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The Association Between KRAS rs712 Polymorphism Within let-7 microRNA-Binding Site and Lung Cancer in the Iranian Population

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Abstract

Introduction: Recent studies have indicated the function of polymorphisms in the miRNAs-binding sites within the target genes in cancer incidence. The aim of this study was to investigate the relationship between KRAS rs712 polymorphism within let-7 microRNA-binding site and lung cancer (LC) risk in the Iranian population.

Materials and Methods: This case-control study was performed among 100 patients with LC and 100 healthy individuals. The rs712 polymorphism in KRAS gene promoter was evaluated using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique and direct sequencing. Statistical analysis was performed using SPSS software and t-test.

Results: The findings of this study revealed that rs712, TT and GT genotypes are significantly associated with LC incidence in the Iranian population (adjusted odds ratio [OR] = 3.556, 95% CI = 1.346–9.379, P = 0.01) and (OR = 2.45, 95% CI = 1.274–4.719, P = 0.01), and rs712 T allele was associated with LC incidence (OR = 1.711, 95% CI = 1.147–2.553, P = 0.01). In addition, our analysis showed an association between the rs712 TT genotype and LC metastasis (P = 0.006).

Conclusions: According to our findings, rs712 T allele is significantly associated with the initiation and progression of LC in the Iranian population and it may be served as a LC screening marker in the future.

Keywords: Lung Cancer, Polymorphism, let-7, rs712

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Introduction

Lung cancer (LC) is the leading cause of death from cancer worldwide. Lack of effective screening and diagnostic methods have caused more than 80% of cancer patients to die during the first five years of their diagnosis.[1] The 2 main types of LC are small cell lung cancer (SCLC) with the origin of neuroendocrine cells, which accounts for about 20% of all LCs, and the most common type of LC is non-small cell lung cancer (NSCLC) with the origin of squamous cell cancer and adenocarcinoma, which accounts for 80% of all LCs.[2] Various factors are involved in LC, among which, cigarette smoking and genetic polymorphisms associated with cancer-related genes have been identified as major risk factors for LC.[3,4]

The MiRNAs are a group of non-encoding RNAs with a length of 25-18 nucleotides that are among the most important regulatory factors for gene expression. The MiRNAs perform their regulatory effect on the expression of target genes by pairing with their complementary regions, often in the 3' UTR region of target gene mRNA.[5] They actually regulate about 60% of all coding genes and play an important role in many biological processes, including cell proliferation and differentiation, immunity, cell death and cancer.[6,7] Recent studies have shown that several miRNAs, including mir126 play a major role in the initiation and progression of LC, and the expression of these miRNAs in LC tissues is different from healthy tissues.[8,9] Most of these miRNAs lead to the overlapping of the expression of cancer-related genes and finally, tumor formation by increasing oncogenes expression and reducing tumor suppressor gene expression.[10-12] Let-7 is a 21 nucleotide miRNA, which was first identified in 2000 in the evaluation of genes involved in *Caenorhabditis elegans* differentiation. Let-7 in humans controls the expression of 11 different genes in humans and is known as a tumor suppressor gene, so its decreased expression is associated with an increased risk of LC.[13,14] It is believed that the Let-7 could decrease KRAS expression through a let-7-KRAS binding located at the 3′ UTRs of KRAS, which has been proved to be one of the most frequently activated oncogenes

of most cancers.[15] The presence of G/T single nucleotide polymorphism in the binding region of let-7 miRNA in the KRAS 3′ UTR region disrupts the binding site of let-7 in the KRAS regulatory region and thus increases the expression of this oncogene.[16]

So far, several studies have reported the association between Rs712 polymorphism and the risk of various cancers, including breast, ovarian and LCs.[17,18] Considering the important function of rs-712 polymorphism in regulating KRAS expression by miRNA let-7, the present study was conducted to investigate the association between KRAS rs712 polymorphism and the risk of LC in the Iranian population.

