Correlation between expression of XB130, AKT and prognosis in non-small cell lung cancer patients

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Abstract

Objective To examine XB130 and AKT expression levels in the pathological tissues of non-small cell lung cancer (NSCLC) followed by analysis of their relationship with other common prognostic indicators.Methods A retrospective analysis of the clinical and pathological characteristics of 120 NSCLC subjects from August 1, 2014 to August 1, 2015 was conducted. The expression of XB130 and AKT was detected by immunohistochemistry. Correlation analysis of XB130 with various other factors was analyzed by the Chi-squared test. Relationship between XB130, AKT and patient overall survival (OS), as well as progression-free survival (PFS) was examined using Cox analysis.Results The XB130 expression was significantly correlated to tumor differentiation (P = 0.004), T staging (P = 0.045), lymphatic metastasis (P = 0.028), and AKT expression (P = 0.043). Univariate analysis of the Cox proportional hazard model revealed that high XB130 expression, lymphatic metastasis, T staging, and high AKT expression were adverse prognostic factors for NSCLC (P < 0.05). Besides, the multivariate analysis demonstrated that high XB130 expression, lymph node metastasis, and high AKT expression were the independent prognostic factors (P < 0.05).Conclusion XB130, AKT, and lymph node metastasis are independent risk factors for NSCLC prognosis. XB130 might affect patient prognosis through AKT. XB130 may be a prognostic marker for the survival and a candidate for the treatment of NSCLC, with potential therapeutic prospects.

Background

Lung cancer is one major malignancy in the world[1, 2], whose morbidity and mortality rates in China in 2015 were 733.3‰ and 610.2‰, respectively. More than 85% of lung cancers are NSCLC[3]. Although early NSCLC can be cured by surgery, about 70% of subjects show metastasis when diagnosed[3]. Moreover, drug resistance has emerged even in the presence of advanced lung cancer treatment, such as molecularly targeted therapy and immunotherapy (reference: Strategies for the treatment of non-small-cell lung cancer after drug resistance to EGFR-TKI). The prognosis of advanced NSCLC patients is poor, showing one-year average survival and lower than 20% 5-year survival rate[4-7]. For lung cancer patients, the currently proven carcinogens include smoking, air pollution, environmental factors, ionizing radiation, ras, myc gene family, etc. (reference: Air pollution and environmental risk factors for altered lung function among adult women of an urban slum area of Delhi: A prevalence study) (reference: ENVIRONMENTAL FACTORS AND p53 MUTATION SPECTRUM IN LUNG CANCER), but there are few studies on the role of XB130 in the occurrence, development, and mechanism of lung cancer. XB130 is a novel cytosolic adaptor protein that stimulates tumor development through regulating gene expression. High XB130 level was reported to be significantly related to cell proliferation, angiogenesis, and poor prognosis in a variety of tumor patients[8-12]. However, there have been few studies about the clinical value of XB130 in cancers including NSCLC. Besides, overactivation of the AKT pathway is an important mechanism for promoting cancer cell proliferation, which mediates the effects of XB130. This study focused on the correlation between XB130, AKT expression in NSCLC tissues and known risk factors, and their relationship with cancer prognosis.

Methods

Clinical data

In our study, 120 non-small cell lung cancer (NSCLC) subjects undergoing radical mastectomy at the Department of Thoracic Surgery, Qingdao City Hospital from August 1, 2014 to August 1, 2015 were enrolled. None of them had any anti-tumor treatment before surgery. All patients finished the follow-up study. There were 32 males and 88 females, aged 42 to 75 years old. Besides, we conducted the staging based on the 7th Edition TNM staging criteria from the American Joint Committee on Cancer (AJCC).

Specimen examination ***Immunohistochemistry two-step method for detecting gene expression in lung cancer tissues*** All paraffin sections were made into 4-um pieces and then stained in strict accordance with the reagent instructions. The EDTA repair solution was used for antigen retrieval. Main reagents: mouse anti-human XB130 antibody (concentration: 1:100; PTG); mouse anti-human AKT antibody (concentration: 1:400; Abcam).

