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Original Article

FasL -844T/C mutation may participate in occurrence and development of pulmonary squamous cell carcinoma in south China

Hongguang Zhao[1,2], Weimin Mao[1,2], Wenhu Chen[3], Peng Du[3], Jiangfeng Qin[3], Chenyu Zhuang[4], Jiali Tong[4], Lifang Wang[3]

[1]Department of Thoracic Surgery, Zhejiang Key Laboratory of Diagnosis and Treatment Technology on Thoracic Oncology (Lung and Esophagus), Zhejiang Cancer Hospital, Hangzhou 310022, China; [2]Wenzhou Medical University, Wenzhou 325035, P. R. China; [3]Department of Biochemistry, Institute of Basic Medical Science, Zhejiang Medical College, Hangzhou 310053, P. R. China; [4]Class 1201/1202, Department of Clinical Medicine, Zhejiang Medicine College, Hangzhou 310053, P. R. China

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Abstract: Fas-FasL system plays an important role in cancer initiation, development and progression. FasL -844T/C mutation has been shown to be significant in the genetic susceptibility to cancer. We explored the relationship between FasL gene -844T/C SNP and pulmonary squamous cell carcinoma (SCC). 330 patients with pulmonary SCC in south China were recruited from July 2007 to Oct 2011 in Zhejiang Cancer Hospital and their clinicopathological data were collected. 297 cases of cancer-free individuals were selected as control group. PCR-RFLP technique was carried out to detect FasL -844T/C single nucleotide polymorphism (SNP). Fas -1377G/A SNP was also detected to investigate whether it interfered with the functional effect of the FasL -844T/C in pulmonary SCC development. χ^2 test and logistic regression were used to analysis the association between FasL -844T/C, Fas -1377G/A polymorphism or other clinicopathological parameters and pulmonary SCC. Gender, smoking and FasL -844 genotypes could increase risk of pulmonary SCC susceptibility mainly in age <60 group. However, only smoking was associated with pulmonary SCC in age <60 group. Compared with TT genotype, FasL -844CC was risk factor for development of pulmonary SCC (adjusted OR=1.518, 95% CI=1.100-2.094, *P*=0.011). After grouping patients with smoking pack-years, it was a risk factor for pulmonary SCC in ≥20 pack-years group. There was no effect modification between FasL -844 SNP and smoking or gender by test of the interaction term (*P*<0.05). FasL -844C/T was significant associated with stage, lymph node metastasis and vascular tumor thrombus in age ≥60. Our results show that FasL -844T/C SNP is associated with the occurrence of pulmonary SCC in heavy smoking and younger people, and the development and metastasis in elder people.

Keywords: FasL gene, SNP, pulmonary squamous cell carcinoma

Introduction

Lung cancer is the leading cause of cancer-related death around the world. Non-small cell lung cancer (NSCLC) is the most common histological type in lung cancers. Squamous cell carcinoma (SCC) is the second-largest subtype of NSCLC, accounts for 30% of NSCLC cases. The incidence and mortality of pulmonary SCC have continued to increase in recent years [1]. Although pulmonary SCC is mainly caused by cigarette smoking, other factors can also cause it.

Molecular mechanisms of tumor development are still the hot spot in cancer research. Apoptosis-related genes play an important role in tumor development. Apoptosis resistance is one of the significant characters in tumor immune evasion. Regulatory defects of moleculars in the apoptosis pathway result in tumorigenesis, tumor cell invasion and metastasis [2, 3]. Mutation of key genes in cell-death pathway would influence the susceptibility to cancer.

Fas-Fasl ligand (FasL) system is a crucial apoptosis pathway [4, 5]. Fas interacts with FasL to transmit a “death signal” to target cells and trigger apoptosis [5-8]. This system also plays an important role in immune evasion [9]. Increased expression of FasL would contribute

to malignant transformation and progression [10].

