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Original Article

PI3K expression predicts overall survival in lung adenocarcinoma

Lei Li[1], Dan Liu[1], Jing Jin[1], Yingying Zhu[1], Lan Yang[1], Juan Song[1], Weimin Li[1], Yan Huang[2]

[1]*Department of Pulmonary and Critical Care Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan, China;* [2]*Health Management Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China*

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Abstract: Background: Phosphoinositide 3-kinase (PI3K)/phosphorylated PI3K (p-PI3K) is considered a hallmark in tumor initiation and progression, but its prognostic value in non-small cell lung cancer (NSCLC) remains controversial. Methods: In the present study, we included 118 NSCLC tissue samples and 13 adjacent normal lung tissue specimens. Immunohistochemical staining was applied to test PI3K/p-PI3K expression. Pearson Chi-squared test and Kaplan-Meier curve were conducted to analyze its correlation with both clinicopathological features and prognosis in NSCLC patients. Results: PI3K/p-PI3K expression in lung cancer tissue differed significantly from that of normal lung tissue ($P < 0.001$). M stage was significantly correlated to PI3K expression ($P = 0.037$), but no significant association was found between p-PI3K expression and clinical characteristics. Neither PI3K nor p-PI3K were correlated to overall survival of NSCLC patients ($P = 0.105$ and $P = 0.190$, respectively). However, it was found in subgroup analysis that lung adenocarcinoma patients with positive PI3K expression had a favorable survival ($P = 0.043$). Notably, this correlation was determined invalid in subsequent multivariate analysis ($P = 0.052$). Conclusions: PI3K could predict the overall survival of lung adenocarcinoma patients, but failed to be an independent prognostic predictor.

Keywords: Immunohistochemistry, non-small cell lung cancer, PI3K, p-PI3K, survival

Introduction

Lung cancer is one of the most frequent malignancies and ranks as the leading cause of cancer-related death worldwide [1, 2]. During the past few decades, various new targets and novel therapies were discovered to improve the outcome of lung cancer, but minimal progress had been achieved [3, 4]. According to data collected from 2004 to 2010 on a global scale, the 5-year survival rate of lung and bronchus cancer remained less than 18% [5]. Recently, increasing numbers of studies gradually focused on exploring reliable markers of prognosis, especially proteins in oncogenic signaling pathways. Such markers were hoped to guide the rational treatment and surveillance for lung cancer patients.

Phosphoinositide 3-kinase (PI3K) is considered as a hallmark of cancer. There are 3 types of PI3K enzymes, including class I, class II and class III [6, 7]. Among them, class I PI3K is the research hotspot nowadays. It has 4 isoforms (p110α, p110β, p110γ and p110δ, encoded by PIK3CA, PIK3B, PIK3G and PIK3D). The former 2 types are expressed ubiquitously, while the later 2 types are only in immune cells [8]. The activation of PI3K can be triggered by growth factor, receptor-coupled tyrosine kinase, and Ras-related GTPases. Activated PI3K is able to catalyze the generation of phosphatidylinositol-3, 4, 5-trisphosphate (PIP_3), a critical lipid second messenger [9, 10]. PIP_3 will then regulate survival, metabolism, proliferation, invasion and apoptosis through several critical effectors, including protein kinase B (AKT) [11, 12], PDK1 (3-phosphoinositide-dependent protein kinase 1)-mTORC2 (mechanistic target of rapamycin complex 2)-SGK (serum and glucocorticoid-regulated kinase) axis [13, 14], Rac signaling pathway [15, 16] and TEC family kinase [17-19]. PI3K as well as PIP_3 play important roles in driving tumor initiation and progression. In normal cells, PIP_3 degrades rapidly and terminates PI3K signaling pathways. But in cancer cells, its amount increases intensively due to elevated activities of PI3K's upstream

oncogenic proteins, activated mutation of PI3K itself, and loss of related tumor suppressors (such as PTEN) [20, 21].

Previous studies have noted altered expression of PI3K in different types of cancers, including esophageal squamous cell carcinoma [22], renal cell carcinoma [23], laryngeal cancer [24], gastric cancer [25], esophageal squamous cell carcinoma [26, 27] and hepatocellular carcinoma [28]. It usually correlated with clinicopathologic features like tumor size, lymphatic metastasis, and stage. It was also demonstrated that PI3K had the potential to be a prognostic predictor of several cancers [29], esophageal squamous carcinoma [27] and prostate cancer [30]. However, its prognostic value in NSCLC still remained elusive.

