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ORIGINAL ARTICLE

A missense mutation (S3660L) in MLL3 gene influences risk of gastric cancer

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Summary

Purpose: *Several studies indicated that the expression level of MLL3 gene in gastric cancer tissue was associated with prognosis, and previous studies also suggested that genetic polymorphisms of MLL3 were related to the risk for gastric cancer. The present study aimed to investigate the association of a missense mutation (S3660L) in the MLL3 gene with gastric cancer risk in a Chinese population.*

Methods: *In the present study, we identified a novel missense mutation in MLL3 gene (S3660L) by directly sequencing method in 48 gastric cancer patients. To further explore the relation between gastric cancer and this mutation, we selected 354 gastric cancer patients and 377 healthy control subjects and designed a case-control study.*

Results: *We found that the AG genotype (14.9 vs 6.40%, odds ratio/OR=2.58, 95% CI: 1.33-4.54, p<0.001) and A allele (7.5 vs 3.2%, OR=2.46, 95% CI: 1.55~5.34, p<0.001) were common in the gastric cancer patients than in the control subjects.*

Conclusion: *We concluded that this novel missense (S3660L) mutation in MLL3 gene is likely to increase the gastric cancer risk.*

Key words: *gastric cancer, genetic variants, missense mutation, MLL3*

Introduction

Previous studies indicated that gastric cancer is a highly aggressive and lethal malignancy which accounts for 8.6% of new cancer patients worldwide. It is estimated that there are 930,000 new gastric cancer cases every year, and about 700,000 patients die annually of gastric cancer [1]. This malignancy is the fourth most common cancer worldwide [2]. Although many studies indicated that the traditional risk factors including nitrates, smoked fish and salted meats, moldy foods containing aflatoxin, and infection with *Helicobacter pylori (H. pylori)* are involved in the development of gastric cancer [3]; however, the mechanisms of gastric carcinogenesis remain largely unclear up to date. Recently, lots of published studies provided evidence that gastric cancer is a multifactorial disorder, resulting from the interaction between enviromental factors and genetic mutations. Several genes, such as cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) gene [4], CYP1B1 gene [5], COX-2 gene [6], are considered to be possibly associated with susceptibility of gastric cancer.

MLL3, a member of the TRX/MLL gene family located to chromosome 7q36.1. [7], was considered as an important poor prognostic factor for gastric cancer; a study has shown that inactivation of MLL3 in mice resulted in epithelial tumor formation, suggesting that MLL3 may be a tumor suppressor gene [8]. Also, MLL3 has been reported to be frequently deleted in myeloid leukemias [9,10]. Moreover, other authors indicate somatic mutations in the MLL3 gene in glioblastoma and pancreatic ductal adenocarcinoma [11]. However, the association of MLL3 mutation with gastric cancer remains unclear.

In the present study, we aimed to identify novel functional mutations of the MLL3 gene and to test any possible association with gastric cancer.

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Table 1. Characteristics of study participants

Characteristics	*Patients*	*Controls*	*p-value*
Number of subjects (%)	354 (100)	377 (100)	
Age, years (mean ± SD)	54.1±11.5	54.6±11.8	0.546
Sex, male (N, %)	217 (61.3)	236 (62.6)	0.875
Smoking (N, %)	273 (77.11)	186 (49.33)	<0.001
Alcohol use (N, %)	203 (57.34)	194 (51.46)	0.734
Family history of cancer (N, %)	48 (13.56)	13 (3.45)	<0.001

SD: standard deviation

Methods

Subjects

This study was approved by the Ethics Committee of the General Hospital of the People's Liberation Army. The study was conducted according to the standards of the Declaration of Helsinki. Written informed consent was obtained from all participants.

A total of 731 genetically unrelated subjects, including 377 healthy control subjects and 354 gastric cancer patients were recruited from the General Hospital of the People's Liberation Army between March 2009 and June 2013. All subjects were Han Chinese as described in the previous study [12]. Briefly, histological demonstration of gastric cancer was confirmed by two pathologists. Subjects with previous malignancy or with metastatic cancer from other origins were excluded. For gastric cancer patients, the clinicopathological variables, including tumor site, tumor area, grade of differentiation, depth of tumor invasion, lymph node metastasis, distant metastasis and TNM stage, were obtained from the medical records as shown in Table 1. None of the patients had undergone radiotherapy or chemotherapy before surgery. Depth of tumor invasion, lymph node metastasis, distant metastasis, and TNM stage were assessed according to the American Joint Committee for Cancer Staging in 2002. The control group consisted of individuals visiting the Hospital for routine examinations. These persons had no gastrointestinal disorders or personal and family history of cancer, which were traced back to ≥3 generations and laterally to 2nd and 3rd degree relatives.

Primers design and MLL3 gene sequencing

Sequence information for use as a reference template was obtained from the Ensembl Genome Browser (Human, number ENSG00000055609). Sequencing primers were designed using Primer Premier 5.0 software (Premier Co, Canada). The sense primer was 5'GCTGGGTTCTGCTTCTCA3', and the antisense primer was 5'TCACCATCAATCCCTGTT3'. The genomic DNA was extracted from peripheral white blood cells according to the methods described by Xie et al. [13]. The polymerase chain reaction (PCR) was undertaken with 50 ng of genomic DNA in a 20 μL solution containing 10 μL of Power Mix (Beijing Biotech, Beijing, China), 9.5 μL of distilled water, and 0.2 mM of each forward and reverse primer. A GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) was used for PCR amplification. Sequencing reactions were performed by BGI-Beijing (Beijing, China; http://www.genomics.cn).

