

rs712 polymorphism within let-7 microRNA-binding site might be involved in the initiation and progression of colorectal cancer in Chinese population

Qiang-Hua Jiang^{1,*}Hong-Xin Peng^{2,3,*}Yi Zhang⁴Peng Tian⁴Zu-Lian Xi⁴Hao Chen⁴

¹Department of Surgery, Dongyang People's Hospital, Dongyang, Zhejiang, ²Medical College, Southeast University, ³Central Laboratory, Nanjing First Hospital, Nanjing, Jiangsu, ⁴Hubei Key Laboratory of Biological Targeted Therapy, Yichang Yiling Hospital, Yichang, Hubei, People's Republic of China

*These authors contributed equally to this work

Abstract: rs712 within 3'-untranslated region of *KRAS* can affect the specific binding between the mRNA and its targeted microRNAs, leading to the activation of *KRAS* oncogene. However, the possible association between the locus and susceptibility to colorectal cancer (CRC) remains unclear. We investigated genotypes of the locus in 586 cases and 476 controls to explore the possible association between them. Results of our case-control study showed that genotypes TT (6.5% vs 2.5%, $P=0.002$, adjusted odds ratio [OR] = 2.810, 95% confidence interval [CI] = 1.342–5.488) and GT/TT (36.5% vs 30.5%, $P=0.038$, adjusted OR = 1.342, 95% CI = 1.030–1.712) and allele T (21.5% vs 6.5%, $P=0.004$, adjusted OR = 1.328, 95% CI = 1.105–1.722) of rs712 were significantly associated with an increased risk of CRC, and the significant association was also observed in the recessive model (TT vs GG/GT, 6.5% vs 2.5%, $P=0.003$, adjusted OR = 0.372, 95% CI = 0.191–0.725). However, there was no association between genotype GT and risk of CRC (30.0% vs 28.0%, $P=0.235$, adjusted OR = 1.210, 95% CI = 0.903–1.548). Furthermore, genotype GT ($P=0.003$) and allele T ($P=0.003$) were significantly associated with poor differentiation, and genotypes GT and TT and allele T were significantly associated with tumor-node-metastases stage III ($P=0.001$ for GT vs GG, $P<0.001$ for TT vs GG, and $P<0.001$ for T vs G) and node metastasis ($P<0.001$ for GT vs GG, $P=0.001$ for TT vs GG, and $P<0.001$ for T vs G), respectively. These findings indicated that allele T and genotypes TT and GT/TT of rs712 might be susceptible factors for CRC, and mutated allele and genotypes of the locus might predict a poor clinical outcome in Chinese population.

Keywords: rs712, CRC, polymorphism, susceptibility

Introduction

Colorectal cancer (CRC) is a complex disease initiated by the interaction of environmental and personal genetic factors. According to the latest report of American Cancer Society, ~132,700 persons will be diagnosed as new CRC cases and 49,700 individuals with CRC will die in 2015.¹ In 2011, there were 310,244 newly diagnosed CRC patients and 149,722 dead CRC cases in the People's Republic of China.² Although there is an increase in the research and development of strategies for its prevention, diagnosis, and therapy, the etiology of CRC cases remains poorly understood, and the clinical outcome of the case is not significantly improved.

It is well known that many causes, such as chromosome instability, loss of heterozygosity, and promoter CpG island methylation, can lead to carcinogenesis and progression of CRC.³ However, these can only account for a small proportion of the cases. Recently, accumulating evidence indicated that single nucleotide polymorphisms (SNPs) of oncogenes and suppressed genes were involved in the onset of CRC.⁴ A recent meta-analysis reported that rs16892766 polymorphism within the region of

Correspondence: Hao Chen
Hubei Key Laboratory of Biological Targeted Therapy, Yichang Yiling Hospital, Yichang 443100, Hubei, People's Republic of China
Tel/fax +86 0717 753 3236
Email clockren@126.com

8q23.3 was significantly associated with its susceptibility to CRC.⁵ Wu et al⁶ reported that C-1306T polymorphism within MMP-2 is a risk factor for CRC susceptibility, especially in Caucasians. These studies suggested that SNP within key gene might be an important research field to explore the cause of CRC individual.

