Original Article

The Expression Profile Evaluation of Mir-125b in Tuberculosis and Non-Small Cell Lung Cancer Patients

Abstract

Background and Aim: Worldwide, lung cancer, specifically nonsmall-cell lung cancer (NSCLC), is one of the most preventable and common cancer types. Tuberculosis (TB) is a major global public health threat, and the most important infectious disease throughout history in the world. Having TB before lung cancer significantly correlates with mortality due to lung cancer. TB and lung cancer have similar symptoms, and TB has symptoms identical to malignancy, so sometimes its diagnosis is confused with lung cancer. The miRNAs are biological molecules that play critical regulatory roles in the physiological and pathological processes. In several research types, many miRNAs have been studied, which showed the alternation of expression in TB and NSCLC separately. In this research paper, the expression of mir-125b was examined, which is commonly altered in TB and NSCLC. **Materials and Methods:** Thirty patients with NSCLC, thirty patients with TB who were new cases, and thirty healthy individuals were contributed to this research. The expression pattern of mir-125b was evaluated and compared in TB and NSCLC patients with healthy controls, using the real-time polymerase chain reaction method. **Results:** As a result, the expression of mir-125b was lower in TB and NSCLC patients than in healthy controls. **Conclusion:** Therefore, mir-125b can be used as a prognostic biomarker in TB and NSCLC diseases. The expression pattern of mir-125b could be useful to follow-up on the biological pathways that have essential roles in these diseases.

Keywords: *Expression profile, mir-125b, nonsmall-cell lung cancer, tuberculosis*

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Introduction

Lung cancer is the second most common cancer among men and is one of the most preventable types of cancer. The types of lung cancer are classified based on the appearance of the cells under the microscope. Nonsmall-cell lung cancer (NSCLC) is also divided into three categories: (1) superficial tissue cancer, (2) mucosal epithelial gland cancer (glandular epithelium), and (3) large-cell lung cancer. Among those with cancer, about 85% are NSCLC, and about 15% are SCLC.[1] Worldwide, lung cancer is the most common cancer in prevalence and mortality in Europe and North America. About 85% of lung cancer cases are associated with smoking. About 40% of lung cancers are "adenocarcinomas," which usually form in the lateral lung tissue. Sclerotic cell carcinoma accounts for about 30% of all lung cancers, and about 9% are "large-cell carcinoma.[2]

Tuberculosis (TB) disease is a major global public health threat[3] and is one of the most important infectious diseases throughout history in the world. Various communities are continually trying to control and combat this disease. One-third of the world population is latently infected with *Mycobacterium tuberculosis*, a notorious intracellular pathogen, with around 1.6 million deaths recorded in 2017.

MicroRNAs are small evolutionarily conserved nucleotide sequences that regulate gene expression after transcription, by mRNA degradation or inhibition of translation.[4] MicroRNAs are involved in many transformative biological processes such as proliferation, development, metabolism, apoptosis,[5] stem cell differentiation, and disease development.[6,7] The aberrant miRNAs expression is also involved in many human diseases and disorders including cancer,[8,9] diabetes, kidney diseases, neurodegenerative diseases, liver diseases, heart diseases, and altered immune system function.[10,11]

Mir-125b is one of the miRNAs which shows expression changes in TB and NSCLC. Some researchers have shown

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that the expression of mir-125b is decreased TB and NSCLC.[12,13] The results of other studies showed that the function of mir-125b in NSCLC is in the apoptosis pathway,[14] proliferation, invasion, and migration of NSCLC cells.[15,16] In addition, TB affects the nature of the immune response.[13] The research results have shown that mir-125b can be used as a prognostic biomarker in NSCLC and TB.[17] Since the results of previous studies on the blood and tissue samples in NSCLC patients have shown different results in terms of an alteration of miR125b expression profile in different stages of the disease, in this research study, the expression pattern of miR125b in plasma samples of new-case patients was been examined.

Materials and Methods

This research has been approved by the research committee of Biological Science School, Varamin-Pishva Branch, Islamic Azad University, and confirmed in the Ethics Committee of Pasteur Institute of Iran. Furthermore, a written informed consent form was obtained from participants.

