Transcription Factor KLF4: A Potential Biomarker of Non-small Cell Lung Cancertranscription Factor KLF4: A Potential Biomarker of Non-small Cell Lung Cancer

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Research

Keywords: KLF4, NSCLC, Immunohistochemistry

Posted Date: September 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-64121/v1

Abstract

Background Krüppel-like factor 4 (KLF4) is a member of the zinc finger transcription factor family and plays an important role in cell proliferation, differentiation, apoptosis, embryonic development, organ formation, and tumor invasion. There is little research on the clinicopathological characteristics and KLF4 expression in non-small cell lung cancer (NSCLC).

Methods We conducted a retrospective study to further explore the correlation between KLF4 expression and clinicopathological features of NSCLC by immunohistochemical staining.

Results This study included 81 males (52.3%), 74 females (47.7%), and 101 (65.2%) ages <65, 54 (34.8%) ages ≥65 years. A total number of 127 cases (81.9%) were adenocarcinoma, 26 cases (16.8%) were squamous cell carcinoma, and 2 cases (1.3%) were large cell carcinoma. There were 140 patients (89.7%) in T1-T2 phase and 15 patients in T3-T4 phase; 130 patients (83.9%) in N0 phase and 25 patients (16.1%) in N1-N3 phase. There were no distant metastases in 154 patients (99.4%), and only 1 patient (0.6%) was in M1 phase. In terms of clinical stage, 142 patients (91.6%) in stage I-II and 13 patients (8.4%) in stage III-IV. The number of patients with smoking history was 40 (25.8%). KLF4 expression in NSCLC tissues was significantly decreased compared with normal lung tissues (P<0.001); KLF4 expression in adenocarcinoma was lower than that in normal lung tissues (P<0.001), while there was no significant difference in quamous cell carcinoma (P=0.314). Higher KLF4 expression was significantly associated with clinicopathological features of NSCLC such as squamous cell carcinoma (P<0.001), later T stage (T3-T4 stage) (P=0.013), lymph node metastasis (P=0.011), later clinical stage (III-IV stage) (P=0.001). KLF4 expression was not associated with age (P=0.082); there was no significant difference in KLF4 expression between male and female patients with adenocarcinoma (P=0.709). It was also found that KLF4 was positively correlated with Ki-67 expression in patients with adenocarcinoma (r=0.272, P=0.002).

Conclusions Aboval all, the tumor suppressor gene KLF4 might become a potential biomarker and a new therapeutic target for lung cancer patients.

1. Introduction

After entering the 21st century, despite the progress in the diagnosis and treatment of lung cancer, the prognosis of lung cancer is still poor due to the lack of early diagnosis, and the 5-year survival rate is still very low, about only 18%[1]. There were 18.1 million new cancer cases worldwide in 2018, of which 11.6% were lung cancer patients, while 18.4% of 9.6 million cancer deaths died from lung cancer so lung cancer is the most common type of cancer diagnosed and the leading cause of cancer death[2]. With the development of our economy, the accumulation of factors such as air pollution, tobacco and occupational exposure, the incidence and mortality of lung cancer are on the rise, which brings huge social and economic burden[3]. Non-small cell lung cancer (NSCLC) accounted for about 80% of the total lung cancer[4]. Patients with non-small cell lung cancer are often diagnosed later in clinical stage,

resulting in poor prognosis. Therefore, the related mechanism of lung cancer development needs to be further studied in order to provide more support for early diagnosis and early treatment in clinical work.

