ORIGINAL ARTICLE

Received: January 2018
Accepted: May 2018

The Association Between rs11671784 Polymorphism in pre-miR-27a and Lung Cancer

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ABSTRACT

Background: MicroRNAs are one of the most remarkable controlling agents of gene expression. MiR-27a is an important onco-miR which has an increased expression and oncogenic role in many types of cancer through controlling tumor-suppressor genes such as FOXO1, TP53, RYBP, and FADD. Moreover, it has a dual role in some types of cancer and decreased expression in some neoplasms, including non-small cell lung cancer (NSCLC) playing a tumor-suppressing role by controlling pro-to-oncogenes such as IGF-1, EGFR, KRAS, PPFP, MYT1, and CYP1B1. Polymorphisms in pre-miRNA can affect miRNA processing and expression. There are two polymorphisms "rs11671784" and "rs895819" within the loop region of pre-miR-27a. In 2013, the association between rs895819 and decreased expression of miR-27a alongside an increased risk of NSCLC was determined. Hence, the rs11671784 polymorphism was selected for this study. This study aims to investigate the association between the rs11671784 polymorphism within the loop region of pre-miR-27a and lung cancer susceptibility.

Methods: This case-control study was conducted on genomic DNA from the blood samples of 110 healthy subjects and 70 patients collected from the Omid Hospital of Isfahan using the salting-out method. The genotype of rs11671784 was determined using appropriate primers and the Restriction Fragment Length Polymorphism (RFLP) technique. A statistical analysis was performed using the Power Marker, SPSS version-23 software and the SISA website.

Results: This study proved that the presence of the T allele at the polymorphic position can reduce the risk of lung cancer up to 6.7 fold (OR=0.15, p=0.039, 95% CI=0.019-1.2), likely because of its effect in pre-miR-27a processing. In fact, only one patient was found with the T allele, compared to ten in healthy subjects, who was a smoker suffering adenocarcinoma. The U allele observed in the pre-miR-27a, compared to the C allele, can reduce the free energy (ΔG) by 0.8 kcal/mol and stabilize the structure, and accordingly, may lead to an increase in miR-27a.

Conclusion: The present study examines the correlation between rs11671784 and lung cancer for the first time, which suggests that rs11671784 could be known as a biomarker for resistance to lung cancer among the studied population. However, further studies are needed to determine the effect of this polymorphism on the expression of miR-27a.

Keywords: Lung cancer, EGFR, microRNAs, Polymorphism, miR-27a, RFLP

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BCCR
2018; 10(2):3-15
www.bccrjournal.com

INTRODUCTION:

Lung cancer is the most common type of cancer, with new cases being registered every year (13% of all new cases of cancer). This cancer is responsible for 19% of mortality resulting from cancer. Owing to late diagnosis, the five-year survival rate of this cancer is less than 15% and it is one of the most difficult types of cancer to treat[1]. Lung cancer is defined as the uncontrolled cell growth of the epithelial cells in the lungs. It is histologically classified into two main types: small cell lung cancer (SCLC) (15-20% cases of lung cancer) and non-small cell lung cancer (NSCLC) (80-85%). NSCLC is divided into three types, namely, adenocarcinoma, squamous cell carcinoma (SCC), and large-cell lung cancer[2,3]. Genetic alterations that lead to the activation of the proto-oncogenes or the inactivation of tumor suppressor genes are important factors in lung cancer[4]. Micro-RNAs are regulators of gene expression. They can effectively contribute to enhanced performance of oncogenes or decreased functions of tumor suppressors, through increasing or decreasing expression. The Micro-RNA expression separates the normal tissues from the tumour tissues, as well as the different tumour types[5]. An increased expression of the oncogenic micro-RNAs or a decreased expression of the tumour suppressor micro-RNAs is involved in causing cancer[6].