Single nucleotide polymorphisms (SNPs) of many genes are associated with the susceptibility to cancer. Fas and FasL genes mutation has been shown to increase the risk of many types of cancers [11-15]. Because Fas-FasL system plays an important role in cancer initiation, development and progression, their SNPs have been showed to be significant in the genetic susceptibility to cancer.

The clinical significances of FasL -844T/C and Fas -1377G/A mutation in esophageal carcinoma and pulmonary adenocarcinoma were studied in our prior researches [16, 17]. Now, we will analyze whether FasL -844T/C and Fas -1377G/A SNPs would influence the susceptibility or clinicopathological factor of pulmonary SCC in this research.

Methods

Subjects

330 cases of pulmonary SCC were selected from Zhejiang Cancer Hospital, affiliated hospital of Zhejiang Medical College, China, between July 2007 and October 2011. 297 cases of cancer-free individuals who had been randomly selected from medical examination center were chosen as control group. Sex and age were not restricted. All subjects were unrelated ethnic Han Chinese who came from South China. Each subject signed an informed consent to participate in this research and to allow his biological samples to be analyzed. This research had been approved by Medical Ethics Committee, Zhejiang Medical College.

All patients had detailed clinicopathological data and underwent radical surgery. Tumor and lymph node metastasis staging was according to the 7th AJCC Classification System on the basis of postoperative pathological diagnosis.

Genotyping

3 mL of peripheral blood was collected from each subject, and genomic DNA was extracted using Blood Genome DNA Extraction Kit (Takara Biotechnology Co. Ltd., Dalian, China).

FasL -844T/C polymorphism was detected using PCR-RFLP with forward primer 5'-CAGCTACTCGG AGGCCAAG-3' and reverse primer 5'-GCTCTGAGGGGAGAGACCAT-3'. PCR products were amplified in the following condition: 1 cycle of 2 min at 95°C, 35 cycles of 30 sec at 94°C, 30 sec at 62°C, and 45 sec at 72°C, followed by 7 min at 72°C. Products were digested with BsrDI (Fermentas, Thermo Fisher Scientific Inc.) at 55°C for 4 h. All products were electrophoresed with 3% MS-6 Agarose (Takara Biotechnology Co. Ltd., Dalian) and then visualized with GelRed (Biotium Company, U.S) staining. The PCR product amplified for these loci was 410 bp. -844T was distinguished with BsrDI restriction enzymes, resulting in 232 bp and 168 bp fragments. The experiment was performed by two people independently in a blind way. More than 10 percent of samples were randomly selected for identification and the results were 100 per cent concordant.

Fas -1377G/A polymorphism was also detected using PCR-RFLP with forward primer 5'-TGTGTGCACAAGGCTGGCGC-3' and reverse primer 5'-TGCATCTGTCACTGCACTTACCACCA-3'. The PCR product amplified for these loci was 122 bp. To introduce a restriction endonuclease site, 3'end of forward primer was changed from CAC to CGC, which created a BstUI restriction enzyme cutting site. The amplification process was the same as above. After amplification, for Fas -1377G allele, BstUI (New England Biolabs, Beverly, MA) digestion generated two fragments, 104 bp and 18 bp.

Statistical analyses

Statistical analyses were carried out using SPSS 16.0 (SPSS, Chicago, IL). Differences were considered statically significant with $P<0.05$.

Departure from Hardy-Weinberg equilibrium (HWE) in control group was examined with χ^2 test to check for genotyping error. Age and sex in these two groups were compared with T-test and χ^2 test, respectively.

