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Original Research

Potential risk assessment of miR-143 gene polymorphisms rs41291957 and rs353292 in colorectal cancer

Mehvish Naseer Ahmed[1, 2], Qaisar Mansoor[1*], Ruqia Mehmood Baig[2*], Tehreem Tahir[1], Samina Asghar Abbasi[1, 2]

[1] Institute of Biomedical and Genetic Engineering (IBGE), 24- Mauve Area, G-9/1, Islamabad Pakistan
[2] Department of Zoology, PMAS-Arid Agriculture University, Rawalpindi, Pakistan

[*]**Correspondence to:** qmibge@gmail.com

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Abstract: Colorectal cancer is a life-threatening and therapeutically challenging disease. Increasingly it is being deciphered that genetic and epigenetic mutations play a central role in cancer onset and progression. Excitingly, discovery of non-coding RNAs is considered to be a milestone in molecular oncology and emerging evidence is deepening our understanding about pivotal role of miRNAs in carcinogenesis. miR-143 has been experimentally verified to play an instrumental role as tumor suppressor. Recent studies suggest that single nucleotide polymorphisms rs41291957 and rs353292 in miR-143 may associate with the progression and or development of colorectal cancer. In present study 400 Pakistani subjects participated including 200 colorectal cancer patients and 200 age and gender matched healthy individuals. Blood samples and clinical information of the confirmed patients was collected from cancer diagnosis and treatment hospitals in Pakistan. The polymorphisms rs41291957 and rs353292 were genotyped in patients and controls by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and results were validated by Sanger sequencing. The association of the SNPs within the study group was analyzed by χ^2 test with p value < 0.05 as significant. Odds ratio was calculated with 95% confidence interval.Genetic predisposition to cancer was observed in presence of characteristic rs45291957 polymorphism. χ^2 test results show strongly significant association mi-RNA rs45291957 SNP with colorectal cancer p value 0.0111 (<0.05) along with the statistically significant correlation tested by odds ratio with 95% confidence interval. However, no significant correlation (p value 0.6683) could be found for the association of rs353292 with colorectal cancer in Pakistani population. The present study for the first time gave evidence of miR-143 rs41291957 involvement in colorectal cancer patients of Pakistani population. This target can be a useful molecular tool for the prognosis and treatment targets for colorectal cancer in Pakistani population. rs353292 genetic association can be explored for different cancers in Pakistan to completely rule out its role in cancer.

Key words: MicroRNA; Cancer; Signaling.

Introduction

Colorectal cancer is a life-threatening and therapeutically challenging disease. Increasingly it is being deciphered that genetic and epigenetic mutations play a central role in cancer onset and progression (1,2,3).

MicroRNA discovery has paradigmatically shifted our conceptual approach towards central dogma of molecular biology. Overwhelmingly increasing high-impact researches have helped us to re-conceptualize our knowledge about the intermediate steps between transcription and translation (4,5,6). miRNAs have revolutionized the field of molecular oncology and scientists have witnessed tremendous advancements in the underlying mechanisms which centrally regulate cancer onset and progression. Single-nucleotide polymorphisms (SNPs) are single base-pair alterations in the DNA sequences that showcase a major source of genetic heterogeneities. SNP-based, genome-wide, large-scale association studies have leveraged our understanding related to tumor heterogeneity to another level (7,8). Technological advancements have enabled detection of many polymorphisms that can be used for an effective evaluation of the risk of various common traits, including human cancers.

miR-143 has captivated attention of cancer biologists because of its ability to inhibit prevent cancer progression. Accumulating evidence has started to unveil how miR-143 targets different oncogenic mRNAs and how long non-coding RNAs sponge miR-143 away to potentiate expression of oncogenes (9).

miR-143 has emerged as a critical miRNA reportedly involved in regulation of cancers. Efficacy of miR-143 with a longer passenger strand is currently being tested (10).