NSCLC, especially when in the form of adenocarcinoma can be inherited[7]. So far, a total of 213 genes have been identified as being linked to lung cancer[8]. The most frequent type of lung cancer, adenocarcinoma with a prevalence of 40%, is the most common type of cancer among women and non-smokers[9,10]. The most frequent genetic alterations observed in these patients are mutations in the KRAS proto-oncogene (Kirsten rat sarcoma viral oncogene homolog) that can be found in smokers. The mutations and amplification of the EGFR proto-oncogene (Epidermal growth factor receptor) are prevalent in NSCLC (and especially in adenocarcinomas among non-smokers). These genes activate signalling pathways involved in growth, differentiation, survival, proliferation, invasion, angiogenesis, and metastasis (9,10). Two ACUGUGA sequences are observed in the 3'-untranslated-region of the EGFR that are the binding sites of miR-27a, b. An A13 repeat, which, according to the study performed by Yuan et al in 2009, is associated with increased expression of EGFR, is also located between these two regions[11]. MiR-27a, b, in addition to the binding sites on the EGFR transcript, also has three binding sites at the 3'UTR of the KRAS[12].

MiR-27a is one of the most important onco-miRs, with an oncogenic role and increased expression in many types of cancer such as breast cancer, serous ovarian cancer, uterine leiomyoma (ULM), hepatocellular carcinoma (HCC), acute myeloid leukemia (AML), kidney cancer, and gastric cancer[13]. This miRNA induces epithelial-mesenchymal transition and metastasis through the inhibition of the Adenomatous Polyposis Coli (APC) protein[14]. Its oncogenic role has also been affirmed by its inhibition of tumor-suppressor genes such as FOXO1, TP53, RYBP, FADD (involved in apoptosis), and the telomerase inhibitor has proved the presence of Microcephalin 1 (MCPH1)[15,16]. Moreover, in various types of cancer, including oral squamous cell carcinoma (OSCC), colorectal cancer, malignant melanoma, acute promyelocytic leukemia (APL), and lung cancer, expression decreases and the molecule also plays a tumor-suppressing role by controlling proto-oncogenes such as IGF-1, EGFR, PPFP, MYT1, and CYP1B1. This results in the inhibition of cell growth and proliferation[15]. In the case of prostate cancer, both increased and decreased expressions of miRNA have been observed in different conditions[13]. It has been found that its expression decreases in NSCLC[17,18]. It has also been observed that miR-27a is up-regulated in SV40 ST-transformed human bronchial epithelial cells, which may be indicative of its dual role in lung cancer[19].

The expression of this miRNA at different stages of lung development is different among mice[20]. MiR-27a down-regulates EGFR and MET, and its expression is controlled by MET[17]. The Hsa-mir-27a gene is located at the chromosome region, "19p13.13", in the area of the gene cluster "miR-23a ~ 27a ~ 24-2". These miRNAs are encoded as primary transcripts that are separated after processing via Drosha. Seventy-eight nucleotide precursor-miRNA creates miR-27a after gaining maturity. The three micro-RNAs act in almost the same pathways, and are therefore expressed at the same time. However, it has also been observed that the down-regulation of miR-27a occurs independently of the other two[13]. Single nucleotide polymorphisms (SNP) in the miRNA can affect its processing and expression, as well as its binding to the target mRNA, through which it plays a role in the initiation and progression of various types of cancer, or is associated with drug resistance[21,22]. There are two polymorphisms, namely "rs11671784" and "rs895819" within the loop region of pre-miR-27a. In 2013, the association between rs895819 and decreased expression of miR-27a and increased risk of NSCLC was determined[17]. Hence, the rs11671784 polymorphism within the pre-mir-27a was selected. This study aims to investigate the association between the rs11671784 polymorphism and the risk of lung cancer using the PCR-RFLP technique.

METHODS:

This case-control study approach was studied using blood samples obtained from 110 control subjects without any personal or hereditary sign of lung cancer and 70 volunteer patients suffering from lung cancer. The blood samples were collected from the Omid Hospital of Isfahan. Blood collection tubes containing EDTA anticoagulant were used. Information regarding personal information such as age, gender, smoking status, and type of lung cancer was also collected from existing medical files. All patients were found to be in the metastatic stages III or IV. The genomic DNA was extracted from blood samples using the Miller salting-out method[23]. Spectrophotometry was used to measure the purity and concentration of the extracted DNA. The quality of the extracted genomic DNA was determined using agarose gel electrophoresis.

