EXPRESSION AND POTENTIAL ROLE OF miR200b AND miR1274a IN LUNG CANCER PATIENTS

AKCİĞER KANSERLİ HASTALARDA miR200b VE miR1274a EKSPRESYONLARI VE POTANSİYEL ROLLERİ

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Öz

Amaç: Akciğer kanseri genetik veya çevresel etkenler nedeniyle hava yolu epitel hücrelerinin kontrolsüz çoğalmasıyla oluşan ölümcül bir hastalıktır. En sık görülen kanser tiplerinden biridir ve kanser sebepli ölümler arasında ilk sırada yer almaktadır. Etiyolojisindeki en önemli neden sigara kullanımı ve tütün dumanı maruziyetidir. Mikro RNA'lar (miRNA) saç tokası yapısı içeren, yaklaşık 18-24 nükleotidlik kısa ve kodlamayan RNA'lar olup, diğer genler gibi DNA üzerinden transkribe edilir ve proteine dönüştürülmeden küçük RNA molekülleri halinde gen regülasyonunda görev alırlar. Yapılan çalışmalarda miRNA ekspresyon seviyelerinin malignant tümörlerin gelişimi ve ilerlemesi ile ilişkili olduğu ve miRNA'ların onkogen veya tümör süpresör etki gösterebileceği belirlenmiştir. Çalışmamızda da miR200b ve miR1274a ekspresyonlarının akciğer kanseri ile ilişkisinin belirlenmesi amaçlanmıştır. Gereç ve Yöntemler: Çalışmamıza 90 sağlıklı kontrol birey ve 90 akciğer kanseri hastası dahil edildi. EDTA'lı tüplere alınan kan örneklerinden periferal mononükleer hücreler (PBMC) izole edildi. Mononükleer hücreler total RNA izolasyonunda kullanıldı. miRNA ekspresyonları qRT-PCR (kantitatif gerçek zamanlı-PCR) ile belirlendi ve sonuçlarımız uygun istatistiksel yöntemler ile değerlendirildi. Bulgular: Araştırmamız kapsamında akciğer kanser riski üzerine etkinliğini araştırdığımız miR200b ve miR1274a'nın her ikisi de akciğer kanserli bireylerde anlamlı oranda düşük ekspresyon özelliği göstermiştir (p=0.005 ve p=0.021). Tartışma: Araştırmamız sonuçlarına göre miR200b ve miR1274a'nın akciğer kanserinde tümör süpresör özellik gösterdiği düşünülmektedir.

Anahtar Kelimeler

Akciğer Kanseri; miR200b; miR1274a; qRT-PCR

Abstract

Aim: Lung cancer (LC) is a fatal disease characterized by uncontrolled proliferation of airway epithelial cells and caused by genetic or environmental factors. LC is one of the most common types of cancer and the leading cause of cancer-induced deaths. The most important factors in the etiology of LC are smoking and exposure to tobacco smoke. Micro RNAs (miRNAs) are short and non-coding RNAs that contain hairpin structure and approximately 18-24 nucleotides. Like the other genes, miRNAs are transcribed from DNA and involved in gene regulation without converting to protein. Previous studies have determined that miRNA expression levels are associated with the development and progression of malignant tumors and that miRNAs can act as oncogene or tumor suppressors. The aim of our study was to determine any association between expression levels of miR200b and miR1274a and lung cancer. Material and Method: Ninety controls and ninety LC patients were included in our study. Total RNA isolation was performed in peripheral blood mononuclear cells (PBMC) isolated from whole blood collected with ETDA; miRNA expressions were evaluated with qRT-PCR (quantitative Real Time-PCR). Results were evaluated by appropriate statistical methods. Results: We evaluated the effect of miR200b and miR1274a expression levels on lung cancer risk and found decreased levels of these miRNA expression levels in lung cancer (p=0.005 and p=0.021). Discussion: We conclude that miR200b and miR1274a have a tumor suppressor function in lung cancer.

