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Genetic variant in the 3'-untranslated region of *VEGFR1* gene influences chronic obstructive pulmonary disease and lung cancer development in Chinese population

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Lung inflammation and epithelial to mesenchymal transition (EMT) are two pathogenic features for the two contextual diseases: chronic obstructive pulmonary disease (COPD) and lung cancer. *VEGFR1* (or *FLT1*) plays a certain role in promoting tumour growth, inflammation and EMT. To simultaneously test the association between the single nucleotide polymorphisms (SNPs) in *VEGFR1* and risk of COPD and lung cancer would reveal genetic mechanisms shared by these two diseases and joint aetiology. We conducted a two-population hospital-based case–control study. Three potential functional SNPs (rs664393, rs7326277 and rs9554314) were genotyped in southern Chinese and validated in eastern Chinese to explore their associations with COPD risk in 1511 COPD patients and 1677 normal lung function controls, and with lung cancer risk in 1559 lung cancer cases and 1679 cancer-free controls. We also detected the function of the promising SNP. Individuals carrying the rs7326277C (CT+CC) variant genotypes of *VEGFR1* had a significant decrease in risk of both COPD (OR = 0.78; 95% CI = 0.68–0.90) and lung cancer (OR = 0.79; 95% CI = 0.64–0.98), compared with those carrying the rs7326277TT genotype. Functional assays further showed that the rs7326277C genotypes had lower transcriptional activity and caused decreased VEGFR expression, compared with the rs7326277TT genotype. However, no significant association was observed for the other two SNPs (rs664393 and rs9554314) and either COPD or lung cancer risk. Our data suggested that the rs7326277C variant of *VEGFR1* could reduce both COPD and lung cancer risk by lowering VEGFR1 mRNA expression; the SNP might be a common susceptible locus for both COPD and lung cancer.

Introduction

Chronic obstructive pulmonary disease (COPD) and lung cancer are two leading causes of morbidity and mortality in China and worldwide (1,2). Smoking is the major risk factor for both diseases. Epidemiological studies (3–7) and our previous studies (8) have indicated that COPD is an independent risk factor of lung cancer. Furthermore, these two pulmonary diseases share some pathological mechanisms (3,9–11). The fact that about 20–30% of smokers develop COPD and 10–15% of smokers develop lung cancer (12,13), suggests that the genetic factors might affect the risk of both diseases. Several genome-wide association studies (GWAS) found that many single nucleotide polymorphisms (SNPs) are associated with COPD and lung cancer susceptibility (14–17). Among these SNPs, some genetic loci are shared by lung cancer and COPD. To elucidate those common susceptible factors for COPD and lung cancer would be beneficial to the prevention of these two diseases, especially to lung cancer prevention of COPD patients who are at high risk of lung cancer.

Vascular endothelial growth factor (*VEGF*) is one of the most important stimulating angiogenesis factors. Tumor angiogenesis reflects tumor growth, invasion and metastasis ability. The interaction of *VEGF* and its receptors (*VEGFR1/FLT1* and *KDR/FLK1*) is the key to the regulation of tumor angiogenesis. *VEGFR1* (or *FLT1*), one of three tyrosine kinase receptors for *VEGF*, is a cell membrane–bound tyrosine kinas receptor that binds to *VEGF*. *VEGFR1* is a critical mediator of tumor angiogenesis, which plays a crucial role on the development, invasion and migration of tumours, especially of malignant tumors (18,19). Several studies have showed that VEGFR1 is highly expressed in lung cancer tissues (20,21).

