HMGA2 rs968697 T>C Polymorphism is Associated With The Risk of Colorectal Cancer

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Abstract

Background: Recently, a genetic polymorphism (rs968697 T>C) in *HMGA2* gene has been reported to be associated with hepatoblastoma risk. However, no studies reported the effect of the polymorphism on the risk of colorectal cancer (CRC). The study aimed to explore whether rs968697 polymorphism had a significant impact on CRC risk.

Methods: A total of 500 CRC patients and 500 age and gender matched healthy individuals were genotyped by Sanger sequencing. Quantitative real-time PCR technology was used to detect the relative expression of *HMGA2* gene in 30 pairs of primary CRC and adjacent non-cancerous tissues.

Results: *HMGA2* rs968697 polymorphism was significantly associated with CRC risk (CC vs. TT: OR=0.20, 95%CI=0.06-0.70, P=0.01; (CC+CT) vs. TT: OR=0.71, 95%CI=0.53-0.96, P=0.02; CC vs. (CT+TT): OR=0.21, 95%CI=0.06-0.73, P=0.01; C vs. T: OR=0.67, 95%CI=0.51-0.89, P<0.01). The analysis based on tumor stage indicated that *HMGA2* rs968697 polymorphism was significantly associated with CRC tumor stage. In addition, the genotype-tissue expression showed that the rs968697 polymorphism was related to *HMGA2* gene expression. The in silico analysis showed that rs968697 polymorphism located in the promoter region of *HMGA2* gene could affect the binding of transcription factors.

Conclusion: Our study suggested that *HMGA2* rs968697 polymorphism was associated with CRC risk and might serve as a reliable biomarker to detect CRC risk.

Running head: *HMGA2* rs968697 T>C polymorphism and colorectal cancer

1. Introduction

High mobility group AT-hook 2 (*HMGA2*) gene is located on chromosome12q14.3, and encodes an architectural transcription factor that belongs to the non-histone chromosomal high mobility group protein family. The protein can act as an oncogenic protein closely linked to aggressive tumor behavior and poor clinical outcomes [1–3]. Thereinto the promoting role of HMGA2 in the occurrence and development of colorectal cancer (CRC) has been reported [4–5]. For instance, HMGA2 over-expression was associated not only with metastasis but also with reduced survival rates of CRC patients [4]. HMGA2 could promote intestinal tumorigenesis by facilitating MDM2-mediated ubiquitination and degradation of p53 [5]. In recent years, a case-control study also investigated the association of a genetic polymorphism (rs968697 T > C) in the cancer-related gene with cancer risk, and found that the polymorphism was significantly associated with hepatoblastoma risk.

In view of the significance of rs968697 polymorphism and the critical role of *HMGA2* gene in CRC, we speculated that *HMGA2* rs968697 polymorphism might be involved in CRC risk. However, no studies reported the effect of the polymorphism on CRC risk. Thus, a case-control study was conducted to assess the strength of the effect.

2. Methods And Materials

2.1 Peripheral blood sample collection

Peripheral blood samples of 500 CRC patients and 500 age and gender matched healthy individuals were collected from Xuhui District Central Hospital of Shanghai between October 2017 and January 2021. The mean age of CRC patients and healthy individuals was 59.1 ± 7.4 and 59.4 ± 7.7 years, respectively. The ratio of male and female was 53:47 in both CRC patients and healthy individuals. Diagnosis of all patients was histopathologically confirmed. Healthy individuals were cancer-free individuals living in the same residential area and seeking for routine physical examination. This study protocol was approved by the Institutional Review Board of the Hospital. All enrolled subjects provided written informed consent in the study.

2.2 Genotyping

Genomic DNA was extracted from peripheral blood samples by using the TIANamp genomic DNA Kit (Tiangen). The concentration and quality of genomic DNA were determined by using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Genotyping was performed by Sanger sequencing.

2.3 The relationship between rs968697 T > C polymorphism and *HMGA2* gene expression

Total RNA was extracted from 30 pairs of primary CRC and adjacent non-cancerous tissues by using the RNAsimple total RNA kit (Tiangen) according to the manufacturer's instructions. The cDNA was synthesized using ReverTra Ace qPCR RT Kit (TOYOBO). Quantitative Real-time PCR was performed using Roche FastStart Universal SYBR Green Master (Rox). The expression level of *HMGA2* gene was normalized to the internal control GAPDH. The $2^{-\Delta\Delta Ct}$ method was used to determine *HMGA2* gene expression. The specific primer pairs for HMGA2 and GAPDH were 5'-CAAGTTGTTCAGAAGAAGCC-3' (forward), 5'-GGCAATACAG AATAAGTGGTC-3'(reverse); and 5'-GTCTCCTCTGACTTCAACA-3' (forward), 5'-TGAGGG TCTCTCTCTTCCT-3' (reverse), respectively.

