Interleukin-10 (*IL-10*) Promoter Genotypes Are Associated with Lung Cancer Risk in Taiwan Males and Smokers

TE-CHUN HSIA[1,2,3*], WEN-SHIN CHANG[2,4*], SHINN-JYE LIANG[1,2*], WEI-CHUN CHEN[1,2], CHIH-YEN TU[1,2], HUNG-JEN CHEN[1,3], MEI-DUE YANG[2], CHIA-WEN TSAI[2], CHIN-MU HSU[2], CHANG-HAI TSAI[2] and DA-TIAN BAU[2,4]

[1]*Department of Internal Medicine and* [2]*Terry Fox Cancer Research Laboratory, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[3]*Department of Respiratory Therapy and* [4]*Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan, R.O.C.*

Abstract. *Interleukin-10 (IL-10) is an immunosuppressive cytokine involved in carcinogenesis via immune escape. The present study aimed at evaluating the contribution of IL-10 promoter A-1082G (rs1800896), T-819C (rs3021097), A-592C (rs1800872) genetic polymorphisms to the risk of lung cancer in Taiwan. Associations of three IL-10 polymorphic genotypes with lung cancer risk were investigated among 358 lung cancer patients and 716 age- and gender-matched healthy controls. In addition, the genetic-lifestyle interaction was also examined. The results showed that the percentages of TT, TC and CC for IL-10 T-819C genotypes were differentially represented as 59.2%, 35.8% and 5.0% in the lung-cancer patient group and 52.0%, 37.0% and 11.0% in the non-cancer control group, respectively (p for trend=0.0025). The CC genotype carriers were of lower risk for lung cancer (OR=0.4, 95% CI=0.23-0.69, p=0.0005). Further stratification of the population by gender and smoking behavior showed that the IL-10 T-819C genotype conducted a protective effect on lung cancer susceptibility, which was obvious among males and smokers (p=0.0003 and 0.0004, respectively). The CC and TC genotypes of IL-10 T-819C compared to the TT genotype may have a protective effect on lung cancer risk in Taiwan, particularly among males and smokers.*

*These Authors contributed equally to this study.

Correspondence to: Da-Tian Bau, Terry Fox Cancer Research Laboratory, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422052121 (ext. 7534), e-mail: datian@mail.cmuh.org.tw; artbau2@gmail.com

Key Words: Polymorphism, genotype, interleukin-10, lung cancer, smoking, Taiwan.

Lung cancer is one of the most fatal cancers and non-small cell lung cancer (NSCLC) is the most common type of it. The most well-established environmental factor for lung cancer of tobacco use. In literature, it is reported that various carcinogens contained in cigarette smoke may produce reactive oxygen species that can induce DNA adducts and strand breaks in the genome. However, there were also some studies that showed that only 10-15% of all smokers actually develop lung cancer during their life, suggesting that individual susceptibility to carcinogens in cigarette smoke can vary among different populations (1, 2). In the past years, molecular epidemiological studies showed that specific genotypes were associated with higher risk among cigarette smokers than non-smokers (3-8) or *vice versa* (9-13). The revealing of gene-environment interactions on lung cancer risk, especially among smokers and non-smokers, are one of the hot issues in lung cancer study.

Interleukin-10 (IL-10) is produced mainly by macrophages and T-lymphocytes, which plays a central role in both anti-inflammation and immunosuppression. Animal and *in vitro* studies have shown that higher levels of IL-10 expression were associated with smaller tumors and reduced metastasis (14). Genetic polymorphisms found in the regulatory sites, especially the promoter region, were believed to affect the expression of gene-encoded proteins and associate with cancer susceptibility and prognosis responses. In addition, polymorphisms in inflammation genes have also been shown to influence pain, depression and fatigue after clinical surgery (15-17). Furthermore, tumor immune surveillance studies have reported an association between IL-10 and tumorigenesis processes of several human cancers including lymphoma (18, 19), myeloma (20), thyroid (21), colon (22, 23), prostate (24, 25), breast (26), gastric (27) and lung cancer (28-30). As for lung cancer, several studies have indicated that loss or dysfunction of IL-10 in lung tumor sites may promote tumor progression and result in poor clinical outcomes in the patients; however, opposite effects

Table I. *Distribution of selected demographic data of the 358 lung cancer patients and 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 the Chi-square test. SD, Standard deviation.

Table II. *The primer sequences, polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) conditions for Interleukin-10 A-1082G, T-819C and A-592C genotyping work.*

Polymorphisms (locations)	Primer sequences	Restriction enzyme	SNP sequence	DNA fragment size (bp)
A-1082G (rs1800896)	F: 5'-CTCGCTGCAACCCAACTGGC-3'	*Mnl* I	A	139 bp
	R: 5'-TCTTACCTATCCCTACTTCC-3'		G	106 + 33 bp
T-819C (rs3021097)	F: 5'-TCATTCTATGTGCTGGAGAT-3'	*Mae* III	T	209 bp
	R: 5'-TGGGGGAAGTGGGTAAGAGT-3'		C	125 + 84 bp
A-592C (rs1800872)	F: 5'-GGTGAGCACTACCTGACTAG-3'	*Rsa* I	C	412 bp
	R: 5'-CCTAGGTCACAGTGACGTGG-3'		A	236 + 176 bp

*F and R indicate forward and reverse primers, respectively. SNP, Single nucleotide polymorphism.

have also been reported in other studies (31-37). Interestingly, the absence of IL-10 expression has been associated with poor outcome in early stage of NSCLC (32, 33). On the contrary, in late-stage NSCLC, the presence of IL-10-positive macrophages at the tumor margins serves as an indicator of poor prognostic outcome (31). In addition, high serum IL-10 levels were associated with shorter survival times among advanced lung cancer patients (34).

