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Bioinformatics Study and Experimental Evaluation of miR-182, and miR-34 Expression Profiles in Tuberculosis and Lung Cancer

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Abstract

Background: Lung cancer is one of the most dangerous cancers and tuberculosis is one of the deadliest infectious diseases in the world. Many studies have confirmed the connection between lung cancer and tuberculosis, and also the microRNAs (miRNAs) that play a major role in the development of these two diseases. This study aims to use different databases to find effective miRNAs and their role in different genes in lung and tuberculosis diseases. It also aims to determine the role of miR-34a and miR-182 in lung cancer and tuberculosis.

Methods: Using the Gene Expression Omnibus (GEO) database, the influential miRNA databases were studied in the two diseases. Finally, considering bioinformatics results and literature studies, two miR-34a and miR-182 were selected. The role of these miRNAs and their target genes was carefully evaluated using bioinformatics. The expression of miRNAs in the plasma of patients with lung cancer and tuberculosis and healthy individuals was investigated.

Results: According to the GEO database, miR-34a and miR-182 are miRNAs that affect tuberculosis and lung cancer. By checking the miRBase, miRcode, DIANA, miRDB, galaxy, Kyoto Encyclopedia of Genes and Genomes databases, the role of these miRNAs on genes and different molecular pathways and their effect on these miRNAs were mentioned. The results of the present study showed that the expression of miR-34a and miR-182 was lower than that of healthy people. The p-value for miR-182 was <0.0001 and for miR-34a was 0.3380.

Conclusion: Reducing the expression pattern of these miRNAs indicates their role in lung cancer and tuberculosis occurrence. Therefore, these miRNAs can be used as a biomarker for prognosis, diagnosis, and treatment methods.

Keywords: Lung Cancer; Tuberculosis; MicroRNAs; Expression Profiles; Bioinformatics

Introduction

Tuberculosis (TB) is one of the deadliest infectious diseases in the world. In 2019, Cohen et al.[1] stated that 1.4 million people in the world are at risk of TB. About 25% of cancer deaths are due to lung cancer, which leads to more deaths than other common cancers, including breast, prostate, and colorectal cancers. The onset and

progression of lung cancer result from the accumulation of genetic changes, including point mutations, deletions, translocations, or amplification, of dynamic epigenetic changes influenced by environmental factors[2].

TB may increase the risk of lung cancer through severe and long-term pulmonary inflammation, which leads to host tissue damage, fibrosis, scar formation, and genetic changes[3]. In general, it can be said that TB is a risk factors for lung cancer[4]. Radiological findings and clinical symptoms (such as persistent cough, hemoptysis, weight loss, fever, chest pain, shortness of breath, and loss of appetite) can be considered as similarities between TB and lung cancer[5]. Thus, the identification and development of a non-invasive or minimally invasive blood biomarker, methylated genes[6], and circulating DNA[7], such as tumor cells or microRNAs (miRNAs) could provide a new approach to the initial clinical diagnosis of non-small cell lung cancer (NSCLC).

miRNAs control several cellular processes, including inhibition of stability (mRNAs), control genes involved in cellular processes, and participate in the regulation of proliferation, differentiation, migration, and apoptosis[8]. Depending on the type of tumor, miRNAs in cancer can either suppress the tumor or oncogene or play only one role[9].

The nuclear factor κB (NF-κB) pathway is activated in all types of cancers by causing inflammation. Conjugation of ubiquitin (Ub) is involved in almost all signaling steps of NF-κB activation. For example, signaling mediators such as tumor necrosis factor (TNF) receptor–associated factors (TRAFs) and receptor interacting protein (RIP), are rapidly modified by K63-linked poly-Ub chains and lead to the activation of NF-κB by (TNF-α or interleukin 1β [IL-1β]) and transforming growth factor β (TGF-β)-activated kinase 1 (TAK1) and IκB kinase (IKK). The accumulation of K48-linked ubiquitination/degradation of inhibitor of nuclear factor kappa B (IκBα) leads to the activation of IKK complex, phosphorylates IκBs, nuclear transport and NF-κB activation. Ub deconjugation mediated by deubiquitinases including CYLD lysine 63 deubiquitinase (CYLD) and A20, TNIP1 and optineurin (OPTN) negatively controls NF-κB signaling. NF-κB seems to be an oncogenic mediator of tumors because NF-κB, which is suppressed by TGF-β in normal cells, controls TGF-β in cancer cells. miR-182 is induced by TGF-β and targets genes that act as negative regulators of NF-κB, leading to NF-κB activation in glioma[10].

MiR-182 is located at the 7q31-34 locus adjacent to *Met*, BRAF oncogene. miR-182 suppresses the *Met* gene and is known as a tumor suppressor gene in the lung. By inhibiting epithelial-mesenchymal transition (EMT) caused by hepatocyte growth factor (HGF), it inhibits lung cancer metastasis. Meanwhile, miR-182 shows a difference in the primary type of lung tumor and its metastasis. In the process of EMT, epithelial cells change their polarization capabilities to migratory and invasive capabilities. This process is accompanied by a decrease in intra-epithelial proteins such as E-cadherin (tumor suppressor genes) and zonula occludens-1 (ZO-1), and an increase in mesenchymal proteins fibronectin and N-cadherin. miR-182 increases the level of E-cadherin and decreases the level of Snail, which inhibits EMT and plays a role in metastasis. Lung cancer EMT and metastasis are suppressed by miR-182 by targeting HGF/Met/Snail signaling. HGF induces EMT. binds to its Met receptor and leads to increased HGF/Met in lung cancer. HGF initiates EMT by suppressing E-cadherin through EMT regulators such as Snail and Slug by activating HGF/Met signaling[11].

