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Investigation of the Association between a Genetic Variant in MiR-196a-2 Gene and the Risk of Lung Cancer in the Iranian Population

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Abstract

Introduction: MicroRNAs (miRNAs) play an important role in the expression of their target genes. The single-nucleotide polymorphisms (SNPs) in miRNAs may affect their function and expression. The aim of the present study was to investigate the association between miR-196a2 rs116614913 polymorphism and non-small cell lung cancer (NSCLC) in the Iranian population.

Materials and Methods: This case-control study was performed among 103 lung cancer patients and 100 healthy controls. The polymerase chain-reaction restriction fragment length polymorphism (PCR-RFLP) method and direct sequencing were used for miR-196a2 polymorphism genotyping. Statistical analyses were performed using SPSS software and t test method.

Results: According to the findings of this study, there was no significant association between rs11614913 polymorphisms and the risk of lung cancer in codominant model (CT vs. CC: OR=0.67, TT vs. CC: OR=0.74, CT + CC vs. CC: OR=1.133), dominant model (CT+TT vs. CC: OR=0.657) and recessive model (TT vs. CC+CT: OR=0.88). In addition, there was no relationship between the clinicopathological characteristics of patients and controls.

Conclusions: In summary, findings indicated no significant association between miR-196a2 rs11614913 polymorphisms and lung cancer in the Iranian population. Further studies with larger sample sizes are recommended to verify these findings.

Keywords: Lung Cancer, Polymorphism, miR-196a2

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Introduction

Lung cancer is one of the leading causes of death from cancer worldwide and the five-year survival rate of these patients is less than 15%. It is categorized in two main subgroups according to the type of tumor cells; more than 85% of lung cancer cases are classified as non-small cell lung cancer (NSCLC) and the others are small cell lung cancer (SCLC).[1,2] Identification of novel genetic biomarkers for early diagnosis of lung cancer can reduce the mortality rate of the disease.[3,4] MicroRNAs (miRNAs) are small non-coding RNAs and each miRNA can regulate several target genes.[5,6] So far, more than 1000 miRNA have been identified in human beings. They play a role in various physiologic and pathologic processes including cell proliferation, call differentiation, apoptosis and carcinogenesis.[7,8] Single-Nucleotide Polymorphisms (SNPs) located in miRNA genes may affect the expression and final structure of the miRNAs.[9,10] In previous research, a relationship was found between rs712 Polymorphism within let-7 microRNA-Binding site and lung cancer.[11] Rs116614913 (C/T) polymorphism in miR-196a2 gene may affect the final structure of this miRNA and its binding to target genes.[12-14] So far, the association between this polymorphism and several cancers, including NSCLC, head and neck carcinoma and gastric cancer has been reported.[2,15,16] However, there are some contradictory reports about the function of rs116614913 alleles in cancer. Han Y. reported that rs116614913 T allele is associated with a decreased risk of hepatocellular carcinoma (HCC) in the Chinese population.[17] On the other hand, Akkız reported a relationship between the T allele and HCC in Turkish population.[18] Considering the importance of new genetic markers for the early diagnosis of lung cancer and various reports on the role of this polymorphism in the development of cancers in this study, we decided to investigate the association between this polymorphism and lung cancer in the Iranian population.

Materials and Methods

Sample Collection

This case-control study included 103 patients with NSCLC and 100 healthy controls among patients who referred to

Tabriz Emam Reza hospital within seven months from July 2017 until January 2018. Four milliliters whole bloods were collected from all samples and were quickly transferred to the laboratory inside the cool box. Then, the samples whose cancer had been confirmed via pathology underwent DNA extraction and the samples whose type of cancer was not NSCLC were set aside. Written informed consent was obtained from all participants and the study was approved by the Ethical Committee of Tabriz University of Medical Sciences (confirmation code: IR.TBZMED.REC.1397.497).

DNA Extraction and Genotyping

Genomic DNA was extracted from all blood samples using salting out method[19] and the DNA was stored at -20°C. The PCR was performed using the following primers to generate a 149-bp product: forward 5′-CACCCAGCAACCCAAAGTCTA-3′ and reverse 5′- CCCTCGACGAAAACCGACT-3′. The PCR reaction was performed with a total volume of 20-µL mixture containing 50 ng genomic DNA, with 10 µM of both primers, 10 µL 2x PCR master mix and 2 µL deionized water. The PCR conditions were 95°C for 5 minutes, followed by 40 cycles of 30 seconds at 95°C, 40 seconds at 58.7°C, and 5 minutes at 72°C, and a final elongation step at 72°C for 10 minutes. Subsequently, a total 10 µL PCR product was digested using 0.2 µL (10 U/µL) restriction enzymes (Thermo Fisher Scientific, Pittsburgh, PA, USA, for miR-196a-2 and sacI) for 16 hours at 37°C. The digested fragments were analyzed by electrophoresis on a 3% Agarose gel and ten random samples were verified and sequenced by Sanger sequencing (Macrogen Inc. Seoul, Korea) (Figure 1).

