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STAT3/p-STAT3 expression is correlated with clinicopathological characteristics and prognosis in non-small cell lung cancer

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Summary. Signal transducer and activator of transcription factor 3 (STAT3)/phosphorylated STAT3 (p-STAT) play a critical role in tumorigenesis, however, there is limited information on its prognostic value in non-small cell lung cancer (NSCLC). To address this question, 239 lung cancer and 71 normal lung tissue samples were obtained in this study. Immunohistochemistry was applied to detect STAT3/p-STAT3 expression. Pearson's Chi-squared test and the Kaplan-Meier method were conducted to evaluate associations with patients' clinical characteristics and survival. According to our results, STAT3/p-STAT3 was significantly upregulated in lung cancer tissue (p<0.001). Moreover, p-STAT3 expression was significantly correlated with age (p=0.046) and pathological types (p=0.037). In survival analysis, STAT3 positivity was negatively associated with survival in patients older than 60 years (p=0.043) but failed to be an independent prognostic factor in multivariate analysis (p=0.083). Therefore, STAT3/p-STAT3 may serve as a critical biomarker in NSCLC.

Key words: NSCLC, prognosis, STAT3

Introduction

As the leading cause of cancer-related death, lung cancer claims an estimated 2.3 million lives worldwide each year. Non-small cell lung cancer (NSCLC) is the primary pathological type, accounting for approximately 85% of total primary lung malignancies (Zou et al., 2020; Le et al., 2021). Much progress has been made in the diagnosis and treatment of lung cancer during the past few decades. However, its prognosis remains unfavorable, with a five-year overall survival rate of less than 20% (Gettinger et al., 2018; Wang et al., 2021). Various proteins have been proven to correlate with the prognosis of NSCLC, including epidermal growth factor receptor (EGFR) (Fan et al., 2017; Zhang et al., 2017a), protein kinase B (AKT) (Liu et al., 2011), mammalian target of rapamycin (mTOR) (Panwar et al., 2023), and Janus kinase 1 (JAK1) (Liu et al., 2017). Nonetheless, their prognostic values remained controversial in different studies. Therefore, it is essential to identify a reliable biomarker to predict the prognosis of NSCLC patients and guide rational treatment.

The signal transducer and activator of transcription factor (STAT) family is a group of various transcription factors. It consists of seven subtypes, including STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6 (Li et al., 2023). All these members share a STAT_α domain, a SH2 domain, a DNA-binding domain, an N-terminal domain, and a C-terminal domain (Li et al., 2022). They are located in the cytoplasm most of the time. When activated, they can be transferred to the nucleus and regulate a variety of cellular events, including proliferation, differentiation, angiogenesis, apoptosis, and drug resistance (Gao et al., 2017). Among the various family members, STAT3 is considered a key factor in tumorigenesis; it can be phosphorylated by VEGFR, EGFR, and IL-6 receptors through JAK2 at the tyrosine 705 site on the C-terminal domain. Afterward, phosphorylated-STAT3 (p-STAT3) translocates into the nucleus and regulates various target genes (Zhu and Zhou, 2015; Banerjee and Resat, 2016). It has been proven that STAT3 and p-STAT3 are related to shorter survival in patients with different cancers, including pancreatic, gastric, and colorectal cancer (Tong et al.,

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Abbreviations. NSCLC, Non-small cell lung cancer; STAT3, Signal transducer and activator of transcription factor 3; p-STAT3, Phosphorylated signal transducer and activator of transcription factor 3

2020); nevertheless, no consensus has been reached in lung cancer (Mohassab et al., 2020).

In this study, we explored the prognostic values of STAT3 and p-STAT3 in NSCLC patients. To address this question, we detected the expression of STAT3/p-STAT3 in both lung cancer and normal lung tissue specimens by immunohistochemistry. In addition, we also collected the clinical and survival information of these patients and determined their correlations with STAT3/p-STAT3 expression by statistical analysis.

Materials and methods

Patients and tissue specimens

From January 2008 to December 2013, 255 participants with complete resections of primary NSCLC were enrolled in this study from the West China Hospital, Sichuan University of China. No preoperative chemotherapy or radiotherapy was given, and subsequent standard therapy was followed according to the National Comprehensive Cancer Network guidelines (NCCN, 2009a,b). Clinical characteristics, including gender, age, histological types, differentiation, tumor size, lymph node metastasis, and distant metastasis, were obtained from the medical records by two physicians independently. Meanwhile, TNM staging was completed according to the tumor-node-metastasis system of the International Union Against Cancer (Olawaiye et al., 2021), and pathology, as well as differentiation, were evaluated according to the NSCLC World Health Organization classification (Padinharayil et al., 2023). In addition, the institutional review board approval for this study was obtained from the Committee on Medical Ethics of West China Hospital.