MiR-34a targets the 3' untranslated region (3'-UTR) of more than 700 genes involved, in normal survival and development. It stimulates some (differentiation, apoptosis) and inhibits others (cell cycle, stemness, or EMT). As a tumor suppressor, it suppresses processes related to cancer progression such as EMT associated with tumor invasion and metastasis by inhibiting transcription factors such as snail family transcriptional repressor 1 (SNAIL), zinc finger E-box binding homeobox (ZEB), and twist family BHLH transcription factor (TWIST), and suppresses inflammatory molecules such as TNF-α and IL-6 which are involved in chronic inflammation and cancer progression. MiR-34a is an important regulator and suppressor of programmed cell death ligand 1 (PD-L1), which regulates the immune response in cancer.

The process of regulating apoptosis is done by suppressing anti-apoptotic proteins by miR-34a, including B-cell lymphoma 2 (Bcl-2) and silent information regulator (SIRT1), which are direct targets of miR-34a. The increase of acetylation and, as a result, the activation of p53 transcription, which leads to p53-dependent apoptosis, is done by inhibiting SIRT1. miR-34a initiates apoptosis by reducing bcl-2, which is associated with the activation of pro-apoptotic proteins Bax and Bak. Cell proliferation, which leads cells from G1 to S and from G2 to M phase, is controlled by miR-34a and proteins such as cyclins and cyclin-dependent kinases. Cell cycle arrest in the G1 or G2 phase is mediated by miR-34a.

MiR-34a controls Wnt, Notch, TGF-β1/Smad signaling in EMT signaling pathways. In EMT, miR-34a sponges ceRNA. Inhibition and progression of cancer

with these methods are done in EMT signaling pathways with miR-34a. EMT regulatory molecules lead to transcriptional repression and upregulation of miR-34a expression. In this study, the expression profile of miRNAs, miR-182, and miR-34a, was investigated. In this research, a bioinformatics study was done on these two miRNAs and their related genes in miRPathDB, Gene Expression Omnibus (GEO), miRBase, miRcode, DIANA databases, miRDB, galaxy, and Kyoto Encyclopedia of Genes and Genomes (KEGG). After that, based on obtained results from bioinformatics analysis, the expression pattern of selected miRNAs in the blood plasma of TB and NSCLC patients, compared to healthy people, was evaluated[12].

Materials and Methods

1. Databases

In recent years, several bioinformatics tools have been developed to manage the micro RNA-related data. In this research firstly several bioinformatics tools (including mirBase, University of California Santa Cruz [UCSC], mirpathDB.2, galaxy, DIANA, miRcode, Ensembl, KEGG) were used for miRNAs data management. Then, regarding bioinformatics results and literature studies, miR-34a and miR-182 were selected to confirm their expression profiles in plasma samples of TB and lung cancer patients compared to normal individuals. The information obtained from each database and the link to connect to them is listed in Table 1.

2. Sampling

The samples of this research were carried out based on the research plan approved by the Department of Genetics, Faculty of Advanced Science and Technology, Tehran Islamic Azad University of Science and Research, the ethical code is IR.PII.REC.1400.016. Informed consent was obtained from all subjects involved in the study.

The blood samples of TB patients were taken from the Mycobacteriology and Pulmonary Research Department of the Pasteur Institute of Iran. Thirty samples of NSCLC patients were received from Dr. Masih Daneshvari Hospital and 30 blood samples from normal people were used as control group samples.

Inclusion criteria were the new cases of men and

Table 1. Databases that are related to microRNAs and their applications

Database	Link	Description
mirBase	https://www.mirbase.org/	According to the number of reads in each miR, miR activity is determined in part 5p or 3p.
UCSC	https://genome.ucsc.edu/	Was used for the effect and non-effect of microRNAs on the expression of the target genes.
mirpathDB.2	https://mpd.bioinf.uni-sb.de/	Finding target genes of microRNAs in autophagy and apoptosis pathway
galaxy	https://usegalaxy.org/	Finding long non-coding RNA that sponges the desired microRNAs
miRDB	http://mirdb.org/	Lists the number of genes that affect each miR
DIANA	http://diana.imis.athenainnovation.gr/DianaTools/index.php	Is an advanced web server and database tool for testing and analyzing microRNA function
miRcode	http://www.mircode.org/	Conservation, seed position, transcript region, of each miR is expressed.
Ensembl	https://asia.ensembl.org/index.html	Looking for different versions of long non-coding RNA and different types of splices on miR-34a and miR-182 necessary studies have been done and the results are given in the results section.
KEGG	https://www.genome.jp/kegg/pathway.html	Understanding the functions, high-level applications of the biological system including (cell, organism and ecosystem, from molecular level information, large-scale molecular data sets). It is produced and displayed by genome sequencing and other high-throughput experimental technologies.

