

RESEARCH COMMUNICATION

Case-control Study of Single Nucleotide Polymorphisms of PSCA and MUC1 Genes with Gastric Cancer in a Chinese

Fang Li, Mei-Zuo Zhong*, Jian-Huang Li, Wei Liu¹, Bin Li

Abstract

Aims: A case-control study of 300 gastric cancer patients and 300 controls was conducted to investigate whether the polymorphisms rs2294008 in PSCA and rs2070803 in MUC1 might be associated with risk of gastric cancer in a Chinese population. **Methods:** Single nucleotide polymorphisms (SNPs) were genotyped using the Sequenom MassARRAY platform. **Results:** The data showed that the rs2294008 TT genotype increased gastric cancer risk to an adjusted odds ratio (OR) of 2.26 (95% CI 1.25-4.07), TC to 1.72 (95% CI 1.23-2.42) and TC/TT to 1.81 (95% CI 1.31-2.50), while the rs2070803 GA genotype was associated with a decrease in risk to an adjusted OR of 0.42 (95% CI 0.28-0.62) and rs2070803 GA/AA to 0.46 (95% CI 0.32-0.67). Further stratification analysis revealed that rs2294008 in PSCA consistently increased risk of both intestinal and diffuse-type gastric cancers. The effect of rs2070803 in MUC1 was noteworthy also consistent with both subtypes. **Conclusions:** Our study suggested rs2294008 in the PSCA gene to be associated with increased risk of gastric cancer and rs2070803 in MUC1 to play a protective role in a Chinese population.

Keywords: Gastric cancer - prostate stem-cell antigen - muc 1 - single nucleotide polymorphism - Chinese population

Asian Pacific J Cancer Prev, 13, 2593-2596

Introduction

Gastric cancer is the second most common cause of cancer-related death worldwide as a result of the complex interaction of environmental conditions and genetic and epigenetic abnormalities (Cesar et al., 2002). However, genetic factors substantially contribute to the gastric carcinogenesis (Micev & Cosic-Micev, 2011).

PSCA is a 123-amino-acid cell membrane glycoprotein belonging to the LY-6/Thy-1 family of cell surface antigens and is expressed in lots of cancers (Bahrenberg et al., 2000). It was reported that PSCA had an in vitro cell-proliferation inhibitory activity (Sala et al., 2011). MUC1 is a transmembrane mucin localized to the apical membranes of normal secretory epithelial cell (Gendler, 2001), protecting the underlying epithelium by forming a mucous barrier with secreted mucins and signaling the presence of adverse conditions in the extracellular environment (Yin et al., 2003; Byrd & Bresalier, 2004). Single nucleotide polymorphisms (SNPs) have attracted considerable attention in recent years as potential markers for predicting disease susceptibility and for guiding individualized therapeutic regimens (Micev & Cosic-Micev, 2011). Currently, SNPs have been widely used as defacto standards for excellent and robust genetic markers which could themselves be functionally responsible for disease susceptibility (Yoshida et al., 2010).

A genome wide association study (GWAS) indentified three single nucleotide polymorphisms (SNPs) associated

with diffuse gastric cancer; two of which were rs2294008 in PSCA and rs2070803 in MUC1. A following study considered MUC1 a major susceptibility gene and the SNP with MUC1 (rs2070803) was significantly associated with diffuse-type gastric cancer (Sakamoto et al. 2008; Saeki et al., 2011). In the present study, we evaluated the association of the two SNPs (rs2294008 in PSCA and rs2070803 in MUC1) with gastric cancer susceptibility in a Chinese population.

Materials and Methods

Study Subjects

Three hundred pathologically confirmed gastric cancer patients were enrolled at the Xiangya Hospital of Central South University and Tumor Hospital of Hunan Province, China, between September 2009 and August 2011. Three hundred subjects without tumors were selected randomly from the same geographic region as the patients with cancer. Informed consent was obtained from each participant. Questionnaires were used to obtain baseline characteristics about the study subjects. This study was approved by the Ethical Committee of Xiangya Hospital of Central South University and Tumor Hospital of Hunan Province and conducted according to the principles of the Declaration of Helsinki.

