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Original Article

Prognostic value of KIF2A and TP53 overexpression in non-small cell lung cancer

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Abstract: Kinesin family member 2a (KIF2A), a member of Kinesin-13 family, is involved in the development and progression of several human cancers. However, its expression and prognostic implication in non-small cell lung cancer (NSCLC) remain unknown. In this study, we investigated the expression of KIF2A and TP53 and their prognostic significance in 188 patients with NSCLC. KIF2A and TP53 protein expression were assessed by tissue microarray-based immunohistochemistry (IHC). Real-time PCR was used to evaluate *KIF2A* mRNA expression in 22 pairs of NSCLC and matched tumor-adjacent tissues. We observed significantly elevated *KIF2A* transcripts in NSCLC tissues. Moreover, IHC expression levels of both KIF2A and TP53 protein were significantly increased in NSCLC specimens compared with non-tumorous lung tissues. High KIF2A protein expression in NSCLC was related to TNM stage (P<0.001), tumor status (T) (P=0.022), lymph node metastasis (N) (P=0.006) and gender (P=0.019). High expression of both KIF2A and TP53 was related to TNM stage (P=0.001) and N stage (P=0.007). Further study revealed the positive correlation between KIF2A and TP53 expression in NSCLC (r=0.340, P<0.001). Multivariate analyses suggested that KIF2A and TP53 were independent prognostic factors for patients with NSCLC. These results suggest that high KIF2A or TP53 expression is associated with poor prognosis in NSCLC, and the combined detection of KIF2A and TP53 may be helpful in predicting the prognosis of NSCLC patients.

Keywords: KIF2A, TP53, tissue microarray, immunohistochemistry, NSCLC, prognosis

Introduction

Lung cancer is the leading cause of cancer-related death worldwide, consisting of two major histological subtypes, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Approximately 85% of the lung cancer patients are diagnosed with NSCLC [1]. Despite great advance in diagnostic techniques and treatment methods (e.g. surgical resection, targeted therapy, chemotherapy, and radiotherapy), the long-term survival of lung cancer is still unsatisfactory, with the 5-year survival rate of <15% due to late diagnosis, cancer metastasis, and resistance to treatment [2, 3]. In order to improve the management of lung cancer patients, it is urgent to identify new prognostic biomarkers that can provide information on disease course after standard treatments and help to select proper treatments for NSCLC patients.

Kinesin superfamily protein 2A (KIF2A), a microtubule-based motor protein, belongs to the Kinesin-13 family that consists of KIF2A, KIF2B, and KIF2C/mitotic centromere-associated kinesin (MCAK). The Kinesin-13 family are M-type non-motile microtubule depolymerase [4] and known to modulate MT dynamics during multiple important cellular processes such as mitosis [5], axonal branching [6], cytokinesis [7] and ciliogenesis [8]. Microtubule (MT) depolymerizing activities of KIF2A were demonstrated *in vitro* [6, 9]. KIF2A is also found to be essential for accurate chromosome segregation and the formation of bipolar spindle during mitosis and meiosis [10]. KIF2A specifically localizes to spindle poles in human cells during mitosis. Cells deficient in KIF2A form aberrant monopolar spindles rather than normal bipolar spindles, leading to a block in cell cycle progression [11]. Moreover, monopolar spindles could cause the loss or gain of chromosomes in daughter cells [10], resulting in significant changes in the migration and proliferation of tumor cells (refs). Previous studies indicated that cytoskeletal recombination plays a major role in the migra-

tion of neoplastic cells [12]. As a critical cytoskeleton, microtubule is essential for mitotic activity of cells and is also vital for tumor cells to invade adjacent tissues and distant metastasis (refs). The depolymerization and decrease of microtubule is related to the metastatic potentiality of malignant tumors [13-15]. Up to now, few studies have reported the relationship between KIF2A and the prognosis of malignant tumors.

