Research Article

MicroRNA Binding Site Polymorphisms are Associated with the Development of Gastric Cancer

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Abstract

MicroRNAs (miRNAs) binding to the 3'-untranslated regions (3'-UTRs) of messenger RNAs (mRNAs), affect several cellular mechanisms such as translation, differentiation, tumorigenesis, carcinogenesis and apoptosis. The occurrence of genetic polymorphisms in 3'-UTRs of target genes affect the binding affinity of miRNAs with the target genes resulting in their altered expression. The current case-control study of 100 samples (50% cancer patients and 50% health persons as control) was aimed to evaluate the genotyping of microRNA single nucleotide polymorphisms (SNPs) located at the binding site of the 3'-UTR of C14orf101 (rs4901706) and mir-124 (- rs531564) genes and their correlations with gastric cancer (GC) development. The Statistical analysis results indicated the significant association of AG development risk with the SNPs located in the 3'-UTR of C14orf101 and mir-124. It could be concluded from the results that these genes are associated with the gastric adenocarcinoma.

***Keywords*:** Carcinogenesis, C14orf101 polymorphism, Mir-124, Gastric cancer development

Introduction

Gastric cancer is regarded as one of the most common malignancies in the world and is the second leading cause of death around the world. According to the World Health Organization (WHO), around one million people in the world were diagnosed with gastric cancer in 2012, and about 70% live in developing countries, especially in eastern Asia, accounting for 6.8% of all cancers. About 50% of gastric cancers are malignant. There are also 723,000 deaths from gastric cancer every year, equivalent to 8.7% of all deaths (Abediankenari et al., 2013).
Considering the high prevalence of gastric cancer in Khorasan Razavi province of Iran and its genetic variations with other regional countries, the current study has been designed to conclude the effectiveness of GC treatment and prevention in the region (Barni et al., 1988). In this study, genomic DNA of 50 gastric cancer patients and 50 healthy controls was extracted from paraffin blocks in gastric. Thereafter, genotyping of C14orf101 and mir-124 genes was performed using PCR-RFLP and statistical analysis using Medcalc software.

The alternative name of C14orF101 is TMR260 (NCBI gene number; rs4901706) which is responsible to translate a transmembrane protein, TMEM260, located on the short arm of chromosome 14 (Compare et al., 2010).The Mir-124 gene (NCBI gene number; rs531564) is located on the short arm of chromosome 8. In many studies, the altered expression of Mir-124 has been observed having no effect on the differentiation of the neuronal cells but affects the types of cancers, especially gastrointestinal (GI) cancers (Tepes, 2009).
The current study was aimed to evaluate the association of MicroRNA binding sites polymorphisms with the development of gastric cancer. For this purpose, 50 cancer patients were included in this study along with the 50 normal healthy persons as control.

Materials and Methods

Case-control study was designed to investigate the relationship between miRNA polymorphisms in the 3'-untranslated regions (3'-UTRs) of C14orf101 and mir-124 genes of

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the under treatment gastric cancer patients at Ghayem Hospital, Mashhad, Khorasan Razavi, Iran. 50 confirmed patient samples of 2017 (5 women and 45 males aged 50-65 having gastric adenocarcinoma) were taken from pathology department after consultation with the statistics expert in the form of paraffin blocks. Along with the cancer patients samples, 50 paraffin blocks of healthy subjects (those whose pathology results are not difficult and non-cancerous) were also taken as control. Paraffin tissues were dehydrated and genomic DNA was extracted as per kit manufacturer's instruction (ParsTus DNA extraction kit, Cat No.A101211).

Primers Used

The primers were synthesized by Bioneer Company, South Korea as described in Table 1 (Li et al., 2012).

Table 1. Primers Used

Gene	RS	Primer Sequences	Enzyme	PCR Product Size
c140f101	Rs - 4901706	Forward:5-CTGAAGTG CTGTTATCG GAAACC-3 Reverse :5-GCCCTTAC ACAGTTCA TGACCAA-3	Bsp143 (Sau3)	494= 314+ 180
mir-124	Rs-531364	Forward: 5-CATTGTCTG TGTGATTG GGGGA-3 Reverse : 5-AAACACAG TCACGGAG GAAGGTG-3	Alw26I (BsmA)	682= 500+182

To determine the precise binding locus of C14 orf101 and mir-124 genes, their sequences were first determined by NCBI and primers were designed using GeneRunner software and their specificity were determined via BLAST protocol. NEB Cutter software was used to determine the limited enzymes for RFLP reactions.

