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ORIGINAL RESEARCH

Expression and prognostic role of IKBKE and TBK1 in stage I non-small cell lung cancer

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Background: The inhibitors of nuclear factor kappa-B kinase subunit epsilon (*IKBKE*) and TANK-binding kinase 1 (*TBK1*) are important members of the nonclassical IKK family that share the kinase domain. They are important oncogenes for activation of several signaling pathways in several tumors. This study aims to explore the expression of IKBKE and TBK1 and their prognostic role in stage I non-small cell lung cancer (NSCLC).

Patients and methods: A total of 142 surgically resected stage I NSCLC patients were enrolled and immunohistochemistry of IKBKE and TBK1 was performed.

Results: IKBKE and TBK1 were expressed in 121 (85.2%) and 114 (80.3%) of stage I NSCLC patients respectively. IKBKE expression was significantly associated with TBK1 expression (P=0.004). Furthermore, multivariate regression analyses showed there was a significant relationship between patients with risk factors, the recurrence pattern of metastasis and IKBKE+/TBK1+ co-expression (P=0.032 and P=0.022, respectively). In Kaplan–Meier survival curve analyses, the IKBKE+/TBK1+ co-expression subgroup was significantly associated with poor overall survival (P=0.014).

Conclusions: This is the first study to investigate the relationship between IKBKE and TBK1 expression and clinicopathologic characteristics in stage I NSCLC patients. IKBKE+/TBK1+ co-expression was significantly obvious in patients with risk factors and with recurrence pattern of distant metastasis. Furthermore, IKBKE+/TBK1+ is also an effective prognostic predictor for poor overall survival.

Keywords: IKBKE, TBK1, NSCLC, prognosis, cancer

Introduction

Lung cancer is the leading cause of cancer-related death worldwide. Non-small cell lung cancer(NSCLC) accounts for 80–85% of all lung cancer cases.[1,2] The primary treatment for stage I NSCLC is surgical resection, but at least 30% of patients experienced recurrence within 5 years.[3] Adjuvant chemotherapy is recommended for patients with risk factors, such as poor histologic differentiation, vascular invasion, wedge resection, tumors >4 cm, incomplete lymph node sampling, and visceral pleural invasion.[4] Even after receiving adjuvant chemotherapy, the 5-year survival rate is only 60–90% due to local recurrence or distant metastasis.[5,6] Therefore, we defined high-risk patients as tending to local recurrence or distant metastasis. It is challenging to screen true high-risk patients for adjuvant chemotherapy, and to avoid unnecessary adjuvant chemotherapy in low-risk patients to reduce the survival reduction caused by treatment-related toxicity.

The IKK family contains five protein factors. IKKα, IKKβ, and IKKγ belong to canonical group, and the other two (IKBKE and TBK1) belong to non-canonical

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group.[7] The amino acid sequence analysis confirmed that IKBKE has 67% homology with TBK1.[8] IKBKE is highly expressed in human normal pancreatic tissue, thyroid tissue, spleen, and peripheral blood leukocytes, and can be up-regulated by multiple cytokines such as tumor necrosis factor alpha (TNF-α), IL-1, IL-6, and interferon-γ.[9] TBK1 is constitutively expressed in immune cells, brain, lungs, gastrointestinal tract, and reproductive organs.[10] Studies have demonstrated that IKBEK and TBK1 need to bind to scaffold proteins to form a protease complex to activate downstream protein factors, thereby activating the *NF-κB* pathway. TNAK (TNF receptor-associated factor family member-associated NF-κB activator), NAP1 (NAK associated protein 1), and SINTBAD (similar to NAP and TBK1 adaptor) are the main scaffold proteins.[11–17] Several studies have shown that IKBKE was overexpressed in breast cancer, glioma, gastric cancer, pancreatic cancer, ovarian cancer, renal cancer, and lung cancer and was associated with poor prognosis.[18–24] IKBKE and TBK1 may play important roles in tumorigenesis, proliferation, and angiogenesis.[25,26] Nevertheless, the association between IKBKE and TBK1 expression and clinicopathological characteristics and its role in the prognosis of stage I NSCLC has not been reported.

