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Original Article

Role of *ERCC5* His1104Asp and His46His gene polymorphisms in the development of gastric cancer risk in a Chinese Han population

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Abstract: We carried out a hospital based case-control study to assess the association of two functional gene polymorphisms in *ERCC5* (His1104Asp and His46His) in the development of gastric cancer in a Chinese population. A total of 194 gastric cancer patients and 225 control subjects were collected between May 2012 and May 2015. Polymerase chain reaction coupled with restriction fragment length polymorphism (PCR-RFLP) assay was carried out to genotype the *ERCC5* His1104Asp and His46His gene polymorphism. Using unconditional multiple logistic regression analysis, individuals carrying with the TT genotype of *ERCC5* His46His were significantly associated with a moderate risk of gastric cancer compared to the GG genotype (OR=2.03, 95% CI=1.10-3.74; *P* value =0.005). In dominant model, individuals with the CT+TT genotype of *ERCC5* His46His was significantly associated with a light risk of gastric cancer compared to the CC genotype (OR=1.59, 95% CI=1.03-2.46; *P* value =0.03). However, *ERCC5* His1104Asp genetic variation could not affect the risk of gastric cancer using unconditional multiple logistic regression analysis. In conclusion, we demonstrated that *ERCC5* His46His gene polymorphism contributes to the susceptibility of gastric cancer. These findings need to be validated in larger, preferably population-based, studies including different ethnic groups.

Keywords: *ERCC5*, His1104Asp, His46His, polymorphism, gastric cancer

Introduction

Gastric cancer (GC) is a highly lethal cancer, with increasing incidence worldwide [1]. It is reported that morbidity of gastric cancer are highest in developing countries, and half the world total occurs in Eastern Asia (mainly in China) [2, 3]. Long-term infection of *Helicobacter pylori* infection is identified to be an important risk factor for the susceptibility to gastric cancer [4, 5]. Previous studies have indicated that some environmental and dietary factors could influence the gastric cancer risk, such as a family history of cancer, alcohol consumption, obesity, and high sodium intake [6]. However, individuals who exposed to the same risk factors of gastric cancer do not always develop this cancer, which suggests that molecular mechanisms are involved in the susceptibility to this cancer. Poor understanding on molecular mechanisms underlying tumorigenesis of GC leads to lack of effective treatment [7]. Intensive studies focus on identification of GC related genes, such as *PRKAA1*, *APE1*, *XRCC1*, *LMP2*, *LMP7*, *miR-146a* and insulin like growth facror-1 [8-12].

Excision repair cross-complementing rodent repair deficiency, complementation group 5 (*ERCC5*), is an important member of a family of enzymes, and this protein includes DNaseIV/flap structure-specific endonuclease 1 (*FEN1*) group of structure-specific nucleases and has function in NER [13]. *ERCC5* gene polymorphisms are reported to have an important role in the development of several kinds of cancers [14-18]. Currently, only few studies have reported the association between *ERCC5* gene polymorphisms and gastric cancer risk [19, 20]. Thus, we carried out a hospital based case-control study to assess the association of two functional gene polymorphisms in *ERCC5* (His1104Asp and His46His) in the development of gastric cancer in a Chinese population.

Table 1. The primers, length of digested fragment and restriction enzymes as well as digested fragments of ERCC5 His1104Asp and His46His

ERCC5	SNP	Primers (5'-3')	Lengths of the PCR products	Restriction enzyme	Digested fragment
His1104Asp	rs17655	GACCTGCCTCTCAGAATCAT CCTCGCACGTCTTAGTTT	271	NlaIII	CC: 227 bp, 44 bp; CG: 271 bp, 227 bp, 44 bp; GG: 271 bp
His46His	rs1800975	GCAGTATGTGAATAGGGGTAACAAG CTGTTTCTTCAATAGTGGAGCATCC	377 bp	NcoI	CC: 254 bp, 123 bp; CT: 123 bp, 254 bp, 377 bp; TT: 377 bp

Materials and methods

Subjects

In our study population, all analyses were restricted to Han Chinese. A total of 194 gastric cancer patients were selected from our hospital between May 2012 and May 2015. All the gastric cancer obtained endoscopy examination and confirmed by pathology. All the gastric cancer patients were primary gastric cancer. All the gastric cancer patients had no previous history of other cancer, serious liver and kidney diseases, or prior chemotherapy or radiotherapy. There were no age, sex, or disease stage restrictions for case recruitment.

