

Relationship of ERCC5 genetic polymorphisms with metastasis and recurrence of gastric cancer

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SUMMARY

OBJECTIVE: This study aims to explore the role of *ERCC5* genetic polymorphisms in gastric cancer and their relationship with metastasis and recurrence of gastric cancer.

METHODS: A total of 200 patients with gastric cancer and 133 healthy subjects were enrolled. MassARRAY iPLEX® technology was used to genotype *ERCC5* rs2016073, rs751402, rs2094258, rs2296147, and rs2296148 between the control group and the gastric cancer group. The relationship of *ERCC5* genetic polymorphisms with metastasis and recurrence of gastric cancer was explored. The differences in sociodemographic characteristics between patients with gastric cancer and control subjects were compared using the chi-square test. The genetic loci between the control group and the gastric cancer group were analyzed by the chi-square test.

RESULTS: There was no significant difference in the metastasis of gastric cancer between males and females ($p=0.628$), but there was a significant difference in the metastasis of gastric cancer ($p=0.005$). Patients aged ≤ 60 years and >60 years showed no significant difference in the metastasis of gastric cancer ($p=0.420$), but there was a significant difference in the recurrence of gastric cancer ($p<0.001$). The loci rs2016073, rs751402, and rs2094258 in the gastric cancer group showed no significant differences compared with the control group ($p=0.194$), and the loci rs2296147 and rs2296148 showed significant differences.

CONCLUSIONS: The results suggested that *ERCC5* polymorphisms (e.g., rs201607, rs751402, rs2094258, rs2296147, and rs2296148) may be associated with metastasis and recurrence of gastric cancer.

KEYWORDS: ERCC5. Genetic polymorphism. Metastasis. Recurrence. Gastric cancer.

INTRODUCTION

Gastric cancer is the second most common cause of cancer-related deaths in the world, the epidemiology of which has changed within the past decades¹. Gastric cancer remains a considerable health burden throughout the world². Metastasis is a crucial impediment to the successful treatment of gastric cancer³. Recurrence of gastric cancer after 10 years of surgical resection is highly rare, and local recurrence is common after radical surgery⁴. Many studies have explored biomarkers

to monitor metastasis and recurrence of gastric cancer⁵⁻⁷, but further study is still needed.

The common polymorphic variant in the 5'-untranslated region of the excision repair cross-complementation group 5 (*ERCC5*) gene was described to generate an upstream open reading frame that regulates both the basal *ERCC5* expression and its ability to be synthesized following DNA damage⁸. Genetic variations in *ERCC5* might influence individual vulnerability to gastric cancer⁹.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: the study was supported by the Scientific Research Plan Project of Health Industry of Gansu Province (GSWSKY2018-22).

Received on August 04, 2021. Accepted on September 07, 2021.

A previous study indicated that *ERCC5* rs2094258 polymorphism may contribute to the risk of breast cancer¹⁰. Our previous study found that *ERCC5* rs2094258 and rs751402 polymorphisms were not associated with the development of gastric cancer in co-dominant, dominant, and recessive models¹¹. But in a meta-analysis, it was demonstrated that rs751402 C>T, rs2296147 T>C, and rs873601 G>A polymorphisms of *ERCC5* gene were associated with the susceptibility of gastric cancer¹². In another study, the *ERCC5* rs751402 gene polymorphism may influence the susceptibility to gastric cancer in the Chinese population¹³.

In this study, the roles of *ERCC5* genes (e.g., rs2016073, rs751402, rs2094258, rs2296147, and rs2296148) in gastric cancer were explored, and their relationship with metastasis and recurrence of gastric cancer was studied by MassARRAY iPLEX[®] technology.

METHODS

Study population

In this study, 333 subjects were enrolled from our hospital between October 2012 and December 2014, including 200 patients with gastric cancer who were newly diagnosed as case group and 133 subjects as control group. The case group included 170 men and 30 women, with age ranging from 39 to 78 years (56.9±10.1). The control group included 80 men and 53 women, with age ranging from 20 to 87 years (49.4±14.2). The percentage of metastasis and recurrence in the case group was 9.0% and 18.0%, respectively. Exclusion criteria for patients with gastric cancer were primary tumors outside the esophagus, other malignant neoplasms, recurrent tumors, and serious liver and kidney diseases. Exclusion criteria for the control group were a history of malignant cancers, serious liver and kidney diseases, and digestive system disease. The basic medical data of patients with gastric cancer were obtained from medical records. A standardized questionnaire including sociodemographic characteristics was used to interview both patients and controls. Before the study, all subjects signed the informed consent, and this study was approved by our hospital.

