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Original Article

Genetic Variation in MiRNA Processing Machinery Genes and Susceptibility to Colorectal Cancer in the Iranian Population

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Abstract

Background: We aimed to elucidate the potential correlation between single-nucleotide polymorphisms (SNPs) in miRNA machinery genes and colorectal cancer (CRC) risk in an Iranian cohort.

Methods: We conducted a robust case–control study involving 507 participants, which included 213 patients diagnosed with CRC and 294 healthy controls at Research Institute for Gastroenterology and Liver Diseases in Tehran Province, Iran in 2018. The study focused on genotyping four specific SNPs, *RAN* (rs14035), *GEMIN3* (rs197412), *GEMIN4* (rs2740348), and *Dicer* (rs3742330), using advanced ARMS-PCR and Tetra-primer ARMS-PCR techniques.

Results: Notably, our investigation revealed the significant inverse association between the C/C genotype of rs197412 in the *GEMIN3* gene and CRC risk (OR=0.54, 95% CI=0.33-0.87; *P*=0.0087). In stark contrast, the T/T genotype of rs14035 in the *RAN* gene was strongly associated with a heightened risk of developing CRC (OR=4.44, 95% CI=2.60-7.57, *P*<0.0001). Furthermore, we found that the G/G genotype of rs2740348 in *GEMIN4* posed an increased risk for CRC (OR=2.9, 95% CI=1.44-5.85, *P*=0.0041) and it has a major effect on CRC risk in our population. The alleles and genotypes of rs3742330 in *Dicer*, however, did not exhibit a significant correlation with CRC.

Conclusion: Our study provides compelling evidence that SNPs within miRNA processing genes significantly contribute to susceptibility to CRC among the Iranian population. Our research not only contributes to the growing body of miRNA-related genetic studies but also opens avenues for population-specific risk assessment and personalized medicine approaches in cancer therapy.

Keywords: Colorectal cancer; Single-nucleotide polymorphisms, Genetics

Introduction

Colorectal cancer (CRC) is a significant global health challenge and is the third deadliest cancer, with approximately 1.2 million new cases annually (1). There is a sex disparity, as cancer is more prevalent in women (614,000 annual cases) than in men, for whom it is the third most common cancer, with 746,000 new cases each year (2). The increasing incidence of CRC in Iran highlights its increasing public health impact (3). Although a diverse range of risk factors, including lifestyle choices, medical conditions (i.e., diabetes mellitus), and genetic and epigenetic factors, contribute to CRC, the exact mechanisms initiating and driving CRC progression are still not fully understood (4). Recent studies have emphasized the crucial role of microRNAs (miRNAs), small noncoding RNA molecules that regulate gene expression after transcription. MiRNAs modulate gene expression by binding to messenger RNAs, either inhibiting their translation or triggering mRNA degradation, which can lead to cancer (5, 6). The production of functional miRNAs involves complex processes, beginning with the synthesis of primary miRNA transcripts in the nucleus, followed by their export to the cytoplasm, where they are processed by *Dicer* into miRNA duplexes. These duplexes form part of the miRNA-induced silencing complex (miRISC), which targets specific mRNAs for gene regulation (7, 8). Additionally, single-nucleotide polymorphisms (SNPs) in miRNA processing genes or binding sites, termed miR-SNPs, are significant in gene expression regulation and have implications for disease prognosis and treatment (8-12).

Dicer is essential for RNA interference and gene silencing mechanisms. Genetic variants in these gene contributing to the onset and progression of colorectal cancer. Studies investigating CRC patients have revealed that high expression of the *DICER*1 gene is associated with increased sensitivity to bevacizumab-based therapy (11-15). *GEMIN3* and *GEMIN4* are integral parts of the microRNA ribonucleoprotein complex and play key roles in miRNA stability and function. *GEMIN4*, in particular, belongs to the GEMIN protein family and is involved in various pathological mechanisms. *GEMIN4* forms part of a complex that selectively binds to miRNAs, leading to the formation of an RNA-induced silencing complex (RISC) (16). Therefore, alterations in *GEMIN4* can influence the expression of multiple miRNAs, which are significantly associated with various aggressive tumors (17-19). Research by Wu et al. revealed that polymorphisms in *GEMIN4* genes, specifically rs2740348 and rs7813, are linked to increased cancer risk and could serve as novel biomarkers for cancer risk prediction (20). A meta-analysis encompassing six studies indicated a significant association between the *GEMIN4* and heightened cancer risk, whereas the rs197412 polymorphism in *GEMIN3* did not exhibit a similar correlation with cancer risk (21).