Immunohistochemical staining procedures The negative control group was also immunohistochemically stained. The procedure was the same as the experimental group, and only the primary antibody was substituted by the PBS buffer. Then the counting process was performed by two pathologists, and the tissue sections were counted separately by the double-blind method. When the results of the grouping were inconsistent, the third pathologist recounted and grouped. According to the result determination method, all the slices were grouped, and the obtained data was used for statistical analysis.

Result judgment

RBM3 staining

Five high-magnification fields were randomly selected, and 100 cells were counted. The depth of coloration and the number of positive cells were determined. Staining intensity scores: colorless for 0, light yellow for 1, brown for 2, and tan for 3. Positive cell percentage scoring criteria: 25% of positive staining cells were low expression, and >25% were high expression.

AKT staining Five high-magnification fields were randomly selected, and 200 cells were counted. The staining intensity of cancer cells and the proportion of stained cells were comprehensively scored. The film was read by a pathologist in a blinded way. Once the cytoplasm and/or nucleus were stained, it was judged to be a positive cell. Staining intensity: unresponsive cells for 0, pale yellow for 1, brown yellow for

2, and sepia for 3. The stained cell percentage indicated: < 10% was 0, < 20% was 1 point, < 50% was 2 points, and ≥ 50% was 3 points. Staining intensity multiplied by stained cell percentage was used for evaluating the AKT level. The integral 0 to 2 and 3 to 9 were classified into negative and positive expression, respectively.

Statistical methodSPSS 21.0 software was used to perform the statistical analysis. Correlation comparisons were conducted with the chi-square test. Cox regression analysis was applied for the survival analysis. $P < 0.05$ was statistically significant.

Results

XB130 and AKT expression The representative images of immunohistochemical staining are shown in Fig. 1. There were 31 cases of high AKT expression and 24 cases of low AKT expression in 55 patients with high expression of XB130; there were 41 cases of low AKT expression and 24 cases of high AKT expression in 65 cases of low expression of XB130, with significant difference ($P = 0.043$). This finding indicates that the expression of AKT in most subjects with high XB130 expression is also high, with their expression levels significantly correlated. Besides, the XB130 expression was also significantly related to tumor differentiation ($P = 0.004$), T staging ($P = 0.045$), and lymphatic metastasis ($P = 0.028$) (Table 1).

XB130, AKT, and lymph node metastasis are independent prognostic factors affecting PFS and OS We then performed the univariate analysis of Cox proportional hazard model (Table 2). Overall survival (OS) and progression-free survival (PFS) in subjects having high XB130 level were significantly less than those in subjects having low XB130 level ($P = 0.000$), indicating that patients having high XB130 expression were more likely to have worse survival and prognosis and high XB130 expression is one of the adverse factors affecting the prognosis of NSCLC. Significant differences in OS and PFS were discovered between subjects with and without lymphatic metastasis ($P = 0.000$). Subjects having lymphatic metastasis had worse prognosis than subjects without lymphatic metastasis, indicating that lymphatic metastasis affects the prognosis of NSCLC. Besides, OS ($P = 0.002$) and PFS ($P = 0.003$) were significantly different between subjects having low AKT level and those having high AKT level. Subjects with low AKT level had a lower risk, indicating that high expression of AKT is one of the factors affecting the prognosis of NSCLC. Taken together, high XB130 expression, lymphatic metastasis, and high AKT expression were adverse risk factors for NSCLC prognosis ($P < 0.05$).We then conducted the multivariate analysis of Cox proportional hazard model (Table 3). Patients having high XB130 or AKT expression had a worse prognosis ($P < 0.05$), indicating that high XB130 level and high AKT level were independent risk factors for the prognosis of NSCLC, which is not affected by age, gender, lymph node metastasis, T staging, smoking or not, and the degree of differentiation. When comparing to the patients with non-lymph node metastasis, those with lymphatic metastasis had a worse prognosis ($P = 0.000$), indicating that lymphatic metastasis is an independent prognostic factor, not affected by age, gender, smoking or

