ANTICANCER RESEARCH *37*: 3563-3567 (2017)
doi:10.21873/anticanres.11726

The Contribution of *MMP-8* Promoter Polymorphisms in Lung Cancer

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Abstract. *Background/Aim: Accumulated evidence has supported the hypothesis that the functional polymorphisms of matrix metalloproteinases (MMP) were associated with the risk of various types of cancer. However, few reports have studied the contribution of MMP-8 genotypes to either diagnostic or prognostic potential in lung cancer. In this study, we focused on the contribution of a polymorphism in the promoter region of MMP-8 (C-799T) and two non-synonymous polymorphisms (Val436Ala and Lys460Thr) to lung cancer risk. Patients and Methods: Genomic DNA was isolated from peripheral blood of 358 patients with lung cancer and 716 non-cancer healthy individuals. MMP-8 C-799T (rs11225395), Val436Ala (rs34009635) and Lys460Thr (rs35866072) polymorphic genotypes of each subject were determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: The results showed that the three polymorphisms were not significantly associated with increased risk of lung cancer in the overall investigated population. Furthermore, when the analyses were stratified according to age, sex, status of smoking and drinking, pack-years of smoking and family history of lung cancer, there was also no significant association between these genotypes and increased lung cancer risk. Conclusion: The polymorphisms MMP-8 C-799T, Val436Ala and Lys460Thr may not play a major role in mediating personal susceptibility to lung cancer in Taiwan.*

Worldwide, lung cancer has been the leading cause of cancer mortality for many years (1, 2). Even though personalized antitumor therapies, mainly based on *epidermal growth factor receptor* (*EGFR*) genotype, are being developed, the prognosis of patients with lung cancer remains unsatisfying, with a 5-year survival rate of less than 20% (1). Thus, other useful genomic markers may strengthen the current predictive systems for revealing the personalized lung etiology for each patient about their genetic and environmental factors and personalized therapeutic strategies.

Matrix metalloproteinases (MMPs), a family of zinc and calcium-dependent proteolytic enzymes are in charge of regulating the aggravation of extracellular matrix and basement membranes (3-5). MMPs play an important role in many physiological and pathological events during carcinogenesis, such as cell proliferation, differentiation, apoptosis, invasion, migration, metastasis, angiogenesis and immune surveillance (6). Previous literature indicated that functional polymorphisms of *MMPs* play a role in determining inter-individual differences of susceptibility to several types of cancer (7-14).

In literature, the C-799T genotypes of *MMP-8* were reported to be associated with breast cancer (15) and the electrophoretic mobility shift assays revealed differences in nuclear protein binding to oligonucleotides representing the C-799T genotypes (16). The promoter constructs containing the CT and TT genotypes at the C-799T had a 3-fold greater activity in chorion-like trophoblast cells compared to the constructs containing the C alleles (16). However, the role of *MMP-8* genotypes was seldom examined in lung cancer (17).

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Key Words: Genotype, lung cancer, *MMP-8*, nonsynonymous, polymorphism, promoter.

Table I. *Distribution of selected demographics of the 358 patients with lung cancer and the 716 matched controls.*

Characteristics	Controls (n=716)			Patients (n=358)			*p*-Value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			64.8 (6.8)			64.0 (6.9)	0.5871
Gender							0.3642
Male	488	68.1%		254	70.9%		
Female	228	31.9%		104	29.1%		0.3642
Smoking status							
Ever smokers	563	78.6%		293	81.8%		
Non-smokers	153	21.4%		65	18.2%		0.2282

[a]Based on Chi-square test; SCC, squamous cell carcinoma; SD, standard deviation.

