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Association of *TIMP-2* Rs8179090 Genotypes With Lung Cancer Risk in Taiwan

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Abstract. *Background/Aim: The tissue inhibitor of metalloproteinase-2 (TIMP-2) is a critical inhibitor of matrix metalloproteinases (MMPs). Along with MMPs, TIMP-2 regulates the breakdown and remodeling of the extracellular matrix (ECM) and basement membranes. This study investigated the role of genotypes of the TIMP-2 -418G/C (rs8179090) single nucleotide polymorphism on lung risk. Materials and Methods: A total of 358 lung cancer patients and 716 healthy subjects were recruited in this study. Genotypes were identified via the polymerase chain reaction-restriction fragment length polymorphism methodology. Results: The distribution of alleles and genotype frequencies of TIMP-2 -418G/C genotypes between the two groups were compared and no statistically significant difference (p>0.05) was found. The heterozygous and homozygous variant genotypes showed no differential distribution between the control and case groups (p>0.05). Conclusion: TIMP-2 -418G/C variants might not be associated with lung cancer susceptibility and could not serve as predictors.*

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Lung cancer is the leading cause of cancer-related mortality worldwide for both genders (1). There are approximately 2.1 million newly diagnosed patients with lung cancer and approximately 1.8 million annual deaths. (2). Among the different histopathologic types, non-small cell lung cancer (NSCLC) is the main type of lung cancer, accounting for approximately 80% of all cases, including adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma (3, 4). Despite the rapid developments in therapeutic drugs and hospital care, there are no obvious clinical manifestations in the early stage of NSCLC, and frequently metastases are identified in most NSCLC patients at the time of diagnosis. As a result, currently, NSCLC cases have a relatively poor prognosis, and the 5-year survival rates are only approximately 20% (5). According to information listed above, it is of critical importance to identify practical biomarkers for effective early diagnosis of NSCLC.

There is evidence that genetics play a role in lung cancer development (6), and statistical data showed that heritability contributes to lung cancer genetic risk by approximately 8% (7). Smoking has been reported to induce gene mutations, which may contribute to an extremely heavy mutation load and lung cancer development (8, 9). Therefore, smoking together with the unrevealed genetic factors can all contribute to lung cancer risk.

Tissue inhibitor of metalloproteinase-2 (TIMP-2), a 21-KD endogenous protein, is a major inhibitor of matrix metalloproteinase-2 (MMP-2) (10), a critical enzyme in the regulation of the proliferative and metastatic behaviors of tumor cells (11). In literature, MMP-2 is frequently reported

to be over-expressed in tumor tissues (12, 13), and its regulation and signaling may determine a poor prognosis (14-16). TIMP-2 forms a complex with MMP-2 more effectively than TIMP-1 and regulates its proteolytic activity. In 2000, Ara *et al*. demonstrated that *TIMP-2* mutations can influence its binding with MMP-2, leading to carcinogenesis (17). Epidemiological studies on *TIMP-2* are limited and inconclusive. In 2014, Yaykaşli *et al*. reported that *TIMP-2* genotypes are associated with prostate cancer risk (18). In 2015, Zhang *et al*. showed that *TIMP-2* genotypes can contribute to gastric cancer development (19). In 2020, *TIMP-2* genotypes were found to significantly associate with an increased risk of colorectal cancer (20). On the contrary, Wang *et al*. have provided evidence showing that *TIMP-2* gene polymorphisms are not associated with an increased risk of breast cancer (21). However, surprisingly, there is no literature on the effect of *TIMP-2* genotypes on lung cancer. This point may be explained that TIMP-2 is so essential and its substrates are so complicated that the expression level could not be dramatically altered (22). Only slight regulation, such as polymorphism(s) could be happened, while the key point(s) is not found yet. In this study, we examined the genotypes of *TIMP-2* rs8179090 polymorphism and evaluated their contribution to the risk of lung cancer in Taiwan.