In this study, the median follow-up time of the patients was 39.0 months, ranging from 1 to 60 months. Considering inadequate tissue and missing information, only 239 lung cancer tissue samples and 71 adjacent normal lung tissue specimens were finally included.

Immunohistochemistry

All tissue samples were collected during surgery, then fixed in 10% formalin and embedded in paraffin within 12 to 24 hours. Subsequently, tissues were sliced into 4 μm sections, then deparaffinated, hydrated, and blocked with xylene, graded ethanol in distilled water, and 3% H_2O_2 in 100% methanol, respectively. Afterward, the blocked slides would finish antigen retrieval at 95°C for 30 minutes with Tri/ethylenediaminetetraacetic and were incubated with specific primary antigens at 4°C overnight. Whereafter, slides were then incubated with secondary antibodies for 30 minutes. At last, Harris hematoxylin was used to counterstain these sections. The primary and secondary antibodies used were as follows: STAT3 (AF6294, Affinity Biosciences), p-STAT3 (AF3294, Affinity Biosciences), and goat anti-rabbit IgG (Dako, Shanghai, China).

Immunohistochemical scoring

Evaluation of the slides was undertaken by two experienced pathologists independently, blinded to the patient's information. The dual rate semi-quantitative method, considering both the intensity and fraction of immunostaining, was used to obtain a score. In this method, fraction scores were divided into four categories: 3 (>50% positive cells), 2 (20-50%), 1 (10-20%), and 0 (<10%); and intensity scores were also divided into four categories: 3 (dark brown staining), 2 (readily appreciable brown), 1 (barely detectable), and 0 (no appreciable staining). By multiplying both above scores, the total score was achieved. Slides scoring <2 were defined as negative, while 2-9 were positive.

Statistical analysis

The statistical analysis was undertaken using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Pearson's Chi-squared test was performed to calculate the correlation between STAT3/p-STAT3 expression and clinical characteristics, such as gender, age, pathological types, differentiation, and TNM stage. Simultaneously, the Kaplan-Meier method was used to draw survival curves, and both the log-rank test and Cox regression analysis (univariate) were performed to determine the significance regarding five-year survival. Finally, Cox regression analysis (multivariate) was conducted to determine independent prognostic factors. Results were considered significant when $p<0.05$.

Results

STAT3 and p-STAT3 expression in lung cancer and normal lung tissue

The expression of STAT3/p-STAT3 in both normal lung and lung cancer tissue is shown in Figure 1. Of the 239 lung cancer specimens, 159 (66.5%) had STAT3-positive expression and 80 (33.5%) had STAT3-negative expression; while 147 (61.5%) specimens had p-STAT3-

Table 1. Expression levels of STAT3 and p-STAT3 in lung cancer tissue and normal lung tissue.

Protein	Expression level	Lung cancer tissue No. (%)	Normal lung tissue No. (%)	*p*-value
STAT3	N	80(33.5)	38(53.5)	<0.001
	P	159(66.5)	33(46.5)	
p-STAT3	N	92(38.5)	57(80.3)	<0.001
	P	147(61.5)	14(19.7)	

N, negative; P, positive.

positive expression and 92 (38.5%) had p-STAT3-negative expression.

Moreover, the different expressions of STAT3/p-STAT3 in lung cancer tissue and normal lung tissue were also analyzed. As shown in Table 1, both STAT3 and p-STAT3 were significantly highly expressed in lung cancer tissue ($p<0.001$ and $p<0.001$, respectively).

Relationship between STAT3/p-STAT3 expression and clinicopathological features

The main clinicopathological characteristics of 239 participants are summarized in Table 2. p-STAT3 expression was significantly associated with age ($p=0.046$); as participants became older, the percentage of p-STAT3-positive expression was significantly increased. Conversely, no significant correlation was found between STAT3 expression and age. Additionally, the positive expression of p-STAT3 was also significantly altered between different pathological types ($p=0.037$). Based on the pathological types, the percentage of p-STAT3-positive expression was 54.5% in adenocarcinoma (ADC) and 67.8% in non-ADC. However, there were no significant correlations between STAT3/p-STAT3 and other clinicopathological features, such as gender, differentiation, T stage, N stage, M stage, and TNM stage.

