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Relationship between glutathione S-transferase M1 gene polymorphism and gastric cancer

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ABSTRACT

This experiment was designed to investigate the relationship between glutathione S-transferase M1 (GSTM1) gene polymorphism and gastric cancer (GC). For this purpose, from January 2013 to December 2014, 116 patients with GC diagnosed in the Department of Gastroenterology of our hospital and 120 healthy people in the physical examination center were selected as the research objects. 116 patients with GC served as the observation group and 120 healthy people in the physical examination center served as the control group. Collect and isolate the peripheral blood nucleated cells of the subjects, obtain the GSTM1 gene polymorphism by sequencing, analyze the differences of GSTM1 genotype between the two groups, compare the differences of clinicopathological characteristics of patients with different genotypes in the observation group, look for the survival relative risk factors of patients with GC, and analyze the risk factors of death risk of GC by multivariate Cox risk proportional regression. Results showed that the proportion of GSTM1 (-) in the observation group (62.07%) was raised compared with the control group (48.33%) ($p<0.05$). There was a correlation between GSTM1 gene polymorphism and smoking, TNM stage differentiation and GSTM1 gene polymorphism in the observation group. The specific analysis found that the proportion of non-smoking, stage I-III and low differentiation in the GSTM1 (-) group was raised compared with that in the GSTM1 (+) group ($p<0.05$). TNM stage, differentiation degree and GSTM1 gene polymorphism were correlated with the median survival time of patients with GC ($p<0.05$). Further multivariate Cox risk proportional regression analysis showed that TNM stage IV, low differentiation and GSTM1 (-) the relative risk coefficients of death in patients with GC were stage I - III, high/medium differentiation and GSTM1, respectively (+) patients were 1.75, 1.46, and 2.14 times higher. In conclusion, GSTM1 gene polymorphism is associated with susceptibility to GC, and the GSTM1 deletion genotype is an unfavourable factor for poor prognosis in patients with GC.

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Introduction

Gastric cancer (GC), which mainly originates from gastric mucosal epithelium, is the malignant tumors that threaten human health, ranking fifth in the global malignant tumor incidence rate (1). Changes in dietary structure and environment, Helicobacter pylori infection, genetics, etc. are all important inducements leading to the occurrence of GC. Only a few patients in the early phase of GC have upper gastrointestinal symptoms such as nausea and vomiting, while patients in the advanced phase of GC often have upper gastrointestinal symptoms such as epigastric discomfort and fullness after eating (2). With the proliferation and metastasis of tumor cells, patients have symptoms such as increased epigastric pain, anorexia, fatigue, etc., while patients with advanced GC have anemia, emaciation, and even cachexia (3). At present, the mechanism of GC is not clear, and the general view is that it is the result of the synergy of genetics, living habits and environment. Glutathione s-transferasem1 (GSTM1), as a key phase II detoxification metabolic enzyme, can catalyze the carcinogen with electrophilic properties to bind glutathione, form a hydrophilic polymer, and then excrete out of the body so as to prevent electrophilic DNA carcinogens from combining with DNA and other macromolecules to form DNA adducts, causing genetic material toxicity damage and even inducing carcinogenesis (4). Studies found that GSTM1 in different individuals is mainly GSTM1 gene deletion and GSTM1 gene carrying two genotypes. The change of genotype may affect the inactivation ability of individuals to carcinogens and increase the risk of cancer in some individuals exposed to the adverse environment (5). GSTM1 gene deletion has been reported to be associated with susceptibility to cervical cancer (6), lung cancer (7), nasopharyngeal carcinoma (8) and liver cancer (9). However, there are few reports on the susceptibility to GC and the prognosis of patients with GC. Our study aims to explore the correlation between GSTM1 gene polymorphism and the susceptibility to GC and the prognosis of patients.