TP53, one of the most important tumor suppressor genes, has been reported to be mutated in over 50% of all human malignant tumors [16], with about 50% of NSCLC harboring *TP53* mutations [17, 18]. The prognostic implication of TP53 protein expression in NSCLC has been extensively studied. Many clinical studies suggest that TP53 alterations are associated with a poor prognosis in NSCLC and patients with mutant *TP53* may be relatively more resistant to radiotherapy and chemotherapy [19, 20]. However, there is no definitive evidence that the *TP53* status could play a significant role in patients with NSCLC. Wild-type TP53 is usually undetectable by standard immunohistochemistry, since it has a very short half-life. In contrast, mutant TP53 protein is stabilized and has a prolonged half-life, thus the TP53 protein detected by immunohistochemistry was believed to be the mutated TP53 protein [21, 22].

Although many studies have attempted to determine the prognostic values of KIF2A and TP53 in various cancers (refs), the prognostic role of KIF2A in NSCLC hasn't been evaluated. In the present study, we assessed the expression levels of KIF2A and TP53, and analyzed their correlation with clinicopathologic features and prognosis in NSCLC. We also examined whether the combined detection of KIF2A and P53 was prognostic in NSCLC.

Materials and methods

Patients and tissue samples

We enrolled 188 NSCLC patients who underwent surgical therapy at the Affiliated Hospital of Nantong University, Jiangsu, China from 2004 to 2009. At the time of surgery, the mean age of patients was 62.8 years (range, 35-83 years). No patient received chemotherapy, radiotherapy or immunotherapy prior to the operation. Formalin-fixed, paraffin-embedded NSCLC samples (*n*=188) and matched peritumoral specimens (*n*=53) were retrieved for the study. Clinical data were obtained for each participant from archived medical records at the hospital, including sex, age, smoking status, histological type, tumor size, tumor differentiation, tumor status (T), lymph node metastasis (N), distant metastasis (M), and TNM stage. All patients were followed up by telephone. Tumors were staged according to the guidelines of the 8th edition of TNM staging in lung cancer [23]. Informed consent was obtained from each patient prior to surgical operation. The study protocol was approved by the Human Research Ethics Committee of the Affiliated Hospital of Nantong University, Jiangsu Province, China.

qRT-PCR analysis

Total RNA was extracted from 44 freshly frozen lung tissues, including 22 NSCLC tissues and 22 matched non-cancerous tissue samples. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was conducted with LightCycler Fast Start DNA Master SYBR Green I Kit (Roche Diagnostics, Tokyo, Japan) as previously described [24]. KIF2A-specific primers were as follows: forward, 5'-GCCGAATACATCAAGCAAT-3' and reverse, 5'-CTCTCCAGGTCAATCTCTT-3' (109-bp). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a normalization control (forward primer, 5'-TGCACCACCAACTGCTTAGC-3'; reverse primer, 3'-GGCATGGACTGTGGTCATGAG-5'). Reverse transcription was conducted by incubation for 30 min at 42°C, followed by Taq polymerase activation for 2 min at 94°C. Target gene fragments were amplified through 35 cycles at the following parameters: 95°C for 20 s, 56°C for 20 s, and 72°C for 30 s. All measurements were performed in triplicate.

Tissue microarray (TMA) construction and immunohistochemistry (IHC)

NSCLC tissue specimens and matched tumor-adjacent tissues were prepared and used to construct TMAs as previously described [25]. IHC was performed as previously described [26]. KIF2A was detected by mouse anti-human KIF2A monoclonal antibody (5 μg/ml, ab55383, Abcam), and TP53 was detected by rabbit anti-human TP53 polyclonal antibody (dilution 1:100, M3629, DAKO). A slide in which primary

