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Reduced expression of Krüppel-like factor 17 is related to tumor growth and poor prognosis in lung adenocarcinoma

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ABSTRACT

Krüppel-like factor 17 (KLF17), a new member of the Krüppel-like factors (KLFs), has been reported to be a negative regulator of epithelial-mesenchymal transition (EMT) and metastasis in breast cancer. However, the biological role and clinical significance of KLF17 in lung adenocarcinoma has been less clear. In the present study, we showed that KLF17 expression was decreased in lung adenocarcinoma. Reduced expression of KLF17 was correlated significantly with a short survival time in patients with lung adenocarcinoma ($P < 0.0001$). Moreover, KLF17 expression was an independent prognostic indicator for patients with lung adenocarcinoma. KLF17 expression level was correlated with the tumor stage ($P = 0.016$) and tumor size ($P = 0.001$) in lung adenocarcinoma. Overexpression of KLF17 inhibited cell growth in A549 and PC-9 cell lines. In conclusion, it is possible that KLF17 inhibits tumor growth in lung adenocarcinoma. The reduced expression of KLF17 is a valuable prognostic indicator for patients with lung adenocarcinoma, and KLF17 could be a novel target for treatment of lung adenocarcinoma.

1. Introduction

Lung cancer is one of the most common cancers worldwide. It is the leading and second leading cause of cancer-related death in men and women, respectively [1]. However, the mortality rate of lung cancer is gradually decreasing in men, while it is increasing in women [2,3]. Currently, the incidence of lung adenocarcinoma (AC) is increased among the three major histological types of non-small cell lung cancer (NSCLC) [4,5]. Although progress has been made in the prevention, early detection and treatment of lung adenocarcinoma, the overall 5-year survival rates of patients are less than 14% in the US and are even lower (5–10%) in Europe and in other countries [6]. Hence, it is necessary to identify new and effective biomarkers for early diagnosis and prognosis of lung adenocarcinoma.

Krüppel-like factors (KLFs), which include KLF1 – KLF17 [7], are a subfamily of the mammalian Sp/KLF (specificity protein/Krüppel-like factor) zinc-finger protein family. They play important roles in transcription by binding via their highly conserved DNA-binding domains (DBDs) or C-termini to related G/C and CACCC boxes of target genes [8]. KLFs regulate vital cellular processes, including differentiation, proliferation, development and apoptosis [9], and stimulate differentiated cells to regress to stem cells [10]. Furthermore, many KLFs are implicated in tumor cell proliferation [11,12], invasion and metastasis [13,14]. They function as activators or suppressors as determined by the gene promoters and regulator proteins they bind [15,16]. In addition, some KLFs show different tissue distributions and posttranslational regulations and can even exert opposing effects in different tissues. Thus, the roles of KLFs are controversial and may be dependent on the tissue and cell type.

KLF17 is a new member of Sp/KLFs [8]. Gumireddy et al. [17] and Iwanicki et al. [18] reported that KLF17 expression was decreased in invasive breast cancer cell lines and in breast cancer tissues with lymph node metastasis. In their studies, knockdown of KLF17 expression in non-invasive breast cancer cell lines promoted epithelial-mesenchymal transition (EMT) in vitro and lung metastasis in vivo. Furthermore, statistical analysis indicates that KLF17 expression is an effective predictor for lymph node metastasis in breast cancer. However, little is known about KLF17 expression in lung adenocarcinoma and in particular about whether it can act as a prognostic indicator for lung adenocarcinoma.

In the present study, we found that KLF17 expression was lower in adenocarcinoma cell lines and lung adenocarcinoma tissues than those of immortal bronchial epithelial cells and adjacent normal

Abbreviations: EMT, epithelial-mesenchymal transition; MTD, maximum tumor diameter; KLF17, Krüppel-like factor 17.

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lung tissues, respectively. Reduced expression of KLF17 was an independent indicator of poor prognosis for patients with lung adenocarcinoma. The overexpression of KLF17 in lung adenocarcinoma cell lines inhibited cells growth. Taken together, our findings suggest that KLF17 may be a tumor suppressor gene in lung adenocarcinoma and that it may be a novel biomarker of prognosis in patients with lung adenocarcinoma.

2. Materials and methods

2.1. Cell lines and cell culture

An immortal human bronchial epithelial cell line (16HBE) was a gift from the Cancer Prevention Center of Sun Yat-sen University. Human lung adenocarcinoma cell lines, including A549, H322, H1299 and PC-9, were purchased from Cell Bank, Chinese Academy of Sciences (Shanghai, China). All cell lines were cultured in Dulbecco's modified Eagle medium (DMEM, Gibco BRL, Carlsbad, CA) supplemented with 10% fetal bovine serum (Gibco BRL), 100 U/ml penicillin G sodium and 100 μg/ml streptomycin sulfate (Sigma, Saint Louis, MO) and incubated at 37 °C in an atmosphere containing 5% CO_2.

2.2. Real time-PCR analysis

Total RNA was isolated from cells, primary lung cancer and adjacent normal lung tissues using Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. cDNA was then synthesized using PrimeScript RT reagent Kit (Invitrogen). Specific primers were used for KLF17 (forward: 5′-GCTCTGGAGTGCACACCTCTT-3′ and reverse: 5′-CAGCATCTCTGCGCTGTGA-3′). Specific primers for β-actin were used to control for amplification (forward: 5-TCGTCCACCGCAAATGCTTCTAG-3′) and (reverse: 5′-ACTGCTGTCACCTTCACCGTTCC-3′). Then equal amounts of loaded cDNA were detected in the presence of the SYBR® Premix Ex TaqTMkit (Invitrogen) using an ABI7900 PRISM (Applied Biosystems, Sunnyvale, CA).