The epithelial to mesenchymal transition (EMT) is a process initially observed in embryonic development in which cells lose epithelial characteristics and gain mesenchymal properties such as increased motility and invasion (22). Recent research suggests that EMT is also important in tumor progression (22,23). Previous studies have established a link between VEGFR1 and EMT in colon/pancreatic/hepatocellular carcinoma cell organoids (24–26). Currently, recognized common pathological mechanisms for both lung cancer and COPD are the long-term inflammatory process (9,10) and the epithelial–mesenchymal transition (EMT), which are also thought to cause lung carcinogenesis during the COPD period (11). Studies have found that VEGF and sVEGFR1 (soluble VEGFR1) are involved in the development of abnormal pulmonary vascular remodelling in patients with COPD (27).

The human *VEGFR1* gene (OMIM: 165070) is also called *FLT1*, which is located in chromosome 13q12 and encodes a member of the vascular endothelial growth factor receptor (VEGFR) family. A previous study has identified one SNP in the promoter of *VEGFR1* gene to be associated with breast cancer (28) and some GWAS have found that the intron SNP of *VEGFR1* was associated with acute lung injury caused by infection (29), which is one of risk factors for both lung cancer and COPD. However, the association between the SNPs of *VEGFR1* and chronic pulmonary diseases is unclear.

Based on the above point of view, we hypothesised that the SNPs in *VEGFR1* may influence the susceptibility of both COPD and lung cancer. In this study, we tested the associations

between three putative functional SNPs (rs7326277T>C, rs9554314A>C and rs664393C>T) of *VEGFR1* and COPD risk in a total of 1511 COPD patients and 1677 normal lung function controls, as well as lung cancer risk with a total of 1559 lung cancer cases and 1679 cancer-free controls. We further performed a series of biological assays to identify the biological effects of the promising SNP.

Materials and Methods

Study subjects and data collection

Respective case–control studies were conducted for COPD and lung cancer in two stages. The study population of COPD and lung cancer has been described previously (30–36). Briefly, the discovery set included 1025 COPD cases and 1061 normal lung function controls, and 1056 lung cancer cases and 1056 cancer-free controls of southern Chinese. The validation set comprised 486 COPD cases and 616 normal lung function controls, and 503 lung cancer cases and 623 normal lung function controls. The detailed information on subjects' collection is presented in supplementary Appendix. Moreover, participants' demographic characters and related variables, such as age, sex, smoking status and drinking status, were obtained during face-to-face interviews after a written informed consent was signed. Lung cancer was diagnosed according to the standard clinical criteria with pathologic confirmation from surgery, biopsy or cytology samples. Definition of COPD and its severity stages were according to the global initiative for chronic obstructive lung disease (2). The detailed definition of selected variables is presented in supplementary Appendix. The study was approved by the institutional review boards of Guangzhou Medical University and Soochow University.

SNP selection

The SNPs of *VEGFR1* that are predicted to be functional with location on the promoter region (supposed to be 2000 bp upstream of the transcriptional start site of the *VEGFR1* gene), exons and 3'-untranslated region (3'-UTR) were selected in this study. According to the dbSNP database (http://www.ncbi.nlm.nih.gov/), we found that there are three putatively functional SNPs of *VEGFR1* with a minor allele frequency >5% in Chinese population (i.e. rs664393C>T in promoter region and rs7326277T>C and rs9554314A>C in 3'-UTR). So, we chose the three SNPs in this study.

Genotype and phenotype detection

The Taqman allelic discrimination assay was used to detect the genotypes of the SNPs rs7326277T>C and rs664393C>T. Primers and probes were designed using the Primer Express 3.0 (Applied Biosystems) and synthesised by Shanghai GeneCore Biotechnologies (Shanghai, China) as shown in supplementary Table S1. The polymerase chain reaction (PCR) was performed in the ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster City, CA, USA) and the genotypes were automatically determined by the Sequence Detection Systems software 2.0.1 (Applied Biosystems). To confirm the genotyping results, we randomly selected 10% samples to repeat by Taqman assay and, as expected, the results were all 100% concordant. The primers and probes of rs9554314A>C could not been found by the Primer Express 3.0 (Applied Biosystems), so PCR–restriction fragment length polymorphism method was used to identify this SNP's genotypes with primers [5'-AGG CTC ACT AGG GAA TGT GCT G-3' (forward) and 5'-TTT CTC CAG TTG GGA CTC AGG A-3' (reverse)] and the enzyme BseGI (New England BioLabs, Ipswich, MA). The *VEGFR1* expression level was detected in mRNA level by real-time PCR as previously described (35). The detailed protocol is described in supplementary Appendix.