UCSC: University of California Santa Cruz; KEGG: Kyoto Encyclopedia of Genes and Genomes.

women aged 25 to 55 years diagnosed with the disease. For TB, blood samples were taken from those who underwent the polymerase chain reaction (PCR) and sputum tests for final confirmation. Exclusion criteria included a history of anti-TB drugs, corticosteroids or other nonsteroidal anti-inflammatory, anti-cancer, or hepatitis drugs, and co-infection with HIV. Healthy individuals did not have any history of autoimmune, rheumatic, neuropsychiatric, endocrine or malignant diseases. A 5 ml volume of venous blood was collected from participants after overnight fasting using 0.05 M ethylenediaminetetraacetic acid (EDTA) tubes. In less than an hour, they were transferred to the laboratory while being in indirect contact with ice. Peripheral blood mononuclear cells (PBMCs) were isolated by the Ficoll-Hypaque gradients (Lymphoprep, Nycomed, Oslo, Norway). Plasma was separated by centrifugation at a speed of 7,000 ×g for 10 minutes from blood samples.

3. RNA extraction

RNA extraction from PBMC cells, was done by use of the kit (No. 11 828 665 001, Roche, Mannheim, Germany) and also using 400 μL Lysis/Binding Buffer. Lysis buffer was added to cells that were suspended in phosphate buffered saline. Then the mixture was poured into a filter tube and centrifuged. Dnase and Dnase I incubation buffer was added to the precipitate and kept at 15°C to 25°C for 15 minutes. Washing buffer solutions (I to III) were added in sequence and centrifuged again. Elution Buffer was added and centrifuged for the last time.

Purification and concentration of RNAs extracted were read by NanoDrop (Thermo Scientific, Waltham, MA, USA) for further evaluation. Purification with absorbance A260/280 and concentration of RNAs was determined in ng/μL.

4. cDNA synthesis and quantitative reverse transcription polymerase chain reaction

The reverse transcription-primer was used as a stable stem-loop structure that eliminates the need for enzyme proliferation that increases the target cDNA from ~22 main to 60 nt[13] ~22 to >60 nt, and stem-loops bind to the 3' ends of this miRNA to form a hairpin[14]. Dedicated forward primer and universal reverse primer were designed for miR-specific quantitative reverse transcription PCR (RT-qPCR). The sequence of (5'-3') primers of miRNAs is shown in Table 2. cDNA was synthesized by the transcriptor first strand cDNA synthesis kit (No. 04379012001, Roche) and with 1 to 5 μL RNA for a 20 μL reaction in a 0.5 mL sterile nuclease-free microtubule, thin-walled PCR tube. Stem-loop primer was used instead of oligo-dT primer and random hexamer primer. cDNAs were taken on gel %2 after extraction. Reverse transcriptase quantitative PCR analysis of miRNAs was performed with cyber Green qPCR Master Mix (Takara, Kusatsu, Japan) and miR-16 was considered as reference gene and internal control[15].

5. Data and statistical analysis

Obtained results from RT-qPCR expression change studies were analyzed using the t-test in GraphPad Prism software version 8 (GraphPad, San Diego, CA, USA). Fold change and p-value statistical analysis of blood miRNA expression in patients and normal individuals and relative expression for target genes were calculated using ΔΔCT (ΔΔCt formula $R=2^{-\Delta\Delta Ct}$) method.

Results

1. GEO

In the present study, miRNA microarray profiles that are common in TB and lung cancer and had differenc-

Table 2. The 5' → 3' sequence of microRNAs specific primers

miRNA name	Sequence (5' → 3)
miRNA-16 forward	CCGGAGTAGCAGCACGTAAAT
miRNA-16 revers	ATCCAGTGCAGGGTCCGA
miRNA-16 stem-loop	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGCCAA
miRNA-182 forward	CCTAGATAAACCGTTACCAT
miRNA-182 revers	ATCCAGTGCAGGGTCCGA
miRNA-182 stem-loop	GTCGT ATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCACAC
miRNA-34a forward	CCTAGATTGGCAGTGTCTTA
miRNA-34a revers	ATCCAGTGCAGGGTCCGAGTGCAGGGTCCGAGGT
miRNA-34a stem-loop	GTCGT ATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACAACC

es in expression in TB and lung cancer tissues were obtained from the GEO database. Articles related to lung cancer in blood samples named GSE76462, GSE25508, GSE102298, GSE84040, GSE25019, GSE76462, and articles related to TB in blood samples named GSE29190, GSE39163, GSE29190 were found in GEO. Based on logarithm folding change (logFC) and p-value, the necessary studies were performed among the found articles, and finally, we obtained a series of common miRNAs with logFC above 2 or less than −2 and with a p-value less than 5%. GEO, with GEO Series (GSE) number: GSE102298 from NSCLC and GSE39163 samples was found in TB samples in the peripheral blood of different patients with TB and lung cancer, and common miRNAs were placed in both samples of TB and lung cancer. Finally, the selected miRNAs were miRNAs (182, 34a) with a p-value of less than 0.05 and a logFC of 2 or less than −2 in the lung, TB, and normal tissue diseases reported in this study (Table 3). The result was a significant change in the expression of miRNAs.

