of their target genes expression by binding to the AGTCAAAT octame sequence in DNA [10]. Human OCT4 has 5 exons. The cytogenetic position is 6p21.31 and in the complex of tissue adaptation. This gene has OCT4B and OCT4A isoforms [11, 12]. Widespread researches on lung cancer markers, it hasn't found still possibility in using a single marker that can be used clinically for early detection, recurrence and metastasis, determining prognosis, or response to treatment, and be acceptable for its sensitivity and specificity.

SOX2 expression factor profile to maintain the self-renewal state of cancer stem cell is similar to embryonic stem cells expression profile. Many studies have shown the potential association of these transcription factors with various cancers, including lung cancer. Since lung cancer is the leading cause of cancer deaths in the world, the identification of the molecular basis of this disease helps to diagnose and treat the disease. In this research, the SOX2 gene expression pattern has been studied considering their potential role in lung cancer. In this study, the pattern of expression of NANOG, SOX2 and OCT4 genes has been investigated considering their potential role in lung cancer, and whether it is possible to use these three factors as a biomarker to detect lung cancer.

MATERIALS AND METHODS

For evaluating the expression of SOX2, NANOG and OCT4 genes from 30 patients with lung cancer, a NSCLC blood sample was taken. Patients entered to the study after being diagnosed with lung cancer by pathologists and with their complete satisfaction. To prevent RNA degradation was poured in tubes containing RNA stabilizing agents then were stored in a freezer with -70°C.

After RNA extraction, spectrophotometric method is used to evaluate the quantity and quality of extracted RNA. In this method, the optical absorption rate is measured by the RNA bases. The existing bases in the structure of the nucleic acids in the wavelength of 260 nm have the highest absorbance for the UV light, so the absorption of RNA samples is called at the wavelength. Most of the extracted samples had absorption higher than 1000 ng/µl. It is suitable for other molecular analyzes such as Real Time PCR.

RNA treatment with DNase enzyme was performed according to the Fermentas Company's instructions and then cDNA was synthesized. Gene Runner software was used to design the primers used in this research.

In order to achieve the proper temperature of the specific primers function, the PCR reaction was performed at three temperatures of 60, 62 and 64. To carry out the Real-Time PCR reaction, its program was pre-designed for the device. This reaction was performed on plates or ABI strips. In this research, GAPDH gene was used as internal control.

Real-time PCR products are run on the gel to confirm the proper reproduction of target genes. In this study, agarose and acrylamide gels were used to confirm the final products.

Statistical analysis of the results obtained in this study was also done by Graph Pad software. In all statistical analyzes, P-value <0.05 was considered as a significant level of data.

RESULTS

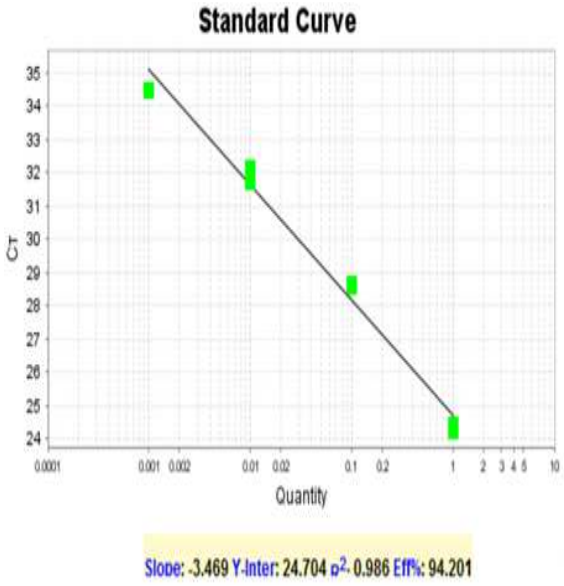

Figure 1: The standard curve related to the proliferation of SOX2 gene.

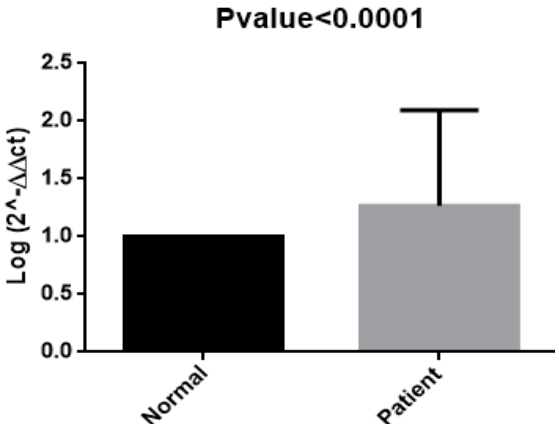

Figure 2: The expression level of SOX2 gene in normal and patient subjects

The results for SOX2 gene expression by Real Time PCR technique

At first, the PCR efficiency was calculated by different dilutions of the cDNA. Efficiency of 94% and slope of -3.469 indicates the suitability of PCR for performing the next steps of PCR.