PCR reaction were performed for locus of C14 orf101 and mir-124 genes according to the PCR amplification protocol shown in table 2. All PCR amplification reactions were conducted for 36 cycles at 3 phases (denaturation, annealing and extension)

Table 2. PCR amplification protocol

PCR Amplification Protocol for c14orf101			
Denaturation	94°C	5 min	P1
	94°C	30 s	Cycles: P2+36
Annealing	64°C	40 s	
Extension	72°C	40 s	
	72°C	10 min	P3

PCR Amplification Protocol for Mir -124			
Denaturation	94°C	5 min	P1
	94°C	30 s	Cycles: P2+ 36
Annealing	65°C	40 s	
Extension	72°C	40 s	
	72°C	10 min	P3

Result

Evaluation of PCR by Electrophoresis

RFLP-PCR is a method that has been tested for PCR production in the presence of limited enzymes and the size of the components created using gel electrophoresis was checked alongside the marker (Lerner et al., 1958).

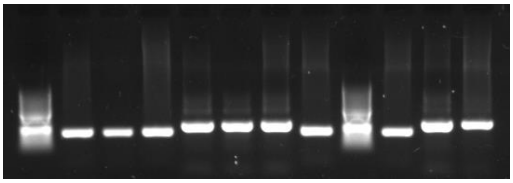


Figure 1. Agarose gel 1% extruded genomic DNA of the patient

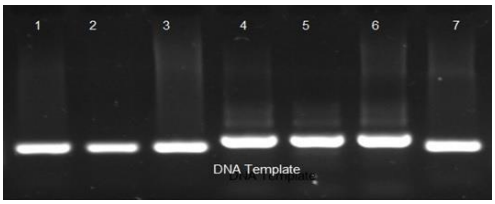


Figure 2. Agarose gel 1% extruded genomic DNA from healthy people

Results of Polymerase Chain Reaction (PCR) Promoter of C14 orf101 and Mir-124 Genes:

After examining the quality of DNA extracted from the control and patients, polymerase chain reaction (PCR) was performed based on the protocol. PCR product of C14orf101 and mir-124 has been yield as a 500-bp and 700-bp fragment, as shown in Figure 3 and 4. These fragments contain the

region in which the desired polymorphism occurs.

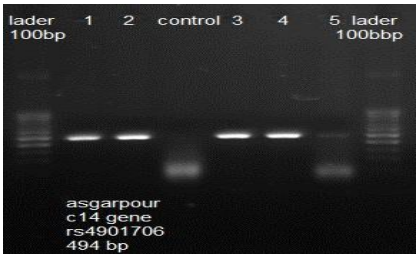


Figure 3. Agarose gel electrophoresis of the PCR product of C14orf101 gene.

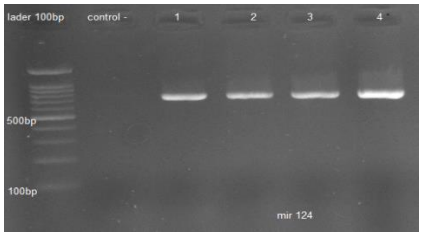
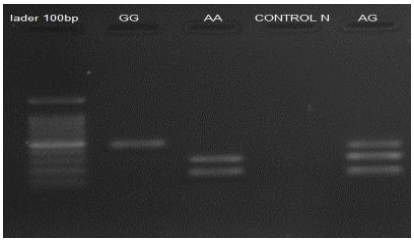


Figure 4. Agarose gel electrophoresis of the PCR product of mir-124 gene

Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP)

The primers sequences and the restriction enzyme for analysis of of C14orf101 and mir-124 gene polymorphisms were described in table 1. Figure 5 shows results from PCR-RFLP.