Materials and Methods

Patient population. This study was approved by the ethics committee of China Medical University Hospital (DMR100-IRB-284). Written informed consent was obtained from all study subjects. We enrolled 358 lung cancer patients and 712 healthy non-cancer controls. Only Chinese patients diagnosed via pathological diagnosis were included. Cases with any other type of cancer, possible tumors, severe infectious disease, and immune disease were excluded. The control group was recruited from the physical examination center of the hospital during the same period. Demographics of the cohort and controls have also been used in our previous study (23) and are summarized in Table I.

Genotyping methodology of the TIMP-2 rs8179090 polymorphism. Peripheral blood was collected from each participant and genomic DNA was extracted within 24 h by using the Genome DNA Extraction Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions (23-25). The single nucleotide polymorphism (SNP) *TIMP-2* rs8179090 was genotyped by using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) methodology as previously described (26, 27). The primer sequences of *TIMP-2* rs8179090 were designed by Terry Fox Cancer Research Lab and the forward and reverse primer pair was: TTCTAAGGCCTCCATTTGAA and GTTCTTCCAGGACACCAGGC, respectively. The PCR reaction was performed in a total volume of 25 μl and the PCR conditions were as follows: pre-denaturation at 94˚C for 2 min; followed by 35 cycles denaturation at 94˚C for 20 s, annealing at 57˚C for 20 s, extension at 72˚C for 20 s; and finally, extension at 72˚C for 20 min. The PCR products were examined by 3% agarose gel electrophoresis. The PCR products were digested by *Mnl* I; the digestible G allele produced two fragments of 117 and 119 bps, whereas the undigested T allele was of 236 bp. Random samples of the PCR products were chosen for direct sequencing to verify the accuracy and reliability of the genotyping results.

Statistical methodology. The Student's *t*-test was adopted for the comparisons of the distributions of ages between the case and control groups. The Pearson's Chi-square methodology was used to examine the distribution pattern of *TIMP-2* genotypes and the interaction between *TIMP-2* genotypes and smoking status. The contribution of *TIMP-2* genotypes to lung cancer risk was also estimated by the odds ratios (ORs) and the 95% confidence intervals (CIs). *p*-Values less than 0.05 were considered to indicate statistically significant differences.

Results

The distributions of age, gender, and smoking status in the selected 358 lung cancer cases and 716 healthy controls are presented in Table I. Additionally, the histology of the lung cancer cases is also shown in Table I. During selection of healthy controls, we applied matching strategies for age, gender, and smoking status, and the results showed that there was no difference in the distributions of age, gender, and smoking behavior between the cases and controls (*p*-Values all >0.05) (Table I). We have to emphasize that since we matched the frequency of smoking behaviors in addition to age and gender, the percentage of smokers in the control became as high as 78.6% and this percentage is not representative of the Taiwanese population. Also, this matching strategy could not show whether smoking is a lung cancer risk factor. Regarding the histopathologic subtypes, 60.9% (218 cases) were adenocarcinoma, 29.6% (106 cases) were squamous cell carcinoma, and 9.5% (34 cases) were other types.

The genotypic distributions of *TIMP-2* rs8179090 among the 716 controls and the 358 patients with lung cancer are presented and analyzed in Table II and Table III, respectively. The *p*-Value for Hardy-Weinberg equilibrium analysis was 0.9917 for the control group. The results showed that the genotypes of *TIMP-2* rs8179090 were not distributed differently between the lung cancer and healthy control groups (*p* for trend=0.5167) (Table II). In detail, the *TIMP-2* rs8179090 heterozygous CG and homozygous CC genotypes were negatively associated with an altered lung cancer risk, compared with the wild-type homozygous GG genotype (OR=1.02 and 1.60, 95%CI=0.75-1.37 and 0.71-3.56, *p*=0.9204 and 0.2505, respectively; Table II). In the recessive model, a 1.60-fold non-significant increase in lung cancer risk was observed for the CC genotype carriers at *TIMP-2* rs8179090 compared with those carrying the GG+CG genotypes (OR=1.60, 95%CI=0.71-3.54, *p*=0.2523). In the dominant model, there was a non-significant 1.06-fold increase in lung cancer risk for the CG+CC genotype carriers at *TIMP-2* rs8179090, compared with GG carriers (OR=1.06, 95%CI=0.80-1.41, *p*=0.6952).