Genotyping

Genomic DNA was extracted from peripheral blood

Department of Oncology, Xiangya Hospital, Central South University, Changsha, Hunan, China *For correspondence: 844569232@qq.com

using Whole Blood Gene DNA Isolation Kit (GenMagBio, Beijing, China), according to the manufacturer's instructions. Genotyping of SNPs was done using MassARRAY-IPLEX technology and matrix-assisted laser desorption ionization time of flight mass spectrometry platform (MALDI-TOF-MS). (Sequenom Inc., San Diego, CA). PCR primers were designed using the Assay Design 3.1 program (Sequenom). Analysis and scoring were performed using the program Typer 3.4 (Sequenom).

Statistical analysis

Chi-square test was used to compare demographic distributions, allele and genotype frequencies between cases and controls. Hardy-Weinberg equilibrium was checked for controls using the goodness-of-fit χ^2 test. The associations between the genotypes and risk of gastric cancer were estimated by calculating the odds ratios (ORs) with 95% confidence intervals (CIs) using unconditional logistic regression analyses adjusted for age and gender. All tests were conducted at the $P = 0.05$ level of significance, using SPSS 17.0 software package (SPSS, Chicago, IL)

Results

Demographic features of the subjects are shown in Table 1. There was no significant difference in the distribution of age ($P = 0.60$) and sex ($P = 0.18$) between the case and control subjects. Genotype frequencies for the two SNPs conformed to the Hardy-Weinberg equilibrium in controls ($P = 0.65$ for rs2294008 and $P = 0.70$ for rs2070803).

Allelic frequencies of the two SNPs between gastric cancer patients and controls are summarized in Table 2. The allele frequencies of rs2294008-T and rs2070803-A were significantly different between the cases and controls ($P < 0.001$, adjusted OR = 1.59, 95% CI 1.24-2.03 for

Table 1. Characterization of Gastric Cancer Patients and the Controls

		Patients, n(%)	Controls, n(%)	P-value
Variable				
Overall		300	300	
Gender	Male	192 (64.3)	175 (58.3)	0.18
	Female	108 (35.7)	125 (41.7)	
Age(year)	Mean±SD	52.2±11.8	51.7±12.0	0.6
	Lauren	211 (70.3)		
classification	Intestinal			
	Diffuse	77 (25.7)		
	Unclassified	12 (4.0)		

Table 4. Association of PSCA and MUC1 Genotype with 2 Major Types of Gastric Cancer

Genotype		Diffuse, n(%)	OR(95% CI)	P	Intestinal, n(%)	OR(95% CI)	P
rs2294008	CC	15 (19.5)	1		100 (47.4)	1	
	TC	40 (51.9)	2.18 (1.24-3.83)	0.007	101 (47.9)	1.73 (1.20-2.51)	0.004
	TT	22 (28.6)	5.12 (2.36-11.1)	<0.001	10 (4.7)	1.60 (0.81-3.16)	0.175
	TT/TC	62 (80.5)	2.65 (1.56-4.49)	<0.001	111 (52.6)	1.71 (1.20-2.44)	0.003
rs2070803	GG	62 (80.5)	1		172 (81.5)	1	
	GA	13 (16.9)	0.40 (0.21-0.79)	0.008	30 (14.2)	0.40 (0.25-0.62)	<0.001
	AA	2 (2.6)	1.28 (0.39-4.22)	0.687	9 (4.3)	0.80 (0.30-0.14)	0.655
	AA/GA	15 (19.5)	0.49 (0.27-0.89)	0.019	39 (18.5)	0.43 (0.29-0.66)	<0.001

OR, odds ratio adjusted for age and sex; CI, confidence interval

rs2294008 and $P < 0.001$, adjusted OR = 0.57, 95% CI 0.41-0.78 for rs2070803).

