Contribution of *Interleukin-4* Genotypes to Lung Cancer Risk in Taiwan

WEN-SHIN CHANG[1,2*], SHOU-CHENG WANG[3*], CHIN-LIANG CHUANG[3*], HONG-XUE JI[1,2], CHIEH-LUN HSIAO[1,2], CHIN-MU HSU[1], CHIA-WEN TSAI[1], SHIH-PING LIU[4], PEI-CHEN HSU[5], YEN-LI LO[6] and DA-TIAN BAU[1,2,7]

[1]*Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[2]*Clinical Medical Science, China Medical University, Taichung, Taiwan, R.O.C.;*
[3]*Taichung Armed Forces General Hospital, Taichung, Taiwan, R.O.C.;*
[4]*Basic Medical Science, China Medical University, Taichung, Taiwan, R.O.C.;*
[5]*Departments of Pediatrics, Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan, Taiwan, R.O.C.;*
[6]*Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, R.O.C.;*
[7]*Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan, R.O.C.*

Abstract. *Aim: Lung cancer is the leading cause of cancer-related death worldwide. Interleukin-4 (IL-4) is a typical pleiotropic T helper 2 cytokine involved in immunology during carcinogenesis. The present study aimed at evaluating the contribution of IL-4 promoter T-1099G (rs2243248), C-589T (rs2243250), C-33T (rs2070874) genetic polymorphisms to the risk of lung cancer in Taiwan. Materials and Methods: The contributions of the promoter IL-4 polymorphic genotypes to lung cancer risk were investigated in 358 lung cancer patients and 716 age- and gender-matched healthy controls. In addition, the interaction between IL-4 and individual smoking status was also evaluated. Results: The percentages of CC, CT and TT for IL-4 C-589T genotypes were differentially represented as 69.0%, 26.5% and 4.5% in the lung-cancer patient group and 61.3%, 30.4% and 8.3% in the non-cancer control group, respectively (p=0.0156). The TT genotype carriers were of lower risk for lung cancer (odds ratio (OR)=0.48, 95% confidence interval (CI)=0.27-0.86, p=0.0106) than the CC genotype carriers. We also analyzed the allelic frequency distributions and the results showed that the T allele of IL-4 C-589T conducted a protective effect on lung cancer susceptibility (p=0.0022). On the contrary, there was no difference in the distribution of genotypic or allelic frequencies among patients and controls for the IL-4 promoter T-1099G and C-33T. Conclusion: The TT genotype of IL-4 C-589T compared to the CC wild-type genotype may have a protective effect on lung cancer risk in Taiwan and may serve as an early detection and prediction marker.*

Statistically, lung cancer has been the leading cause of cancer-related mortality for years worldwide, and non-small cell lung cancer (NSCLC) is the most common type, accounting for about 80% of lung cancer cases (1, 2). Although the development of anti-tumor therapy is rapid, the prognosis of lung cancer patients remains poor, with a 5-year survival rate of less than 20% (3). The most well-established environmental factor for lung cancer is the individual consumption of tobacco, which is also useful for prognosis prediction (4, 5). Carcinogens contained in cigarettes have been related to elevated reactive oxygen species, DNA adducts and stand breaks in lung cells. However, there also exist certain studies showing that only 10-15% of all smokers actually develop lung cancer during their lifetime, suggesting that individual susceptibility to carcinogens in cigarette smoke can vary in different individuals (6, 7). In the past decade, studies have shown that specific genotypes contributed to higher risk of lung cancer for cigarette smokers than non-smokers (8-15) or *vice versa* (16-19). Therefore, investigation of gene-environment interactions on lung cancer risk especially for smokers and non-smokers is an important issue to reveal lung cancer etiology.

Interleukin-4 (IL-4) is mainly produced by marcrophages and T lymphocytes, and plays a broad role in both anti-

*These Authors contributed equally to this study.

Correspondence to: Da-Tian Bau, Terry Fox Cancer Research Laboratory, Department of Medical Research, 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: Genotype, *interleukin-4*, lung, polymorphism, Taiwan.