differentiation. It was worth noting that the univariate analysis of Cox proportional hazard model demonstrated that T staging affected the prognosis of NSCLC, but multivariate analysis showed that T staging did not affect the NSCLC prognosis. The possible reason is that T staging has a lower impact on prognosis than lymph node metastasis, high XB130 expression, and high AKT expression, and the prognostic effect of T-stage is interfered by the confounders.

Discussion

Lung cancer is very malignant due to high morbidity and mortality. More than 1 million suffers die from lung cancer yearly, and about 85% of them die because of NSCLC, seriously endangering human life and health. Traditional treatments for NSCLC include surgery, radiotherapy, chemotherapy, and combination chemotherapy with radiotherapy and chemotherapy (Signal Transduction and Targeted Therapy 3, Article number: 28 (2018)). With the continuous improvement of technology, molecularly targeted therapy is gradually accepted. Despite this, the 5-year survival rate is still low (15%), so further research on the occurrence, development, and mechanism of lung cancer is the current focus. Here, we examined the XB130 expression in the pathological tissues of NSCLC and its relationship with OS and PFS, and suggested that it promoted the lung cancer cell proliferation via the PI3K/AKT pathway. These findings provide new ideas and directions for inhibiting tumor proliferation and evaluating disease prognosis, progression in the future.As reported, XB130 is involved not only in cell proliferation, survival, invasion[9, 13, 14] but also in signal transduction[15]. Controlled by Rac and cytoskeleton[13], XB130 activates c-Src and PI3K/AKT pathway[14], which in turn regulates cytoskeleton[13]. These signaling pathways are critical for the lung cancer progression[16, 17]. As an adapter protein, XB130 is crucial for signal transduction, cancer cell proliferation, survival, and tumorigenesis. It binds the F-actin network of lamellipodia and enhances the cancer cell motility and invasiveness. XB130 is phosphorylated by Src and other tyrosine kinases, and its interaction with Src results in the SRE transactivation of AP-1. XB130 also has a YxxM motif, a kinase binding to the p85a subunit of PI3K, through which the AKT is subsequently activated by its SH2 domain. Down-regulating XB130 can affect various molecules downstream of AKT. XB130 inhibition also decreases the expression levels of multiple genes involved in cell proliferation and/or survival. The effects of XB130 in lung cancer are mediated by AKT, which is an important signaling molecule for tumorigenesis and development. PI3K/AKT stimulates cancer cell proliferation, suppresses apoptosis and increases cell invasion and metastasis by directly phosphorylating multiple transcription factors. PI3K/AKT activation is prevalent in human malignancies, and its inhibition is important for the prevention and treatment of malignant tumors. AKT is a central mediator for the inhibition and induction of apoptosis-sensitive chemotherapeutic drugs. More and more experiments have shown that inhibition of Akt expression, especially activation of Akt (p-Akt), can block the PI3K/Akt classical signaling pathway to improve the treatment effect. Here, the XB130 expression was significantly related to AKT expression, and XB130 may affect patient prognosis through AKT. High XB130 level is significantly related to cell proliferation, angiogenesis, and poor prognosis in a variety of tumor patients[8-12]. However, there have been few studies about the clinical value of XB130 in cancers including NSCLC. To study the relationship between XB130 expression and prognosis of gastric cancer,