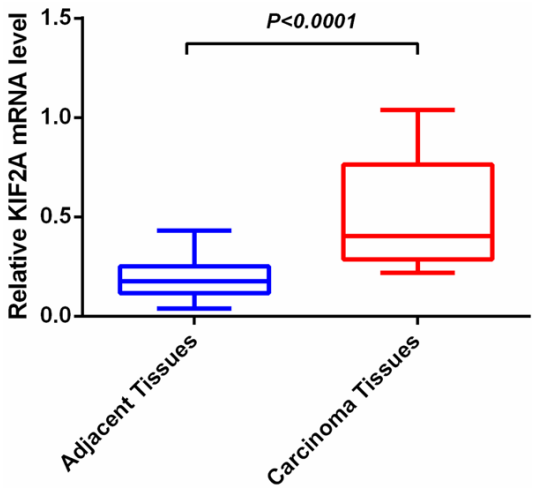

Figure 1. Expression of KIF2A mRNA in NSCLC and adjacent non-cancerous tissues. qRT-PCR was performed to evaluate KIF2A mRNA expression levels in NSCLC (cancer) compared with tumor adjacent (normal) tissues. Normalized to GAPDH mRNA levels, the KIF2A mRNA level in NSCLC tissues was significantly higher than that in peritumoural tissues ($P<0.05$). Error bar is the standard error.

antibody was replaced by phosphate-buffered saline served as negative controls.

Immunostained slides were evaluated by two pathologists who were blinded to the clinical characteristics of the patients. The expression of KIF2A and TP53 was scored using the semi-quantitative H-score method, taking into account of both the staining intensity and the percentage of positively stained cells. The staining intensity was scored as 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The percentage of KIF2A- or TP53-positive cells was also determined ranging from 0% to 100%. The product of the intensity and percentage score was used as the final KIF2A/TP53 staining score, with a minimum value of 0 showing negative staining and a maximum value of 300 showing 100% of cells with strong staining intensity.

Statistical analysis

The Wilcoxon non-parametric signed-rank test was used to compare the expression levels of *KIF2A* and *TP53* mRNA in fresh-frozen NSCLC tissues and matched tumor-adjacent tissues. To analyze the impacts of the KIF2A and TP53 expression on the prognosis of NSCLC, the continuous IHC expression scores of the two proteins were dichotomized (low vs. high) using specific cutoff points. The cutoff points were calculated using the X-tile software program (The Rimm Lab at Yale University; http://www.tissuearray.org/rimmlab) as previously described [27]. Statistical optimal cutoff value of protein expression levels for NSCLC was selected based on minimum *P* value from log-rank χ^2 statistics in term of overall survival. χ^2 test was used to evaluate whether KIF2A and TP53 expression levels were correlated with clinicopathologic parameters in NSCLC. The correlation between KIF2A and P53 expression was tested using Spearman's test. The survival curves were calculated using the Kaplan-Meier method, and the log-rank test was used to compare the survival curves. Prognostic significant factors identified in the univariate Cox's regression model were subsequently evaluated using the multivariate Cox's regression model. For all analyses, differences were considered to be statistically significant when *P* value was less than 0.05. All statistical analyses were carried out using SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

Evaluation of KIF2A mRNA expression by qPCR

Relative expression of *KIF2A* mRNA in NSCLC and matched peritumoral tissues were quantified by qRT-PCR. After normalized to *GAPDH*, *KIF2A* mRNA expression levels was found to be significantly elevated in NSCLC tissues ($n=22$) when compared with paired non-cancerous tissues ($n=22$) (0.507±0.0564 vs. 0.190±0.0207, $P<0.001$) (**Figure 1**).

Expression of KIF2A and TP53 protein in NSCLC detected by immunohistochemistry

We examined KIF2A and TP53 protein expression in 188 NSCLC tissues and 51 matched adjacent normal lung tissues by immunohistochemistry. Positive staining of KIF2A was mainly localized in the cytoplasm of tumor cells (**Figure 2**), while positive staining of TP53 was visualized in the nuclei. In subsequent analyses, KIF2A and TP53 protein expression levels were dichotomized using cutoff values determined by X-tile program, with 100 for KIF2A (low expression: score ≤100; high expression: score >100) and 80 for TP53 (low expression: score ≤80; high expression: score >80). High

