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Association of *ERBB4* genetic polymorphism with the risk and prognosis of non-small cell lung cancer in Chinese Han population

A population-based case–control study

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Abstract

The aim of this study was to explore the association of rs1836724 single-nucleotide polymorphism (SNP) of ERBB4 with risk and prognosis of non-small cell lung cancer (NSCLC) in the Chinese Han population.

The genotype of rs1836724 SNP of *ERBB4* from 258 patients with NSCLC and 200 noncancer controls were detected the TaqMan-MGB probes real-time fluorescence polymerase chain reaction. The distribution of genotype and alleles between the 2 groups was compared, and the association between clinicopathological characteristic and rs1836724 SNP was analyzed. Prognosis and influencing factors were analyzed by Kaplan-Meier and Cox regression analysis.

There were significant differences in the genotype and allele distribution of *ERBB4* rs1836724 between the NSCLC group and control group ($P < .05$). And CC genotype of rs1836724 was associated with increased risk of NSCLC in the Chinese Han population. Rs1836724 SNP was associated with TNM stage and lymph nodal metastasis ($P = .001$, $P = .007$). The median follow-up was 29 months, and the progression-free survival and overall survival of 258 NSCLC patients were 27.91% and 31.39%, respectively. Patients with GG genotype of rs1836724 had poor progression-free survival and overall survival. Rs1836724 SNP was an independent prognostic marker of NSCLC patients, CC genotype had a high risk of poor prognosis (odds ratio = 1.587, 95% confidence interval: 1.079–2.335, $P = .019$).

In Chinese Han populations, rs1836724 SNP of *ERBB4* may contribute toward the increased risk and poor prognosis of NSCLC.

Abbreviations: NSCLC = non-small cell lung cancer, OS = overall survival, PFS = progression-free survival, SNP = single-nucleotide polymorphism.

Keywords: *ERBB4*, non-small cell lung cancer, prognosis, risk

1. Introduction

Lung cancer is the leading cause of cancer-related morbidity and mortality in the worldwide for both men and women.[1] It was reported that the number of new lung cancer was 2.1 million and 1.8 million deaths predicted in 2018.[2] Non-small cell lung cancer (NSCLC) is the main histopathologic type of lung cancer, accounting for 80% of all lung cancer cases.[3] There are no obvious clinical manifestations in the early stage of NSCLC, and metastasis has already occurred in most NSCLC patients when they were diagnosed. Despite therapeutic progress, NSCLC patients remain have a poor prognosis, and the 5-year survival rate is only approximately 20%.[4] So it is of utmost importance to identify a novel marker of the early diagnosis and prognosis for patients with NSCLC.

ERBB4 is one of the member of epidermal growth factor receptor subfamily, which has been found to be closely related to the growth, development of cells, and play important roles in the occurrence and development of various tumors.[5] And *ERBB4* is emerging as an increasingly important player in the genesis and progression of human cancers. *ERBB4* is abnormally expressed in various human cancers, which may strongly link to the growth and survival of cancers, such as breast cancer, lung cancer, ovary cancer, and colon cancer.[6–9] The previous studies found that *ERBB4* increases the proliferation potential of lung cancer cells,[10] and serum *ERBB4* mRNA expression was associated with distant metastasis and TNM stages, as one of the predictive

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factor for metastasis and poor overall survival of lung adenocarcinoma patients.[11] And it was reported that *ERBB4* polymorphism might contribute to increased risk of various human cancers, such as colorectal and breast cancers,[12,13] hepatocellular carcinoma,[14] and esophageal squamous cell carcinoma.[15] However, rarely studies have examined the significance of *ERBB4* polymorphism on the risk and prognosis of NSCLC.

The present study was aimed to explore the correlation of the single-nucleotide polymorphism (SNP) of *ERBB4*, rs1836724, which might be the potential target sequence of miRNA, with the risk and prognosis of NSCLC in the Han Chinese population.