A random sample of 225 healthy unrelated individuals was recruited between May 2012 and May 2015 from the physical examination center at our hospital. A detailed recruitment and exclusion criteria were used. Generally, subjects with a history of cancer, any digestive diseases, chronic diseases and severe endocrinological, metabolic, and nutritional diseases were excluded from this study. All the control subjects were confirmed to be free of gastric cancer through endoscopy examination.

The demographic and dietary characteristics of selected subjects were collected from a self-designed questionnaire, and consisted of sex, age, BMI, tobacco smoking and alcohol consumption. The tumor stage was collected from medical records. The *Helicobacter pylori* infection was diagnosed by serology test. Gastric cancer patients and control subjects gave their written informed consent prior to participating into our study, according to the procedures approved by Ethical Review Board in our Hospital.

DNA extraction and genotyping

Five ml peripheral blood was obtained from each study subject, and the blood samples were stored in tube with EDTA. The DNA was isolated using the TIANamp Blood DNA Kit (Tiangen, Beijing, China), according to the instructions of manufacturer, and the isolated DNA was kept at -20°C until using. Polymerase chain reaction coupled with restriction fragment length polymorphism (PCR-RFLP) assay was carried out to genotype the *ERCC5* His1104Asp and His46His gene polymorphism. The primers, length of digested fragment and restriction enzymes as well as digested fragments of *ERCC5* His1104Asp and His46His were shown in **Table 1**. The PCR reaction was performed in a 15-μl reaction solution with 10 μl 5× buffer, 1.5 μl $MgCl_2$, 0.3 μl dNTP, 0.25 μl each primer and 0.2 μl Taq polymerase. The *ERCC5* His1104Asp and His46His were amplified as follows: denaturation at 95°C for 5 min, and then 40 cycles of 95°C for 15 s, 58°C for 30 s, and finally 72°C for 45 s. The products of PCR reaction was stored at 4°C and was confirmed by electrophoresis in a 2% agarose gel stained with ethidium bromide, and then visualized under an ultraviolet light.

Statistical analysis

For categorical characteristics, data are expressed as frequencies and percentage. Differences of demographic and dietary factors as well as genotype frequencies between gastric cancer patients and control subjects were compared using chi-square test and univariate analysis. Genotypic frequencies in control subjects for each SNP were tested for departure from Hardy-Weinberg equilibrium (HWE) using a Chi-square (χ^2)-test with one degree of freedom. Multiple logistic regression analysis was taken to assess the association between *ERCC5* His1104Asp and His46His gene polymorphisms and gastric cancer risk. Odds ratio (OR) and their related 95% confidence intervals (CIs) were calculated by unconditional logistic regression analyses adjusted for potential confounding factors. The three genetic models

Table 2. Baseline information of study subjects

Variables	Patients	%	Controls	%	Chi-square test	*P* value	OR (95% CI)	*P* value
Age, years								
<60	67	34.54	131	58.22			1.0 (Reference)	-
≥60	127	65.46	94	41.78	23.45	<0.001	2.64 (1.74-4.01)	<0.001
Sex								
Female	63	32.47	102	45.33			1.0 (Reference)	-
Male	131	67.53	123	54.67	7.22	0.01	1.72 (1.14-2.62)	0.01
BMI								
<24	121	62.37	148	65.78			1.0 (Reference)	-
≥24	73	37.63	77	34.22	0.53	0.47	1.16 (0.76-1.76)	0.47
Tobacco smoking								
No	120	61.86	147	65.33			1.0 (Reference)	-
Yes	74	38.14	78	34.67	0.55	0.46	1.16 (0.76-1.77)	0.46
Alcohol consumption								
No	117	60.31	152	67.56			1.0 (Reference)	-
Yes	77	39.69	73	32.44	2.38	0.12	1.37 (0.90-2.09)	0.12
Family history of cancer								
No	169	87.11	208	92.44			1.0 (Reference)	-
Yes	25	12.89	17	7.56	3.28	0.07	1.81 (0.90-3.69)	0.07
Helicobacter pylori infection								
No	70	36.08	136	60.44			1.0 (Reference)	-
Yes	124	63.92	89	39.56	24.74	<0.001	2.71 (1.79-4.11)	<0.001
Clinical stage								
I-II	83	42.78						
III-IV	111	57.22						