RNA interference and luciferase assays

Because only the SNP rs7326277T>C showed a promising association with both diseases, we constructed two reporter genes comprising the 3'-UTR of *VEGFR1* with different rs7326277T>C alleles. The protocol for construction of the two luciferase reporter genes is presented in supplementary Appendix. The *VEGFR1 in vitro* luciferase assays were performed first without any microRNA treatment. A549 (a human lung cancer cell line) and 16HBE (a human bronchial epithelial cell line) were seeded into 24-well plates at 1×10^5 cells/well and cultured at 37°C in 5% CO_2 for 24 h. The cells then were transiently transfected with 1.5 μg of reporter plasmids (T or C allele) alone using Lipofectamine 2000 according to the protocol (Invitrogen, Carlsbad, CA, USA). The activities of reporter genes with renilla luciferase and the internal standard firefly luciferase were quantified by a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). Independent triplicate experiments were done for each plasmid construct. Meanwhile, we chose two microRNAs miR-339-5p and miR-136-5p that are predicted to have a binding stie in 3′-UTR of VEGFR1 near this SNP for adding the RNA interference assay to show their effect interacting with the SNP. The mimics and inhibitors of miR-339-5p and miR-136-5p synthesised by GenePharma Co (Shanghai, China) were co-transferred to the luciferase reporters to show the effect of microRNAs on *VEGFR1* reporter genes *in vitro*.

Statistical analysis

Differences in the distributions of demographic characteristics and genotypes between the cases and controls were evaluated using the chi-square test. The association between each SNP and diseases risk was estimated using an unconditional logistic regression model with adjustment for surrounding factors. A multiplicative interaction was suggested to detect the possible gene–environment interaction (34). Homogeneity test was performed using the Breslow–Day test. A power test for the interaction was performed using the SAS macro PowerGxE as suggested by the previously published article (37). The statistical power was calculated by the PS Software (37). The One-way ANOVA tests, Student's *t* test was used to analyse *VEGFR1* expression in lung tissues with different genotypes. The Student's *t* test was also used to examine the difference in levels of luciferase reporter gene expression between different constructs. All tests were two-sided by using the SAS software (version 9.3; SAS Institute, Cary, NC, USA). $P < 0.05$ was considered to be statistically significant.

Results

Demographic characteristics of the study population

As shown in supplementary Tables S2 and S3, as expected, the distributions of age and sex in both COPD and lung cancer controls were well matched with their patients. There were more current smokers and former smokers in both COPD and lung cancer patients than controls for both data sets ($P < 0.001$ for all), and the sick smokers consumed more cigarettes pack years than healthy smokers. Moreover, COPD and lung cancer patients had a significantly higher prevalence of pre-existent lung diseases than controls in both data sets ($P < 0.05$).

VEGFR1 genotypes and COPD or lung cancer risk

The observed genotype frequencies of the three SNPs were all in Hardy–Weinberg equilibrium in controls ($P > 0.05$ for all).

For COPD, in the discovery set, the frequency distribution of genotypes of rs7326277 differed significantly between COPD cases and controls ($P = 0.01$). Rs7326277 CT genotype was associated with a decreased risk of COPD compared with wild homozygote (OR = 0.78; 95% CI = 0.65–0.94). In dominant genetic models, subjects carrying rs7326277CT+CC had a 22% decrease in risk of COPD (95% CI = 0.65–0.93), compared with subjects carrying rs7326277TT. No significant association was observed for the other two SNPs (rs664393 and rs9554314). In the validation set, the results about associations between SNPs in *VEGFR1* and COPD were similar to the results in the discovery set, confirming the above findings (Table 1).