Polymorphism of rs41291957 with its Mutant allele (G>A) in colorectal cancer patients had greater risk to produce a larger tumor size as compared to those patients which don't have mutant allele (11). Such findings suggested that Single nucleotide polymorphisms in the promoter region of miR-143/145 might be interconnected to the etiology of colorectal cancer. Mutant genotypes or alleles of rs41291957 and rs353292 were significantly associated with an increased risk of colorectal cancer compared with the wild genotypes or alleles. rs41291957 was found to be associated with a significantly reduced risk of Non-Hodgkin Lymphoma in Caucasian population (12). A significantly greater

risk of colorectal cancer was found to be associated with heterozygous (CT) genotype compared to CC genotype ($p < 0.001$). Heterozygous and mutant CT/TT genotype carriers had a 1.62-fold increased risk of colorectal cancer compared to CC genotype carriers. Moreover, T allele of rs353292 was associated with a significantly greater risk of colorectal cancer development as compared to C allele (13).

Materials and Methods

Subjects

Two hundred (200) patients diagnosed for colorectal cancer including colon and rectum etiology along with age and gender matched healthy individuals, participated in the study. The patients were ascertained from cancer hospitals and tertiary care hospitals of Pakistan. 5ml blood sample was taken from the participants after informed consent. The relevant clinical data was also obtained from the patients.

Genotyping

miR-143 SNPs rs412919457 and rs353292 were genotyped by PCR-RFLP technique. For the purpose of genotyping, genomic DNA was isolated by GeneJET Genomic DNA Purification Kit (Thermo Scientific, Lithuania). The PCR was performed in a final reaction volume of 25µL containing 1X $(NH_4)_2SO_4$ PCR buffer, 1.5mM $MgCl_2$, 0.4mM dNTPs, 1U *Taq* DNA polymerase enzyme (Thermo Scientific, Lithuania) with 2µL DNA (20ηg/ml) using left 5'tgccattgtttgcacaactt'3 and right 5'ccaactgaccagagatgcag'3 primers for rs41291957 and left 5'gagcgtagcccaagaggaa'3 and right 5'agcaatcaattgtctccaacc'3 primer for rs353292. The amplified PCR product was checked on 2% w/v agarose gel. Subsequently PCR products of rs41291957 and rs353292 were digested by restriction enzymes *MSPI* (Thermo Scientific, Lithuania) and *BseMI* (Thermo Scientific, Lithuania) respectively according to the manufacturer's protocol. The digested products were visualized on 2% w/v agarose gel.

Sequencing

The genotyping by PCR-RFLP was validated by Sanger sequencing method using ABI Genetic Analyzer 3500 (Hitachi, Japan).

Statistical Analysis

Statistical analysis was done by SPSS (version 20). *p value* < 0.05 for χ^2 test was considered as statistically significant. Odds ratio (OR) was calculated with 95% confidence interval (CI).

Results

Subjects

The patients were presented with both colon and rectum cancer etiology. The mean age range of patients was 49. In the present study group, it was seen that colorectal cancer was more prevalent in males (66.7%) than females (33.3%) and it has a higher frequency in age group 16 to 50 years than old age i.e. 60 to 80 years. Histopathologically the metastasis classification of the patients revealed; lymph node metastasis (12.7%), liver metastasis (7.00%), bone marrow and blood metastasis was (0.5%) and lung metastasis (12.4%). Adenocarcinoma was (30.7%) present in patients. Most frequently cancer stage in patients stage 2 (35.4%) and stage 3 (26%). Stage 1 frequency was 19.3 % and stage 4 was 19.2% in colorectal cancer patients (Table 1). Most prevalent cancer type was colon 58.3%, 39.6% of the patients were affected with rectum cancer and only 2.1% were having involvement of both the rectum and colon (Table.1).

miR-143 rs41291957 Genotyping Results:

χ² Test

The genotype frequencies of rs41291957 were in

Table 1. Age, Gender and Clinical hallmarks of the Patients.