2.3. Western blotting analysis

Western blotting was performed as previously described [19] with anti-human KLF17 antibody (1:200, Abgent, CA) or anti-GAPDH (1:3000, Genescript, NJ).

2.4. Patient information and samples

Sixty paraffin-embedded lung adenocarcinoma samples from patients admitted between 2006 and 2007 were selected from The First Affiliated Hospital of Sun Yat-sen University archives. Complete clinical information, including the five-year survival time and outcomes, were obtained for this study. All patients were diagnosed pathologically according to WHO criteria [20]. Furthermore, the initial maximum tumor diameter (MTD) of each patient was recorded, which was calculated using a Computed tomography (CT) scan. Written consents from patients and approval of the Institutional Research Ethics Committee of The First Affiliated Hospital of Sun Yat-sen University were obtained. The clinical information on all patients is summarized in Table 1. All paraffin-embedded samples were cut into 4 μm sections. Four paired lung adenocarcinoma samples and adjacent tissues (located 3 cm away from the cancer margin) and two normal lung tissues from patients with pulmonary angioma were acquired in 2011 and were used for analysis of KLF17 expression.

2.5. Immunohistochemistry

Immunohistochemistry was performed as described previously [19] with antibody for KLF17 (1:100; Sigma, St. Louis, MO). The scoring of IHC sections was completed by two independent observers who were blind to patient status and outcome. The method for scoring the intensity of staining (intensity score) and distribution of positively stained tumor cells (distribution score) has been described previously [19]. Briefly, the intensity score is classified as follows: 0 (no staining); 1 (light yellow), 2 (yellow brown), and 3 (brown). The distribution score was graded using the following criteria: 0 (0%), 1 (<10%), 2 (10–50%), and 3 (>50%). The staining index (intensity score × distribution score), which was scored as 0, 1, 2, 3, 4, 6 and 9, was used to calculate the expression level of the KLF17 protein. The optimal cutoff values of KLF17 were chosen based on statistical analysis using the log-rank test with respect to overall survival. In our study, staining index scores of $\geqslant 6$ and $\leqslant 4$ were regarded as high and low KLF17 expression levels, respectively.

2.6. Transient infection, colony formation and Cell Counting Kit-8 (CCK-8) assay

The full-length recombinant human KLF17 cDNA (NM_173484; 1170 bp) in the plasmid pEZ-M15-KLF17, which contains yellow fluorescent protein (YFP), was purchased from GeneCopoeia, Inc. (MD). A549 or PC-9 cells were transfected with pEZ-M15-KLF17 or the pEZ-M15 vector (control) using polyJet DNA transfection reagent (SignaGen, Laboratories, Gaithersburg, MD) according to the manufacturer's instructions. After 72 h of transfection, the presence of the KLF17 protein in the transfected A549 or PC-9 cells was confirmed using western blotting analysis. Colony formation assays were performed as described previously [21]. For the CCK-8 assay, A549 or PC-9 cells were seeded at a density of 0.8×10^4 per well in a 96-well plate. After a 24-h incubation, the cells were transfected with pEZ-M15-KLF17 or the control vector, as described above. These cells or parental cells were then analyzed every day using a Cell Counting Kit-8 assay (Dojindo, Kumamoto, Japan) according to the manufacturer's protocol.

2.7. Statistical analysis

All experimental data were analyzed using the SPSS 16.0 statistical software package (SPSS Inc., Chicago, IL). Kaplan–Meier analysis and the log-rank test were performed to determine the difference between two different KLF17 expression groups. Various clinical risk factors for survival were initially analyzed using univariate analysis and the significant variables were then analyzed using multivariate analysis in the Cox proportional hazards regression model. The Mann–Whitney U test was performed to analyze the correlation between clinicopathologic characteristics and KLF17 expression. The initial MTD between different KLF17 expression groups was analyzed using the Wilcoxon rank sum test. The in vitro observations were analyzed using the Student's t test or one-way analysis of variance. The data from at least two independent experiments were expressed as the mean ± standard deviation (SD). $P < 0.05$ was considered statistically significant.

3. Results

3.1. KLF17 expression is decreased in human lung adenocarcinoma cell lines and lung adenocarcinoma tissues

Real time-PCR and western blotting were used to determine KLF17 mRNA and protein expression in 16HBE, A549, H322, H1299 and PC-9 cell lines. As shown in Fig. 1A and C, both the mRNA and protein levels of KLF17 in lung adenocarcinoma cell lines were lower than in the immortal human bronchial epithelial cell line (16HBE cells). The mRNA was reduced by up to 7.81-fold (Fig. 1A). In partic-

Table 1
Demographic and clinicopathologic characteristics of patients with lung adenocarcinoma.