For lung cancer, the effects of rs7326277 variants (dominant genetic model) on risk of lung cancer were found in both the discovery set (OR = 0.78; 95% CI = 0.62–1.00) and the validation set (OR = 0.80; 95% CI = 0.67–0.96). And, there was no significant association between the other two SNPs (rs664393 and rs9554314) and lung cancer risk.

In addition, because the associations of the adverse genotype in the two data sets were homogeneous ($P = 0.721$), we merged the two sets. Similarly, rs7326277 variant genotypes of *VEGFR1* (dominant genetic model) had a 22% decrease in risk of COPD (OR = 0.78; 95% CI = 0.68–0.90) and a 21% decrease in risk of lung cancer (OR = 0.79; 95% CI = 0.64–0.98).

Table I. Associations between the genotypes of *VEGFR1* gene and COPD as well as lung cancer risk

Genotypes	COPD case–control study						Lung cancer case–control study					
	Discovery set (southern Chinese)			Validation set (eastern Chinese)			Discovery set (southern Chinese)			Validation set (eastern Chinese)		
	Cases, *n* (%)	Controls, *n* (%)	Adjusted OR (95% CI)[a]	Cases, *n* (%)	Controls, *n* (%)	Adjusted OR (95% CI)[a]	Cases, *n* (%)	Controls, *n* (%)	Adjusted OR (95% CI)[a]	Cases, *n* (%)	Controls, *n* (%)	Adjusted OR (95% CI)[a]
Total no. of subjects	1025	1061		486	616		1056	1056		503	623	
rs7326277T>C												
TT	447 (43.6)	402 (37.9)	1.00 (ref.)	229 (47.1)	254 (41.2)	1.00 (ref.)	452 (42.8)	403 (38.2)	1.00 (ref.)	232 (46.1)	252 (40.4)	1.00 (ref.)
CT	456 (44.5)	516 (48.6)	**0.78 (0.65–0.94)**	211 (43.4)	293 (47.6)	0.78 (0.61–1.01)	481 (45.5)	516 (48.9)	**0.80 (0.68–0.99)**	218 (43.4)	298 (47.9)	**0.78 (0.61–1.02)**
CC	122 (11.9)	143 (13.5)	0.77 (0.59–1.02)	46 (9.5)	69 (11.2)	0.72 (0.48–1.10)	123 (11.7)	136 (12.9)	0.79 (0.60-1.04)	53 (10.5)	73 (11.7)	0.78 (0.52–1.16)
P value[b]			**0.01**			0.06			0.09			0.16
Dominant model												
TT	447 (43.6)	402 (37.9)	**0.78 (0.65–0.93)**	229 (47.1)	254 (41.2)	**0.77 (0.60–0.98)**	452 (42.8)	403 (38.2)	**0.80 (0.67–0.96)**	232 (46.1)	252 (40.4)	**0.78 (0.62–1.00)**
CT+CC	578 (56.4)	659 (62.1)		257 (52.9)	362 (58.8)		604 (57.2)	653 (61.8)		271 (53.9)	371 (59.6)	
Recessive model												
TT+CT	903 (88.1)	918 (86.5)	0.88 (0.68–1.15)	440 (90.5)	547 (88.8)	0.82 (0.55–1.22)	933 (88.3)	920 (87.1)	0.87 (0.68–1.13)	450 (89.5)	550 (88.3)	0.88 (0.60–1.28)
CC	122 (11.9)	143 (13.5)		46 (9.5)	69 (11.2)		123 (11.7)	136 (12.9)		53 (10.5)	73 (11.7)	
rs9554314A>C												
AA	487 (47.5)	479 (45.1)	1.00 (ref.)				513 (48.6)	503 (47.6)	1.00 (ref.)			
CA	428 (41.8)	459 (43.3)	0.92 (0.77–1.11)				434 (41.1)	433 (41.0)	0.99 (0.83–1.19)			
CC	110 (10.7)	123 (11.6)	0.89 (0.66–1.18)				109 (10.3)	120 (11.4)	0.89 (0.67-1.19)			
P value[b]			0.27						0.50			
rs664393C>T												
CC	570 (55.6)	596 (56.2)	1.00 (ref.)				590 (55.9)	609 (57.7)	1.00 (ref.)			
CT	384 (37.5)	396 (37.3)	1.02 (0.85–1.22)				400 (37.9)	380 (36.0)	1.10 (0.92–1.32)			
TT	71 (6.9)	69 (6.5)	1.11 (0.78–1.59)				66 (6.3)	67 (6.3)	1.02 (0.72–1.47)			
P value[b]			0.72						0.52			