Materials and Methods

Patients

A total of 200 participants were enrolled in the present study. The case group consisted of 100 clinically and pathologically confirmed stage I–III LC patients and 100 healthy individuals (confirmed by a health checkup) were selected as the controls, and were recruited in Masih Daneshvary hospital in Tehran from January 2017 to December 2018. About 3 mL peripheral blood was obtained from each individual and stored at -80°C. The clinical data, such as the individual's age, gender, and status of smoking and drinking and pathological features were collected from each person. A written informed consent was signed by each participant.

DNA Extraction and Genotyping

Genomic DNA was extracted from 200 µL peripheral blood using a standard DNA isolation kit (CinnaGen, Iran) according to the manufacturer's protocol and was stored at -70°C for future use. The let-7a KRAS rs712 polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method using restriction enzyme (Tag1). The primer sequences and reaction conditions were previously described by Takamizawa et al.[19] Digested fragments were analyzed by electrophoresis on 1.5% agarose gel for 60 minutes at 100v. In addition, genotyping accuracy was confirmed by direct sequencing of 5% of PCR products (Genetic analyzer 3130, ABI) . All the sequencing results were found to be completely identical with the PCR-RFLP results.

Statistical Analysis

The obtained data were analyzed using the SPSS software version 22. The observed genotype frequencies were compared between the controls and cases by determining the Hardy-Weinberg equilibrium (HWE) using the chi-square test. Two-sided χ^2 tests were used to evaluate the differences in the genotype and allele frequencies of the rs712 polymorphisms between cases and controls. The association between the *KRAS* rs712 polymorphism and LC risk was expressed as the odds ratio (OR) and 95% CI. The *P* value < 0.05 was considered to be statistically significant.

Results

Genotyping

The PCR products (10 µL) were digested using TaqI restriction enzyme. After enzyme treatment the 426-bp band shows the T allele (undigested), and the 154-bp and 272-bp bands show the G allele (digested).

Characteristics of Study Subjects

The clinical characteristics of 100 cases and 100 controls of this study are summarized in Table 1. The average age was 53.2 ± 10.9 years and 56.1 ± 13.2 years for the LC patients and control subjects respectively. The cases and controls were suggested to be adequately matched in terms of the age and age at menarche. The clinical records of the patients revealed that 78% of the patients were at the stages I and II of tumor and 22% of the patients were at stages III and IV. Patients with well and poor differentiation were 79% and 21%, respectively. The frequency of smokers and non-smokers in the LC group was 37% and 63%, respectively, and was 42% and 58% in the control group, respectively. Also and the distribution difference between the 2 groups was not significant ($P = 0.344$).

Association Between the rs712 Polymorphism and LC

The frequency of genotype and allele of the let-7-*KRAS* rs712 polymorphism are summarized in Table 2. The frequency of GG, GT, and TT genotypes of the let-7-*KRAS* rs712 polymorphism in the LC patients group was 19%, 65% and 16%, respectively, and the genotype frequency in the control group was 38%, 53% and 9%, respectively. There was a significant difference between the 2 groups in the comparison between GT and GG (65% vs 53%, $P = 0.010$, adjusted OR = 2.45, 95% CI = 1.247–4.719). Furthermore, TT genotype in cases was significantly higher than that of controls and it was significantly associated with LC (16% vs 9%, $P = 0.015$ OR = 3.556, 95% CI = 1.346–9.379). The frequency of T allele (48.5% vs 35.5%, $P = 0.011$, adjusted OR = 1.711, 95% CI = 1.147–2.553) was significantly higher in cases compared to the control group (Table 2).

Association Between the rs712 Polymorphism and Clinical Features of LC Patients

The effect of the rs712 polymorphism on the clinical features of LC patients is summarized in Table 2. The rs712 polymorphism was not significantly associated with the N-stage. The TT genotype was not associated with stage III and IV ($P = 0.263$ for TT vs GG), but a significant association was observed between the TT genotype and node metastasis ($P = 0.006$ for TT vs GG). These findings suggest that the T allele of rs712 would be associated with LC progression in the Iranian population.

