ORIGINAL RESEARCH

Association of *MDM4* Gene rs4245739 Polymorphism with the Risk and Clinical Characteristics of Colorectal Cancer in a Chinese Han Population

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Background: Studies show that MDM4 may play a pivotal role in colorectal cancer (CRC). Recently, a host of studies suggest that MDM4 gene rs4245739 polymorphism may modify the risk of different cancers.

Methods: In this study, we were interested whether MDM4 gene rs4245739 polymorphism correlated with the risk and clinical characteristics of CRC. Logistic regression was adopted to estimate the association of rs4245739 polymorphism and CRC risk.

Results: We enrolled 444 CRC patients and 530 controls and found MDM4 gene rs4245739 polymorphism may decrease the risk of CRC. Stratified analyses uncovered that this variant was connected to a less risk of CRC in females, non-drinkers, non-smokers, and people under 60 years old. Additionally, rs4245739 polymorphism was related to TNM staging, pathological type, tumor size, and location of CRC. Furthermore, this polymorphism was significantly linked with the survival of CRC.

Conclusion: Totally, this study suggests that MDM4 rs4245739 polymorphism is linked with the risk and clinical characteristics of CRC.

Keywords: *MDM4*, colorectal cancer, case–control study, rs4245739, polymorphism

Introduction

Colorectal cancer (CRC) is one of the most frequently diagnosed cancers and is the fourth most mortal cancer with an annual rate of almost 900,000 CRC-related deaths worldwide.[1] The global new CRC patients are predicted to roar by 60% to above 2,200,000 in 2030, with 1,100,000 deaths caused by CRC.[2] CRC ranks the 5th and 4th most dominant cancer in males and females in China, respectively.[3] Up to date, the pathogenesis of CRC is still unclear. Both genetic factors and environmental risk factors including poor diets, obesity, alcohol consumption, smoking, and lacking of exercise were reportedly associated with CRC risk.[4,5] A host of studies have identified novel gene loci associated with CRC susceptibility.[6–11]

P53, a tumor suppressor gene, plays a crucial role in multiple physiological processes, including cell cycle initiation and arrest, DNA lesion repair, signal pathway of apoptosis, autophagy, metabolism, and oxidative status.[12,13] The main process of p53 degradation is ubiquitin-mediated proteolysis. One of the ubiquitin-labeled p53 enzymes is mouse double minute (MDM)-2 protein. MDM2 and its homolog MDM4 have very similar protein structures and both have an N-terminus,

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a region containing p53-binding domain.[14] The activity of p53 is negatively regulated by the interaction between MDM2 and MDM4.[15–17] MDM4 can directly connect to MDM2 to suppress its decomposition, which impacts the inhibitory effects of MDM2 on p53 activity.[18] MDM4 is associated with tumor formation via restraining p53 tumor suppressor activity.[19–21] Double knockdown of MDM4 and MDM2 can enhance the antitumor activity of 5-fluorouracil in colon cancer cells.[22] MDM4 may be critical in colorectal carcinogenesis.[23]

MDM4 gene is located on chromosome 1q32. Recently, some studies investigated the potential link between MDM4 gene rs4245739 polymorphism and the risk of various cancers.[24,25] Among these studies, only the Norwegian study by Gansmo et al probed into the connection between MDM4 gene rs4245739 polymorphism and CRC risk; however, they found no connection.[26] In addition, no Chinese study interpreted the relationship between CRC risk and MDM4 gene rs4245739 polymorphism among Chinese individuals. Thus, we performed this study to address the connection between this variant and CRC susceptibility in a Chinese population.

Patients and Methods
Subjects
Totally 444 CRC patients and 530 volunteers were enrolled from Dalian Municipal Central Hospital and Nantong Third People's Hospital. No CRC patient had undergone radio- or chemo-therapy. Diagnosis of CRC was made histopathologically. Clinicopathologic data of all participants were acquired from medical records. Qualified controls were chosen from the same area within the same period. All enrolled participants were more than 18 years old. Approval was given by the Ethics committees of the tested Hospitals, and Declaration of Helsinki was followed. All subjects provided written informed consent.