2.4 Bioinformatics analysis

RegulomeDB (www.regulomedb.org/regulome-search/) is a database annotating SNPs with known and predicted regulatory elements in the intergenic regions of the human genome [6]. SNPinfo (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html) is a web-based tool integrating genome-wide association studies and candidate gene information into functional SNP selection for genetic association studies [7]. In this study, RegulomeDB and SNPinfo were used to explore the latent function of HMGA2 rs968697 polymorphism. For RegulomeDB database, POLR2A ChIP-Seq data from human colon cancer cell (HCT116) was analyzed.

2.5 Statistical analysis

In control group, Hardy-Weinberg equilibrium (HWE) of the genotype distribution was analyzed using the chi-square goodness-of-fit test. The association between *HMGA2* rs968697 polymorphism and CRC risk was evaluated using logistic regression analysis. The association between *HMGA2* rs968697 polymorphism and CRC tumor stage was analyzed using the chi-square test. The expression levels of *HMGA2* gene were compared among different genotype groups by using the one-way ANOVA. Statistical analyses were performed using SPSS 22.0 software. P value less than 0.05 was considered statistically significant.

3. Results

3.1 *HMGA2* rs968697 was significantly associated with CRC risk

As shown in Table 1, the genotype distribution of *HMGA2* rs968697 polymorphism was 371/115/14 (TT/CT/CC) in control group, which conformed to HWE (P = 0.17). In CRC patients, the genotype distribution of *HMGA2* rs968697 polymorphism was 400/97/3 (TT/CT/CC). The comparison results showed that *HMGA2* rs968697 polymorphism was significantly associated with CRC risk. The individuals carrying rs968697 CC genotype had a decreased risk of CRC compared with those carrying the TT genotype (OR = 0.20; 95%CI = 0.06–0.70; P = 0.01). The individuals carrying rs968697 CC and CT genotype had a decreased risk of CRC compared with those carrying the TT genotype (OR = 0.71; 95%CI = 0.53–0.96; P = 0.02). The individuals carrying rs968697 CC genotype had a decreased risk of CRC compared with those carrying the CT and TT genotype (OR = 0.21; 95%CI = 0.06–0.73; P = 0.01). The individuals carrying rs968697 C allele had a decreased risk of CRC compared with those carrying the T allele (OR = 0.67; 95%CI = 0.51–0.89; P < 0.01). Furthermore, the analysis based on tumor stage indicated that rs968697 polymorphism was significantly associated with CRC tumor stage (Table 2).

Table 1
The association of *HMGA2* rs968697 polymorphism with CRC risk

Genotype/allele	Cases (N = 500)	Controls (N = 500)	Comparison	[a]OR (95%CI)	[a]P
TT	400 (80.0%)	371 (74.2%)	CT vs. TT	0.77 (0.57–1.05)	0.10
CT	97 (19.4%)	115 (23.0%)	CC vs. TT	0.20 (0.06–0.70)	0.01
CC	3 (0.6%)	14 (2.8%)	(CC + CT) vs. TT	0.71 (0.53–0.96)	0.02
T	897 (89.7%)	857 (85.7%)	CC vs. (CT + TT)	0.21 (0.06–0.73)	0.01
C	103 (10.3%)	143 (14.3%)	C vs. T	0.67 (0.51–0.89)	< 0.01

[a]: adjusted for gender and age; OR: Odds ratio; CI: Confidence interval.

Table 2
The association of *HMGA2* rs968697 polymorphism with CRC tumor stage

Genotype/allele	I + II (N = 265)	III + IV (N = 235)	P
TT	198 (74.7%)	202 (86.0%)	0.01
CT	65 (24.5%)	32 (13.6%)	
CC	2 (0.8%)	1 (0.4%)	
T	461 (87.0%)	436 (92.8%)	< 0.01
C	69 (13.0%)	34 (7.2%)	

3.2 Rs968697 polymorphism was related to *HMGA2* gene expression

As shown in Fig. 1, the genotype-tissue expression showed that rs968697 polymorphism was related to the expression of *HMGA2* gene. *HMGA2* gene had a higher expression level in CRC and adjacent non-cancerous tissues with rs968697 TT genotype than in CRC and adjacent non-cancerous tissues with rs968697 CC genotype.

3.3 Rs968697 polymorphism affected the binding of transcription factors

The in silico analysis showed that rs968697 polymorphism located in the promoter region of *HMGA2* gene affected the binding of transcription factors, such as DBP, CDPCR3, POLR2A and TAXCREB (Table 3).

Table 3
Bioinformatics analysis for the latent function of *HMGA2* rs968697 polymorphism.