DNA extraction and single-nucleotide polymorphisms genotyping

DNA from peripheral blood samples that were collected from patients was extracted using TIANamp Blood DNA Kit (Tiangen, Beijing, China), according to the instructions

of the manufacturer. The *ERCC5* rs2016073, rs751402, rs2094258, rs2296147, and rs2296148 were genotyped by MassARRAY iPLEX[®] technology (Shanghai Genechem Co., Ltd.). The polymerase chain reaction (PCR) fragments of the investigated polymorphisms were subsequently digested with their specific restriction enzyme. The PCR began with an initial denaturation at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 60 s, and a final extension at 72°C for 5 min. After desalted with resin, the Typer software automatically interpreted the molecular weight peaks detected by the mass spectrometry, and the transformation showed the molecular mass spectrum peak map corresponding to the single-nucleotide polymorphism (SNP) site. The PCR primers were as follows:

rs2016073	ACGTTGGATGCTCCTTTGGAAAGGCTTATC (2nd-PCR) ACGTTGGATGAAGCAGGAAGGGCTCTAGG (1st-PCR)
rs751402	ACGTTGGATGGTATTAGACGGAACCGAGC (2nd-PCR) ACGTTGGATGAAACAGCCAGAAGATGTCCC (1st-PCR)
rs2094258	ACGTTGGATGCAATTTCCCGTACTTCTG (2nd-PCR) ACGTTGGATGAACTCAGTGAAGGCTGAC (1st-PCR)
rs2296147	ACGTTGGATGCAGACGTTTGGGCTAAGC (2nd-PCR) ACGTTGGATGAACACGTCTCAGCAGCTGTC (1st-PCR)
rs2296148	ACGTTGGATGATTCTTCTACGACGGACTGC (2nd-PCR) ACGTTGGATGCTTTGTTGTAGGAGCAGG (1st-PCR)

Statistical analysis

Statistical analyses were performed using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). The differences in sociodemographic characteristics between patients with metastasis and recurrence of gastric cancer were compared using the chi-square test. Genetic loci between the control group and the gastric cancer group were analyzed using the chi-square test. All tests were two-sided. A p-value <0.05 was considered statistically significant.

Ethical issue and informed consent

The authors declare that they have no conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study. The study protocol and the comprehensive written informed consent used in this study protocol were reviewed.

RESULTS

Relationship of gender and age with metastasis and recurrence of gastric cancer

In patients with metastasis, there were 16 males and 2 females. In patients without metastasis, there were 154 males and 28 females. There was no significant difference in the metastasis of gastric cancer between males and females ($p=0.628$). In patients with recurrence, there were 36 males and 0 female. In patients without recurrence, there were 134 males and 30 females. Results showed that the recurrence rate of males was higher than that of females ($p=0.005$). Among patients aged ≤ 60 years, patients with metastasis were 13, and patients without metastasis were 114. Among patients aged >60 years, patients with metastasis were 5, and patients without metastasis were 68. There was no significant difference in age for the metastasis of gastric cancer. Among patients aged ≤ 60 years, patients with recurrence were 0, and patients without recurrence were 126. Among patients >60 years, patients with recurrence were 36, and patients without recurrence were 60. There was a significant difference in age for the recurrence of gastric cancer ($p<0.001$) (Table 1).

Comparison of genetic loci between the control group and the gastric cancer group

As for loci rs2016073, rs751402, and rs2094258, there were no significant differences between the control group and the gastric cancer group ($p=0.194$, $p=0.257$, and $p=0.686$, respectively). As for loci rs2296147 and rs2296148, there were significant differences between the control group and the gastric cancer group ($p<0.001$ and $p=0.005$, respectively) (Table 2).

Relationship of gene loci with metastasis and recurrence of gastric cancer

As for locus rs2016073, there was a significant difference between different genotypes and gastric cancer metastasis

Table 1. Relationship of gender and age with metastasis and recurrence of gastric cancer.