Table I. *Distribution of selected demographic data of the 358 lung cancer patients and their 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.

inflammation and immunosuppression. IL-4 not only controls the activation of B cells but also the maturation of T helper 2 cells (20, 21). IL-4 has been reported to mediate an antitumor effect (22-24). Elevated levels of IL-4 expression were associated with tumors in breast cancer, renal cell cancer, prostate cancer, colon cancer and lung cancer (25). In a knockout mice model, it was found that tumor growth was delayed in IL-$4^{-/-}$ recipient mice challenged with lung cancer cell transplantation (26). All the above evidence indicated that the altered expression levels may play a role in lung carcinogenesis.

To date, the genotypic contribution of *IL-4* to cancer is not well-studied. As for lung cancer, *IL-4* rs2243250 single nucleotide polymorphisms (SNPs) have been found to associate with a reduced risk of non-small cell lung cancer (NSCLC) among Portuguese (27). Similarly, the CC and CT genotypes of *IL-4* rs2243250 were also lower in patients with NSCLC than in healthy controls among people in Beijing (28). Furthermore, the genotypic frequencies of CC and CT genotypes of *IL-4* rs2243250 were found to be associated with lung cancer susceptibility only between female squamous-cell carcinoma (SCC) patients and female controls, but not between cases and controls, adenocarcinoma patients and controls, and between male SCC patients and male controls in China (29). However, previous groups have focused on only one promoter SNP of *IL-4*, which could be strengthened by recruiting other SNPs, especially in the promoter region controlling the expression levels of IL-4. In the current study, we aimed to investigate the contribution of *IL-4* promoter T-1099G (rs2243248), C-589T (rs2243250), C-33T (rs2070874) genetic polymorphisms to the risk of lung cancer in Taiwan.

Materials and Methods

Study population. 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 samples of their peripheral blood. Twice as many non-lung cancer healthy volunteers as controls were selected by matching for age, gender and smoking behavior after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of the controls included previous malignancy, metastasized cancer from other or unknown origin, and any genetic or familial diseases. The genotyping 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.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotyping conditions. Genomic DNA from the peripheral blood leucocytes of each patient and control subject was prepared using the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed as our previous articles (10, 11, 30). 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 forward and reverse primers and the restriction enzymes for each SNP are summarized in Table II. The genotype analysis was performed by two researchers independently and blindly. About five percent of the samples for each SNP were randomly selected for direct sequencing and the results from PCR-RFLP and direct sequencing were 100% concordant.

Statistical analyses. All 716 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 genotype frequencies of *IL-4* SNPs in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's Chi-square test was used to compare the distribution of the *IL-4* genotypes between cases and controls. The associations between the *IL-4* 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.

Table II. *Summary of primer sequences, polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP) for interleukin-4 (IL-4) promoter G-1099T, C-589T and C-33T sites.*

Polymorphisms (locations)	Primer sequences	Restriction enzyme	SNP sequence	DNA fragment size (bp)
T-1099G	F: 5'-GGTCCTTACGTTCACTGCTG-3'	*Sfc* I	G	262 bp
(rs2243248)	R: 5'-GGCTCAAGTGCTCCTCCTAC-3'		T	135 + 127 bp
C-589T	F: 5'-TAAACTTGGGAGAACATGGT-3'	*Ava* II	T	237 bp
(rs2243250)	R: 5'-TGGGGAAAGATAGAGTAATA-3'		C	219 + 18 bp
C-33T	F: 5'-CTGGAAGAGAGGTGCTGATT-3'	*BsmA* I	C	334 bp
(rs2070874)	R: 5'-ACTCACCTTCTGCTCTGTGA-3'		T	169 + 165 bp

*F and R indicate forward and reverse primers, respectively; the forward primer for C-589T was specifically designed at the G underlined for the cutting of Ava II restriction endonuclease.

