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Original Article

MMP-2 and *MMP-9* gene polymorphisms associated with cervical cancer risk

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Abstract: Aim: To inquire into whether there is any relationship between *MMP-2* rs2285053 and *MMP-9* rs3918242 polymorphisms and the onset risk of cervical cancer (CC) and invasion and metastasis. Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was utilized to examine the frequency and distribution of *MMP-2* rs2285053 and *MMP-9* rs3918242 polymorphisms in 150 CC patients and 120 healthy individuals who were matched therewith in age and gender, and the CC risk and invasion and metastasis condition of individuals carrying different genotypes of the two single nucleotide polymorphisms (SNPs) were compared. The data were processed by SPSS 18.0 software. Results: The differences of genotype distribution frequencies of the two SNPs between the two groups were not apparent ($P > 0.05$), and no significant relevance was found between the two SNPs and the lymphatic metastasis of CC. The CC risk for those carrying CC/TT (rs2285053) and CT/TT (rs3918242), CC (rs2285053) and CC (rs3918242), and CC (rs2285053) and CT/TT (rs3918242) respectively was 0.135, 1.138, and 1.182 times as much as that for those carrying CT/TT (rs2285053) and CC (rs3918242) (95% CI=0.028-0.660; 95% CI=0.615-2.103; 95% CI=0.558-2.502), reflecting that the two SNPs had interactions there between. Conclusions: *MMP-2* rs2285053 and *MMP-9* rs3918242 polymorphisms may have correlations with CC susceptibility.

Keywords: *MMP-2*, *MMP-9*, cervical cancer, polymorphism

Introduction

Characterized by high incidence, easy metastasis and recurring, cervical cancer (CC) is a very common gynecologic malignant tumor; and the morbidity thereof ranks the second among female malignant tumors, and is at the first place among women genital tract malignancies in China [1, 2]. The occurrence and development of CC is a rather complicated biological process, and etiology and pathogenesis thereof still remain unclear. Until now, many factors like virus infection, sexual activities, marital status, childbirth, economic and sanitary conditions, unbalanced diet have been observed to be relevant to the risk of CC [3-8]. In addition, the infection of human papillomavirus (HPV) has been verified to be the main cause of CC occurrence by a large number of studies [9, 10]. Although many females are exposed to the same carcinogenic factors, only a few of them finally develop the disease, which shows that genetic factors are involved in the occurrence risk of CC and genetic susceptibility is related to the pathogenesis of CC.

MMP-2 and *MMP-9* both belong to collagenase IV, and play important roles in tumor cell proliferation, angiogenesis and invasion and metastasis process. *MMP-2* rs2285053, *MMP-2* rs243865 and *MMP-9* rs3918242 polymorphisms can affect the expression level of proteins by changing the transcriptional activity of genes, and this is associated with the onset of tumors [11, 12]. It has been shown by reports that the three single nucleotide polymorphisms (SNPs) are correlated with the risk of a variety of malignancies such as lung cancer, breast cancer, gastric cancer, esophagus cancer, and colorectal cancer, but the relationship of the three SNPs with CC risk has not been reported yet [13-17]. This study applied polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to detect and analyze *MMP-2* rs2285053 and *MMP-9* rs3918242 polymorphisms of 150 CC cases

Table 1. Primer sequences of *MMP-2* rs2285053 and *MMP-9* rs3918242 polymorphisms

SNP	Primer (5'→3')	Length	Annealing temperature
MMP-2 rs2285053	F: ATAGGGTAAACCTCCCCACATT	300 bp	56°C
	R: GGTAAAATGAGGCTGAGACCTG		
MMP-9 rs3918242	F: GCCTGGCACATAGTAGGCCC	435 bp	60°C
	R: TTCCTAGCCAGCCGGCATC		

and 120 controls so as to find out whether any linkage existed between the two SNPs and the CC occurrence risk.