The association of STAT3/p-STAT3 expression with overall survival of NSCLC patients

The Kaplan-Meier curve was used to determine the correlation between STAT3/p-STAT3 expression and the five-year median survival rate of NSCLC patients. As shown in Figure 2, no significant correlations were found between patients' overall survival and STAT3/p-STAT3 expression ($p=0.515$ and $p=0.926$, respectively). The five-year median survival rate in patients with negative STAT3/p-STAT3 expression was similar to patients with positive STAT3/p-STAT3 expression.

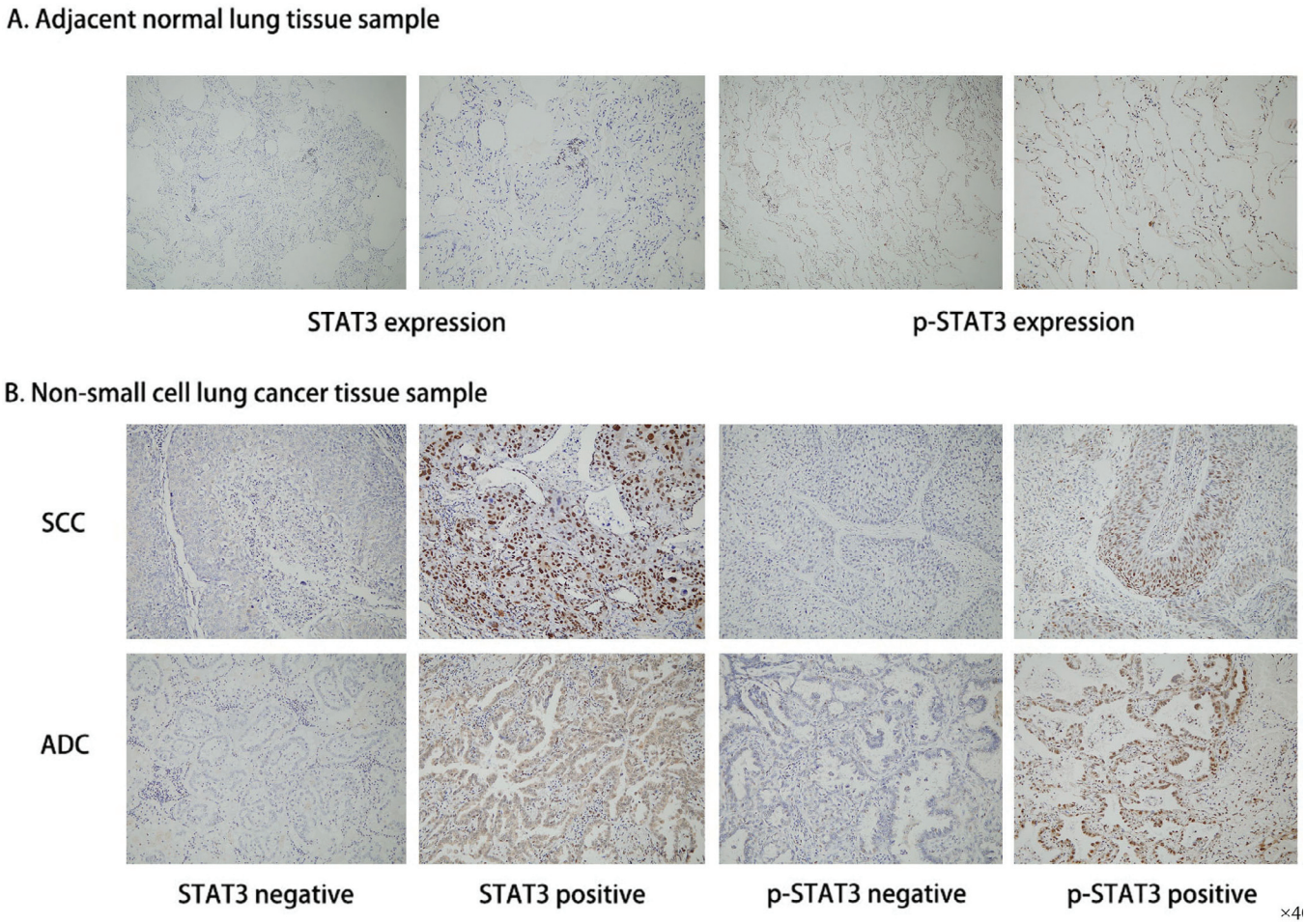

Fig. 1. Expression of STAT3 and p-STAT3 in normal lung tissue samples **(A)** and non-small cell lung carcinoma samples **(B)**. **A.** Immunohistochemical staining of each protein. **B.** Immunohistochemical staining of negative and positive samples in both Squamous cell carcinoma (SCC) and adenocarcinoma (ADC). × 400.