Blood Collecting and Genotyping
Peripheral blood (2 mL) was collected from all participants, and DNA was isolated from its leukocytes using a DNA purifying Kit (Tiangen Biotech) as instructed by the manufacturer. A matrix-supported laser desorption/ionization time-of-flight mass spectrometer on a MassARRAY system (Sequenom, San Diego, CA, USA) was adopted for genotyping. The primers of GTAGTACGAACATAAAAATGCATT TATCCA (forward) and ATTTTCAAATAATGTGGTAAG

TGAGCG (reverse) were used for nucleotide extension. Each PCR involved a mixture (25 ul) of genotyping assays (20×, 1.25 ul), DNA (20 ng) and genotyping master mix (2×, 12.5 ul). PCR procedures were denaturing at 96 °C, 5 min; 35 cycles, 96 °C for 30 s, annealing at 57 °C, 40 s; elongating at 72 °C for 5 min. Genotyping accuracy was guaranteed by randomly choosing 1/10 of the specimens for secondary testing.[27] The results were 100% consistent.

Statistical Analyses
All statistical analyses were carried out on SPSS 22.0 (SPSS Inc., Chicago, USA) at the significance level of $P < 0.05$. Categorical and continuous data were examined by Chi-square (χ^2) test and Student's t-test, respectively. Hardy–Weinberg equilibrium (HWE) was assessed using χ^2-test. The genotype and allele type allocations between groups were compared via logistic regression by calculating the odds ratios (ORs) and 95% confidence intervals (CIs) with or without adjustment. Stratification was done by sex, age, drinking and smoking status. Additionally, the exposure combined models were assessed by logistic regression. Overall survival (OS) was defined by the Kaplan–Meier approach.

Results
Characteristics of Subjects
Demographic and clinical information of the subjects is listed in Table 1. No differences between the two groups were identified for age, smoking, sex, or alcohol. In terms of site of cancer, there were 290 with rectal cancer and 154 patients with colon cancer. The 444 CRC patients consisted of 95.4% adenocarcinoma (424), 3.2% squamous cell carcinoma (14), and 1.4% other types (6). We also investigated tumor node metastasis (TNM) stage, tumor size, and family history of CRC patients.

Connection Between CRC Risk and MDM4 Gene rs4245739 Polymorphism
The genotype and allele allocations of the tested polymorphism differed considerably between the CRC patients and controls (Table 2). The HWE test showed no evident bias in genotypic frequency among the controls. Individuals with AC and CC genotype were at lower risk of developing CRC (AC vs AA: OR, 0.76; 95%CI, 0.57-1.00; $P = 0.046$; CC vs AA: OR, 0.47; 95% CI, 0.23–0.97; $P = 0.036$). The presence of CC+AC genotype or C allele demonstrated a significantly lower risk for CRC. These results were also

Table 1 Demographic Information and Risk Factors for Colorectal Cancer and Control

Characteristics	Case (N=444)	Control (N=530)	P
Age	56.45±8.42	55.68±9.03	0.169
Sex			0.580
Male	299(67.3%)	348(65.7%)	
Female	145(32.7%)	182(34.3%)	
Smoking			0.570
Yes	194(43.6%)	222(41.8%)	
No	250(56.4%)	308(58.2%)	
Alcohol			0.408
Yes	263(59.2%)	300(56.6%)	
No	181(40.8%)	230(43.4%)	
Family history			
Yes	77(17.3%)		
No	367(82.7%)		
TNM stage			
I + II	228(51.3%)		
III+IV	216(48.7%)		
Tumor size			
>5 cm	264(59.4%)		
≤5 cm	180(40.6%)		
Pathological type			
Adenocarcinoma	424(95.4%)		
Squamous cell carcinoma	14(3.2%)		
Others	6(1.4%)		
Location of colorectal cancer			
Rectal cancer	290(65.3%)		
Colon cancer	154(34.7%)		

Abbreviation: TNM, tumor node metastasis.

true in dominant and homozygote models after age and gender adjustment. We then further evaluated the role of MDM4 gene rs4245739 in the risk of CRC stratified by sex,

age, alcohol and smoking (Table 3). Non-drinkers, non-smokers, women, and youngsters (age <60 years) were found with significantly less risk of CRC.