Statistical Analysis

Deviations in the distribution of categorical variables of allelic and genotypic frequencies in rs11614913 and rs2910164 polymorphisms were evaluated by Student's *t* test and chi-square test between patients and controls. *P* values under 0.05 were considered statistically significant in all calculations. Statistical tests of this study were done using SPSS software (v.16.0, Chicago, IL).

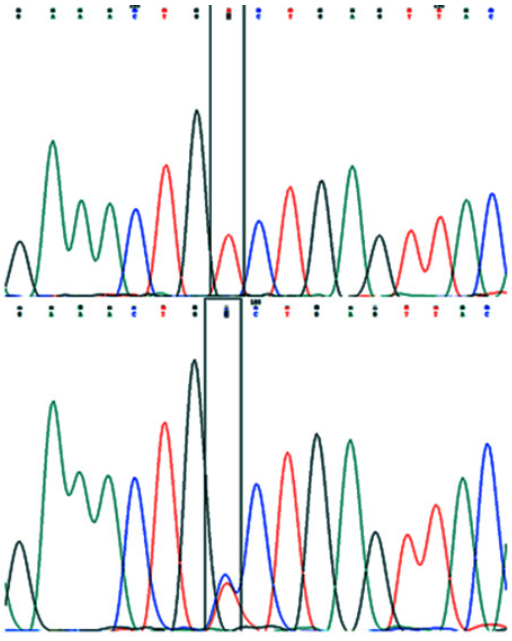

Figure 1. Results of miR196-a2 rs11614913T>C Direct Sequencing. PCR-RFLP was used for rs11614913T>C genotyping and direct sequencing was used for validation of RFLP results.

Results

The Relationship Between the rs116614913 and Clinical Features of Patients

In evaluation of the patient's age, no significant change was observed between patients and controls, ($P > 0.05$). On the other hand, male cases were four times more than female cases ($P = 0.01$). Regarding the type of tumor, patients with squamous cell carcinomas and subsequently adenocarcinoma comprised most of the cases. The mean age of men was 62.26 ± 9.88 and 64.64 ± 10.45 years in case and control groups, respectively, and the mean age of women was 60.13±11.38 and 63.15 ± 9.27 years in case and control groups, respectively (Table 1).

MiR-196a2 Genotypes and Their Association With the Risk of Lung Cancer

The results of determination of rs11614913T > C genotypes by PCR-RFLP and sequencing are shown in Figures 1 and 2. The genotypes of miR196-a2 rs11614913T > C for all patients and controls are shown in Table 2. No significant association was observed between the CT, TT and CC genotypes of rs11614913T>C polymorphism and the risk of lung cancer. The association between different genotype models of rs11614913T>C polymorphism and the risk of lung cancer was investigated as follows: codominant model (CT vs. CC: OR=0.67, TT vs. CC: OR = 0.74, CT + CC vs. CC: OR = 1.133), dominant model (CT+TT vs. CC: OR=0.657) and recessive model (TT vs. CC+CT: OR = 0.88). In addition, there was no significant association between the allelic frequencies of rs11614913T>C polymorphism and the risk of lung cancer (C vs T, adjusted OR = 1.39, 95% CI: 0.77-2.50, *P* = 0. 27) (Table 2; Figure 3).

Discussion

Mutation and polymorphism of MiRNAs genes can change the transcription of miRNAs and may be associated with different diseases and cancers.[20,21] MiR-196a2 consists of two distinct mature miRNAs (miR-196a-3P and miR-196a-5P) that are processed from an early loop. SNPs in miRNAs genes

Table 1. Clinopathological Characteristics of NSCLC Patients and Controls

Characteristic	Patient (n=103)	Control (n=100)	*P* Value
Age (mean ± SD)	61.79±10.22	63.93±9.88	0.13
Male * (%)	80 (77.7)	52 (52.0)	0.01
Female (%)	23 (22.3)	48 (48.0)	0.03
Adenocarcinoma	36 (34.0)	-	
Squamous cells carcinoma	46 (44.7)	-	
Large cells carcinoma	3 (2.9)	-	
Other and unclassified carcinoma	18 (17.5)	-	

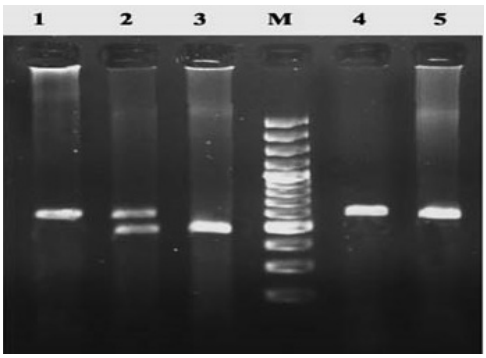

Figure 2. Gel Electrophoresis of the miR196-a2 rs11614913T>C Polymorphism After Enzyme Digestion. D: 100bp DNA marker; Lanes 1, 4 and 5, are TT; Lane 3 is CC and Lane 2 is CT genotype.