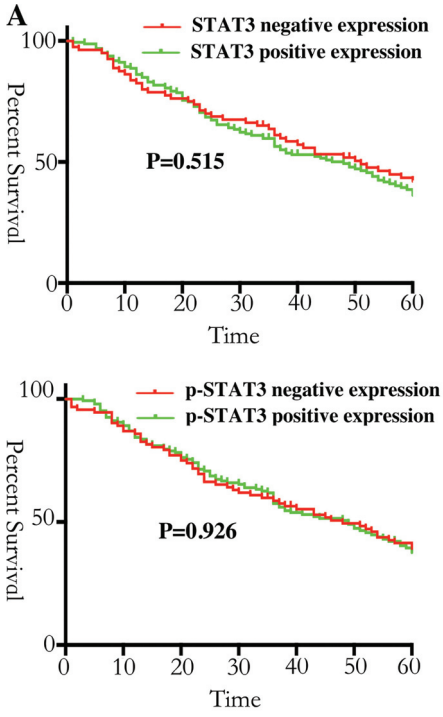

Fig. 2. Associations between STAT3/p-STAT3 expression status and overall survival in non-small cell lung cancer (NSCLC). **A.** Survival of STAT3 negative and positive expression. **B.** Survival of p-STAT3 negative and positive expression

Whereafter, the subgroup analysis in patients with negative/positive STAT3 expression (Fig. 3) was conducted using a Kaplan-Meier curve. Intriguingly, our results determined that patients with positive STAT3 expression had a shorter survival time than those with negative STAT3 expression in patients older than 60 years (p=0.043). Meanwhile, in patients with poor differentiation, there was a clear trend of shorter survival times in patients with positive rather than negative STAT3 expression; however, no significant difference was found (p=0.061). Additionally, no significant relationships were found in other subgroups, including patients younger than 60 years (p=0.229), male (p=0.188), female (p=0.357), ADC (p=0.565), non-ADC (p=0.832), good/moderate differentiation (p=0.734), N0 (p=0.333), N1/2/3 (p=0.447), stage 1/2 (0.186), stage 3/4 (p=0.609). Notably, since only 11 patients were detected with distant metastases, subgroup analyses of M0 and M1 were not conducted.

Subsequently, the aforementioned subgroup analysis was also conducted in patients with negative/positive p-STAT3 expression (Fig. 4). Nonetheless, no significant difference was found in either subgroups, including male (p=0.721), female (p=0.633), ADC (p=0.938), non-ADC (p=0.954), poor differentiation (p=0.601), good/moderate differentiation (p=0.878), N0 (p=0.951), N1/2/3 (p=0.776), younger than 60 (p=0.548), older than 60 (p=0.711), stage 1/2 (p=0.868), and stage 3/4 (p=0.556).

Table 2. Association between STAT3/p-STAT3 expression and clinical features of 239 patients.