There has been no previous study to investigate the combined effects of cigarette smoking and *IL-10* genotypes on lung cancer risk. Therefore, in the present study, we aimed at revealing the genotypic frequencies of genotypes of promoter polymorphism of *IL-10* and focusing on the association of *IL-10* genotypes with lung cancer susceptibility among Taiwan never- and ever-smokers.

Materials and Methods

Investigated population and sample collection. Three hundred and fifty-eight patients diagnosed with lung cancer were recruited at the Outpatient Clinics of General Surgery at the China Medical University Hospital during 2005-2008. The clinical characteristics of patients, including histological details, were all graded and defined by expert surgeons. All participants voluntarily completed a self-administered questionnaire and provided 5 ml of their peripheral blood samples. Twice as many non-lung cancer healthy volunteers were selected as controls by matching for age, gender and smoking behavior after initial random sampling from the Health Examination Cohort of our Hospital. The exclusion criteria of the controls included previous malignancy, metastasized cancer from other or unknown origin and any genetic or familial diseases. Our 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 (Table I).

PCR-restriction fragment length polymorphism genotyping conditions. Genomic DNA of each participant was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed, as previously describerd (5, 6, 38). The polymerase chain reaction (PCR) cycling conditions were: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s; and a final extension at 72˚C for 10 min. The sequences of primers for PCR and the specific restriction enzymes for each DNA product are listed in Table II. The genotype analysis was performed by two researchers independently and blindly. Five percent of the samples were randomly selected for direct sequencing and the results were 100% concordant.

Statistical analyses. All 716 of the controls and 358 cases with genotypic and clinical data were analyzed. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the

Table III. *Distribution of IL-10 genotypes among the 358 lung cancer patients and the 716 matched controls.*

Genotype	Controls		Patients		OR (95% CI)	*p*-Value[a]
	n	%	n	%		
A-1082G						
AA	561	78.4%	273	76.3%	1.00 (reference)	
AG	130	18.1%	69	19.3%	1.09 (0.79-1.51)	0.6155
GG	25	3.5%	16	4.4%	1.32 (0.69-2.50)	0.3998
P_{trend}						0.6398
T-819C						
TT	372	52.0%	212	59.2%	1.00 (reference)	
TC	265	37.0%	128	35.8%	0.85 (0.65-1.11)	0.2446
CC	79	11.0%	18	5.0%	0.40 (0.23-0.69)	0.0005*
P_{trend}						0.0025*
A-592C						
AA	368	51.4%	173	48.3%	1.00 (reference)	
AC	277	38.7%	145	40.5%	1.11 (0.85-1.46)	0.4480
CC	71	9.9%	40	11.2%	1.20 (0.78-1.84)	0.4371
P_{trend}						0.6028

[a]Based on the Chi-square test; *$p<0.05$. OR, odds ratio; CI, conficence interval.

genotype frequencies of *IL-10* single-nucleotide polymorphisms in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. The Student's t test was applied for continuous data analysis. The Pearson's Chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the *IL-10* genotypes between cases and controls. The associations between the *IL-10* polymorphisms and lung cancer risk were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from logistic regression analysis with the adjustment for possible confounders. $p<0.05$ was considered statistically significant and all statistical tests were two-sided.

Results

The frequency distributions of age, gender and smoking status for the 358 lung cancer patients and 716 non-cancer controls are presented in Table I. We have applied frequency matching to recruit the non-cancer healthy controls, thus the distributions of age and gender were comparable between the control and case groups (Table I). As for the smoking lifestyle, we have to notice that, among the very high percent of smokers (81.8%), we have chosen similar numbers ($p>0.05$) of smokers and controls (78.6%) for stratification and comparison (Table I).

The distributions of the *IL-10* genotypes at A-1082G (rs1800896), T-819C (rs3021097), A-592C (rs1800872) among the controls and the lung cancer patients are presented and analyzed in Table III. There was no association between the genotype of either A-1082G or A-592C and lung cancer risk. However, the genotypes of *IL-10* T-819C were differently distributed between lung cancer and healthy control groups (p for trend=0.0025) (Table III). In detail, the *IL-10* T-819C heterozygous TC and homozygous CC genotypes seemed to be associated with decreased lung cancer risk (OR=0.85, 95%CI=0.65-1.11, p=0.2446; OR=0.40, 95%CI=0.23-0.69, p=0.0005, respectively), with the later being statistically significant (Table III).