[a]Adjusted in a logistic regression model that included age, sex, smoking status and drinking status.
[b]The chi-square test for the genotypes distribution between cases and controls.

Stratification analysis

We further explored the effects of different environmental stratification factors on the association between rs7326277 genotypes and risk of COPD or lung cancer. There was significant interaction (multiplication model: $P = 0.04$) between this SNP and smoking status on the risk of COPD ($P = 0.04$) with the OR value equalling to 0.69 (95% CI = 0.57–0.83) in the stratum of non-smoking and no significant effect observed (OR = 0.94, 95% CI = 0.76–1.17) in the stratum of smoking. However, there was no such interaction effect on the risk of lung cancer (Tables 2 and 3). In addition, for other stratification factors, we did not find any significant differences for associations between rs7326277 variant genotypes and risk of COPD or lung cancer in each stratum (homogeneity test $P > 0.05$ for all).

mRNA levels of VEGFR1 expression

As shown in Figure 1A, the mRNA level of *VEGFR1* was significantly lower in lung cancer tissues with rs7326277C genotypes than in those with rs7326277TT genotype ($P = 0.035$), but the difference in their adjacent normal tissues was approaching borderline statistical significance ($P = 0.069$).

Luciferase activity

As shown in Figure 1B, the transcription activity of the reporter gene integrating the *VEGFR1* 3'-UTR with rs7326277C allele was significantly lower than that with T allele both in A549 cell and in 16HBE cell (*P* values are both <0.001). However, because the selected microRNAs are not directly bound to this SNP in the 3'-UTR of *VEGFR1* according to bioinformatics analysis, the mimics and inhibitors of miR-339-5p and miR-136-5p failed to exert any effect on the reporter genes either with rs7326277T allele or C allele both in A549 cell and in 16HBE cell ($P > 0.05$ for all; supplementary Figure S1).

Discussion

In this study, we found that individuals carrying the rs7326277C variant genotypes of *VEGFR1* had a significant decrease in risk of both COPD and lung cancer compared with those carrying rs7326277TT in Chinese population. The protective effect of rs7326277C allele in the development of COPD or lung cancer was reconfirmed by the next biological assays including quantitative real-time-PCR and luciferase activity. However, no significant associations between other two SNPs (rs9554314T>G and rs664393C>T) and COPD or lung cancer were found. To best of our knowledge, this is the first study to reveal the associations between SNPs in the *VEGFR1* gene and risks of both COPD and lung cancer.

COPD and lung cancer have common risk factors and have been considered to have a common pathogenesis, such as the long-term inflammatory process (9,10) and the EMT (11). EMT was recently originally found in COPD and now has been regarded as a precancerous condition for a variety of epithelial cancers, including lung cancer (11,38,39). Recent studies demonstrated that *VEGFR1* promotes tumor growth, metastasis and inflammation (40,41). Meanwhile, *VEGFR1* was found to play a regulatory role on the process of EMT (24–26). Here, our results suggested that the rs7326277C variant genotypes would cause a low mRNA expression. The result was corresponding with the association that the variant could decrease the risk of both COPD and lung cancer.