The expression of SOX2 gene in normal and patient people

Expression level of SOX2 gene in peripheral blood of NSCLC and healthy subjects was investigated as control by Real Time PCR. The graph pad software was used to analyze the data.

As shown in Figure 2, the expression of SOX2 in patients with lung cancer is significantly higher than normal. Due to the fact that the dispersion of the expression increase was different among different samples, in order to draw a chart of logarithmic variations were taken on the basis of 10. After analyzing the results, using Graph pad software and t-test, the value of P-value <0.0001 was obtained, which is low with a confidence level of 5%. This value of P-value indicates the high expression of SOX2 in patients.

The results for NANOG gene expression by Real Time PCR technique

First, PCR efficiency for the NANOG gene was calculated by making 1 to 5 cDNA dilutions. 98% efficiency and 3.351 slope indicates the suitability of PCR performance for further steps (Fig. 3).

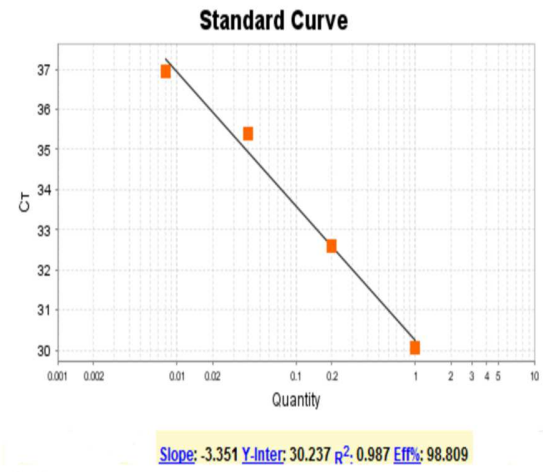

Figure 3: The standard curve for the proliferation of the NANOG gene

NANOG expression in patients and normal subjects

Real-time PCR technique was used to test the expression of NANOG gene in normal and patient subjects. The results were also evaluated by the Graph Pad Software.

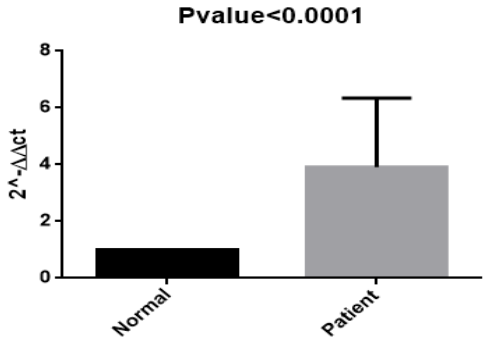

Figure 4: NANOG expression in normal and patient subjects

As shown in Fig. 4, NANOG expression level in NSCLC subjects is higher than normal subjects. Analysis of the results of Real Time PCR showed that NANOG has increased significantly in patients compared with normal people. CtΔΔ was used to test the difference between normal and patient. Statistical analysis was performed by Graph Pad software using t-test. In analyzing the statistical data in this study, P-value <0.05 was considered as a significant level. P-value <0.0001 in this

experiment shows a significant level of the obtained data. Figure 4-7 shows the expression of the NANOG gene expression in the examined patient samples compared to normal. As shown in the figure, the expression of the NANOG gene in patients has increased compared to normal.

Results of OCT4 gene expression analysis by Real Time PCR

First, PCR efficiency for the OCT4 gene was calculated by making 1 to 5 cDNA dilutions. 106% efficiency and -3.183 slope indicates the suitability of PCR performance for further steps.

OCT4 gene expression in patients and normal subjects

The OCT4 expression level in NSCLC patient samples and healthy subjects were done as control by real time PCR techniques.

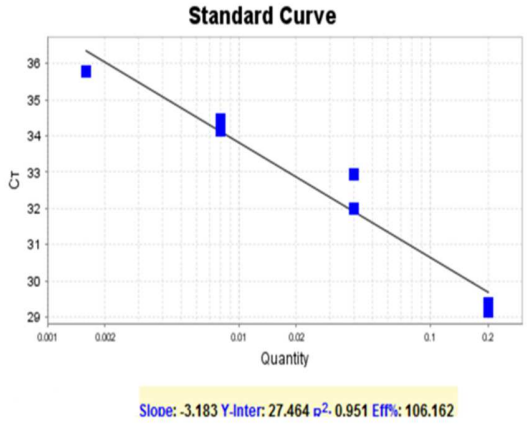

Figure 5: Figure 3: The standard curve for the proliferation of the OCT4 gene

As shown in Figure 6, the OCT4 expression level in patients is increased compared with normal individuals. Analysis of the results obtained by Real Time PCR showed that OCT4 in patients compared with normal people showed a significant increase. The statistical analysis of the data was also done by Graph Pad software using t-test. The significance level was considered to be 5% and P-value <0.0001 in this test. This lower value of 5% confidence level indicates the significance level of the data. Changes in OCT4 expression in patients compared to normal are presented in Fig. 6.