Keywords

Lung Cancer; miR200b; miR1274a; qRT-PCR

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Introduction

Lung cancer is a fatal disease caused by uncontrolled proliferation of lung tissue cells. The disease is the most common cancer type and the leading cause of cancer-related death worldwide [1]. Lung cancer has been histologically categorized by the World Health Organization (WHO) according to the tissue origin. Since 95% of lung tumors originate from the bronchial epithelium, these group of tumors are called bronchogenic carcinomas [2]. Lung cancer is divided into two major subtypes, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), which vary in their biology, treatment, and prognosis [3]. There are many factors in the etiology of lung cancer such as active and passive smoking; occupational exposure to materials such as asbestos, nickel, chromium, and arsenic; radiation exposure; and indoor and outdoor air pollution [4].

Family history and hereditary factors can also increase the risk of developing lung cancer. Functional changes that occur in oncogenic or tumor suppressor genes, which can be part of or within genes that control important physiological pathways such as cell proliferation, DNA replication, and DNA repair, can cause cancer and can be inherited [5,6]. These pathways, however, are also controlled by "mitotically and/or meiotically heritable changes in gene expression that occur without a change in DNA sequence," that is, with epigenetic regulation [7,8]. Epigenetic mechanisms can be classified as DNA methylation, histone modifications, and non-coding RNAs [8,9].

MiRNAs, a class of non-coding RNAs, are short, non-coding RNAs of about 18-24 nucleotides that contain the hairpin structure and are transcribed through DNA like other genes. They are involved in gene regulation as small RNA molecules without being converted to protein [10-14]. MiRNAs reduce the expression of the target gene by base pairing with 3' UTR regions of mRNAs or by preventing target mRNA degradation or interrupting translation [10-13].

MiRNAs exert tissue-specific expression and are identified as oncogenic or tumor suppressor miRNAs according to the molecular pathway characteristics of the mRNA they are targeting. In a variety of cancer cases, miRNAs with increased expression have been described as oncogenic miRNAs or oncomirs, and these group miRNAs often function as an uncontrolled growth enhancer and/or anti-apoptotic pathway in cancer types [14-16].

In contrast to oncomirs, miRNAs that are effective on the expression of oncogenes are referred to as tumor suppressor miRNAs. Tumor suppressor miRNAs inhibit tumor formation by suppressing oncogenes and increasing the activity of the differentiating genes. Thus, decreased expression of tumor suppressor miRNAs leads to increased expression of oncogenes and tumorigenesis [17].

In our previous study, we reported a relationship between lung cancer and 35 genetic variations of miRNA genes targeting DNA methyltransferases and Methyl-CpG-binding proteins, and reported that the rs318039 variant of the miR1274a gene is associated with lung cancer subtypes. Although we did not find a relationship between the rs72563729 variant of the miR200b gene from the investigated variations and lung cancer, it was suggested that miR200b expression changes, which is a tumor suppressor miRNA, may be associated with lung cancer [6].

The miR-200 family has 5 members (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) and comes together as two transcripts of polystistronic pri-miRNA. MiR200b-200a-429 is located on chromosome 1p36, and miR200c-141 is located on chromosome 12p13. MiR200 family is a family of miRNAs reported to have tumor suppressor function in many types of cancer, including breast cancer, colorectal cancer, pancreatic cancer, gastric cancer, and endometrial carcinoma [18].

MiR1274a, among cancer-related miRNAs such as miR200, has been reported to be associated with proliferation in breast cancer [19], is responsible for epithelial-mesenchymal transformation, and increases expression in human gastric tumors [20]. Functional effects have not yet been fully determined and there is a limited number of studies in the literature.

The aim of this study was to determine the expression levels of miR1274a and miR200b in patients with lung cancer who have not yet been determined to be associated with lung cancer, miRNAs that have not yet been determined to be associated with lung cancer.