In the present study, we explored the expression of both PI3K and phosphorylated PI3K (p-PI3K) in NSCLC tissue samples as well as adjacent normal pulmonary tissue specimens by immunohistochemical staining. Additionally, we collected the clinicopathological and prognostic information of these patients, then assessed their correlation with PI3K/p-PI3K expression.

Materials and methods

Tissue collection

From January 2008 to December 2013, 251 NSCLC patients with a median age of 60 years old (range, 34 to 86 years old) were enrolled in this study. Their lung cancer tissue samples were obtained from Pathology Department of West China Hospital, Sichuan University. Additionally, adjacent normal pulmonary tissue specimens (4-5 μm sections) were also resected in the same block if available. Considering incomplete information, tissue deficiency and loss to follow-up, only 118 NSCLC tissue samples and 13 normal lung tissue specimens were finally included.

Clinical information of these patients, such as age, gender, pathologic type, differentiation and tumor size, were collected from the electronic medical records. Staging was conducted independently by two sophisticated physicians, according to tumor-node-metastasis system of International Union Against Cancer [31]. All patients were treatment- naïve prior to resection and underwent standard therapy after surgery according to National Comprehensive Cancer Network Clinical Practice Guidelines for Oncology, NSCLC, 2004 [32]. Institutional review board approval for the present study was obtained from West China Hospital, and written informed consent was acquired from every participant enrolled in this study.

Immunohistochemical staining (IHC)

Immunohistochemical analysis was conducted to explore altered expression of PI3K/p-PI3K in NSCLC tissue. All of these tissue samples were obtained during surgery. Subsequently, the formalin-fixed, paraffin-embedded tissue were conducted within 12 to 24 hours after surgery. Before IHC, sections were freshly cut and finished the procedures of deparaffinization, hydration and blocking. Then, sections were incubated in a moist chamber with specific primary antibody against PI3K (ab191606, Abcam) or p-PI3K (ab182651, Abcam) at 4°C overnight. After washing, the membranes were incubated with secondary antibody (goat anti-rabbit IgG, Dako, Shanghai, China) at room temperature for 30 min.

Scoring system

The PI3K/p-PI3K immunoreactivity was evaluated by two sophisticated pathologists independently, according to a previously described semi-quantitative system. This scoring system took both the fraction and intensity of immunostaining into account. The intensity scores were divided into 4 types: 3 (dark brown), 2 (readily appreciable brown), 1 (barely detectable) and 0 (no appreciable). The fraction scores were also divided into 4 types: 3 (defined as > 50% positive cells), 2 (defined as 20-50% positive cells), 1 (defined as 10-20% positive cells) and 0 (defined as < 10% positive cells). The total score was determined by multiplying both of the scores. A tissue sample was considered as positive if the score was ≥ 2.

Statistical analysis

Pearson Chi-square test was used to determine the correlation between PI3K/p-PI3K expression and clinicopathological characteristics of the patients. Simultaneously, Kaplan-Meier curve was conducted to identify patients' survival according to PI3K/p-PI3K expression, and survival based on clinical characteristics were performed using Cox regression model (univariate). Additionally, multivariate analysis was also conducted using Cox regression model (multi-