Recently, some biomarkers have also provided new perspectives for targeted therapy in lung cancer patients, such as human epidermal growth factor receptor 2 (Her2), phosphatase and tensin-like protein (PTEN) and Krüppel like transcription factor 4 (KLF4), etc. Of which KLF4 was first identified by Shields et al. in 1996[5, 6], also known as gastrointestinal enrichment Krüppel like transcription factor, is a member of the KLF family and belongs to the larger Sp1 like transcription factor family[7-9]. KLF4 is a eukaryotic zinc finger protein transcript with binding site specificity, which is involved in the regulation of cell proliferation, differentiation, apoptosis and somatic reprogramming. KLF4 has gradually received widespread attention due to the diversity of its biological functions, and many studies have explored the role of KLF4 in lung cancer. Fadous-Khalife et al. reported that KLF4 expression is related to the clinicopathological characteristics of lung cancer patients and is a potential biomarker of lung cancer[10], but its research has some shortcomings such as the small number of included tissue samples. Our preliminary basic research found that KLF4 can inhibit the metastasis of NSCLC[11]. In order to further study the expression of KLF4 in NSCLC and its clinicopathological correlation with NSCLC, this study intends to include surgical tissue samples from 155 patients with NSCLC, and draw conclusions by KLF4 immunohistochemical staining and statistical analysis.

2. Materials And Methods

2.1 Patients and samples

Patients hospitalized in Peking University First Hospital Thoracic Surgery Department from September, 2018 to Febrary, 2019 and diagnosed with non-small cell lung cancer were included. They all did not receive chemotherapy or radiotherapy before surgey and their clinical data was complete. A tatal of 155 patients with NSCLC were included in the present study. Clinical information was obtained from hospital records, including age at diagnosis, gender, smoking history, surgical stage, histopathological classification and so on. Pathological claasification was performed based on the criteria of the World Health Organization (WHO), and staging was performed according to the 8th edition of TNM classification for lung cancer published by International Association for Study of Lung Cancer((IASLC)/Union for International Cancer Control (UICC).This study was approved by the Clinical Research Ethics Committee of the Peking University

First Hospital, Beijing, China. Ethical review number is No. (2019) SCI (91). The informed consents obtained from participants were both informed and written. The participants must be above the age of 18, diagnosed with non-small cell lung

cancer with complete clinical data, and signed the informed consent.

2.2 Immunohistochemistry staining

Surgical specimens were fixed in 10% formalin and embedded in parafin. Four-micrometer sections from NSCLC tumors were deparaffinized and antigen retrieval was performed by microwaving. Immunohistochemistry staining was performed using KLF4 monoclonal antibody (Abcam, Cat. No. Ab215036). The adjacent normal tissues were used as positive controls and negative controls were used PBS as primary antibody.

2.3 Evaluation of immunohhistochemistry staining

For all the sections only nuclear positive staining was assessed. A combined score based on the intensity of nuclear positive staining and the percentage of positive labeled cells was defined. The intensity of nuclear staining was assessed as follows: 0, no positive staining; 1, mild ; 2, moderate; 3, strong. The percentage of labeled cells was distributed as follows: 0, no positive cells; 1, <30% of tumor cells are positive; 2, 30%-60% of tumor cells are positive; 3, >60% of tumor cells are positive.

2.4 Statistical analysis

Statistical analysis was performed using SPSS 25.0. The median and quartile were used to describe the grade data. The expression of KLF4 between tumor tissues and adjacent normal tissues was compared by nonparametric test of paired data. Correlation between KLF4 expression and clinicopathological characteristics was analyzed by nonparametric test of two independent samples. Chi square test or Fisher exact test was performed to analyze the correlation beetween Ki67 exprssion and clinicopathological characteristics. The correlation between KLF4 expression and Ki67 expression was analyzed by Spearman rank correlation test. $P < 0.05$ was considered significant.

3. Results

3.1 Patient characteristics

This study selected 155 patients who met the study criteria between September 2018 and February 2019. Patient characteristics were shown in Table 1.

Table 1. Patient characteristics.

Characteristics	No.（%）
Gender	
Male	81（52.3%）
Female	74 (47.7%)
Age	
＜65	101（65.2%）
≥65	54（34.8%）
Pathological classification	
Adenocarcinoma	127（80.9%）
Squamous	26（16.6%）
Large cell carcinoma	2（1.3%）
Characteristics	No.（%）
T stage	
Tis	1（0.6%）
T1	110（71.0%）
T2	29（18.7%）
T3	14（9.0%）
T4	1（0.6%）
N stage	
N0	130(83.9%)
N1	18(11.6%)
N2	6(3.9%)
N3	1(0.6%)
M stage	
M0	154(99.4%)
M1	1(0.6%)
Clinical stage	
I	105（67.7%）
II	37（23.9%）