Association of FasL -844T/C, Fas -1377G/A SNP or other clinicopathological data and pulmonary SCC were analyzed with χ^2 test. Pulmonary SCC risk was estimated as odds ratios (OR) and 95% confidence intervals (CI) with conditional logistic regression or multinomial logistic regression controlling for age, and

Table 1. Baseline clinical characteristics and the genotypic and allelic frequencies of SNPs of cases and controls

		Cases (%) n=330	Controls (%) n=297	χ^2	*P*
Gender	Male	314	179	113.186	0.000**
	Female	16	118		
Age	<60	134	192	36.194	0.000**
	≥60	196	105		
Smoking (pack-years)	<20	49	234	258.066	0.000**
	≥20	281	63		
FasL -844 *Genotypes*	TT	32 (9.7)	52 (17.5)	15.510	0.000**
	TC	121 (36.7)	128 (43.1)		
	CC	177 (53.6)	117 (39.4)		
Alleles	T allele	185 (28.0)	232 (39.1)	17.127	0.000**
	C allele	475 (72.0)	362 (60.9)		
Fas -1377 *Genotypes*	GG	133 (40.3)	121 (40.7)	2.652	0.266
	GA	140 (42.4)	138 (46.5)		
	AA	57 (17.3)	38 (12.8)		
Alleles	G allele	406 (61.5)	380 (64.0)	0.807	0.369
	A allele	254 (38.5)	214 (36.0)		

**$P<0.01$.

Table 2. Main effects of individual risk factors on pulmonary SCC risk by Logistic regression

Variable	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Age	1.006 (0.638-1.587)	0.979	-	-
Gender	0.077 (0.044-0.134)	0.000	0.416 (0.217-0.799)†	0.008
Smoking	4.615 (3.757-5.670)	0.000	3.910 (3.037-5.034)‡	0.000
FasL -*844* locus	1.577 (1.254-1.983)	0.000	1.469 (1.090-1.980)§	0.011

†Adjusting for age, smoking and FasL -844 locus; ‡Adjusting for age, gender and FasL -844 locus; §Adjusting for age, gender and smoking status.

gender. This codominant model was defined as heterozygotes (1 variant genotype) versus wild-type (0 variant genotype) or homozygotes (2 variant genotype) versus wild-type. Effect modification was test by the interaction term.

The relationships between these SNPs or smoking and clinicopathological data were analyzed with χ^2 test for univariate analyses, then binary logistic regression (Ascendant Wald method) for multivariate analyses with each clinicopathological data as dependant variable and with genotypes of FasL -844T/C polymorphism or Fas -1377G/A polymorphism or smoking with other clinicopathological data as covariates. The clinicopathological data included tumor volume (diameter less than or equal to 3 cm versus greater than 3 cm), tumor location (central bronchogenic versus peripheral), differentiation (well-moderately differentiated versus poorly differentiated), stage (I versus II-III), T grade (T1-T2 versus T3-T4), visceral pleura invasion, lymphonode metastasis, and vascular tumor thrombus (positive versus negative).

Results

Gene polymorphisms and susceptibility to pulmonary SCC

The FasL -844 and Fas -1377 genotypes frequencies were agreed with Hardy-Weinberg equilibrium in control groups (χ^2=2.663, *P*=0.103; χ^2 =0.019, P=0.890, respectively). Base-line clinical characteristics of cases and controls are summarized in **Table 1**. Cases were older and had more males than controls. Gender (adjusted OR=0.416, 95% CI=0.217-0.799, P=0.008), smoking (adjusted OR=3.910, 95% CI= 3.037-5.034, P=0.000) and FasL -844 Genotypes (adjusted OR=1.469, 95% CI=1.090-1.980, P=0.011) were associated with increased risk of pulmonary SCC susceptibility in main effect analysis (**Table 2**).

There was no effect modification between FasL -844 SNP and smoking or gender by test of the interaction term (*P*<0.05).

Associations between the SNPs with pulmonary SCC

The associations of the genotypes and risk of pulmonary SCC were analyzed with Logistic regression analysis (**Table 3**). When adjusting for age, gender and smoking, FasL gene -844 CC genotype was associated with an increased

Table 3. Association of genotypic and allelic frequencies of FasL -844 with pulmonary SCC risk

		Crude OR (95% CI)	*P*	Adjusted OR# (95% CI)	*P*
FasL -844 Genotypes	TT	1		1	
	TC	1.536 (0.926-2.547)	0.096	1.677 (0.882-3.191)	0.115
	CC	1.568 (1.222-2.012)	0.000**	1.518 (1.100-2.094)	0.011*
Alleles	T allele	1		1	
	C allele	1.431 (1.088-1.882)	0.010**	1.341 (0.933-1.926)	0.113

*$P<0.05$, **$P<0.01$. #Adjusting for age, gender and smoking.