The study population included thirty healthy controls, thirty TB new case patients, and 30 new NSCLC patients. All of the participants were between the ages of 25 and 55 years, before treatment and without any history of surgery. The characteristics of the patients of the present study are summarized in Table 1.

After sampling peripheral blood from the patient's cubital vein, the samples were kept in the tubes containing an anticoagulant (ethylenediaminetetraacetic acid). Then, the samples were centrifuged with a speed of 7000 g for 10 min to separate the plasma. *Total RNA was then isolated* from plasma by the RNA blood kit (Roche, Germany). The quality of RNA was determined using the NanoDrop (Thermo scientific).

The cDNA was synthesized by Transcriptor First Strand cDNA Synthesis Kit (Roche, No. 04 379 012 001, Germany). To transcription the miRNAs into the first-strand cDNA, the stem-loop primer was used. The stem-loop primer binds to the 3′ end of the miRNA and lengths the cDNA,[18] as shown in Figure 1.

The expression pattern of mir-16 as a reference gene and mir-125b was analyzed in TB patients, NSCLC patients and healthy controls by real-time polymerase chain reaction (PCR) in Rotor-Gene 6000 (Corbett Research, Australia). The LightCycler FastStart DNA Master PLUS Kit SYBR Green I (Roche, Germany) was used for real-time PCR. Primer sequences were designed by the primer express software to exclude amplification of mir-125b and the reference gene mir-16 [Table 2].

Statistical analysis

Data were analyzed by SPSS (version 20) and Graph Pad Prism 6 Software (San Diego, California, USA). Therefore, the fold change and *P* value were calculated to determine the expression changes of mir-125b in TB, NSCLC Patients

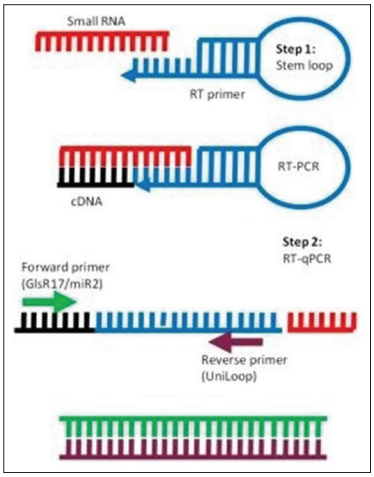

Figure 1: Stem-loop quantitative reverse transcription reverse transcription-quantitative polymerase chain reaction design to identify miRNAs[19]

Table 1: Demographical information of study participants with tuberculosis, non-small cell lung cancer, and normal control

Patients group	Age (years)	Hepatitis (%)	HIV (%)	Family history of TB (%)	Family history of NSCLC (%)	NonIranian (%)	Smoking (%)	Sex (male/female) (%)
TB	25-55	13	15	30	25	35	35	47/53
NSCLC	25-55	5	5	15	10	10	70	95/5

TB: Tuberculosis, NSCLC: Nonsmall cell lung cancer

Table 2: The 5' → 3 sequences of micro RNAs specific primers

miRNA name	Sequence (5' → 3')
miRNA-16 forward	CCGGAGTAGCAGCACGTAAAT
miRNA-16 reverse	ATCCAGTGCAGGGTCCGA
miRNA-16 stem lope	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGCCAA
miRNA-125b forward	CCTAGATTCCCTGAGACCCT
miRNA-125b reverse	ATCCAGTGCAGGGTCCGA
miRNA-125b stem lope	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCACAA

compared to healthy controls. Analysis of variance test was used for comparison of patients groups.

Results

The statistical analysis was performed to calculate the expression pattern of mir-125b in NSCLC (n = 30), TB (n = 30), and normal (n = 30) subjects. According to Figure 2, the expression levels of plasma miR-125b were significantly different in TB and NSCLC patients than in healthy controls.

Statistical analysis using SPSS software (Version 20) (San Diego-California,USA) showed that the expression level of miR-125b in the patients with NSCLC was significantly lower than that in the control subjects (P < 0.0001). Furthermore, miR-125b showed significantly lower expression in patients with TB than in the control individuals (P < 0.0001). The results also showed that the expression changes of mir-125 were significantly similar in TB and NSCLC diseases, which showed that miR-125b had considerable prognostic power to the screening of NSCLC and TB.

