



The Association of *pre-mir-196a2* T/C Polymorphism and Risk of Gastric Cancer in Ardabil, Iran

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Abstract

Micro-RNAs are a large group of non-coding RNAs of 18-25 nucleotides in length. Polymorphisms in the human pre-mir-RNAs could change the efficiency of target cell and can also play a role in cell cycle regulation and cancer. The aim of this study is to investigate the association of rs11614913 T/C polymorphism with gastric cancer risk in Ardabil Province. In a case-control study, this polymorphism was evaluated by PCR-RFLP method. For this purpose, the genomic DNA was extracted from blood samples of 50 subjects with gastric cancer and 50 healthy controls. The 149-bp amplified fragments in PCR were treated with *MspI*. In tumor samples, the frequencies of CC, TC, and TT genotypes were 14%, 40%, and 46%, respectively whereas these ratios were 8%, 28%, and 64%, respectively. The frequency of CC genotype in case group was somewhat different from the control group (14% vs. 8%), But this difference was not statistically significant (p -value = 0.18). In this study, we did not find any significant association between rs11614913 T/C polymorphism and gastric cancer risk in Ardabil Province.

Key words: Pre-mir-196a2; Gastric cancer; Polymorphism

Introduction

MicroRNAs (miRNA) are evolutionary conserved non-coding RNA molecules of 18-25 nucleotides in length. MicroRNAs are implicated in the control of physiological and pathological cellular processes (Hüttenhofer *et al.*, 2005). Recent evidence indicate that pre-mir-196a2 can play as a mediator in a wide spectrum of biological processes, including cell proliferation, differentiation, cell death, immune response and tumorigenesis (Li *et al.*, 2005). In the last decades, the association between single nucleotide polymorphisms and gastric cancer has been investigated worldwide. Recently, a newly discovered class of polymorphisms at miRNAs and miRNA target sites have been reported (McLean *et al.*, 2014). Gastric cancer (GC) is one of the most common cancers and a leading cause of cancer-related death worldwide (Parkin *et al.*, 2002). There is a wide variation in the prevalence of gastric cancer in different areas and Ardabil province has been reported to have the highest incidence rate in the country (Moradpour Hesari *et al.*, 2015). Several mutations that occurred in proto-oncogenes have role in the promotion, development and

progression of gastric cancer (Tahara, 1993). Due to the interactions between genetic and environmental factors, the importance of genetic variations on cancer susceptibility could vary among different populations (Shields *et al.*, 2000). The aim of the present case-control study, is to investigate the effect of pre-mir-196a2 T/C polymorphism on gastric cancer susceptibility in Ardabil province, Iran.

Materials and Methods

Samples

This hospital – based study population includes 50 patients with gastric cancer and 50 cancers – free controls. All cases were newly diagnosed and histopathologically confirmed gastric cancer. Cases were diagnosed with primary gastric cancer and the secondary recurrent tumors were excluded. Controls were age and gender matched healthy volunteers that had no current or previously diagnosed cancer. These information were obtained using a structured questionnaire. The tumor location was obtained from histopathology records of the gastric cancer patients. 5 ml of venous blood from each subject was drawn into a coded tube containing EDTA.

Genotyping

Genomic DNA was isolated from 5 ml of whole blood using a DNA extraction kit (manual Archive pure DNA purification) and stored at -20°C . The Mir - 196a2 polymorphism was determined by polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) using following primers: 3'-CCCCTTCCCTTCTCCTCCAGATA-5', 3'-GCCGAAAACCGACTGATGTAAGT-5'. Briefly, 20 μl PCR mixture containing 300ng genomic DNA with 0.25 μM of both primers, 2 μl 10xPCR buffer, 1.5 mM MgCl₂, 0.1 mM dNTPs and 1U *Taq* DNA polymerase (Cinnagen Co.). The 149bp PCR products were digested using the 5' U of the restriction enzyme *MspI* (Thermo science co.) at 5 $^{\circ}\text{C}$ followed by a run in 3% agarose gel. In the presence of the wild-type T allele, the PCR product remained intact. The PCR products with 149 bp digested with *MspI* into 2 fragments of 125 and 24 bp, respectively. To eliminate any discrepancy, all analyses were performed blindly without the knowledge of the case-control status. In addition, 10% of samples were randomly selected and genotyped in duplicate and the result showed 100% concordant.

Results

Study characteristics

The mean age of the patient group was 66.5 years (range, 37-86 years), and there were no statistically significant differences in the distributions of age and gender between cases and controls. The mean age of controls was 62.1 years (range, 45-80 years). The characteristics of the participants are presented in Table 1. Table 2 shows the distribution of CC genotype and its statistical relationships with TC, TT, TT+TC, and CC+TC among the case and control groups. The distribution of this polymorphism in the control group was in Hardy-Weinberg equilibrium. The frequency of genotypes was 7 (14%), 20 (40%) and 23 (46%) among cases and 4 (8%), 14 (28%) and 32(46%) among controls for C/C, T/C, and T/T, respectively. The 149bp amplified fragment was digested with *MspI* (Thermo science co.). The intact fragment indicates the T allele. However, substitution of C allele by T allele tends to create an *MspI* restriction site. Thus, digestion of the C allele yielded 125 and 24bp fragments.