KRAS is one of the most important oncogene, which is located at 12p12.1. It is a member of *RAS* family and encodes a GDP/GTP-binding protein that belongs to a small GTPase superfamily.⁷ The product is involved in RAF/MEK/MAPK, AKT, and ERK signal transduction pathways and regulates cell proliferation and differentiation in many kinds of cancer cell lines, including CRC cell lines. Moreover, high frequency of *KRAS* mutation had been examined in many kinds of solid cancer, such as CRC, thyroid cancer, and non-small-cell lung cancer.^{8–10} In CRC, a frequency of 59% CRC patients were detected as *KRAS*-mutated cases.¹¹ Furthermore, genetic variation of *KRAS* is involved in tumorigenesis and progression of CRC as well as response to antiepidermal growth factor receptor monoclonal antibody in metastatic CRC.^{12–14} rs712 has been reported to be significantly associated with the risk of cancer.¹² Blons et al¹⁴ reported that *KRAS* exon 2 mutation was involved in the clinical outcome of CRC and might be an independent prognostic biomarker to predict recurrence in resected stage III distal colon cancer patients undergoing adjuvant therapy. Additionally, cetuximab has been recommended to be used in the treatment for metastatic CRC patients with wild-type *KRAS*.¹⁵

Let-7, the first discovered microRNA containing let-7a–g and I, acted as a tumor suppressor in many kinds of malignancies including CRC.¹⁶ It can bind to specific seed regions in mRNA 3′-untranslated region (UTR) of *KRAS*, and the downregulated expression can affect its function in the processes of CRC carcinogenesis and progression. There are ten let-7 complementary binding sites (LCSs) in 3′-UTR of *KRAS* mRNA.¹⁷ rs712, which is located in LCS1 of *KRAS* 3′-UTR,¹⁷ has been intensively investigated in many kinds of malignancies, such as gastric cancer, oral squamous cell carcinoma, and nasopharyngeal carcinoma.^{12,18} So, we speculated that the locus of *KRAS* may also associate with CRC. Thus, we performed this case–control study to investigate the effect of rs712 within *KRAS* on the susceptibility to CRC in Chinese population.

Materials and methods

A total of 1,062 participants were enrolled in the present study. The case group consisted of 586 clinically and pathologically confirmed tumor-node-metastasis stage I–III CRC patients,

and 476 healthy checkup individuals were selected as the controls. All of them were recruited from Yichang Yiling Hospital, Dongyang People’s Hospital, and Nanjing First Hospital between January 2012 and December 2014. Meanwhile, 1 mL peripheral blood from each individual was collected. Clinical data, such as individual’s age, sex, and pathological features, were collected from clinical medical record. Written informed consent was signed by each participant, and this study was approved by the ethics committee of Yichang Yiling Hospital, Hubei, People’s Republic of China.

Genomic DNA was extracted from 200 μ L peripheral blood using CWBIO genomic DNA extracted kit (CWBIO, Beijing, People’s Republic of China) according to the manufacturer’s protocol and stored at -80°C for detection. Ultraviolet spectrophotometer (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ, USA) was used to examine the concentration and purity of the extracted DNA. Polymerase chain reaction–restriction fragment length polymorphism was selected to detect rs712 genotype. The restriction enzyme, reaction, and agarose electrophoresis condition were used as reported by Jin et al.¹⁹ Meanwhile, 5% samples were randomly selected to DNA sequencing.

Genotype frequencies of two groups were obtained by counting. GenAIEx 6.5 software (Peakall R and Smouse SE, Australian National University) was used to test Hardy–Weinberg equilibrium (HWE) in the control group.²⁰ Two-sided Student’s *t*-test was selected to compare the differences in the quantitative data, and χ^2 -test was used to analyze the rs712 genotype or allele frequency differences between the two groups. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were selected to estimate the strength of association between rs712 and risk of CRC. All statistics were performed using SPSS software 17.0 (SPSS Inc., Chicago, IL, USA), and $P < 0.05$ was considered as statistically significant.