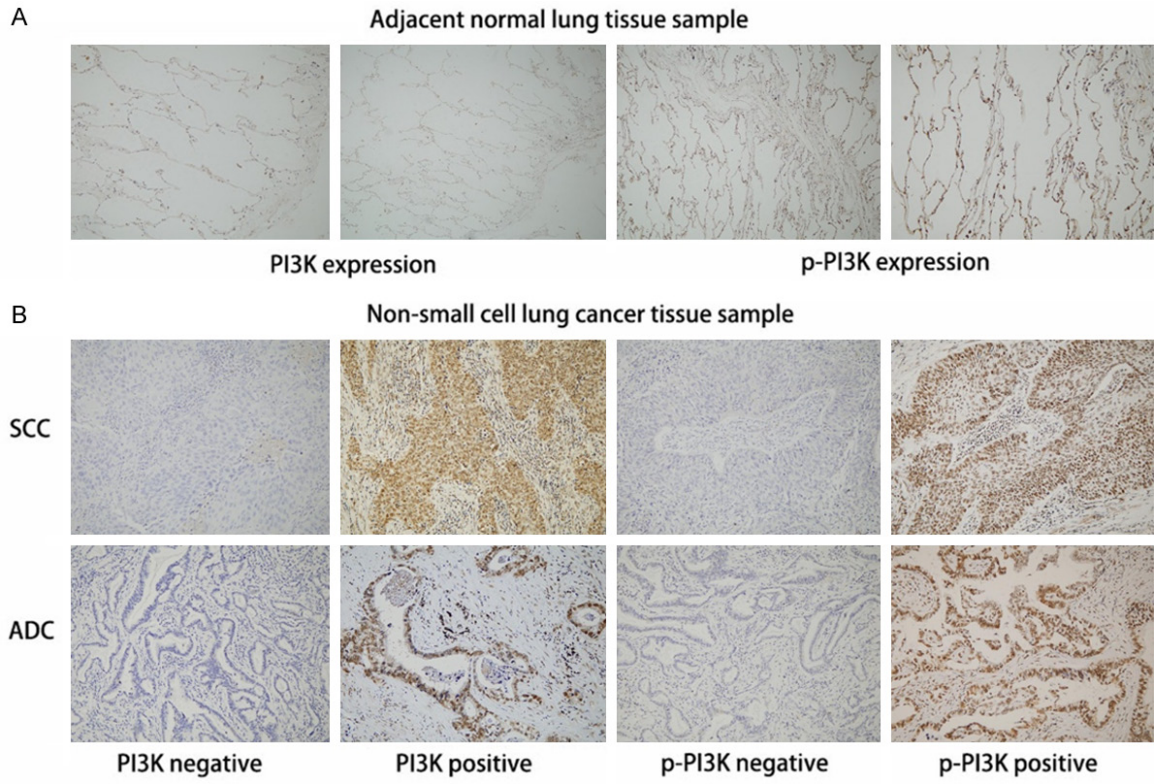

Figure 1. PI3K and p-PI3K expression levels in adjacent normal lung tissue samples (A) and non-small cell lung cancer tissue samples (B) are shown. (B) Immunohistochemical staining of negative and positive in both SCC and ADC are shown for each protein. Original magnification, ×400.

Table 1. Expression levels of PI3K and p-PI3K in NSCLC tissue samples and adjacent normal lung tissue samples

Protein	Expression level	Lung cancer tissue No. (%)	Normal lung tissue No. (%)	*P* value
PI3K	N	98 (83.1)	8 (61.5)	< 0.001*
	P	20 (16.9)	5 (38.5)	
p-PI3K	N	24 (20.2)	0 (0.0)	< 0.001*
	P	94 (79.7)	13 (100.0)	

N, negative; P, positive. *: p < 0.001, with statistical significance.

variate). Only significant markers identified in univariate analysis were included in this procedure. All statistical analysis was conducted in SPSS 16.0 (SPSS Inc., Chicago, IL, USA). P < 0.05, was considered significant.

Results

PI3K and p-PI3K expression in non-small cell lung cancer tissue and normal lung tissue samples

Typical IHC staining images of PI3K/p-PI3K expression in lung cancer tissue samples and normal lung tissue samples are shown in **Figure 1**. In NSCLC tissue samples, 16.9% (20/118) exhibited positive PI3K expression, while 79.7% (94/118) exhibited positive p-PI3K expression (**Table 1**). In contrast, 38.5% (5/13) were PI3K positive and 100.0% (13/13) were p-PI3K positive in normal lung tissue samples (**Table 1**). The differences between NSCLC tissue and normal lung tissue were highly significant (both P < 0.001).

Relationships between PI3K/p-PI3K expression and clinicopathologic characteristics

The main clinicopathologic features of 118 NSCLC patients are summarized in **Table 2**. In the present study, only M stage was proven significantly correlated to PI3K expression (P = 0.037). It was found that the PI3K positive rate increased significantly in NSCLC patients with distant metastasis. Notably, the p-PI3K positive rate also increased in NSCLC patients with distant metastasis, and no negative p-PI3K expression was found in the M0 group, but the relationship was not significant (P = 0.352). Nonetheless, no significant correlations were

Table 2. Correlations between PI3K/p-PI3K expression and clinicopathological features of 118 NSCLC patients