In the National Health Insurance Research Database with 33,919 lung cancer patients collected during 2002 to 2008, nearly two thirds of the patients were men (39). During these years, there is an increasing trend of gender ratio for the female lung cancer patients in Taiwan. Therefore, we were interested in whether the genotype of *IL-10* T-819C contributed to the gender difference of lung cancer susceptibility. Thus, the stratification by gender showed that the genotypes of *IL-10* T-819C were differently distributed among the males (p=0.0003) but not females (p=0.9868) (Table IV).

The interaction of the genotype of *IL-10* T-819C and cigarette smoking lifestyle of the participants was of our interest since lung cancer is one of the smoking-related cancers. The results in Table V showed that the genotypic distribution of the variant genotypes of *IL-10* T-819C was significantly different between lung cancer and control groups who were ever smokers (p=0.0004) but not different in the case among the non-smokers (p=0.8698) (Table V). Overall, it seemed that there was a synergistic impact of *IL-10* T-819C genotype and smoking lifestyle on lung cancer risk.

Table IV. *Distribution of IL-10 T-819C genotypes among patients with lung cancer after stratification by gender.*

Variable	T-819C genotype			
	TT (%)	TC (%)	CC (%)	*p*-Value[a]
Males				
Controls	258 (52.9%)	171 (35.0%)	59 (12.1%)	
Cases	159 (62.6%)	86 (33.9%)	9 (3.5%)	0.0003*
Females				
Controls	114 (50.0%)	94 (41.2%)	20 (8.8%)	
Cases	53 (51.0%)	42 (40.4%)	9 (8.6%)	0.9868

[a]Based on the Chi-square test. *$p<0.05$.

Table V. *Distribution of IL-10 T-819C genotypes among patients with lung cancer after stratification by personal smoking habits.*

Variable	T-819C genotype			
	TT (%)	TC (%)	CC (%)	*p*-Value[a]
Smokers				
Controls	297 (52.8%)	201 (35.7%)	65 (11.5%)	
Cases	179 (61.1%)	103 (35.1%)	11 (3.8%)	0.0004*
Non-smokers				
Controls	75 (49.0%)	64 (41.8%)	14 (9.2%)	
Cases	33 (50.8%)	25 (38.5%)	7 (10.7%)	0.8698

[a]Based on the Chi-square test. *$p<0.05$.

Discussion

In the present investigation, the contribution of three single nucleotide polymorphisms at the promoter region of *IL-10* (A-1082G, T-819C and A-592C) to lung cancer risk was evaluated in a central Taiwan population. No obvious differential distribution in the genotypes of A-1082G or A-592C was found. However, the CC genotype of *IL-10* T-819C was significantly associated with a decreased risk of lung cancer (Table III). This genotype (CC) was also found to be associated with higher risk of type-2 diabetes mellitus and higher level of *IL-10* production (40). In addition, the haplotypes of IL-10 A-1082G, T-819C and A-592C were determined among lung cancer patients and IL-10 mRNA levels were found to be significantly higher in tumors with the non-ATA haplotype than with the ATA haplotype (41). All the evidence above showed that the C allele at *IL-10* T-819C is closely related to a higher level of *IL-10* mRNA and IL-10 protein.

The present study has also examined the interaction of *IL-10* genotypes with gender and smoking lifestyle on lung cancer risk in Taiwan. We found that the association between IL-10 T-819C genotype with lung cancer risk was obvious among males but not among the females (Table IV). As for the smoking lifestyle, it is shown that the association between *IL-10* T-819C genotype with lung cancer risk was obvious, especially among ever-smokers. However, there was no such differential genotypic distribution for the non-smokers (Table V). In 2007, Cesar-Neto and his colleagues found that smoking behavior decreased the levels of IL-10 and other cytokines such as IL-1α, IL-8, tumor necrosis factors (TNF)α, matrix metalloproteinase (MMP)-8 and osteoprotegerin in sites with periodontitis (42).

The association between alteration in IL-10 expression and carcinogenesis has long been explained by dysregulation in immune suppression and tumor immune surveillance (44-47). In the present study, we demonstrated that the TT genotype synergistically increased the risk of lung cancer among smokers but not the non-smokers (Table V). The possible mechanism is that the TT-inherited *IL-10* genotype and smoking lifestyle were both related to a decrease of IL-10. However, the direct evidence of altered IL-10 and consequence events in lung cancer carcinogenesis could not be assessed in cell culture models since the cell-cell interactions of immune suppression and tumor immune surveillance should be taken into serious consideration.

In conclusion, our findings suggest that C allele at the *IL-10* T-819C promoter polymorphic site is associated with lower lung cancer risk, especially among males and smokers.

Conflicts of Interest

The Authors declare no interest conflict with any person or company.

Acknowledgements

We appreciate Hong-Xue Ji, Chieh-Lun Hsiao, Chia-En Miao, Lin-Lin Hou, Liang-Yi Lin and Tissue-bank of China Medical University Hospital for their excellent technical assistance. This study was supported by research grants from Terry Fox Cancer Research Foundation and Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW103-TDU-B-212-113002).

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Received September 9, 2014
Revised September 23, 2014
Accepted September 26, 2014