Table III. *Distribution of interleukin-4 (IL-4) genotypes among the 358 lung cancer patients and the 716 matched controls.*

Genotype	Controls		Patients		OR (95% CI)	*p*-Value[a]
	n	%	n	%		
T-1099G						
TT	595	83.1%	309	86.3%	1.00 (reference)	
TG	108	15.1%	43	12.0%	0.77 (0.52-1.12)	0.1918
GG	13	1.8%	6	1.7%	0.89 (0.33-2.36)	1.0000
P_{trend}						0.3825
C-589T						
CC	439	61.3%	247	69.0%	1.00 (reference)	
CT	218	30.4%	95	26.5%	0.77 (0.58-1.03)	0.0848
TT	59	8.3%	16	4.5%	0.48 (0.27-0.86)	0.0106*
P_{trend}						0.0156*
C-33T						
CC	453	63.3%	238	66.5%	1.00 (reference)	
CT	223	31.1%	101	28.2%	0.86 (0.65-1.14)	0.3181
TT	40	5.6%	19	5.3%	0.90 (0.51-1.60)	0.7767
P_{trend}						0.5777

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

Results

The frequency distributions of basic characters such as age, gender, and smoking status for the 358 lung cancer patients and 716 non-cancer controls are summarized and compared in Table I. Since we have applied frequency matching to recruit the non-cancer healthy controls, there was no difference in the distributions of age and gender between the control and case groups (Table I).

The distributions of the *IL-4* genotypes at promoter T-1099G (rs2243248), C-589T (rs2243250), C-33T (rs2070874) among the non-cancer controls and the lung cancer patients are presented and statistically analyzed in Table III. There was no association between the genotype of either T-1099G or C-33T and lung cancer risk. However, the genotypes of *IL-4* rs2243250 were differently distributed between lung cancer and non-cancer control groups (p=0.0156) (Table III). In detail, the *IL-4* rs2243250 heterozygous CT and homozygous TT genotype seemed to be associated with decreased lung cancer risk (OR=0.77, 95% CI=0.58-1.03, p=0.0848; OR=0.48, 95% CI=0.27-0.86, p=0.0106, respectively) and only the later was statistically significant (Table III).

To confirm the above findings, the analysis of allelic frequency distribution for the three *IL-4* promoter SNPs was also conducted and results are summarized in Table IV. Supporting the findings that homozygous TT genotype of *IL-4* rs2243250 was associated with decreased lung cancer risk, the allele T was 17.7% in the cases, significantly lower than that of 23.5% in controls (p=0.0022). However, there was no significant difference in the allelic frequencies of rs2243248 or rs2070874 between controls and lung cancer cases (Table IV).

Table IV. *Distribution of interleukin-4 (IL-4) allelic frequencies among 358 lung cancer patients and 716 matched controls.*

Allele	Controls	%	Patients	%	*p*-Value[a]
T-1099G					
Allele T	1298	90.6%	661	92.3%	0.2254
Allele G	134	9.4%	55	7.7%	
C-589T					
Allele C	1096	76.5%	589	82.3%	0.0022*
Allele T	336	23.5%	127	17.7%	
C-33T					
Allele C	1129	78.8%	577	80.6%	0.3652
Allele T	303	21.2%	139	19.4%	

[a]*p*-Value based on chi-square test. *Statistically significant.

Discussion

In the current study, the contribution of three SNPs at the promoter region of *IL-4* (T-1099G, C-589T and C-33T) regarding lung cancer risk was evaluated in a Chinese population. No obvious differential distribution in the genotypes of T-1099G or C-33T was found. However, the TT genotype of IL-4 C-589T (rs2243250) was significantly associated with a decreased risk of lung cancer (Table III). It is consistent with previous findings that *IL-4* rs2243250 plays an important role in the determination of lung cancer susceptibility (27-29). In existing literature, the CT or CC genotype at *IL-4* rs2243250 was associated with a decreased expression of IL-4 (31, 32), and allele C was also found to be associated with lower expression of *IL-4* (33, 34). All the above evidence showed that the C allele at *IL-4* rs2243250 may be responsible for the regulation of IL-10 mRNA and protein expression.