III	12（7.7%）
IV	1（0.6%）
Smoking	
Yes	40（25.8%）
No	115（74.2%）

3.2 KLF4 was decreased in NSCLC tissues

The median (Q25, Q75) of KLF4 expression in tumor tissues was 2 (0, 4), of which in adjacent normal tissues was 5 (4, 5). The expression of KLF4 was significantly decreased in tumor tissues compared with control (P<0.001). As for lung adenocarcinoma, KLF4 was significantly decreased in tumor tissues (P<0.001). KLF4 expression was not significant differentially expressed in tumor tissues compared with control tissues (P=0.341), shown in Table 2.

Table 2. KLF4 expression in NSCLC tissures and normal tissues.

Classification（No.）	KLF4 expression Median (Q_{25}, Q_{75})	*P* (compared with normal)
Normal（155）	5（4, 5）	
NSCLC（155）	2（0, 4）	（0.001
Adenocarcinoma（127）	0（0, 3）	（0.001
Squamous（26）	5（4, 5）	0.314

3.3 The expression of KLF4 was higher in squamous tumor tissues than adenocarcinoma tissues

The median (Q25, Q75) of KLF4 expression was 0 (0, 3) in adenocarcinoma tissues, of which in control tissues was 5 (4, 5). KLF4 expression was significantly different between lung adenocarcinoma tumor samples and squamous tumors samples (P<0.001), shown in Table 3. Immunohistochemical staining was shown in Figure 1.

Figure 1. Immunohistochemical analysis of KLF4 expression in adenocarcinoma and squamous cell carcinoma. (A)(B)(C) KLF4 positive staining in squamous cell carcinoma; (D)(E)(F) KLF4 positive staining in adenocarcinoma.

3.4 The expression of KLF4 and clinicopathological characteristics

KLF4 expression in different T stages of tumor tissues were also significantly different (P=0.013), shown in Table 2. The median of KLF4 expression (Q25,Q75) was 0 (0,3) and 4 (0,4) in tissue samples of lung

cancer patients during the T1-T2 and T3-T4 stage, respectively. Further stratification analysis revealed that there was no significant difference of KLF4 expression in adenocarcinoma or squamous cell carcinoma.

The median (Q25,Q75) expression of KLF4 in N0 and N1-N3 patients was 0(0,3.25) and 3(1,4), respectively. The expression was significantly higher in patients with lymph node metastasis than in those without lymph node metastasis (P=0.011). Further stratification analysis revealed that there was no significant difference of KLF4 expression in adenocarcinoma or squamous cell carcinoma.

The median KLF4 expression (Q25,Q75) was 0(0,3) and 4(2.5,4) in patients with stage I-II and stage III-IV, respectively. KLF4 expression was significantly higher in patients with clinical stage III-IV than in patients with stage I-II (P=0.001). The further analysis showed that 7 adenocarcinoma patients with III-IV stage had higher expression than 120 patients with I-II stage (P=0.013); there was no significant difference in squamous cell carcinoma patients (P=0.744).

We next detect the relationship between KLF4 expression and clinicopathologic variables. No significant correlation was found between KLF4 expression and age, gender.

Table 3. Relationship between KLF4 expression and clinicopathological features of NSCLC.

Characteristics（No.）	KLF4 expression Median(Q25, Q75)	*P*
Gender		0.003
Male（81）	2（0, 3）	
Female（74）	0（0, 4）	
Age		0.082
＜65（101）	0（0, 3）	
≥65（54）	2.5（0, 4）	
Pathological classification		＜0.001
Adenocarcinoma（127）	0（0, 3）	
Squamous（26）	5（4, 5）	
T stage		0.013
T1-T2（140）	0（0, 3）	
T3-T4（15）	4（0, 4）	
N stage		0.011
N0（130）	0（0, 3.25）	
N1-N3（25）	3（1, 4）	
Clinical stage		0.001
I-II （142）	0（0, 3）	
III-IV （13）	4（2.5, 4）	

There was a positive correlation between tumor tissue KLF4 expression and Ki-67 proliferation index in non-small cell lung cancer (r=0.512, P<0.001), shown in Figure 2A. There was also a positive correlation between KLF4 expression and Ki-67 proliferation index in adenocarcinoma tumor tissues (r=0.272, Pr=0.002), shown in figure 2B. No correlation was found between KLF4 expression and Ki-67 proliferation index in 26 squamous cell carcinoma tumor tissues (r=0.063, Pr=0.759), shown in Figure 2C.