Genotype frequencies of polymorphism of PSCA and MUC1 for patient and control groups are summarized in Table 3. We found that the TC (adjusted OR = 1.72, 95% CI 1.23-2.42, $P = 0.002$), TT (adjusted OR = 2.26, 95% CI 1.25-4.07, $P = 0.007$) and TC/TT (adjusted OR = 1.81, 95% CI 1.31-2.50, $P < 0.001$) genotypes of rs2294008 were associated with a significantly increased risk of gastric cancer compared with the CC genotype. When compared with the GG genotype, the GA (adjusted OR = 0.42, 95% CI 0.28-0.62, $P < 0.001$) and GA/AA (adjusted OR = 0.46, 95% CI 0.32-0.67, $P < 0.001$) genotypes of rs2070803 were associated with a significantly lower risk of gastric cancer.

The associations between the two SNPs and gastric cancer were further evaluated by stratified analysis of histological type. As shown in Table 4, a significant association was observed between rs2294008 polymorphism and risk of both intestinal (TC: adjusted OR = 1.73, 95% CI 1.20-2.51; TC/TT: adjusted OR = 1.71, 95% CI 1.20-2.44, $P = 0.003$) and diffuse-type (TT: adjusted OR = 5.12, 95% CI 2.36-11.11, $P < 0.001$; TC: adjusted OR = 2.18, 95% CI 1.24-3.83, $P = 0.007$; TT/TC: adjusted OR = 2.65, 95% CI 1.56-4.49, $P < 0.001$) gastric cancer. Meanwhile, a statistically significantly protective

Table 2. Frequencies of PSCA and MUC1 Allele in Gastric Cancer Patients and Healthy Controls

	Allele	MAF	OR(95% CI)	P-value	
	Patients (%)	Controls (%)			
rs2294008	T	0.35	0.26	1.59 (1.24-2.03)	<0.001
rs2070803	A	0.12	0.19	0.57 (0.41-0.78)	<0.001

MAF, Minor allele frequency; OR, odds ratio adjusted for age and sex; CI, confidence interval

Table 3. Association Between PSCA and MUC1 Genotype and Risk of Gastric Cancer

Genotype	Control, n(%)	Case, n(%)	OR(95% CI)	P-value	
rs2294008	CC	168 (56.0)	124 (41.3)	1	
	TC	111 (37.0)	141 (47.0)	1.72 (1.23-2.42)	0.002
	TT	21 (7.0)	35 (11.7)	2.26 (1.25-4.07)	0.007
	TT/TC	132 (44.0)	176 (58.7)	1.81 (1.31-2.50)	<0.001
rs2070803	GG	195 (65.0)	240 (80.0)	1	
	GA	95 (31.7)	49 (16.3)	0.42 (0.28-0.62)	<0.001
	AA	10 (3.3)	11 (3.7)	0.89 (0.37-2.15)	0.802
	AA/AG	105 (35.0)	60 (20.0)	0.46 (0.32-0.67)	<0.001

OR, odds ratio adjusted for age and sex; CI, confidence interval

effect of the rs2070803 genotypes on gastric cancer risk was observed in both intestinal (GA: adjusted OR = 0.40, 95% CI = 0.25-0.62, $P < 0.001$; GA + AA: adjusted OR = 0.43, 95% CI 0.29-0.66, $P < 0.001$) and diffuse-type (GA: adjusted OR = 0.40, 95% CI 0.21-0.79, $P = 0.008$; GA/AA: adjusted OR = 0.49, 95% CI 0.27-0.89, $P = 0.019$) gastric cancer.

Discussion

In this study, we investigated the associations of genetic variants of rs2294008 in PSCA and rs2070803 in MUC1 with gastric cancer susceptibility in an independent case-control study with 300 GC cases and 300 controls in a Chinese population. We found that PSCA rs2294008 was significantly associated with increased risk of gastric cancer but MUC1 rs2070803 might be a protective factor for gastric cancer among Chinese populations.