Clinicopathologic characteristics	No. of cases (%)	Clinicopathologic characteristics	No. of cases (%)
Age (y)		T1	15 (25.0)
⩽65	39 (65.0)	T2	27 (45.0)
>65	21 (35.0)	T3	7 (11.7)
Gender		T4	11 (18.3)
Male	36 (60.0)	N classification	
Female	24 (40.0)	N0	34 (56.6)
Tumor location		N1	10 (16.7)
Left upper lobe	12 (20.0)	N2–N3	16 (26.7)
Left lower lobe	17 (28.3)	M classification	
Right upper lobe	17 (28.3)	M0	50 (83.3)
Right middle lobe	4 (6.7)	M1	10 (16.7)
Right lower lobe	10 (16.7)	Smoking	
Differentiation		Yes	29 (48.3)
Poor	24 (40.0)	No	31 (51.7)
Moderate	32 (53.3)	KLF17 expression	
Well	4 (6.7)	Negative	5 (8.3)
Pathological stage		Positive	55 (91.7)
I	30 (50.0)	Low expression	32 (53.3)
II	6 (10.0)	High expression	23 (38.4)
III	14 (23.3)	Vital status (as follow up)	
IV	10 (16.7)	Death	32(53.3)
T classification		Alive	28(46.7)

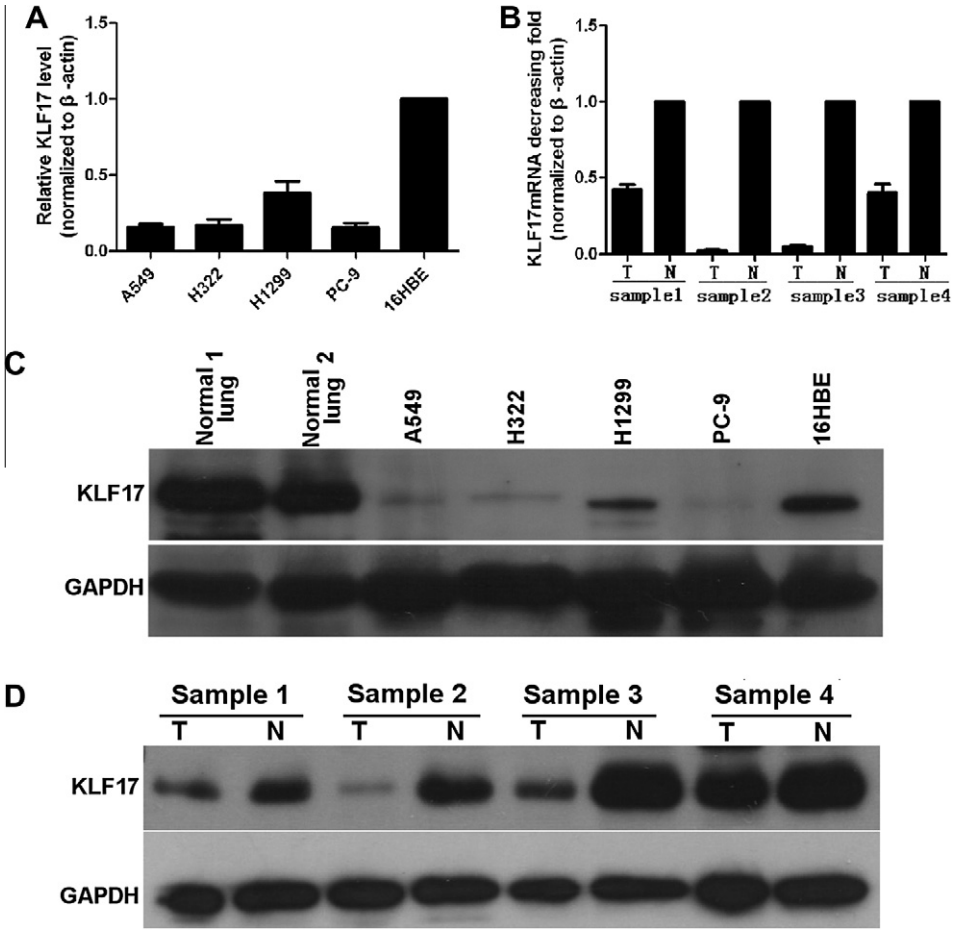

Fig. 1. KLF17 is down-regulated in lung adenocarcinoma cell lines and lung adenocarcinoma tissues. (A) The expression of KLF17 mRNA in human bronchial epithelial cells (16HBE) and lung adenocarcinoma cell lines (A549, H322, H1299, PC-9) was analyzed using real time-PCR. (B) The KLF17 mRNA in lung adenocarcinoma (tumor, T) and matched normal lung tissue (N) from the same patient was analyzed using real time-PCR. β-Actin was used as an internal control. (C) Western blotting analysis showed that KLF17 protein expression in A549, H322, H1299 and PC-9 cells was lower than in 16HBE cells and human normal lung tissues. (D) Western blotting analysis of the protein level of KLF17 in paired tissues (Tumor, T and normal, N) from same patient.

ular, the protein level of KLF17 in human normal lung tissues was significantly higher than that of lung adenocarcinoma cell lines. Furthermore, the expression levels of both KLF17 mRNA (Fig. 1B) and protein (Fig. 1D) were lower in tumor tissues than in adjacent lung

tissues. The cancer-adjacent tissue/tumor ratio of KLF17 mRNA in four paired tissues was decreased and varied from 2.5-fold to 50-fold reduced (Fig. 1B). These results indicated that KLF17 expression, at both the protein and mRNA level, was decreased in lung adenocarcinoma cell lines and lung adenocarcinoma tissues.