Correlation Between MDM4 Gene rs4245739 and Clinicopathological Data of CRC Patients

Then, the connection between the tested polymorphism and clinical data of CRC patients was assessed. MDM4 gene rs4245739 polymorphism was connected to the histological grade, TNM stage, and tumor size of CRC (Table 4).

Survival Analysis of MDM4 Gene rs4245739 Polymorphism with CRC Patients

We explored the relationship between this variant and the prognosis of CRC patients. For the tested polymorphism, Kaplan-Meier single-factor analysis showed AC genotype carriers relative to AA genotype enjoyed significantly better OS (HR, 0.66, 95% CI, 0.47–0.93; log-rank P = 0.018, Figure 1).

Discussion

Herein, this study showed that MDM4 gene rs4245739 polymorphism was related to decreased risk and prognosis for CRC in Chinese subjects. Stratified analyses indicated the C allele from this single nucleotide polymorphism (SNP) has a protective role in CRC among females, non-drinkers, non-smokers, and those at age <60 years. Furthermore, the tested polymorphism was linked with the survival of CRC.

Table 2 Genotype Frequencies of MDM4 Gene Rs4245739 Polymorphism in Cases and Controls

Models	Genotype	Case (n, %)	Control (n, %)	OR (95% CI)	*P*-value	*OR (95% CI)	*P*-value
Co-dominant	AA	304(68.6%)	323(61.2%)	1.00(reference)	–	1.00(reference)	
Heterozygote	AC	128(28.9%)	180(34.1%)	**0.76(0.57–1.00)**	**0.046**	**0.70(0.52–0.94)**	**0.019**
Homozygote	CC	11(2.5%)	25(4.7%)	**0.47(0.23–0.97)**	**0.036**	**0.43(0.21–0.90)**	**0.025**
Dominant	AA	304(68.6%)	323(61.2%)	1.00(reference)	–	1.00(reference)	
	CC+AC	139(31.4%)	205(38.8%)	**0.72(0.55–0.94)**	**0.016**	**0.67(0.50–0.90)**	**0.006**
Recessive	AC+AA	432(96.8%)	503(95.3%)	1.00(reference)	–	1.00(reference)	
	CC	11(2.5%)	25(4.7%)	0.51(0.25–1.05)	0.064	0.50(0.24–1.03)	0.059
Allele	A	736(83.1%)	826(78.2%)	1.00(reference)	–	1.00(reference)	
	C	150(16.9%)	230(21.8%)	**0.73(0.58–0.92)**	**0.007**	–	–

Notes: The genotyping was successful in 443 cases and 528 controls for rs4245739 polymorphism; Bold values are statistically significant (P < 0.05). *Adjusting for age and sex.

Table 3 Stratified Analyses Between MDM4 Gene Rs4245739 Polymorphism and the Risk of Colorectal Cancer