Table II. *Summary of primer sequences, specific restriction enzymes, PCR and digestion product sizes.*

Polymorphism (rs number)	Primer sequences	Restriction enzyme	SNP sequence	DNA fragment size (bp)
C-799T	F: 5'-CCATCTTCACATAGCCTTGG-3'	*Sfc* I	T	285 bp
(rs11225395)	R: 5'-CCTTGTCTTCTGCCTGTGAA-3'		C	172+113 bp
Lys460Thr	F: 5'-GGATTACAGGCATTAGCCAC-3'	*Nla* III	A	332 bp
(rs35866072)	R: 5'-CGAAAATGCATGCTGAACTTCC-3'		C	245+87 bp
Val436Ala	F: 5'-GGATTACAGGCATTAGCCAC-3'	*Bbs* I	C	264 bp
(rs34009635)	R: 5'-GCCATATCTACAGTTAAGCCAT-3'		T	162+102 bp

*F and R indicate forward and reverse primers, respectively.

The current study aimed at investigating the contribution of *MMP-8* C-799T, Val436Ala and Lys460Thr polymorphisms to the susceptibility of lung cancer in Taiwan.

Materials and Methods

Patients and controls. Three hundred and fifty-eight patients were histologically confirmed with lung cancer and recruited by the surgery team at the Outpatient Clinics of General Surgery at the China Medical University Hospital as previously described (18-20). The exclusion criteria of the patient group were any patient with history of any other malignancy and pulmonary diseases, such as chronic obstructive pulmonary disease (COPD), pneumothorax and asthma. All participants voluntarily completed a self-administered questionnaire and provided a 3- to 5-ml sample of peripheral blood. At the same time, seven hundred and sixteen non-lung cancer healthy volunteers as controls were selected by matching for age, gender and smoking behavior after initial random sampling from the databank of Health Examination Cohort of China Medical University Hospital with more than 10,000 subjects. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin and any genetic or familial diseases. The study was approved by the Institutional Review Board of the China Medical University Hospital with the document coded DMR100-IRB-284 and written informed consent was obtained from all participants. Selected recorded characteristics of the subjects in case and control groups are summarized and compared in Table I.

Methodology of MMP-8 genotyping. Genomic DNA from peripheral blood of each participant was extracted, aliquoted and stored as previously described (21-23). Briefly, the primers for *MMP-8* C-799T, Val436Ala and Lys460Thr polymorphisms were custom designed by our team (shown in Table II) and the genotyping polymerase chain reaction (PCR) cycling conditions *via* My Cycler (Biorad, Hercules, CA, USA) for *MMP-8* were: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 57˚C for 30 s and 72˚C for 30 s and a final extension at 72˚C for 10 min.

Statistical analysis. Goodness-of-fit test was used in order to ensure that the controls recruited were representative of the general population and to exclude the possibility of genotyping error. Pearson's Chi-square test without Yates' correction or Fisher's exact test was used to compare the distribution of the *MMP-8* genotypes between case and control groups. The associations between the *MMP-8* polymorphisms and lung cancer risk were estimated by computing odds ratios (ORs) as well as their 95% confidence intervals (CIs) from unconditional logistic regression analysis with the adjustment for possible confounding factors.

Results

Basic characteristics compared between the lung cancer patient and control groups. The frequency distributions of the characteristics for lung cancer among the case subjects and control are summarized and compared in Table I. No

Table III. *Distributions of matrix metalloproteinase-8 (MMP-8) genotypic frequencies among the lung cancer cases and the control group.*

	Cases (%)	Controls (%)	Adjusted OR (95% CI)[a]	*p*-Value[b]
C-799T				
CC (wildtype)	188 (52.5)	351 (49.0)	1.00 (reference)	
CT	130 (36.3)	273 (38.1)	0.87 (0.68-1.23)	0.3999
TT	40 (11.2)	92 (12.9)	0.79 (0.51-1.24)	0.3198
CT+TT	170 (47.5)	365 (51.0)	0.83 (0.59-1.17)	0.2807
p_{trend}				0.5130
Lys460Thr				
AA (wildtype)	355 (99.2)	711 (99.4)	1.00 (reference)	
AC	3 (0.8)	4 (0.6)	1.53 (0.38-6.81)	0.5931
CC	0 (0.0)	0 (0.0)	--	
Val436Ala				
TT (wildtype)	358 (100.0)	716 (100.0)	1.00 (reference)	
CT	0 (0.0)	0 (0.0)	--	
CC	0 (0.0)	0 (0.0)	--	
p_{trend}				

OR, Odds ratio; CI, confidence interval. [a]Data has been adjusted with confounding factors include age, gender and smoking status. [b]Based on Chi-square test without Yates' correction or Fisher's exact test; *p<0.05.