The *RAN* gene, is key for pre-miRNA translocation from the nucleus to the cytoplasm (22, 23). Overexpressed in various cancer types, including colon and ovarian cancer (24-26), *RAN* also influences cancer progression via the PI3K pathway (27). A meta-analysis revealed that the *RAN* SNP rs14035 is associated with reduced cancer risk, highlighting its potential as a cancer risk biomarker and emphasizing the role of miRNA machinery gene variations in understanding cancer pathogenesis and developing targeted treatments (28). Hence, understanding miRNA machinery gene variants provides not only a clearer picture of the molecular pathogenesis of CRC but also aids in the development of potential biomarkers for early detection and personalized treatment strategies.

In our research, we focused on the relationship between SNPs in genes vital for miRNA biogenesis and susceptibility to colorectal cancer in the Iranian population. We analyzed four specific SNPs, *RAN* (rs14035), *GEMIN3* (rs197412), *GEMIN4* (rs2740348), and *Dicer* (rs3742330), in a group of Iranian CRC patients and controls. The

aim of this study was to deepen the understanding of the roles of these SNPs in CRC development within this demographic cohort by comparing our findings with global data. Our findings indicate that SNPs in miRNA processing genes are significantly linked to CRC susceptibility in the Iranian population. This underscores the need for broader genetic screenings and further studies across different populations to validate and refine these findings, which could lead to more personalized and effective CRC screening methods.

Materials and Methods

Ethics statement

This study was approved by the ethics committees of Shahid Beheshti University of Medical Science (SBMU) and Research Institute for Gastroenterology and Liver Diseases (IR.SBMU.RIGLD.REC.1396.182). Written informed consent was obtained from all the subjects who participated in this project.

Participants

In this case–control study, we meticulously selected 213 individuals diagnosed with sporadic colorectal cancer (CRC) at Research Institute for Gastroenterology and Liver Diseases in Tehran Province, Iran in 2018. These cases were confirmed through rigorous clinical examination, colonoscopy, and histopathological analysis of biopsy-obtained tissues. To enhance the reliability of our findings, we excluded patients who had previously received radiation or chemotherapy, thereby eliminating confounding treatment effects. Clinical information, including tumor size, stage of cancer, degree of differentiation, and metastasis status, was also meticulously collected.

The control cohort consisted of 294 non-cancer individuals who were carefully matched with the CRC patients in terms of sex, age, and demographic characteristics. We ensured that the control subjects were free from any familial history of cancer or inflammatory diseases.

Data on epidemiological variables were comprehensively collected for all participants to facilitate a multivariate analysis of CRC risk factors. The rigorous matching of cases and controls in terms of geographical residence and ethnicity ensures the minimization of potential confounders related to environmental and genetic backgrounds.

DNA extraction and genotyping

Peripheral blood was used for DNA extraction from each patient and control subject using the standard salting-out method (29). We programmed each primer using the primers online software (available from http://primer1.soton.ac.uk/primer1.html) (30).

Amplification refractory mutation systems polymerase chain reaction (ARMS-PCR) was used for genotyping the rs197412 *GEMIN3*, rs14035 *RAN*, and rs2740348 *GEMIN4* polymorphisms, and tetra-primer-ARMS-PCR (TP-ARMS-PCR) was used for genotyping rs3742330 in *Dicer*1.

Amplification was carried out on a Gene Tool thermocycler (Eppendorf). The PCR program described in Table 1.