SNP	Predictive tools	Transcription factors
rs968697	SNPinfo	ATF6, DBP, CDPCR3, DR3, NRSF, PAX8, PPARA, SZF11, TAXCREB
	RegulomeDB	POLR2A

4. Discussion

As one of the most common cancers, CRC was estimated to cause over 1.8 million new cases and 881,000 deaths in 2018 [8]. Although the molecular etiology of CRC is still unknown, its onset is significantly associated with genetic variants. Single nueleotide polymorphism (SNP) is one of the most common genetic variants and involves altered cancer risk [9–11]. In the current study, we conducted a case-control study investigating the association between *HMGA2* rs968697 polymorphism and CRC risk,

and found that rs968697 C allele could reduce CRC risk compared with the T allele. To the best of our knowledge, this is the first epidemiological study exploring the association of *HMGA2* rs968697 polymorphism with CRC risk in the Chinese population. Furthermore, we also found that rs968697 polymorphism was related to the expression of HMGA2 gene in CRC and adjacent non-cancerous tissues. Bioinformatics analysis showed that the rs968697 polymorphism had an effect on the binding of transcription factors to the promoter of *HMGA2* gene, which provided a possible molecular explanation for the effect of the genetic polymorphism on CRC risk. However, the detailed mechanism still needs to be verified by further well-designed experiment. It is worthy of note that the current sample size is relative small, which may not obtain sufficient statistical power. Therefore, further replication studies are necessary to fully establish the association between *HMGA2* rs968697 polymorphism and CRC risk.

5. Conclusion

Our molecular epidemiological findings demonstrated a significant association of *HMGA2* rs968697 polymorphism with CRC risk. The rs968697 polymorphism may serve as a potential biomarker for CRC risk.

Declarations

Ethical approval and consent to participate

The study was approved by the Ethics Committee of Yancheng Teachers' University, and all participants provided written informed consent.

Authors contributions and consent to publish

ZL drafted the manuscript. SZ collected clinical samples. JY and XW were responsible for the figures and tables. XG critically revised the manuscript. All authors approved the final manuscript.

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Competing Interests

The authors declare no conflict of interest.

Availability of data and materials

The data and materials used or analysed during the current study are available on request to the corresponding author.

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Figures

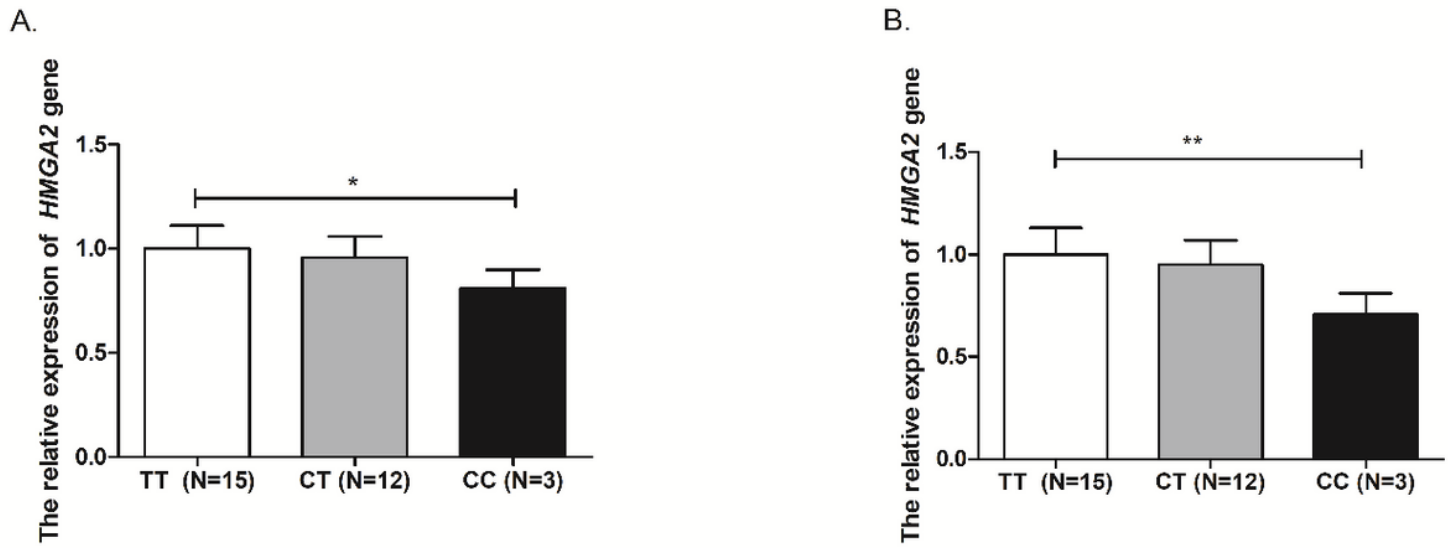

Figure 1

The relationship between rs968697 polymorphism and the expression of HMGA2 gene in 30 pairs of primary CRC and adjacent non-cancerous tissues (A: adjacent non-cancerous tissues; B: CRC tissues; *:P<0.05; **:P<0.01).