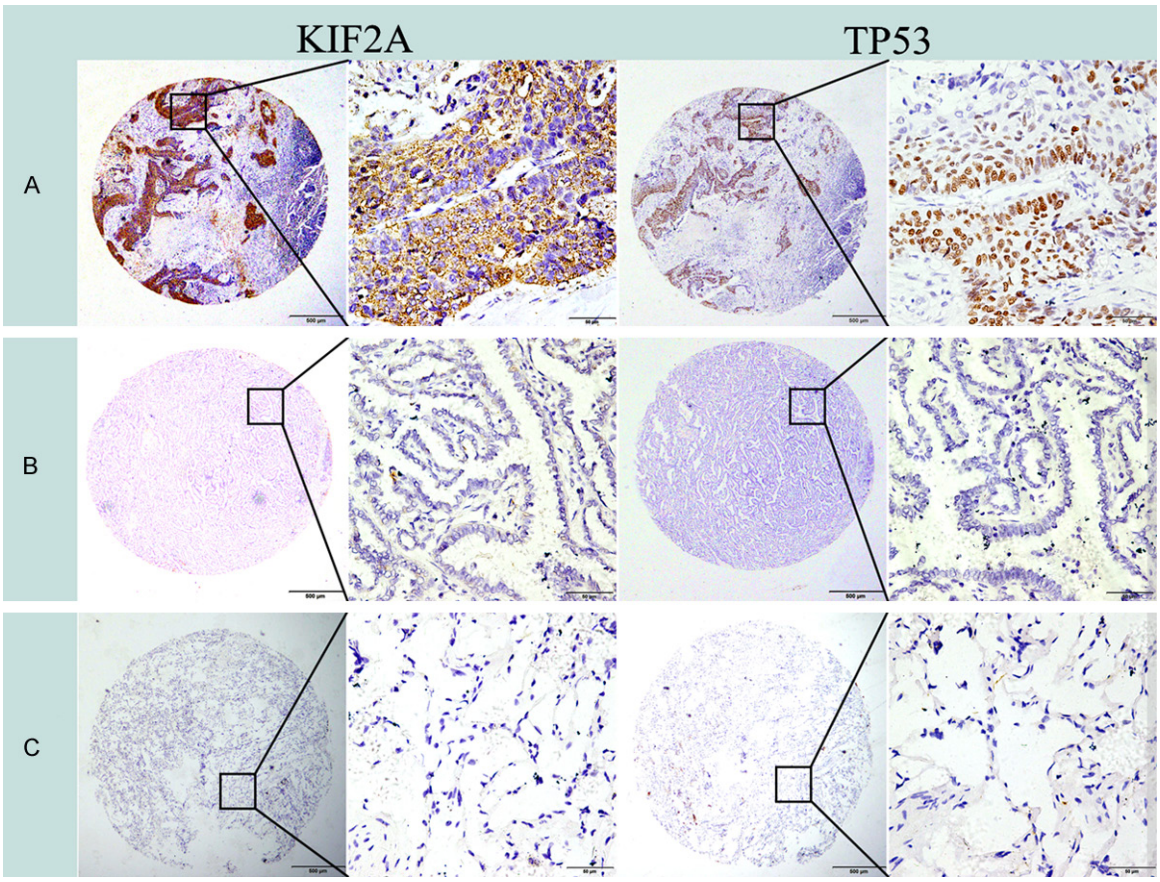

Figure 2. Representative patterns of KIF2A and TP53 protein expression in NSCLC and adjacent non-cancerous tissues. Row A, positive immunohistochemical staining of KIF2A and TP53 in NSCLC. Row B, negative immunohistochemical staining of KIF2A and TP53 in NSCLC. Row C, negative immunohistochemical staining of KIF2A and TP53 in tumor adjacent non-cancerous tissue. Originalmagnification ×40 (bar=500 μm) in columns 1 and 3, and ×400 (bar=50 μm) in columns 2 and 4.