Variables		PI3K		*P* value	p-PI3K		*P* value
		Negative (n = 98)	Positive (n = 20)		Negative (n = 24)	Positive (n = 94)	
Age	≤ 60 (n = 64)	50 (78.1)	14 (21.9)	0.121	15 (23.4)	49 (76.6)	0.363
	> 60 (n = 54)	48 (88.9)	6 (11.1)		9 (16.7)	45 (83.3)	
Gender	Male (n = 82)	69 (84.1)	13 (15.9)	0.632	60 (35.9)	107 (64.1)	0.873
	Female (n = 36)	29 (80.6)	7 (19.4)		7 (19.4)	29 (80.6)	
Histology	ADC (n = 58)	52 (89.7)	6 (10.3)	0.124	16 (27.6)	42 (72.4)	0.067
	Non-ADC (n = 58)	46 (79.3)	12 (20.7)		8 (13.8)	50 (86.2)	
	Missing (n = 2)	0 (0.0)	2 (100.0)		0 (0.0)	2 (100.0)	
Differentiation	Poor (n = 33)	29 (87.9)	4 (12.1)	0.133	7 (21.2)	26 (78.8)	0.298
	Well/moderate (n = 54)	52 (96.3)	2 (3.7)		17 (31.5)	37 (68.5)	
	Missing (n = 31)	17 (54.8)	14 (45.2)		0 (0.0)	31 (100.0)	
pT stage	1/2 (n = 79)	66 (83.5)	13 (16.5)	0.244	20 (25.3)	59 (74.7)	0.122
	3/4 (n = 27)	25 (92.6)	2 (7.4)		3 (11.1)	24 (88.9)	
	Missing (n = 12)	7 (58.3)	5 (41.7)		1 (8.3)	11 (91.7)	
pN stage	0 (n = 60)	52 (85.0)	9 (15.0)	0.775	12 (20.0)	48 (80.0)	0.628
	1/2/3 (n = 46)	40 (87.0)	6 (13.0)		11 (23.9)	35 (76.1)	
	Missing (n = 12)	7 (58.3)	5 (41.7)		1 (8.3)	11 (91.7)	
pM stage	0 (n = 103)	89 (86.4)	14 (13.6)	0.037*	23 (22.3)	80 (77.7)	0.352
	1 (n = 3)	2 (66.7)	1 (33.3)		0 (0.0)	3 (100.0)	
	Missing (n = 12)	7 (58.3)	5 (41.7)		1 (8.3)	11 (91.7)	
Stage	1/2 (n = 69)	60 (87.0)	9 (13.0)	0.655	17 (24.6)	52 (75.4)	0.917
	3/4 (n = 37)	31 (83.8)	6 (16.2)		6 (16.2)	31 (83.8)	
	Missing (n = 12)	7 (58.3)	5 (41.7)		1 (8.3)	11 (91.7)	

ADC, adenocarcinoma; Non-ADC, non-adenocarcinoma, mainly including squamous cell carcinoma, adenosquamous carcinoma, and large cell carcinoma; *, P < 0.05.

Table 3. Univariate Cox regression analysis of overall survival in lung adenocarcinoma patients

Variables	HR	*P* value	95% CI
Gender (male vs. female)	1.737	0.151	0.818-3.687
Age (≤ 60 vs. > 60 years old)	0.825	0.604	0.399-1.706
Differentiation status (low vs. moderate to well)	1.170	0.782	0.483-2.838
T stage (1/2 vs. 3/4)	0.498	0.107	0.214-1.162
N stage (N0 vs. N1/N2/N3)	0.316	0.024*	0.116-0.860
M stage (M0 vs. M1)	0.832	0.794	0.192-3.536
TNM stage (1/2 vs. 3/4)	0.564	0.168	0.250-1.273

*, P < 0.05.

found between PI3K/p-PI3K expression and other characteristic, including age, gender, histologic type, differentiation, and TNM stage.

Correlations between clinicopathological characteristics and prognosis of NSCLC patients

The results of Cox regression analysis (univariate) are shown in **Table 3**. The survival time differed significantly by T stage (P = 0.004), N stage (P = 0.001) and TNM stage (P = 0.002). Other features, including gender (P = 0.303), age (P = 0.600), histological types (P = 0.727), differentiation (P = 0.509) and M stage (P = 0.844) were not significantly correlated to NSCLC patients' survival.