Discussion

In this study, the association between the let-7-KRAS rs712 and the risk of LC was investigated in the Iranian population. A significant association was found between the KRAS rs712 polymorphism T allele and increased risk of LC. In addition, a relationship was found between TT and GT genotypes and the risk of LC, and our stratified analysis indicated that LC patients with the KRAS rs712 TT genotype were more likely to have the clinical stages III or IV of the disease and metastasis and they were three times more susceptible to

Table 1. Association of Rs712 G/T *KRAS* Gene Polymorphisms and the Risk of Lung Cancer

Model	Genotype	Case (100)	Control (100)	OR and 95% CI	*P* Value
Codominant	GG	19 (19%)	38 (38%)	---	
	GT	65 (65%)	53 (53%)	2.45 (1.274- 4.719)	0.010
	TT	16 (16%)	9 (9%)	3.556 (1.346-9.379)	0.015
Dominant	GG	19 (19%)	38 (38%)	---	
	GT/TT	81 (81%)	62 (62%)	2.613 (1.381- 4.941)	0.005
Recessive	TT	16 (16%)	9 (9%)	---	
	GG/GT	84 (84%)	91 (91%)	0.519 (0.222- 1.216)	0.199
Allele	G	103(51.5%)	129(64.5%)	---	
	T	97 (48.5%)	71 (35.5%)	1.711 (1.147 – 2.553)	0.011

Table 2. Clinical and Pathological Characteristics of Lung Cancer Patients

Variable	Genotype			*P* Value	
	GG	GT	TT	GT vs GG	TT vs GG
Male	10	47	9	0.161	0.979
Female	9	18	7	---	
Stage					
III + IV	3	12	7	0.997	0.263
I+ II	16	53	9	---	---
Metastasis					
Yes	3	12	10	0.751	0.006
No	16	53	6	---	---
Smoking					
Yes	7	28	7	0.792	0.739
No	12	37	9	---	---

LC compared to GG genotypes. These findings support the hypothesis that the let-7-KRAS rs712 may serve as a diagnostic marker for LC screening. For the first time in 2004, Takamizawa et al reported that reduced expression of the let-7-miRNA is associated with LC.[19] Further studies reported that the association between let-7 and LC was due to changes in the expression of *KRAS*, *HMGA2* and *MYC* genes that were applied through let7 miRNA.[20,21] The KRAS is an important human oncogene, which encodes a major member of the small GTPase family that acts as intracellular signal transducer of the MAPK pathway. The oncogenic KRAS mutation, which leads to changes in KRAS expression, is correlated with the incidence and progression of several human cancers including LC.[22,23] The KRAS 3′ UTR contains multiple let-7 complementary sites (LCSs), which enables let-7 to regulate KRAS expression, so the let-7 miRNA family are reported as KRAS negative regulators.[24] It has been reported that variant allele at the LCS6 site truly affects the regulation of *KRAS* expression *in vitro* and increases the expression levels of *KRAS*, so tumors containing the variant allele have lower *let-7* levels and they are associated with prognosis in NSCLC.[25] Kim et al sequenced the complete region of the 3' UTR of KRAS in multiple non-small cell LCs and epithelial ovarian cancers and reported that rs712 may have a functional role in the regulation of KRAS by disrupting the complementary sites of let-7.[26] Peng et al performed a case-control study on rs712 among Chinese LC patients and did not find any significant association between the patients with LC and healthy controls.[27]

Conclusions

Although the precise molecular mechanism of the association between the KRAS rs712 polymorphism and LC is still unknown, according to the findings of this study and also previous reports, it can be concluded that the KRAS rs712 T allele may disrupt the let-7 binding site, which leads to high expression of KRAS and ultimately increases susceptibility to LC. In summary, rs712 within 3′ UTR of KRAS was associated with the initiation and progression of LC and the rs712 T allele may be considered as susceptible and prognostic LC biomarkers for the Iranian population. According to the best of our knowledge, this research was the first study which evaluated the association between this polymorphism and LC in the Iranian population. At the end further studies with more samples are recommended to confirm the findings of this study.

Authors' Contributions

All Authors contributed equally to this research.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

Ethical Approval

The protocol used in this experiment was confirmed by the institutional Medical Ethics Commission of Baqiyatallah University of Medical Sciences.

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