Primer sequences and amplicon size (bp) for SNP amplification described in Table 2**.** We used distilled water instead of extracted DNA as the negative control. After PCR amplification, the PCR products were subjected to 1.5% agarose gel electrophoresis and separated exactly (Fig. 1). The gel electrophoresis mixture contained a red safe stain in 0.5X TBE (Tris/Borate/EDTA). All genotyping was performed randomly without any data about the sample or control.

Table 1: PCR program for ARMS-PCR and Tetra-ARMS-PCR for SNP amplification

GENE	*SNP*	*Initial denaturation (4min)*	*Denaturation Temperature (30sec)*	*Cycle number*	*Annealing temperature ℃*	*Annealing Time*	*Extension temperature*	*Extension Time*	*Elongation temperature*	*Elongation Time*
RAN	rs14035	94 ℃	94 ℃	33	60 ℃	1 min	72 ℃	1min	72 ℃	5 min
GEMIN3	rs197412	95 ℃	95 ℃	32	55 ℃	30sec	72 ℃	30sec	72 ℃	5 min
GEMIN4	rs2740348	95 ℃	95 ℃	30	64 ℃	30sec	72 ℃	30sec	72 ℃	5 min
DICER	rs3742330	95 ℃	95 ℃	32	60.5 ℃	30sec	72 ℃	30sec	72 ℃	5 min

Table 2: Primer sequences and amplicon size (bp) for SNP amplification

Gene	*SNP*	*Primer*	*Primer sequence (5′ → 3′)*	*Amplicon size*
RAN		WE	ACTGATGTTCCATCCTGTTTGTG	177 bp
	rs14035	MR	ACTGATGTTCCATCCTGTTTGTA	177 bp
	(C/T)	CF	CACCTTCATATTGGCTAGGT322	
	rs197412	WR	CAGGGACTCTCTGTTCTG	
GEMIN3	(T/C)	MR	CAGGGACTCTCTGTTCTA	153 bp
		CF	TTGCTGAATTGGTAGAGGAT	153 bp
GEMIN4		WE		
	rs2740348	MR	CTCAACACCAAGTCTGGCCG	168 bp
	(G/C)	CF	CTCAACACCAAGTCTGGCCC	168 bp
			GGATATCACAGCTTCCATTG	
DICER				
	rs3742330	FI (G		187 bp
	(A/G)	Allele)	GCTTCAATCTTGTGTAAAGGGATTCGG	142 bp
		RI (A	AAATATTGGATCTTGCTCTGTTAGGGGGT	273 bp
		Allele)	TCTTCTGCAGATAATGCAAATGGGTTAAA	
		FO	TTTGGTTCATGAATCCAGGTGTTCC	
		RO		

Statistical analysis

The chi-square test and Hardy–Weinberg equilibrium were used to calculate the frequencies of alleles and genotypes by using SNPStats online software (http://bioinfo.iconcologia.net/SNPstats) (31) and MEDCALC online software (https://www.medcalc.org/calc/odds_ratio.php). A $P< 0.05$ was presumed to indicate statistical significance. The dominant and recessive inheritance models were applied to the analysis of genotypes.

Results

Functional prediction of miRNA machinery gene SNPs among Iranian patients

A total of 213 patients with CRC (119 men and 94 women) were distinguished at the Institute for Gastroenterology and Liver Disease (Taleghani General Hospital); the average age was 53± 14.6 years, and 294 noncancerous individuals (164 men and 129 women), who were 50.2±15.2 years old on average without any history of hereditary or serious disease, were enrolled in this study as a control group. All the control individuals had a similar ethnicity (Iranian population). Clinical characteristics and demographic variables are shown in Table 3.

Table 3: Demographic data and clinical characteristics of patients and controls for CRC risk

Variable	*CRC (n=213)*	*HC (n=294)*
Median age (yr)	53.1+-14.6	50.2+-15.2
Sex		
Male	119	165
Female	94	129
Primary tumor location %		NA
Colon	68%	
Rectum	21%	
Cecum	11%	
Differentiation %		NA
Well-differentiated	42%	
Moderator-Differentiated	24%	
Poorly differentiated	4%	
Not differentiated	30%	
Clinical stages and TNM %		NA
I	11%	
II	54%	
IIIC	26%	
IV	9%	

All genotype dissemination in noncancerous people was compatible with that expected according to the Hardy–Weinberg equilibrium model. Next, we evaluated the association between the risk of CRC and the rs197412 *GEMIN3*, rs14035 *RAN*, rs3742330 *Dicer* 1, and rs2740348 *GEMIN4* polymorphisms in the Iranian population using dominant and recessive inheritance models (Table 4). The results of 1.5% agarose gel electrophoresis are presented in Figs. 1-4.