The PCR-RFLP technique was used to study the rs11671784 polymorphism in the hsa-miR-27a gene. The gene sequence was obtained from the NCBI database and the primers were designed using the Oligo7 software. The best primers were selected based on their length (18-30 nucleotides), the fragment length (200-700 bp), appropriate Tm (55-64˚C), similar Tm (difference less than 3˚C), the percentage of guanine and cytosine bases (40-60%), appropriate ΔG, lack of hairpins, self-annealing, and duplex primers or self-complementary on the 3' base. In order to ensure that coupling of the selected primer occured with no other areas of the human genome, the blast was done using the NCBI database. The designed primers were ordered by the Sepahan Teb. Inc as a forward primer, GGGATTTCCAACCGACCCTG (Tm=64.43˚C), and a reverse primer, GGTCAACCCAGCCTGATACCG (Tm=64.57˚C). The AvaII restriction enzyme cutting the GGwCC sequence was selected from the SGD database.

Polymerase chain reaction (PCR) was performed using the Ependorff Mastercycler AG PCR device in a volume of 25µl using 250ng of genomic DNA, 10pmol of each primer, 2.5µl of PCR buffer (10X), 1µl of 50-mM Magnesium Chloride, 0.5µl of 10-mM dNTP, and an enzyme unit of the SmarTaq DNA Polymerase obtained from CinnaGen, Iran company. DNA amplification was carried out with 5 minutes of initial denaturation at 94°C, and 33 cycles of denaturation at 94°C for 40 seconds, primers annealing at 64°C for 40 seconds, and a 40-second extension at 72°C, with a final extension at 72°C for 10 minutes. Afterwards, 5 µl of the PCR product was transferred to a new vial containing 1µl of Red buffer and 0.2µl AvaII enzyme, brought to a volume of 10 µl. The micro-tubes, containing these materials,

were incubated at 37˚C for 16 hours, and finally, electrophoresis was performed on 1.5% agarose gel.

The genotypes in the patients and control subjects were determined using the RFLP technique. The genotypes and allele frequencies of the rs11671784 polymorphism in the study groups were also calculated. The primers were designed in such a way that a restriction site along with the position of the SNP in the PCR product, was present to control enzyme activity. The bands 367, 258, 120 bp were observed among individuals with the C allele due to the cutting position. Similarly, among individuals with the T allele, due to there being only one cutting site, the bands 488 and 258 bp was observed. In heterozygous individuals with both alleles, bands 488, 367, 258, and 120 bp were observed after digestion. After that, each digested product was loaded onto the agarose gel alongside the primary control products.

Next, the χ^2-test and the odds ratio (OR) with a 95% confidence interval (CI) were calculated as an indicator of the association between polymorphism and the risk of lung cancer, using the Internet service SISA and the SPSS software (version 23), with $p<0.05$ being considered as significant. The Hardy-Weinberg equilibrium was analyzed in the studied groups using Power Marker (version 3.25). The free energy (ΔG) was calculated and the secondary structure of the pre-miR-27a was determined using the MicroRNA SNP Database and the RNAfold web server.

RESULTS:

To measure the purity and concentration of the extracted DNA, the absorption ratio of A260/A280 (nucleic acids to protein absorption ratio) was calculated using the spectrophotometer, which was between 1.8 and 2. The quality of the extracted genomic DNA was determined using agarose gel electrophoresis (**Figure 1**). In

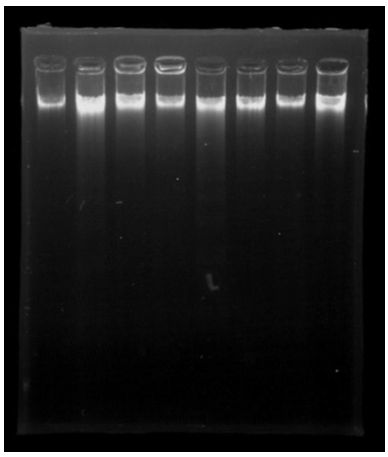

Figure 1. Agarose gel electrophoresis of a number of genomic DNAs extracted from control and patient blood samples on 1% agarose gel at a constant voltage of 100 v. DNA bands were visible through Ethidium bromide staining and UV light.