Patients and methods

Patient selection

A totlal of 142 tissue samples were retrospectively collected from patients who were pathologically diagnosed with stage I NSCLC between January 2004 and June 2012 in Shandong Cancer hospital. All of them received anatomical segmentectomy or standard lobectomy via open thoracotomy and did not receive other treatments except for surgery. This retrospective study was approved by the ethics committee at Shandong Cancer Hospital and Institute. All patients have provided written informed consent, and this study was conducted in accordance with the Declaration of Helsinki.

Immunohistochemistry

Tumor tissue samples were obtained from 142 surgically resected stage I NSCLC patients. Surgically resected specimens were fixed in formalin and embedded in paraffin. Then, 4-μm paraffin-embedded sections were dewaxed and heated to 95°C for 20 mins for antigen retrieval in 10 mmol/L citrate buffer. Then, the sections were cleared of endogenous peroxidase activity and were blocked with 5% BSA for 30 mins in room temperature (RT). Then, all sections were incubated at 4°C overnight with primary anti-IKBKE (ab7891, Abcam, IHC 1:100, Cambridge, MA) or anti-TBK1 (ab109735, Abcam, IHC 1:400, Cambridge, MA). Then, the specimens were incubated with biotinylated secondary antibodies for 1 hr in RT. After incubation with ABC-peroxidase for 1 hr then all sections were colored using a DAB Kit (ZSGB-BIO, People's Republic of China) and counterstained with hematoxylin. All IHC results were evaluated by two pathologists. Double-blind readings were performed by two pathologists at the unknown tumor grade, and 10 fields were randomly collected from each pathological section, and the percentage of positive cells and the intensity of staining were scored: 1) coloring cells accounted for <5% cell count is 0; 5–25% is 1 point; 26–50% is 2 points; 51–75% is 3 points; and >75% is 4 points; 2) no staining is 0 points; the light yellow is 1 point; the brown and yellow is 2 points; the sepia is 3 points; 3) the two points are multiplied to form a positive grade: 0 is negative (-), 1–4 is weakly positive (+), 5–8 is positive (++), and 9–12 is strongly positive (+++).

Statistical analysis

The categorical variables were tested using Pearson's chi-squared tests or Fisher's exact test, and continuous variables were expressed as means±SD and were analyzed using independent *t*-test. The significant ($P<0.05$) clinicopathological characteristics in univariate analysis were enrolled in multivariate logistic regression analysis, and multivariate logistic regression analysis was used to confirm the independent clinicopathological characteristics which were associated with IKBKE and TBK1 expression. The Kaplan–Meier was used to analyze progression-free survival (PFS) and OS, and the survival curves of four subgroups were compared by log-rank test. All analysis was two-sided, and $P<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS (version 20.0).

Results

Expression of IKBKE and TBK1 and their association in stage I NSCLC patients

Among the 142 enrolled stage I NSCLC patients, 48 (33.8%) patients were squamous cell carcinoma, and 65 patients (45.8%) were adenocarcinoma. Twenty-nine patients were other types of NSCLC including 8 large cell carcinoma, 5 atypical carcinoid tumor, 5 pleomorphic carcinoma, 9 adenosquamous carcinoma, and 2 mucinous epidermoid carcinoma. IKBKE and TBK1 expression was

found in 85.2% (n=121) and 80.3% (n=114) of stage I NSCLC patients, respectively. The association between IKBKE and TBK1 expression and clinicopathological characteristics is summarized in Table 1. IKBKE and TBK1 expression was associated with risk factors (P=0.021 and P=0.014). From Table 1 we found patients with risk factors showed increased IKBKE and TBK1 expression compared to patients who had no risk factors. IKBKE expression was observed in 91.3% and 77.4% of patients who had risk factors compared to patients who had no risk factors. Besides, TBK1 expression was observed in 87.5% and 71.0% in these two subgroups. In addition, TBK1 expression was significantly associated with recurrence pattern of metastasis compared to local recurrence (P<0.001) and TBK1 expression was found in 89.6% of patients with the recurrence pattern of metastasis compared to 64.0% of patients with the recurrence pattern of local recurrence. We further explored the correlation between IKBKE expression and TBK1 expression and found there was a significant correlation between IKBKE and TBK1 expression (P=0.004), as 84.3% (102/121) of tumors with IKBKE positive expression simultaneously showed TBK1 positive expression (Table 2).