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Materials and Methods

General Information

116 patients with GC diagnosed in the Department of Gastroenterology of our hospital and 120 healthy people in the physical examination center were selected as the research objects (from January 2013 to December 2014). 116 patients with GC served as the observation group, and 120 healthy people in the physical examination center served as the control group. The general data were collected, including gender, BMI, etc. In the observation group, there were 70 males and 46 females, aged from 39 to 78 years, with an average of (57.38 ± 13.57) years, and the BMI was (21.24 ± 5.36) kg/m^2; in the control group, there were 64 males and 56 females, aged from 36 to 75 years, with an average of (54.64 ± 14.61) years, and the BMI was (22.47 ± 6.72) kg / m^2. Statistics showed that there was no difference in general data between the two groups (P>0.05).

Grouping criteria: ①All patients met the diagnostic criteria of GC in the clinical diagnosis and treatment guidelines of the Chinese Medical Association (2021 version) (10), and were confirmed by pathology; ②Patients with an estimated survival of ≥ 3 months; ③ Patients and their families gave informed consent and signed the consent form.

Exclusion criteria: ①Patients with severe liver and kidney dysfunction and mental disease;②Patients with other primary tumors; ③Incomplete research materials; ④Patients who died of non-gastric cancer.

Sample Collection and Processing

In this study, about 8ml of fasting peripheral blood of the subjects in the two groups was collected. Blood was collected from the elbow vein before treatment or medication, centrifuged in a centrifuge (3000 rpm/min) for 5min within 1.5h, and the middle nucleated cell layer was separated into a new 1.5ml centrifuge tube for genomic DNA extraction.

DNA Extraction of Genome

A genomic DNA extraction kit was used to extract genomic DNA. All steps are carried out in strict accordance with the standard operation of the Thermo Fisher kit. The detailed operation steps are as follows: add 200ul protease solution to the centrifuge tube according to the sample volume, add 1ml of peripheral blood nucleated cell layer sample and buffer Ge, mix well with a vortex oscillator for 1min, and place at 65 ℃ for 10min. Add 2ml absolute ethanol to the sample, mix well, transfer to the adsorption column, then add 1.5ml buffer, centrifuge at 3000 rpm/min for 1min. Add 200ul elution buffer to the adsorption column, and the obtained solution is the genomic DNA of the peripheral blood of the two groups. If the purity of DNA detected by the spectrophotometer was 1.8 to 2.0, it is a qualified sample for subsequent experiments.

PCR Amplification and GSTM1 Gene Polymorphism Analysis

The GSTM1 gene was amplified by PCR. The total PCR reaction system was 25 μl. PCR reaction conditions were 95 °C for 5 min, (95 °C 35S, 54 °C 45s, 72 °C 30s) × 45 cycles, 72 °C for 5min. The polymorphic site primer was the upstream primer of GSTM1 gene polymorphism region (5'-3') 'GAACTCCCTGAAAAGCTAAAGC', downstream primer (5'-3') 'GTTGGGCTCAAATATACGGTGG'. The PCR reaction products were sent to Biotechnology Co., Ltd. for sequencing, and the distribution of GSTM1 gene polymorphism in the two groups was obtained through analysis. GSTM1 (+) is an amplification fragment with a molecular weight of 215bp in the amplification product, that is, GSTM1 carries the genotype, while GSTM1 (-) expresses the amplification fragment corresponding to the deletion in the DNA sample, that is, GSTM1 deletion genotype.

Statistical Analysis

Use IBM SPSS software (23.0) for statistical analysis. All measurement data conform to a normal distribution, expressed by ($\bar{x} \pm s$), and the SNK-q test is used for comparison between groups; the counting data are expressed in percentage, and the comparison between groups adopts χ^2 Inspection. The survival rate was analyzed by log-rank test, and the multivariate Cox proportional hazard regression model was used to evaluate the risk factors affecting the prognosis of GC patients. P<0.05 was regarded as a significant difference.

Results

Comparison of GSTM1 Genotype Frequency between Two Groups

The proportion of GSTM1 (-) in the observation group (62.07%) was higher than that in the control group (48.33%) (p<0.05) (Table 1).