Accordingly, the results obtained from a statistical analysis demonstrated that the T/T genotype of the rs14035 RAN gene enhanced the risk of CRC in the considered people (OR =4.44 (2.60-7.57), 95% CI, P = 0.001). In addition, genotype frequencies displayed meaningful differences under the dominant model (P = 0.0001; OR = 2.92; 95% CI, 1.69-5.03).

In addition, the dominant model of rs197412 (C/C genotype) in the *GEMIN3* gene reduced CRC risk in the studied people (OR = 0.54 (0.33-0.87), 95% CI, *P*= 0.0087), and the rs197412 T/T genotype in the *GEMIN3* gene did not have a punctual effect on CRC risk in the carrier person (*P* =0.37; odds ratio [OR] =1.29 (0.74-2.23), 95% confidence interval [CI].

Table 4: Allele and genotype frequencies of 4 mir-SNPs in CRC patients & controls

Gene name	*SNPs*	*Genotype*	*Disease=0, (Control N (%))*	*Disease=1 (Case N (%))*	*OR (95% CI)*	*P-value*
RAN	rs14035(C/T)					
		C/C	147 (36.8)	18 (16.7)	1.00	
		C/T	216 (54.1)	57 (52.8)	2.16 (1.22-3.81)	0.0001
		T/T	36 (9)	33 (30.6)	7.49 (3.79-14.78)	
		C/C vs. C/T-T/T			2.92 (1.69-5.03)	0.0001
		TT vs. C/C-C/T			4.44 (2.60-7.57)	0.0001
GEMIN4	rs2740348(G/C)					
		C/C	156(39.1)	27(25)	1.00(reference)	
		G/C	222(55.6.3)	66(61.1)	1.72(1.05-2.81)	0.0001
		G/G	21(5.3)	15(13/9)	4.13(1.89-8.99)	
		C/C vs. G/C-G/G			1.93(1.19-3.11)	0.0057
		C/C-G/C vs. G/G			2.90(1.44-5.85)	0.0041
GEMIN3						
	rs197412(T/C)	C/C	246 (61.6)	81 (75)	1	
		C/T	90(22.6)	6 (5.6)	0.20 (0.09-0.48)	0.0001
		T/T	63(15.8)	21 (19.4)	1.01(0.58-1.76)	
		C/C vs. C/T-T/T			0.54 (0.33-0.87)	0.0087
		C/C-C/T vs. T/T			1.29 (0.74-2.23)	0.37
DICER	rs3742330(A/G)	G/G	410 (81.6)	375 (74.7)	1.00	0.28
		A/G	91(18.4)	127 (25.4)	1.51 (0.72-3.16)	

Moreover, in *GEMIN4*, the rs 2740348 C/C genotype was significantly different among the genotypes according to the dominant model (*P* = .0057; OR = 1.93; 95% CI, 1.19-3.11). Moreover, the G/G genotype increased the risk of CRC in our studied cohort (*P* = .0041; OR = 2.90; 95% CI, 1.44-5.85). In addition, according to the *DICER* gene rs3742330 genotyping result, no meaningful relationship was detected between the rs3742330 allele and the risk of CRC according to any of the inheritance models in this study (*P* =0.28; odds ratio [OR]=1.51 (0.72-3.16); 95% confidence interval [CI]).

The frequencies of the alleles and genotypes in the controls and CRC patients are reported in Table 4. To validate the accuracy of our findings, we repeated genotyping by random selection 30% of the time without providing any information about the sample situation (case or control). Fortunately, the rate of accuracy was 100%.

