Tumor Biol. (2013) 34:3159–3163
DOI 10.1007/s13277-013-0885-x

RESEARCH ARTICLE

A let-7 binding site polymorphism rs712 in the *KRAS* 3′ UTR is associated with an increased risk of gastric cancer

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Received: 26 April 2013 / Accepted: 22 May 2013 / Published online: 2 June 2013

Abstract Recently, single nucleotide polymorphisms in let-7 miRNA binding site in 3′ untranslated region (UTR) of *KRAS* mRNA have been found to be associated with the cancer risk. In this study, we genotyped the frequency of *KRAS* rs712 to test its effect on gastric cancer (GC) risk in a hospital-based case–control study in a Chinese population, with 181 histologically confirmed GC patients and 674 cancer-free controls, using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay. The TT genotype of rs712 was associated with an increased risk of GC when taking GG genotype as a reference (adjusted odds ratio (OR)=3.05, 95 % confidence interval (CI), 1.53–6.08). Similarly, the T allele of rs712 was associated with a statistically significant increase in susceptibility compared with G allele (adjusted OR=1.44, 95 % CI, 1.10–1.90). Our data demonstrated that the T allele of the let-7 binding site polymorphism rs712 in *KRAS* 3′ UTR was associated with a significantly increased risk of GC, suggesting that the *KRAS* rs712 polymorphism may be a genetic marker for the development of GC.

Keywords Let-7 · *KRAS* · Polymorphism · Gastric cancer

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Introduction

Gastric cancer (GC) is the most frequently occurring cancer worldwide, and it was estimated that there would be 21,320 new cases and 10,540 deaths over world in 2012 [1]. As other cancers, GC is a complex trait caused by both genetic and environmental factors. In addition to the exogenous factors, like eating habits and *Helicobacter pylori* (*H. pylori*) infection, the genetic polymorphisms in the DNA repair and cell proliferation processes are also crucial in the development of GC [2, 3]. Also like other cancers, the low 5-year survival rate of GC is due to late diagnosis. Therefore, the genetic biomarker of GC, which could screen out high-risk population and subsequently achieve early diagnosis, is of important value.

The *KRAS* gene belongs to the mammalian *ras* gene family, and it encodes a protein that is a member of the small GTPase superfamily [4]. *KRAS* polymorphisms have been reported to be related to the risk and survival of various cancer patients including GC [5, 6]. Recently, the lethal-7 (let-7) binding site polymorphisms in the *KRAS* 3′ untranslated region (UTR) have been demonstrated to be

associated with cancer risk and patients survival in several tumors [7–10].

MicroRNAs are small noncoding RNAs that regulate gene expression by base pairing with the 3′ UTR of target mRNA. MicroRNA-related polymorphism was defined as a polymorphism at or near a microRNA binding site of functional genes, and it can affect gene expression by interfering with microRNA function [11]. The let-7 family of microRNAs could regulate KRAS activity by binding to the 3′ UTR of human *KRAS* gene [12]. The let-7-related polymorphisms may influence the binding ability of *KRAS* gene with let-7 microRNAs and impact the following *KRAS* transcription. Researchers reported that the let-7 binding site polymorphisms regulated KRAS expression in in vitro model and are also associated with the cancer risk in non-small-cell lung cancer patients as well as survival in oral cancer [7, 8].

To date, most reports about let-7 polymorphism concentrated on LCS6 that was associated with cancer risk and survival [13–15]. There is only one study on the correlation of rs712 and cancer risk reporting a role of rs712 polymorphism on susceptibility to oral squamous cell carcinoma (OSCC), and there is no research on the association of rs712 polymorphism and GC risk [16]. In this study, we genotyped the frequency of *KRAS* rs712 polymorphism to test its importance on GC risk in a hospital-based case–control study in a Chinese population.

Materials and methods

Study population

This study comprised 181 patients with histologically confirmed GC and 674 cancer-free controls. All participants were selected from the Han ethnic group and recruited from the Luoyang Central Hospital between July 2010 and June 2012. Demographic and clinical information, including age, gender, differentiation status, clinical stage, and metastasis status, was obtained using short questionnaire and clinical medical records. Hospital-based controls were drawn from the same population and matched to the cases by gender, age (±3 years), and ethnicity. Each participant signed a written informed consent and donated 2 ml of venous blood for genomic DNA extraction. Approval for the experiments was obtained from the institutional review board of the hospital.