IKBKE and TBK1 co-expression in stage I NSCLC patients

We divided all patients into four subgroups according to IKBKE and TBK1 expression as follows: IKBKE-/TBK1- (n=9, 6.3%); IKBKE+/TBK1- (n=19, 13.4%); IKBKE-/TBK1+ (n=12, 8.5%); IKBKE+/TBK1+ (n=102, 71.8%). The representative examples of four subgroups are shown in Figure 1. We then explored the association of IKBKE and TBK1 co-expression and clinicopathological characteristics (Table 3). Patients with risk factors and patients with the recurrence pattern of metastasis had more frequency in the IKBKE+/TBK1+ subgroup than in other three subgroups (P=0.042 and P=0.004, respectively). In addition, we did not find significant association between other clinicopathological characteristics and the expression of IKBKE and TBK1. In multivariate regression analysis of clinicopathological characteristics for IKBKE and TBK1 co-expression, we found patients with risk factors and the recurrence pattern of metastasis were significantly related to IKBKE+/TBK1+ co-expression (P=0.032 and P=0.022, respectively). The adjusted OR of patients with risk factors was 1.633, with a 95% CI 1.043–2.557 when compared with patients with no risk factors. The adjusted OR of patients with recurrence pattern of metastasis was 1.670, with a 95% CI 1.078–2.587 when compared with patients with recurrence pattern of local recurrence (Table 4).

The prognostic significance of IKBKE and TBK1 co-expression in stage I NSCLC patients

The median follow-up period for OS and PFS was 1,442 days (range 488–3,239 days) and 1,021 days (range 107 −2,989 days), respectively. During the follow-up period, the recurrence rate was 89.4% (n=127). Survival was analyzed using the Kaplan–Meier method with stratification of IKBKE and TBK1 expression. The results showed that IKBKE+/TBK1+ co-expression subgroup was significantly associated with poor OS (median 1,351 days; 95 CI % 1,178–1,565; P=0.014) than those in IKBKE-/TBK1- subgroup (median 2,378 days; 95 CI% 1,924–4,291), IKBKE+/TBK1- subgroup (median 1,554 days; 95 CI% 959–2,148), and IKBKE-/TBK1+ subgroup (median 1,643 days; 95 CI% 1,529–1,836). However, the IKBKE+/TBK1+ co-expression subgroup did not show significant association with poor PFS than the other three subgroups (P=0.109), although the Kaplan–Meier survival curve showed this tendency (Figure 2).

Discussion

Our study tried to investigate IKBKE and TBK1 expression in stage I NSCLC and the association between their expression and clinicopathological characteristics. We found that IKBKE and TBK1 expressed in 85.2% and 80.3% of stage I NSCLC, respectively, and IKBKE and TBK1 co-expressed in 71.8% of all cases. Identifying high-risk patients who need postoperative adjuvant chemotherapy to improve OS and reduce the toxicity of unnecessary chemotherapy is a key element in the whole treatment management of early-stage NSCLC. Our study demonstrated that IKBKE and TBK1 might play an important role in tumorigenesis and proliferation in stage I NSCLC. IKBKE and TBK1 may be good molecular targets for combination therapy.

Targeted therapy is the treatment of precise targeting molecular “targets” by small molecule drugs that specifically bind to these targets. Protein kinases are important signal messengers to modulate cell life activities and play an important role in cell proliferation, survival, apoptosis, metabolism, transcription, and differentiation. IKBKE and

Table 1 The association of IKBKE and TBK1 expression and clinicopathological characteristics