In the stratification analysis, the association between the rs7326277C variant genotypes and risk of COPD and lung cancer were significant in non-smokers but not in smokers. In addition, the rs7326277C variant genotypes significantly interacted with non-smoking on decreasing COPD risk. This indicated

Table II. Stratification analysis of the VEGFR1 rs7326277T>C genotypes by selected variables in COPD patients and controls

	Controls (n = 1677)				Adjusted OR (95% CI)[a]	P_{homo}[b]	P_{inter}[d]
	TT, n (%)	CT+CC, n (%)	TT, n (%)	CT+CC, n (%)	CT+CC vs. TT		
Age (years)							
≤60	319 (45.3)	385 (54.7)	323 (40.4)	476 (59.6)	**0.78 (0.63–0.96)**	0.67	0.80
>60	357 (44.2)	450 (55.8)	333 (.9)	545 (62.1)	**0.77 (0.63–0.95)**		
Sex							
Male	392 (44.4)	491 (55.6)	406 (41.3)	577 (58.7)	0.87 (0.72–1.05)	0.08	0.07
Female	284 (45.2)	344 (54.8)	250 (36.0)	444 (64.0)	**0.68 (0.54–0.85)**		
Smoking status							
No	376 (47.2)	421 (52.8)	393 (38.3)	633 (61.7)	**0.69 (0.57–0.83)**	**0.04**	**0.04**
Yes	300 (42.0)	414 (58.0)	263 (40.4)	388 (59.6)	0.94 (0.76–1.17)		
Pack-years smoked							
0	375 (47.2)	419 (52.8)	391 (38.3)	631 (61.7)	**0.69 (0.57–0.83)**	0.05	0.06
<20	95 (43.2)	125 (56.8)	114 (41.9)	158 (58.1)	0.95 (0.66–1.36)		
≥20	206 (41.5)	291 (58.6)	151 (39.4)	232 (60.6)	0.91 (0.70–1.21)		
Drinking status							
No	548 (44.6)	680 (55.4)	525 (39.2)	816 (60.9)	**0.79 (0.67–0.92)**	0.86	0.88
Yes	128 (45.2)	155 (54.8)	131 (39.0)	205 (61.0)	0.75 (0.54–1.05)		
GOLD stages							
I	249 (44.2)	314 (55.8)	656 (39.1)	1021 (60.9)	**0.80 (0.66–0.97)**	0.77	0.79
II	242 (44.0)	308 (56.0)			**0.81 (0.66–0.98)**		
III	91 (48.7)	96 (51.3)			**0.68 (0.50–0.94)**		
IV	32 (43.2)	42 (56.8)			0.90 (0.56–1.45)		

Bold type: statistically significant, $P < 0.05$.

[a]ORs were adjusted for age, sex, smoking status and alcohol use in a logistic regression models.

[b]*P* value for the homogeneity test in each stratum was tested by the Breslow–Day test.

[c]*P* value of test for the multiplicative interaction between the rs7326277T>C genotypes and selected variables on COPD risk in logistic regression models.

Table III. Stratification analysis of the *VEGFR1* rs7326277T>C genotypes by selected variables in lung cancer patients and controls