2. miRBase

miRNAs were collected in miRBase (https://mirbase.org/), the main online repository of miRNA sequences and annotations. Considering the number of readings in the miRBase database per million, it was found that the readings in the miR-34a and 182 were higher in the 5p range. Knowing the activity of more miRNAs in the 3p or 5p region helps to design primers and continue research with other databases such as miRDB. Stem-loop (5'-3') region sequences and mature sequences from the database, miRpathDB: miRBase for miR (182-5p/34a-5p), and we obtained the seed sequence from miRpathDB for primer design are listed in Supplementary Table S1.

3. KEGG

KEGG maps pathways of molecular interaction, reaction, and relationship networks were manually drawn, including (metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, human diseases, and drug development). From the KEGG database, cancer pathway (https://www.genome.jp/kegg-bin/show_pathway?map05200), apoptosis occurring in NSCLC lung cancer (https://www.genome.jp/pathway/map05223+C15493), apoptosis in TB (https://www.genome.jp/pathway/hsa05152), autophagy (https://www.genome.jp/pathway/map04136+C00350), apoptosis (https://www.genome.jp/pathway/map04210), the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway (https://www.genome.jp/pathway/map04630), and the effect of these miRs on the genes of pathways related to apoptosis and autophagy in other databases were studied. Considering that the genes found are involved in the apoptosis and autophagy pathways and miR-34a and miR-182 are effective on these genes in these pathways. It can be concluded that the desired miRNAs are effective in the pathways of autophagy and apoptosis.

4. MirpathDB.2

From miRpathDB, in addition to finding miR sequences, autophagy and apoptosis pathway genes that are targeted by miR-34a and miR-182 are listed in the Supplementary Table S2 which shows experimental evidence of related genes to miR-34a (https://mpd.bioinf.uni-sb.de/mirna.html?mirna=hsa-miR-34a-5p&organism=hsa) and miR-182 (https://mpd.bioinf.uni-sb.de/mirna.html?mirna=hsa-miR-182-5p&organism=hsa).

5. UCSC

The effects of miRNAs on the target mRNA are mediated either by fully connecting the 3'-UTR or incompletely with the 5'-UTR to be regulated and/or degraded. Since many miRNAs can connect to the 3'-UTR of mRNAs and the effect is greater in the 3'-UTR region, the effect of miRNAs on the 3'-UTR regions was investigated. The genes targeted by miR-34a and miR-182 in TB and lung cancer were studied from the KEGG and miRPathDB2 databases as well as the UCSC database and also articles (Supplementary Table S3). In the UCSC database, all genes involved in TB inflammatory and autophagy

Table 3. LogFC and p-values obtained (miR-34a and miR-182) from the GEO database

MicroRNA	NSCLC		Tuberculosis	
	LogFC	p-value	LogFC	p-value
miR-182	−2.644	0.0532743	1.55	1.63E-03
miR-34a	2.632	0.4051477	−2.62	2.01E-03

LogFC: logarithm folding change; GEO: Gene Expression Omnibus; NSCLC: non-small cell lung cancer.

responses and pathways of apoptosis in NSCLC in the metastasis, cell growth, tumor invasion, cell migration, and cell proliferation and are targeted by miR-34a, miR-182 were studied with a focus on time and direction targeting. To investigate the mechanism of gene silencing, the target miRNAs must be aligned with the target genes, and from this point of view, the effect of miRNAs was investigated.

6. MiRcode

The seed position on the chromosome and transcript region shows this region and conservation in organisms such as Primates, Mammals, and other organisms (http://www.mircode.org/?gene=&mirfam=miR-182&class=&cons=&trregion=) (http://www.mircode.org/?gene=&mirfam=miR-34ac%2F34bc-5p%2F449abc%2F449c-5p&class=&cons=&trregion=).

7. DIANA

DIANA-tool (http://diana.imis.athena-innovation.gr/DianaTools/) is an advanced web server and database tool for testing and analyzing the function of miRNAs. It shows details of the miRNA itself, Gene, PubMed links of (miRNA and Gene) and a view of the UCSC graphic and related diseases, and even the miRNA sequence of the desired miRNAs. DIANA-microT-CDS is the 5th version of the microT algorithm that is specifically focused on a set of positive and negative miRNA recognition elements (MREs) located in both the 3'-UTR and CDS regions. Using the microT-CDS (http://www.microrna.gr/microT-CDS) from DIANA algorithm hsa-miR-34a-5p, hsa-miR-182-5p was investigated. It demonstrates the binding region of the transcript with the desired miRNA for hsa-miR-34a-5p (https://dianalab.e-ce.uth.gr/html/dianauniverse/index.php?r=microT_CDS/results&keywords=hsa-miR-34a-5p&genes=&mirnas=hsa-miR-34a-5p%20&descr=&threshold=0.7), and for hsa-miR-182-5p (https://dianalab.e-ce.uth.gr/html/dianauniverse/index.php?r=microT_CDS/results&keywords=hsa-miR-182-5p&genes=&mirnas=hsa-miR-182-5p%20&descr=&threshold=0.7) were found 1,108 and 1,710 targets respectively. The found information of miRs is added in (Supplementary Tables S4, S5). As seen in these tables, miR-34 is involved in lung neoplasms and miR-182 in carcinoma, non-small-cell lung, and lung neoplasms.