Variable		IKBKE expression			TBK1 expression		
	Total cases	Positive	Negative	*P*	Positive	Negative	*P*
	N=142(%)	N=121(85.2%)	N=21(14.8%)		N=114(80.3%)	N=28(19.7%)	
Gender				0.789			0.642
Male	91 (64.1%)	77 (84.6%)	14 (15.4%)		72(79.1%)	19(20.9%)	
Female	51 (35.9%)	44 (86.3%)	7 (13.7%)		42(82.4%)	9(17.6%)	
Age				0.064			0.609
≤60	67 (47.2%)	61 (91.0%)	6 (9.0%)		55(82.1%)	12(17.9%)	
>60	75 (52.8%)	60 (80.0%)	15 (20.0%)		59(78.7%)	16(21.3%)	
Differentiation				0.469			0.940
Poor	37 (26.1%)	31 (83.8%)	6 (16.2%)		29(78.4%)	8(21.6%)	
Moderate	62 (43.7%)	51 (82.3%)	11 (17.7%)		50(80.6%)	12(19.4%)	
Well	43 (30.2%)	39 (90.7%)	4 (9.3%)		35(81.4%)	8(18.6%)	
Histologic type				0.577			0.723
Squamous carcinoma	48 (33.8%)	43 (89.6%)	5 (10.4%)		37(77.1%)	11(22.9%)	
Adenocarcinoma	65 (45.8%)	54 (83.1%)	11 (16.9%)		54(83.1%)	11(16.9%)	
Large cell carcinoma	8 (5.6%)	7 (87.5%)	1 (12.5%)		6(75.0%)	2(25.0%)	
Atypical carcinoid tumor	5 (3.5%)	4 (80.0%)	1 (20.0%)		4(80.0%)	1(20.0%)	
Pleomorphic carcinoma	5 (3.5%)	5 (100.0%)	0 (0%)		4(80.0%)	1(20.0%)	
Adenosquamous carcinoma	9 (6.4%)	6 (66.7%)	3 (33.3%)		8(88.9%)	1(11.1%)	
Mucinous epidermoid carcinoma	2 (1.4%)	2 (100.0%)	0 (0%)		1(50.0%)	1(50.0%)	
Risk factors				0.021			0.014
Yes	80 (56.3%)	73 (91.3%)	7 (8.7%)		70(87.5%)	10(12.5%)	
No	62 (43.7%)	48 (77.4%)	14 (22.6%)		44(71.0%)	18(29.0%)	
Recurrence pattern				0.319			<0.001
Local	50 (38.7%)	41 (82.0%)	9 (18.0%)		32(64.0%)	18(36.0%)	
Metastasis	77 (54.2%)	68 (88.3%)	9 (11.7%)		69(89.6%)	8(10.4%)	

Table 2 The association between IKBKE and TBK1 expression

Variable	IKBKE expression		
	Positive (n=121)	Negative (n=21)	*P*
TBK1 expression			0.004
Positive (n=114)	102 (84.3%)	12 (57.1%)	
Negative (n=28)	19 (15.7%)	9 (42.9%)	

TBK1 are serine/threonine protein kinases which is part of IKK family.[9] Ma et al demonstrated the activity of TBK1 is strongly promoted by phosphorylation at Ser 172.[27] In breast cancer, as an oncogene, IKBKE is regulated by K63-linked polyubiquitination at lysine 30 and lysine 401 and controlled by the cIAP1/cIAP2/TRAF2 E3 ligase complex ubiquitinates itself.[28] Although IKBKE and TBK1 were characterized as activators of *NF-κB* pathway, several studies indicated non-canonical IKKs (IKBKE and TBK1) were sufficient, but not essential for *NF-κB* activation.[18,29] *NF-κB* pathway plays a pivotal role in the development of various tumors. In addition to *NF-κB* pathway, IKBKE and TBK1 also activated AKT by direct phosphorylation of AKT to promote cell proliferation and survival.[30] In addition, Saxton et al[31] found mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) controls metabolic processes and phosphorylates key substrates to promote oncoprotein-induced cell proliferation and survival. Yu et al[32] showed TBK1 can regulate AKT-mTORC1 signaling axis. Bodur et al[33] recently demonstrated TBK1 can directly phosphorylate mTORC1 at Ser 2159 to promote its tumorigenic activity. Recent studies have confirmed that IKBKE can directly phosphorylate signal transducers and activators of transcription 3 (STAT3), knocking down IKBKE can significantly reduce the content of STAT3 and phosphorylated STAT3, and affect the proliferation and progression of lymphoma through STAT3 signaling pathway.[34] Furthermore, several studies have explored the tumorigenic function of IKBKE and TBK1 in various cancers. Qin et al[35] indicated silencing IKBKE using synthetic siRNA can reduce focus formation potential and clonogenicity in human breast cancer cells. Li et al[36] verified that IKBKE overexpression in glioma is positively correlated to the grade of glioma. Silencing IKBKE can significantly inhibit tumor cell proliferation and invasion, and lead to cell cycle arrest. Lu et al demonstrated IKBKE plays an important role in regulating cell proliferation, invasion and epithelial-mesenchymal transitionof glioblastoma cells in vitro and in vivo.[37] Besides, Niederberger et al[38] found IKBKE/TBK1-sensitive acute myeloid leukemia (AML) cells tend to have MYC oncogenic signatures and inhibition of IKBKE/TBK1 can downregulate the MYC oncogenic pathway in AML cell lines. Accumulating evidence indicates the oncogenic potential of IKBKE and TBK1 is an important basis for them as targets in targeted therapy. Recent studies have shown that CYT387, a small molecule inhibitor that is traditionally thought to inhibit JAK/STAT signal transduction pathway, could inhibit IKBKE kinase activity and become a newly discovered small inhibitor of IKBKE. Experiments demonstrated CYT387 could significantly