2. Materials and methods

2.1. Subjects

According to the reference to obtain the expected relative risk and minor allele frequency, the sample size was calculated with the calculation formula in the ⟨Medical statistics⟩.[16,17] A total of 258 patients who diagnosed as NSCLC at Changzhi People's Hospital from January, 2015 to December 2017 were collected as the NSCLC group, including 173 males and 85 females. Including criteria were: underwent surgical resection, and treated with routine chemotherapy after the operation; did not receive chemotherapy or radiotherapy before surgical resection; unrelated Han Chinese; w0ithout previous malignant disease or other primary tumor. All patients had complete clinical information and postoperative pathological reports including the pathological types, TNM stages, and the degrees of tumor differentiation. The pathological types and TNM stages were determined based on classification and pathology of lung cancer.[18] A total of 258 NSCLC patients were followed up every 2 months in the first year after surgery and every 3 to 6 months thereafter by routine examination of outpatient or phone calls. Recurrence and survival status were recorded by regular rechecks and end point of follow-up was defined as January 2020.

Two hundred noncancer and unrelated controls, consisting of 120 males and 80 females who underwent physical examination, were randomly selected as control group, during the same period. The basic information on the age, sex, smoking history, or family history of all participants was obtained from hospital records. All participants signed the informed consent and the present study was approved by the ethics committee of People's Hospital of Changzhi.

2.2. SNP genotyping

Peripheral venous blood samples from each participant were collected to analyze genotyping. Genomic DNA was extracted from peripheral venous blood using Takara blood genome DNA extraction Kit in accordance with the manufacturer's protocol. The specific primer and TaqMan MGB double fluorescent probes that countered rs1836724 SNP were designed and synthesized by ABI company. Genomic DNA as the template and sterilized water as negative control, SNP genotypes of rs1836724 were detected on Applied Biosystems 7500 sequence Detection System (ABI, USA) by real-time fluorescence polymerase chain reaction. And the genotype was validated by gene sequencing methods.

2.3. Statistical analysis

SPSS19.0 software (SPSS Inc, Chicago, IL) was used to analyze the data. The characteristics and genotype frequencies between NSCLC group and control group were compared using χ^2 or independent t test. χ^2 value was calculated to examine the Hardy-Weinberg equilibrium. The correlation between genotype and characteristics of NSCLC patients was analyzed by χ^2 test. Survival curves were estimated with Kaplan-Meier method and compared with the log-rank test. Cox regression analysis was performed to analyze the potential prognostic factors for NSCLC patients. $P < .05$ was considered significant difference.

Table 1

Characteristics between NSCLC group and control group.

	NSCLC group (n = 258)	Control group (n = 200)	χ^2/t	*P*
Age, y ()	56.38 ± 8.39	55.15 ± 8.94	1.518	.130
BMI, kg/m^2 ($\overline{x} \pm s$)	23.12 ± 1.30	23.35 ± 1.45	1.504	.133
Sex (male/female)	174/84	120/80	2.714	.099
Smoking, n (%)	140 (54.3)	88 (44.0)	4.747	.029
Family history, n (%)	72 (27.9)	36 (18.0)	6.136	.013

BMI = body mass index, NSCLC = non-small cell lung cancer.

3. Results

3.1. Characteristics between patients with NSCLC and controls

The characteristics of the patients with NSCLC and normal controls were displayed in Table 1. There was no significant difference in age, BMI, and sex between NSCLC group and control group, whereas the smoking and family history of NSCLC was significantly higher in the NSCLC group than that in the control group ($P < .05$).

3.2. Association of ERBB4 SNP with risk of NSCLC

The distributions of genotype and alleles of rs1836724 of *ERBB4* in NSCLC group and control group were shown in Table 2. The genotype frequencies in the controls were both in agreement with the Hardy-Weinberg equilibrium ($P > .05$). The genotype frequency of CC in NSCLC patients was higher than that in control group ($P < .05$). And Logistic regression analysis revealed that CC genotype of rs1836724 was correlated with a significant increased risk of NSCLC after adjustment of general data, and C allele of rs1836724 had a higher risk of NSCLC, respectively. The results indicated that the rs1836724 of *ERBB4* was potentially contributed to the increased risk of NSCLC in the Chinese Han population.

3.3. Association of ERBB4 SNP with clinicopathological characteristics in NSCLC patients

The distribution of demographic factors and clinicopathological characteristics in different genotype of rs1836724 in NSCLC patients were presented in Table 3. There was a significant association between rs1836724 SNP and TNM stage, lymph nodal metastasis ($P = .001$ and $P = .007$, respectively), whereas there was no significant correlation between rs1836724 SNP and demographic factors.