Material and Method

Study group and collection of samples

Ninety lung cancer patients and 90 healthy control subjects were included in the study. The Ethics Committee for Clinical Research approved our work and the content of the study is in accordance with the Helsinki Declaration Principles. The study was described in detail and signed informed consent forms were obtained from all patients.

PBMC collection and RNA isolation

Blood samples were collected in EDTA-treated tubes and PBMCs were isolated by standard Ficoll density-gradient centrifugation by using Biocoll Separating Solution (D = 1.077g / ml, Biochrom, Berlin, Germany). Briefly, 5 mL of venous blood samples were diluated in 1:1 ratio sterile phosphate buffer saline (1XPBS) and phase was formed by adding Ficoll to the same volume of blood. The tubes were centrifuged at 400xg for 30 min (4Co), then the opaque white mononuclear cells in the middle (buffy coat) between plasma and Ficoll were collected. After the washing with PBS, PBMCs were stored at -80 °C until RNA isolation.

Total RNA was isolated from PBMCs using the mirVANA miRNA Isolation Kit (Ambion, Austin, TX, USA) in accordance with the manufacturer's protocol. Isolated RNA samples were assessed for purity with a Thermo Scientific NanoDrop (TM) 1000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA) and stored.

qRT-PCR

Following the kit procedure, the RNA concentrations were adjusted to 5ng/1µL and then passed to the cDNA synthesis stage. Exiqon miRCURY LNATM Universal cDNA Synthesis Kit II (Exiqon, Vedbaek, Denmark) was used for cDNA synthesis. The cDNA synthesis reaction was prepared in the following manner with a total volume of 10 µl; 5x reaction buffer (2µl), nuclease-free water (4.5µl), enzyme mix (1µl), synthetic RNA spike (0.5µl), template total RNA (5ng /µl, 2µl). The total of 10 µl reaction mixture was incubated for 60 min at 42 °C and 5 min at 95 °C, respectively. Prior to qRT-PCR amplification, the cDNA reaction products were diluted 80x (395 µL nuclease-free water + 5 µL

cDNA). The qRT-PCR reaction was prepared in a total volume of 10 µl as follows: PCR master mix (5µl), PCR primer mix (1µl), diluted cDNA template (4µl). RT-PCR analysis was performed by the LightCycler® Nano System (Roche Diagnostics, Mannheim, Germany) with the "Exiqon miRCURY LNATM Universal RT microRNA PCR primers" and the SYBR® Green master mix (Exiqon, Vedbaek, Denmark). The amplification conditions are indicated in Table 1. Primer sets for a total of 3 gene regions, hsa-mir-1274a (product no: 206999), hsa-mir-200b-3p (product no: 206071), and housekeeping gene SNORD48 (product no: 203903) were obtained from Exiqon (Vedbaek, Denmark).

Raw data, expressed as threshold cycle (Ct) values, were computed with the LightCycler® Nano SW 1.1 (Roche Diagnostics, Mannheim, Germany). Relative expression was calculated with the comparative Ct method (2−ΔCt) as follows; Relative ratio= 2−ΔCt (ΔCt miR1274a – ΔCt SNORD48) and 2−ΔCt (ΔCt miR200b-3p – ΔCt SNORD48)

Statistical analysis

Expression levels of miR200b-3p and miR1274a were determined by one-way ANOVA using the IBM SPSS Statistics 21 Software; p value <0.05 was considered significant and the results were expressed as mean ± standard deviation.

Results

Expression levels of SNORD48, miR200b-3p and miR1274a of lung cancer and control individuals were determined in this study and the miR200b-3p and miR1274a expressions were normalized to SNORD48 expression as the housekeeping gene. When miRNA expression levels were compared, miR200b-3p expression levels of lung cancer patients were significantly lower than in the control (p = 0.005) (Graph 1).