Materials and methods

Research subjects

The cases were 150 CC patients aged 22-75 and the controls were 120 healthy objects aged 20-79. The cases with a mean age of 49 were selected from Linyi People's Hospital from September, 2010 to December, 2014 and were pathologically diagnosed with CC. The controls with an average age of 45.5 were randomly recruited from healthy individuals who got regular check-ups in the corresponding hospital. All the subjects agreed to provide 5 ml fresh peripheral blood. All the blood samples were preserved at -20°C for 6 h. The Linyi People's Hospital medical ethics committee had given approval to our study.

Methods

Extraction of DNA

Phenol-chloroform method was adopted to extract the genomic DNA, which was then dissolved in TE buffer. After quantitative analysis of the mixture by ultraviolet spectrophotometer, the mixture was stored at -20°C for further using.

Selection of SNPs

The rs2285053 and rs3918242 polymorphisms in the promoter region of *MMP-2* and *MMP-9* respectively were selected for detection and analysis after logging in the dbSNPs Database (http: //www.ncbi.nlm.nih.gov/sites/entrez).

Genotyping

We carried out the genotyping of *MMP-2* rs2285053 and *MMP-9* rs3918242 polymorphisms with PCR-RFLP technique. Primer designing was performed utilizing Primer 5.0 software, and primer synthesizing was accomplished by Shanghai Sangon Biotech CO., Ltd (**Table 1**). The amplification target fragments were respectively of 300 bp and 435 bp. The amplification process was completed with MJPTC-200 type PCR amplification instrument. The 20 μl PCR reaction system contained genomic DNA (50 ng), forward primer (12.5 pmol/L), reverse primer (12.5 pmol/L), dNTP (0.1 mmol/L), 1×PCR buffer solution (50 mmol/L KCl, 10 mmol/L Tris-HCl and 0.1% Triton X-100), $MgCl_2$ (1.8 mmol/L), and Taq enzyme (1.0 U). The following were steps included in the PCR reaction process: 5 min initial denaturation at 94°C; 34 cycles of 40 s denaturation at 94°C, annealing of the exon and the promoter at 61°C, and 30 s extension at 72°C; and 7 min final extension. The PCR productions were observed with 2% agarose gel electrophoresis (AGE). The PCR products of the two SNPs, 5 U of restriction endonuclease Hinf I and Sph I, 1.0 μl buffer solution, and double distilled water were mixed together to reach a total volume of 10 μl, and the 10 μl mixture was put at 37°C overnight for digestion of the PCR productions. The digestion results were detected using 3.0% agarose gel. Three fragments of 300 bp, 254 bp, and 46 bp respectively were obtained from the amplification products of rs2285053, and lengths of the three fragments obtained from the PCR products of rs3918242 polymorphism were respectively 435 bp, 247 bp, and 188 bp. 10% of the samples were randomly selected and we carried out the digestion process again with double blood method so as to verify the accuracy of the former digestion process, and the results obtained this time were completely the same as the former results.

Statistical methods

SPSS 18.0 software was adopted to process the data. Hardy-Weinberg equilibrium (HWE) examinations of the frequency distributions of the genotypes of the two SNPs in both the cases and the controls were conducted. χ^2 test was employed to complete comparison of genotype and allele frequencies between two groups. We selected the nonconditioned Logistic regression method to calculate the odds ratios (ORs) with their 95% confidence intervals (95% CIs) that were used to represent

Table 2. Frequencies of genotypes and alleles of the two polymorphisms

		Case n=150	Control n=120	*P*	OR (95% CI)
MMP-2 rs2285053	CC	116 (77.3)	82 (68.3)	-	1.00
	CT	34 (22.7)	38 (31.7)	0.099	0.632 (0.368-1.088)
	TT	0	0	-	-
	C	266 (88.7)	202 (84.2)	-	1.00
	T	34 (11.3)	38 (15.8)	0.129	0.679 (0.413-1.117)
MMP-9 rs3918242	CC	116 (77.3)	86 (71.7)	0.324	0.741 (0.427-1.286)
	CT	30 (20.0)	34 (28.3)	-	1.00*
	TT	4 (2.7)	0	-	-
	C	262 (86.0)	203 (85.8)	0.611	0.866 (0.526-1.424)
	T	38 (14.0)	34 (14.2)	-	1.00

This was the calculation result for CT and TT genotypes of rs3918242 together.