Table I. *Summary of demographics of the 358 patients with lung cancer and the 716 matched controls (derived from reference 23).*

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							
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 without Yates' correction; SCC: Squamous cell carcinoma; SD: standard deviation.

Table II. *TIMP-2 rs8179090 genotypes among the 358 patients with lung cancer and 716 healthy controls.*

Genotype	Controls		Patients		OR (95%CI)	*p*-Value[a]
	n	%	n	%		
rs8179090						
GG	530	74.0%	261	72.9%	1.00 (Reference)	
CG	172	24.0%	86	24.0%	1.02 (0.75-1.37)	0.9204
CC	14	2.0%	11	3.1%	1.60 (0.71-3.56)	0.2505
p_{trend}						0.5167
p_{HWE}						0.9917
Carrier comparison						
GG +CG	702	98.0%	347	96.9%	1.00 (Reference)	
CC	14	2.0%	11	3.1%	1.60 (0.71-3.54)	0.2523
GG	530	74.0%	261	72.9%	1.00 (Reference)	
CG+CC	186	26.0%	97	27.1%	1.06 (0.80-1.41)	0.6952

[a]Based on chi-square test without Yates's correction; OR: odds ratio; CI: confidence interval; p_{trend}: *p*-value for trend analysis; pHWE: *p*-Value for Hardy-Weinberg equilibrium analysis.

Table III. *Distribution of allelic frequencies for TIMP-2 rs8179090 among the 358 patients with lung cancer and 716 healthy controls.*

Allele	Controls, n	%	Patients, n	%	OR (95%CI)	*p*-Value[a]
rs8179090						
G	1232	86.0%	608	84.9%	1.00 (Reference)	
C	200	14.0%	108	15.1%	1.09 (0.85-1.41)	0.4861

[a]Based on chi-square test without Yates's correction; OR: odds ratio; CI: confidence interval.

To validate the results in Table II, an allelic frequency distribution analysis for the *TIMP-2* rs8179090 was performed and the results are shown in Table III. The variant C allele was 15.1% in the lung cancer group and 14.0% in the control group (OR=1.09, 95%CI=0.85-1.41). There was no significant difference in the allelic frequencies of *TIMP-2* rs8179090 between the two groups (p=0.4861, Table III).

Table IV. *TIMP-2 rs8179090 genotype in lung cancer risk after stratification by smoking status.*

Genotype	Non-smokers, n		OR (95%CI)[a]	aOR (95%CI)[b]	*p*-Value	Smokers, n		OR (95%CI)[a]	aOR (95%CI)[b]	*p*-Value
	Controls	Cases				Controls	Cases			
GG	116	45	1.00 (ref)	1.00 (ref)		414	216	1.00 (ref)	1.00 (ref)	
CG	33	17	1.33 (0.67-2.62)	1.23 (0.74-2.37)	0.4120	139	69	0.95 (0.68-1.33)	0.99 (0.74-1.28)	0.7690
CC	4	3	1.93 (0.42-8.98)	1.58 (0.66-6.74)	0.3927	10	8	1.53 (0.60-3.94)	1.68 (0.65-3.15)	0.3716
Total	153	65				563	293			
p_{trend}					0.5339					0.6255

[a]By multivariate logistic regression analysis; [b]by multivariate logistic regression analysis after adjusted of age and gender; p_{trend}: *p*-Value for trend analysis; CI: confidence interval; aOR: adjusted odds ratio.

As mentioned above, smoking behavior is a well-known risk factor of lung cancer worldwide. Therefore, we were interested in examining the interactions between *TIMP-2* rs8179090 genotypes and smoking habit. The data showed that among the non-smokers, those with *TIMP-2* rs8179090 CG and CC genotypes were at 1.33- and 1.93-fold odds of having lung cancer (95%CI=0.67-2.62 and 0.42-8.98, *p*=0.4120 and 0.3927, respectively). Among smokers, those with *TIMP-2* rs8179090 CG and CC genotypes were at 0.95- and 1.53-fold odds of having lung cancer (95%CI=0.68-1.33 and 0.60-3.94, *p*=0.7690 and 0.3716). However, the differences did not reach statistical significance. After adjusting for age, gender, and alcohol drinking status, there were still no statistically significant differences between the two variant genotypes at *TIMP-2* rs8179090, among non-smokers and smokers (Table IV).