Discussion

Cancer is one of the major health problems and the leading cause of death in developed countries and accounts for a high percentage of deaths worldwide.[20] Exclusively, lung cancer is the most common cause of cancer-related deaths among men and women.[21] TB is one of the most important infectious diseases of the century that can affect all body organs, but the lungs are more likely to develop TB.[22] MiRNAs are involved in various physiological and developmental processes such as insulin secretion, apoptosis, hematopoiesis, brain morphogenesis, or tissue differentiation. They are also involved in the immune system and viral diseases.[23-25] Several types of research have been done to investigate the expression profile pattern of different miRNAs in lung cancer and TB.[26] In a study conducted in 2016, the diagnostic value of 29 microRNAs for the diagnosis of TB in children was investigated. Fifteen cases of microRNAs were overexpressed, and 14 cases of microRNAs were downregulated. MiR-1, miR-155, miR 31, miR 146a, miR 10a, miR 125b, and miR 150 have decreased expression and miR 29 has increased expression.[27] The present study results also show that miR-125 has a reduced expression in TB patients, which is in agreement with this study.

By reviewing the KEGG database, we found that the microRNAs have a critical regulatory pathway in lung cancer cancers. Decreasing and increasing the expression of each can have a significant impact on tumorigenesis, angiogenesis, and metastasis. That way, as shown in Figure 3, the downregulation of miR-125b leads to metastasis.

On the other hand, other studies on lung cancer and TB have also been investigated in other miRNAs and reported as diagnostic biomarkers. For example, in 2016, a study by Zhu *et al*. On altered miRNA expression of miR-182, miR-183, and miR-210, miR-126, in smokers, pneumonia

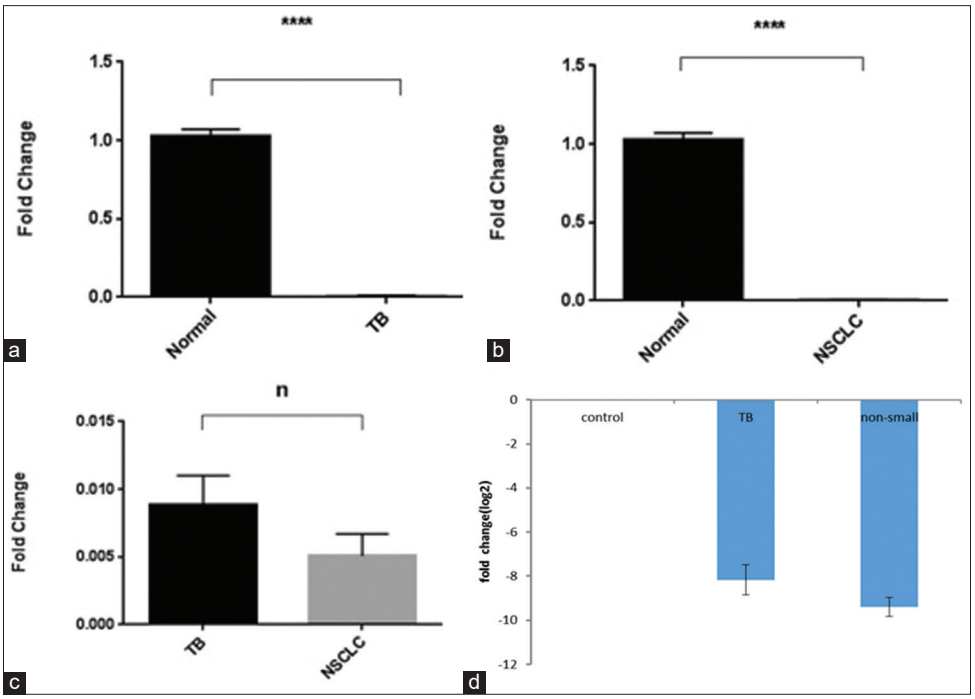

Figure 2: MiRNA-125 expression patterns in both tuberculosis and nonsmall-cell lung cancer patients are significantly lower than normal individual (a) tuberculosis and normal, (b) nonsmall-cell lung cancer and normal (c) tuberculosis and nonsmall-cell lung cancer, and (d) tuberculosis and nonsmall-cell lung cancer in comparison with normal individuals