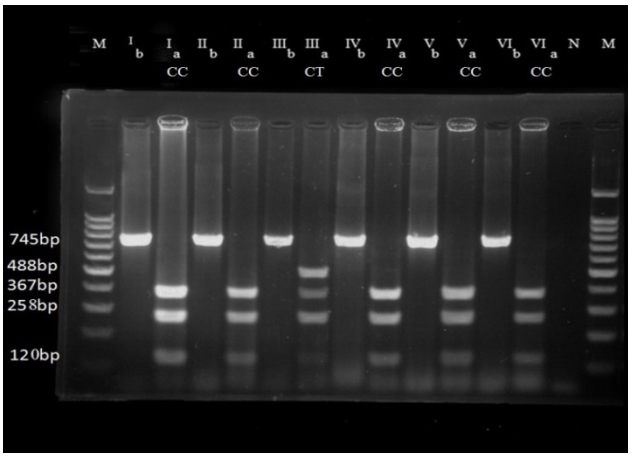

Figure 2. **Genotyping of the rs11671784 polymorphism (C> T) in patients and control subjects using RFLP technique.** M: marker 100 bp. N: negative control - no DNA. a: after digestion. b: before digestion. Homozygous dominant contain have 367, 258, 120 bp bands and heterozygous samples contain 488, 367, 258, 120 bp bands. Examples I to III are from healthy subjects and IV to VI are from patients. Electrophoresis was performed in 1.5% agarose gel at a constant voltage of 60 V.

this polymorphism, participants with the CC and CT genotypes were observed in contrast to the rare genotype "TT" (**Figure 2**).

The genotype and allele frequencies of this polymorphism are expressed in Table 1. According to the calculations, the degree of heterozygosity in the case and control subjects was 0.09 and 0.014, respectively, and the degree of expected heterozygosity (or 2pq in the Hardy-Weinberg equilibrium) was 0.084 and 0.014, respectively. No significant difference was found between observed and expected results. The Hardy-Weinberg equilibrium was tested in this study, based on the Fisher's exact test, using the Power Marker software. The exact p-value was greater than 0.05 among the patients, the control subjects, and the entire studied population. Thus, the allele ratio and homozygous-heterozygous frequency ratio in successive generations are constant and are not affected by population modifiers, including mutation, natural selection, migration, gene flow, and genetic drift.

The odds ratio (OR) of the T allele in the case of lung cancer was equal to 0.15 and the value of p was observed to be less than 0.05, using the internet service, SISA. For the CT genotype, the OR was equal to 0.145 and the p-value was calculated to be less than 0.05. The T allele could reduce the risk of lung cancer 6.7 fold among the subjects that were included in the study. Moreover, the CT genotype could also reduce the risk of lung cancer 6.9 fold (**Table 1**). The frequency of smokers and gender was also calculated among cases and controls, along with its relationship with lung cancer and the polymorphism ($p<0.05$) (**Table 1**).

Associations between genotypes, age, gender, smoking and risk of lung cancer

Variables		Case (%)	Control (%)	Chi-Square value	OR [95% CI]	p-value
Genotype:	CT	1 (1.4)	10 (9)	4.377	0.145 [0.018,1.158]	0.036
	CC	69 (98.6)	100 (91)			
Allele:	**T**	1 (0.7)	10 (4.5)	4.239	0.15 [0.019,1.2]	0.039
	C	139 (99.3)	210 (95.5)			
Gender:	**Male**	45 (69.2)	55 (50)	6.17	2.25 [1.18,4.3]	0.013
	Female	20 (30.8)	55 (50)			
Age:	>50	44 (72.1)	26 (32.1)	22.31	5.48 [2.64,11.35]	2.0E-6
	<50	17 (27.9)	55 (67.9)			
	>60	25 (41)	4 (4.9)	27.82	13.37 [4.33,41.27]	0
	<60	36 (59)	77 (95.1)			
	>70	6 (9.8)	1 (1.2)	5.49	8.73 [1.02,74.5]	0.019
	<70	55 (90.2)	80 (98.8)			
Smoking status:	Yes	30 (50.8)	8 (8.4)	35.25	11.25 [4.64,27.3]	0
	No	29 (49.2)	87 (91.6)			

