The Contribution of Matrix Metalloproteinase-1 Promoter Genotypes in Taiwan Lung Cancer Risk

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Abstract. *Background/Aim: Up-regulation of metalloproteinase (MMP) proteins has been shown in various types of solid cancers and the genotype of MMP1 has been associated with the risk of solid cancers. The contribution of MMP1 genotype to lung cancer has been investigated in various countries, though, to our knowledge, not in Taiwan. Therefore, in this study, we focused on the contribution of a polymorphism in the promoter region of MMP1 to lung cancer risk in Taiwan population. Patients and Methods: Genomic DNA was isolated from peripheral blood of 358 patients with lung cancer and 716 healthy individuals (non-cancer patients). MMP1 rs1799750 polymorphic genotypes of each sample were determined using the typical methodology of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: The percentages of 2G/2G, 1G/2G, and 1G/1G for MMP1 -1607 genotypes were 34.4%, 41.3% and 24.3% in the disease group and 33.9%, 44.0%, and 22.1% in the control group (p trend=0.6298), respectively. The results of carrier comparisons in dominant and recessive models also support the findings that 1G or 2G appears not to be a determinant allelic biomarker for Taiwan lung cancer. Conclusion: The MMP1 -1607 1G allele is a non-significant protective biomarker for lung cancer in Taiwan.*

For many years, lung cancer has been the most common and leading cause of cancer mortality all over the world (1, 2). Although there is a rapid development of personalized therapies and medicine, the prognosis of patients with lung cancer remains poor, with a 5-year survival rate of less than 20% (3). Thus, novel predictive and prognostic markers might strengthen the current genomic predictive systems, in order to reveal the personalized etiology of lung cancer.

Matrix metalloproteinases (MMPs), also known as matrixins, are a family of calcium-dependent enzymes in charge of regulating the homeostasis of extracellular matrix contents (4-6). It has been reported that MMPs are involved in many tumorigenesis events, such as cell proliferation, differentiation, apoptosis, invasion, migration, metastasis, angiogenesis and immune surveillance (7). Previous literature indicated that some *MMP* polymorphisms, especially those implicated in gene expression regulation, play a key role in determining inter-individual differences of susceptibility to several types of cancer (8-16), while some others do not (17-19).

In the literature, the commonly investigated rs1799750 genotypes at the promoter region of *MMP1* were reported to be associated with lung cancer in populations of America (20, 21) and China (22). In particular, in patients with high grade (larger or equal to 2) lung cancer, the AG/GG genotypes were associated with higher risk of lung injury due to radiotherapy (23). In addition, it was reported that lung cancer patients carrying the *MMP1* polymorphism rs1144393 (AG/GG) exhibited higher MMP1 protein levels in lung and plasma tissues, compared to patients with the

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wild-type (AA) genotype (23). However, the contribution of *MMP1* genotypes to lung cancer still need to be validated in other populations. Thus, the current study aimed to investigate the contribution of *MMP1* genotypes to the susceptibility of lung cancer in Taiwan.

Materials and Methods

Collection of 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 (19, 24). 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, 760 non-lung cancer healthy volunteers as controls were selected as controls, matched 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 individuals. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other known or unknown origin, and any genetic or familial diseases. The study was approved by the Institutional Review Board of the China Medical University Hospital (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 MMP1 genotyping. Genomic DNA from peripheral blood of each participant was extracted aliquoted and stored long-term at –80˚C as previously described (19). The sequences of primers and the restriction enzymes for *MMP1* promoter -1607 genotyping were the same as in our recently published *MMP1* rs1799750 genotyping methodology (9, 25, 26). Briefly, the forward and reverse primers for MMP1 promoter -1607 genotyping were 5'-TGACTTTTAAAACATAGTCTATGT-3' and 5'-GATTGATTTGAG ATAAGTCATAGC-3', respectively. The genotyping polymerase chain reaction (PCR) cycling conditions *via* My Cycler (Biorad, Hercules, CA, USA) for *MMP1* were: 1 cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 58˚C for 30 s and 72˚C for 30 s; final extension step at 72˚C for 10 min. After amplification, PCR products were subject to digestion with *Alu I* restriction endonuclease (New England BioLabs, Ipswich, MA, USA) for 2 h at 37˚C and separation by 3% agarose gel electrophoresis. The genotypes were identified as homozygous 2G/2G (269 bp), heterozygous 1G/2G (269, 241 and 28 bp) and homozygous 1G/1G (241 and 28 bp). The genotyping process was performed by two independent and blinded researchers, with results being 100% concordant.