Variables		STAT		*p*-value	p-STAT3		*p*-value
		Negative (n=80)	Positive (n=159)		Negative (n=92)	Positive (n=147)	
Age	≤60 (n=126)	41 (32.5)	85 (67.5)	0.747	56 (44.4)	70 (55.6)	0.046*
	>60 (n=113)	39 (34.5)	74 (65.5)		36 (31.9)	77 (68.1)	
Gender	Man (n=167)	53 (31.7)	114 (68.3)	0.386	60 (35.9)	107 (64.1)	0.214
	Woman (n=72)	27 (37.5)	45 (62.5)		32 (44.4)	40 (55.6)	
Histology	ADC (n=121)	46 (38.0)	75 (62.0)	0.170	55 (45.5)	66 (54.5)	0.037*
	Non-ADC (n=115)	34 (29.6)	81 (70.4)		37 (32.2)	78 (67.8)	
	Missing (n=3)	0 (0.0)	3 (100.0)		0 (0.0)	3 (100.0)	
Differentiation	Poor (n=72)	22 (30.6)	50 (69.4)	0.251	27 (37.5)	45 (62.5)	0.170
	Good/moderate (n=124)	48 (38.7)	76 (61.3)		59 (47.6)	65 (52.4)	
	Missing (n=43)	10 (23.3)	33 (76.7)		6 (14.0)	37 (86.0)	
pT stage	1, 2 (n=155)	57 (36.8)	98 (63.2)	0.283	73 (40.6)	92 (59.4)	0.929
	3, 4 (n=65)	19 (29.2)	46 (70.8)		26 (40.0)	39 (60.0)	
	Missing (n=19)	4 (21.1)	15 (78.9)		3 (15.8)	16 (84.2)	
pN stage	0 (n=118)	37 (31.4)	81 (68.8)	0.285	45 (38.1)	73 (61.9)	0.451
	1, 2, 3 (n=102)	39 (38.2)	63 (61.8)		44 (43.1)	58 (56.9)	
	Missing (n=19)	4 (21.1)	15 (78.9)		3 (15.8)	16 (84.2)	
pM stage	0 (n=209)	71 (34.0)	138 (66.0)	0.435	87 (41.6)	122 (58.4)	0.123
	1 (n=11)	5 (45.5)	6 (54.5)		2 (18.2)	9 (81.8)	
	Missing (n=19)	4 (21.1)	15 (78.9)		3 (15.8)	16 (84.2)	
Stage	1, 2 (n=140)	48 (34.3)	92 (65.7)	0.915	57 (40.7)	83 (59.3)	0.917
	3, 4 (n=80)	28 (35.0)	52 (65.0)		32 (40.0)	48 (60.0)	
	Missing (n=19)	4 (21.1)	15 (78.9)		3 (15.8)	16 (84.2)	

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

Multivariate analysis

Finally, we conducted a multivariate Cox regression analysis to further evaluate the independent prognostic values of STAT3 expression in patients older than 60 years. Variables in this model were those previously determined to be influential, including the N stage ($p<0.001$) and TNM stage ($p<0.001$, Table 3). As shown in Table 4, only N stages were independent prognostic factors for NSCLC patients older than 60 years of age ($p=0.004$). However, no significant correlations were found between five-year survival and TNM stage ($p=0.101$) or STAT3 expression ($p=0.083$).

Table 3. Univariate Cox regression analysis of overall survival in patients older than 60 years.

Variables	HR	*p*-value	95% CI
Gender	1.057	0.841	0.616-1.815
Histological types (ADC/non-ADC)	0.723	0.188	0.445-1.172
Differentiation status (low/moderate to well)	1.208	0.508	0.690-2.113
T stage (1, 2/3, 4)	0.630	0.086	0.371-1.068
N stage (N0/N1, N2, N3)	0.324	0.000**	0.192-0.546
M stage (M0/M1)	0.340	0.075	0.104-1.116
TNM stage (1, 2/3, 4)	0.371	0.000**	0.221-0.632

**, $p<0.001$

Table 4. Multivariate Cox regression analysis of overall survival in patients older than 60 years.

Variables	HR	*p*-value	95% CI
N stage (N0/N1, N2, N3)	0.408	0.004*	0.223-0.746
TNM stage (1, 2/3, 4)	0.606	0.101	0.333-1.103
STAT3 (negative/positive)	0.605	0.083	0.343-1.069

*, $p<0.05$

Discussion

In this study, the expression levels of STAT3 and p-STAT3 were detected in 239 lung cancer and 71 normal lung tissue samples. In the malignant specimens, 66.5%