411 patients were selected as subjects[12]. They found that both mRNA and protein of XB130 were detected in normal gastric tissues. The OS was significantly shorter in subjects having high XB130 compared with those having low XB130. The XB130 expression predicts the sensitivity of tumors to several clinical drugs. 5-fluorouracil (5-FU), cisplatin, and irinotecan inhibited the activity of wild-type and XB130-knockout gastric cancer cells in a concentration-dependent manner, with cisplatin, irinotecan and 5-FU being more sensitive to XB130-knockout and wild-type cells, respectively. In 5-FU treatment, the survival rate was higher in subjects having high XB130 level when comparing to those having low level, suggesting that the reduction in XB130 protein level is a prognostic factor for gastric cancer patients with short survival time, high recurrence rate, and chemotherapy response. To elucidate the prognostic role of XB130 in pancreatic ductal adenocarcinoma (PDAC), a retrospective analysis of 76 PDAC subjects who underwent continuous surgical resection was performed[18]. The expression of XB130 in paraffin-embedded tumor sections was detected by immunohistochemistry and the correlation between XB130 expression and clinicopathological indicators was assessed. They found that XB130 was significantly up-regulated in PDAC relative to normal pancreatic tissue; enhanced XB130 level was associated with lymphatic metastasis, tumor nodular metastasis (TNM) stage, and tumor grade; the survival rate was significantly lower in patients having high XB130 level when comparing to those having low XB130 level; high XB130 level, tumor scale, long-distance metastasis, TNM stage, and lymph node metastasis were considered to be independent risk factors that affect prognosis by univariate analysis of the Cox proportional hazard model; multivariate analysis demonstrated that high XB130 level and long-distance metastasis were significant risk factors. Taken together, XB130 is believed to be a potential pathological indicator for predicting PDAC patient prognosis. In NSCLC, our study showed the similar results. In summary, the expression of XB130 was significantly related to the tumor differentiation degree, lymphatic metastasis, T staging, and AKT expression. High XB130 expression, high AKT expression, and lymphatic metastasis were independent prognostic factors for NSCLC. XB130 may be an important clinical prognostic marker. XB130 might regulate the survival and prognosis of NSCLC by affecting the AKT signaling pathway. We believe that XB130 might become a promising therapeutic drug target for NSCLC treatment, and inhibition of XB130 expression may become one of the methods for treating tumors.

Conclusions

XB130 may become a potential therapeutic target for the treatment of NSCLC in the future.

Declarations

Ethics approval and consent to participate

Our study was approved by Qingdao Municipal Hospital (approval no. QSL2014015067). All patients provided written informed consent prior to enrollment in the study.

Consent for publication

Written informed consent was obtained from all participants.

Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they had no competing interests.

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Authors' contributions

Conception and design: LC.
Analysis and interpretation of data: XYZ.

Drafting the article: ZLH

Revising it critically for important intellectual content: KG,YL,CYG,TLZ.

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Tables

Table 1. Relationship between XB130 and clinical pathological parameters

	n=128	XB130 (n=128)		χ^2	*P*
		poor (n=65)	rich (n=55)		
Age				2.719	0.142
≤55	60	28	32		
55	60	37	23		
Gender				0.076	0.838
Male	32	18	14		
Female	88	47	41		
Smoking				0.040	1.000
Yes	84	45	39		
No	36	20	16		
Degree of tumor differentiation				8.685	0.004
Medium + high differentiation	59	40	19		
Poor differentiation	61	25	36		
Lymphatic metastasis				5.211	0.028
Presence (N+)	55	36	19		
Absence (N0)	65	29	36		
T staging				6.214	0.045
T (1)	54	36	18		
T (2)	31	14	17		
T (3)	35	15	20		
AKT				4.535	0.043
Low expression	65	41	24		
High expression	55	24	31		