3.4. The effect of ERBB4 SNP on prognosis of NSCLC

A total of 258 NSCLC patients were followed up for 11 to 60 months, and the median follow-up was 29 months. Kaplan-Meier survival curves and log-rank test of progression-free survival

Table 2

Distribution of genotypes and alleles of rs1836724 of *ERBB4* in NSCLC group and control group, n (%).

		NSCLC group (n = 258)	Control group (n = 200)	*P*	OR (95% CI)	*P*
Genotype	TT	94 (36.43)	78 (39.00)	.014	1	
	TC	100 (38.76)	94 (47.00)		0.883 (0.585–1.332)	.553
	CC	64 (24.81)	28 (14.00)		1.897 (1.110–3.242)	.019
Alleles	T	288 (55.81)	250 (62.50)	.041	1	
	C	228 (44.19)	150 (37.50)		1.274 (1.105–1.831)	.045

CI = confidence interval, NSCLC = non-small cell lung cancer, OR = odds ratio.

(PFS) and overall survival (OS) were used to examine the effect of rs1836724 of *ERBB4* on prognosis of NSCLC patients. The rates of PFS and OS were 27.91% (72/258) and 31.39% (81/258), respectively. As shown in Figure 1, the Kaplan-Meier survival curves displayed that patients with GG genotype of rs1836724 had poor PFS and OS, compared with CC genotype carriers(all $P < .05$).

To test whether the rs1836724 was an independent prognostic marker for the prognosis of the NSCLC patients, the univariate and multivariate Cox regression model was used to identify markers that predict the prognosis of NSCLC. As shown in Table 4, univariate Cox regression indicated that high TNM stage, and CC genotype of rs1836724 had a higher risk of recurrence and death, which was associated with poor prognosis.

Furthermore, multivariate Cox regression (forward: condition) adjusted for statistically significant prognostic markers was performed to control the impact of TNM stage, analyzing whether rs1836724 was still associated with the prognosis (Table 5). The results showed that the risk of recurrence in patients with CC was 1.587-fold, higher than TT carriers (95% confidence interval 1.083–2.288; $P = .017$), and the risk of death for the CT and CC genotype were higher than that for TT genotype. The result indicated that rs1836724 SNP was an independent prognostic marker for NSCLC patients, and patients with CC genotype had a higher risk of poor prognosis. In addition, TNM stage also was an independent prognostic marker for NSCLC patients.

Table 3

Association between rs1836724 of *ERBB4* and clinicopathological characteristic in NSCLC patients.

Index	TT (94)	TC (100)	CC (64)	*P*
Age, y				.575
<60	59	68	45	
≥60	35	32	19	
BMI, kg/m^2				.429
<23	27	36	24	
≥23	67	64	40	
Sex				.268
Male	67	69	38	
Female	27	31	26	
Smoking				.728
Yes	54	53	33	
No	40	47	31	
Family history				.290
Yes	28	31	13	
No	66	86	54	
Pathological type				.371
Adenocarcinoma	41	46	35	
Squamous	53	54	29	
TNM stage				.001
I + II	55	68	25	
III + IV	39	32	39	
Tumor size, cm				.247
≤3	53	58	29	
>3	41	42	35	
Lymph node metastasis				.007
Yes	35	34	37	
No	59	66	27	

BMI = body mass index, NSCLC = non-small cell lung cancer.

4. Discussion

The epidermal growth factor receptors (EGFRs) tyrosine kinase, especially EGFR and ERBB2, are over-expressed or somatically mutated in many cancers, such as breast cancer, ovarian cancer, and lung cancer.[19] ERBB4, as a member of EGFR tyrosine kinase subfamily, its expression, and mutations are also involved in the genesis and progression of many human cancers. For example, Donoghue et al[20] found that increased ERBB4 activation was associated with increased proliferation, angiogenesis, tumorigenicity, which might be a potential prognostic and therapeutic target in glioblastoma. Kim et al[21] indicated that increased expression of ERBB4 was associated with poor prognosis of breast cancer, and ERBB4 expression was identified as an indicator of long-term prognosis in patients with triple negative breast cancer. Some researches found that multiple SNPs of the *ERBB4* were associated with the diseases including cancers. Kurppa et al[22] found that the promoter polymorphism of *ERBB4* were associated with the risk of poor distant disease-free survival of breast cancer. Yu et al[14] suggested that rs6147150 of *ERBB4* might be involved in the pathogenesis of hepatocellular carcinoma, and Gao et al[23] also verified that rs6147150 would potentially be a novel biomarker for colorectal cancer susceptibility. The presence of activating driver mutations of *ERBB4* has been demonstrated in in adenocarcinoma of NSCLC, which provided an evidence for the oncogenic role of *ERBB4* in lung cancer. However, it was not clear about the association between *ERBB4* polymorphism and increased risk or poor prognosis of NSCLC.