Mean Age	Patients		49.8167 years
	Controls		53.1257 years
Gender	Patients	Male	66.7 %
		Female	33.3 %
Cancer Stage (%)	I		19.3 %
	II		35.4 %
	III		26.09 %
	IV		19.2 %
Cancer Type (%)	Colon		58.3 %
	Rectum		39.6 %
	Colon+ rectum		2.1 %
	Undetermined		20.8 %
Metastasis (%)	Lymph node metastasis		12.5 %
	Liver metastasis		7.00 %
	Blood metastasis		0.5 %
	Bone marrow metastasis		0.5 %
	Lung metastasis		12.4 %
	Tumor Reached serosa		15.6 %
	Adenocarcinoma		30.7 %

Hardy-Weinberg equilibrium. The observed frequencies of wild type homozygous genotype GG was 74.17% and 90.24%, minor allele homozygous AA genotypes were 4.945% and 1.2195% and heterozygous AG genotype frequency was 20.8791% and 8.5365% in patients and controls respectively. rs41291957 polymorphism with the *p* value 0.0111 (<0.05) of the χ^2 test was statistically significant in patients (Table 2). Furthermore, the prevalence of the risk allele genotypes i.e. AG/AA were more recurrent (25.8241%) in the patients of colorectal cancer as compared to the healthy controls (9.756%). This difference of heterozygous and rare allele homozygous genotypes was statistically significant *p* value 0.0029, <0.05 (Table 3).

Odds Ratio

The association of rs41291957 with colorectal cancer was estimated using the odds OR with 95% confi-

Table 2. Genotype Frequencies, Hardy-Weinberg Equilibrium (H-W), χ^2 Test, and Odds Ratio (OR) with 95% Confidence Interval (CI) calculations for colorectal cancer patients and controls.

Genotypes	Patients observed frequency (%)	Expected H-W frequency (%)	Control Observed frequency (%)	Expected H-W frequency (%)	χ^2 Test (*p* value)	OR (95% CI)	*p*-value
rs41291957	-	-	-	-	0.0111*	-	-
GG	74.1758	71.54	90.2439	89.3256		0.3162 (0.1433-0.6979)	0.003235*
AG	20.8791	25.9	8.5365	10.3732		2.6878 (1.1638-6.2074)	0.017455*
AA	4.945	2.34	1.2195	0.3012		5.2105 (0.5976-45.428)	0*
	p-value = 0.14069**		*p*-value = 0.208555**			-	-
rs353292	-	-	-	-	0.6683**		
GG	23.583	26.0525	22.3529	27.4083		1.1196 (0.5792-2.1644)	0.74014**
AG	54.1666	49.9782	60	49.8892		0.7826 (0.4466-1.3715)	0.3928**
AA	21.875	23.9692	17.6470	22.7024		1.211 (0.6006-2.4418)	0.59022**
	p-value = 0.7038**		*p*-value = 0.128266**				

*Significant, **Non-Significant

Table 3. Analysis of association of risk allele and rare allele genotypes with colorectal cancer along with wild and risk allele frequencies, χ^2 test and OR with 95% CI.

Genotypes & Alleles	Frequency in Patients (%)	Frequency in Controls (%)	χ^2 test	OR (95% CI)	*P* value
rs41291957				-	-
Genotypes			0.00296806*		
GG	74.1758	90.2439		0.3162 (0.1433-0.6979)	0.003235*
AG/AA	25.8241	9.756		3.1622 (1.4329-6.9781)	0.003236*
Alleles			0.0221*		
G	0.84615	0.9451			
A	0.15384	0.0549			
rs353292					
Genotypes			0.7884467**		
GG	23.9583	22.3529		1.1196 (0.5792-2.1644)	0.740144**
AG/AA	76.0416	77.6470		0.8932 (0.462-1.7266)	0.740144**
Alleles			0.853707**		
G	51.0416	52.3529			
A	48.9583	47.6470			

