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Genotype GG of rs895819 Functional Polymorphism Within *miR-27a* Might Increase Genetic Susceptibility to Colorectal Cancer in Han Chinese Population

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Background: MicroRNA-27a (*miR-27a*) is supposed to be an oncogene in various types of cancers, and genetic variation of *miR-27a* might result in aberrant expression and abnormal second structure of mature-miR-27a, contributing to elevated genetic risk and poor prognosis for colorectal cancer (CRC). *Methods:* In order to explore the possible association between rs895819 within *miR-27a* and CRC in Han Chinese population, we investigated the genotype distributions of rs895819 in 508 CRC cases and 562 healthy check-up controls using TaqMan genotype discrimination system, and analyzed the possible association between them. Odds ratio (OR) and 95% confidential interval (95% CI) were used to assess the strength between allele and genotype of the locus and risk of CRC. *Results:* In our study, we found that genotype GG of rs895819 was significantly associated with an increased risk for CRC (17.1% vs. 11.6%, adjusted OR = 1.546, 95% CI = 1.070–2.236), and allele A carrier (AA/AG) was significantly associated with a decreased risk for CRC (82.9% vs. 89.4%, adjusted OR = 0.63, 95% CI = 0.446–0.893). In addition, a significant association was observed between genotype GG and larger tumor size (>5 cm; $P < 0.001$), and allele G was significantly associated with higher pathological stage (TNM-III) ($P = 0.008$). *Conclusion:* These results indicated that miR-27a might be involved in the development and progression of CRC, genotype GG within rs895819 might be a genetic susceptible factor for CRC. Further multicentral, large sample size, and well-designed epidemiological study as well as functional study are warrant to verify our findings. J. Clin. Lab. Anal. **30**:351–355, 2016. © 2015 Wiley Periodicals, Inc.

Key words: colorectal cancer; polymorphism; rs895819

INTRODUCTION

The incidence of colorectal cancer (CRC) has significantly increased in recent decades worldwide, with high morbidity and mortality observed both in developed and developing countries. According to a CRC study in 2014, approximately 71,830 men and 65,000 women have been diagnosed with CRC, and 26,270 men and 24,040 women have died of the disease in the United States (1). In China, a total of 274,841 persons were diagnosed as new CRC patients and 132,110 patients died in 2010 (2). Although it is essential to understand the mechanism of CRC carcinogenesis, the precise mechanisms remain largely unknown. It is well known that CRC is one of the complicated diseases with an intense cross-talk of genetic and environmental factors. Evidences suggest that genetic variation and aberrant epigenetics of CRC-related genes are closely associated with CRC carcinogenesis (3, 4).

MicroRNA-27a (*miR-27a*), which is located in 19p13.13, including an exon, is considered as a candidate

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CRC-related susceptible gene. It comprises a small noncoding RNA with length of 22 bp. It has been identified as an oncogene in many kinds of cancers, including CRC (5). It could be involve in the cell proliferation, metastasis, and drug resistance in cancer cell lines by specially binding to 3′-UTR of its target genes. Significantly upregulated miR-27a was observed in gastric cancer; pancreatic carcinoma; laryngeal tumor tissues; and RKO, SW480, and HT29 cell lines (5–9). It is reported that miR-27a could regulate cell proliferation and division, colony formation, and migration in pancreatic cell line (8). Also, it also could regulate the drug resistance of esophageal, gastric, and CRC cell line as well as leukemia (10–13). Furthermore, it was found that miR-27a could promote metastasis in gastric and CRC cells by inducing epithelial–mesenchymal transition (6, 14). Thus, *miR-27a* is supposed to be a candidate gene that is involved in colorectal carcinogenesis.