PI3K/p-PI3K expression and overall survival of NSCLC patients

The correlations between PI3K/p-PI3K expression and NSCLC patients' 5-year median survival rate were determined by Kaplan-Meier curve, (**Figure 2**). Neither PI3K (P = 0.105) nor p-PI3K (P = 0.190) were significant prognostic factors for NSCLC patients.

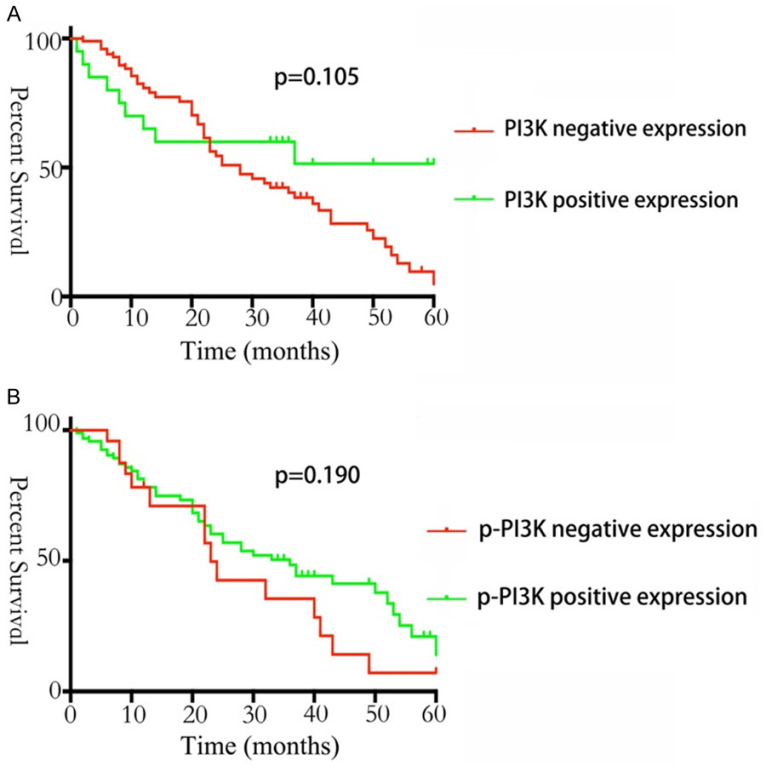

Figure 2. Correlation between PI3K/p-PI3K expression and overall survival in non-small cell lung cancer (NSCLC). A. Survival of PI3K negative and positive expression; B. Survival of p-PI3K negative and positive expression.

Subsequently, we performed subgroup analysis according to clinical characteristics in NSCLC patients with positive/negative PI3K expression (**Figure 3**). It was determined that PI3K could serve a prognostic factor for favorable survival in lung adenocarcinoma patients (P = 0.043), but no significant relationship was found in patients with other pathologic types (P = 0.581). Similarly, no significant correlations were found in other subgroups, including male (P = 0.256), female (P = 0.303), poor differentiation (P = 0.765), well/moderate differentiation (P = 0.234), N0 (P = 0.780), N1/2/3 (P = 0.826), ≤ 60 years old (P = 0.175), > 60 years old (P = 0.437), stage 1/2 (P = 0.396), stage3/4 (P = 0.608).

In the subgroup analysis of NSCLC patients with negative/positive p-PI3K analysis, no significant associations were found between p-PI3K expression and patients' 5-year median survival rate (**Figure 4**).

Multivariate analysis

Multivariate analysis was conducted to further determine the prognostic values of PI3K expression in lung adenocarcinoma patients. Other variables included in this analysis were clinical features proved to be significant in Cox regression analysis (univariate). As shown in **Table 3**, only N stage was eligible (P = 0.024). Other factors, including gender (P = 0.151), age (P = 0.604), differentiation (P = 0.728), T stage (P = 0.107), M stage (P = 0.794) and TNM stage (P = 0.168) were excluded.

In multivariate analysis (**Table 4**), no independent prognostic factors for lung adenocarcinoma patients were determined. Neither PI3K expression (P = 0.052) nor N stage (P = 0.184) were significantly correlated to 5-year survival of lung adenocarcinoma patients.