Variables	(Case/Control)			AC vs AA	CC vs AA	CC vs AA+AC	CC+AC vs AA
	AA	AC	CC				
Sex							
Male	229/255	64/80	6/13	0.89(0.61–1.30); 0.545	0.51(0.19–1.37); 0.177	0.53(0.20–1.41); 0.194	0.84(0.59–1.20); 0.333
Female	75/68	64/100	5/12	**0.58(0.37–0.91); 0.018**	0.38(0.13–1.13); 0.073	0.50(0.17–1.46); 0.200	**0.56 (0.36–0.87); 0.010**
Smoking							
Yes	120/132	68/78	5/10	0.96(0.64–1.44); 0.841	0.55(0.18–1.66); 0.281	0.56(0.19–1.66); 0.289	0.91(0.61–1.36); 0.651
No	184/191	60/102	6/15	**0.61(0.42–0.89); 0.010**	0.42(0.16–1.09); 0.067	0.48(0.18–1.26); 0.127	**0.59(0.41–0.84); 0.004**
Alcohol							
Yes	144/152	113/138	5/8	0.86(0.62–1.21); 0.397	0.66(0.21–2.06); 0.472	0.71(0.23–2.18); 0.543	0.85(0.61–1.19); 0.350
No	160/171	15/42	6/17	**0.38(0.20–0.72); 0.002**	0.38(0.15–0.98); 0.039	0.43(0.17–1.11); 0.074	**0.38 (0.22–0.66); <0.001**
Age (years)							
<60	193/183	54/96	6/16	**0.53(0.36–0.79); 0.001**	**0.36(0.14–0.93); 0.028**	0.42(0.16–1.10); 0.070	0.51(0.35–0.74); 0.000
≥60	111/140	74/84	5/9	1.11(0.75–1.66); 0.605	0.70(0.23–2.15); 0.532	0.67(0.22–2.04); 0.481	1.07(0.73–1.58); 0.729

Note: Bold values are statistically significant ($P < 0.05$).

Several studies focused on the connection between MDM4 gene rs4245739 polymorphism and cancer risk. Zhou et al firstly observed that MDM4 rs4245739 polymorphism decreased the risk of esophageal squamous cell carcinoma (ESCC) and assumed that rs4245739 polymorphism can interrupt the miRNA-regulated gene regulation, which can modify ESCC risk.[28] They subsequently reported an association between MDM4 rs4245739 polymorphism and a lower risk of breast cancer (BC) in a study with 1,100 BC patients and 1,400 controls in China.[29] As for BC, conflicting results were obtained in other studies. Gansmo et al indicated that C allele of rs4245739 polymorphism reduced the risk for BC marginally in a population from Norway.[26] Two studies from Iran did not obtain any association between this SNP and BC risk.[30,31] However, a genome-wide association study with 10,707 BC and 76,646 controls identified rs4245739 polymorphism as an important estrogen receptor (ER) negative–specific BC risk locus.[32] Differences in BC types, sample sizes and races may

Table 4 The Associations Between MDM4 Rs4245739 Polymorphism and Clinical Characteristics of Colorectal Cancer

Characteristics	Genotype Distributions			
	AA	AC	CC	AC+CC
TNM stage				
III+IV/ I + II	160/144	51/77	5/6	56/83
OR (95% CI); P-value	1.0 (reference)	**0.60(0.39–0.91); 0.015**	0.75(0.22–2.51); 0.640	**0.61(0.40–0.91); 0.016**
Tumor size				
>5 cm/≤5 cm	198/106	60/68	6/5	66/73
OR (95% CI); P-value	1.0 (reference)	**0.47(0.31–0.72); 0.000**	0.64(0.19–2.15); 0.689	**0.48(0.32–0.73); 0.000**
Family history				
Yes/No	48/256	26/102	3/8	29/110
OR (95% CI); P-value	1.0 (reference)	1.36(0.80–2.31); 0.255	2.00(0.51–7.81); 0.549	1.41(0.84–2.35); 0.191
Pathological type				
Adenocarcinoma/Not	294/10	121/7	8/3	129/10
OR (95% CI); P-value	1.0 (reference)	0.59(0.22–1.58); 0.287	**0.09(0.02–0.39); 0.008**	0.44(0.18–1.07); 0.066
Location of colorectal cancer				
Rectal cancer/colon cancer	211/93	73/55	6/5	79/60
OR (95% CI); P-value	1.0 (reference)	**0.59(0.38–0.90); 0.013**	0.53(0.16–1.78); 0.475	**0.58(0.38–0.88); 0.010**