KIF2A expression was noted in 42.55% of (80/188) tumor specimens, when compared with only 11.76% (6/51) in matched peritumoral tissues. χ^2 test analysis revealed that a significantly larger percentage of tumor tissues had high KIF2A levels than that of peritumoral tissues (χ^2=16.51, P<0.001). Similarly, NSCLC samples (44.15%) significantly more frequently showed high TP53 expression than matched non-tumorous tissues (9.80%) (χ^2=20.34, P< 0.001). Besides, the Spearman correlation test demonstrated that the expression levels of KIF2A and TP53 protein in NSCLC were positively correlated (r=0.340, P<0.001).

Correlation of KIF2A and TP53 expression levels with clinicopathological features in NSCLC

To determine the role of KIF2A and TP53 in the development of NSCLC, we analyzed their association with major demographic and clinicopathological variables of NSCLC patients. KIF2A expression was significantly associated with gender (P=0.019), tumor status (T) (P= 0.022), lymph node metastasis (N) (P=0.006), and TNM stage (P<0.001). In contrast, no significant association was observed with age, histological type, and tumor differentiation (**Table 1**). TP53 showed a positive correlation with lymph node metastasis (N) (P=0.041) and TNM stage (P=0.004), but not with the rest variables (**Table 1**). Given the positive correlation between KIF2A and TP53 expression in NSCLC (r= 0.340, P<0.001). We speculated that patients with both high expression levels of KIF2A and P53 might represent a subgroup with a worse outcome. We then divided patients into four groups based on levels of the two protein in NSCLC specimens: group 1, tumors with low expression of the two proteins ($KIF2A^-/P53^-$, 76 cases); group 2, tumors with high expression of

Table 1. Association of high expression of KIF2A and TP53 with clinicopathological characteristics in NSCLC patients

Groups	n	KIF2A expression			TP53 expression			KIF2A$^+$/TP53$^+$ expression		
		High (%)	Pearson χ^2	*P* value	High (%)	Pearson χ^2	*P* value	KIF2A$^+$/TP53$^+$ (%)	Pearson χ^2	*P* value
Total	188	80 (42.55)			83 (44.15)			51		
Age										
<60 years	64	28 (43.75)	0.057	0.812	32 (50.00)	1.347	0.246	20 (31.25)	0.834	0.361
≥60 years	124	52 (41.94)			51 (41.13)			31 (25.00)		
Gender										
Male	147	56 (38.10)	5.480	0.019*	63 (42.86)	0.456	0.499	36 (24.49)	2.373	0.123
Female	41	24 (58.54)			13 (31.71)			15 (36.59)		
Differentiation										
Low grade	64	26 (40.63)	0.178	0.673	31 (48.44)	1.111	0.292	16 (25.00)	0.091	0.763
Middle and high grade	107	47 (43.93)			43 (40.19)			29 (27.10)		
Others	17	7			4			6		
Histological type										
Squamous cell carcinoma	86	36 (41.86)	4.543	0.103	42 (48.84)	3.605	0.165	25 (29.07)	3.124	0.210
Adenocarcinoma	48	26 (54.17)			23 (47.92)			16 (33.33)		
Others[a]	54	18 (33.33)			18 (33.33)			10 (18.52)		
T										
Tis+T1	60	20 (33.33)	7.636	0.022*	23 (38.33)	2.373	0.305	15 (25.00)	2.525	0.283
T2	81	34 (41.98)			35 (43.21)			20 (24.69)		
T3+T4	43	26 (60.47)			23 (53.49)			16 (37.21)		
Unknown	4	0			0					
N										
N0	103	33 (32.04)	10.339	0.006*	37 (35.92)	6.406	0.041*	19 (18.45)	9.972	0.007*
N1	46	25 (54.35)			24 (52.17)			15 (32.61)		
N2	39	22 (56.41)			22 (56.41)			17 (43.59)		
TNM stage										
0-I	76	20 (26.32)	16.000	<0.001*	23 (30.26)	11.040	0.004*	11 (14.47)	14.102	0.001*
II	59	31 (52.54)			29 (49.15)			18 (30.51)		
III-IV	49	29 (59.18)			29 (59.18)			22 (44.90)		
Unknown	4	0			0					

*$P<0.05$; [a]others, Adenosquamous carcinoma and others.