3.2. The reduced expression of KLF17 in archived lung adenocarcinoma tissues was correlated with poor patient prognosis

To examine whether reduced expression of KLF17 was correlated with the prognosis of patients with lung adenocarcinoma, 60 cases of archived paraffin-embedded samples were assayed using immunohistochemistry, and the corresponding clinical information was analyzed retrospectively. Low expression of KLF17 was found in 37 of 60 (61.7%) cases, and high expression of KLF17 was detected in 23 of 60 (38.4%) cases (Fig. 2A–E). Furthermore, highly expressed KLF17 was mainly localized to the cytoplasm of the region of lung adenocarcinoma (Fig. 2A-inset). Additionally, there were nine cases with lymph node metastasis in 23 cases that showed high expression of KLF17 (39.1%) localized to the cytoplasm. To distinguish immunostaining with KLF17 antibody from non-specific background staining, the KLF17 antibody was replaced with PBS, and these sections were used as a negative control. As shown in Fig. 2D and E, these results indicated that the KLF17 protein was specifically detected by the KLF17 antibody. Using the optimal cut-off value for the immunostaining score, we calculated the correlation between KLF17 expression and the survival time of patients with lung adenocarcinoma. Log-rank analysis demonstrated that the survival time was significantly different between the high- and low-KLF17 expression groups ($P < 0.0001$). Kaplan–Meier analysis indicated that the cumulative 5-year survival rate in the high-KLF17 expression group was 87% (95% CI: 0.772–0.968), whereas it was 17.6% (95% CI: 0.031–0.321) in the low-KLF17 expression group (Fig. 2F). These results demonstrated that low expression of KLF17 protein was associated with a short survival time in patients with lung adenocarcinoma.

3.3. Low expression of KLF17 was an independent indicator of poor prognosis in patients with lung adenocarcinoma

We examined the effects of KLF17 protein expression and other clinical indicators, including pathological stage, tumor