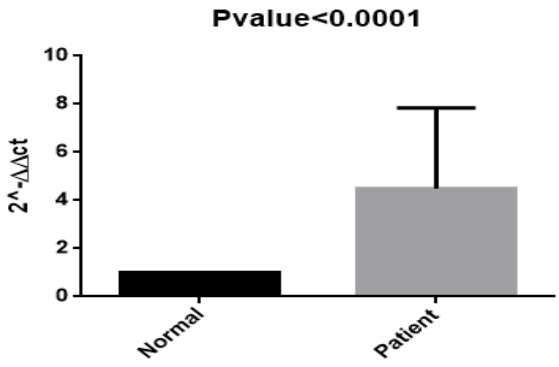

Figure 6: OCT4 expression level in normal and patient subjects

DISCUSSION AND CONCLUSION

Among all types of cancers, lung cancer is one of the deadliest cancers, and is the leading cause of cancer deaths [13]. Most lung cancers are diagnosed in advanced stages of the disease. For this reason, there is no chance of surgical treatment [14]. Many efforts are being made to find a suitable molecular bio marker to detect and anticipate lung cancer. Factors such as SOX2, OCT4, and NANOG are suggested to identify and confirm lung stem cells [15]. The NANOG gene was first identified as a gene is present in the previous embryo implantation and also in powerful cells in the culture medium [16]. NANOG regulates the power of the stem cells and the cell fate through its interaction with the transcription factors SOX2 and OCT4 [17]. Chio *et al*., showed that increasing the simultaneous expression of both OCT4 and NANOG genes causes the appearance of cancerous stem cells in lung adenocarcinoma cells [18]. Jeter *et al*., examined the potential tumorous function of NANOG in prostate cancer. The results showed that NANOG induction induced drug resistance in MCF-7 breast cancer cell line and tumor recurrence in Du145 prostate cancer cell line [17]. In the first NANOG-related and gastric cancer study, it was shown that NANOG1 and the false gene of NANOGP8 have a higher expression in the primary gastric tumors compared to the marginal epithelium. Subsequently in the recent years, OCT4 has been recognized as a major regulator in inducing and maintaining cellular proliferation with vital roles in the primary stages of differentiation [19]. In a trial conducted by Ralf et al., the expression of the OCT4 powerful factor was studied in tumor samples of lung adenocarcinoma and it was observed that this

gene has a high expression in several tumor samples. Identifying the ulcerative factor expression in several tumor samples indicates the role of this gene in tumorigenic activity [20]. While there has been an increase in the expression of OCT4 in several cancer cell and tumor cell lines, there is little information about the expression and performance of different types of OCT4 species in cancer stem cells [21]. In a study by RDE Maria *et al*, it was concluded that there is a small subset of undifferentiated cells in NSCLC and SCLC tumor cells that exhibit high expression of proliferative factors such as NANOG and OCT4, and again the genes role have been confirmed in tumorigenicity [22]. Increasing SOX2 expression has been observed in all types of lung cancer. SOX2 proliferation was higher than NSCLC in SCLC type lung cancer [23]. Rudin et al showed SOX2 proliferation in 27% of SCLC specimens. In addition, the level of SOX2 expression in NSCLC is significantly higher than lung normal samples and other tumors. Increasing the expression of SOX2 as well as the proliferation of SOX2 is a predictive factor for lung cancer [23].

SOX2 is associated with invasive behavior of the lung tumors. In the present study, the level of SOX2 expression was evaluated by immunohistochemistry. Expression of this protein was detected in 50% of NSCLC tumor samples. The increasing was more detected in older and male subjects, and did not correlate with the degree and stage of the tumor [24]. Other studies have shown that proliferation and following increased expression of SOX2 protein is one of the most important mechanisms for the initiation and progression of lung cancer tumors type SCC. The frequency of proliferation of SOX2 in lung SCC is reported 20% to 60% (23) [25]. The studies have reported that powerful factors of OCT4, SOX2, and NANOG are potentially diagnostic markers for lung cancer. In a study by Eva Sodja et al, The expression of SOX2, OCT4 and NANOG genes in peripheral blood of patients with SCLC was investigated. The analysis of the results showed that the level of SOX2 expression in the peripheral blood of SCLC patients was significantly higher than the expression of this gene in normal individuals. There was a significant correlation between the level of expression of mRNA in SOX2 gene and the number of metastatic sites. There was no significant difference in expression of OCT4 and NANOG between normal individuals and those with cancer [26]. In this study, the expression of OCT4, NANOG and SOX2 in peripheral blood of patients with NSCLC was investigated. Analysis of the results showed that the expression of OCT4, NANOG and SOX2 was significantly higher in the peripheral blood than the normal subjects. According to the increased expression of genes in lung cancer, it seems that these genes have a tumorigenic role in the development of lung cancer. By considering the expression of these genes in lung cancer, these genes can be used as suitable biomarkers for the diagnosis of lung cancer. In order to obtain more accurate results, it is suggested that this research be done at a wider level and with more samples.

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