KIF2A and low expression of TP53 (KIF2A$^+$/TP53$^-$, 29 cases); group 3, tumors with low KIF2A expression and high TP53 expression (KIF2A$^-$/P53$^+$, 32 cases), and group 4, tumors with high expression of two proteins (KIF2A$^+$/P53$^+$, 51 cases). We found that tumors with high KIF2A and high TP53 expression were significantly positively related to lymph node metastasis (N) (*P*=0.007) and TNM stage (*P*=0.001).

Prognostic value of KIF2A and TP53 protein expression in NSCLC

We used the Cox's proportional hazards regression model to further explore the impacts of KIF2A and TP53 on NSCLC survival. Univariate analysis showed high KIF2A expression (*P*< 0.001), high TP53 expression (*P*<0.001), tumor TNM stage (*P*=0.012), lymph node metastasis (N) (*P*=0.007) and high expression of both KIF2A and TP53 (*P*<0.001) were significantly negatively associated with 5-year survival of NSCLC patients. Multivariate analysis further demonstrated that high KIF2A expression (*P*=0.014) and high TP53 expression (*P*=0.006) were independent prognostic factors for poor survival of NSCLC (**Table 2**). Kaplan-Meier survival curves revealed that NSCLC patients with KIF2A$^+$ tumors had significantly poorer survival than those with KIF2A$^-$tumors (**Figure 3**). Similarly, high TP53 levels also conferred survival disadvantage in NSCLC patients. It was also notable that subgroup with the KIF2A$^+$/P53$^+$ phenotype had the poorest prognosis among all the four subgroups, as shown by the shortest survival time (**Figure 3**).

Table 2. Univariate and multivariate analysis of different prognostic factors for 5-year survival in patients with NSCLC

Variable	Univariate analysis			Multivariate analysis		
	HR	*P*	95% CI	HR	*P*	95% CI
KIF2A expression	3.080	<0.001*	2.086-4.549	3.261	0.014*	1.270-8.368
High vs. low						
TP53 expression	2.303	<0.001*	1.567-3.385	1.796	0.006*	1.182-2.730
High vs. low						
KIF2A/TP53 expression	1.463	<0.001*	1.251-1.712	0.901	0.620	0.596-1.362
$KIF2A^+/TP53^-$ vs. $KIF2A^+/TP53^+$ vs. $KIF2A^-/TP53^+$ vs. $KIF2A^-/TP53^-$						
Age (years)	0.886	0.542	0.601-1.307			
≤60 vs. >60						
Gender	0.903	0.666	0.570-1.433			
Male vs. female						
Differentiation	1.003	0.985	0.717-1.403			
Well and moderate vs. poor						
Histological type	0.935	0.607	0.723-1.209			
Sq vs. Ad vs. others						
T	1.011	0.934	0.782-1.307			
Tis+T1 vs. T2 vs. T3+T4						
N	1.356	0.007*	1.088-1.691	1.266	0.317	0.797-2.011
N0 vs. N1 vs. N2						
TNM stage	1.346	0.012*	1.068-1.698	0.856	0.536	0.523-1.401
0-I vs. II vs. III-IV						

*$P<0.05$.