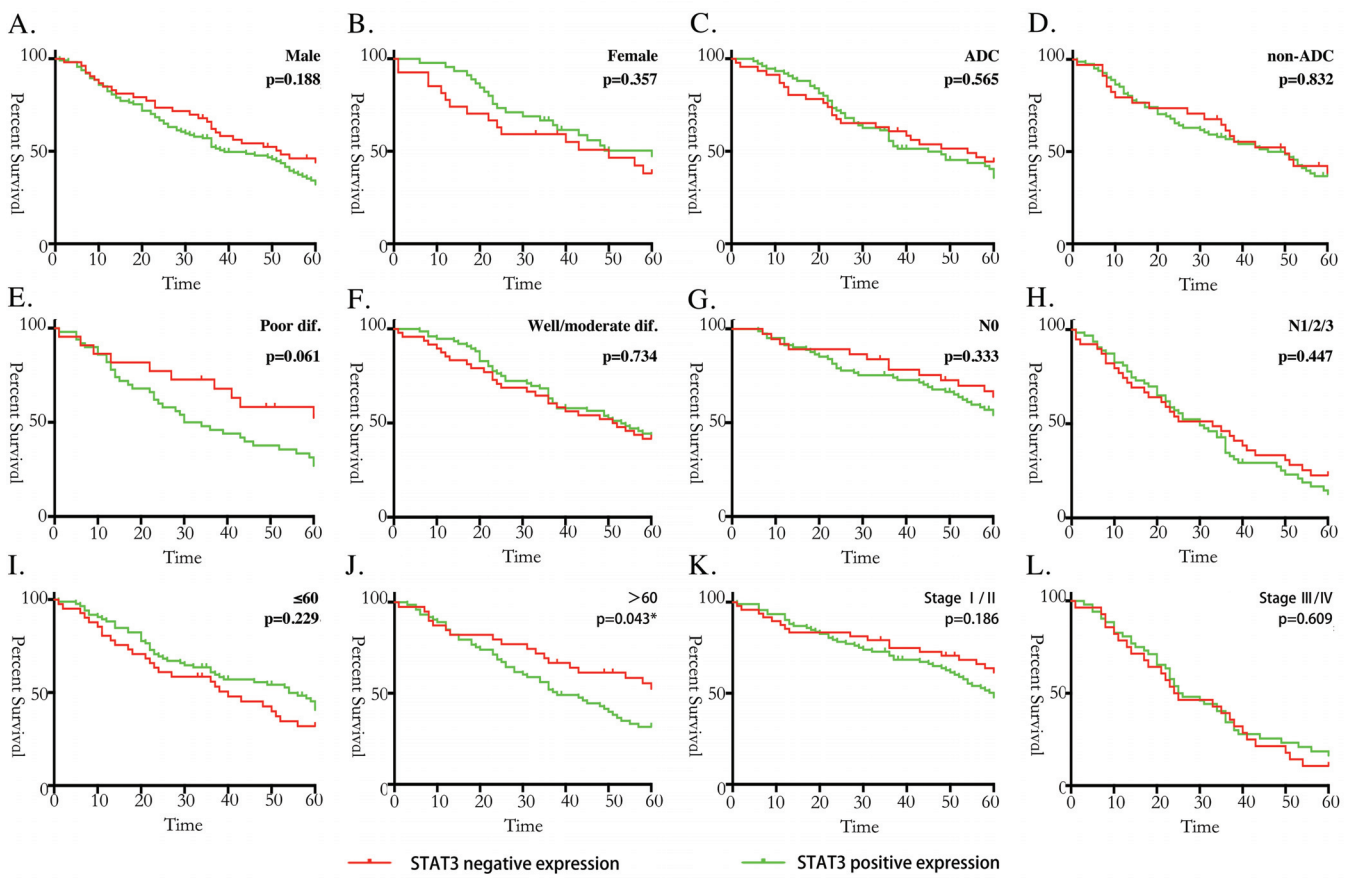

Fig. 3. Kaplan-Meier curves for patient survival according to STAT3 expression. The survival analysis is stratified by STAT3-negative and STAT3-positive expression in Men **(A)**, Women **(B)**, ADC **(C)**, non-ADC **(D)**, poor differentiation **(E)**, good/moderate differentiation **(F)**, N0 **(G)**, N1/2/3 **(H)**, ≤60 years old **(I)**, >60 years old **(J)**, Stage I/II **(K)**, stage III/IV **(L)**, respectively.

and 61.5% showed positive expression for STAT3 and p-STAT3, respectively; while in the normal lung tissue specimens, only 46.5% and 19.7% had STAT3- and p-STAT3-positive expression. Both proteins were significantly increasingly expressed in lung cancer tissue samples. Meanwhile, the associations between STAT3/p-STAT3 expression and patients' clinicopathological characteristics were determined. Only age and pathological types were significantly correlated with p-STAT3 expression but no significant relationships were found between STAT3 expression and clinical characteristics. Additionally, the prognostic values of STAT3 and p-STAT3 were also analyzed. No significant correlations were found between their expression levels and patients' overall survival. Intriguingly, it was determined that STAT3-positive expression predicted a shorter survival time in NSCLC patients older than 60 years.