	Patients (n = 1559)		Controls (n = 1679)		Adjusted OR (95% CI)[a]	P_{homo}[b]	P_{inter}[c]
	TT, n (%)	CT+CC, n (%)	TT, n (%)	CT+CC, n (%)	CT+CC vs. TT		
Age (years)							
≤60	361 (44.6)	448 (55.4)	357 (40.2)	530 (59.8)	0.80 (0.68–1.01)	0.65	0.64
>60	323 (43.1)	427 (56.9)	298 (37.6)	495 (62.4)	**0.78 (0.64–0.97)**		
Sex							
Male	476 (43.6)	615 (56.4)	467 (39.4)	718 (60.6)	**0.81 (0.70–0.98)**	0.76	0.84
Female	208 (44.4)	260 (55.6)	188 (38.1)	306 (61.9)	**0.77 (0.60–0.99)**		
Smoking status							
No	332 (45.2)	403 (54.8)	362 (39.6)	552 (60.4)	**0.79 (0.64–0.96)**	0.65	0.5
Yes	352 (42.7)	472 (57.3)	293 (38.3)	472 (61.7)	0.82 (0.77–1.01)		
Pack-years smoked							
0	332 (45.2)	403 (54.8)	362 (39.6)	552 (60.4)	**0.79 (0.64–0.96)**	0.69	0.62
<20	85 (42.5)	115 (57.5)	110 (38.5)	176 (61.5)	0.83 (0.58–1.21)		
≥20	267 (42.8)	357 (57.2)	183 (38.2)	296 (61.8)	0.81 (0.63–1.03)		
Drinking status							
No	563 (44.5)	703 (55.5)	529 (39.6)	808 (60.4)	**0.80 (0.68–0.95)**	0.70	0.68
Yes	121 (41.3)	172 (58.7)	126 (36.8)	216 (63.2)	0.82 (0.60–1.13)		
Pre-existing COPD							
No	592 (44.1)	750 (55.9)	594 (39.3)	916 (60.7)	**0.81 (0.69–0.95)**	0.71	0.66
Yes	92 (42.4)	125 (57.6)	61 (36.1)	108 (63.9)	0.76 (0.50–1.15)		
Histological types							
Adenocarcinoma	290 (47.2)	325 (52.8)	655 (39.0)	1024 (61.0)	**0.71 (0.59–0.86)**	0.57	–
Squamous cell carcinoma	232 (44.0)	295 (56.0)			0.88 (0.73–1.07)		
Large cell carcinoma	28 (42.4)	38 (57.6)			0.85 (0.51–1.41)		
Small cell lung cancer	74 (38.3)	119 (61.7)			1.01 (0.75–1.38)		
Other carcinomas	54 (37.5)	90 (62.5)			1.05 (0.74–1.50)		
Stages							
I	89 (44.5)	111 (55.5)	655 (39.0)	1024 (61.0)	0.78 (0.58–1.06)	0.62	–
II	66 (44.9)	81 (55.1)			0.77 (0.54–1.09)		
III	206 (42.0)	284 (58.0)			0.87 (0.70–1.07)		
IV	323 (44.7)	399 (55.3)			**0.78 (0.65–0.94)**		

Bold type: statistically significant, $P < 0.05$.
[a]ORs were adjusted for age, sex, smoking status and alcohol use in a logistic regression models.
[b]P value for the homogeneity test in each stratum was tested by Breslow–Day test.
[c]P value of test for the multiplicative interaction between rs7326277T>C genotypes and selected variables on lung cancer risk in logistic regression models.

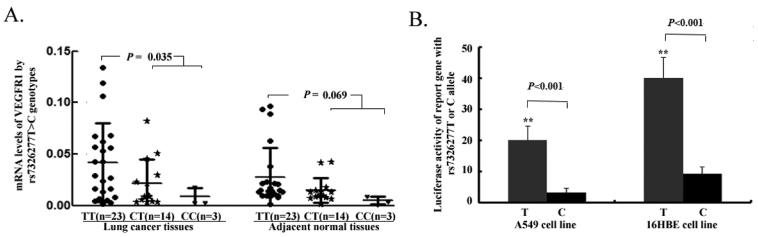

Fig. 1. (A) mRNA levels of *VEGFR1* expressions in lung tissues or adjacent normal tissues by rs7326277T>C genotypes; (B) the luciferase activity of the reporter gene integrated the *VEGFR1* 3'-UTR with rs7326277C allele or T allele in both A549 cell and 16HBE cell.

that the rs7326277C variant genotypes companying with healthy behaviours, such as non-smoking, would play a greater role on decreasing the development of COPD. However, such interaction effect was not found for lung cancer. Moreover, the study power for the significant interaction was relatively small (22.76%; two-sided test, α = 0.05). This might be due to the limited sample size. However, we could not rule out the possibility that the result was achieved by chance. So, validations in other ethnics and large samples are needed.