Discussion

KIF2A is demonstrated to be MT-based motor protein. MTs, as one type of the vital components of the cytoskeleton, play an important role in cell mitosis, migration, and signaling transduction [28]. Previous study reported that the overexpression of Kinesin-13 proteins could cause a moderate increase in the frequency of abnormal multipolar and monopolar spindles, which may further contribute to the gain or loss of chromosomes in daughter cells [29]. Ganem et al. reported that the cell cycle progression was inhibited in KIF2A-deficient cells and these cells formed monopolar spindles instead of normal bipolar spindles in mitosis, thereby leading to the mis-segregation of chromosome [10]. Moreover, the prognostic values of KIF2A in various cancers have also been investigated. In breast cancer, patients with higher KIF2A expression were found to have poorer survival. Additionally, *in vitro* experiments revealed that silencing of *KIF2A* gene reduced cell proliferation, invasion, and migration [30]. Fan et al. found that KIF2A was significantly more highly expressed in colorectal cancer tissue than in the corresponding adjacent non-tumorous tissues, and that KIF2A levels were inversely related with the survival time of colorectal cancer patients [31]. The levels of KIF2A in squamous cell carcinoma of the oral tongue (SCCOT) were also reported to exceed that in the non-tumorous tissues. The same study also observed that silencing of *KIF2A* gene induced apoptosis of SCCOT cells [32]. Consistently, in the present study, we demonstrated the preferential expression of KIF2A in NSCLC tissues compared with that in the adjacent non-cancerous tissues, and the survival disadvantage was observed in patients with tumors expressing high levels of KIF2A.

It was recently reported that KIF2A is an upstream regulator of the phosphatidylinositol (PtdIns)-3-Kinase (PI3K)/AKT signaling pathway [32]. PI3K is a lipid kinas that coverts PtdIns (4, 5) P2 (PIP2) to PtdIns (3, 4, 5) P3 (PIP3), a second messenger, which subsequently activates downstream signaling cascade and regulates multiple important cellular events, such as proliferation, survival, and invasion [33]. Previous studies have shown that the activation of PI3K/AKt pathway is a frequent event in many types of human cancer [34, 35], including NSCLC [36]. PI3K and phosphorylated (p)-AKt were also independent adverse prognostic biomark-

