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Mir146a Polymorphism in Gastric, Colon and Rectum Cancers#

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Research Article

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ABSTRACT

miRNAs are short non-coding RNAs and are involved in many mechanisms in cancer. It is possible that miRNAs act as oncogenes or tumour suppressors. miRNAs are also known to play a role in processes such as inflammation, apoptosis, cell growth and differentiation in cancer. In this study, it was aimed to determine the polymorphism of the mir146a gene in stomach, colon and rectum cancers, which are frequently seen in the Turkish population. For this purpose, rs2910164 and rs2961920, which are single nucleotide polymorphisms occurring in the mir146a gene, were investigated in blood samples taken from a total of 212 patients with 73 stomach cancer, 76 colon cancer and 63 rectum cancer, and 77 healthy control individuals. Polymorphism analysis was carried out using the RT-PCR method. Statistical analysis of the obtained data was analyzed using Khi ($\chi 2$) and logistic regression tests. Significant results were obtained when patients who consumed alcoholic beverages were compared with controls ($p<0.05$). In the Turkish population, rs2961920 polymorphism of the mir146a gene was found to be more significant in gastric and colon cancers compared to controls ($p<0.05$). It was determined that there was a significant correlation between GG+CG and CC genotypes and rs2910164 polymorphism of the mir146a gene in individuals with gastric cancer in the same population ($\chi 2$: 5.49 p: 0.019). Again, a significant correlation was found between rs2910164 polymorphism and GG+CC and CG genotyping in gastric cancer ($\chi 2$:5.39, p: 0.020). As a result, it was determined that it may be effective in terms of stomach and colon cancer with the single nucleotide polymorphisms in the mir146a gene in the Turkish population. It is thought that the possible roles of the mir146a gene in cancer should be supported by further studies.

Keywords: Colon cancer, Gastric cancer, mir146a, polymorphism, Rectum cancer, Turkish population

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Introduction

Cancer is defined as the uncontrolled division of cells by the combination of many complex processes (Farber, 1984). It is thought that environmental factors that can affect the genetic structure may affect genetic changes and carcinogenesis in colons that are genetically susceptible to cancer development (Murray, 1996). In Turkey, deaths from cancer take second place after cardiovascular diseases. The most common types of cancer are listed as lung, breast, prostate, colorectal and stomach cancers. The worldwide incidence of colorectal has been estimated to be higher in women than in men (Karahasanoğlu, 2011). Colorectal cancers occur both as hereditary (~5%) and sporadic (~95%). Gastric cancer ranks fourth among other cancers in the matter of incidence and mortality worldwide. The incidence of gastric cancer has been estimated to be higher in men than in women (Karahasanoğlu, 2011). Recent studies have noted that single-stranded RNAs molecule (miRNAs) are included in the modulation of many basic cellular functions. It has also been shown that outside of normal conditions, miRNA levels in the cell are associated with the development of cancer in humans (Wijnhoven et al., 2007). It is known that miRNAs are short, non-coding RNAs that are amplified

from exon and intron regions in the genome, and do not turn into proteins, but have many roles in the organism (Kim, 2005; Saydam et al., 2011). miRNAs can prevent protein formation by causing mRNA degradation. Thanks to this feature, miRNAs can regulate the pathways related to the development, proliferation, invasion, cell cycle and apoptosis of cells in cancer. In case of disruption of miRNA functions, it can cause diseases such as cancer (Ruvkun, 2001). Changes in protein-coding oncogenes or tumour suppressor genes cause cancer. In recent years, it has been reported that the genetic causes of cancer are more complex with the demonstration that miRNAs are also effective in tumour formation (Calin and Croce, 2006). More than 50% of cancer-associated genomic domains or fragile regions are composed of genes encoding miRNA, suggesting that miRNAs are important in the pathogenesis of cancer (Calin et al., 2004). It has been demonstrated that *mir146a* has a role in the control of the inherited immune response and future immunological responses. (Jazdzewski et al., 2008; Xu et al., 2008; Shen et al., 2008; Hu et al., 2009; Hauptman et al., 2013).

In this study, we aimed to analyse the relationship between *mir146a* (rs2961920 and rs2910164) polymorphism and colorectal and gastric cancers in our population.