Results

A_{260}/A_{280} value of each sample was in an interval of 1.8–2.1, thus all 586 cases and 476 healthy checkup individuals were included in our study. The baseline characteristics of the two groups were listed in Table 1. As shown in Table 1, there was no significant difference in age, sex, and status of smoking and drinking in the case and control groups. In the case group, 332 and 254 patients were colon and rectum cancer cases, respectively. Patients with well and poor differentiation were 412 and 174, respectively. The frequency distributions of tumor-node-metastasis stage (I–II/III), invasion (T1–2/T3–4), and node metastasis (N0/N1–3) were 69.6% and 30.4%, 47.8% and 52.2%, and 69.6% and 30.4%, respectively.

Table 1 Baseline characteristics of case and control groups

Variables	Cases (586)	Percentage (%)	Controls (476)	Percentage (%)	P-value
Age (years, M $\pm$ SD)	56.2 $\pm$ 13.5		56.8 $\pm$ 11.3		0.798
Male/female	398/188	67.9/32.1	312/164	65.5/34.5	0.414
Smoking (yes/no)	223/363	38.1/61.9	181/295	38.0/62.0	0.992
Drinking (yes/no)	198/388	33.8/66.2	144/332	30.3/69.7	0.321
Location (colon/rectum)	332/254	56.7/43.3			
Differentiation (well/poor)	412/174	70.3/29.7			
Stage (I + II/III)	408/178	69.6/30.4			
Invasion (T1 + T2/T3 + T4)	280/306	47.8/52.2			
Node metastasis (N0/N1–3)	408/178	69.6/30.4			

Abbreviations: M, mean; SD, standard deviation.

P-value of HWE in the control group was 0.753, suggesting that the genotype distribution of the locus was fit for HWE. The frequencies of genotypes GG, GT, and TT were 63.5%, 30.0%, and 6.5% in cases and 69.5%, 28.0%, and 2.5% in controls, respectively. There was no difference between the two groups in comparison of GT vs TT (30.0% vs 28.0%, $P=0.235$, adjusted OR = 1.210, 95% CI = 0.903–1.548) (Table 2). Furthermore, genotype TT distribution (6.5% vs 2.5%, $P=0.002$, adjusted OR = 2.810, 95% CI = 1.342–5.488) in cases was significantly higher than that in controls. Frequencies of allele T (21.5% vs 16.5%, $P=1.328$, adjusted OR = 1.328, 95% CI = 1.105–1.722) and allele T carrier (GT/TT) (36.5% vs 30.5%, $P=0.038$, adjusted OR = 1.342, 95% CI = 1.030–1.712) were significantly higher in cases compared to controls (Table 2). In addition, distribution of genotype allele G carrier (GG/GT) was significantly lower in cases than controls (93.5% vs 97.5%, adjusted OR = 0.372, 95% CI = 0.191–0.725) (Table 2).

The association between rs712 and clinical baseline characteristics was described in Table 3. There was no difference between rs712 genotype and allele frequency in colon and rectum cancer subgroups ($P=0.853$ for GT vs GG, $P=0.795$ for TT vs GG, and $P=0.946$ for T vs G). No association was found between rs712 and invasion depth in the case group ($P=0.885$

for GT vs GG, $P=0.460$ for TT vs GG, and $P=0.530$ for T vs G), whereas genotype GT ($P=0.003$) and allele T ($P=0.003$) were significantly associated with poor differentiation; genotypes GT and TT and allele T were significantly associated with stage III ($P=0.001$ for GT vs GG, $P<0.001$ for TT vs GG, and $P<0.001$ for T vs G) and node metastasis ($P<0.001$ for GT vs GG, $P=0.001$ for TT vs GG, and $P<0.001$ for T vs G) in the case group, suggesting that rs712 would be associated with CRC progression in Chinese population.

Discussion

KRAS, encoding a member of small GTPase superfamily proteins, is an important oncogene in the initiation of CRC.²¹ Variations within *KRAS* have been reported to be significantly associated with susceptibility, drug resistance, and poor prognosis in patients with CRC.^{12,22,23} Recently, some studies reported the association between SNP of *KRAS* and susceptibility to CRC. rs61764370 within *KRAS* 3'-UTR was not related to the risk of ovarian or breast cancer.²⁴ Significant associations were observed between rs712 and susceptibility to papillary thyroid cancer, gastric cancer, and oral squamous cell carcinoma.^{18,19,25} However, few studies reported the association between rs712 and the risk of CRC, and the possible association between them in Chinese population remains