Table 2. Univariate analysis of XB130 and OS, PFS

	OS		PFS	
	hr (95% CI)	*P*	hr (95% CI)	*P*
Age				
≤ 55	0.674 (0.429-1.058)	0.087	0.701 (0.450-1.091)	0.115
> 55	1.000 (Ref.)		1.000 (Ref.)	
XB130				
Low expression	0.240 (0.148-0.390)	0.000	0.261 (0.163-0.417)	0.000
High expression	1.000 (Ref.)		1.000 (Ref.)	
Gender				
Male	0.667 (0.389-1.144)	0.141	0.687 (0.406-1.162)	0.162
Female	1.000 (Ref.)		1.000 (Ref.)	
Smoking				
Yes	1.034 (0.629-1.698)	0.896	0.984 (0.601-1.610)	0.947
No	1.000 (Ref.)		1.000 (Ref.)	
Degree of tumor differentiation				
Medium + high differentiation	0.716 (0.454-1.130)	0.151	0.685 (0.438-1.073)	0.098
Poor differentiation	1.000 (Ref.)		1.000 (Ref.)	
Lymphatic metastasis				
Absence (N0)	0.223 (0.133-0.374)	0.000	0.235 (0.143-0.386)	0.000
Presence (N1+N2)	1.000 (Ref.)		1.000 (Ref.)	
T staging				
T1	0.518 (0.301-0.891)	0.017	0.555 (0.326-0.946)	0.030
T2	0.831 (0.469-1.474)	0.528	0.819 (0.462-1.453)	0.495
T3	1.000 (Ref.)		1.000 (Ref.)	

T1	1.000 (Ref.)		1.000 (Ref.)	
T2	1.605 (0.928-2.776)	0.090	1.475 (0.862-2.521)	0.156
T3	1.931 (1.122-3.322)	0.017	1.800 (1.058-3.065)	0.030
AKT				
Low expression	0.489 (0.311-0.771)	0.002	0.514 (0.330-0.803)	0.003
High expression	1.000 (Ref.)		1.000 (Ref.)	

Table 3. Multivariate analysis of XB130 and OS, PFS

	OS		PFS	
	hr (95% CI)	*P*	hr (95% CI)	*P*
Age				
≤ 55	0.614 (0.360-1.047)	0.073	0.656 (0.384-1.120)	0.123
> 55	1.000 (Ref.)		1.000 (Ref.)	
Gender				
Male	0.489 (0.226-1.061)	0.070	0.573 (0.263-1.247)	0.161
Female	1.000 (Ref.)		1.000 (Ref.)	
Smoking				
Yes	0.979 (0.392-2.445)	0.965	0.909 (0.366-2.255)	0.837
No	1.000 (Ref.)		1.000 (Ref.)	
Degree of tumor differentiation				
Medium + high differentiation	1.063 (0.597-1.895)	0.835	0.986 (0.561-1.733)	0.986
Poor differentiation	1.000 (Ref.)		1.000 (Ref.)	
Lymphatic metastasis				
Absence (N0)	0.169 (0.092-0.312)	0.000	0.181 (0.100-0.326)	0.000
Presence (N1+N2)	1.000 (Ref.)		1.000 (Ref.)	
T staging				
T1	0.805（0.393-1.649）	0.553	0.811 (0.395-1.666)	0.569
T2	1.546 (0.697-3.425)	0.284	1.407 (0.640-3.097)	0.396
T3	1.000 (Ref.)		1.000 (Ref.)	
T1	1.000 (Ref.)		1.000 (Ref.)	
T2	1.921 (1.011-3.649)	0.051	1.734 (0.918-3.275)	0.090
T3	1.243 (0.607-2.547)	0.553	1.232 (0.600-2.529)	0.569
AKT				

Low expression	0.282 (0.165-0.484)	0.000	0.318 (0.187-0.543)	0.000
High expression	1.000 (Ref.)		1.000 (Ref.)	
XB130				
Low expression	0.327 (0.177-0.603)	0.000	0.345 (0.189-0.632)	0.001
High expression	1.000 (Ref)		1.000 (Ref.)	