*Significant, **Non-Significant

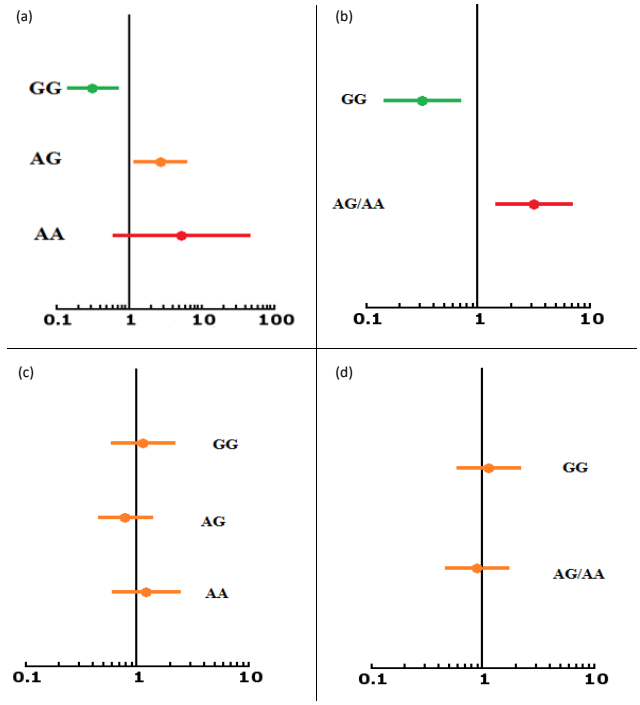

Figure 1. Forest plots for Odds Ratio results. **a, b;** Forest plot 95%CI miR-143 rs41291957 GG (Homozygous dominant genotype) AG (Heterozygous genotype, significantly associated with CRC risk) AA (homozygous recessive genotype, significantly associated with CRC risk), **Figure c, d**; Forest plot of rs353292 at 95% CI GG (Homozygous dominant genotype) AG (Heterozygous genotype) AA (homozygous recessive genotype, non-significant).

dence (CI). The OR strength of rare allele homozygotes AA 5.2105 (95% CI 0.5976-45.428) and heterozygous AG 2.6878 (95% CI 1.1638-6.2074) further marked the significant (p value 0, < 0.05 and p value 0.017455, < 0.05 respectively) contribution with the disease association (Table 2). The overall prevalence of risk allele genotypes AG/AA showed statistically significant (p value 0.0032, <0.05) OR 3.1622 (95% CI 1.4329-6.9781) as shown in table 3. While GG homozygote OR of 0.3162 (95% CI 0.1433-0.6979) with p value 0.0032 was observed in control group (Table 2). The forest plot for the OR with 95% CI are shown in Figure 1 (a&b).

miR-143 rs353292 Genotyping Results:

χ² Test

The genotyping results showed that the polymorphism for rs353292 SNP were in Hardy Weinberg equilibrium. The frequency of homozygous genotype GG (23.583%) did not show significant difference as compared to healthy group (22.3529%). Likewise, no remarkable difference was observed for the heterozygous AG and rare homozygotes AA by χ^2 analysis with p value <0.05 as significance borderline (Table 2).

Odds Ratio

The slight differences in genotype frequencies of rs353292 was checked for the OR strength with 95% CI. The OR of rare AA homozygote was 1.211(95% CI 0.6006-2.4418), OR for AG heterozygote 0.7826 (95% CI 0.4466-1.3715) as in table 2, and the genotypes with risk allele i.e. AG/AA OR value was 0.8932 (95% CI 0.462- 1.7266) as shown in table 3. The GG homozygote OR 1.1196 (95% CI 0.5792-2.1644) was observed (table 2, 3). The strength of the OR value with 95% CI did not reveal any significant association of the SNP with the disease. The forest plots for the OR data are shown in figure 1 (c & d).

DNA Sequencing

Direct DNA sequencing was done for the validation of PCR-RFLP genotyping by random selection of the samples for each of the SNP i.e. miR-143 rs41291957 and miR-143 rs353292 (Figure 2). The sequencing validated the success of PCR-RFLP genotyping strategy.