(dominant, recessive and additive) were applied to assess the association of the *ERCC5* His1104Asp and His46His gene polymorphisms with the gastric cancer risk. All the analysis was conducted with the SPSS statistical package software, version 17.0 (SPSS Inc, Chicago, IL, USA). *P* value <0.05 was considered statistically significant.

Results

A total of 194 gastric cancer patients (63 females and 131 males, median age: 62.52 years) and 225 control subjects (102 females and 123 males, median age: 50.45 years) were included into the current study (**Table 2**). The basic characteristics of the gastric cancer patients and control subjects were listed in **Table 1**. By chi-square test and univariate analysis, gastric cancer patients were more likely to have higher age (OR=2.64, 95% CI=1.74-4.01), be males (OR=1.72, OR=1.14-2.62), and suffer from *Helicobacter pylori* infection (OR=2.71, 95% CI=1.79-4.11). However, no significant difference was found between gastric cancer patients and control subjects in terms of BMI, tobacco smoking, alcohol consumption and family history of cancer. Out of 194 gastric cancer patients, there were 83 (42.78%) patients in I-II stage, and 111 (57.22%) patients in III-IV stage.

The genotype distributions of *ERCC5* His1104Asp and His46His in controls agreed with the Hardy-Weinberg equilibrium (*P* values for HWE were 0.07 and 0.97 for *ERCC5* His1104Asp and His46His, respectively) (**Table 3**). A statistically significant difference was found in the genotype distributions of *ERCC5* His46His between gastric cancer patients and control subjects using chi-square test (χ^2=6.38, *P*=0.02), but no statistically significant difference was observed in the genotype distributions of *ERCC5* His1104Asp between the two groups.

Using unconditional multiple logistic regression analysis, individuals carrying with the TT genotype of *ERCC5* His46His were significantly

Table 3. Genotype distributions of ERCC5 His1104Asp and His46His gene polymorphisms

ERCC5	Cases	%	Controls	%	Chi-square test	*P* value	*P* value for HWE in controls
His1104Asp							
CC	73	37.63	94	41.78			
CG	100	51.55	112	49.78			
GG	21	10.82	19	8.44	1.13	0.57	0.07
His46His							
CC	55	28.35	87	38.67			
CT	98	50.52	106	47.11			
TT	41	21.13	32	14.22	6.38	0.04	0.97

Table 4. Association between ERCC5 His1104Asp and His46His gene polymorphisms and gastric cancer risk

ERCC5	Cases	%	Controls	%	OR (95% CI)[1]	*P* value
His1104Asp						
Co-dominant						
CC	73	37.63	94	41.78	1.0 (Ref.)	-
CG	100	51.55	112	49.78	1.15 (0.75-1.77)	0.50
GG	21	10.82	19	8.44	1.42 (0.67-3.02)	0.32
Dominant						
CC	73	37.63	94	41.78	1.0 (Ref.)	-
CG+GG	121	62.37	131	58.22	1.19 (0.79-1.80)	0.39
Recessive						
CC+CG	173	89.18	206	91.56	1.0 (Ref.)	-
GG	21	10.82	19	8.44	1.32 (0.65-2.68)	0.41
His46His						
Co-dominant						
CC	55	28.35	87	38.67	1.0 (Ref.)	-
CT	98	50.52	106	47.11	1.46 (0.93-2.32)	0.09
TT	41	21.13	32	14.22	2.03 (1.10-3.74)	0.01
Dominant						
CC	55	28.35	87	38.67	1.0 (Ref.)	-
CT+TT	139	71.65	138	61.33	1.59 (1.03-2.46)	0.03
Recessive						
CC+CT	153	78.87	193	85.78	1.0 (Ref.)	-
TT	41	21.13	32	14.22	1.62 (0.94-2.78)	0.06