Genotyping

Genomic DNA was extracted from the peripheral blood samples by using a DNA isolation kit (Bioteke, Beijing, China) according to the instruction manual. The polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay was performed to identify the *KRAS* rs712 T/G genotype. The PCR primer was designed as follows: sense primer, 5′-ATGACAGTGGAAGTTTTTTTTCCTC-3′ and antisense primer, 5′-GAATCATCATCAGGAAGCCCAT-3′. The 25-μL PCR reaction mixture was composed of 50 ng template DNA, 2.5 μL 10×PCR buffer, 3.0 μL dNTPs, 1.5 mmol/L $MgCl_2$, 0.5 μL of each primer, and 1.0 U of *Taq* DNA polymerase. The PCR conditions were 94 °C for 2 min, followed by 35 cycles for 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C, with a final elongation at 72 °C for 10 min. The PCR product was digested 4 h at 65 °C with *Taq* I restriction enzyme (New England BioLabs Inc; Beverly, MA, USA). The digested fragments were separated on 6 % polyacrylamide gel and stained with argent nitrate. To confirm the accuracy of the genotyping method, randomly selected PCR products were analyzed by DNA sequencing analysis, and the results were completely identical.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was evaluated by chi-square test to compare the observed genotype frequencies among the controls. Association between the *KRAS* rs712 polymorphism and GC risk was estimated by computing odds ratios (ORs) and 95 % confidence intervals (CIs). Data were adjusted according to age and gender using binary logistic regression analysis. Two-sided χ^2 tests of statistical significance were performed using the SPSS software version 19.0 (SPSS Inc, Chicago, IL, USA), and value of $P<0.05$ was considered as statistically significant.

Results

The characteristics of cases and controls are summarized in Table 1. The cases and controls appeared to be adequately matched in terms of age and gender. The genotype and allele frequencies of the rs712 are presented in Table 2. The genotype frequencies in the controls were in agreement with the HWE model. The genotype frequencies of the rs712 were 58.0, 33.1, and 8.8 % for the GG, GT, and TT genotypes, respectively, among the GC group and 65.6, 31.3, and 3.1 % among the controls, respectively. When taking the GG genotype as a reference, we found that the TT genotype was associated with an increased risk of GC (adjusted OR=3.05, 95 % CI, 1.53–6.08). Similarly, the T allele was associated with a statistically significant increase in susceptibility to GC when taking the G allele as a reference (adjusted OR=1.44, 95 % CI, 1.10–1.90). Taken together, these data suggest that the let-7a *KRAS* rs712 T allele may be a putative risk factor for the development of GC.

Table 1 Characteristics of the GC patients and healthy controls

	GC (n=181, %)	Control (n=674, %)
Gender		
Male	119 (65.7)	422 (62.6)
Female	62 (34.3)	252 (37.4)
Age (mean±SD, years)	57.7±12.2	55.8±11.9
Differentiation status		
Well to moderate	69 (38.1)	
Poor to undifferentiated	112 (61.9)	
Clinical stage		
I–II	81 (44.8)	
III–IV	100 (55.2)	
Metastasis		
Yes	77 (42.5)	
No	104 (57.5)	

GC gastric cancer, *SD* standard deviation

Discussion

In the present study, we investigated the *KRAS* rs712 polymorphism in 181 histologically confirmed GC patients and 674 cancer-free controls and demonstrated that the T allele of the let-7 binding site polymorphism rs712 in *KRAS* 3′ UTR was associated with a significantly increased risk of GC.

MicroRNAs (miRNAs) can act as trans-acting factors to suppress translation or induce mRNA degradation of target genes, and they are globally found to regulate genes expression in various cancers as oncogenes or tumor suppressors. The let-7 miRNA family functions as tumor suppressors in many malignant tumors including GC, and their expressions were also down-regulated in various cancers [17–19]. Let-7 was reported to target and down-regulate RAS by binding to specific sites in the 3′ UTR of the *KRAS* mRNA and then exert its tumor-suppressing function [12, 20]. Over-expression of let-7a can inhibit the growth of transplanted lung cancer in nude mice model [20]. Also, expression of let-7 is related to cancer survival in the anti-EGFR monoclonal antibodies-treated metastatic colorectal cancer patients, in which let-7 showed significant association with overall survival [21, 22].