Discussion

In present study we provided evidence for the first time that the SNP rs41291957 in the promoter region of miR-143 associated with colorectal cancer susceptibility in Pakistani population. The findings being reported were derived from the statistically significant χ^2 test. The strength of the association was further observed at the common homozygous (GG), heterozygous (AG) and rare homozygous (AA) genotypes; with the widest forest plot (Figure 2, a) for rare homozygotes in colorectal cancer patients (OR 5.2105, 95% CI 0.5976 – 45.428). Likewise, the confidence for the strong association of the risk allele genotypes (AG/AA) with colorectal cancer; significance level was comparable to the wild type allele genotype (GG) dominance in healthy individuals. These results were determined by the statistically significant ($p < 0.05$) χ^2 test and OR calculation. Moreover, it was interesting to note the risk allele (A) predominance in the patients as compared to controls group, p value 0.0221, <0.05 (Table 3). However no statistically significant hallmarks were observed for the association of rs353292 with colorectal cancer in present study. On the other hand, previously this particular genetic polymorphism has also been reported for disease association in study (14).

It was reported in a study, that risk allele genotypes of rs41291957 and rs353292 were significantly associa-

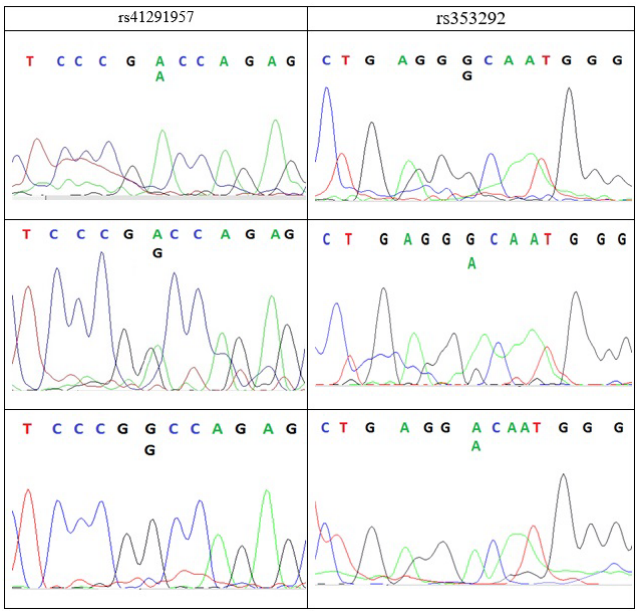

Figure 2. Sequence Analysis of the PCR-RFLP genotyping Results. miR-143 rs41291957 (GG; wt/wt, AG; wt/mt, AA; mt/mt); rs353292 (GG; wt/wt, AG; wt/mt , AA; mt/mt).

ted with increased risks of colorectal cancer in Chinese population as compared to the wild type genotypes or alleles (11*). In a follow up study, endometriosis was found significantly (p value 0.04, <0.05) susceptible to rs41291957 single nucleotide polymorphism in Iranian population (15). Furthermore, broadening the spectrum of potential involvement of rs353292 SNP in other etiology, it was depicted in a study that, the null association of the SNP in conotruncal heart defects (16). Similarly, in another study it was reported that, the polymorphism of miR-143/145 and the risk and severity of cardiovascular disease in the Chinese Han people which showed that the (A) allele of the rs353292 polymorphism is associated with serum high sensitivity C-reactive protein (hs-CRP) levels in Coronary heart disease patients, and it may affect the occurrence and development of MI by up-regulation of CRP gene through miR-143/145 (17).

Elaborated research has identified some additional SNPs of miR-143 rs3733845 and rs3733846 to be associated with an increased risk of lung adenocarcinoma (18).

The findings of the present study and amalgam of scientific literature rationalizes the potential involvement of miR-143 SNPs especially rs41291957 in cancer. This candidate has been proved as disease prognostic and diagnostic genetic marker for colorectal cancer.

In conclusion, miR-143 single nucleotide polymorphism rs41291957 has been found strongly associated with colorectal cancer. This genetic marker can be regarded as potential risk candidate for early predication, disease prognosis and treatment target in colorectal cancer.

References

1. Farooqi AA, de la Roche M, Djamgoz MBA, Siddik ZH. Overview of the oncogenic signaling pathways in colorectal cancer: Mechanistic insights. Semin Cancer Biol. 2019; 58:65-79.