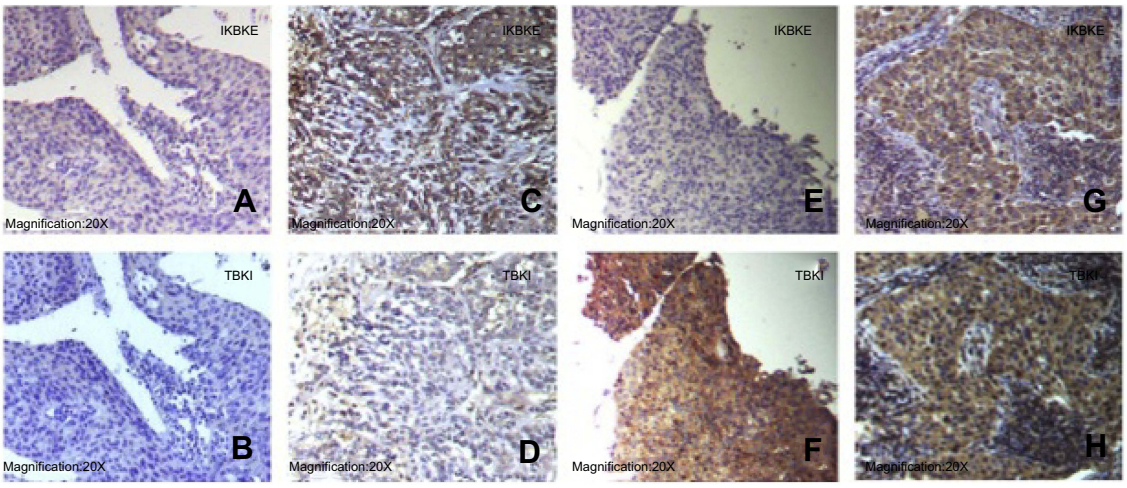

Figure 1 Representative examples of the four subgroups. A and B, E and F, G and H were moderately differentiated squamous cell carcinoma. C and D were large cell lung cancer. IKBKE and TBK were cytoplasmic stain. A and B: IKBKE+/TBK+. C and D: IKBKE+/TBK−. E and F: IKBKE−/TBK+. G and H: IKBKE−/TBK−.

Table 3 The association of IKBKE and TBK1 co-expression and clinicopathological characteristics

Variable	IKBKE-/TBK1- IKBKE+/TBK- IKBKE-/TBK+			IKBKE+/TBK1+	*P*
	N=9 (6.3%)	N=19 (13.4%)	N=12 (8.5%)	N=102 (71.8%)	
Gender					0.961
Male	6 (6.6%)	13 (14.3%)	8 (8.8%)	64 (70.3%)	
Female	3 (5.9%)	6 (11.8%)	4 (7.8%)	38 (74.5%)	
Age					0.114
≤60	1 (1.5%)	11 (16.4%)	5 (7.5%)	50 (74.6%)	
>60	8 (10.7%)	8 (10.7%)	7 (9.3%)	52 (69.3%)	
Differentiation					0.937
Poor	3 (8.1%)	5 (13.5%)	3 (8.1%)	26 (70.3%)	
Moderate	4 (6.4%)	8 (12.9%)	7 (11.3%)	43 (69.4%)	
Well	2 (4.7%)	6 (14.0%)	2 (4.6%)	33 (76.7%)	
Histologic type					0.548
Squamous carcinoma	4 (8.3%)	7 (14.6%)	1 (2.1%)	36 (75.0%)	
Adenocarcinoma	3 (4.6%)	8 (12.3%)	8 (12.3%)	46 (70.8%)	
Large cell carcinoma	1 (12.5%)	1 (12.5%)	0 (0%)	6 (75.0%)	
Atypical carcinoid tumor	0 (0%)	1 (20.0%)	1 (20.0%)	3 (60.0%)	
Pleomorphic carcinoma	0 (0%)	1 (20.0%)	0 (0%)	4 (80.0%)	
Adenosquamous carcinoma	1 (11.1%)	0 (0%)	2 (22.2%)	6 (66.7%)	
Mucinous epidermoid carcinoma	0 (0%)	1 (50.0%)	0 (0%)	1 (50.0%)	
Risk factors					0.042
Yes	3 (3.7%)	7 (8.8%)	5 (6.2%)	65 (81.3%)	
No	6 (9.7%)	12 (19.3%)	7 (11.3%)	37 (59.7%)	
Recurrence pattern					0.004
Local	6 (12.0%)	12 (24.0%)	3 (6.0%)	29 (58.0%)	
Metastasis	1 (1.3%)	7 (9.1%)	8 (10.4%)	61 (79.2%)	