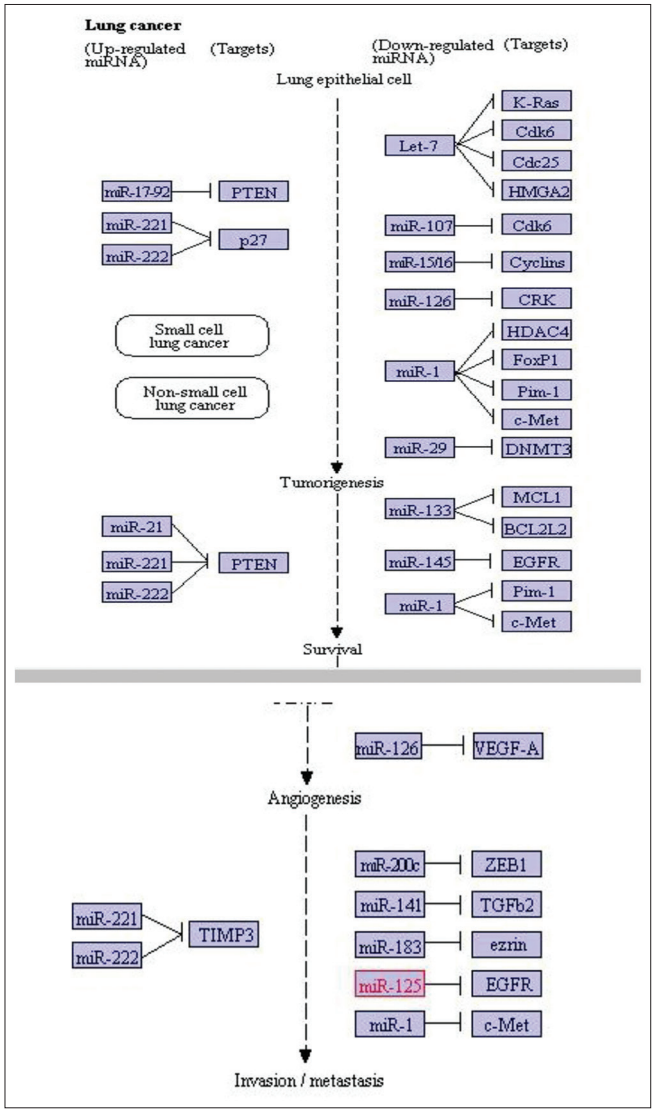

Figure 3: The data obtained from Kyoto Encyclopedia of Genes and Genomes from the roles of Micro RNAs in lung cancer

patients, people with NSCLC, people with gastric cancer, and normal individuals were studied, and the results of their research showed that these four miRNAs could be used as a diagnostic biomarker for lung cancer.[28] However, some researchers reported that the mir 125 b has a high expression level in NSCLC tissues. The results of a study by Wang *et al*. in 2017 on the expression of MiR-125b in NSCLC showed that miR-125b expression in NSCLC tissue was higher than that in paracarcinoma tissue.[29]

In comparison, our study was done on plasma sample sources of TB and NSCLC patients. Besides, Yuxia *et al*. analyzed serum levels of miR-125b in 193 patients with different stages of NSCLC in 2012. They found that serum miR-125b was consistently expressed in the nontumor group and was significantly associated with the NSCLC stage, and also patients with high miR-125b expression displayed a substantially poorer prognosis compared with patients with low expression ($P < 0.0001$).[17]

Studies have shown that it has been found that markers for lung cancer and TB have been found so far. In the present study, miR-125b is described as a prognostic even may be diagnostic biomarker in lung cancer and TB, which must be checked in a large number of patients in larger study groups. Furthermore, the expression profile if mir 125 b should be evaluated at different stages of these diseases in both the blood and tissue samples. Be more accurately assessed to have a diagnostic value. On the other hand, several studies have shown that TB and lung cancer overlap in many cases and TB is a precursor to lung cancer. Hence, mir 125 b could be used for screening of TB and NSCLC patients. It has been often said that TB can be a risk factor for lung cancer. People with TB usually have drug resistance and in many cases, develop lung cancer, so this biomarker can help follow-up on the TB patient to prevent getting lung cancer or lung cancer patient from the risk of metastasis.

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Conflicts of interest

There are no conflicts of interest.

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