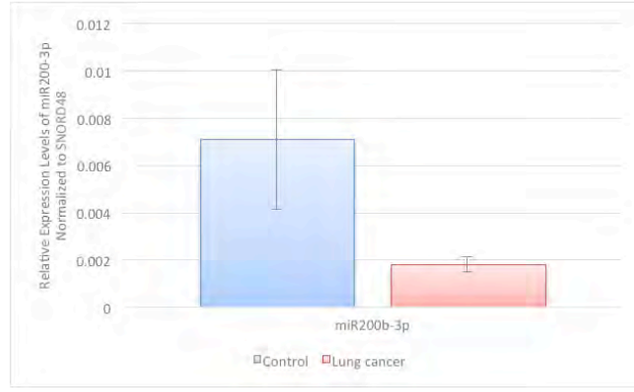
Similarly, expression of miR1274a was significantly reduced in lung cancer patients when compared to control (p = 0.021) (Graph 2).

Discussion

Lung cancer is the most common types of cancer and is the most frequent cause of cancer deaths in both males and females. Among lung cancer causes, smoking and tobacco smoke exposure are the first order. Tobacco causes 80-90% of lung malignant lesions, while exposure to physical and chemical carcinogens is another effect in the etiology [21-23].

Recognition of the importance of non-coding miRNAs in relation to tumor progression and progression is increasing day by day. While some miRNAs are identified as oncogenes (miR-17-92, miR-155 and miR-21) and others as tumor suppressor (miR-15a, miR-16a and let-7) [24].

The MiR200 family consists of five members: miR-200a, miR-200b, miR-200c, miR-141, and miR-429 [25]. MiR200b is an important member of the miR200 family. It has been indicated that miR200 inhibits cell invasion and metastasis in lung adenocarcinomas [24]. MiR200 has been reported to be a key regulator of cancer formation and metastasis prevention due to its key role in epithelial-mesenchymal transformation (EMT) [26,27]. Similarly, miR200b and miR200c expressions were also reported to be associated with carcinogenesis in gastric cancer tissues and cell lines, and downregulated in these tissues



Graphic 1. The miR200b-3p expression levels of controls individuals and patients with lung cancer.



Graphic 2. The miR1274a expression levels of controls individuals and patients with lung cancer.

[18]. Unlike these studies, Lin et al. evaluated serum miR200 expression in metastatic NSCLC patients and reported that serum miR200 expressions were not associated with lung cancer metastasis, SCLC, and adenocarcinoma risk [28].

In our study, miR200b-3p expression levels of lung cancer patients were significantly lower than in control subjects, and miR200b was downregulated in lung cancer. Our results support studies that emphasize that miR200b is a tumor suppressor [18,25,26].

There is no study investigating miR1274a expression levels and lung cancer risk. However, Janssen and colleagues have found that miR1274a is associated with proliferation in breast cancer [19].

Wang et al. have reported that miR1274a is involved in EMT and that expression in human gastric tumors is increased. However, overexpression of miR1274a has been shown to activate PI3K / Akt signaling and to increase expression of cyclin D1, MMP-2 and MMP-9 [20].

There is a limited number studies in the literature for miR1274a and our study showed that expression of miR1274a in lung cancer patients decreased significantly in comparison with the control.

Lung cancer is a fatal disease that is known to be caused by various carcinogens such as asbestos and especially cigarette smoke. Also, genetic factors have an important role in the etiology. One of the main goals of contemporary cancer research is to identify the molecular causes of lung cancer and to develop treatments for these targets, because lung cancer is the leading cause of cancer deaths.

Both miR200b and miR1274a, which we investigated for efficacy on lung cancer risk in our study, showed significantly low expression characteristics in lung cancer patients. It is well known that miR200b is a tumor suppressor, and our results show that miR1274a also has tumor suppressor properties. It is predicted that miR200b and miR1274a levels will be the candidates to be investigated and used as molecular biomarkers because of the ability to identify peripheral cancers.

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Competing interests

The authors declare that they have no competing interests.

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