Clinicopathological Characteristics of GSTM1 (-) and GSTM1 (+) Patients in the Observation Group

The study found that the GSTM1 gene polymorphism was correlated with smoking and TNM phase differentiation in the observation group. The specific analysis found that the proportion of non-smoking, phase I-III and poorly differentiated patients in the GSTM1 (-) group was significantly higher than that in the GSTM1 (+) group (p<0.05) (Table 2).

Risky Factors Affecting the Survival of Patients with GC

The study found that TNM phase, differentiation degree and GSTM1 gene polymorphism were correlated with the median survival time of GC patients (P<0.05). The median survival time of phase I-III GC patients was higher than that of phase IV patients, and the median survival time of

Table 1. Comparison of GSTM1 Genotype Frequency between Observation Group and Control Group.

GSTM1genotype	Observation group	Control group	χ^2	*P*
GSTM1(-)	72	58	4.4978	0.0339
GSTM1(+)	44	62		
Total	116	120		

highly / moderately differentiated GC patients was higher than that of poorly differentiated patients, while GSTM1 (+) the median survival time of patients with GC was significantly higher than that of GSTM1 (-) patients (Table 3).

Cox Regression Analysis of Mortality Risk of GC

Further multivariate Cox proportional regression analysis showed that TNM phase, degree of differentiation and GSTM1 gene polymorphism were risk factors for poor prognosis and death in patients with GC. TNM phase IV, low differentiation and GSTM1, relative risk coefficients of death in (-) GC patients were phase Ⅰ - Ⅲ, high/medium differentiation and GSTM1, respectively (+) patients are 1.75, 1.46 and 2.14 times (Table 4).

Discussion

GC is the most common tumor in China, and its prognosis is relatively poor. About 170000 people die of GCr every year, and its mortality ranks first among all kinds of malignant tumors (11), and its etiology and pathogenesis have not been completely clear. Generally, when carcinogens or carcinogen precursors in the environment enter the human body, under the biotransformation of phase I metabolic enzymes, and then through the biodegradation or transformation of phase II metabolic enzymes, they are absorbed or excreted by the human body, but will not be enriched in the organism, so as to avoid inducing the occurrence of cancer (12).

Glutathione-S-transferase (GSTM1) gene is a member of the GST superfamily, which encodes enzymes responsible for scavenging free radicals and other carcinogens. The activity of these enzymes may be different due to GSTM1 gene polymorphism, which eventually leads to differences in the possibility of cancer among individuals (13). As a key enzyme involved in the biological metabolism of a variety of carcinogens, the homozygous

Table 2. Clinicopathological Characteristics of GSTM1 (-) and GSTM1 (+) Patients in the Observation Group.

Index		n	Genotype		χ^2	*P*
			GSTM1(+) (n=44)	GSTM1(-) (n=72)	GSTM1(+) (n=44)	GSTM1(-) (n=72)
Age	≥60	67	24	43	0.300	0.584
	<60	49	20	29		
Gender	Male	70	26	44	0.046	0.829
	Female	46	18	28		
Tumor size	≥5cm	43	16	27	0.015	0.902
	<5cm	73	28	45		
Part	Cardia	49	19	30	0.156	0.925
	Gastric body	15	5	10		
	Pylorus	52	20	32		
Smoke	Yes	46	23	23	4.716	0.030
	No	70	21	49		
Drink	Yes	73	26	47	0.448	0.503
	No	43	18	25		
TNMPhase	Phase I-III	77	24	53	4.448	0.034
	Phase IV	39	20	19		
Degree of differentiation	High/medium	73	33	40	4.426	0.035
	Low	43	11	32		

Table 3. Risky Factors Affecting the Survival of Patients with GC.