Table 4. Risk of pulmonary SCC associated with FasL -844 genotypes by smoking

Smoking (*pack-years*)	FasL -844	Cases (%) n=330	Controls (%) n=297	Crude OR (95% CI)	P	Adjusted OR# (95% CI)	P
<20	TT+TC	25	141	1		1	
<20	CC	24	93	1.455 (0.784-2.701)	0.234	1.248 (0.659-2.363)	0.497
≥20	TT+TC	128	39	4.302 (3.258-5.682)	0.000**	3.139 (2.221-4.434)	0.000**
≥20	CC	153	24	3.301 (2.698-4.038)	0.000**	2.655 (2.053-3.433)	0.000**
≥20	TT+TC	128	39	1		1	
≥20	CC	153	24	1.942 (1.109-3.401)	0.020*	1.855 (1.053-3.265)	0.032*

*$P<0.05$, **$P<0.01$. #Adjusting for age and gender.

Table 5. Relationships between pulmonary SCC clinicopathological parameters and FasL -844T/C SNPs

n (%)		Cases n=330			χ^2	*P*
		TT	TC	CC		
Age (year)	<60	13	51	71	0.124	0.940
	≥60	19	70	106		
Sex	male	30	114	170	0.673	0.714
	female	2	7	7		
Tumor length	≤3 cm	10	36	43	1.417	0.492
	>3 cm	22	85	134		
Location	Central bronchogenic	23	88	129	0.014	0.993
	Peripheral	9	33	48		
Differentiation	Well-moderately differentiated	25	73	107	3.858	0.145
	Poorly differentiated	7	48	70		
Stage	I	15	39	41	8.537	0.014*
	II-III	17	82	136		
Visceral pleura invasion	Yes	11	50	91	4.893	0.087
	No	21	71	86		
Lymphonode metastasis	Yes	20	58	71	6.082	0.048*
	No	12	63	106		
Vascular tumor thrombus	Yes	29	106	122	17.894	0.000**
	No	3	15	55		

*$P<0.05$, **$P<0.01$.

risk for development of pulmonary SCC (adjusted OR=1.518, 95% CI=1.100-2.094, *P*=0.011) compared with TT genotype. The C haplotype was not a risk factor for this carcinoma (adjusted OR=1.341, 95% CI=0.933-1.926, *P*=0.113) compared with the T haplotype.

Table 6. Risk of tumor stage, lymphonode metastasis and vascular tumor thrombus according to FasL -844 genotype in pulmonary SCC

Geno-types	Stage			Lymphonode metastasis			Vascular tumor thrombus		
	aOR#	CI	*P*	aOR#	CI	*P*	aOR#	CI	*P*
TT	1			1			1		
TC	1.869	0.832-4.198	0.130	1.839	0.806-4.196	0.147	1.365	0.370-5.040	0.641
CC	1.740	1.175-2.578	0.006**	1.608	1.086-2.382	0.018*	2.128	1.146-3.954	0.017*

*P<0.05, **P<0.01. #Adjusting for age and gender.