Note: Bold values are statistically significant ($P <0.05$).
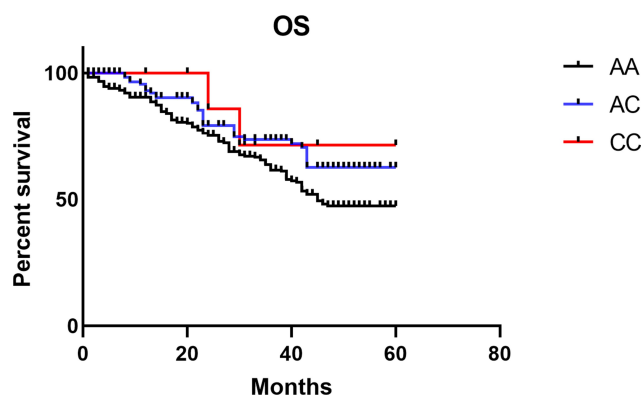**Abbreviations:** TNM, tumor node metastasis.

OS

Figure 1 Kaplan–Meier analysis of overall survival of CRC patients with MDM4 gene rs4245739 polymorphism.

contribute to these inconsistent findings regarding BC. As for other types of cancers, Fan et al showed MDM4 rs4245739 polymorphism decreased the risk of non-Hodgkin lymphoma.[33] Gao et al observed that this SNP increased susceptibility to small cell lung cancer.[34] Mohammad Khanlou et al. revealed that MDM4 rs4245739 polymorphism did not associate with the risk of thyroid cancer among Iranian-Azeri patients.[35] Different from the study by Gansmo et al from Norway,[26] we recognized a relationship of MDM4 rs4245739 polymorphism with decreased risk for CRC in Chinese Han population. Gansmo et al suggested this SNP was not associated with CRC risk,[26] but they only investigated colon cancer and ignored rectal cancer.[26] Obviously, the sample sizes and ethnicities were both different between our study and the Norwegian study. Another point was that eating habits and living environments were different. These above factors may explain the conflicting findings. Due to these paradoxical results, Wang et al conducted a meta-analysis to address this issue and found that rs4245739 polymorphism decreased the risk of overall cancer,[36] which was in line with our study.

Next, the stratified analyses of some factors found that MDM4 rs4245739 polymorphism correlated with a lower risk of CRC in females, non-smokers, non-drinkers, and those at age <60 years old, which suggested these exposure risk factors probably interact with the rs4245739 polymorphism. In addition, we evaluated the link of this polymorphism with clinicopathological data of CRC patients. The tested polymorphism in CRC patients was correlated to the tumor size, TNM stage, pathological type, and location of CRC. Reportedly, the MDM-4 oncogene rs4245739 SNP set up an unsuitable miR-191 target location and was related to both overall and disease-free survivals of ovarian cancer among Caucasians.[37] MDM4 rs4245739 AC/CC genotypes were also significantly related to better overall, disease-specific, and

disease-free survival.[38] We observed that AC genotype carriers showed better OS compared with AA genotype carriers. As far as we know, we uncover a connection of rs4245739 polymorphism with the survival of CRC for the first time.

This study harbors some limitations. First, the moderately large sample size may decrease the power value of this study. Second, only one SNP in the MDM4 gene was investigated. Third, there were insufficient follow-up data of CRC patients. Fourth, we only recruited the Han Chinese population. Last, functional experiments should be conducted to further investigate the roles of this SNP in the pathogenesis of CRC.

To sum up, MDM4 gene rs4245739 polymorphism is linked with the risk and prognosis of CRC, and the C allele has a protective role in CRC risk and prognosis particularly. Further researches in other populations are warranted to validate these findings.

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Disclosure

The authors report no conflicts of interest for this work.

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