Material and Methods

Study Population

The sanction obtained from the local ethics committee for conducting research was acquired and an informed consent form from the volunteers before collecting biological material was also received. Volunteers were directed to a form containing demographic information, which includes age, gender, occupation, tobacco use, family history of cancer, and alcohol consumption. In our study, individuals with 73 gastric cancer (GC), 63 rectum cancer (RC), and 76 colon cancer (CC) who appealed to Sivas Cumhuriyet University Faculty of Medicine, Department of General Surgery (Sivas, Turkey) were comprised in the patient group, and 77 healthy individuals were included in the control group. GC, RC and CC were diagnosed according to WHO guidelines and histological classification was done (https://www.iarc.fr/en/publications/pdfs-online/pat-gen/bb2/bb2-chap3).pdf. Healthy individuals without any chronic disease, living in the same region and with no family history of cancer were included in the control group, regardless of gender and age. All volunteers included in the study are individuals born and living in Turkey.

DNA Isolation

DNA isolation for polymorphism analysis was performed from approximately 2 ml of peripheral blood taken from the patient and control groups into a citrate tube. The DNA isolation process was made from whole blood as soon as the blood reached the laboratory (Gong et al., 2012).

Mir146A genotyping

Hydrolysis probes were used for the identified rs2910164 (C/G) and rs2961920 (A/C) polymorphisms of the mir146a gene. A Real-time Genotyping kit (PrimerDesign Ltd., Southampton, UK) was provided for the single nucleotide polymorphism (SNPs). Initial denaturation is required for reverse transcription polymerase chain reaction (RT-PCR) for 8 minutes at 95°C, 15 cycles of continued denaturation at 95°C for 10 seconds, 60 seconds of extension at 60°C, followed by denaturation at 95°C. Further, 10-second 40 cycles and the other extension at 68°C for 60 seconds were done. Fluorogenic reads obtained from RT-PCR were obtained from two channels, viz yellow and orange. RT-PCR products were amplified. Then, allelic discrimination testing was performed.

Statistical Analysis

The analysis of the obtained data was applied using a statistical package for the social sciences program (SPSS), viz statistical analysis software (version 22.0; SPSS, Inc., Chicago, IL, USA). Odd ratios (ORs) and confidence intervals (95%) of the polymorphism data were calculated by unconditional logistic regression analysis. In addition, logistic regression was used to evaluate the relationship between genotypes, age and gender. Using the Student's test, demographic information from the patient and control groups was examined. To test the deviation from Hardy-Weinberg equilibrium, $\chi 2$ or Fischer's exact test (two-sided) was used to determine sex distributions, correlation with alleles and genotypes relative to controls. Pearson's $\chi 2$ test was used to detect differences in genotype frequencies and allelic status between volunteers in the control and patient groups.

Results

Demographic Characteristics of the Populations Studied

In this study, the gastric, colon and rectum cancer patient groups, as well as the control group, were composed of 73, 76, 63 and 77 individuals respectively. The responses of patients and controls to the questions and the use of empirical data were applied in a statistical analysis of the relationship between mir146a rs2961920 and rs2910164 polymorphism and gastric, colon and rectal cancer. The characteristics of gastric, colon and rectal cancer patients and controls such as gender, age range, age average, smoking status, alcohol use and family history of cancer are given in Table 1.

Table 1. Characteristics of the Colon, Gastric, and Rectum cancer patients and controls

Variable	Controls n(%)	Colon Cancer n(%)	Rectum Cancer n(%)	Gastric Cancer n(%)
Sample size	77	76	63	73
Sex				
Males	41 (53.2)	39 (51.3)	33 (52.4)	60 (82.2)
Females	36 (46.8)	37 (48.7)	30 (47.6)	13 (17.8)
Age(year)				
Range	48-90	34-85	43-83	40-85
Means±SD				
Males	63.07±6.12	62.10±9.97	66.00±9.34	60.28±9.85
Females	66.55±9.32	66.81±10.77	63.86±10.11	60.15±12.60
Smoking History				
Smoker	28 (36.4)	22(28.9)	22 (34.9)	35 (47.9)
Males	25 (89.3)	20 (90.9)	20 (90.9)	33 (94.2)
Females	3 (10.7)	2 (9.1)	2 (9.1)	2 (5.8)
Alcoholic Drink Consumption				
Yes	2 (2.6)	16 (21.1)	8 (12.7)	20 (27.0)
Males	2 (100.0)	13 (81.2)	6 (75.0)	18 (90.0)
Females	0 (0.0)	3 (18.8)	2 (25.0)	2 (10.0)
Family history of cancer	16 (20.8)	9 (11.8)	8 (12.7)	7 (9.5)

Comparison of Alcohol Consumption of Gastric, Colon, Rectum Cancer Patients and Controls