Table 4 Multivariate regression analysis of clinicopathological characteristics for IKBKE and TBK1 co-expression

Variables	*P*	OR	95% CI
Risk factors	0.032	1.633	1.043–2.557
Recurrence pattern	0.022	1.670	1.078–2.587

inhibit the proliferation of NSCLC lines in vitro, block the development and progression of NSCLC induced by IKBKE and TBK1 mediated KRAS-driven tumorigenesis.[39] Considering the high positive expression rate of IKBKE and TBK1 in stage I NSCLC, targeted therapy targeting IKBKE and TBK1 might be a potential strategy to suppress cancer development.

In our study, we explored the correlation between the following clinicopathological characteristics, including gender, age, differentiation, histologic type, risk factors, and recurrence pattern and IKBKE/TBK1 expression status. We found IKBKE+/TBK1+ expressed in 71.8% of all cases, suggesting that IKBKE and TBK1 may play a pivotal role in NSCLC initiation. The multivariate regression analysis was applied to select prediction characteristics for IKBKE+/TBK+ expression in stage I NSCLC and the multivariate analysis demonstrated patients with risk factors and the recurrence pattern of metastasis were significantly related to IKBKE +/TBK1+ co-expression (*P*=0.032 and *P*=0.022, respectively).

In addition, we did not find significant association between other clinicopathological characteristics and the expression of IKBKE and TBK1. The stage I NSCLC patients with risk factors were optimal candidates to receive adjuvant chemotherapy, whereas the locoregional or systemic recurrence was still high in these patients.[5] Even in stage I NSCLC patients with no risk factors, the locoregional or systemic recurrence also could not be ignored. Therefore, how to explore effective treatments to prolong the OS time in high-risk patients remains challenging. Our research provides a new perspective for screening high-risk patients. We found stage I NSCLC patients with risk factors were significantly related to IKBKE+/TBK1+ co-expression (*P*=0.032). Furthermore, we found the recurrence pattern of metastasis was also significantly related to IKBKE+/TBK1+ co-expression (*P*=0.022). Recently, studies have shown IKBKE and TBK1 could phosphorylate AKT to activate AKT pathway,[30,40] and AKT1 regulates pathological angiogenesis, vascular maturation, and permeability.[41] All these may explain why the patients with IKBKE+/TBK1+ co-expression were prone to recurrence with metastasis in our study and the IKBKE+/TBK1+ subgroup has shorter OS than other subgroups (*P*=0.014), whereas we did not find statistically significant PFS differences between the four subgroups (*P*=0.109). Li et al showed IKBKE