Our study demonstrated that STAT3/p-STAT3 were aberrantly expressed in lung cancer. This finding indicated the critical role of STAT3 in tumorigenesis, which had been proven previously (Johnson et al., 2018; Wingelhofer et al., 2018). In recent years, abundant studies have been trying to provide insights into the mechanism for such a process. In Lu L's study, whole-transcriptome profiling revealed 2251 direct target genes of STAT3 in diffuse large B-cell lymphoma. By regulating diverse oncogenic signaling pathways (such as NF-κB and AKT), cancer cell survival, proliferation, invasion, and migration were promoted (Lu et al., 2018). This conclusion was similar to Ramu A's study on oral cancer (Ramu et al., 2017). Meanwhile, another study on prostate cancer reported that STAT3 activation promoted the expression of pituitary tumor transforming gene 1 (*PTTG1*), then increased cancer stem cell populations and induced epithelial-mesenchymal transition (EMT) (Huang et al., 2018). Besides the aforementioned studies, many other types of research have also been conducted on other types of cancers, including colorectal (Liang et al., 2017), liver (Yu et al., 2017a), renal cell (Zhang et al., 2017b), large cell lung (Yu et al., 2017b), and neuroblastoma (Hadjidaniel et al., 2017). In these studies, various proteins and signaling pathways (such as RING-finger protein 6 (Liang et al., 2017), Eukaryotic