	Metastasis			Recurrence		
	Yes	No	p-value	Yes	No	p-value
Gender						
Male	16	154	0.628	36	134	0.005
Female	2	28		0	30	
Age, year						
≤ 60	13	114	0.420	0	126	<0.001
>60	5	68		36	60	

($p=0.021$). As for locus rs751402, there was a significant difference between different genotypes and gastric cancer metastasis ($p=0.021$). As for locus rs2094258, there was a significant difference between different genotypes and gastric cancer metastasis ($p<0.001$). As for locus rs2296147, there was a significant difference between different genotypes and gastric cancer metastasis ($p<0.001$). There was no significant difference between different genotypes of rs2296148 locus and gastric cancer metastasis ($p=0.197$) (Table 3).

As for locus rs2016073, there was no significant difference between different genotypes and gastric cancer recurrence. As for locus rs751402, there was no significant difference between different genotypes and gastric cancer recurrence ($p=0.094$). As for locus rs2094258, there was a significant difference between different genotypes and gastric cancer recurrence ($p=0.021$). As for locus rs2296147, there was no significant difference between different genotypes and gastric cancer recurrence ($p=0.434$). As for locus rs2296148, there was a significant difference between different genotypes and gastric cancer recurrence ($p<0.001$) (Table 3).

DISCUSSION

Functions pertaining to DNA repair and synthesis are believed to play a critical role in cancer development and seem to be affected by genetic polymorphisms¹⁴. Several SNPs in *ERCC5* gene have been identified and have been studied for their association with different cancer risk, such as rs751402 C>T, rs2296147 T>C, rs873601 G>A, rs2094258 C>T, rs1047768 T>C, rs17655G>C, rs2018836G>A, and rs3818356G>A^{12,15-17}. In this study, a case-control study was performed to investigate the association of *ERCC5* genetic polymorphisms with metastasis and recurrence of gastric cancer.

In a previous study, *ERCC5*-763 A>G (rs2016073) may be associated with chemosensitivity of oxaliplatin-based chemotherapy in Chinese patients with advanced colorectal cancer¹⁸. In our study, locus rs2016073 showed no significant differences between the control group and the gastric cancer group and between different genotypes and gastric cancer recurrence, but a significant difference between different genotypes and gastric cancer metastasis was observed. So, results indicated that locus rs2016073 may play a role in gastric cancer, especially in metastasis.

Our results found that locus rs751402 in the gastric cancer group showed no significant difference in the control group and in the gastric cancer recurrence group, which was consistent with our previous study¹¹. Another study using unconditional multiple logistic regression analysis revealed that the AA genotype of rs751402 significantly increased risk of gastric cancer compared with the GG genotype¹⁶. Stratification by cancer type

Table 2. Comparison of genetic loci between the control group and the gastric cancer group.

Loci	Single-nucleotide polymorphisms	Control group	Gastric cancer group	χ^2	p-value
rs2016073	A	67	90	3.281	0.194
	G	20	22		
	AG	46	88		
rs751402	A	20	22	2.72	0.257
	G	67	90		
	GA	48	88		
rs2094258	C	47	44	0.753	0.686
	T	16	21		
	CT	72	76		
rs2296147	C	4	7	17.11	<0.001
	T	76	153		
	CT	55	40		
rs2296148	C	128	170	7.902	0.005
	TC	7	30		

Table 3. Relationship of gene loci with metastasis and recurrence of gastric cancer.

Loci	Single-nucleotide polymorphisms	Metastasis		χ^2	p-value	Recurrence		χ^2	p-value
		Yes	No			Yes	No		
rs2016073	G	22	0	7.758	0.021	1	21	4.721	0.094
	A	89	1			15	75		
	GA	80	8			21	67		
rs751402	AG	80	8	7.758	0.021	21	67	4.721	0.094
	G	89	1			15	75		
	A	22	0			1	21		
rs2094258	C	52	9	21.475	<0.001	9	52	7.758	0.021
	CT	109	0			28	81		
	T	30	0			0	30		
rs2296147	C	6	1	31.131	<0.001	0	7	1.67	0.434
	CT	32	8			8	32		
	T	153	0			29	124		
rs2296148	C	161	9	1.663	0.197	22	148	23.227	<0.001
	TC	30	0			15	15		

indicated that rs751402 polymorphism may increase the risk of gastric cancer and hepatocellular carcinoma, which was further confirmed by a false-positive report probability analysis¹⁹. We found that locus rs751402 was related to gastric cancer metastasis. So, all these results indicated that locus rs751402 may play a role in gastric cancer, especially in metastasis.