Table 7. Genotypic and allelic frequencies of FasL gene -844 in subgroups according to age

N (%)			Genotype (Frequency)			Allele (Frequency)	
			TT	TC	CC	T	C
<60	Cases	134	13 (9.7)	52 (38.8)	69 (51.5)	78 (29.1)	190 (70.9)
	Control	192	35 (18.2)	83 (43.2)	74 (38.5)	153 (39.8)	231 (60.2)
χ^2				7.288		7.957	
P				0.026*		0.005**	
≥60	Cases	196	19 (9.7)	69 (35.2)	108 (55.1)	107 (27.3)	285 (72.7)
	Control	105	17 (16.2)	45 (42.9)	43 (40.9)	79 (37.6)	131 (62.4)
χ^2				6.199		6.825	
P				0.045*		0.009**	

**P*<0.05, ***P*<0.01.

Risk of pulmonary SCC was evaluated by combining smoking and FasL -844 genotypes (**Table 4**). FasL -844CC was not a risk factor for pulmonary SCC in less than 20 pack-years group, but was a risk factor in more than or equal to 20 pack-years group.

The relationships between clinicopathological significance and the SNPs

The relationships between pulmonary SCC clinicopathological data and FasL -844 SNP were compared (**Table 5**). There were no significant differences between the gene polymorphism and age, gender, tumor length, location, differentiation, and visceral pleura invasion. While FasL -844C/T was significant associated with stage, lymph node metastasis and vascular tumor thrombus. FasL CC versus TT was dangerous, adjusted OR=1.740 (1.175-2.578), *P*=0.006; adjusted OR=1.608 (1.086-2.382), *P*=0.018; adjusted OR=2.128 (1.146-3.954), *P*=0.017, respectively (**Table 6**). However, there were no association of Fas -1377 SNP or smoking and pulmonary SCC clinicopathological parameters (P<0.05) (data not showed).

Association of FASL -844T/C polymorphism and pulmonary SCC stratified by age

All cases and control were stratified by age to explore the association of FASL -844T/C polymorphism and pulmonary SCC (**Table 7**). Genotype frequencies in control subgroups of different age are agreed with frequencies under the Hardy-Weinberg equilibrium (χ^2=1.852, *P*=0.174 and χ^2=0792, *P*=0.373, respectively).

For those age <60 years, the frequencies of the TT, TC and CC genotypes were 9.7%, 38.8% and 51.5%, respectively, among the cases and 18.2%, 43.2% and 38.5%, respectively, among the controls. The frequencies of C alleles in patients and controls of this subgroup were 70.9% and 60.2%, respectively, which had statistical difference. Furthermore, smoking and FasL -844 locus were risk factor and gender was a protective factor for pulmonary SCC in multivariate analyses (**Table 8**).

For those age ≥60, genotypes or alleles were significantly different between these two groups: 9.7% (TT), 35.2% (TC) and 55.1% (CC) for the cases, and 16.2% (TT), 42.9% (TC) and 40.9% (CC) for the controls, respectively. 72.7% and 62.4% for C alleles in cases and controls respectively. In multivariate analyses, smoking was a risk factor for pulmonary SCC, however, gender and FasL -844 locus were not risk factor for pulmonary SCC (**Table 8**).

We conducted further analyses to explore whether FasL -844T/C polymorphism was asso-

Table 8. Main effects of individual risk factors on pulmonary SCC risk by Logistic regression in subgroups according to age

Age	Variable	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
<60	Gender	0.069 (0.029-0.163)	0.000**	0.239 (0.094-0.606)†	0.003**
	Smoking	4.501 (3.362-6.026)	0.000**	3.675 (2.686-5.029)‡	0.000**
	FasL -844 locus	1.555 (1.125-2.150)	0.008**	1.571 (1.039-2.376)§	0.032*
≥60	Gender	0.087 (0.041-0.185)	0.000**	1.000 (0.322-3.110)†	1.000
	Smoking	4.892 (3.442-6.954)	0.000**	4.839 (3.010-7.779)‡	0.000**
	FasL -844 locus	1.536 (1.090-2.162)	0.014*	1.432 (0.928-2.211)§	0.105

**P*<0.05, ***P*<0.01. †Adjusting for smoking and FasL -844 locus; ‡Adjusting for gender and FasL -844 locus; §Adjusting for gender and smoking status.