In order to investigate the relationship between age and risk of lung cancer, Levene's test was performed, using the SPSS software (version 23), to verify the homogeneity of the two communities. The significant value of p (<0.05) represents the non-equal variances and heterogeneity of the studied samples. An independent t-test was performed to compare the means of the two communities that are significant (<0.05) and also to represent the association of age with the occurrence of lung cancer (**Table 2**). In order to investigate the relationship between age and the incidence of the disease, the age frequency of the studied individuals and the correlation between age groups of less and greater than 50 years old suffering from lung cancer was determined, the Chi-square test was applied to the events, and the odds ratio was determined. According to the OR, the risk of lung cancer at the age of 50 or older is 5.475 fold greater. Since the p-value is much smaller than 0.05, this relationship was significant among the studied population. Besides, with a p-value of 0, the risk of lung cancer in people over 60 years is 13.4 times greater than people under 60 years (**Table 1**). In order to investigate the relationship between age and the polymorphism in the study, Levene's test and t-test were performed that showed no association with age of onset and this genetic variation (**Table 2**).

The relationship between the rs11671784 polymorphism with types of lung cancer was evaluated, using the SPSS software and the SISA. Due to the low number of people detected with the kind of lung cancer where the p-value was greater than 0.05, the observed associations weren't significant (**Table 3**). Also, the relationship of the rs11671784 polymorphism with gender was investigated and the p-value was observed to be greater than 0.05, which was a sign of independence of the gender from the studied SNP (see **Table 3**). The number of people suffering from lung cancer is shown in **Table 3**, based on their gender and type of lung can-

Table 2. Levene's test and t-test to evaluate the relationship between age and incidence of lung cancer, alleles and genotypes.

Age	Levene's Test		t-test					
	F	Significant	t	df	Significant	Mean difference	Standard error difference	95% CI
For lung cancer susceptibility Equal variances assumed	5.076	0.026						
Equal variances not assumed			-7.845	138.5	0.000	-15.64926	1.9948	[-19.695,-11.6034]
For rs11671784 alleles Equal variances assumed	0.014	0.906	1.258	240	0.209	6.87821	5.4654	[-3.888,17.6445]
Equal variances not assumed								
For rs11671784 genotypes Equal variances assumed	0.385	0.536	0.068	119	0.946	0.37168	5.44764	[-10.415,11.1586]
Equal variances not assumed								

Table 3. Genotypes associated with gender, smoking, and type of lung cancer

Variables	Case (%)		Chi-Square value	p-value	Smoking		Chi-Square value	p-value
	CC (%)	CT (%)			Yes (%)	No (%)		
Kind of cancer:			1.478	0.7			11.73	0.008
Adeno carcinoma	17 (39.5)	1 (100)	-	-	7 (26.9)	12 (60)	5.101	0.03
SCC	13 (30.2)	0 (0)	-	-	8 (30.8)	4 (20)	0.68	0.37
NSCLC*	6 (14)	0 (0)	-	-	2 (7.7)	4 (20)	1.51	0.24
SCLC	7 (16.3)	0 (0)	-	-	9 (34.6)	0 (0)	12.52	0.005
Gender:			0.422	0.5			22.46	0
Male	40 (70.2)	1 (100)	-	-	28 (93.3)	9 (33.3)		
Female	17 (29.8)	0 (0)	-	-	2 (6.7)	18 (66.7)		
Smoking status:			1.1	0.3				
Yes	24 (47.1)	1 (100)	-	-				
No	27 (52.9)	0 (0)	-	-				

Table 3. Continue...

Variables	OR [95% CI]	Gender		Chi-Square value	p-value	OR [95% CI]
		Male (%)	Female (%)			
Kind of cancer:				6.174	0.01	
Adeno carcinoma	0.246 [0.07,0.8]	10 (27)	9 (64.3)	6.03	0.014	0.2 [0.06,0.76]
SCC	1.78 [0.45,7]	13 (35.1)	2 (14.3)	2.13	0.14	3.25 [0.63,16.8]
NSCLC*	0.33 [0.05,2.4]	6 (16.2)	1 (7.1)	0.706	0.4	2.5 [0.275,23]
SCLC	-	8 (21.6)	2 (14.3)	0.18	0.67	1.66 [0.3,8.96]
Gender:	0.036					
Male	[0.007,0.185]					
Female						
Smoking status:						
Yes						
No						