Lung cancer is a gender-related cancer. In the National Health Insurance Research Database of Taiwan containing 33,919 lung cancer patients recorded during 2002 to 2008, nearly two thirds of the patients were males (35). During recent years, there is an increasing trend of gender ratio for the female lung cancer patients in Taiwan and the prevalence and mortality rates for female non-small cell adenocarcinoma are very high in Taiwan. Therefore, we were interested to see whether the genotype of *IL-4* rs2243250 contributed to the gender difference of lung cancer susceptibility. After stratification by the gender, it was found that the genotypes of *IL-4* rs2243250 were not differently distributed among males, but TT genotype was lower than CC genotype between the groups of female patients and non-cancer female subjects. From another angle, lung cancer is also a smoking-related cancer. Therefore, the interaction of the genotype of *IL-4* rs2243250 and the cigarette smoking lifestyle of the participants was analyzed but the results showed that the genotypic distribution of the variant genotypes of *IL-4* rs2243250 was not significantly different between lung cancer and control groups who were ever-smokers, but different in the case among the non-smokers. In 2007, Cesar-Neto and his colleagues found that smoking behavior decreased the levels of IL-10, IL-1α, IL-8, TNFα, MMP-8 and osteoprotegerin in sites with periodontitis, but not IL-4 (36). To date, there exists no direct evidence showing that an altered expression of IL-4 is essential in lung cancer carcinogenesis in any cell culture models since the cell-cell interactions of immune suppression and tumor immune surveillance networks are very complicated. Moreover, the detail mechanism of the contribution of *IL-4* rs2243250 genotype to non-smoker lung cancer development needs further investigations.

In conclusion, the T allele at the *IL-4* rs2243250 promoter polymorphic site was associated with lower lung cancer risk, especially among patients that were females and non-smokers.

Acknowledgements

The Authors declare no conflicts of interest. We appreciate Tissue bank of China Medical University Hospital for their excellent technical assistance. This study was supported by research grants from Taichung Armed Forces General Hospital (104A10 and 104A11), Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW104-TDU-B-212-113002).

References

1 Siegel RL, Miller KD and Jemal A: Cancer statistics, 2015. CA Cancer J Clin *65*: 5-29, 2015.

2 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin *65*: 87-108, 2015.

3 Li Z, Chen L and Qin Z: Paradoxical roles of IL-4 in tumor immunity. Cell Mol Immunol *6*: 415-422, 2009.

4 Hasegawa Y, Ando M, Maemondo M, Yamamoto S, Isa S, Saka H, Kubo A, Kawaguchi T, Takada M, Rosell R, Kurata T and Ou SH: The role of smoking status on the progression-free survival of non-small cell lung cancer patients harboring activating epidermal growth factor receptor (EGFR) mutations receiving first-line EGFR tyrosine kinase inhibitor versus platinum doublet chemotherapy: a meta-analysis of prospective randomized trials. Oncologist *20*: 307-315, 2015.

5 Bhatt VR, Batra R, Silberstein PT, Loberiza FR, Jr. and Ganti AK: Effect of smoking on survival from non-small cell lung cancer: a retrospective Veterans' Affairs Central Cancer Registry (VACCR) cohort analysis. Med Oncol *32*: 339, 2015.

6 Shields PG and Harris CC: Cancer risk and low-penetrance susceptibility genes in gene-environment interactions. J Clin Oncol *18*: 2309-2315, 2000.