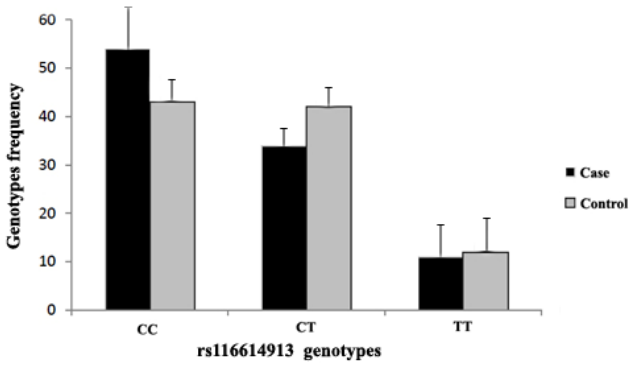

Figure 3. Frequency DIAGRAM of rs116614913 Polymorphism Genotypes in Patients and Control Groups.

can potentially affect the targeting of miRNAs.[22,23] Hoffman et al showed that the expression of mature miR-196a2 in cells with a miR-196a2-C allele increased 9.3 folds, but in cells with pre-miR-196a2-T allele increased only 4.4-fold and the C rs11614913 allele increased the levels of miR-196a2 in lung cancer tissue.[24] In this study, the association between miR196-a2 rs11614913T>C polymorphism and susceptibility of lung cancer was investigated. According to findings, there was no significant association between the rs11614913T>C polymorphism T allele and increased risk of LC (OR = 0.729 $P = 0.139$). In addition, no relationship was found between CT and TT genotypes and the risk of lung cancer. Mir196-a2 can target numerous genes that can contribute to the inhibition of cell proliferation and carcinogenesis.[25] There are several controversial reports about the function of miR196-a2 rs11614913T>C SNP in different cancers. Fang et al reported that miR196-a2 rs11614913T>C polymorphism is associated with lung cancer and significantly affects platinum-based chemotherapy response in Chinese patients.[26] In another study by Yin et al, no correlation was found between miR196-a2 rs11614913T>C polymorphism and the risk of lung cancer in Chinese population.[27] These findings were consistent with the present findings of the present. Parlayan et al reported an association between this polymorphism and colorectal cancer.[28] In a similar study by Hezova et al, no significant relationship was reported between miR196-a2 rs11614913T>C polymorphism and colorectal cancer susceptibility.[29] On the other hand, a recent meta-analysis reported a significant association between miR196-a2 rs11614913T>C polymorphism and lung cancer risk.[30] According to our findings, there was no significant association between the rs11614913T>C polymorphism T allele and CT or TT genotypes and the risk of lung cancer in Iranian population. These results were consistent with the results of Yin et al study on the Chinese population.[27]

Conclusions

In summary, the findings of this study suggest no significant relationship between the miR-196a2 rs11614913 polymorphism and the risk of lung cancer in the Iranian population. There was a sample size limitation in this study. Hence, it is suggested to carry out further studies with larger

Table 2. Distributions of miR-196-a2 Alleles and Genotypes Frequencies Among the Groups

Genotype	Case (%) (n=103)	Control (n=100)	OR (95%CI)	*P* Value
Codominant				
CC	56 (54.4)	45 (45)	1	---
CT	35 (34.0)	42 (42)	0.67 (0.37-1.22)	0.22
TT	12 (11.6)	13 (13)	0.74 (0.31-1.75)	0.50
CT+CC	91 (90)	87(87)	1.133 (0.498-2.577)	0.83
Dominant				
CC	56 (54.4)	45 (45)	1	---
CT+TT	45 (44)	55 (55)	0.657 (380-1.239)	0.13
Recessive				
CC+ CT	91 (88.4)	87 (87)	1	---
TT	12 (11.6)	13 (13)	0.88 (0.38- 2.00)	0.77
Allele				
C	147 (71.3)	132(66)	1	---
T	59 (28.7)	68 (34)	0.729 (0.48-1.10)	0.139

OR= odds ratio and CI= confidence interval for odds ratio.

sample sizes in order to achieve more accurate results. To the best of our knowledge, this is the first study that investigated the association between rs11614913T>C polymorphism and risk of lung cancer in the Iranian population.

Authors' Contributions

ES and MS involved in the conceptualization, design, and methodology of the study; AS participated in patient selection; AD assisted in gene analysis; ES participated in statistical analysis, critically reviewed, and finalized the manuscript.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

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