8. MiRDB

miRDB (http://mirdb.org/) lists the number of genes that affect each miR, 1,266 target hsa-miR-182-5p (https://mirdb.org/cgi-bin/search.cgi) genes, and 899 genes for hsa-miR-34a-5p (https://mirdb.org/cgi-bin/search.cgi) are listed in this database (Supplementary Table S6). It also shows the type of function, 3'-UTR sequence and miRNA sequence, seed location, 3'-UTR length, NCBI gene ID, and even gene symbol in relation to each gene. The effect of miR-34a and miR-182 on genes (forkhead box O3 [FOXO3], B-cell lymphoma 2 [BCL2], IL-6, TNF-α, and Bax) that are important to us in previous and future studies have been shown. As can be seen in this, the miRDB database shows the target genes *FOXO3*, *BCL2*, and *IL-6* for miR-182, and the target genes for miR-34a are not displayed. (+) will indicates the influence of miR on the desired gene and (–) absence of miR effect on that gene. *TNF-*α, *Bax*, *IL-6*, *FOXO3*, and *Bcl2* gene expression profiles have been examined in previous studies by Sheikhpour et al.[16] Also, results of their other study showed that in PBMCs of TB patients compared to the control group, the expression of type 2 dopamine receptor genes decreased.

9. DRD2, UCSC

The miRNAs that target the 3'-UTR region of the dopamine receptor D2 (*DRD2*) gene were evaluated in articles and UCSC with confirmation on the GEO database from TB and NSCLC data. The frequency and adjustment of miRNAs with the *DRD2* gene are listed in Supplementary Table S7. In this table, the ID names (GSE) of the articles and the number of repeats of targeting on the 3'-UTR region of the *DRD2* gene on USCS and logFC and p-value from GEO are presented. These data were searched in GEO only in blood samples of TB and NSCLC diseases.

10. Evaluation of long non-coding RNA to investigate interferences with miRNA function in apoptosis

By sponging miRNAs individually or several miRNAs simultaneously, long non-coding RNA (lncRNA) will leads to the lack of effect of miRNAs on their target genes. Therefore, we decided to investigate the effect of lncRNA on miRNAs. From the galaxy lncRNA database, RNAs that sponge the desired miRNAs in the path of autophagy and apoptosis were found. The results obtained from the galaxy are given in the Supplementary Table S8 with some SRX7431053 studies. lncRNAs obtained from the galaxy at UCSC were investigated for their effects on miR-34a, 182. The only lncRNA that simultaneously spangles both miRNAs, ENSG00000254154.8 named CRYZL2P-SEC16B, affects miR-34a four times and miR-182 once. The information of ENSG00000254154.8 which is located in chromosome 1 and in the region 77,928,788–178,038,007 in the reverse strand was

analyzed in Ensemble. The supplementary information is given in the Supplementary Table S9. Three transcripts ENSG00000254154.8 from Ensemble are shown in the address of https://asia.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000254154;r=1:177928788-178038007. The results indicate that one of the factors that prevent expression is lncRNAs, which are lncRNAs that sponge miR-34a ENSG00000249196.6, ENSG00000249835.2, ENSG00000254154.8 and lncRNA which sponges miR-182. ENSG00000241158.7, ENSG00000254154.8, ENSG00000278893.1 act inconsistently with the target gene and stop microRNAs.

11. The results of the patient's demographical information

The demographic information of the patients showed that approximately 40% of the lung cancer patients were smokers. Equal numbers of both sexes were smokers. They ranged in age from 25 to 55 years, with a mean age of 35 in lung cancer patients, 31 in TB, and 38 in healthy individuals. Weight loss and coughing were among the symptoms in more than 80% of patients.

12. MiR-182 and miR-34a expression patterns studies

The statistical analysis results of miRNA expression, including the change of parity and p-value with t-test, were performed in Graph Pad Prism software version 8. Both miR-182 and miR-34a show lower expression in TB and NSCLC patients than in healthy individuals. As shown in Figure 1, the mean expression of miR-182 in NSCLC and TB patients shows a decrease compared to healthy subjects ($p \leq 0.0001$) and the frequency of miR-182 was significant. Also, the p-value related to miR-34a in patients with TB and NSCLC compared to healthy subjects was 0.3380; the decrease in expression can be seen in Figure 2.

13. Statistical analysis and receiver operating characteristics curve analysis

Receiver operating characteristics (ROC) analysis was performed to investigate the test accuracy and diagnostic value of miR-182 and miR-34a in TB and NSCLC patients. The accuracy of the test was measured by the area under the ROC curve (AUC). ROC curve analysis shows miR-34a and miR-182 in NSCLC patients (AUC=0.6429) and TB patients (AUC=0.9341), respectively. In TB patients, AUC=0.5231 and AUC=0.8325 were recorded in miR-34a and miR-182, respectively (Figure 3). Therefore, these results showed good test detection power for miR-182.