In the present study, the SNP of *ERBB4*, rs1836724 was detected to analyze the association between the polymorphism of *ERBB4* and NSCLC. The results found that NSCLC patients had higher frequencies of CC genotype of rs1836724 than normal controls, suggesting rs1836724 of *ERBB4* contributed to the increased risk of NSCLC. Although there was correlation between TNM stage and lymph nodal metastasis, rs1836724 SNP was associated with TNM stage and lymph nodal metastasis, respectively, in patients with NSCLC. Kaplan-Meier survival curves and log-rank test indicated that NSCLC patients with CC genotype of rs1836724 had poor PFS and OS, than TT

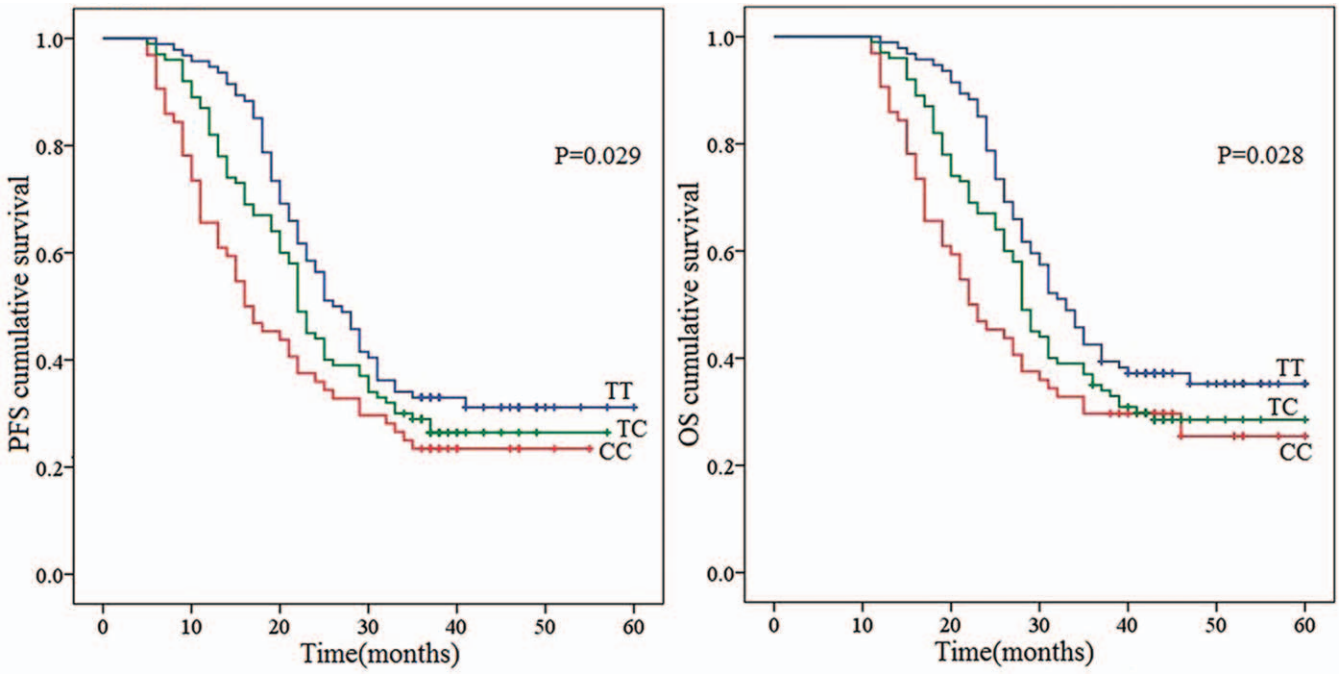

Figure 1. Kaplan-Meier curves of PFS and OS in non-small cell lung cancer patient with respect to rs1836724 of *ERBB4*. OS = overall survival, PFS = progression-free survival.

genotype. And multivariate Cox regression revealed CC genotype of rs1836724 was an independent risk factor for prognosis of NSCLC. In the existing studies, it was verified that rs1836724 polymorphism was associated with the risk and prognosis of other cancers. For example, Bagheri et al[24] demonstrated that rs1836724 polymorphism in 3'-UTR of *ERBB4* was associated with the risk of breast cancer. Wei et al[25] indicated the ERBB4 rs1836724 polymorphism, which impacted the miRNA binding sites, was associated with the overall survival of ovarian cancer. To the best of our knowledge, this is the first study to demonstrate

Table 4

Univariate Cox regression analysis in NSCLC patients.