7 Shields PG: Molecular epidemiology of smoking and lung cancer. Oncogene *21*: 6870-6876, 2002.

8 Ji YN, Wang Q and Suo LJ: CYP1A1 Ile462Val polymorphism contributes to lung cancer susceptibility among lung squamous

carcinoma and smokers: a meta-analysis. PLoS One *7*: e43397, 2012.
9 Spitz MR, Gorlov IP, Dong Q, Wu X, Chen W, Chang DW, Etzel CJ, Caporaso NE, Zhao Y, Christiani DC, Brennan P, Albanes D, Shi J, Thun M, Landi MT and Amos CI: Multistage analysis of variants in the inflammation pathway and lung cancer risk in smokers. Cancer Epidemiol Biomarkers Prev *21*: 1213-1221, 2012.
10 Hsia TC, Chang WS, Liang SJ, Chen WC, Tu CY, Chen HJ, Yang MD, Tsai CW, Hsu CM, Tsai CH and Bau DT: Interleukin-10 (IL-10) promoter genotypes are associated with lung cancer risk in Taiwan males and smokers. Anticancer Res *34*: 7039-7044, 2014.
11 Hsia TC, Chang WS, Chen WC, Liang SJ, Tu CY, Chen HJ, Liang JA, Tsai CW, Hsu CM, Tsai CH and Bau DT: Genotype of DNA double-strand break repair gene XRCC7 is associated with lung cancer risk in Taiwan males and smokers. Anticancer Res *34*: 7001-7005, 2014.
12 Hsia TC, Tsai CW, Liang SJ, Chang WS, Lin LY, Chen WC, Tu CY, Tsai CH and Bau DT: Effects of ataxia telangiectasia mutated (ATM) genotypes and smoking habits on lung cancer risk in Taiwan. Anticancer Res *33*: 4067-4071, 2013.
13 Chen WC, Tsai CW, Hsia TC, Chang WS, Lin LY, Liang SJ, Tu CY, Cheng WE, Chen HJ, Wang SM and Bau DT: The contribution of DNA apurinic/apyrimidinic endonuclease genotype and smoking habit to Taiwan lung cancer risk. Anticancer Res *33*: 2775-2778, 2013.
14 Liu CJ, Hsia TC, Tsai RY, Sun SS, Wang CH, Lin CC, Tsai CW, Huang CY, Hsu CM and Bau DT: The joint effect of hOGG1 single nucleotide polymorphism and smoking habit on lung cancer in Taiwan. Anticancer Res *30*: 4141-4145, 2010.
15 Liu CJ, Hsia TC, Wang RF, Tsai CW, Chu CC, Hang LW, Wang CH, Lee HZ, Tsai RY and Bau DT: Interaction of cyclooxygenase 2 genotype and smoking habit in Taiwanese lung cancer patients. Anticancer Res *30*: 1195-1199, 2010.
16 Govindan R: Lung cancer in never smokers: a new hot area of research. Lancet Oncol 11: 304-305, 2010.
17 Liu YL, Xu Y, Li F, Chen H and Guo SL: CYP2A6 deletion polymorphism is associated with decreased susceptibility of lung cancer in Asian smokers: a meta-analysis. Tumour Biol *34*: 2651-2657, 2013.
18 Lo YL, Hsiao CF, Jou YS, Chang GC, Tsai YH, Su WC, Chen KY, Chen YM, Huang MS, Hsieh WS, Chen CJ and Hsiung CA: Polymorphisms of MLH1 and MSH2 genes and the risk of lung cancer among never smokers. Lung Cancer *72*: 280-286, 2011.
19 Wu X, Wang L, Ye Y, Aakre JA, Pu X, Chang GC, Yang PC, Roth JA, Marks RS, Lippman SM, Chang JY, Lu C, Deschamps C, Su WC, Wang WC, Huang MS, Chang DW, Li Y, Pankratz VS, Minna JD, Hong WK, Hildebrandt MA, Hsiung CA and Yang P: Genome-wide association study of genetic predictors of overall survival for non-small cell lung cancer in never smokers. Cancer Res *73*: 4028-4038, 2013.
20 Paul WE: Interleukin-4: a prototypic immunoregulatory lymphokine. Blood *77*: 1859-1870, 1991.