Table 9. Association of clinicopathological parameters and FasL -844 SNPs in subgroups according to age

	<60		≥60	
	χ^2	*P*	χ^2	*P*
Sex	0.939	0.625	3.015	0.221
Volum	0.197	0.906	3.345	0.188
Differentation	4.036	0.133	1.344	0.511
Location	0.574	0.751	0.532	0.766
Visceral pleura	2.722	0.256	2.096	0.351
Stage	5.411	0.248	9.950	0.041*
Lymphnode metastasis	2.148	0.342	9.490	0.009**
Vascular tumor thrombus	6.576	0.037*	11.791	0.003**

**P*<0.05, ***P*<0.01.

ciated with clinicopathological parameters in the selected population of patients (**Table 9**). Then, multivariate analysis was carried out (**Table 10**). For those ≥60, FasL -844CC genotype showed significant associations with stage, lymph node metastasis and vascular tumor thrombus with TT as reference (OR=1.780, 95% CI=1.066-2.973, *P*=0.027; OR=1.852, 95% CI=1.090-3.148, *P*=0.023; OR=3.105, 95% CI=1.081-8.924, *P*=0.035; respectively). For those <60, FasL -844CC genotype were not associated with vascular tumor thrombus (*P*=0.314).

Discussion

Fas-Fas ligand (FasL) system has been recognized as the main pathway for the induction of apoptosis in cells and tissues. FasL is a 40 kD transmembrane glycoprotein, which is classified as a type II protein of the TNF family. FasL mainly exists in cytotoxic T lymphocyte (CTL), NK cells and some immune privileged site, such as testis and eye. It can trigger cell death signal cascade by crosslinking with its receptor, Fas [10].

Fas-FasL system can participate in immune evasion. Lower expression of Fas and/or higher expression of FasL is discovered in many kinds of tumors, which can counterattack T lymphocyte by FasL crosslinking with Fas and decrease injury by suicide or T cell killing [18-21]. Furthermore, FasL-Fas can also mediate activation-induced cell death (AICD) in the tumour microenvironment, which can lead to apoptosis among the tumor-infiltrating lymphocytes [22, 23].

Overexpression of FasL had been reported in multiple human carcinomas, such as breast carcinomas [24], gastric carcinoma [25], esophageal carcinoma [26], hepatocellular carcinoma [27], melanoma [28], colorectal carcinoma [29], pancreatic carcinomas [30], as well as lung carcinoma [31]. Expression of FasL was an early event and an early stage remark in carcinogenesis [32, 33].

There was association between expression of FasL and clinicopathological parameters. A significant association was found between FasL and worse tumor differentiation [34, 35]. FasL immunostaining in tumor cells correlated with tumor stage [36, 37] and lymph node metastasis [38]. These findings all suggest that FasL is related to carcinogenesis, tumor development and progress.

FasL -844T/C locates in a putative binding motif for a transcription factor, CAAT/enhancer-binding protein β, and compared with the -844 T allele, the -844 C allele significantly increases basal expression of FasL [39], suggesting that FasL -844T/C polymorphism may influence FasL expression and FasL-mediated signaling, and ultimately, contributing to the susceptibility to cancer.

Table 10. Risk of tumor stage, lymphonode metastasis and vascular tumor thrombus according to FasL -844 genotype in subgroups according to age

		Age	TT	TC	CC
Stage	aOR#	≥60	1	1.243	1.780
	CI			0.448-3.449	1.066-2.973
	P			0.677	0.027*
Lymphnode metastasis	aOR#		1	1.491	1.852
	CI			0.505-4.401	1.090-3.148
	P			0.469	0.023*
Vascular tumor thrombus	aOR#		1	2.738	3.105
	CI			0.324-23.169	1.081-8.924
	P			0.355	0.035*
	aOR#	<60	1	0.750	1.507
	CI			0.133-4.236	0.678-3.352
	P			0.745	0.314

*$P<0.05$, #Adjusting for age and gender.