Table 3. Relationship between the two SNPs and lymphatic metastasis

SNP	*MMP-2* rs2285053		*MMP-9* rs3918242	
Genotype	CC	CT/TT	CC	CT/TT
Lymphatic metastasis	23	4	24	3
Non-lymphatic metastasis	93	30	92	31
P	0.324		0.134	
OR (95% CI)	0.539 (0.173-1.684)		0.371 (0.104-1.317)	

the relative risk of CC. The effect modification model was applied to analyze interactions between *MMP-2* rs2285053 and *MMP-9* rs3918242. The level of significant differences was $P < 0.05$.

Results

General features of the research subjects

The CC patients had an age range of 22-75 and an average age of 49; and the age range and average age of the healthy controls were 20-79 and 45.5. The age differences of the two groups were found with no significance in statistics ($P > 0.05$).

HWE test

The genotypes of *MMP-2* rs2285053 and *MMP-9* rs3918242 in the case subjects and the control subjects were in conformity with HWE ($P > 0.05$), suggesting the significant representativeness of all subjects.

Analysis of genotype distributions of MMP-2 rs2285053 and MMP-9 rs3918242

The frequencies of CC, CT, C allele and T allele of *MMP-2* rs2285053 in case and control groups were respectively, 77.3% vs. 22.7%, 68.3% vs. 31.7%, 88.7% vs. 11.3%, and 84.2% vs. 15.8% (**Table 2**), but no obvious differences in the genotype frequencies between cases and controls were found ($P > 0.05$). These results indicated that *MMP-2* rs2285053 polymorphism has no significant association with CC susceptibility. The analysis results of the relevance between *MMP-2* rs2285053 and lymphatic metastasis conditions showed that no distinct correlation existed there between (OR=0.539, 95% CI=0.173-1.684) (**Table 3**).

As described in **Table 3**, the frequencies of the three genotypes (CC, CT, and TT) and alleles (C and T) of *MMP-9* rs3918242 were respectively 77.3%, 20.0%, 2.7% and 86.0% and 14.0% in cases and 71.7%, 28.3%, 0%, and 85.8%, 14.2% in controls, and discrepancies in distributions of the genotypes and alleles between the CC patients and the healthy participators were not obvious ($P > 0.05$). In addition, the CC risk of those who carry CT and TT genotypes was 0.741 times as much as that of CC genotype carriers. All these showed that the *MMP-9* rs3918242 polymorphism had no apparent linkage with the occurrence of CC. Furthermore, the association analysis of *MMP-9* rs3918242 and lymphatic metastasis status showed that rs3918242 was not significantly correlated with lymphatic metastasis (OR=0.371, 95% CI=0.104-1.317).

Influences of interactions between the two SNPs on CC risk

We put the low frequency genotype TT and CT of *MMP-9* rs3918242 together for analysis

Table 4. Interactions of the two SNPs on CC risk

Genotype		Case	Control	OR (95% CI)
MMP-2 rs2285053	*MMP-9* rs3918242			
CT/TT	CC	32 (17.5)	26 (21.7)	1.00
CT/TT	CT/TT	2 (5.0)	12 (10.0)	0.135 (0.028-0.660)
CC	CC	84 (57.5)	60 (50.0)	1.138 (0.615-2.103)
CC	CT/TT	32 (20.0)	22 (18.3)	1.182 (0.558-2.502)

(**Table 4**). The TT/CT of *MMP-2* rs2285053 together with CC of *MMP-9* rs3918242 was set into the low activity group. The results revealed that the carriers of *MMP-2* rs2285053 TT/CT and *MMP-9* rs3918242 TT/CT, rs2285053 CC and rs3918242 CC, and rs2285053 CC and rs3918242 TT/CT respectively had 0.135, 1.138, and 1.182 times as much the CC risk as the carriers of *MMP-2* rs2285053 TT/CT and *MMP-9* rs3918242 CC. Therefore, interactions existing between the two SNPs may have an influence on CC occurrence, and the carriers of *MMP-2* rs2285053 TT/CT and *MMP-9* rs3918242 TT/CT may have lower risk of CC.