PSCA was detected in prostate, pancreatic, renal, bladder and gastric cancer (Reiter et al., 1998; Bahrenberg et al., 2000; Argani et al., 2001; Elsamman et al., 2006), and was reported to had a tumor suppressor-like activity, and is involved in the regulation of gastric epithelial-cell proliferation (Sakamoto et al., 2008). It was reported that the risk allele T of rs2294008 is associated with lower transcriptional activity of PSCA (Sakamoto et al., 2008), but the precise role and function of PSCA is still unknown.

PSCA rs2294008 was first described associated with diffuse-type gastric cancer in Japanese and Korean populations (Sakamoto et al., 2008) and the association was validated in different Asian populations, such as Chinese and Korean populations (Matsuo et al., 2009; Song et al., 2011; Zeng et al., 2011). Recent studies in Caucasians and Chinese population indicated that PSCA rs2294008 was a similar risk factor for both the diffuse and the intestinal-types of gastric cancer (Lu et al., 2010; Sala et al., 2011), which is in line with our results. A meta-analysis in East Asian studies showed an association between rs2294008 and gastric cancer (adjusted OR = 1.84) (Lu et al., 2010).

MUC1 was reported to function as a growth factor receptor in human embryonic stem cells (Hikita et al., 2008). MUC1 is expressed at high levels over the entire surface of diverse types of carcinoma cells and may promote the malignant epithelial-mesenchymal transition (EMT) phenotype by disrupting polarity and cell-cell interactions (Kufe, 2009). It is also supposed that MUC1 has a protective function against environmental insults and tumorigenesis in normal epithelial cells which keep maintaining their cell polarity (Kufe, 2009).

MUC1 is a highly polymorphic mucin and genetic variants in MUC1 have been associated with the risk of gastric cancer in previous studies with a candidate gene approach (Xu et al., 2009; Jia et al., 2010). A gene-centric genome-wide association study (GWAS) identified a significant association of rs2070803 polymorphism in MUC1 with diffuse-type gastric cancer ($P = 1.2 \times 10^{-6}$, adjusted OR = 1.62, CI 1.33-1.98) (Byrd & Bresalier, 2004). Their following study validated that rs2070803 allele-A was significantly associated with diffuse-type of gastric cancer, and rs2070803 polymorphism in MUC1

might be used to identify individuals at risk for diffuse-type of gastric cancer ($P = 4.33 \times 10^{-13}$; adjusted OR = 1.71 by meta-analysis of the studies) (Sakamoto et al., 2008).

To date, no prior study has examined gastric cancer risk for rs2070803 SNP among Chinese populations. A statistically significant protective association was found between rs2070803 SNP and gastric cancer in our study. The result we obtained from this study suggests a relation between rs2070803 polymorphism and gastric cancer. Accumulation of these studies is an important contributing factor to understand the association between MUC1 gene polymorphism and gastric cancer.

Our study design was conducted regardless of smoking habit, alcohol drinking, and H. pylori infection of gastric cancer. The lack of association between these two polymorphisms and environmental factors strengthen the independent impact of polymorphisms on etiology of gastric cancer development. And this phenomenon may simply reflect dominant nature of this polymorphism in the pathogenesis of gastric cancer.

In conclusion, the data from this study of a Chinese population provided evidence that polymorphism in PSCA is involved in susceptibility to gastric cancer. MUC1 rs2070803 polymorphism may have a protective effect during tumor progression. Further valuation on the molecular mechanisms by which PSCA involved in susceptibility to gastric cancer and the exploration of the pleiotropic functions of MUC1 in diverse ethnic populations and larger number of patients is needed.

Acknowledgements

The author(s) declare that they have no competing interests.