		PFS		OS	
		OR (95% CI)	*P*	OR (95% CI)	*P*
Age, y	<60				
	≥60	1.018 (0.750–1.381)	.909	1.068 (0.779–1.464)	.682
BMI, kg/m^2	<23				
	≥23	1.244 (0.923–1.677)	.152	1.331 (0.983–1.803)	.064
Sex	Male				
	Female	1.088 (0.798–1.484)	.593	1.093 (0.795–1.503)	.583
Smoking	Yes				
	No	0.917 (0.686–1.224)	.555	1.089 (0.810–1.465)	.571
Family history	Yes				
	No	0.723 (0.518–1.008)	.056	0.757 (0.539–1.062)	.107
Pathological type	Adenocarcinoma				
	Squamous	1.067 (0.800–1.423)	.660	1.066 (0.793–1.432)	.674
TNM stage	I + II				
	III + IV	1.485 (1.113–1.982)	.007	1.550 (1.154–2.083)	.004
Tumor size, cm	≤3				
	>3	1.020 (0.764–1.361)	.895	1.036 (0.771–1.392)	.814
Lymph node metastasis	No				
	Yes	1.217 (0.910–1.627)	.185	1.274 (0.947–1.714)	.110
rs1836724	TT		.035		.034
	TC	1.251 (0.894–1.751)	.146	1.012 (0.828–1.238)	.904
	CC	1.637 (1.128–2.376)	.010	1.280 (1.023–1.603)	.031

BMI = body mass index, CI = confidence interval, NSCLC = non-small cell lung cancer, OR = odds ratio; OS = overall survival; PFS = progression-free survival.

Table 5

Conditional multivariate Cox regression analysis in NSCLC patients.

		PFS		OS	
		OR (95% CI)	*P*	OR (95% CI)	*P*
TNM stage	I + II				
	III + IV	1.504 (1.113–2.032)	.008		.003
rs1836724	TT		.042		.034
	TC	1.373 (0.974–1.937)	.071	1.455 (1.023–2.068)	.037
	CC	1.574 (1.083–2.288)	.017	1.587 (1.079–2.335)	.019

CI = confidence interval, NSCLC = non-small cell lung cancer, OR = odds ratio; OS = overall survival; PFS = progression-free survival.

the association between rs1836724 of *ERBB4* and risk, prognosis of NSCLC, which provide a new direction for the prevention, treatment, and prognosis of NSCLC.

However, there are still some limitations to be considered, including the small sample size which might lead to the deviation of results. A large population size in Han or other ethnic populations should be validated in follow-up studies to determine the significance of *ERBB4* in NSCLC. And many NSCLC patients, who only received chemotherapy or received chemotherapy before surgical surgical resection, were ruled out. To better verify the association of ERBB4 SNP with the risk of NSCLC, all primary NSCLC patients should be collected to analyze. Second, the exact molecular mechanism of the effect of rs1836724 of *ERBB4* on the risk, prognosis of NSCLC still need to be revealed in the further studies. In addition, There were many marginally significant results (*P* value between .01 and .05), which might affect the credibility of the results. So the correlation between ERBB4 and risk or prognosis of NSCLC should be correlation for multiple testing in a large population in the further study.

In conclusion, SNP of *ERBB4,* rs1836724, may contribute to the increased risk and poor prognosis of NSCLC in the Chinese Han population.

Author contributions

Conceptualization: Wan-ping Wang.
Data curation: Xia-zhen Wang, Ding Wei.
Investigation: Hai-bo Bian.
Validation: Liang Liu.
Writing – original draft: Wan-ping Wang.
Writing – review & editing: Hai-bo Bian, Xia-zhen Wang, Liang Liu, Ding Wei.

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