The locus rs2094258 as an important polymorphism plays an important role in many cancers^{15,20}. Positive *Helicobacter pylori* individuals with rs2094258 TT genotypes demonstrated an increased risk of gastric cancer²¹. The rs2094258 polymorphism may be associated with an increased risk of gastric cancer in Southern China²². The rs2094258 interacted

with rs2094258 and metabolic gene *GSTP1* rs1695 and formed the basis for various inter-individual susceptibilities to atrophic gastritis²³. Our results indicated that locus rs2094258 was significantly related to metastasis and recurrence of gastric cancer. The above results demonstrated that locus rs2094258 may be a critical DNA repair gene for gastric cancer.

The locus rs2296147 showed a significant difference between the control group and the gastric cancer group and was significantly related to metastasis of gastric cancer, but not to the recurrence of gastric cancer. A case-control study showed that the polymorphic locus on *ERCC5* rs2296147 could reduce the risk of esophageal cancer, which will help further understand the pathogenesis of esophageal cancer²⁴. *ERCC5* rs2296147 C variant genotypes were associated with a significantly lower esophageal squamous cell carcinoma risk²⁵. The rs2296147 polymorphism might alter the risk of developing gastric cancer, especially the diffuse subtype²⁵. Therefore, the rs2296147 polymorphism could be used as surrogate markers for gastric cancer.

The locus rs2296148 showed no significant difference between the control group and the gastric cancer group and was not significantly related to metastasis of gastric cancer, but showed a significant difference in the recurrence of gastric cancer. Until now, there is no relevant literature report about locus rs2296148 for gastric cancer. Our results indicated that locus rs2296148 may be related to the recurrence of gastric cancer.

CONCLUSIONS

In this study, five *ERCC5* gene polymorphisms were explored. The results found that two loci (rs2296147 and rs2296148) in gastric cancer were significantly different from the control group, four loci (rs201607, rs751402, rs2094258, and rs2296147) were related to metastasis of gastric cancer, and two loci (rs2094258 and rs2296148) were related to the recurrence of gastric cancer. *ERCC5* gene polymorphisms may perform important functions

in gastric cancer and its metastasis and recurrence. Due to the small sample size, further validation by larger population-based case-control studies is needed.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All participants signed the informed consent, and this study was approved by the Gansu Provincial Hospital.

IMPACT STATEMENT

This study aims to explore the role of *ERCC5* genetic polymorphisms in gastric cancer. Genotype *ERCC5* rs2016073, rs751402, rs2094258, rs2296147, and rs2296148 were detected. The loci rs2016073, rs751402, and rs2094258 in the gastric cancer group showed no significant differences compared with the control group, and loci rs2296147 and rs2296148 showed significant differences. *ERCC5* polymorphisms (e.g., rs201607, rs751402, rs2094258, rs2296147, and rs2296148) may be associated with metastasis and recurrence of gastric cancer. *ERCC5* gene polymorphisms may perform important functions in gastric cancer and its metastasis and recurrence.

AUTHORS' CONTRIBUTIONS

SW: Conceptualization, Data curation, Resources, Writing-original draft, Writing-review & editing. **XC:** Conceptualization, Data curation, Investigation, Resources. **YF:** Conceptualization, Data curation, Formal analysis, Investigation, Resources. **HZ:** Conceptualization, Data curation, Formal analysis, Investigation, Resources. **WL:** Data curation, Formal analysis, Investigation, Methodology, Resources. **XS:** Data curation, Formal analysis, Investigation, Methodology, Resources. **XM:** Data curation, Formal analysis, Investigation, Methodology, Resources. **SC:** Data curation, Formal analysis, Investigation, Methodology, Resources. **JL:** Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Resources, Supervision, Writing-original draft, Writing-review & editing.

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