Figure 2. (A) Scatter map of KLF4 and Ki-67 of NSCLC. (B) Scatter map of KLF4 and Ki-67 of lung adenocarcinoma. (C) Scatter map of KLF4 and Ki-67 of lung squamous cell carcinoma.

4. Discussion

Lung cancer has caused a heavy economic and health burden in China. Recently, the diagnosis and treatment of lung cancer has been greatly improved in the early stage, and there are also problems of metastasis, recurrence, drug resistance and so on. Therefore, it is particularly important to find better biomarkers and new therapeutic targets for lung cancer. KLF4 is a nuclear transcription factor and plays a tumor inhibitory role in lung cancer according to multiple clinical and experimental evidence. A total of 155 NSCLC patients were included in this study to explore KLF4 expression and its correlation with clinicopathological features.

The results showed that the expression of KLF4 in tumor tissues of NSCLC was significantly lower than that of normal lung tissues; the down-regulation of KLF4 expression may be associated with promoter hypermethylation, loss of heterozygosity in KLF4 loci or point mutations in the coding region[12]. Meanwhile, KLF4 expression in T3-T4 stage was higher than that in T1-T2 stage patients. The further analysis showed that there was no significant difference in adenocarcinoma patients and squamous cell carcinoma patients, separately. However, a study from Shaoli Li et al. included 60 patients found that the proportion of KLF4 low expression increased significantly in the T3, T4 group[13]. A recently published study[14] showed that there was no significant difference in KLF4 expression in tumor tissues of patients with stage T1-T2 and stage T3-T4 NSCLC. Our results showed that KLF4 expression was significantly higher in patients with N1-N3 stage. The further analysis showed that there was no significant difference in adenocarcinoma patients and squamous cell carcinoma patients, separately. Previous studies have shown[15-17] lower KLF4 expression in patients with lymph node metastasis. The expression of KLF4 in this study was significantly higher in patients with III-IV stage than in patients with I-II stage. KLF4 expression of patients with III-IV adenocarcinoma was higher than that of 120 patients with I-II adenocarcinoma; there was no significant difference in KLF4 expression between 20 patients with I-II and 6 patients with III-IV squamous cell carcinoma. Studies by Shaoli Li, Wenxian Hu, Zhang Zhiping, and others suggested that decreased KLF4 expression in patients with NSCLC following staging progression[13-15]. Marie Claude Fadous-Khalife's study found that KLF4 expression was absent in the stage I, and the expression of KLF4 was significantly higher in patients with II, III, IV stages[12]. The potential gene silencing due to hypermethylation might contribute to the lower KLF4 expression. However, the specific mechanism needs to be elucidated by further studies in the future. Increased KLF4 expression in II, III and IV stages may be associated with reduced tumor differentiation and increased invasiveness[12]. As for correlation between KLF4 expression and distant metastasis of NSCLC, no statistical analysis was carried out. Therec was only one of 155 patients in this study was M1 because patients with distant metastasis generally did not choose surgical treatment. While a study incorporating 50 NSCLC patients with stage M0 and 10 patients with stage M1 showed that KLF4 expression decreased with distant metastasis[13]. The preliminary statistical analysis of this study showed significant differences in KLF4 expression between men and women, but further stratified analysis revealed no correlation between KLF4 expression and sex in adenocarcinoma. This study does not include the female squamous cell carcinoma patient, failing to further analyze whether there is a difference in KLF4 expression between male and female lung squamous cell carcinoma patients.