Fig. 1: 1.5% agarose gel electrophoresis of four single nucleotide polymorphisms genotyping in *GEMIN3* gene. Lane 1,3,5: mutant allele, lane2,4: wild type allele, lane 6: positive control, Lane7: Negative control, Lane8: DNA Ladder 100bp

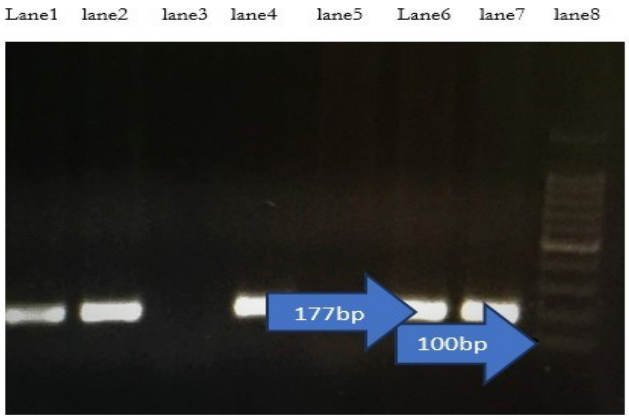

Fig. 2: 1.5% agarose gel electrophoresis of four single nucleotide polymorphisms genotyping in *RAN* gene. Lane 1: mutant allele (177bp), lane2,4,6: wild type allele (177bp), lane3,5: negative control, lane 7: positive control (177bp), lane8:100-bpDNALadder (ARMS-PCR was used)

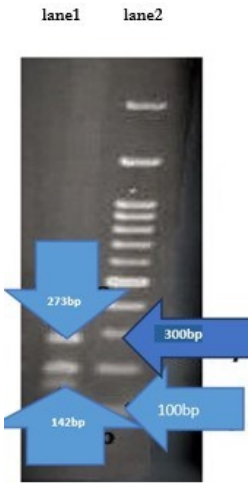

Fig. 3: 1.5% agarose gel electrophoresis of four single nucleotide polymorphisms genotyping of rs3742330 in DICER gene. Lane 1:AG Genotype, lane2:100-bpDNALadder (TETRA-ARMS-PCR technique was used

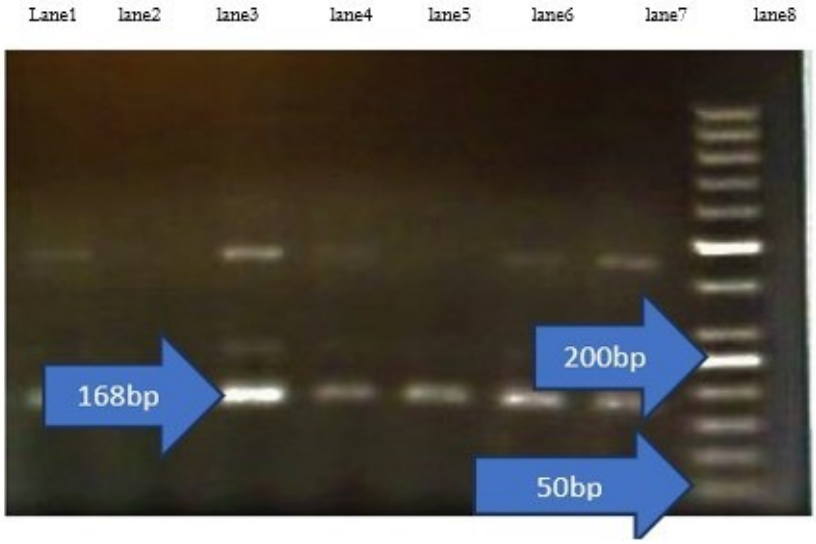

Fig. 4: 1.5% agarose gel electrophoresis of genotyping in rs2740348 in GEMIN4 gene. Lane 1,5,7: mutant allele, lane 2: negative control, lane 3: positive control, lane 4,6: wild type allele, lane8: 50-bp DNA ladder (ARMS-PCR technique was used)