Discussion

The regulation of the components of the extracellular matrix is very complex. For instance, there are tens of types of MMPs that metabolize various substrates, such as collagenases, gelatinases, matrilysins, stromelysins, and others (28-31). MMPs are also thought to play a critical role in cellular activities such as proliferation, differentiation, angiogenesis, apoptosis and metastatic behaviors (32, 33). TIMP-2 acts as an inhibitor of several MMPs. Typically, TIMPs inhibit MMPs, but different TIMPs inhibit different MMPs more effectively than others. For instance, TIMP-1 inhibits MMP-1, MMP-3, MMP-7, MMP-9, and TIMP-1 inhibits MMP-3 better than TIMP-2, while TIMP-2 inhibits MMP-2 more effectively than TIMP-1, TIMP-3 and TIMP-4 (34). Since TIMP-2 plays a major role in the metabolism of MMPs, subtle genetic variants of *TIMP-2* may cause an imbalance between extracellular matrix contents, promoting carcinogenesis. Therefore, variations in *TIMP-2* genotypes, such as those of *TIMP-2* rs8179090 polymorphism, may be associated with lung cancer risk.

To our surprise, there were no published articles on the role of *TIMP-2* genotypes in lung cancer risk. Therefore, we first examined the contribution of *TIMP-2* rs8179090 genotypes to lung cancer risk. The results showed that the genotypes GG, GC, and CC at *TIMP-2* rs8179090 among healthy Taiwanese were 74%, 24%, and 2%, respectively (Table II). In the lung cancer group, *TIMP-2* rs8179090 CC seemed to have a higher frequency (3.1%) but was not statistically significant (Table II). Allelic frequency analysis indicated that the variant C allele may not contribute to lung cancer risk (Table III). Cigarette smoking is the major environmental contributor to lung cancer risk (35, 36), however, its interaction with specific genotypes is seldomly examined, not to mention *TIMP-2* rs8179090. The present study is the first to reveal the interaction between *TIMP-2* rs8179090 genotypes and smoking in lung cancer risk.

This study opened new directions of research. First, it would be interesting to examine whether *TIMP-2* rs8179090 genotypes are correlated with metastatic status, which should be affected by the composition of the extracellular matrix. We examined the possibility for *TIMP-2* rs8179090 genotypes to predict tumor size, stage, and metastasis, but no significant association was found (data not shown). One explanation for the lack of significance is that the sample size of CC genotypes of *TIMP-2* rs8179090 was extremely small. Second, it is also important to examine the differential expression of *TIMP-2* mRNA and protein among the controls and cases according to various *TIMP-2* rs8179090 genotypes. Third, the size of the sample should be increased to further validate our current findings, such as those indicating a possible association of *TIMP-2* rs8179090 genotypes with smoking. Forth, it is also critical to investigate the effect of other polymorphic sites on protein function. For instance, rs4789936 and rs2003241 are intronic polymorphic sites of *TIMP-2*, which have never been investigated with regard to their contributions to lung cancer either. We cannot exclude the possibility that SNP(s) other than rs8179090, can serve as predictive marker(s) for lung cancer. Last, the role of *TIMP-*

2 rs8179090 genotypes in cancer risk determination is inconclusive, and additional studies are required to validate the current findings. For instance, in colorectal cancer (20), the C allele in *TIMP-2* rs8179090 is associated with decreased cancer risk, while in gastric cancer, the C allele is associated with increased cancer risk (19).

In conclusion, this study provides evidence that the C allele at *TIMP-2* rs8179090 cannot serve as a predictor of lung cancer. In addition, no obvious interaction between smoking status and *TIMP-2* rs8179090 genotype regarding personal susceptibility to lung cancer was observed.

Conflicts of Interest

All the Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Research Design: Liao WC, Huang CW and Hsia TC; Patient and Questionnaire: Liao WC, Hsia TC and Shen YC; Experiment Data analysis: Chang WS and Wang YC; Statistical Analysis: Tsai CW and Yin MC; Manuscript Writing: Tsai CW and Bau DT; Reviewing and Revising: Bau DT.

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