[1]Adjusted for age, sex and *Helicobacter pylori* infection.

associated with a moderate risk of gastric cancer compared to the GG genotype (OR=2.03, 95% CI=1.10-3.74; *P* value =0.005) (**Table 4**). In dominant model, individuals with the CT+TT genotype of *ERCC5* His46His was significantly associated with a light risk of gastric cancer compared to the CC genotype (OR=1.59, 95% CI=1.03-2.46; *P* value =0.03). However, *ERCC5* His1104Asp genetic variation could not affect the risk of gastric cancer using unconditional multiple logistic regression analysis.

Discussion

DNA repair is responsible for maintaining genomic stability in response to the assault of environmental carcinogens that causes DNA damage [21]. If left unrepaired, such DNA damage could cause mutation fixation and initiation of carcinogenesis. Up to now, there are at least five known major DNA repair pathways were found, including above 150 human DNA repair genes, among which the nucleotide-excision repair (NER) pathway is the most versatile and is particularly significant in association with cancer risk [22].

Polymorphisms, which have an effect on the regulation of gene expression, can contribute to the differences between individuals in the susceptibility to a disease and its severity [23]. The regulation of DNA repair is a vital factor in the multistep process of carcinogenesis, and the *ERCC5* gene is an important part of the DNA repair machinery. In the present study, we conducted a study to investigate the *ERCC5* His1104Asp and His46His polymorphisms and development of gastric cancer. We found that *ERCC5* His46His gene polymorphism significantly elevated the risk of gastric cancer in a Chinese Han population.

The authors of previous studies have reported an association between *ERCC5* genetic variations and the development of several kinds of cancer, such as breast cancer, prostate cancer,

bladder cancer, head and neck cancer and colorectal cancer [14-18]. Yang et al. conducted a case-control study in a Chinese population and reported that *ERCC5* rs2296147 gene polymorphism was strongly correlated with an elevated risk of prostate cancer [14]. McCullough et al. revealed a statistically significant association between *ERCC5* His1104Asp genetic variation and a reduced risk of breast cancer [15]. Liu et al. conducted a meta-analysis with eight case-control studies, and reported that CC genotype of *ERCC5* His1104Asp could not influence the risk of bladder cancer [16]. Jiang et al. performed a meta-analysis of eight published studies and reported that *ERCC5* His1104Asp polymorphism is a risk factor for head and neck cancer susceptibility [17]. Du et al. carried out a meta-analysis with 5 published studies and suggested that *ERCC5* His1104Asp gene polymorphism may be associated with the susceptibility of colorectal cancer [18].

Previous study reported that expression of *ERCC5* could regulate transcription and translation, degrade protein and promote methylation [24]. In normal individuals, DNA damage is rare, and thus the expression of *ERCC5* is at low levels. However, various types of environmental carcinogens and endogenous metabolic products may cause DNA damage, thus enhancing the DNA-repair activity of cells and the activities of transcription and translation [25]. A present study indicated that *ERCC5* protein expression was associated with the development, progression and prognosis of gastric cancer [26]. Currently, a recent meta-analysis pooled 13 case-control studies and reported that *ERCC5* His1104Asp polymorphism may be a risk factor for the gastrointestinal cancers [27]. Few studies reported the association between *ERCC5* His46His genetic variation and development of gastric cancer. In our study, we only reported a statistically association between *ERCC5* His46His gene polymorphism and gastric cancer risk, but we did not observe a significant role of *ERCC5* His1104Asp gene polymorphism in the development of this cancer. The discrepancies of our finding with previous studies may be caused by different in populations, selection of subjects and sample sizes.

In conclusion, we demonstrated that *ERCC5* His46His gene polymorphism contributes to the susceptibility of gastric cancer, while *ERCC5* His1104Asp polymorphism does not. These findings need to be validated in larger, preferably population-based, studies including different ethnic groups.

Disclosure of conflict of interest

None.

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