Discussion

miRNAs can regulate approximately 1/3 of the human genome (6). Polymorphism in miRNA processing machinery genes could affect CRC risk. Earlier researchers reported that different types of cancer are related to genetic changes in miRNA processing machinery genes (7). Although genetic alterations in these genes can affect the initiation and development of several types of cancer, the exact role of genetic alterations in miRNA-related genes in CRC has not been determined. Therefore, for the first time, we assessed the relationships between 4 important SNPs, *RAN* (rs14035), *GEMIN3* (rs197412), *GEMINI4* (rs2740348), and *Dicer* (rs3742330), and CRC risk in the Iranian population. The results of our project indicated that the SNPs in the *GEMIN3*, *GEMIN4*, and *RAN* genes could affect CRC risk in Iranian patients with CRC. These SNPs may be considered good prognostic biomarkers for CRC progression and development.

Our study showed that the rs14935 T allele and the rs14035 T/T genotype in the *RAN* gene significantly increased the CRC risk in patients versus noncancerous persons in the studied population. Recent research has indicated that the expression level of *Ran* is elevated in patients with metastatic CRC (32). According to previous studies, XPO5 attaches to pre-miRNA molecules and *RAN* GTPases in the XPO5-RAN-GTP-pre-miRNA complex (33). The rs14035 C/T genotype in *RAN* had an obvious effect on decreasing CRC and the CT+TT genotype in our study increased the risk of CRC. Furthermore, the rs14035 *RAN* gene was shown to be inversely associated with the presence of laryngeal cancer depending on lymph node metastasis (34).

The results of our study indicated that the dominant genotype of *GEMIN4* rs2740348, including the G/G genotype, has an increasing effect on CRC risk. It has been shown that the rs7813 and rs2740348 SNPs in *GEMIN4* can be related to the risk of different types of cancer (21). Multiple studies have demonstrated that the interaction between the RISC complex and *GEMIN3* and *GEMIN4* may affect the degradation of target miRNAs in the cytoplasm (35). rs1971412 of the *GEMIN3* gene in exon 11 changes Ile to Thr by transitioning T to the C nucleotide at the 636 amino acid position and increases the risk of CRC (35).

In this study, the rs197412 C/C genotype in the *GEMIN3* gene had a reduced effect on CRC risk, and the rs197412 C allele in the *GEMIN3* gene

had a protective effect on CRC risk. However, the TT genotype of the *GEMIN3* gene had no significant effect on CRC risk in our population. According to a previous study, the TT allele of rs197412 located in the *GEMIN3* gene significantly increased the risk of CRC (36).

We found that rs3742330 in the *DICER* gene had no relationship with CRC risk in the Iranian population. *Dicer*1 stimulates colon cancer cell invasion and migration via the modulation of tRF-20-MEJB5Y13 expression under hypoxia (37). It was discovered that *Dicer*-related SNPs are associated with the carcinogenesis of CRC (36).

On the other hand, we should notice that there are different limitations in our project. First, we emphasize that this study is a first case-control study on the association of miR-SNPs in the processing machinery genes pathway and CRC progression in the Iranian population. Hence, the results of our project need to be confirmed with more projects. Additionally, the project sample size was not large, and evaluating further DNA samples from CRC patients from our population led to a better assessment of the relationship between the risk of CRC and these miR-SNPs in Iranian people.

Conclusion

This pioneering study represents the first investigation into the link between miRNA processing machinery genes and the onset and progression of colorectal cancer (CRC) in the Iranian population. These findings indicate that miRNAs could serve as promising biomarkers for disease prediction, particularly in cancer. However, research into miR-SNPs within the miRNA machinery gene pathway in CRC is still in its infancy. Despite the limitations of the present study, such as its small sample size and status as an initial case–control investigation in this area, the results provide new insights into the connection between miR-SNPs and CRC risk among Iranians. The use of cost-effective genotyping methods such as ARMS-PCR and Tetra-ARMS-PCR assays were a key feature of this research, as we plan to further assess gene expression in blood samples in subsequent phases. Overall, the study suggested an association between three specific microRNA-related SNPs and CRC risk in the Iranian population, highlighting the need for more extensive research to validate these findings.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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