The SNPs of *VEGFR1* have been reported to be associated with risk and progression of several human diseases; there were several studies investigating on associations between the SNPs of *VEGFR1* and human diseases, such as breast cancer (28,42) and metastatic renal cell carcinoma (43). However, no published study on association between *VEGFR* SNPs and risk of lung cancer or COPD was reported. Moreover, although the GWAS conducted in Chinese or other population for lung cancer did not report any variants in *VEGFR1* as the top hits, which may be due to the stringent criterion of statistical significance ($P < 10^{-5}$), by querying the results of GWAS in Chinese we previously participated in, we found that the frequency of rs7326277T>C genotypes was significantly different between cases and controls (P = 0.016) (44,45). The results were corresponding to the results from this study. Interestingly, one GWAS reported one intron SNP rs9513106 of *VEGFR1* was significantly associated with risk of acute lung injury caused by infection in Americans (29), which is one of the risk factors for both lung cancer and COPD. All these evidences supported that the results found in this study were credible.

Our study has a number of strengths. First of all, we explored the associations between the same SNPs of *VEGFR1* gene and two related pulmonary diseases. As we know, COPD and lung cancer are the most striking smoking-related diseases, and COPD is considered to be an important

risk factor of lung cancer. In our study, this functional SNP rs7326277T>C shared by COPD and lung cancer supported an intrinsic linkage of smoking's effect on these diseases. Furthermore, the subjects carrying rs7326277C variant genotypes without pre-existing COPD would have a protective effect on lung cancer, indicating a possible predisposition to prevention of lung cancer in those genotypes carriers without COPD. Second, our study had two study sets representing two different populations so as to increase external validity. Third, we not only conducted an observational study but also performed consecutive functional assays to verify the biological effects of the SNPs.

There were some limitations in this study. Since the rs7326277 SNP is located in 3'-UTR of *VEGFR1* gene, we conducted the microRNA interference *in vitro* luciferase assays. Before conducting microRNA interference assays, we did not find any microRNA directly binding to this SNP according to bioinformatics analysis, so we selected two microRNAs near this SNP. Consequently, the mimics and inhibitors of selected two SNPs failed to exert any effect on the reporter genes either with rs7326277T allele or C allele in both A549 cell and 16HBE cell. So, we speculated that there could be another biological mechanism for decreasing the risk of COPD and lung cancer of rs7326277CT+CC variant genotypes. For instance, the genetic variant in 3'-UTR of *VEGFR1* gene may change the interaction between the promoter and 3'-UTR, thereby influencing the expression of mRNA level, which should be tested by further functional assays.

As a hospital-based case–control study, it is difficult to avoid information bias. However, with the large sample size and two study populations, we have achieved high statistical powers (88.0% for COPD, 99.7% for lung cancer) and the functional assays also confirmed the associations. Therefore, it appears that our finding is unlikely to have been achieved by chance.

In conclusion, this study found significant associations between a *VEGFR1* polymorphism and risk of COPD or lung cancer in Chinese population. The findings were consistent in COPD and lung cancer. Our results suggested that the genetic variation in *VEGFR1* gene may contribute to the development of both COPD and lung cancer in Chinese population. Validations with larger population-based studies in different ethnic groups are warranted to validate our findings, especially for the biological effects of the polymorphism on COPD.

Supplementary Data

Supplementary Appendix, Tables 1–3 and Figure 1 are available at *Mutagenesis* Online.

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