Discussion

PI3K plays a critical role in driving tumor initiation and progression [8]. In the present study, both PI3K and p-PI3K expression were explored in 118 NSCLC tissue samples and 13 adjacent normal pulmonary tissue specimens. In NSCLC tissue samples, the positive rates of PI3K and p-PI3K were 16.9% (20/118) and 79.7% (94/118), respectively; while in normal tissue samples, the positive rates were 38.5% (5/13) and 100% (13/13). It was determined that the expression of PI3K (P < 0.005) and p-PI3K (P < 0.05) were significantly decreased in NSCLC tissue. Additionally, we also calculated the correlations between PI3K/p-PI3K expression and clinical features of lung cancer patients, including age, gender, histologic types, differentiation, T stage, N stage, M stage and TNM stage. Only M stage was significantly correlated to PI3K expression (P = 0.037). In survival analysis, neither PI3K (P = 0.105) nor p-PI3K (P = 0.190) were significantly correlated with patients' overall survival. Intriguingly, PI3K was determined to be a prognostic factor for favorable survival in adenocarcinoma (P = 0.043).

According to our study, both PI3K and p-PI3K were significantly downregulated in NSCLC tis-

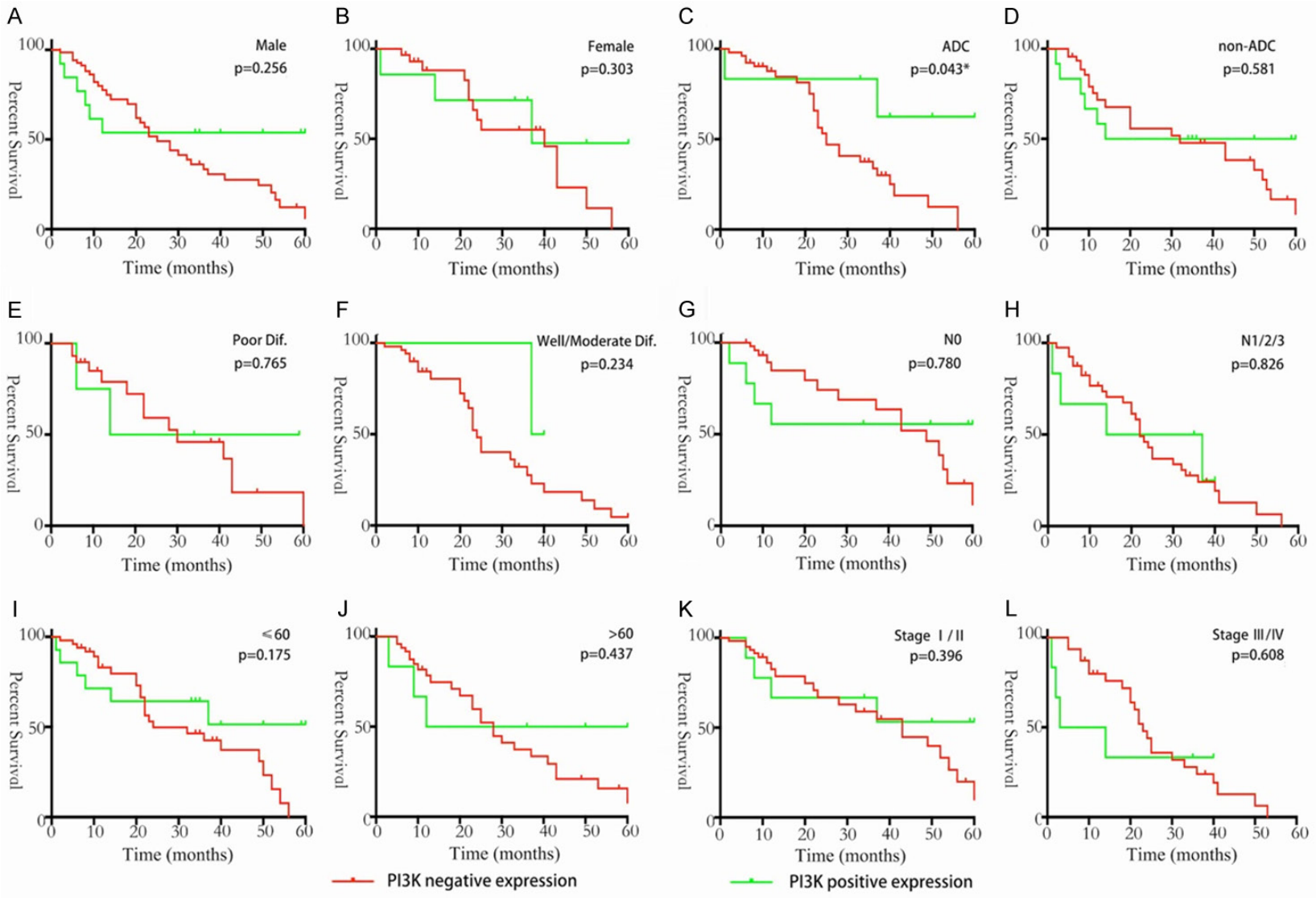

Figure 3. Kaplan-Meier curves for patients' survival according to PI3K expression. The survival analysis is stratified by PI3K negative and PI3K positive expression in male (A), female (B), ADC (C), non-ADC (D), poor differentiation (E), well/moderate differentiation (F), N0 (G), N1/2/3 (H), ≤ 60 years old (I), > 60 years old (J), Stage I-II (K), stage III-IV (L), respectively.