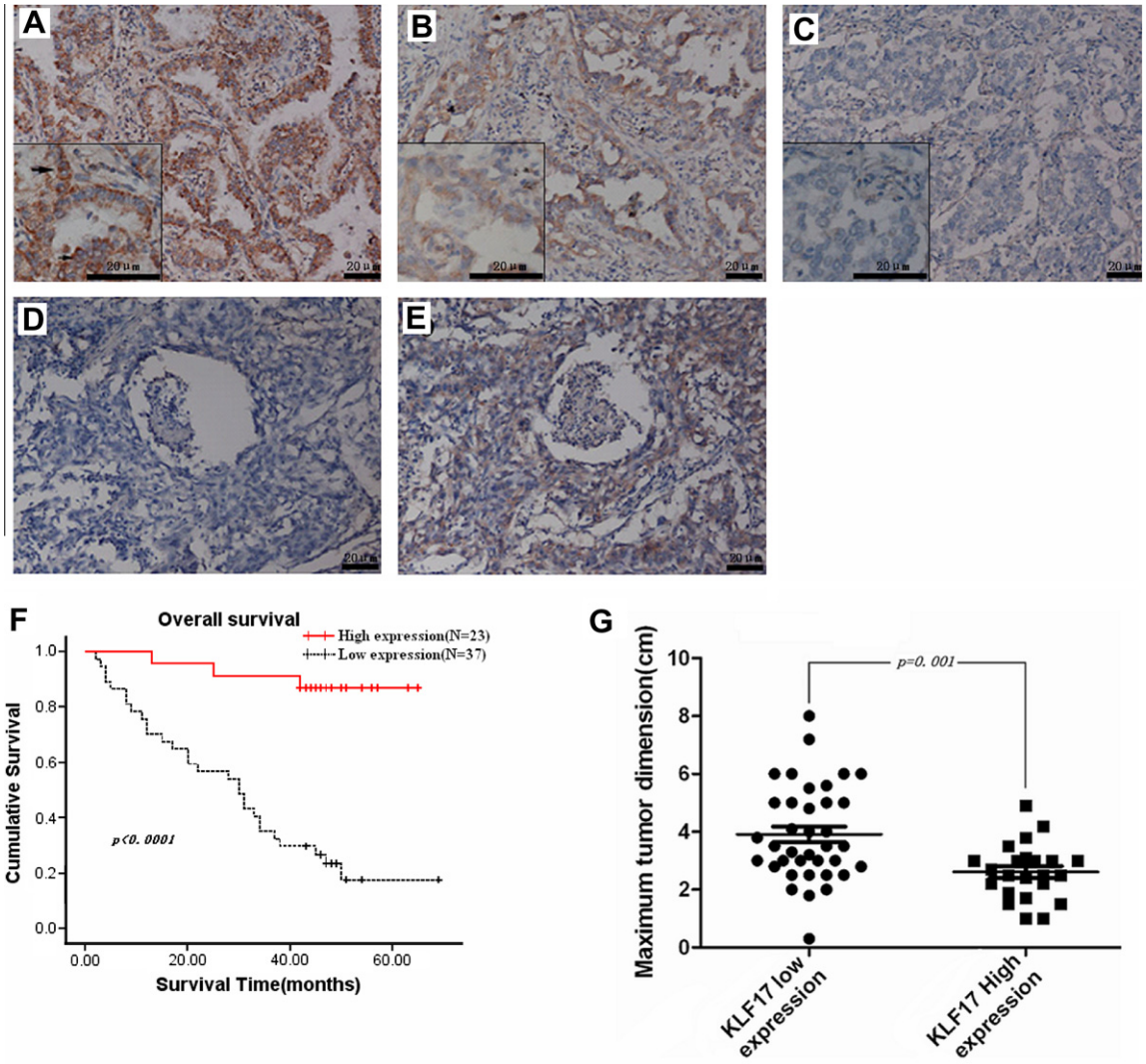

Fig. 2. The expression of KLF17 protein in archived lung adenocarcinoma tissues was correlated with the tumor size and overall survival time of patients. (A–C) Representative images from 60 cases of lung adenocarcinoma with strong immunostaining, moderate staining and negative staining (200×, inset 400×). The KLF17 protein was localized mainly to the tumor cytoplasm of primary lung adenocarcinoma (arrow, inset in A). (D and E) A lung adenocarcinoma section immunostained with PBS (negative control) or using the KLF17 antibody (200×). Scale bar: 20 μm. (F) The overall survival time of patients with lung adenocarcinoma was significantly different between the high (solid red line)- and low (dotted black line)-KLF17 expression groups ($P < 0.0001$). (G) Maximum tumor dimensions (MTDs) of patients with lung adenocarcinoma were significantly different between the high- and low-KLF17 expression groups. Each dot represents the MTD of one patient.

differentiation and tumor, node, metastasis (TNM) staging system [22], on the prognosis of patients with lung adenocarcinoma using univariate and multivariate Cox regression analysis. Initially, the significant variables were selected from all patient clinical indicators using univariate analysis. Multivariate analysis was then used to analyze independent variables. As shown in Table 2, the expression level of KLF17 protein and the M stage were independent indicators of poor prognosis in patients with lung adenocarcinoma. The hazard of death for patients with low KLF17 expression increased 10.431-fold (95% CI: 2.957–36.794) compared with patients with high KLF17 expression ($P < 0.001$), while the hazard of death for patients with tumor metastasis increased 3.484-fold compared with patients without metastasis. These data suggest that the expression level of the KLF17 protein may be a significant prognostic indicator for patients with lung adenocarcinoma.

3.4. The reduced expression of KLF17 was correlated with larger tumor size

Because the expression of KLF17 was a significant prognostic biomarker for patients with lung adenocarcinoma, we next explored the correlation between KLF17 expression and various clinical characteristics. The Mann–Whitney U test indicated that T stage was only significantly correlated with KLF17 expression ($P = 0.016$), whereas age, gender, smoking status, pathological stage, tumor differentiation and TNM stage were not correlated with KLF17 expression ($P > 0.05$, Table. 3). The 7th edition of the TNM lung cancer staging system of indicates that the T stage is mainly categorized according to tumor size [22]. Hence, in our study, we also investigated the difference in MTD between the high- and low-KLF17 expression groups. Wilcoxon rank sum test analysis demonstrated that the MTD was significantly different between these two groups ($P = 0.001$, Fig. 2G). Patients with low KLF17 expression frequently developed larger tumors. The median MTD in the low-KLF17 expression group was 3.5 cm (range: 0.3–8.0 cm); however, the MTD was 2.5 cm (range: 1.0–4.9 cm) in the high-KLF17 expression group. These results indicated that KLF17 may affect tumor growth in patients with lung adenocarcinoma.

3.5. Overexpression of KLF17 in a lung adenocarcinoma cell line inhibits cell growth

To confirm the inhibitory role of KLF17 on the growth of tumor cells, we transfected the plasmids pEZ-M15-KLF17 or pEZ-M15 (control) into A549 or PC-9 cells (the plasmids carried a *neo* gene allowing G418 selection). The transfection efficiency of the pEZ-M15-KLF17 (YFP-KLF17 fusion protein) plasmid in A549 or PC-9 cells was 56.45% and 32.15%, respectively (data not shown). The expressed YFP-KLF17 fusion protein was localized primarily in the nucleus, whereas the YFP protein was found both in the cytoplasm and nucleus of cancer cells (data not shown). Fig. 3A and B show that the KLF17 protein level was significantly increased in A549 or PC-9 cells transfected with the pEZ-M15-KLF17 plasmid compared with the cells transfected with the pEZ-M15 vector. For the colony formation assay, A549 or PC-9 cells were transfected with pEZ-M15-KLF17 and selected with G418 for 3 weeks (cells transfected with the pEZ-M15 vector were used as controls). The number of G418-resistant colonies in cells transfected with the

Table 3
Correlation between KLF17 expression and clinicopathologic characteristics of patients with lung adenocarcinoma.

Characteristics	KLF17 expression Low or none (*N*)	High (*N*)	Mann–Whitney *U* (*P* value)
Age(y)			
⩽65	24	15	0.978
>65	13	8	
Gender			
Male	25	11	0.132
Female	12	12	
Smoking			
No	17	14	0.265
Yes	20	9	
Differentiation			
None/poor	22	5	0.515
Moderate/well	15	18	
Pathological stage			
I	15	15	0.055
II	4	2	
III	10	4	
IV	8	2	
T stage			
T1	5	10	0.016
T2	18	9	
T3	6	1	
T4	8	3	
N stage			
N0	18	16	0.202
N1–N3	19	7	
M stage			
M0	29	21	0.195
M1	8	2	

Table 2
Univariate and multivariate Cox regression analyses of overall survival of patients with lung adenocarcinoma.