cer. Considering a p-value lower than 0.05, the risk of adenocarcinoma among men is 0.2 compared to women in the studied population. In other words, female patients in the study were 5 times more at risk of lung adenocarcinoma than men. Moreover, the relationship between smoking and lung cancer was determined (Table 3). As can be observed, only smokers were affected by SCLC ($p<0.05$) and they were diagnosed with lung adenocarcinoma 4 times less than non-smokers (OR=0.25, $p<0.05$). No significant association between smoking and the studied polymorphism could be observed ($p>0.05$).

DISCUSSION:

This study proved that the presence of the recessive "T" allele at the polymorphic position can reduce the risk of lung cancer 6.7 fold (OR=0.15, p=0.039, 95% CI=0.019-1.2). Due to scarcity in the recessive genotype, the results of the CT genotype and the T allele, associated with lung cancer, were almost identical. The association of the SNP with the type of lung cancer was also examined. However, the result wasn't significant due to missing data and the lack of a larger sample size. In fact, only one patient was found with a CT genotype, who was a smoker suffering from adenocarcinoma. In this study, the frequency of the recessive allele in patients was 0.007, which is similar to the global value and the degree of heterozygosity of 0.014 shows a significant difference from the healthy population. The European population frequency of the T allele is 0.018[24]. In this study, the frequency of alleles C and T among control subjects were 0.955 and 0.045, respectively. Recessive homozygous individuals (TT) are rare in this polymorphism. The study also calculated the degree of heterozygosity as 0.09 among control subjects, which is closer to the heterozygosity of 0.04, observed in the European population[24]. Besides, the observed and the expected rates of heterozygosity were almost equal (2pq in healthy subjects and i 0.084 and 0.014 in patients, respectively), which indicated the presence of the Hardy-Weinberg equilibrium in the studied population and was also an indication of the generalization of the results to the next generation. The balance was confirmed with a p-value of higher than 0.05 using the Power Marker software.

Although lung cancer isn't the most common type of cancer in Iran, its numbers are on the rise. On the basis of mortality, lung cancer is the deadliest cancer,and 30% of deaths from cancer happen due to lung cancer. Unlike western countries that diagnose 20-30% of the cases at an early stage, in Iran diagnosis usually occurs after progression and metastasis, and at this stage, the chance of 5-year survival is 3.3%[1]. The age of onset of the disease in Iran is 60, 10 years lower than the average age of developing lung cancer around the world[25]. The strongest association observed in this study is between the age of over 60 years and incidence of lung cancer (OR=13.4 and p=0, 95% CI=4.33-41.27). According to the Knudson multiple-hit hypothesis, several hits (mutations) are necessary for cell transformation and the number of mutations differs in different types of cancer. Gaining five hits in a cell in order to proceed to lung cancer is a time-consuming process, so this type of cancer is known as an aging disease. According to the study, gender is associated with lung cancer and it was observed that studied men are 2.25 times more likely to develop lung cancer than women. The study also examined the relationship between gen-

der and type of cancer. With a p-value of 0.01, studied women are 5 times more likely to develop lung adenocarcinoma than men. In examining the relationship between smoking and lung cancer, the smokers' risk of lung cancer is 11.25 times more than others (p=0). The real rate of the effect of smoking on lung cancer is 25. However, in this study, owing to the small sample size and large amount of missing data, the OR was undervalued. The relationship between smoking and type of lung cancer was also determined. The incidence of lung adenocarcinoma among smokers was observed 4 times less than non-smokers (p=0.03) and only smokers were affected by SCLC (p=0.005), likely due to the large amount of missing data.

In a study conducted by Song et al. in 2014, association of the rs11671784 polymorphism with a reduced risk of gastric cancer was determined in the Chinese population[26]. In 2015, Deng et al. found that the polymorphism is also associated with reduced sensitivity among patients with bladder cancer towards chemotherapeutic drugs by reducing acute myeloid leukemia protein 1 (AML1 or RUNX1)[27].