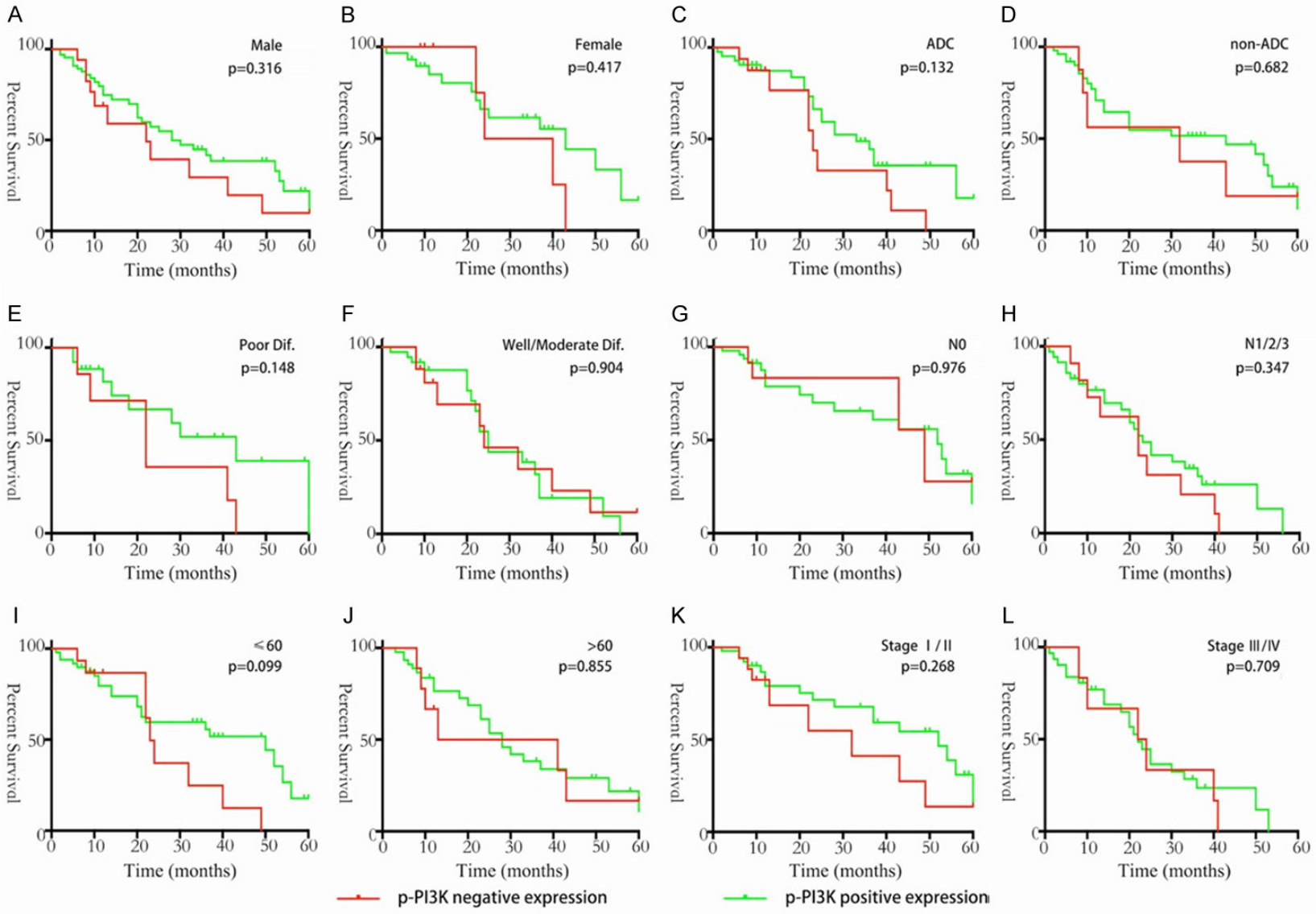

Figure 4. Kaplan-Meier curves for patients' survival according to p-PI3K expression. Survival analysis is stratified by p-PI3K negative and p-PI3K positive expression in male (A), female (B), ADC (C), non-ADC (D), poor differentiation (E), well/moderate differentiation (F), N0 (G), N1/2/3 (H), ≤ 60 years old (I), > 60 years old (J), Stage I-II (K), stage III-IV (L), respectively.

Table 4. Multivariate Cox regression analysis of overall survival in lung adenocarcinoma patients

Variables	HR	*P* value	95% CI
N stage (N0 vs. N1/N2/N3)	0.369	0.052	0.135-1.010
PI3K (negative vs. positive)	2.712	0.184	0.622-11.833

sue samples. However, different conclusions had been reached in previous studies. Ning Wu's study found that PI3K was significantly upregulated in esophageal squamous cell carcinoma tissue samples [22]. In another study about breast cancer, PI3K expression was also increased in patients with axillary lymph node metastasis [33]. This discrepancy between our study and previous studies might arise from various biological mechanisms in different types of cancers. In addition, the unfavorable sample size in the present research might also be influential.

Our study showed that PI3K was significantly associated with M stage in NSCLC. Although identical conclusions had not been determined in previous researche, other significant correlations between PI3K/p-PI3K expression and clinical features were found. Wang discovered that the expression of PI3K was significantly related to axillary lymph node metastasis in stage T1/2 breast cancer [33]. In another study about laryngeal cancer, high PI3K kinase mRNA/protein level was associated with a high tumor front grading (TFG) ($P < 0.05$) [24]. Similar correlations were also determined in NSCLC. It was identified that upregulation of PI3Kp110α was significantly associated with smoking status of NSCLC patients, pathologic type, N stage and TNM stage [34]. Consensus had not been reached in this field, but it suggests that PI3K/p-PI3K did play a critical role in tumor progression and prognosis.