Discussion

TB and NSCLC are the most common diseases that lead to death and TB can play a role in the development of lung cancer. Moon et al.[4] stated that TB causes chronic obstructive pulmonary disease regardless of smoking. Studies on miRNAs affecting TB and lung cancer and their role in the early progression and diagnosis and even in the treatment of these diseases are

Figure 1. Expression of miR-182 in three groups including patients with tuberculosis (TB), lung cancer, and healthy individuals. NSCLC: non-small cell lung cancer.

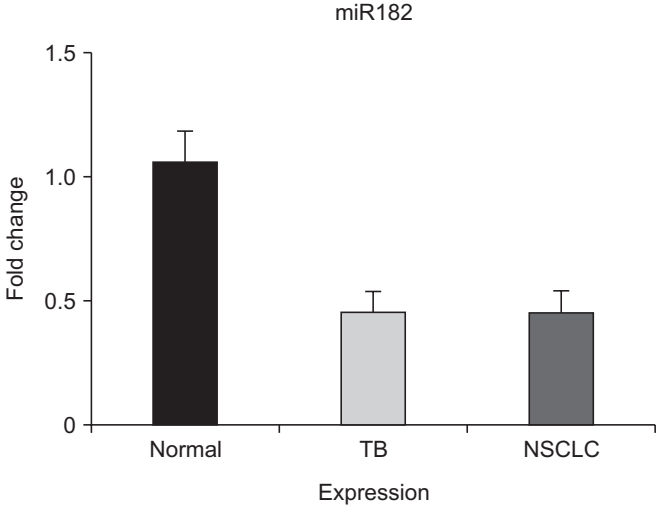


Figure 2. Expression of miR-34a in tuberculosis (TB) and lung cancer patients compared with healthy individuals. NSCLC: non-small cell lung cancer.

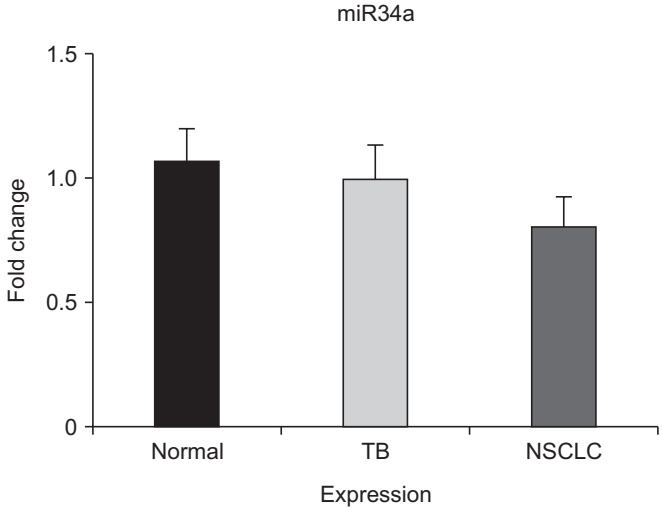
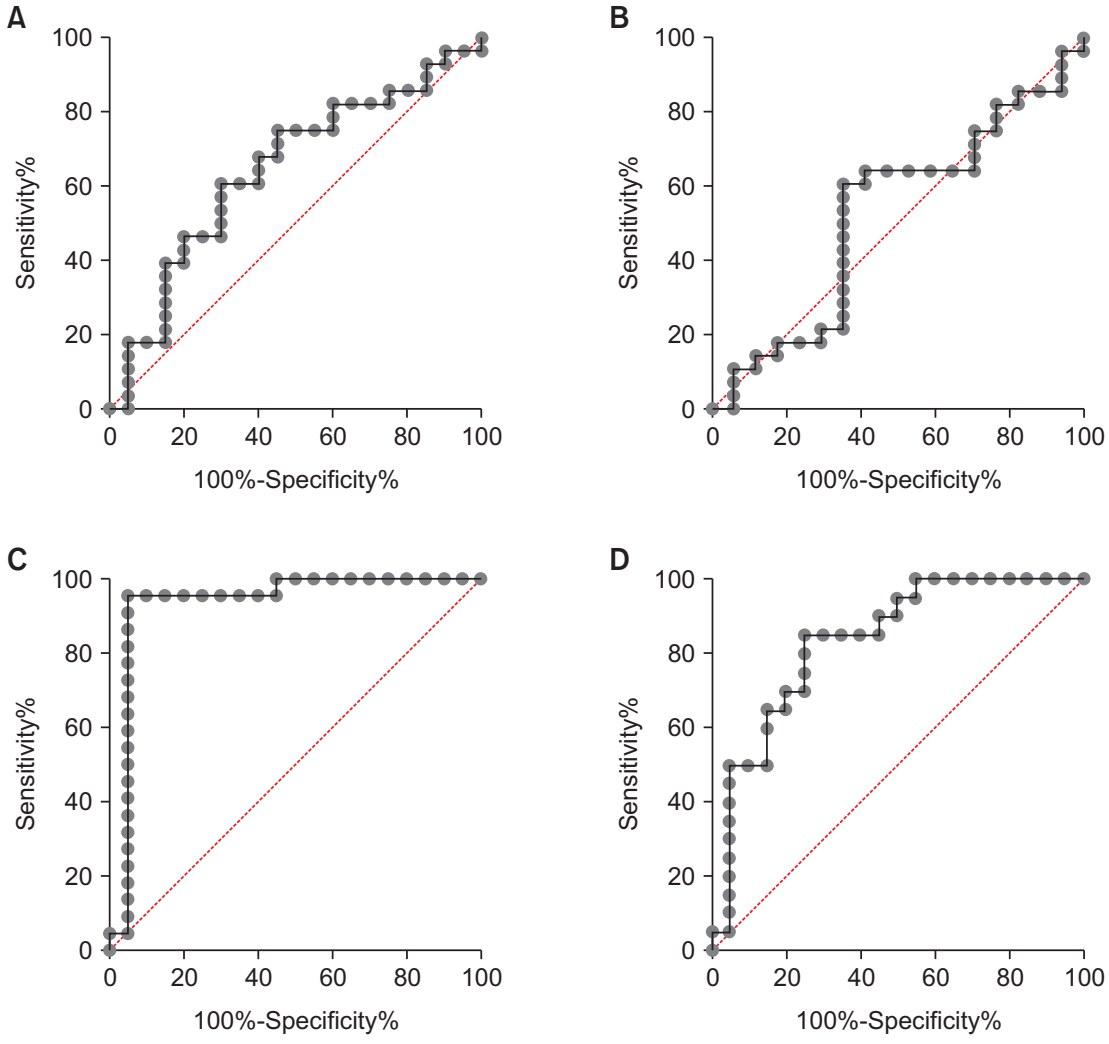