Rs895819, a single nucleotide polymorphism (SNP) locus with an alteration from allele T to G, is located in exon 1 of *miR-27a*. It is suggested that change from allele T to G can influence the maturation of microRNAs (miRNAs) or miRNA-mediated transcriptional regulation (15). Sun et al. (15) reported that allele G carrier (AG/GG) of the locus could increase the susceptible risk for gastric cancer by 1.48 folds in Chinese population. Meanwhile, a recent study conducted by Xiong et al. (16) indicated that allele G, genotype AG, and genotype GG were associated with decreased risk for cervical cancer. Furthermore, the results reported by Kupcinskas et al. (17) suggested that rs895819 was not a genetic susceptible locus for CRC in European subjects. However, Cao et al. (18) reported that genotypes AG and GG had a significantly increased risk of developing CRC compared to AA carriers in Chinese. For this, in order to address the role of rs895819 in susceptibility to CRC, we genotyped the locus in 508 CRC patients and 562 healthy check-up individuals using TaqMan genotyping polymerase chain reaction (PCR) system, and examined the possible association between them.

MATERIALS AND METHODS

Five hundred thirty-two newly diagnosed CRC patients and 578 healthy check-up individuals from February 2010 to August 2014 in the second hospital of Fuzhou were enrolled in the study. All cases were confirmed in accordance with the histological evidence. The controls were healthy check-up individuals recruited from the same hospital. Because of its invasiveness, colonoscopy was not used to exclude the CRC in the controls. But, all subjects in the control group were free from symptoms, and serum tumor protein biomarkers, such as CEA, CA199, and CA50, were normal. All subjects of the study are of Han nationality, which consisted of 95% Chinese population. This study was approved by the ethical committee of Fuzhou Second hospital and written informed consents were obtained from all individuals enrolled in the present study.

The genomic DNA of each participant of the case and control groups were extracted from 200 μl heparin-anticoagulated peripheral blood samples, using Tiangen genomic DNA isolation kit (Tiangen, Beijing, China) in accordance with the manufacturer's instructions. The DNA concentration and purity of each sample were measured by an ultraviolet spectrophotometer (GE Healthcare, USA) and samples with A260/A280 ratio ranged from 1.8 to 2.0 were selected as eligible samples and stored at −20°C. TaqMan allelic discrimination assay was selected to detect genotype of each sample using ABI7500 fluorescence quantitative PCR system (Applied Biosystems, Foster City, CA). The detections were performed in a total volume of 20 μl, which contains 100 ng genomic DNA temple, 10 pM of each primer, 0.2 mM dNTPs, 2.5 μl 10× PCR buffer, 1.5 mmol/l $MgCl_2$, and 0.5 U of Taq polymerase (Tiangen). Primers, probe sequences, reaction conditions used were described previously by Wang et al. (19). In order to confirm the accuracy of the detected results, 5% PCR products were randomly selected to DNA sequencing and the results were 100% consistent.

Allele and genotype frequencies of the locus were obtained by direct counting. Hardy–Weinberg equilibrium (HWE) analysis was conducted using a goodness-of-fit chi-squared test with one degree of freedom, and $P < 0.05$ was considered as a significant departure from HWE (20). Student's *t*-test and chi-squared test were selected for statistical analysis of quantitative and qualitative data, respectively. Crude and adjusted odds ratio (OR) and 95% confidential interval (95% CI) were calculated to estimate the strength of the locus and cancer risk. All the data analyses were performed using SPSS 17.0 statistical software (SPSS Company, Chicago, IL).

RESULTS

Due to genomic DNA purity, 24 cases and 16 control samples were excluded from the study. As a result, a total of 508 cases and 562 controls were enrolled in the study. The detail demographic and baseline characteristics of each group are described in Table 1. As shown in Table 1, there was no significant difference in the result with regard to age, sex, smoking, and drinking in cases and controls. The allele and genotype frequencies of the locus in cases and controls are described in Table 2. The respective genotype frequency of AA, AG, and GG were 48.2%, 34.8%, and 17.1% in the cases, 49.1%, 39.3%, and 11.6% among the controls, respectively. The locus genotype distributions in controls was consistent with the HWE model ($P = 0.053$). No significant distribution difference was found when genotype AG was compared to AA (34.8% vs. 39.3%, $P = 0.417$, adjusted OR = 0.878, 95%