Discussion

Located on chromosome 16q21, the *MMP-2* gene consists of 13 exons and 12 introns, and structural genes thereof have a total length of 27 kb [18]. It is currently believed that adjustments of the expression and function of *MMP-2* occurs in various different levels such as transcription, secretion, the activation of prozymogen, combinations on the cell surface, as well as interactions with MMPs inhibitors from tumor cells or host cells. *MMP-2* protein has the function of degrading collagen IV of the basilar membrane and many kinds of important bioactive molecules [19]. Early studies on the relationship of *MMP-2* with tumors mostly remain on the protein level. Investigations performed by Nomura and Mori et al. have proved that there exists a linkage between the expression level of *MMP-2* protein and gastric cancer risk [20, 21]. Zhou et al. have found that the rs2285053 polymorphism in the promotor region of *MMP-2* gene can block the combination with Sp1 transcription factors and decrease the activity of the promotor [22]. Additionally, those who have the C-C haplotype made up by *MMP-2* rs243865 and rs2285053 polymorphisms have higher risk of lung cancer than those having the T-T haplotype. Rollin et al. have discovered that there are no significant differences in the genotype frequency of *MMP-2* rs243865 and rs2285053 polymorphisms between non-small cellular lung cancer (NSCLC) patients and healthy controls, patients who carry the T allele of *MMP-2* rs2285053 can live longer than those patients who carry the CC genotype of rs2285053 after the onset of NSCLC, and those CC genotype NSCLC patients have 2.6 times higher the relative risk for mortality [14].

MMP-9 can degrade not only collagenase IV but also some cell growth factors like IL-lβ, IGFBP-3, P substance, MCP-3, and VEGF. Animal experiments demonstrate that *MMP-9* has an association with the occurrence of tumors [23]. Also, *MMP-9* has also been found to be related to the infiltrating and metastasis of many tumors in some case-control studies. An SNP in *MMP-9* that has been frequently studied is the rs3918242 polymorphism. Matsumura et al. find that no significant differences in genotype distributions of *MMP-9* rs3918242 exist between the gastric cancer patients and the controls, but there is an obvious relevance between the SNP and the infiltration, clinical stages, and lymphatic metastasis of gastric cancer ($P < 0.05$), and thus predict that the T allele of the SNP may have a correlation with the infiltration of gastric cancer [13].

According to our study, frequencies of genotypes and alleles of *MMP-2* rs2285053 and *MMP-9* rs3918242 in the case group were similar to those in the control group ($P > 0.05$), showing that there existed no obvious linkage between the two SNPs and CC susceptibility. After we divided the 150 patients into two groups according to lymphatic metastasis conditions, we found that the two SNPs also had no significant correlation with lymphatic metastasis ($P > 0.05$). However, our study was only a preliminary investigation on the correlation of *MMP-2* rs2285053 and *MMP-9* rs3918242 with CC risk and invasion metastasis because of its restricted sample sizes, and people should enlarge the sample sizes in further studies about this topic.

Although no apparent relationship between the two SNPs and CC was found, we discovered that interactions existed between the two SNPs

after we combined the genotypes of the two SNPs for analysis. Possible reasons may be that synergy effects exist between the two SNPs due to the fact that the both *MMP-2* and *MMP-9* are collagenase IV, or the fact that the substrates of *MMP-2* and *MMP-9* are partially the same and can both induce the releasing of VEGF.

In summary, our study demonstrated that no significant linkage existed between *MMP-2* rs2285053 and *MMP-9* rs3918242 polymorphisms and CC risk, but there were interactions between the two SNPs. Future studies should further explore correlations between the two SNPs and pathological grading, clinical stages, survival period, and drug reactions of CC on the basis of enlarged sample sizes.

Disclosure of conflict of interest

None.

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