Table IV. *Allelic frequencies for matrix metalloproteinase-8 (MMP-8) polymorphisms in the lung cancer cases and control groups.*

Polymorphic site Allele	Cases (%) N=716	Controls (%) N=1432	Adjusted OR (95% CI)[a]	*p*-Value[b]
C-799T				
Allele C	506 (70.7)	975 (68.1)	1.00 (reference)	0.2225
Allele T	210 (29.3)	457 (31.9)	0.84 (0.65-1.38)	
Lys460Thr				
Allele A	713 (99.6)	1428 (99.7)	1.00 (reference)	0.5924
Allele C	3 (0.4)	4 (0.3)	1.54 (0.49-7.45)	
Val436Ala				
Allele T	716 (100.0)	1432 (100.0)	1.00 (reference)	
Allele C	0 (0.0)	0 (0.0)	--	

OR, Odds ratio; CI, confidence interval. [a]Data has been adjusted with confounding factors including age, gender and smoking status. [b]Based on Chi-square test without Yates' correction or Fisher's exact test; *p<0.05.

difference was found between the two groups as for age, gender and smoking status (all p>0.05). Additionally, there was no difference between the two groups as for alcohol drinking status (p>0.05) (Table I).

Association analysis of MMP-8 genotypes at C-799T, Val436Ala and Lys460Thr with lung cancer risk. The distributions of genetic frequencies for the *MMP-8* polymorphisms in the lung cancer patients and controls are presented and compared in Table III. First, there were no CT or CC genotypes at *MMP-8* Val436Ala among either the cases or the controls. That is to say, all the subjects were of TT genotype at *MMP-8* Val436Ala (Table III, lowest panel). Second, the ORs with adjusting those possible confounding factors (age, gender and smoking status) for the people carrying variant CT and TT genotypes at *MMP-8* promoter C-799T were 0.87 (95% CI=0.68-1.23, p=0.3999) and 0.79 (95% CI=0.51-1.24, p=0.3198) respectively, compared to those carrying the CC wild-type genotype (Table III, upper panel). The p-value for trend was not significant (p=0.5130) (Table III). In the dominant model (CT plus TT *vs*. CC), the association between *MMP-8* promoter C-799T polymorphism and the risk for lung cancer was still not statistically significant (adjusted OR=0.83, 95% CI=0.59-1.17, p=0.2807) (Table III, upper panel). Third, a very small percentage of Taiwanese people were of heterozygous variant AC genotype at *MMP-8* Lys460Thr (0.8% and 0.6% in lung cancer patient and control groups, respectively) and there was no association between *MMP-8* Lys460Thr AC genotypes and the risk for lung cancer (adjusted OR=1.53, 95% CI=0.38-6.81, p=0.5931) (Table III, medium panel).

Association of MMP-8 allelic types at C-799T, Val436Ala and Lys460Thr and lung cancer risk. The adjusted OR for the subjects carrying the T allele at *MMP-8* promoter C-799T was 0.84 (95% CI=0.65-1.38, p=0.2225), compared to those carrying the C wild-type allele (Table IV). Supporting the findings in Table III, there is no differential distribution of allelic frequencies between lung cancer patient and control groups as for the *MMP-8* promoter C-799T or Lys460Thr (Table IV). As for the allelic frequencies at *MMP-8* Val436Ala and Lys460Thr polymorphic sites, there was no association between their genotypes and increased lung cancer risk (Table IV).