TABLE 1. Baseline Characteristics of Case and Control Groups

Variables	Cases (508)	Percentage (%)	Controls (562)	Percentage (%)	*P*-value
Age (years, $M + SD$)	60.2 ± 10.5		59.8 ± 10.6		0.556
Gender					
Male	293	57.7	329	58.5	0.775
Female	215	42.3	233	41.5	
Smoking					
Yes	235	46.3	259	46.1	0.954
No	273	53.7	303	53.9	
Drinking					
Yes	247	48.6	274	48.8	0.965
No	261	51.4	288	51.2	
Location					
Colon	293	57.7			
Rectum	215	42.3			
Tumor size					
<5 cm	260	51.2			
≥5 cm	248	48.8			
Differentiation					
High/moderate	356	70.1			
Poor/undifferentiated	152	29.9			
TNM					
I/II	311	61.2			
III	197	38.8			
Node metastasis					
N0/N1	422	83.9			
N2	86	16.1			

CI = 0.611–1.150), or in comparison of allele G with A (34.4% vs. 31.2%, $P = 0.113$, adjusted OR = 1.156, 95% CI = 0.965–1.386), indicating that genotype AG and allele G were not associated with CRC. There was no significant difference in dominant model (AG/GG vs. AA; 51.8% vs. 50.9%, $P = 0.773$, adjusted OR = 1.033, 95% CI = 0.809–1.318) or overdominant model (34.6% vs. 37.5%, $P = 0.114$, adjusted OR = 0.806, 95% CI = 0.624–1.040). However, significant frequency differences were examined in comparison to GG vs. AA (17.1% vs. 11.6%, $P = 0.027$, adjusted OR = 1.546, 95% CI = 1.070–2.236), and in recessive model (AA/AG vs. GG, 82.9% vs. 88.4%, $P = 0.009$, adjusted OR = 0.631, 95%CI = 0.446-0.893), suggesting that genotype GG was a susceptible factor with increased risk for CRC, allele A carrier (AA/AG) was a protective factor for colorectal carcinogenesis.

In order to further explore the association between them, we performed the analysis between the locus and clinical pathological characteristics of the cases. There was no significant association between cancer location, cell differentiation, node and distal metastasis, and genotype and allele distributions of rs895819. However, significant associations were observed in allele G carrier (AG/GG; $P = 0.019$), allele G ($P < 0.001$) of rs895819 with larger

TABLE 2. MiR-27 rs895819 Polymorphism Genotype and Allele Distributions in the Cases and Controls

Model	Genotype and allele	Cases	Controls	*P*-value	OR and 95% CI	Adjusted OR and 95% CI[a]
Codominant	AA	245 (48.2%)	275 (48.9%)			
	AG	176 (34.8%)	222 (39.5%)	0.417	0.897 (0.690–1.166)	0.878 (0.671–1.150)
	GG	87 (17.1%)	65 (11.6%)	0.027	1.508 (1.047–2.171)	1.546 (1.070–2.236)
Dominant	AA	245 (48.2%)	275 (48.9%)			
	AG/GG	263 (51.8%)	287 (51.1%)	0.773	1.036 (0.815–1.317)	1.033 (0.809–1.318)
Recessive	GG	87 (17.1%)	65 (11.6%)			
	AA/AG	421 (82.8%)	497 (88.4%)	0.009	0.633 (0.448–0.895)	0.631 (0.446–0.893)
Overdominant	AG	176 (34.6%)	222 (39.5%)			
	AA/GG	332 (65.4%)	340 (60.5%)	0.114	0.818 (0.638–1.049)	0.806 (0.624–1.040)
Allele	Allele A	666 (65.6%)	772 (68.7%)			
	Allele G	350 (34.4%)	352 (31.3%)	0.113	1.157 (0.966–1.387)	1.156 (0.965–1.386)

[a]Adjusted by age, gender, smoking, and drinking.

TABLE 3. Rs895819 Polymorphism and Clinical Pathological Characteristics in the Case Group

	Genotype			*P*-value		Allele		
Variables	AA	AG	GG	[1]	[2]	A	G	*P*-value
Location								
Colon	135	91	44			361	179	
Rectum	110	85	43	0.490	0.467	305	171	0.353
Tumor size								
<5 cm	130	73	30			333	133	
≥5 cm	127	91	57	0.224	0.009	333	217	<0.001
Differentiation								
High/moderate	106	77	43			289	163	
Poor/undifferentiated	139	99	44	0.921	0.321	377	187	0.333
TNM								
I/II	159	105	47			408	184	
III	86	71	40	0.212	0.658	258	166	0.008
Node metastasis								
N0/N1	205	139	78			117	55	
N2	40	37	9	0.219	0.177	549	295	0.454

[1], genotype AG versus AA; [2], genotype GG versus AA.

tumor size (> 5 cm), and allele G ($P = 0.008$) with higher TNM stage (III). The detail association between rs895819 and clinical pathological characteristics are listed in Table 3.