Figure 3. Receiver operating characteristics curves related to (A) miR-34a in non-small cell lung cancer (NSCLC), (B) miR-34a in tuberculosis (TB), (C) miR-182 in NSCLC, and (D) miR-182 in TB.



very few. A study supporting the direct link between TB and lung cancer, particularly adenocarcinoma, was written in 2009[17]. Zheng et al.[18] also stated that people who had TB in the last 20 years had a more than 2.5-fold increased risk of lung cancer. Another study in 2010 found an association between TB and lung cancer and evidence that TB is associated with an increased risk of lung cancer[19]. A population cohort study was conducted in 2011 on the association between TB and lung cancer. The incidence of lung cancer was compared between the two groups (non-cancer subjects and patients with newly diagnosed TB) and it was shown that the risk of lung cancer increases in people with TB[20]. A 2015 case report study by Hammen[21] found that in countries with a high incidence of lung cancer, TB is often misdiagnosed as lung cancer. As can be seen in the articles mentioned, all indicate that people with a history of TB can be at increased risk for lung cancer. miRNAs play a key role in controlling infectious diseases, and recently the potential role of miRNAs in the diagnosis and treatment of TB has been considered[22].

Concerning carcinogenesis miRNAs can act as oncogenes or tumor suppressors depending on the cellular context and the multiple target genes, as they affect silencing[9]. MiR-34a plays a role in tumor suppressor[23] and is regulated in some cancers, including lung cancer[24]. Apoptosis, cell cycle, and differentiation are affected by aberrant expression of miR-34a[25,26] suggesting the possible application of miR-34a in tumor treatment and prognosis, diagnosis, and metastasis pathways[27]. As a result, it plays a regulatory role in carcinogenesis[26]. Tumor suppressors and other genes involved in cell differentiation can be downregulated by overexpression of oncogenic miRNAs, leading to