The *KRAS* gene is an oncogene which promotes tumorgenesis by activation of the RAF/MEK/MAPK pathway [23]. Mutations of *KRAS* have been found to be closely related to various cancers including GC and have played important roles in cancer initiation, metastasis, and prognosis. In these variants, a single amino acid substitution leads to an activating mutation or increased in KRAS expression [5, 24, 25]. Recently, functional single nucleotide polymorphisms in miRNA let-7 binding site in 3′ UTR of *KRAS* mRNA have been found to be associated with various cancer risks, including colorectal cancer, breast cancer, ovarian cancer, and OSCC. Moreover, the polymorphisms may be used to predict treatment outcome, patient survival, and prognosis [13, 14, 26, 27]. In this study, we found for the first time that the let-7 *KRAS* rs712 polymorphism was associated with an increased GC risk.

Previous studies have shown that let-7f can inhibit tumor invasion and metastasis in human GC [17]. However, there are only a few studies about the regulation mechanism of let-7 miRNA in GC. The LCS6 polymorphism (rs61764370) in the let-7 binding site of 3′ UTR in *KRAS* can lead to increased expression of KRAS in vitro and decreased let-7 levels in vivo [7]. The LCS6 variant was associated with poor prognosis in head and neck cancers [28], altered drug response in colon cancer [9, 15], triple-negative breast cancer risk in premenopausal women [29], and reduced survival in oral cancer [8]. To date, the studies of let-7 polymorphisms primarily concentrated on LCS6 site, and there is only one study reporting that the rs712 polymorphism has a significant association with OSCC risk, demonstrating a role for rs712 polymorphism on susceptibility to OSCC [16].

The discoveries on the effects and mechanisms of LCS6 polymorphism in various cancers may provide implications for rs712 mechanism. Based on the let-7 targeting *KRAS* gene, we deduced the mechanisms of the rs712 polymorphism in GC development. Just like rs61764370 (LCS6), the T allele of rs712 might disrupt the binding of let-7 miRNA and *KRAS* 3′ UTR, allow increased KRAS expression, and somehow

Table 2 Genotype distribution of hsa-miR-let7a *KRAS* rs712 T/G in the GC patients and controls

Polymorphism	Genotype	GC, n=181 (%)	Controls, n=674 (%)	Adjusted OR (95 % CI)	Adjusted P value
hsa-miR-let7a *KRAS* rs712 T/G	GG	105 (58.0)	442 (65.6)	1.00 (Ref)	
	GT	60 (33.1)	211 (31.3)	1.18 (0.82–1.69)	0.37
	TT	16 (8.8)	21 (3.1)	3.05 (1.53–6.08)	0.001
	G	270 (74.6)	1,095 (81.2)	1.00 (Ref)	
	T	92 (25.4)	253 (18.8)	1.44 (1.10–1.90)	0.009

Data was adjusted according to age and gender using binary logistic regression analysis

Ref reference value

lower let-7 level, which further result in increased cell growth. Thus, the decreased let-7 levels and increased KRAS expression may in concert promote oncogenesis. These hypotheses need testing in the future studies.

Although we found that the let-7 *KRAS* rs712 polymorphism was associated with an increased risk of GC, some limitations existed in this study. The relatively small sample size, especially in the case group, may have limited power to detect the precise effect of the rs712 polymorphism on GC risk. Moreover, the study design is hospital-based and the selection bias cannot be ruled out. Therefore, further association studies are warranted to confirm the result.

In conclusion, our data suggest that the *KRAS* rs712 polymorphism in let-7 miRNA binding site may be used as a genetic marker for the development of GC. While the mechanism is still unknown, the over-expression of KRAS and down-regulation of let-7 induced by the rs712 polymorphism miRNA-disrupting function might be plausible reasons. Further experiments are needed to verify this hypothesis. With the limitation that patients with the T allele having a high GC sensitivity cannot be defined in this study, we wish that our results may contribute to understand the mechanisms of miRNA-disrupting polymorphisms in cancer biology, which is different from the previously discovered genetic markers for cancer risk.

Acknowledgments This work was supported by the Science & Technology Project of Henan Province (no. 132300410105) and the Ph.D. scientific research foundation of Henan University of Science and Technology (no. 09001492).

Conflicts of interest None

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