Index		Median survival time (months)	95%CI	Log-rank (χ^2)value	*P*-value
Smoke	Yes	60.65	51.34, 69.26	0.354	0.573
	No	63.42	53.75, 72.41		
TNMPhase	Phase I-III	65.68	56.27, 73.67	4.450	0.032
	Phase IV	50.87	42.32, 58.63		
Degree of differentiation	High/medium	67.19	58.26, 75.64	4.835	0.026
	low	48.34	41.68, 56.27		
GSTM1	(-)	50.84	42.47, 58.95	4.256	0.038
	(+)	64.65	56.42, 72.35		

Table 4. Cox Regression Analysis of Mortality Risk of GC.

Index		Bvalue	S.E.	RR	95%CI	Pvalue
Smoke	Yes	0.61	0.49	1.05	0.85, 1.27	0.658
	No			1		
TNMPhase	I-III Phase	0.68	0.15	1.75	1.20, 2.54	0.021
	IV Phase			1		
Degree of differentiation	High/medium	0.39	0.19	1.46	1.06, 2.05	0.035
	low			1		
GSTM1	(+)	0.86	0.26	2.14	1.33, 3.98	0.01
	(-)	0.61	0.49	1.05	0.85, 1.27	0.658

deletion of GST M1 will lead to the failure of the enzymes involved in the metabolic transformation of carcinogens to function normally, which will lead to the accumulation of carcinogens in the body and increase the risk of individual carcinogenic exposure in adverse environments (14). This study found that the proportion of GSTM1 (-) genotype in the observation group (62.07%) was higher than that in the control group (48.33%) (P<0.05). This also indicates that the GSTM1 deletion genotype may be associated with the susceptibility to GC.

It has been confirmed that the GSTM1 deletion genotype is associated with the occurrence of a variety of cancers. Studies have found that the GSTM1 deletion genotype is associated with increased lung cancer risk in Japanese and increased lung adenocarcinoma risk in Asians (15). At the same time, the study confirmed that GSTM1 gene deficiency combined with smoking increased the risk of cancer by 65% compared with the simple smoking group, indicating that GSTM1 gene deficiency and smoking have a synergistic effect on promoting the occurrence of lung cancer (16). It further supports the conclusion that genetic factors and environmental factors work together to promote the process of diseases. In colorectal cancer, GSTM1 and GSTT1 deletion genotypes are associated with an increased risk of colorectal cancer in Asians and Caucasians (17). GSTM1 was down-regulated in the tumor tissues of breast cancer patients. At the same time, the study also found that the down-regulation of GSTM1 promoted the EMT pathway, tumor proliferation, and metastasis in breast cancer tissue samples. It is speculated that GSTM1 may affect the susceptibility to metastasis and invasion of breast cancer through the EMT pathway in terms of mechanism of action (18). To evaluate the association between GSTM1 and GSTT1 deletion and GSTP1 rs1695 polymorphism and the risk of liver cancer, studies found that the deletion of GSTM1 and GSTT1 increased the risk of liver cancer (19). It further supports the reliability of the conclusion that it is related to the susceptibility to GC.

Further research in this study found that there was a correlation between gsm1 gene polymorphism and smoking, and TNM phase differentiation degree in the observation group. Specifically, the proportion of non-smoking, phase I-III and low differentiation degree in the GSTM1 (-) group was significantly higher than that in the GSTM1 (+) group (P<0.05). Moreover, TNM phase, degree of differentiation and GSTM1 gene polymorphism were correlated with the median survival time of patients with GC (P<0.05). Further multivariate Cox risk proportional regression analysis showed that the relative risk coefficients of death in patients with TNM phase IV, low differentiation and GSTM1 (-) gastric cancer were 1.75, 1.46 and 2.14 times higher than those in patients with phase I - III, high/medium differentiation and GSTM1 (+) respectively. These results also suggest the role of the GSTM1 deletion genotype in the prognosis of patients with GC.

Conclusion

In conclusion, GSTM1 gene polymorphism is associated with susceptibility to GC, and the GSTM1 deletion genotype is a risk factor for poor prognosis in patients with GC. But the sample size is small. Moreover, other factors may bias the experimental results. We will further expand the sample size in the later research, and select its associated gene polymorphism for further prospective research, in order to provide more accurate evidence for the treatment and prognosis of GC in the future.

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