DISCUSSION

miRNA is an endogenous small noncoding RNA that contains only 17–25 nucleotides. It can negatively regulate gene expression at the posttranscriptional level predominantly by binding to the 3′-UTR of target mRNAs through nucleotide pairing (21). Although it represents only a small proportion of the genome, it could regulate almost one-third of human genes (22), and exists a wide range of functions in organ growth and development (22), cell proliferation and differentiation (23), and carcinogenesis and metastasis (24,25). Genetic variations of miRNA have been reported to be associated with many kinds of cancers, including CRC. A recent meta-analysis indicated *miR-196a2* rs11614913 and *miR-149* rs2292832 polymorphisms might contribute to susceptibility to CRC (26). A study reported by Zhou et al. suggested that *miR-146a*G>C and *miR-196a2*C>T polymorphisms were genetic susceptible loci for hepatocellular carcinoma (HCC) patients in China population, especially in patients with hepatitis B virus (HBV) infection (27). Moreover, SNP of miRNA, miR-machinery genes, and miRNA-binding site of the targeted gene have also been reported to be associated with susceptibility or prognosis as well as drug resistance of cancer (3, 10).

In the present study, we found that genotype GG of rs895819 within *miR-27a* was associated with a significantly increased susceptibility to the risk of CRC, indicating that genotype GG might be a genetic susceptible factor for CRC. Meanwhile, significant association was found between larger cancer size (>5 cm), higher TNM stage (III), and allele G of rs895819, suggesting that allele G was associated with a higher risk for cancer progression. These findings demonstrated that rs895819 might be involved in colorectal carcinogenesis and progression, those who carry genotype GG or allele G would suffer an increased risk for carcinogenesis and procession of CRC. Our result is consistent with the reports by Cao et al. and Wang et al. (18, 19). Rs895819, which was located in the terminal loop of pre-miRNA region of *miR-27a*, would affect the second structure of mature miR-27a, resulting in abnormal function. It was reported that overrepression of miR-27a was found in colorectal cell lines such as RKO, SW480, and HT29 (5, 6). A relatively higher expression of miR-27a in CRC tissues was examined in patients with the genotype GG or allele G carrier (AG/GG) compared to genotype AA (18). Because *miR-27a* has been suggested to function as an oncogene in CRC (28), a relatively higher expression of miR-27a could direct inhibition expression of PLK2 and ZBTB10 (9, 15), leading to an increase of cell viability and colony formation and inhibition of the late apoptosis (9). This maybe the possible reason why genotype GG of rs895819 was associated with an increased risk for CRC.

The present study, to the best of our knowledge, was the largest sample size to report the association between rs895819 and CRC, and indicated that genotype GG of the locus might be a susceptible factor for risk of CRC. However, several limitations should be addressed as follows. Although the genotype distributions in controls were consistent with HWE, all the subjects in our study were

from one single hospital, which might lead to selection bias. The sample sizes in the case and control groups were 508 and 562, respectively, but the sample size was not large enough to reach a more precise conclusion. Due to lack of CRC tissue corresponding to the blood sample, we did not perform the detection of miR-27a and the expression of the targeted gene and the association between genotypes of rs895819 and the expressions of miR-27a and the targeted gene.

CONCLUSION

The present study indicates that genotype GG of rs895819 within *miR-27a* is significantly associated with CRC, and miR-27a might be involved in colorectal carcinogenesis and progression. Further, multiple central, large-sample size, and well-designed epidemiological study and functional research should be conducted to further validate the finding of the study.

CONFLICT OF INTEREST

The authors have declared no conflict of interests with respect to the authorship and/or publication of this article.

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