A



B

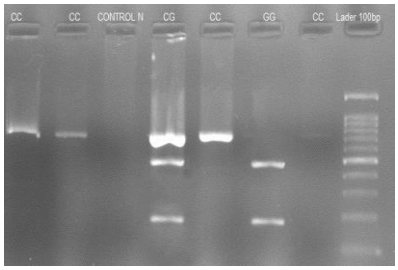


Figure 5. RFLP of C14orf101 (Rs -4901706), and mir-124 (Rs-531364). A: For C14orf101 Rs -4901706 gene polymorphism, the wild type homozygous alleles yielded a 494-bp products, while the mutated type homozygous and heterozygous alleles yielded a 184-314-bp and 182-314-494 bp respectively (B) For mir-124 (Rs-531364) gene polymorphism, the wild type homozygous alleles yielded a 682-bp product, while the mutated type heterozygous and homozygous alleles yielded 182-500-682 bp and 182-500bp products respectively.

Statistical analysis

Table 3 showed the association between the SNPs and cancer risks. For mir-124, patient carrying homozygous CC genotype are 33%. We have been found that the heterozygote CG and homozygote GG are 60% and 7%. Results for control group of the mir-124 gene is described in Table 3.

Table 3. Distribution of different genotype for mir-124 gene in cases and controls.

	Number of patients (45)		
	Genotype	Frequency	Number of Genotypes
Wild type homozygous genotype	CC	15	33%
Wild type heterozygote genotype	CG	27	60%
Mutant type Homozygote genotype	GG	3	7%
	Number of Controls (48)		
	Genotype	Frequency	Number of Genotypes
Wild type homozygous genotype	CC	26	54%
Wild type heterozygote genotype	CG	22	46%
Mutant type Homozygote genotype	GG	0	0

Relationship between genotype of mir-124 polymorphism and gastric cancer diseases has been investigated with Medcalc software. Difference in genotypic frequency between control and patient groups has been studied by Chi-square test.

The value of Chi- square=6/371 with p-value=0.0414 has been obtained. Therefore, according to p-value≤0/05, there is a significant difference in the distribution of the desired polymorphism between the control and patient groups.

In analyses of c140f101 gene polymorphism, we have been found patient carrying homozygous GG, heterozygote GA and homozygote AA genotype are 60%, 30% and 10%. Also, results for control group of the mir-124 gene is described in Table 4.

Table 4. Distribution of different genotype for c140f101 gene in cases and controls.

Number of patients (45)			
	Genotype	Frequency	Number of Genotypes
Wild type homozygous genotype	GG	27	60%
Wild type heterozygote genotype	GA	14	30%
Mutant type Homozygote genotype	AA	4	10%
Number of Controls (48)			
	Genotype	Frequency	Number of Genotypes
Wild type homozygous genotype	GG	43	90%
Wild type heterozygote genotype	GA	5	10%
Mutant type Homozygote genotype	AA	0	0

The relationship between genotypic polymorphism in Table 9 and gastric cancer was assessed by using the Medcalc software. Chi-square test was used to determine the genotypic frequency between two control and patient groups. We obtained Chi-Square= 811.11 with p-value=0.0027. Therefore, considering p-value <0.05, there is a significant difference in the distribution of desired polymorphism between the control and patient groups.

Discussion

Gastric cancer is one of the most common cancers and secondary causes of cancer deaths in the world. Usually, the risk of developing gastric cancer is higher among people with poor nutrition (Babaei et al., 2010). Immigration is one of the most significant factor associated with the risk of stomach cancer indicating that lifestyle factors such as nutritional habits etc are the determinants of stomach cancer (Jemal et al., 2010). It also has been proven that genetic background and polymorphism of alleles in different races are associated with cancer progression. Polymorphism of C14orf101 and mir-124 genes has also been observed to be linked with gastric cancer malignancy (Lin et al., 2015). Considering this relationship (Siewert and Maruyama, 1995), the current research was designed in Mashhad city of Khorasan Razavi with the clinical assistance of Ghayem Hospital. In a similar study performed in 2014 by Mangan Wang and Ping in China, mir-124 genus with rs531564_was found in gastric cancer patients and absent in healthy persons. Therefore, according to the research carried out, the results of the current study are in consistence with the previous published work but discussing the gene behavior in Khorasan Razavi population.

Conclusion

The results of this study showed that the polymorphism of the C14orf101 and mir-124 genes are related to gastric adenocarcinoma and screening of the polymorphisms of this gene could be an important factor in the prognosis of disease, prevention of disease progression, as well as the use of appropriate therapeutic approaches to increase longevity and improve quality life in patients with gastric cancer (Lin et al., 2015).

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