2. Sveen A, Kopetz S, Lothe RA. Biomarker-guided therapy for colorectal cancer: strength in complexity. Nat Rev Clin Oncol. 2020;17(1):11-32.

3. Tenesa A, Dunlop MG. New insights into the aetiology of colorectal cancer from genome-wide association studies. Nat Rev Genet. 2009;10(6):353-8.

4. Farooqi AA, Fuentes-Mattei E, Fayyaz S, Raj P, Goblirsch M, Poltronieri P, Calin GA. Interplay between epigenetic abnormalities and deregulated expression of microRNAs in cancer. Semin Cancer Biol. 2019; 58:47-55.

5. Treiber T, Treiber N, Meister G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. Nat Rev Mol Cell Biol. 2019;20(1):5-20.

6. Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. Nat Rev Cancer. 2015;15(6):321-33.

7. Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. Nat Rev Cancer. 2010;10(6):389-402.

8. Karimzadeh MR, Zarin M, Ehtesham N, Khosravi S, Soosanabadi M, Mosallaei M, Pourdavoud P. MicroRNA binding site polymorphism in inflammatory genes associated with colorectal cancer: literature review and bioinformatics analysis. Cancer Gene Ther. 2020 Mar 23.

9. Farooqi AA, Qureshi MZ, Attar R, Alhewairini SS, Fayyaz S, Sabitaliyevich UY, Duisenbayevich TM, Alaaeddine N. MicroRNA-143 as a new weapon against cancer: overview of the mechanistic insights and long non-coding RNA mediated regulation of miRNA-143 in different cancers. Cell Mol Biol (Noisy-le-grand). 2019;65(6):1-5.

10. Ida H, Tanabe T, Tachibana A. Improved cancer inhibition by miR-143 with a longer passenger strand than natural miR-143. Biochem Biophys Res Commun. 2020;524(4):810-815.

11. Li L, Pan X, Li Z, Bai P, Jin H, Wang T, Song C, Zhang L, Gao L. Association between polymorphisms in the promoter region of miR-143/145 and risk of colorectal cancer. Hum Immunol. 2013; 74(8):993-7.

12. Bradshaw G, Haupt LM, Aquino EM, Lea RA, Sutherland HG, Griffiths LR. Single Nucleotide Polymorphisms in *MIR143* Contribute to Protection Against Non-Hodgkin Lymphoma (NHL) in Caucasian Populations. Genes (Basel). 2019 ;10(3). pii: E185.

13. Yuan F, Sun R, Li L, Jin B, Wang Y, Liang Y, *et al*. A functional variant rs353292 in the flanking region of miR-143/145 contributes to the risk of colorectal cancer. Sci Rep. 2016; 6:30195.

14. Fang R, Xiao T, Fang Z, Sun Y, Li F, Gao Y, *et al*. MicroRNA-143 (miR-143) regulates cancer glycolysis via targeting hexokinase 2 gene. J Biol Chem. 2012; 287(27): 23227-23235.

15. Nimi-Hoveidi E, Kohan L, Hashemi SS. Association of miR-143 rs41291957 and rs4705342 genetic variants with endometriosis risk in infertile women. Feyz. 2016; 20.

16. Wen H, Zhang R, Li Y, Qian H, Yan Z, Chen Y, *et al* Association between functional polymorphisms in the promoter of the miR-143/145 cluster and risk of conotruncal heart defects. Pers Med. 2019; 16(6): 449-455.

17. Chen C, Lu L, Yan S, Yi H, Yao H, Wu D, *et al*. Autophagy and doxorubicin resistance in cancer. Anti-Cancer Drug Des. 2018; 29(1): 1-9.

18. Yang X, Li X, Quan X, Li H, Hao X, Jiang M, Zhou B. Association Between Two Polymorphisms in the Promoter Region of miR-143/miR-145 and the Susceptibility of Lung Cancer in Northeast Chinese Nonsmoking Females. DNA Cell Biol. 2019;38(8):814-823.