Table 2 Genotype and allele distributions of rs712 within *KRAS* in two groups

Model	Genotype and allele	Cases (586)	Controls (476)	P-value	OR and 95% CI	Adjusted OR and 95% CI ^a
Codominant	GG	372 (63.5%)	331 (69.5%)			
	GT	176 (30.0%)	133 (28.0%)	0.235	1.177 (0.899–1.542)	1.210 (0.903–1.548)
	TT	38 (6.5%)	12 (2.5%)	0.002	2.818 (1.448–5.483)	2.810 (1.342–5.488)
Dominant	GG	372 (63.5%)	331 (69.5%)			
	GT/TT	214 (36.5%)	145 (30.5%)	0.038	1.313 (1.015–1.699)	1.342 (1.030–1.712)
Recessive	TT	38 (6.5%)	12 (2.5%)			
	GG/GT	548 (93.5%)	464 (97.5%)	0.003	0.373 (0.193–0.722)	0.372 (0.191–0.725)
Allele	Allele G	920 (78.5%)	795 (83.5%)			
	Allele T	252 (21.5%)	157 (16.5%)	0.004	1.387 (1.112–1.730)	1.328 (1.105–1.722)

Note: ^aOR and 95% CI were adjusted by sex, age, and status of smoking and drinking.

Abbreviations: OR, odds ratio; CI, confidence interval.

Table 3 rs712 within *KRAS* and clinical pathological feature in cases

Variables	Genotype			P-value		Allele		P-value
	GG	GT	TT	GT vs GG	TT vs GG	G	T	
Location								
Colon	204	98	20			506	138	
Rectum	168	78	18	0.853	0.795	414	114	0.946
Differentiation								
Well	278	110	24			666	158	
Poor	94	66	14	0.003	0.123	254	94	0.003
Stage								
I + II	281	109	18			671	145	
III	91	67	20	0.001	<0.001	249	107	<0.001
Invasion depth								
T1 + T2	180	84	16			444	116	0.530
T3 + T4	192	92	22	0.885	0.460	476	136	
Node metastasis								
N0	283	106	19			672	144	
N1–3	89	70	19	<0.001	0.001	248	108	<0.001

unclear. A case–control designed study including 586 CRC clinically and pathologically confirmed CRC patients and 476 healthy individuals was conducted. Our results showed that there was no significant distribution difference between the two groups in comparison of GT vs TT, suggesting that genotype GT of the locus was not associated with the risk of CRC. However, frequencies of allele T and genotypes TT and GT/TT within rs712 were significantly higher in cases than controls, indicating that mutated allele and genotypes of the locus were significantly related to an increased susceptibility to CRC. Furthermore, frequency of genotype GG/GT within the locus was significantly lower in cases than controls, suggesting that genotype GG/GT was negatively associated with CRC and it might be a protective factor for the onset of CRC. Additionally, genotype GT and allele T within rs712 were significantly associated with poor differentiation in cases; genotypes GT and TT and allele T were significantly associated with an increased risk of stage III and node metastasis, respectively, suggesting that rs712 might involve in the progression of CRC, and genotype TT and allele T of the locus could predict poor prognosis in Chinese population. The following interpretations might be accounted for our findings. rs712, which is located in LCS1 of *KRAS* 3'-UTR, alternation of allele G to T, can alter the secondary structure of the mRNA, disrupt let-7 binding site, affect the accessibility of let-7, and influence combining affinity between them.^{26,27} Moreover, lower levels of let-7a, let-7b, and let-7c were detected in the CRC cell line SW480 when it harbored rs61764370, showing that lower level of let-7 might be associated with mutated allele of rs712.²⁸ Consequently, aberrant expression of *KRAS* and low level of let-7 promote

CRC cell proliferation and migration, contributing to carcinogenesis and progression of the disease.²⁸

Conclusion

In summary, rs712 within 3'-UTR of *KRAS* was involved in the initiation and progression of CRC and mutated allele, and genotypes might be used as susceptible and prognostic biomarkers in Chinese population. With the limitations of this study, larger sample size and well-designed studies and function analysis are warranted to further validate our findings.

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Disclosure

The authors report no conflicts of interest in this work.

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