According to the alcohol consumption patients, when the gastric cancer patients and the control group were analyzed, it was determined that the frequency of alcohol use in the patients was 6.5% and 2.6% in the controls. Thus, the alcohol consumption rates of individuals in the patient group were found to be higher. A statistically meaningful difference was observed between the two groups (**χ2: 11.79, p: 0.001**). Alcohol consumption has been identified as a risk factor (**OR:5.43 % 95 CI: 1.91-15.40**) (Table 2). Similarly, the use of alcohol in colon cancer was also found to be a risk factor (**χ^2:12.55, *p*: 0.001**) (**OR: 10.00 % 95 CI: 2.21-45.20**) (Table 2). Finally, it has been determined that the use of alcohol is a risk factor in rectum cancer (**χ^2: 5.31, *p*: 0.021**) (**OR: 5.45 % 95 CI: 1.11-26.69**) (Table 2).

Table 2. Interaction between colon, gastric and rectum cancer and smoking, drinking habit and family history of cancer

	Controls (n:77)	Colon Cancer (n:76)	Rectum Cancer (n:63)	Gastric Cancer (n:73)
Smoking Habit				
Smokers (%)	28 (36.4)	22 (28.9)	22 (34.9)	35 (47.9)
Nonsmokers n (%)	49 (63.6)	54 (71.1)	41 (65.1)	38 (52.1)
χ 2	Ref.	0.95	0.03	0.93
p	-	0.328	0.859	0.333
Crude OR (%95 CI)	-	0.71 (0.36-1.40)	0.93 (0.46-1.88)	0.72 (0.38-1.38)
Adjusted* OR: (%95 CI)	-	0.35 (0.13-0.98)	0.82 (0.31-2.16)	-
Alcoholic Drink Consumption				
Yes (%)	2 (2.6)	16 (21.1)	8 (12.7)	5 (6.5)
No (%)	75 (97.4	60 (78.9)	55 (87.3)	72 (93.5)
χ 2	Ref.	12.55	5.31	11.79
p	-	**0.001***	**0.021***	**0.001**
Crude OR (%95 CI)	-	**10.00 (2.21-45.20)**	**5.45 (1.11-26.69)**	**5.43 (1.91-15.40)**
Adjusted* OR: (%95 CI)	-	-	-	-
Family History of Cancer				
Yes (%)	16 (20.8)	9 (11.8)	8 (12.7)	7 (9.6)
No (%)	61 (79.2	67 (88.2)	55 (87.3)	66 (90.4
χ 2	Ref.	2.23	1.59	3.61
p	-	0.135	0.207	0.057
Crude OR (%95 CI)	-	0.51 (0.21-1.24)	0.55 (0.22-1.39)	0.39 (0.15-1.03)
Adjusted* OR: (%95 CI)	-	0.50 (0.18-1.37)	-	-

Polymorphism Analysis of mir146a in Gastric, Colon and Rectum Cancer Patients

Polymorphism analysis of mir146a rs2961920

In patients with gastric cancer, it was determined that genotypes in the direction of *mir146a* rs2961920 were 0 (0%) AA, 2 (25%) AC and 6 (75%) CC. The genotypes of the individuals in the control group were found to be AA (45.7%), 31 (44.3%) AC and 7 (10. 0%) CC. When gastric cancer patients and controls were analysed with *mir146a* rs2961920 polymorphism by χ2 method, a statistically critical difference was specified in terms of genotype distributions **(χ2: 22.48, *p*: 0.001**). 18 (20%) AA, 44 (61.1%) AC and 10 (13.9%) CC genotypes in terms of *mir146a* rs2961920 polymorphism have been determined in colon cancer patients. A statistically meaningful difference was defined when the colon cancer patients and controls were evaluated by the χ2 method for the distribution of genotype distributions of *mir146a* rs2961920 polymorphism **(χ2: 6.67, *p*: 0.036) (**Table 3).