tumor formation by stimulating proliferation, angiogenesis, and invasion[9]. miR-182 and miR-183 belong to the miR-183 family and are onco-miRNAs that function in tumorigenesis by promoting the growth and migration of tumor cells by targeting the early growth response protein transcription factor 1[28]. In the study of Zhu et al.[29], they stated that the serum level of miR-126 decreased and miR-182, miR-183, and miR-210 significantly increased in NSCLC patients compared to healthy individuals. And also, these miRNAs can differentiate NSCLC or NSCLC at early stages from current smokers without pneumonia and lung cancer or high-sensitivity gastric cancer patients. Therefore, they can serve as tumor biomarkers for early detection of NSCLC[29]. In this study, data from GEO microarrays on lung cancer and TB for meta-analysis to investigate the relationship between miR-34a and miR-182 expression with lung diseases were gathered. In addition, the target genes, KEGG pathway analysis, and other pathways using miRpathDB.2 databases and miR interaction with 3'-UTR of genes at UCSC were investigated. This is the first study to investigate the role of miR-182 and miR-34a together in TB and lung cancer with a dataset from GEO, miRbase, mirpathDB.2, miRcode, DIANA, miRDB, galaxy, reviews and provides significant amounts of data. The results of this study showed that the expression of miR-34a and miR-182 is lower in TB and lung cancer patients, respectively than in healthy individuals. This finding is consistent with the findings of Sun et al.[30] who stated that miR-182 shows a decrease in expression in people with lung cancer. These results are not consistent with the findings of Shen et al.[31] and Wang et al.[32] which show increased expression of miR-182 in sick people compared to healthy people. Meanwhile, Mitchell et al.[33] stated that the severe reduction of miR-182 expression renders it a diagnostic marker. The increased expression of miR-34a in normal people compared to NSCLC and TB and in NSCLC not related to TB indicates the suppressive role of this miR[23]. Since studies show that circulating miRNAs play a role as biomarkers in the development of various diseases and serve for the early diagnosis of lung cancer and TB, miR-182 can be used as a diagnostic marker in cancer and in the absence of mutations. MiR-34a can be mentioned as one of the cancer suppressors, and previous studies[15,34] have stated that not only the type of miR is important in the development of disease, but it is very important to know the target genes in the effect of miR in which stage of the cell cycle. Studies show a dual function for miR-182 that it can act as a tumor suppressor miRNA[22] or oncogenic[35] in increasing cancer cell proliferation, survival, aggressiveness, tumorigenesis, and drug resistance of different tissues. Wang et al.[36] stated that miR-182 has an oncogenic role in lung cancer cells by targeting programmed cell death 4 (*PDCD4*) to increase cell growth and invasion. And the opposite of this practice Zhang et al.[22] stated that miR-182 as a tumor suppressor by targeting cortactin (*CTTN*) inhibits the proliferation and invasion of human lung adenocarcinoma cells. Also, Yang et al.[37] stated that increased invasion, migration, and metastatic activity in lung cancer cell lines will occur through the reduction of *FOXO3* and N-cadherin levels by miR-182 knockdown. According to the information presented, miR-182 shows a different mechanism with tumorigenesis, which is not yet fully understood. Studies showed the inhibitory effect of miR-182 on the growth of several types of tumor cells and the enhancement of the apoptosis pathway. Besides, miR-182 can activate the Met/AKT signaling pathway and play a role in regulating various cell functions including survival, growth, invasion and cell suppression[11]. Various factors play a role in reducing miR-34a expression in NSCLC and TB patients. One of the mechanisms that reduce the expression of miR-34a is the mutation in p53 as well as the gene encoding miR-34a, which are targets for the inactivation of mutations or epigenetics in cancer[38].

One of the influential factors that show the decrease in the expression of both miRNAs can be the presence of lncRNA such as ENSG00000254154.8, which simultaneously sponges miR-34a and miR-182 and justifies the low expression level of both miRNAs in TB and lung cancer. The lncRNAs that sponge miR-34a and miR-182 are listed in the Supplementary Table S8, which explains the downregulation of the expression levels of both miRNAs, which needs further investigation in future experiments. MiRNAs are connected to their target gene sequences with short -circuits of their motifs and can target several tumor stimulants at the same time. This will have side effects for the treatment of tumors. A thorough understanding of the upstream and downstream miRNAs will be very important for successful treatment[39].

In conclusion, this is the first study that simultaneously was done in the field of bioinformatics studies on miR-34a and miR-182 and their effector genes, as well as the effect of lncRNAs on these miRNAs and provides valuable information about the influence of these miRNAs on their effective pathways and mechanisms. The results of the present study indicate that the expression of this miRNA is lower in TB and lung cancer patients than in healthy individuals. Paying attention to this point in the investigation of miRNAs that not only the type of miR but also the knowledge about the target

genes is very important in understanding the disease.

The miRNAs examined in this experiment are among the key miRNAs in the timely and quick diagnosis of the disease from other diseases and the differential diagnosis of TB from lung cancer. Since people with a history of TB have a greater risk of getting lung cancer, miR-34a and miR-182 can be used as prognostic as well as diagnostic and therapeutical biomarkers.

Authors' Contributions

Conceptualization: Alimardanian L, Sheikhpour M. Methodology: Alimardanian L, Sheikhpour M. Formal analysis: Soltani BM, Irani S. Data curation: Alimardanian L, Irani S. Project administration: Alimardanian L, Sheikhpour M. Validation: Alimardanian L, Sheikhpour M. Investigation: Irani S. Writing - original draft preparation: Alimardanian L, Sheikhpour M. Writing - review and editing: Alimardanian L, Sheikhpour M. Approval of final manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Supplementary Material

Supplementary material can be found in the journal homepage (http://www.e-trd.org).

Supplementary Table S1. Mature sequence and stem-loop (5'-3') and seed region (miR-182-5p, miR-34a-5p) from miRbase and miRpathDB databases.

Supplementary Table S2. Target genes of miR-34a, 182 that found from mirpathDB.2.

Supplementary Table S3. Investigated miR-34a, miR-182 targeted genes in UCSC.

Supplementary Table S4. Information miR-34a-5p in DIANA.

Supplementary Table S5. Information miR-182-5p in DIANA.

Supplementary Table S6. The number of genes affecting each miR is listed from the miRDB database.

Supplementary Table S7. Available miR of the 3'UTR region of the *DRD2* gene from the UCSC database.

Supplementary Table S8. Long non-coding RNAs found from the galaxy in lung cancer.

Supplementary Table S9. LncRNA (ENSG0000025 4154.8) in Ensemble.

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