References

- Argani P, Rosty C, Reiter RE, et al (2001). Discovery of new markers of cancer through serial analysis of gene expression: Prostate stem cell antigen is overexpressed in pancreatic adenocarcinoma. *Cancer Res*, **61**, 4320-4.
- Bahrenberg G, Brauers A, Joost HG et al (2000). Reduced expression of pscA, a member of the ly-6 family of cell surface antigens, in bladder, esophagus, and stomach tumors. *Biochem Biophys Res Commun*, **275**, 783-8.
- Byrd JC, Bresalier RS (2004). Mucins and mucin binding proteins in colorectal cancer. *Cancer Metastasis Rev*, **23**, 77-99.
- Cesar AC, Silva AE, Tajara EH (2002). Genetics and environmental factors in gastric carcinogenesis. *Arg Gastroenterol*, **39**, 253-9.
- Elsamman EM, Fukumori T, Tanimoto S, et al (2006). The expression of prostate stem cell antigen in human clear cell renal cell carcinoma: A quantitative reverse transcriptase-polymerase chain reaction analysis. *Bju International*, **98**, 668-73.
- Gendler SJ (2001). Muc1, the renaissance molecule. *J Mammary Gland Biol Neoplasia*, **6**, 339-53.
- Hikita ST, Kosik KS, Clegg DO, et al (2008). Muc1* mediates the growth of human pluripotent stem cells. *PLoS One*, **3**, e3312.
- Jia YB, Persson C, Hou LF, et al (2010). A comprehensive analysis of common genetic variation in muc1, muc5ac,

- muc6 genes and risk of stomach cancer. *Cancer Causes Control*, **21**, 313-21.
- Kufe DW (2009). Mucins in cancer: Function, prognosis and therapy. *Nat Rev Cancer*, **9**, 874-85.
- Lu Y, Chen JJ, Ding YB, et al (2010). Genetic variation of psca gene is associated with the risk of both diffuse- and intestinal-type gastric cancer in a chinese population. *International Journal of Cancer*, **127**, 2183-9.
- Matsuo K, Tajima K, Suzuki T, et al (2009). Association of prostate stem cell antigen gene polymorphisms with the risk of stomach cancer in japanese. *Int J Cancer*, **125**, 1961-4.
- Micev M, Cosic-Micev M (2011). [pathology and pathobiology of the gastric carcinoma]. *Acta Chir Jugosl*, **58**, 39-52.
- Reiter RE, Gu Z, Watabe T, et al (1998). Prostate stem cell antigen: A cell surface marker overexpressed in prostate cancer. *Proc Natl Acad Sci USA*, **95**, 1735-40.
- Sala N, Munoz X, Travier N, et al (2011). Prostate stem-cell antigen gene is associated with diffuse and intestinal gastric cancer in caucasians: Results from the epic-eurgast study. *Int J Cancer*, **130**, 2417-27.
- Sakamoto H, Yoshimura K, Saeki N, et al (2008). Genetic variation in psca is associated with susceptibility to diffuse-type gastric cancer. *Nature Genetics*, **40**, 730-40.
- Saeki N, Saito A, Choi IJ, et al (2011). A functional single nucleotide polymorphism in mucin 1, at chromosome 1q22, determines susceptibility to diffuse-type gastric cancer. *Gastroenterology*, **140**, 892-902.
- Song HR, Kim HN, Piao JM, et al (2011). Association of a common genetic variant in prostate stem-cell antigen with gastric cancer susceptibility in a korean population. *Mol Carcinog*, **50**, 871-5.
- Xu Q, Yuan Y, Sun LP, et al (2009). Risk of gastric cancer is associated with the muc1 568 a/g polymorphism. *Int J Oncol*, **35**, 1313-20.
- Yin L, Li Y, Ren J, Kuwahara H, et al (2003). Human muc1 carcinoma antigen regulates intracellular oxidant levels and the apoptotic response to oxidative stress. *J Biol Chem*, **278**, 35458-64.
- Yoshida T, Ono H, Kuchiba A, et al (2010). Genome-wide germline analyses on cancer susceptibility and gemdbj database: Gastric cancer as an example. *Cancer Sci*, **101**, 1582-9.
- Zeng Z, Wu X, Chen F, et al (2011). Polymorphisms in prostate stem cell antigen gene rs2294008 increase gastric cancer risk in chinese. *Mol Carcinog*, **50**, 353-8.