Discussion

In the literature, the genotypes of SNPs at promoter region of many *MMP* genes were found to be associated with the risk of several types of cancer (7-14). However, only Gonzalez-Arriaga's team investigated one polymorphism in *MMP-8* as risk factor for lung cancer (17). In that work, the samples were composed of 501 lung cancer cases and 501 healthy controls, all of whom were Caucasian. The authors examined only the C+17G genotypes of *MMP-8* gene and found that the G variant allele was associated with a decreased risk of developing lung cancer, while there was no contribution of the particular polymorphism to the overall survival rates for the lung cancer patients (17). In the present study, we examined the genotypes of specific *MMP-8* polymorphisms among a Taiwanese population and assessed

whether there was an association between the genotypes of the promoter region of *MMP-8* (C-799T) and two nonsynonymous polymorphisms (Val436Ala and Lys460Thr) with lung cancer risk. The results showed that no significant association was observed and our findings suggest that these three *MMP-8* polymorphisms may not play a critical role in mediating susceptibility to lung cancer (Tables III and IV).

We focused on the genotypes of promoter region of *MMP-8* (C-799T) based on certain observations; some studies in mice models showed that mutant mice deficient in MMP-8 were more susceptible to develop skin cancer (24, 25). This evidence from knockout mice models strongly suggested that MMP-8 may play a protective role against carcinogenesis (24, 25). Furthermore, there exist certain positive epidemiological findings, which reported that genotypes of promoter regions of other MMPs such as MMP-1 and MMP-9, may serve as promising markers for the prediction of lung cancer susceptibility and prognosis (26-28). From the viewpoint of protein expression and function, elevated MMP-8 might increase cell adhesion by rearrangement of cytoskeleton actin, thus decreasing cell invasion (29). However, there is lack of epidemiological studies investigating the role of polymorphisms in the promoter region of *MMP-8* to the susceptibility of cancers.

In the current study, the results summarized that the genotypes of *MMP-8* C-799T (Tables III and IV) in addition to two nonsynonymous polymorphisms (Val436Ala and Lys460Thr) (Tables III and IV) were not associated with lung cancer risk in the Taiwanese population. Furthermore, when the analyses were stratified by age, sex, status of smoking and drinking, pack-years of smoking, and family history of lung cancer, no significant association between these genotypes and lung cancer risk was observed (data not shown). In 2016, we also revealed that the genotypes of tissue inhibitors of metalloproteinase 1 (*TIMP-1*), which is reported to be in charge of regulation the expression level of extracellular MMP-1, was also associated with lung cancer susceptibility (21). The biological function of MMPs and the TIMPs apparently is more complex than being proved only to involve in controlling the proliferation and metastasis of cancer cells (30, 31), so further studies would be required to elucidate the molecular mechanisms implicated in these complex processes. The phenotypic data including the expression levels of MMP-8 at mRNA or protein are not currently available for further analysis. The complete correlation of patient status, genotype and phenotype would be very helpful to reveal the role of MMP-8 in lung carcinogenesis.

In conclusion, this is the first study to investigate the role of *MMP-8* promoter together with nonsynonymous polymorphic genotypes in lung carcinogenesis. Our results suggest that the genotypes of promoter region C-799T and non-synonymous Val436Ala and Lys460Thr at *MMP-8*, do not significantly confer susceptibility to lung cancer in a Taiwanese population. Further studies elucidating the contribution of the genotypes of other members of *MMP* family to lung cancer risk are needed.

Acknowledgements

The Authors declare no conflicts of interest. The Authors are grateful to Hsin-Ting Li and Shiou-Ting Yen for their excellent technical assistance. All the participants in this study are appreciated. This study was supported mainly by China Medical University to Dr. Hsia (DMR-CELL-17001), by Asia University to Dr. Bau, Dr. Hsia, and Dr. Chao (CMU105-ASIA-17) and partially by research grant from Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW106-TDU-B-212-113004). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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Received April 24, 2017
Revised May 13, 2017
Accepted May 26, 2017