Univariate analysis				Multivariate analysis		
Variables	No. of patients	SE	*P* value	Variables	HR (95% CI)	*P* value
KLF17 expression				KLF17 expression		
None or low	37	0.610	<0.001	None or low	10.431(2.957–36.794)	<0.001
High	23			High	1.000	
Differentiation						
None/poor	27	0.359	0.041		NA	
Moderate/well	33					
Pathological stage						
I–II	36	0.357	0.009		NA	
III–IV	24					
T stage						
T1–T2	42	0.362	0.015		NA	
T3–T4	18					
N stage						
N0	34	0.358	0.026		NA	
N1–N3	26					
M stage				M stage		
M0	50	0.413	0.019	M0	1.000	0.038
M1	10			M1	3.484 (1.074–11.364)	

Note: NA indicates that the data were not available

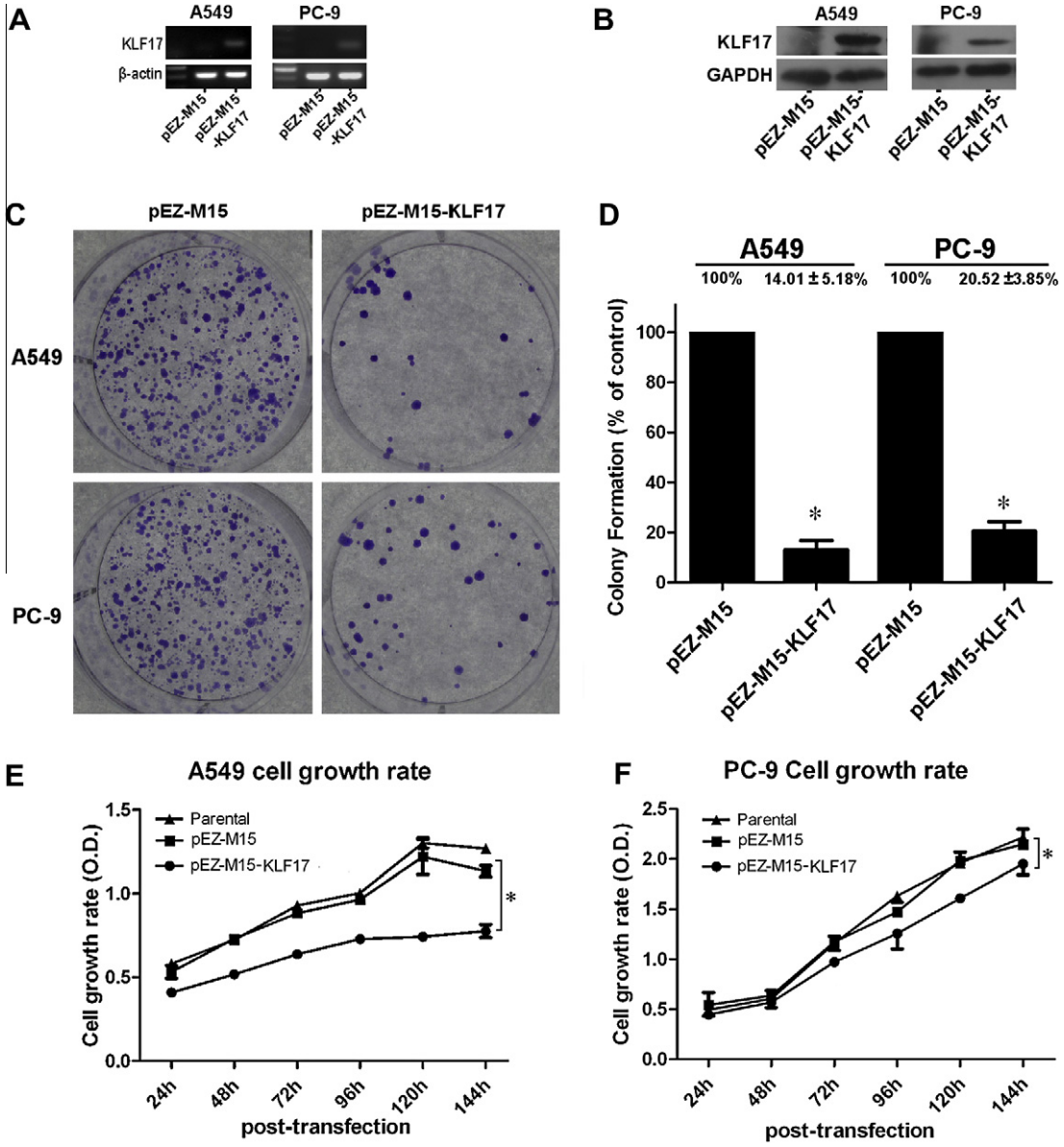

Fig. 3. Overexpression of KLF17 in A549 or PC-9 cells inhibited growth of the cells. *A-B*, Using RT-PCR (A) and western blotting (B), we found that KLF17 expression was increased in A549 or PC-9 cells transfected with the pEZ-M15-KLF17 vector. *C-D*, KLF17 suppressed colony formation in A549 and PC-9 cells. Representative cases are shown from A549 and PC-9 cells transfected with the pEZ-M15-KLF17 (right) and control pEZ-M15 (left) vector (C). The graph shows that the number of colonies formed following pEZ-M15-KLF17 transfection was markedly less than the number following transfection with the control vector (*$P < 0.05$, D). *E-F*, The growth curve of A549 and PC-9 cells transfected with pEZ-M15-KLF17 was lower than that of A549 and PC-9 cells transfected with the control vector or parental cells after 24 h of transfection. The data were expressed as the mean ± SD of three experiments. *$P < 0.05$.