Statistical analysis. Pearson's Chi-square test without Yates' correction or Fisher's exact test was used to compare the distribution of the *MMP1* -1607 genotypes between case and control groups. The associations between the *MMP1* -1607 genotypes and lung cancer risk were estimated by computing odds ratios (ORs) as well as their 95% confidence intervals (CIs) using unconditional logistic regression analysis after the adjustment for possible confounding factors.

Results

Basic characteristics and their distributions among the lung cancer patients and healthy controls. The frequency distributions of the demographic and disease characteristics among lung cancer patients and control group were compared and summarized in Table I. The results showed no obvious difference between the two groups in regard to age, gender, and smoking status (all *p*>0.05). Among lung cancer cases, 60.9%, 29.6% and 9.5% were of adenocarcinoma, squamous cell carcinoma, and other types, respectively, according to histological viewpoint (Table I).

Association analysis of MMP1 -1607 genotypes with lung cancer risk. The distributions of genetic frequencies for the *MMP1* -1607 genotypes among lung cancer patients and controls were compared and presented in Table II. Compared to the wild-type genotype (2G/2G) as the reference group, there was no obvious increased risk for the 1G/2G or 1G/1G groups, even after adjustment for the confounding factors including age, gender, and smoking status (adjusted OR=0.91 and 0.93, 95% CI=0.67-1.21 and 0.71-1.38, *p*=0.6169 and 0.6273; respectively). Comparison of carriers of the recessive (2G/2G+1G/2G *versus* 1G/1G) and dominant (2G/2G *versus* 1G/1G+1G/2G) models showed a non-significant level of the variant 1G allele to behave as a determinant of lung cancer risk (Table II). The frequencies of the *MMP1* promoter -1607 alleles between lung cancer patients and control group are presented in Table III. Supporting the findings shown in Table II, the variant 1G allele at *MMP1* -1607 was not significantly associated with lung cancer risk (adjusted OR=1.04, 95% CI=0.89-1.23, *p*=0.6897) (Table III).

Discussion

In the literature, overexpression of MMP1 has been reported in many types of tumor tissues and has been demonstrated to be associated with the invasion and metastasis of tumor cells (27-29), and poor prognosis of the disease (29, 30). From a viewpoint, overexpression of MMP1 was partially regulated by the upstream promoter variations, such as the 1G/2G polymorphic site located at -1607, which creates a core recognition sequence for transcription factor binding (31). Promoters containing the *2G* allele display significantly higher transcriptional activity than promoters with *1G*. Kanamori and his colleagues reported that there was a higher frequency of 2G alleles in ovarian cancer patients than in healthy controls, which suggests an association between *MMP1* polymorphism and cancer risk (32). The *MMP1* -1607 polymorphism may propose a possibility for more aggressive matrix degradation, thereby facilitating carcinogenesis (33).

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
Histology							
Adenocarcinoma				218	60.9%		
SCC				106	29.6%		
Other				34	9.5%		

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

Table II. *Distributions of matrix metalloproteinase-1 (MMP1) -1607 genotypic frequencies among lung cancer cases and control group.*

	Cases (%)	Controls (%)	Adjusted OR (95% CI)[a]	*p*-Value[b]
MMP1 -1607				
2G/2G (wild-type)	123 (34.4)	243 (33.9)	1.00 (reference)	
1G/2G	148 (41.3)	315 (44.0)	0.91 (0.67-1.21)	0.6169
1G/1G	87 (24.3)	158 (22.1)	0.93 (0.71-1.38)	0.6273
p for trend				0.6298
Carrier comparison				
2G/2G+1G/2G	271 (75.7)	558 (77.9)	1.00 (reference)	
1G/1G	87 (24.3)	158 (22.1)	1.11 (0.78-1.51)	0.4107
2G/2G	123 (34.4)	243 (33.9)	1.00 (reference)	
1G/1G+1G/2G	235 (65.6)	473 (66.1)	0.95 (0.78-1.77)	0.8914

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 test.