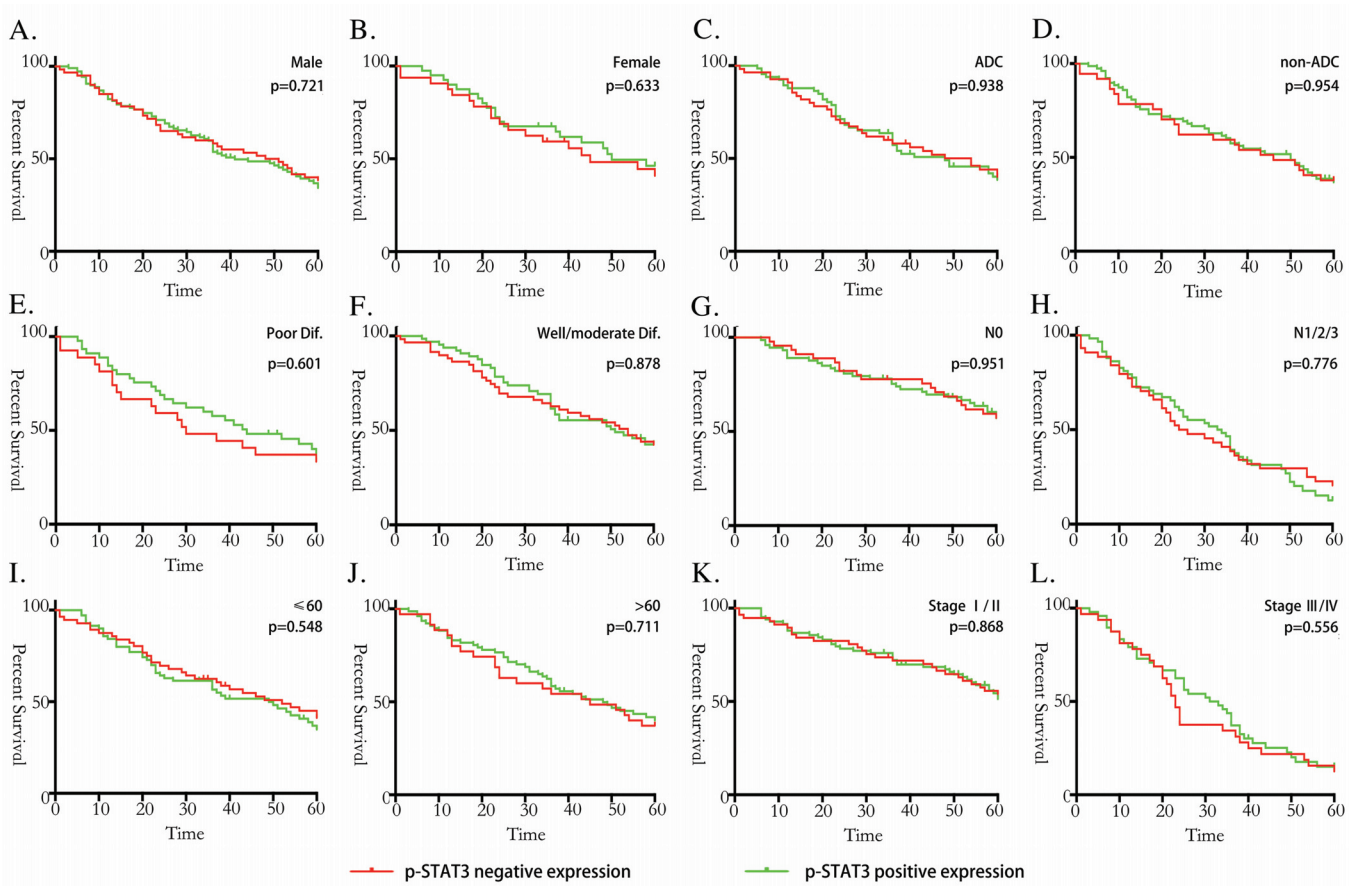

Fig. 4. Kaplan-Meier curves for patients' survival according to p-STAT3 expression. The survival analysis is stratified by p-STAT3-negative and p-STAT3-positive expression in Man **(A)**, Woman **(B)**, ADC **(C)**, non-ADC **(D)**, poor differentiation **(E)**, good/moderate differentiation **(F)**, N0 **(G)**, N1/2/3 **(H)**, ≤60 years old **(I)**, >60 years old **(J)**, Stage I/II **(K)**, stage III/IV **(L)**.

initiation factor 5A2 (Fang et al., 2017), EZH2 (Zhang et al., 2017b), and c-MYC (Hadjidaniel et al., 2017) were involved, indicating that the underlying mechanisms are complicated.

According to the present study, STAT3 was correlated with unfavorable survival. Identical conclusions have been reached in previous studies for several cancers, such as colorectal cancer, nasopharyngeal carcinoma, gastric cancer, and ovarian cancer. A study on colorectal cancer revealed that low levels of nuclear STAT3 predicted a longer disease-specific survival (DSS) and disease-free survival (DFS) (Huang et al., 2017). Furthermore, STAT3 was determined as an independent prognostic signature of poor survival in gastric cancer (Pan et al., 2016). Conversely, a different result arose from a study on ovarian cancer, which identified that STAT3 did not act as a prognostic marker (Li et al., 2017a). This discrepancy might arise from the distinctive tumorigenic mechanisms of different types of malignancy. It is noteworthy that the prognostic value of STAT3 has also been explored in NSCLC. A meta-analysis (Xu and Lu, 2014) determined that high STAT3 expression was a potent predictor of poor prognosis for NSCLC. However, only three studies with less than 200 participants in total were included. Moreover, heterogeneity was very prominent between these studies, including pathological types of lung cancer, antibodies used for immunohistochemistry, and scoring methods. Therefore, this conclusion was not so convincing.

STAT3 plays a pivotal role in regulating diverse cellular processes, encompassing proliferation, survival, differentiation, apoptosis, immune function, and angiogenesis. Phosphorylation of STAT3 at tyrosine 705 by VEGFR, EGFR, and IL-6 receptors is followed by its translocation into the nucleus to modulate an array of target genes (Zhu and Zhou, 2015). Notably, phosphorylation of STAT3 at Y705 and subsequent dimerization are indispensable steps within the canonical JAK-STAT3 signaling pathway (Sgrignani et al., 2018). In comparison with other prognostic markers, such as EGFR and AKT, STAT3 exhibits significant potential as a prognostic indicator.

In the present study, it was determined that p-STAT3 was not a prognostic predictor in NSCLC patients. This conclusion is identical to studies on bladder carcinoma (Zheng et al., 2017) and ovarian cancer (Shang et al., 2017). However, p-STAT3 was proved to be a reliable prognostic factor in other studies. In a study on upper tract urothelial carcinoma, high p-STAT3 expression was correlated with poor recurrence-free survival and overall survival (Li et al., 2017b). Conversely, Bekki H's study informed that positivity for p-STAT3 was significantly correlated with a better prognosis of undifferentiated pleomorphic sarcoma (Bekki et al., 2017). In a meta-analysis including 17 studies and 2346 participants, it was suggested that p-STAT3 overexpression was correlated with poorer overall survival of colorectal cancer patients. Because of these discrepancies, optimized research with a larger sample size should be undertaken to further illuminate the prognostic value of p-STAT3.

Conclusions

STAT3/p-STAT3 expression is correlated with both clinical characteristics and survival in NSCLC. The positive expression of p-STAT3 was significantly correlated with advanced age and non-ADC. Patients with positive STAT3 expression had a shorter survival time than those with negative STAT3 expression in patients older than 60 years. It may help predict the prognosis and guide the appropriate surveillance for NSCLC patients.

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Data Availability. The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest. The authors declared that there is no conflict of interest regarding the publication of this article.

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