Table 3. *Mir146a* (**rs2961920** and **rs2910164**) polymorphisms and odds ratios for colon, gastric and rectum cancer

Genotype	Controls n (%)	Colon Cancer n (%)	Rectum Cancer n (%)	Gastric Cancer n (%)
Rs2961920 (n)	70	62	58	8
AA	32 (45.7)	18 (25.0)	25 (43.1)	0 (00.0)
AC	31 (44.3)	44 (61.1)	30 (51.7)	2 (25.0)
CC	7 (10.0)	10 (13.9)	3 (5.2)	6 (75.0
χ 2	Ref.	6.67	1.36	ND**
p	-	**0.036**	0.506*	ND**
Rs2910164 (n)	77	76	63	73
GG	6 (7.8)	5 (6.6)	4 (6.3)	5 (6.8)
CG	43 (55.8)	52 (68.4)	43 (68.3)	54 (74.0)
CC	28 (36.4)	19 (25.0)	28 (36.4)	14 (19.2)
χ 2	Ref.	2.66	2.29	5.90
p	-	0.264	0.317*	0.052

**Fisher exact test*

Association between Gastric, Colon and Rectum Polymorphism of mir146a rs2910164

When the G and C alleles of gastric cancer patients and controls were assessed, no momentous difference was found (OR: 0.71, 95% CI: 0.34-1.16) (Table 4). When the GG+CG and CC genotypes were evaluated, a statistically significant difference was determined **(OR: 0.41 95% CI: 0.19-0.87)** (Table 4). Finally, when the GG+CC and CG genotypes were compared, a major difference was observed **(OR: 0.44 95% CI: 0.22-0.88)** (Table 4).

Table 4. Genotypic and allelic frequencies of *mir146a* polymorphism (**Rs2910164** (G/C)) in gastric cancer and control subjects.

	Control (n=77)(%)	Gastric Cancer (n=63)(%)	X^2	*p* value	Crude OR (95% CI)	Adjust OR (95% CI)
Rs2910164 *(G /C)*						
G	55(%35,8)	64(%43,8)	Ref.	-	-	
C	99(%64,2)	82(%56,2)	2,07	0,151	0,71(0,34-1,16)	
Codominant						
GG	6(%7,8)	5(%6,8)	Ref.	-	-	
CG	43(%55,8)	54(%74,0)	0,41	0,519	1,50(0,43-5,27)	1,85(0,47-7,25)
CC	28(%36,4)	14(%19,2)	0,55	0,456	0,60(0,15-2,31)	0,73(0,35-1,50)
Dominant						
GG	6(%7,8)	5(%6,8)	Ref.	-	-	
CG+CC	71(%92,2)	68(%93,2)	0,04	0,825	1,14(0,33-3,94)	1,12(0,59-2,15)
Recessive						
GG+CG	49(%63,6)	59(%80,8)	Ref.	-	-	
CC	28(%36,4)	14(%19,2)	5,49	**0,019**	0,41(0,19-0,87)	0,57(0,38-0,86)
Overdominant						
GG+CC	34(%44,2)	19(%26,0)	Ref.	-	-	
CG	43(%55,8)	54(%74,0)	5,39	**0,020**	0,44(0,22-0,88)	0,35(0,16-0,74)

Discussion

In recent years, it has been reported that miRNAs are also effective in tumour formation (Akkız et al., 2010). The genes encoding miRNA are composed of cancer-associated genomic domains or fragile regions. Therefore, miRNAs are thought to be important in the pathogenesis of cancer (Miller et al., 1998). Studies have indicated that miRNAs play a role in the regulation of cell growth and apoptosis mechanism (Cheng et al., 2005; Tanno et al., 2005). In our study, the relationship between two polymorphisms of the mir146a gene in the miRNA family,