Genotyping of novel single nucleotide polymorphisms (SNPs)

The Exon regions in MLL3 gene were sequenced on 48 subjects with gastric cancer. A novel missense mutation (S3660L) causing the change in amino acids from Ser to Leu at codon 3660 was identified. Genotyping for the S3660L variant in the present case–control study was done by PCR amplification of 311bp, followed by restriction digestion with *Bsc4* I (Fermentas, Beijing, China). The sense primer was 5'TTCCTCCAATTTAGGCTCCTCTT3' and the antisense primer was 5'CAGGCATCTCAGAAACTACCTCTACTC3'. The annealing temperature was 62 °C. The PCR product (15 μL) was incubated overnight with *Bsc4* I (5 U) in a total volume of 25 μL at 37 °C, and the resulting fragments were separated on 2.0% agarose gel. Absence of the S3660L variant created a *Bsc4* I site producing two fragments of 241 bp and 70 bp. To confirm the results, we used sequenced genomic DNAs as positive controls in our assays.

Statistics

Analyses were carried out using SPSS version 17.0 (SPSS, Chicago, IL, USA). The Hardy–Weinberg equilibrium was assessed by chi-square analysis. Measurement data are shown as means ±SD, and the differences between gastric cancer patients and control subjects were assessed by independent-sample *t*-test. Differences in enumeration data between gastric cancer patients and control subjects were analyzed using the chi-square test, as were differences in distributions of genotypes and alleles between gastric patients and control subjects. Multivariate Cox logistic regression analysis was used to assess the contribution of the major risk factors. Statistical significance was set at $p<0.05$.

Results

Study population characteristics

In our study, 731 Chinese Han participants

Table 2. The genotype and allele distribution in case and control groups

Group	*N*	*Allele*		*p-value*	*OR (95%CI)*	*Allele*		*p-value*	*OR (95%CI)*
Genotypes, n(F)		*AG*	*GG*			*A*	*G*		
Cases	354	53 (0.149)	301(0.851)	<0.001	2.58 (1.33-4.54)	53 (0.075)	655 (0.925)	<0.001	2.46 (1.55-5 .34)
Controls	377	24 (0.064)	353(0.936)			24 (0.032)	730 (0.968)		

N: number, F: frequency, OR: odds ratio, CI: confidence interval

(354 gastric cancer patients and 377 control subjects) were enrolled. There were no statistically significant differences in age distribution and sex ratio (both p>0.05) between patients and controls. However, the prevalence of family cancer history and smoking in the gastric cancer group was significantly higher than in the control group (p<0.001, p<0.001, respectively). The amounts of alcohol intake were similar between patients and controls (Table 1).

Association of novel mutation in MLL3 with gastric cancer

The genotype distribution in both gastric cancer patients and control subjects were in Hardy–Weinberg equilibrium (both p > 0.05). The frequency of the heterozygote carriers of the AG genotype of MLL3 was significantly higher in gastric cancer patients than in the control subjects (14.9 vs 6.40%, p<0.001). The frequency of the A allele in gastric cancer patients was higher than that in the control subjects [7.5 vs 3.2%, OR=2.46, 95% CI 1.55-5.34, p<0.001] (Table 2). The OR for carriers of the AG genotype for gastric cancer was 2.58 (95% CI 1.33-4.54). By multivariate analysis we adjusted confounders such as smoking, alcohol consumption, and cancer family history, and found that the difference remained significant (p<0.001, OR=2.763, 95% CI 1.230-4.231), indicating that the S3660L mutation was an independent risk factor for gastric cancer (Table 3).

Discussion

In the present study we identified a novel mutation (S3660L) in MLL3 gene and found that the minor allele (A) had a higher frequency in gastric cancer patients than in control subjects in Chinese Han population in China.

The traditional risk factors such as smoking, alcohol consumption, nitrates, smoked fish and salted meats, moldy foods containing aflatoxin, and the infection with *H. pylori* have been reported to influence the development of gastric cancer. Gastric cancer is thought to be a multifactorial disease resulting from the interaction between gene mutation and traditional risk factors. Therefore, much attention has been focused on the association of gene variants with gastric cancer.

The foundation for human studies examining putative causative genes that may be involved in gastric cancer is based on a candidate gene approach. MLL3 is a member of the TRX/MLL gene family and maps to chromosome 7q36.1. It encodes a predicted protein of 4911 amino acids containing two plant homeodomains (PHD), an ATPase alpha/beta signature, a high mobility group, a SET (Suppressor of variegation, Enhancer of Zeste, Trithorax) and two FY (phenylalanine tyrosine) rich domains. PHD and SET domains proteins are chromatin regulators and several of them are altered in cancer [7]. Recently, a few studies over the genetic polymorphisms of MLL3 revealed a positive association with colorectal cancer and pancreatic carcinoma [14,15]. Therefore, the MLL3 gene is thought to be a candidate gene for gastric cancer.

We compared the frequency of S3660L genotypes between case and control groups. The frequency of the AG genotype was significantly higher in gastric cancer patients than in control subjects. This indicated that the risk of gastric cancer was significantly increased in subjects with the A allele. Logistic regression analyses suggested that, after adjustment for other risk factors, the frequency of the AG genotype remained significantly different between gastric cancer patients and control subjects.

In conclusion, the present results indicate that gastric cancer susceptibility is significantly associated with the S3660L mutation.

Table 3. Results from multivariate logistic regression analysis

Variables	*p-value*	*OR*	*95% CI*
Alcohol consumption	0.057	1.753	0.937-3.123
Smoking	0.035	2.323	1.743-3.712
Cancer family history	0.004	2.566	1.621-4.564
S3660L	0.003	2.763	1.230-4.231

OR: odds ratio, CI: confidence interval

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