Polymorphism in the pre-miRNA can influence the ability of Dicer binding. With smaller amounts of pre-miRNA processing, fewer efficient miRNAs can be associated with cancer[28]. This is the pathway, suggested in NSCLC, that shows that the binding of the hepatocyte growth factor(HGF) to the receptor tyrosine kinase, "MET", leads to the activation of ELK1 (transcription factor) and expression of the miR-23a ~ 27a ~ 24-2 cluster. MiR-27a, along with inhibiting EGFR and MET directly via inhibition of Sprouty2 (SPRY2), also inhibits EGFR and MET indirectly, so that MET is controlled by negative feedback[17]. EGFR is a proto-oncogene product that overpowers tumor-specific suppressors such as micro-RNAs. In response to hypoxia, the correlation between EGFR and Argonaute-2 (AGO2) increases so that the phosphorylated tyrosine 393 decreases Dicer binding, micro-RNA processing, and the production of mature micro-RNA. In fact, EGFR works as a regulator of AGO2 through post-translational modification. A large loop in the structure of pre-miRNA is an important regulatory factor in the tyrosine phosphate 393-dependent maturation of miRNA via AGO2[29].

Owing to the different roles of this miRNA in various types of cancer, it is proposed that the present polymorphism affects the AGO2-dependent processing and the expression of miR-27a by changing the loop length of the pre-mir-27a, resulting in a decrease in the risk of lung cancer. The results were observed by testing a small sample size with a low frequency of the SNP. Afterwards, the free energy (ΔG) was calculated. The U allele, compared to the C allele observed in the pre-miR-27a, can reduce ΔG by 0.8 kcal/mol and stabilize the structure, and accordingly, may lead to an increase in miR-27a. Given the role of this tumor suppressor in lung cancer, this polymorphism reduced the risk of lung cancer[30]. The secondary structure of pre-miR-27a with a minimum free energy (MFE) of -37.1 kcal/mol is shown in (**Figure 3**)[31]. As can be seen, the presence of the U allele changes energy levels, resulting in changes in the coupling nucleotides and reducing the loop length of the pre-miR-27a. This decrease can lead to an increase in the processing of this pre-miRNA, whereas increasing the mature miR-27a, which plays a tumour-suppressive role in the NSCLC, may lead to a reduced risk of this type of cancer.