KLF4 can inhibit cell proliferation, resulting in cell cycle arrest at G1/S and G2/M stages. Proliferation index Ki-67 reflects cell proliferation ability. To explore the correlation between KLF4 and Ki-67 expression of NSCLC, immunohistochemistry were performed. Results showed that there was a positive correlation in NSCLC and adenocarcinoma, the correlation in squamous cell carcinoma needs to be confirmed by larger sample size and cytology and animal experiments , because only 26 cases were included in this study.

Above all, there is still a lack of large-scale, multicenter research data on the correlation between KLF4 and NSCLC clinicopathological features such as T stage, N stage, M stage, clinical stage, etc. The number of samples retrieved in the literature is less than 100, and this study included 155 patients and could obtain more objective and accurate results. The KLF4 expression levels of previous studies were classified as positive, negative or high expression, low expression. The KLF4 expression of this study was evaluated as different grades between 0-6 points according to the positive area and intensity of KLF4 positive staining, which could reflect the difference of KLF4 expression more specifically. At the same time, this study adopts the 8th edition of the TNM staging criteria, which has greatly changed compared with 7th edition that used in earlier, to determine the stage of NSCLC.

Our study revealed that KLF4 expression significantly higher in squamous cell carcinoma than in adenocarcinoma. There was no significant difference between 38 squamous cell carcinoma and 40 adenocarcinoma tissues in Mingyue Liu's and Zhiping Zhang's studies[14, 16]. The difference of KLF4 expression in different pathological tissues might attribute to KLF4 biological function dependent on cell microenvironment and tissue specificity[15, 18], and might also associated with DNA mutations, molecular alterations, hypermethylation or miRNAs.

Conclusion

The role and mechanism of KLF4 in NSCLC has not been fully defined at present, and the conclusions of the correlation between KLF4 and the clinicopathological features of NSCLC are not fully unified, which need to be confirmed by larger sample or prospective experiments. Our study showed that overall KLF4 expression in NSCLC decreased compared with normal lung tissue, while KLF4 expression in squamous cell carcinoma was significantly higher than that in adenocarcinoma, and increased with the progression of T stage, N stage, clinical stage, which could be expected to become a new biomarker for the diagnosis of NSCLC.

Abbreviations

KLF4	Krüppel-like factor 4
NSCLC	non-small cell lung cancer

Declarations

Ethics approval and consent to participate

This study was approved by the Clinical Research Ethics Committee of the Peking University First Hospital, Beijing, China. Ethical review number is No. (2019) SCI (91). The informed consents obtained from participants were both informed and written.

Availability of data and materials

All datasets generated for this study are included in the article supplementary material.

Competing interests

The authors of this paper declare no conflict of interest. All authors have approved the manuscript and agree with submission to Diagnostic Pathology.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81670043].

Authors' contributions

All authors contributed equally to this article.

Acknowledgements

Not applicable.

References

[1] Goffin J, Lacchetti C, Ellis P M, et al. First-line systemic chemotherapy in the treatment of advanced non-small cell lung cancer: a systematic review[J]. J Thorac Oncol.2010,5(2): 260-274. Pubmed PMID: 20101151, doi:10.1097/JTO.0b013e3181c6f035.

[2] Barta J A, Powell C A, Wisnivesky J P. Global Epidemiology of Lung Cancer[J]. Ann Glob Health.2019,85(1). Pubmed PMID: 30741509, doi:10.5334/aogh.2419.

[3] Sun D, Cao M, Li H, et al. Cancer burden and trends in China: A review and comparison with Japan and South Korea[J]. Chin J Cancer Res.2020,32(2): 129-139. Pubmed PMID: 32410791, doi:10.21147/j.issn.1000-9604.2020.02.01.

[4] Brambilla E, Travis W D, Colby T V, et al. The new World Health Organization classification of lung tumours[J]. Eur Respir J.2001,18(6): 1059-1068. Pubmed PMID: 11829087, doi:10.1183/09031936.01.00275301.

[5] Shields J M, Christy R J, Yang V W. Identification and characterization of a gene encoding a gut-enriched Kruppel-like factor expressed during growth arrest[J]. J Biol Chem.1996,271(33): 20009-20017. Pubmed PMID: 8702718, doi:10.1074/jbc.271.33.20009.