21 Amsen D, Spilianakis CG and Flavell RA: How are T(H)1 and T(H)2 effector cells made? Curr Opin Immunol *21*: 153-160, 2009.
22 Pardoll DM: Paracrine cytokine adjuvants in cancer immunotherapy. Annu Rev Immunol *13*: 399-415, 1995.
23 Rodolfo M, Zilocchi C, Accornero P, Cappetti B, Arioli I and Colombo MP: IL-4-transduced tumor cell vaccine induces immunoregulatory type 2 CD8 T lymphocytes that cure lung metastases upon adoptive transfer. J Immunol *163*: 1923-1928, 1999.
24 Tepper RI, Pattengale PK and Leder P: Murine interleukin-4 displays potent anti-tumor activity *in vivo*. Cell *57*: 503-512, 1989.
25 Shurin MR, Lu L, Kalinski P, Stewart-Akers AM and Lotze MT: Th1/Th2 balance in cancer, transplantation and pregnancy. Springer Semin Immunopathol *21*: 339-359, 1999.
26 Kemp RA and Ronchese F: Tumor-specific Tc1, but not Tc2, cells deliver protective antitumor immunity. J Immunol *167*: 6497-6502, 2001.
27 Gomes M, Coelho A, Araujo A, Teixeira AL, Catarino R and Medeiros R: Influence of functional genetic polymorphism (-590C/T) in non-small cell lung cancer (NSCLC) development: the paradoxal role of IL-4. Gene *504*: 111-115, 2012.
28 Li X, Shi W, Yu G, Lin L, Yang B, Li J, Guo W, Tang C, Wang H, Gao H, Qin H, Liu Y and Liu X: Interleukin-4 -590T/C polymorphism influences the susceptibility to nonsmall cell lung cancer. DNA Cell Biol *31*: 797-800, 2012.
29 Gu J, Shen Y and Zhang Y: Association between interleukin-4 polymorphisms and environment and nonsmall cell lung cancer in Chinese population. J Cancer Res Ther *10 Suppl*: C135-139, 2014.
30 Chen HJ, Chang WS, Hsia TC, Miao CE, Chen WC, Liang SJ, Chen AC, Chang JG, Tsai CW, Hsu CM, Tsai CH and Bau DT: Contribution of genotype of DNA double-strand break repair gene XRCC3, gender, and smoking behavior to lung cancer risk in Taiwan. Anticancer Res *35*: 3893-3899, 2015.
31 Rosenwasser LJ, Klemm DJ, Dresback JK, Inamura H, Mascali JJ, Klinnert M and Borish L: Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. Clin Exp Allergy *25(Suppl 2)*: 74-78; discussion 95-76, 1995.
32 Nakashima H, Miyake K, Inoue Y, Shimizu S, Akahoshi M, Tanaka Y, Otsuka T and Harada M: Association between IL-4 genotype and IL-4 production in the Japanese population. Genes Immun *3*: 107-109, 2002.
33 Scarel-Caminaga RM, Trevilatto PC, Souza AP, Brito RB and Line SR: Frequencies of the -330 (T --> G) IL-2 and -590 (T --> C) IL-4 gene polymorphisms in a population from south-eastern Brazil. Eur J Immunogenet *29*: 293-296, 2002.
34 Pawlik A, Wrzesniewska J, Florczak M, Gawronska-Szklarz B and Herczynska M: The -590 IL-4 promoter polymorphism in patients with rheumatoid arthritis. Rheumatol Int *26*: 48-51, 2005.
35 Wang BY, Huang JY, Cheng CY, Lin CH, Ko J and Liaw YP: Lung cancer and prognosis in Taiwan: a population-based cancer registry. J Thorac Oncol *8*: 1128-1135, 2013.
36 Cesar-Neto JB, Duarte PM, de Oliveira MC, Tambeli CH, Sallum EA and Nociti FH Jr.: Smoking modulates interleukin-6:interleukin-10 and RANKL: osteoprotegerin ratios in the periodontal tissues. J Periodontal Res *42*: 184-191, 2007.

Received August 5, 2015
Revised September 10, 2015
Accepted September 14, 2015