Table 1. General characteristics of participants.

| Characteristics | Case (n=20) | Control (n=50) |
|---------------------|-------------|----------------|
| Age \leq 50 Years | 4(8%) | 7(14%) |
| Age >50 Years | 46(92%) | 43(86%) |
| Gender | | |
| Female | 18(36%) | 14(28%) |
| Male | 32(64%) | 36(72%) |

Table 2. The *hsa-mir-196a2*T/C genotype distribution OR: Odd ratio; CI: Confidence interval

| Genotypes | Cases (%) | Controls (%) | OR (95% CI) | P Value |
|-----------|-----------|--------------|-----------------|---------|
| CC | 7 (14%) | 4 (8%) | 0.4 (0.1-1.6) | 0.18 |
| TC | 20 (40%) | 14 (28%) | 0.5 (0.2-1.2) | 0.12 |
| CC+TC | 27 (54%) | 28 (56%) | 0.8 (0.4-1.5) | 0.45 |
| TT+TC | 43 (86%) | 46 (92%) | 0.45 (0.2-0.99) | 0.049 |
| TT (ref.) | 23 (46%) | 32 (64%) | 1.00 | - |

If the quantity of restriction enzyme used is inadequate, the homozygous cases may be detected as the heterozygotes. So, to evaluate the accuracy of the restriction experiment, some sequenced samples selected to repeat the

test. All the statistical analyses were performed using SPSS software. The frequency of pre-miR-196a₂ T/C genotypes in the case group were significantly different from those in the control group ($p=0.18$).

Discussion

SNPs are the most common genetic sequence variations in the human genome that affect sequences, coding and splicing, and can influence the cancer susceptibility in population (Nikzad *et al.*, 2015; Soleimani *et al.*, 2016; Karimian and Hosseinzadeh Colagar, 2016). MiRNAs are involved in important biological processes including differentiation, proliferation, apoptosis, angiogenesis and immune response (Shenouda and Alahari, 2009). They can affect miRNA function by modulating the transcription of the primary transcript, pri-miRNA and pre-processing and maturation, or miRNA-mRNA interactions, which could possibly contribute to cancer susceptibility (Ryan *et al.*, 2010; Wiemer, 2007). For miR-196a2 significant association of gastric cancer risk was found in overall analysis (Wang *et al.*, 2012). The high incidence of gastric cancer in Ardabil province, Iran, encouraged us to follow the predisposition and susceptibility factors, including gene polymorphisms. The T/C polymorphism results in a reduction in enzyme activity. The frequency of pre-miR-196a2 T/C genotypes in the case group were significantly different from those in the control groups ($p=0.18$). The frequency of genotypes was 7 (14%), 20 (40%) and 23 (46%) among cases and 4 (8%), 14 (28%) and 32 (46%) among controls for C/C, T/C, and T/T, respectively. The 149 bp amplified fragment was digested with *MspI* (Thermo science co.). The intact fragment indicates the T allele. However, substitution of C allele by T allele tends to create an *MspI* restriction site. Thus, digestion of the C allele yielded 125 and 24 bp fragments. If the quantity of restriction enzyme used is inadequate, the homozygous may be detected as heterozygote, therefore, some sequenced samples were chosen for evaluating the accuracy of digestion. All the statistical analyses were performed using SPSS software. The frequencies of pre-miR-196a2 T/C genotypes in the case groups were significantly different from those in the control groups ($p=0.18$).

Although the incidence of the clinically diagnosed CC genotype patients was higher than the control group (14% versus 8%), but this difference was not statistically significant.

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بررسی ارتباط بین پلی مورفیسم *T/C pre-mir-196a2* و خطر سرطان معده در اردبیل، ایران

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چکیده

میکرو RNAها زیر گروه بزرگی از RNA های غیر کد کننده ۱۸-۲۵ نوکلئوتیدی هستند. پلی مورفیسم های موجود در ژنوم *pre-mir-RNA* -*Mir* انسانی می تواند کارایی سلول هدف را تغییر دهد و همچنین می تواند در تنظیم چرخه سلولی و در سرطانزایی نقش بازی کند. هدف از این مطالعه، بررسی ارتباط پلی مورفیسم *T/C rs11614913* در ژن *Mir-RNA-196a2* و خطر سرطان معده در استان اردبیل می باشد. در این مطالعه موردی-شاهدی این پلی مورفیسم با استفاده از روش *RFLP-PCR* بررسی شد. برای این منظور از نمونه های خون ۵۰ بیمار مبتلا به سرطان معده و ۵۰ فرد سالم استخراج *DNA* صورت گرفت. قطعه تکثیر شده ۱۴۹ جفت بازی محصول *PCR*ی بوسیله آنزیم *MspI* تیمار شد. فراوانی ژنوتیپ های *CC*، *TC* و *TT* بصورت ۱۴٪، ۴۰٪ و ۴۶٪ در میان بیماران و ۸٪، ۲۸٪ و ۶۴٪ در میان نمونه های کنترل بود. فراوانی ژنوتیپ *CC* در گروه مورد، تا اندازه ای متفاوت از گروه کنترل بود (۱۴٪ در مقابل ۸٪) اما این تفاوت از لحاظ آماری معنی دار نبود ($p\text{-value}=0.18$). در این مطالعه بین پلی مورفیسم *rs11614913* *T/C* و خطر ابتلا به سرطان معده در استان اردبیل ارتباط معنادار پیدا نشد.

واژه های کلیدی: سرطان معده؛ میکرو آر.ان.ای ۱۹۶؛ پلی مورفیسم

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