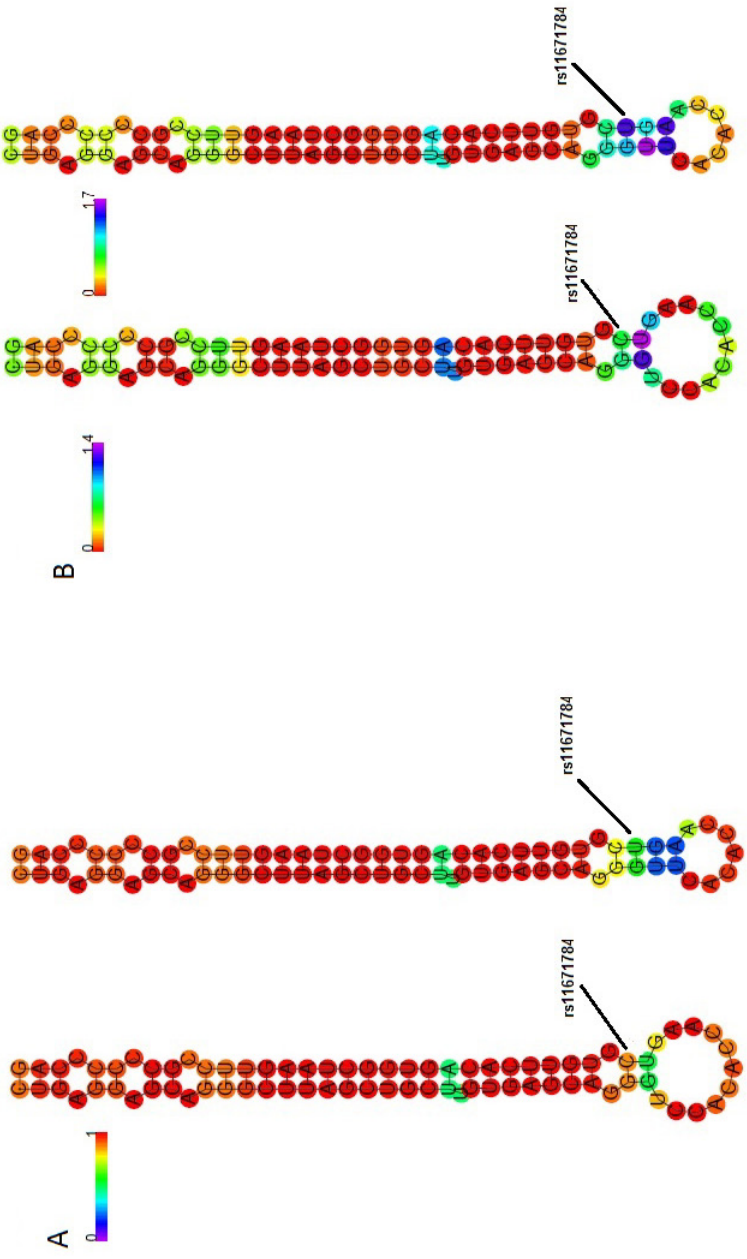

Figure 3. **The secondary structure of pre-miR-27a and the minimum free energy (MEF)**. A: Based on the possibility of base-pairing. Base-pair probabilities between zero and one pair is shown in different colours. B: Based on Positional Entropy. The positional entropy from 0 to 1.4 on the left and 0 to 1.7 on the right, is shown in different colours. U allele (Uracil) decreased the loop length in pre-miR-27a.

There are plenty of stable miRNAs in the serums and other body fluids. Therefore, miRNAs can be used as biomarkers for cancer diagnosis, prognosis, therapeutic effect, prediction of recurrence, and sensitivity to anti-cancer drugs[32]. Through the inhibition of oncogenic miRNAs or decreasing expression using antisense oligonucleotides such as antagomirs (anti-miRs), antisense oligonucleotides methylated on 2' oxygen or locked nucleic acids (LNA), and also by inducing a tumor suppressing miRNA through the transfer of the viral vector AAV (adeno-associated virus), exosomes, plasmids, transposons, and cationic liposomes, cancer progression can be prevented (monoclonal antibodies are provided in cationic liposomes guiding the miRNA to the target organ)[33,34,35,36].

In 2012, Yang and Burwikel proposed DNA sequencing for genotyping rs895819 and rs11671784, since TaqMan allelic discrimination assay appears to be inappropriate for genotyping these adjacent variants with a distance of only 4 nucleotides. They indicated that commercially available pre-designed assays of Applied Biosystems do not consider SNPs with a low allele frequency in their probe design[37]. We recommend the RFLP technique instead of using TaqMan probes.

CONCLUSION:

This study examined the association between the rs11671784 polymorphism and the risk of lung cancer for the first time. In the studied population, the recessive allele "T" can reduce the risk of lung cancer 6.7 fold and be considered as a biomarker of resistance to lung cancer. It is likely that the recessive allele increases the loop length of the pre-miR-27a, which leads to increased AGO2-dependent processing and a higher amount of miR-27a, resulting in a reduced risk of lung cancer. Owing to the small sample size, no significant correlation was observed between the type of lung cancer and the polymorphism. Only one person among the patients was found to carry a recessive allele. Moreover, only the target polymorphism was studied in the DNA of the subjects' blood, whereas a proportion of the disease may be caused by somatic mutations in the patient's lung tissue. It is suggested that the relevance of this polymorphism and different types of lung cancer should be reviewed in larger statistical societies. Besides, the effect of this polymorphism on the expression of miR-27a in the lung cancer cell line, and healthy and cancerous lung tissues should also be evaluated in connection with the U allele and be compared to the verification of the results of the SNP as a biomarker of resistance to lung cancer. Thus, an appropriate treatment can be prepared for patients through changing micro-RNA expression.

CONFLICT OF INTEREST:

The authors have no conflicts of interest to declare.

ACKNOWLEDGEMENT:

The researchers appreciate the support from the Assistant Ministers of Research and Graduate Studies at the University of Isfahan and oncologist Dr. Simin Hemmati. We also thank the patients who took part in the study. We also appreciate the hardworking employees of the Omid Hospital of Isfahan for their cooperation.

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