Several studies demonstrated that FasL polymorphism was associated with an increased risk of cancers, such as pancreatic cancer [40], bladder cancer [15], nasopharyngeal carcinoma [41], ovarian carcinoma [13], esophageal SCC [11, 16]. While, there were still some reports suggested that FasL polymorphism was not associated with an increased risk of cancers, such as melanoma [42], papillary thyroid cancer [43], skin cancer [44], renal cancer [45], gastric cancer [46], oral cancer [47], prostate cancer [48]. However, the associations of FasL -844T/C polymorphism and the risk of human cancers remain inconsistent in some tumors in different studies, which including breast cancer [49-51], cervical cancer [52-54], lung cancer [55-58]. Wang et al. analyzed the relationship of FasL -844T/C polymorphism and breast cancer, and concluded that FasL -844CC genotype significantly increased the risk of breast cancer compared with the TT or TT + TC genotypes [49]. Another report in northern china received similar result [50]. However, no significant relationship was found between this polymorphism and the risk of breast cancer in another report [51]. Sun et al. analyzed the association of FasL -844T/C polymorphism and cervical cancer in northern China [52]. They found a threefold increased risk of cervical cancer among subjects with the FasL -844CC genotype compared with those with the -844TT genotype in a case-control study in Chinese women. However, no significant relationship was found between FasL -844T/C polymorphism and the risk of cervical cancer by other reports [14, 53, 54]. In lung cancer respect, Zhang et al. found that there was a 1.79 fold excess risk of lung cancer for FasL -844CC carriers in northern China [55]. However, no significant association was found by other reporters in Taiwan [56], Canada [57] and Korea [58], respectively. From above reports, we can see that the relationship between FasL -844T/C polymorphisms and the risk of human cancers is different in different areas or between different ethnics.

A meta-analysis was conducted on FasL -844T/C SNP and cancer risk from 19 published studies [59]. It showed FasL -844T allele has a possible protective effect on cancer risk. Another meta-analysis showed that FasL -844C/T polymorphism may be associated with a significantly increased cancer risk [60]. When stratified by ethnicity, it showed a significantly increased risk among Asians but not among Caucasians.

We investigate the risk of FasL -844T/C polymorphism on developing pulmonary SCC in a south China population. Our research reveals that FasL -844CC is a risk factor for pulmonary SCC. Compared with T allele, C allele is a risk factor for pulmonary SCC. This is consistent with Zhang's research in 2005 [55].

Though Sung's research did not find that FasL -844 polymorphism increased the risk of lung cancer, they found that FasL -844CC genotype had higher prevalence in those with advanced tumors than in those with early tumors [56]. We reveal that FasL -844CC is related to later stage of pulmonary SCC. What's more, FasL -844CC is a risk factor for lymph node metastasis and vascular tumor thrombus. This suggests that pulmonary SCC with FasL -844CC has a stronger invasion.

The immune function is weakened with the age increasing. We discover that smoking and FasL -844T/C polymorphism are susceptibility factors to pulmonary SCC for age <60 years patients. However, only smoking is the risk fac-

tor for age ≥60 years patients. Smoking may result in an increasing risk for pulmonary SCC in elder patients due to the longer smoking history, and immune escape by FasL maybe influence less on the basis of weaken immune function in elder patients. However, FasL -844T/C polymorphism is a risk factor for tumor stage, lymphonode metastasis and vascular tumor thrombus for age ≥60 years patients. This suggests FasL can further inhibit the immune function in elder patients, which lead to tumor development.

FasL -844T/C SNP is associated with occurrence, development and metastasis of pulmonary SCC. FasL -844CC is a risk factor in more than or equal to 20 pack-years group. There was no effect modification between FasL -844 SNP and smoking or gender.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Li-Fang Wang, Department of Biochemistry, Institute of Basic Medical Science, Zhejiang Medical College, 481# Binwen Road, Hangzhou 310053, Zhejiang, P. R. China. Tel: +86 571 8769 2750; Fax: +86 571 8769 2750; E-mail: jessie1217@hotmail.com

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