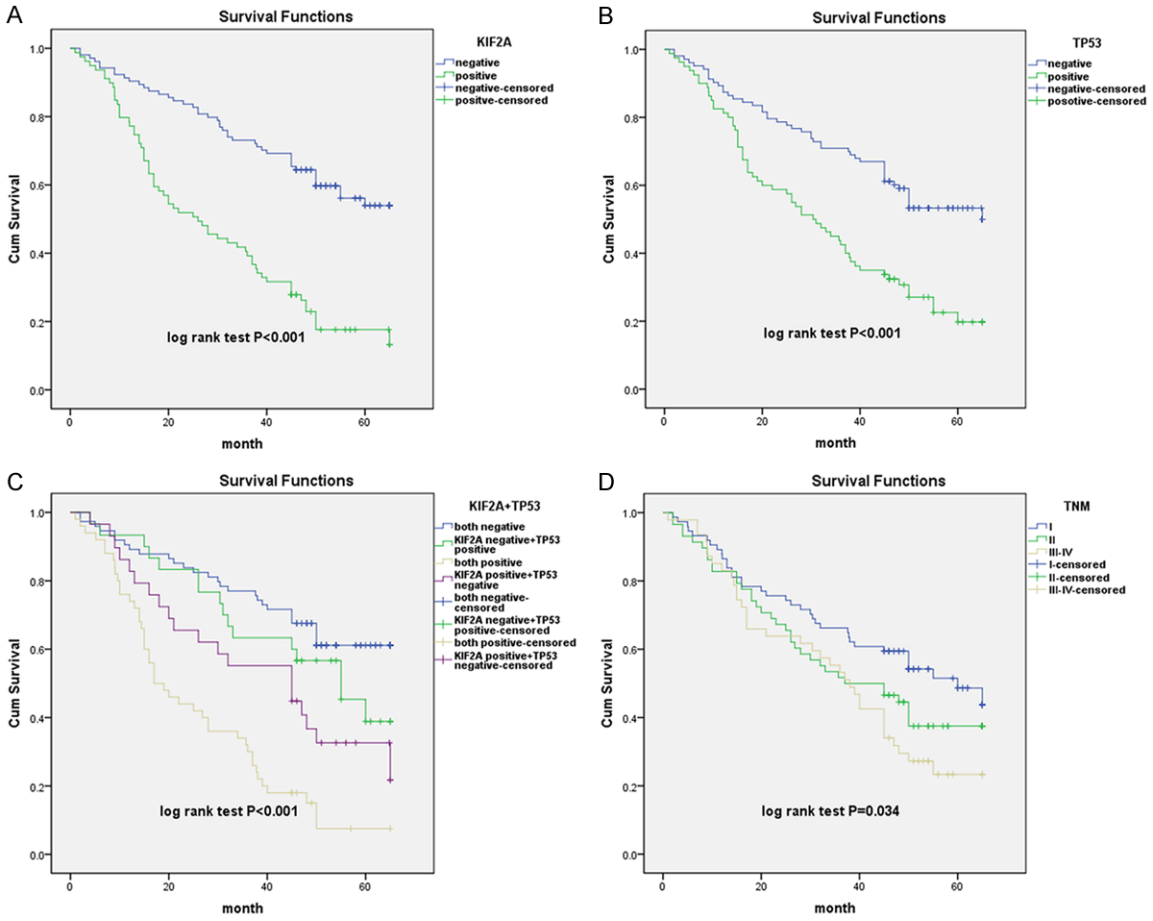

Figure 3. Kaplan-Meier survival analysis for NSCLC patients after surgical therapy. A. Overall survival rate in patients with high KIF2A expression was significantly decreased compared with the low or no expression group. B. Patients with high TP53 expression exhibited significantly poorer survival compared with the low or no expression group. C. Overall survival rate in patients with both high expression of KIF2A and TP53 was significantly lower than that in other groups. D. Advanced TNM stage significantly worsened overall survival when compared with early TNM stage.

ers in NSCLC [36]. Taken together, these studies suggested that KIF2A might partially exert oncogenic effects through PI3K/AKT pathway and be an ideal candidate as the prognostic biomarker and a novel therapeutic target for NSCLC.

The tumor suppressor gene *TP53* is a short-lived transcription factor that plays a major role in the determination of growth arrest or proliferation at cellular level [37]. TP53 participates in regulating transcription [38], apoptosis [39], DNA repair [40], and inhibiting the proliferation of abnormal cells [41]. Accordingly, loss of T53 function in the process of cancer leads to wide-ranging consequences in the tumor cells [42, 43]. *TP53* mutations have been detected in a wild range of tumors and the mutant TP53 may be a new therapeutic target for cancer therapy [44, 45].

In this study, we investigated the expression levels of KIF2A and TP53 and their prognostic implication in NSCLC patients. QRT-PCR analysis revealed KIF2A mRNA expression in NSCLC tissues was significantly higher than that in adjacent non-tumorous tissues. Moreover, we found that both KIF2A and TP53 were more commonly expressed in NSCLC tissues. In addition, the overall survival time of patients with high KIF2A and TP53 expression was significantly shorter than that of patients with low or no expression. High expression of both proteins was correlated with TNM stage and lymph node metastasis. Kaplan-Meier analysis in this study demonstrated that either high expression of

KIF2A or TP53 alone significantly conferred decreased 5-year overall survival. Furthermore, high expression of KIF2A or TP53 alone was identified to be an independent predictive factor for poor NSCLC prognosis.

Despite these interesting findings, our study also has some limitations. The current findings may not be applicable to the general population. Large prospective multi-center studies, involving different ethnicities, are needed to confirm our findings. Moreover, *in vitro* and *in vivo* experiments should be performed to clarify the potential mechanisms underlying the oncogenic effects of KIF2A in NSCLC.

In conclusion, the current study showed significantly increased expression levels of KIF2A and TP53 in NSCLC tissues and their association with unfavorable prognosis. Our results indicated that KIF2A and TP53 might be used as novel prognostic indicator in NSCLC.

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Disclosure of conflict of interest

None.

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