which has been popular recently and has not yet been studied in our country, with gastric, colon and rectal cancers was investigated. In recent studies, it is thought that some SNPs formed in the mir146a gene region may be associated with many cancers. It has been noted that mir146a (rs2910164) single nucleotide polymorphism occurring in the mir146a progenitor sequence is associated with an increased risk of various malignant cancers such as thyroid, liver, prostate and gastric cancer. Some studies have shown the relationship of mir146a with the initiation and progression of colorectal cancer. Su et al. It was determined that in the case of the mir146a (rs2910164) polymorphism in gastric cancer in the Chinese population among 245 gastric cancer patients and 315 healthy controls 46 (19%), 122 (50%), and 77 (32 % (10% of GG controls)), 149 (47%), 134 (43%) had CC, CG, CC, CG and GG genotypes respectively. The comparison of GG and CC genotypes revealed a statistically significant increase in gastric cancer risk. In GG and CG+CC genotypes a statistically significant increase in risk was reported between this polymorphism and gastric cancer (Su and Luo, 2016). In our study, we also found the highest CG genotype in gastric cancer patients and controls. However, in our analysis with reference to the GG genotype, we could not detect a statistically significant difference between the CC genotype and the disease. In addition, we found a statistically significant difference between this polymorphism and gastric cancer in the analysis performed by combining CG+GG genotypes. Therefore, some results of our study were not consistent with the study conducted in the Chinese population. Chae et al. The association between mir146a (rs2910164) polymorphism and colon and rectal cancer was investigated in the Korean population. In this study, 399 colon and rectal cancer patients and 568 healthy control groups were examined. It was determined that among colon cancer patients 90 (41%), 93 (42%), 38 (17%), 165 (29%), 282 (50%), and 121 (21%) had CC, CG, GG control, CC, CG, and GG genotypes respectively. Evaluation of GG and CG genotypes in colon cancer patients and controls demonstrated that there is no statistically significant difference. However, a comparison of GG and CC genotypes revealed that there is a statistically significant increase in risk. In addition, evaluation of GG+CG and CC genotypes demonstrated a statistically significant increase in risk between this polymorphism and colon cancer. In our study, no statistically significant result was found between colon cancer and mir146a (rs2910164) polymorphism among these genotypes. It was determined that among rectal cancer patients 66 (38%), 87 (49%), 23 (13%), 165 (29%), 282 (50%), and 121 (21%) had CC, CG, GG, CC, CG, and GG genotypes respectively. Investigations revealed no statistically significant increased risk in the comparison of GG and CG genotypes in rectal cancer patients and controls. The authors observed a statistically significant increase in risk during the comparison of GG and CC genotypes, as well as GG+CG and CC status (Chae et al., 2013). In our study, it was found that the most common CG genotype was in colon and rectal cancers and controls. We could not detect a significant relationship during the comparison of these genotypes with each other. The evaluation of the data of this study revealed that the relationship between mir146a polymorphism and colorectal cancer was not compatible with the study in the Korean population, which may be caused by the small number of studied samples and the fact that this situation is unique to our society.

A comparison could not be made with the mir146a (rs2961920) polymorphism, since there are no studies conducted in the world or in our country. The study's importance is caused by the fact that it provides new and first information in the literature (Table 5, Table 7).

Environmental factors such as genetic factors that play a role in cancer development also play an important role in increasing the risk of cancer. There are numerous studies investigating the role of lifestyle factors, such as smoking and alcohol use, in the aetiology of colorectal cancer (Giovannucci, 2001; Moskal et al., 2007; Tsong et al., 2007; Hu et al., 2009; Fedirko et al., 2011). It was determined that there was no significant difference between smoking habit and cancer according to our findings in stomach, colon and rectum cancer, which was constituted by our statistical research group. It was thought that the limited number of patients in our study had an effect on these results. In addition, there may not be a statistically significant difference in the Turkish population we studied due to differences between races. It has been reported that alcohol consumption, which is another risk factor for cancer formation, increases the risk of colorectal cancer (Giovannucci, 2004). In our current study, data supporting the results of the mentioned studies were obtained in the statistical analysis of alcohol use in stomach, colon and rectum cancers. Another risk factor is a family history of cancer. It has been stated that the majority of patients with colorectal cancer do not have a family history of colorectal cancer, but it is stated that a family history of colorectal cancer can be up to 20% (Haggar and Boushey, 2009). Our results in this study show that there is no significant relationship between a family history of cancer in the stomach, colon and rectum cancers. In similar studies conducted around the world, a significant relationship was found between a family history of cancer and cancer disease in individuals. The reasons why our findings do not overlap with these studies suggest that the high patient age, the unknown causes of death of family parents living in rural areas and the lack of records may be the biggest factors. In addition, the transition from rural areas to urban life gives rise to the idea that the factors that increase the risk of cancer in the new generation may be more effective.

Conclusion

As a result, we found that the mir146a (rs2961920) polymorphism is a risk factor for gastric and colon cancers, but not for rectal cancer. We found that another polymorphism, mir146a (rs2910164), is not a risk factor for cancers of the stomach, colon, and rectum. In this

study, it was statistically seen that in the Turkish population, alcohol is a significant risk factor for the development of stomach, colon and rectal cancer. This study is important because it is the first study aimed to determine the relationship between mir146a (rs2961920 and rs2910164) polymorphism and stomach, colon and rectum cancers in our country. Increasing the number of individuals in more comprehensive studies like this may contribute to obtaining meaningful results in terms of determining risk factors in the formation of stomach, colon and rectum cancers. Conducting many studies on this subject in Turkey and bringing these studies together can help us achieve more productive results.

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Competing Interests

The authors declare no competing interests.

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