In this study, PI3K was a prognostic factor for favorable survival in lung adenocarcinoma. This prognostic value had been validated in prostate cancer, which showed that patients with negative PI3K expression had shorter time to biochemical progression than those with positive expression [35]. However, most of the previous studies found an opposite conclusion, revealing that PI3K was a prognostic factor for poor survival. A study about gastric cancer determined that patients' overall survival was negatively associated with PI3K expression [29]. Furthermore, this prognostic value remained effective even when exploring it in different isoforms of PI3K. PIK3A mutation was significantly correlated with poor survival of cervical cancer patients, by downregulating the expression level of phosphorylated AKT and its downstream targets [36]. Similarly, PIK3CB/p110β was also significantly correlated with poor survival of glioblastoma [37]. It is noteworthy that some studies of endometrial cancer [38] and breast cancer [39] elucidated no significant associations between PI3K/p-PI3K expression and patients' survival.

Reasons causing this divergence might be varied. Firstly, multiple proteins could induce the biological functions of PI3K, including N-myc interactor (NMI) [40], TRIM22 [41], RAB27B [42], Calponin 2 [43]. This complicated network might regulate the progression and prognosis of tumor, independent of changing PI3K/p-PI3K expression. Secondly, different isoforms of PI3K might exert diverse influences on survival of cancer patients. It was inappropriate tostudy only the total expression of PI3K, without exploring expression levels of different isoforms. Also, the sample size in our study was unfavorable, especially in the subgroup of PI3K positive expression. An optimized cohort study with a promising sample size is needed to further elucidate the relationship between PI3K/p-PI3K expression and prognosis of lung cancer patients.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yan Huang, Health Management Center, West China Hospital, Sichuan University, 37 Guoxue Lane, Wuhou District, Cheng-

du 610000, Sichuan, China. Tel: +86 138821924-79; E-mail: huangyanhy513@163.com

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