Table III. *Allele frequencies of matrix metalloproteinase-1 (MMP1) -1607 1G/2G, in lung cancer cases and in the control group.*

Allele	Cases (%) n=716	Controls (%) n=1432	Adjusted OR (95% CI)[a]	*p*-Value[b]
MMP1 -1607				
Allele 2G	394 (55.0)	801 (55.9)	1.00 (reference)	0.6897
Allele 1G	322 (45.0)	631 (44.1)	1.04 (0.89-1.23)	

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 test.

Lung cancer, the most common cancer worldwide, is characterized by a high death rate, low 5-year survival rate, and high incidence of metastasis (1, 2). In 2001, a large epidemiologic study showed that homozygous 2G/2G genotype at *MMP1* -1607 was associated with increased lung cancer risk in the USA (20). In 2005, Su and his colleagues showed that, among Caucasian lung cancer patients and healthy controls, the 2G allele was associated with increased lung cancer risk in never-smokers and in males (21). In 2011, Liu and his colleagues first showed

that people with the *MMP1* -1607 2G/2G genotype had a 1.71-fold increased risk of lung cancer and invasiveness than those with 1G/1G, among Han Chinese population (22). In 2016, another polymorphic site at *MMP1* promoter region, A-519G, was reported to be associated with controlling of MMP1 expression in lung tissues and plasma, and correlated to the occurrence of higher grade (≥2) of radiation-induced lung injury (23). In this study, they have also investigated the contribution of *MMP1* -1607 polymorphisms to lung cancer risk, but no association was found in a representative sample consisting of 251 lung cancer patients and 193 healthy controls, in China (23). In the current study, results showed that no significant association was observed and our findings suggest that the *MMP1* promoter -1607 polymorphism may not play a critical role in lung cancer susceptibility for Taiwanese (Tables II and III). The findings are consistent with those of Liu's, which has also investigated the contribution of *MMP1* genotypes to lung cancer in Chinese population (23). The inconsistency between Caucasian and Asian populations needs to be further validated by more investigations among variant populations.

Given that age, gender, and smoking may bias the effect of the polymorphism on lung cancer susceptibility, it is logical to speculate that *MMP1* -1607 polymorphism might have interaction with the aforementioned variables. Thus, we have examined the interaction between *MMP1* -1607 genotype and environmental factors by stratification analysis. The results showed that when the population was stratified by age, gender, status of smoking and drinking, pack-years of smoking, and family history of lung cancer, no significant association between *MMP1* polymorphisms and lung cancer risk was observed (data not shown). In 2016, we also revealed that the genotypes of *tissue inhibitor of metalloproteinase 1* (*TIMP-1*) gene, whose coding protein is reported to be responsible for the regulation of extracellular MMP1 activity (34), was associated with lung cancer susceptibility (24). As for another genomic study of *MMP8*, there was no significant association between the genotypes of MMP8 C-799T (rs11225395), Val436Ala (rs34009635), and Lys460Thr (rs35866072) with lung cancer risk in Taiwan (19). Apparently, the role of MMPs and TIMPs in controlling carcinogenesis (35) is more complex, than simply control the proliferation and metastasis of cancer cells (36, 37). Therefore, further studies are required to clarify the molecular mechanisms implicated in these complex processes.

In conclusion, this is the first study to indicate that the genotypes of *MMP1* promoter -1607, do not significantly confer susceptibility to lung cancer in a Taiwanese population. However, further studies elucidating the contribution of the genotypes of other *MMP* family members to lung cancer risk are urgently encouraged in Taiwan.

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