pEZ-M15 vector was set to 100%. As shown in Fig. 3C and D, KLF17 markedly inhibited colony formation of A549 (14.01 ± 5.18%) and PC-9 (20.52 ± 3.85%) cells ($P < 0.05$). Furthermore, the CCK-8 assay demonstrated that the growth rates of A549 or PC-9 cells transfected with the pEZ-M15-KLF17 plasmid were lower than cells transfected with a control vector or parental cells after 24 h of transfection ($P < 0.05$) and that KLF17 inhibited A549 or PC-9 growth in a time-dependent manner (Fig. 3E and F).

4. Discussion

KLF17 suppresses epithelial–mesenchymal transition (EMT) in breast cancer by directly binding to the promoter and inhibiting the transcription of Id1, which is a key regulator of tumorigenesis, EMT, angiogenesis, invasion and metastasis. Enforced KLF17 expression in highly metastatic breast cancer cells inhibits the ability of the cells to metastasize to the lung. In addition, low expression of KLF17 is an independent predictor of lymph node metastasis in breast cancer [17]. However, the expression status of KLF17 in human primary lung adenocarcinoma and its prognostic role for human primary lung adenocarcinoma has been less clear.

In this study, we demonstrate that both the mRNA and protein levels of KLF17 in lung adenocarcinoma cell lines and in primary lung adenocarcinoma tissues were lower than in an immortal human bronchial epithelial cell line and tumor-adjacent lung tissues. These results illustrate that KLF17 is more frequently expressed in human lung tissues and may act as a tumor suppressor in human lung adenocarcinoma. In further investigation of the correlation of KLF17 expression with the prognosis of patients with lung adenocarcinoma, Log-rank analysis demonstrated that the survival

time was significantly different between the high- and low-KLF17 expression groups. Low expression of KLF17 protein in lung adenocarcinoma was associated with a reduced survival time in patients. The 5-year cumulative survival rate of the patients with low KLF17 protein expression (17.6%; 95% CI: 0.031–0.321) was markedly lower than that of the high-KLF17 protein expression group (87.0%; 95% CI: 0.772–0.968). In addition, the risk of death for patients with low KLF17 expression increased by at least 10.431-fold (95% CI: 2.957–36.794) compared with patients with high KLF17 expression, whereas the risk of death for patients with distant tumor metastasis increased by only 3.484-fold (95% CI: 1.074–11.364). These results support the theory that the KLF17 expression level is an important prognostic indicator for patients with lung adenocarcinoma.

We then examined the correlation between KLF17 expression and clinical characteristics to explore the possible role of KLF17 in lung adenocarcinoma. Mann–Whitney U tests revealed that only the tumor T stage was significantly correlated with KLF17 expression ($P = 0.016$). Because tumor T staging reflects mainly the tumor size in the 7th edition of the tumor TNM staging system of lung cancer [22], the maximum tumor dimension (MTD) of the patients was analyzed in our study. Our results indicated that the MTD was significantly different between the high- and low-KLF17 expression groups. Patients with lower expression of KLF17 frequently developed larger tumors. The tumor TNM stage is an independent predictor for overall survival time. Moreover, some evidence [23] indicates that tumor size is an independent prognostic parameter for patients with lung cancer. Taken together, our results suggest that KLF17 is a tumor suppressor and that reduced expression of KLF17 is an important biomarker for poor prognosis of patients with lung adenocarcinoma. However, the role of KLF17 in lung adenocarcinoma is different from its role in breast cancer. The possible reasons underlying this reasons could include (i) the different tissue context and promoters of target genes regulated by KLF17 and (ii) a limitation of our study in that N staging was classified partially according to lymph node enlargement as measured using CT, whereas accumulating evidence indicates that lymph nodes can be normal in size while micrometastasis occurs [24]. Whether KLF17 promotes lymph node metastasis in lung adenocarcinoma requires further investigation.

We then validated the role of KLF17 in lung adenocarcinoma cell lines. Colony formation and CCK-8 assays showed that enforced KLF17 expression in A549 and PC-9 cells inhibited cell growth and that KLF17 suppressed tumor cell growth in a time-dependent manner. In addition, we found that the transfected yellow fluorescent protein (YFP)-KLF17 fusion protein was mainly localized to the nucleus, while KLF17 was localized to the cytoplasm in lung adenocarcinoma tissues. Whether different subcellular distributions of the KLF17 protein induce different functions remains unclear and requires further investigation. KLF17 is chiefly related to KLF1–8 and 12, as analysis of the evolutionary tree indicates that they share a common ancestor [8]. This finding indicates that KLF17 can perform functions similar to KLF1–8 and 12. For example, KLF4 and KLF6 can inhibit tumor cell proliferation [21,25], which is consistent with our results. Based on our results and those of other previous studies [21,25], KLF17 may be a novel tumor growth suppressor of lung adenocarcinoma.