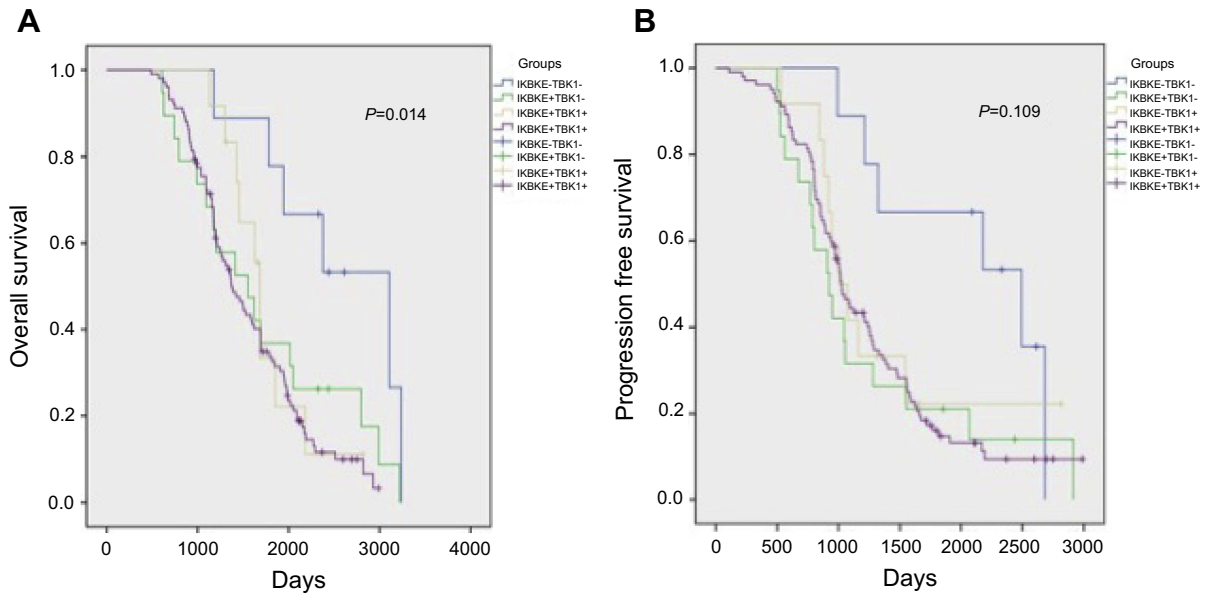

Figure 2 The Kaplan–Meier overall survival (**A**) and progression free survival (**B**) curves for stage I NSCLC according to IKBKE and TBK1 co-expression stratification.
Abbreviations: OS, overall survival; PFS, progression free survival.

and TBK1 dual inhibitors could suppress AKT activation and reduce VEGF expression, leading to impaired angiogenesis and inhibition of tumor growth.[42] Furthermore, several studies have shown that the correlation between high expression of IKBKE and chemotherapy resistance. Guo et al[22] verified that IKBKE is upregulated on mRNA and protein levels in 63 ovarian cancer tissues of 96 ovarian cancer patients, and elevated IKBKE obviously enhances the ability of ovarian cancer cells to resist cisplatin. Besides, Zhang et al[43] demonstrated that PLK4 decreases temozolomidesensitivity by regulating the IKBKE/NF-κB axis which indicates IKBKE could influence glioblastoma proliferation and chemosensitivity. In addition to ovarian cancer and glioblastoma, Guo et al[24] reported that the expression of IKBKE was increased in 98 cases of NSCLC, and the sensitivity of NSCLC cell lines to chemotherapy drugs was increased after silencing IKBKE, suggesting that IKBKE plays a key role in lung cancer and tumor resistance. Zhang et al[44] also demonstrated that TBK1 contributes to tumor invasion and might be a driving factor for metastatic spread of breast cancer. Thus, IKBKE/TBK1 dual inhibitors combined with adjuvant chemotherapy may be a potential strategy for high-risk stage I NSCLC patients.

Apparently, our study has several limitations that should be considered. The retrospective character of this study exists inevitable bias. Considering the limited amount of tissue, we cannot test as many as possible relevant proteins. Moreover, when the patient relapsed, we were unable to obtain the specimen, but only analyzed the specimen that was surgically removed at the initial diagnosis, which limited our understanding of the changes in tumor molecular characteristics.

In conclusion, the current study provides further insights for tumor targeted therapy and the landscape of kinase proteins IKBKE and TBK1 in various cancers. To our knowledge, our study is the first study to investigate the relationships between expression of IKBKE and TBK1 and clinicopathologic characteristics in stage I NSCLC patients and their predictive significance on patients' survival. We found IKBKE+/TBK1+ co-expression was significantly obvious in patients with risk factors and the recurrence pattern of metastasis, and is an effective prognostic predictor for poor OS. Of note, further investigation is required to identify optimal patients who likely obtain benefit from IKBKE and TBK1 dual inhibitors therapy.

Ethical statement

All procedures performed in studies involving human participants were in accordance with ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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