Figures

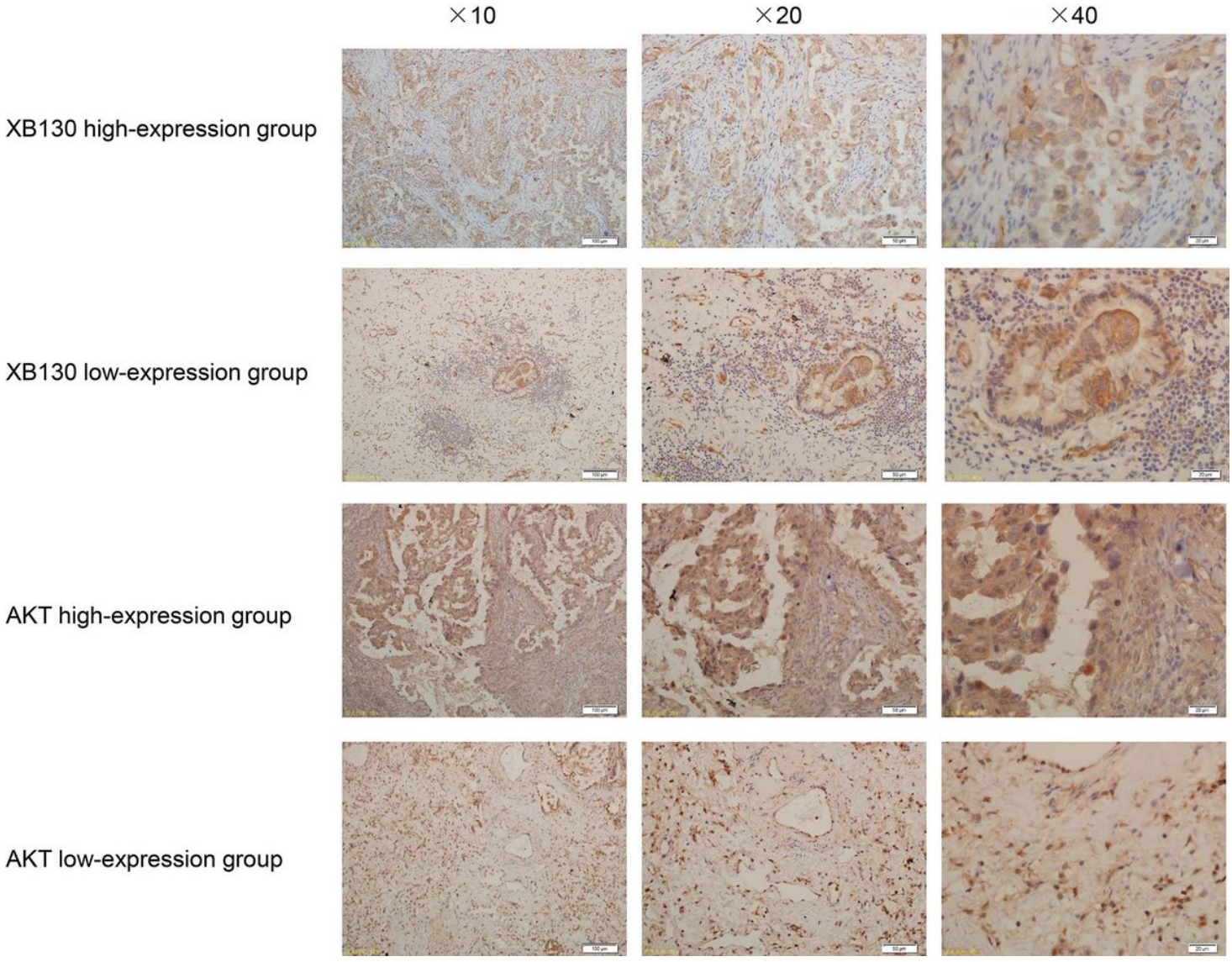

Figure 1

Immunohistochemical staining of XB130 and AKT in lung cancer tissues.