In conclusion, we initially evaluated the possible role of KLF17 in human lung adenocarcinoma and the possibility of using the KLF17 as a prognostic indicator for patients with lung adenocarcinoma. Our data demonstrate that KLF17 protein expression was reduced in lung adenocarcinoma and was an independent prognostic indicator for survival time of patients with lung adenocarcinoma. The expression level of KLF17 was correlated with tumor size, and enforced KLF17 expression inhibited cancer cell growth. Thus, KLF17 not only was an indicator for patient prognosis but also could act as a target for therapeutics.

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References

[1] J. Ahmedin, B. Freddie, M.C. Melissa, et al., Global Cancer Statistics, CA CANCER J. CLIN. 61 (2011) 69–90.
[2] M. Malvezzi, A. Arfé, P. Bertuccio, et al., European cancer mortality predictions for the year 2011, Ann. Oncol. 22 (2011) 947–956.
[3] J. Ahmedin, S. Rebecca, X. Jiaquan, et al., Cancer Statistics, 2010, CA Cancer J. Clin. 60 (2010) 277–300.
[4] N. Wang, W.Q. Chen, W.X. Zhu, et al., Incidence trends and pathological characteristics of lung cancer in urban Beijing during period of 1998 – 2007, Zhonghua Yu Fang Yi Xue Za Zhi 45 (2011) 248–254.
[5] C.M. Sagerup, M. Smastuen, T.B. Johannesen, et al., Sex-specific trends in lung cancer incidence and survival: a population study of 40, 118 cases, Thorax 66 (2011) 301–307.
[6] W.K. Lam, D.N. Watkins, Lung cancer: future directions, Respiratory 12 (2007) 471–477.
[7] G. Suske, E. Bruford, S. Philipsen, Mammalian SP/KLF transcription factors: Bring in the family, Genomics 85 (2005) 551–556.
[8] V. Jane van, A.C. Linda, G.R.Q. Kate, et al., Human KLF17 is a new member of the Sp/KLF family of transcription factors, Genomics 87 (2006) 474–482.
[9] A.R. Black, J.D. Black, J. Azizkhan-Clifford, Sp1 and Kruppel-like factor family of transcription factors in cell growth regulation and cancer, J. Cell Physiol. 188 (2001) 143–160.
[10] T. Kazutoshi, T. Koji, O. Mari, et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (2007) 861–872.
[11] X. Wang, H. Lu, A.M. Urvalek, et al., KLF8 promotes human breast cancer cell invasion and metastasis by transcriptional activation of MMP9, Oncogene 30 (2011) 1901–1911.
[12] F. Yu, J. Li, H. Chen, et al., Kruppel-like factor 4 (KLF4) is required for maintenance of breast cancer stem cells and for cell migration and invasion, Oncogene 30 (2011) 2161–2172.
[13] J. Kaczynski, T. Cook, R. Urrutia, Sp-and Krüppel-like transcription factors, Genome Biol. 4 (2003) 206.
[14] G. Lomberk, R. Urrutia, The family feud: turning off Sp1 by Sp1-like KLF proteins, Biochem. J. 392 (2005) 1–11.
[15] H.-Q. Zheng, Z. Zhou, J. Huang, et al., Kruppellike factor 5 promotes breast cell proliferation partially through upregulating the transcription of fibroblast growth factor binding protein 1, Oncogene 28 (2009) 3702–3713.
[16] B.J. Henson, S.M. Gollin, Overexpression of *KLF13* and *FGFR3* in oral cancer cells, Cytogenet. Genome Res. 128 (2010) 192–198.
[17] K. Gumireddy, A. Li, P.A. Gimotty, et al., KLF17 is a negative regulator of epithelial– mesenchymal transition and metastasis in breast cancer, Nat. Cell Biol. 11 (2009) 1297–1304.
[18] M.P. Iwanicki, J.S. Brugge, Transcriptional regulation of metastatic [Id] entity by KLF17, Genome Biol. 10 (2009) 244.
[19] L. Jun, Z. Nu, S. Li-Bing, et al., Astrocyte elevated gene-1 is a novel prognostic marker for breast cancer progression and overall patient survival, Clin. Cancer Res. 14 (2008) 3319–3326.
[20] W.D. Travis, E. Brambilla, H.K. Müller-Hermelink, C.C. Harris (Eds.), WHO classification of tumors. Pathology and genetics of tumors of the lung, pleura, thymus and heart, IARC Press, Lyon, 2004.
[21] I. Genshi, U. Mika, K. Masashi, et al., Krüppel-like factor 6 is frequently down-regulated and induces apoptosis in non-small cell lung cancer cells, Clin. Cancer Res. 64 (2004) 3838–3843.
[22] Goldstraw P (2009).International Association for the Study of Lung Cancer. Staging Manual in Thoracic Oncology. Orange Park, FL: RX Press Ed.
[23] T. Yano, Y. Morodomi, K. Ito, et al., Verification of the newly proposed T category (seventh edition of the tumor, node, and metastasis classification) from a clinicopathological viewpoint in non-small cell lung cancer-special reference to tumor size, J. Thorac. Oncol. 5 (2010) 45–48.
[24] K. Miyoshi, T. Mimura, K. Iwanaga, et al., Surgical treatment of clinical n1 non-small cell lung cancer: ongoing controversy over diagnosis and prognosis, Surg. Today 40 (2010) 428–432.
[25] H. Wenxian, H. WayneL, L. Hong, et al., Putative tumor-suppressor function of Krüppel-like factor 4 in primary lung carcinoma, Clin. Cancer Res. 15 (2009) 5688–5695.