[6] Shields J M, Yang V W. Two potent nuclear localization signals in the gut-enriched Krüppel-like factor define a subfamily of closely related Krüppel proteins[J]. J Biol Chem.1997,272(29): 18504-18507. Pubmed PMID: 9218496, doi:10.1074/jbc.272.29.18504.

[7] Nakahara Y, Northcott P A, Li M, et al. Genetic and epigenetic inactivation of Kruppel-like factor 4 in medulloblastoma[J]. Neoplasia.2010,12(1): 20-27. Pubmed PMID: 20072650, doi:10.1593/neo.91122.

[8] Cho Y G, Song J H, Kim C J, et al. Genetic and epigenetic analysis of the KLF4 gene in gastric cancer[J]. Apmis.2007,115(7): 802-808. Pubmed PMID: 17614846, doi:10.1111/j.1600-0463.2007.apm_643.x.

[9] Dang D T, Pevsner J, Yang V W. The biology of the mammalian Krüppel-like family of transcription factors[J]. Int J Biochem Cell Biol.2000,32(11-12): 1103-1121. Pubmed PMID: 11137451, doi:10.1016/s1357-2725(00)00059-5.

[10] Yang W T, Zheng P S. Promoter hypermethylation of KLF4 inactivates its tumor suppressor function in cervical carcinogenesis[J]. PLoS One.2014,9(2): e88827. Pubmed PMID: 24551169, doi:10.1371/journal.pone.0088827.

[11] Wu Y, Lin L, Wang X, et al. Overexpression of Krüppel-Like Factor 4 Suppresses Migration and Invasion of Non-Small Cell Lung Cancer Through c-Jun-NH2-Terminal Kinase/Epithelial-Mesenchymal Transition Signaling Pathway[J]. Frontiers in Pharmacology.2020,10(1512). doi:10.3389/fphar.2019.01512. https://www.frontiersin.org/article/10.3389/fphar.2019.01512

[12] Fadous-Khalifé M C, Aloulou N, Jalbout M, et al. Krüppel-like factor 4: A new potential biomarker of lung cancer[J]. Mol Clin Oncol.2016,5(1): 35-40. Pubmed PMID: 27330761, doi:10.3892/mco.2016.883.

[13] Li S, Huang L, Gu J, et al. Restoration of KLF4 Inhibits Invasion and Metastases of Lung Adenocarcinoma through Suppressing MMP2[J]. J Cancer.2017,8(17): 3480-3489. Pubmed PMID: 29151932, doi:10.7150/jca.21241.

[14] Liu M, Li X, Peng K Z, et al. Subcellular localization of Klf4 in non-small cell lung cancer and its clinical significance[J]. Biomed Pharmacother.2018,99(480-485. Pubmed PMID: 29665649, doi:10.1016/j.biopha.2018.01.090.

[15] Hu W, Jia Y, Xiao X, et al. KLF4 downregulates hTERT expression and telomerase activity to inhibit lung carcinoma growth[J]. Oncotarget.2016,7(33): 52870-52887. Pubmed PMID: 27153563, doi:10.18632/oncotarget.9141.

[16] Zhou Y, Hofstetter W L, He Y, et al. KLF4 inhibition of lung cancer cell invasion by suppression of SPARC expression[J]. Cancer Biol Ther.2010,9(7): 507-513. Pubmed PMID: 20215880, doi:10.4161/cbt.9.7.11106.

[17] Whitlock N C, Bahn J H, Lee S H, et al. Resveratrol-induced apoptosis is mediated by early growth response-1, Krüppel-like factor 4, and activating transcription factor 3[J]. Cancer Prev Res (Phila).2011,4(1): 116-127. Pubmed PMID: 21205742, doi:10.1158/1940-6207.Capr-10-0218.

[18] Wong C W, Hou P S, Tseng S F, et al. Krüppel-like transcription factor 4 contributes to maintenance of telomerase activity in stem cells[J]. Stem Cells.2010,28(9): 1510-1517. Pubmed PMID: 20629177, doi:10.1002/stem.477.

Figures

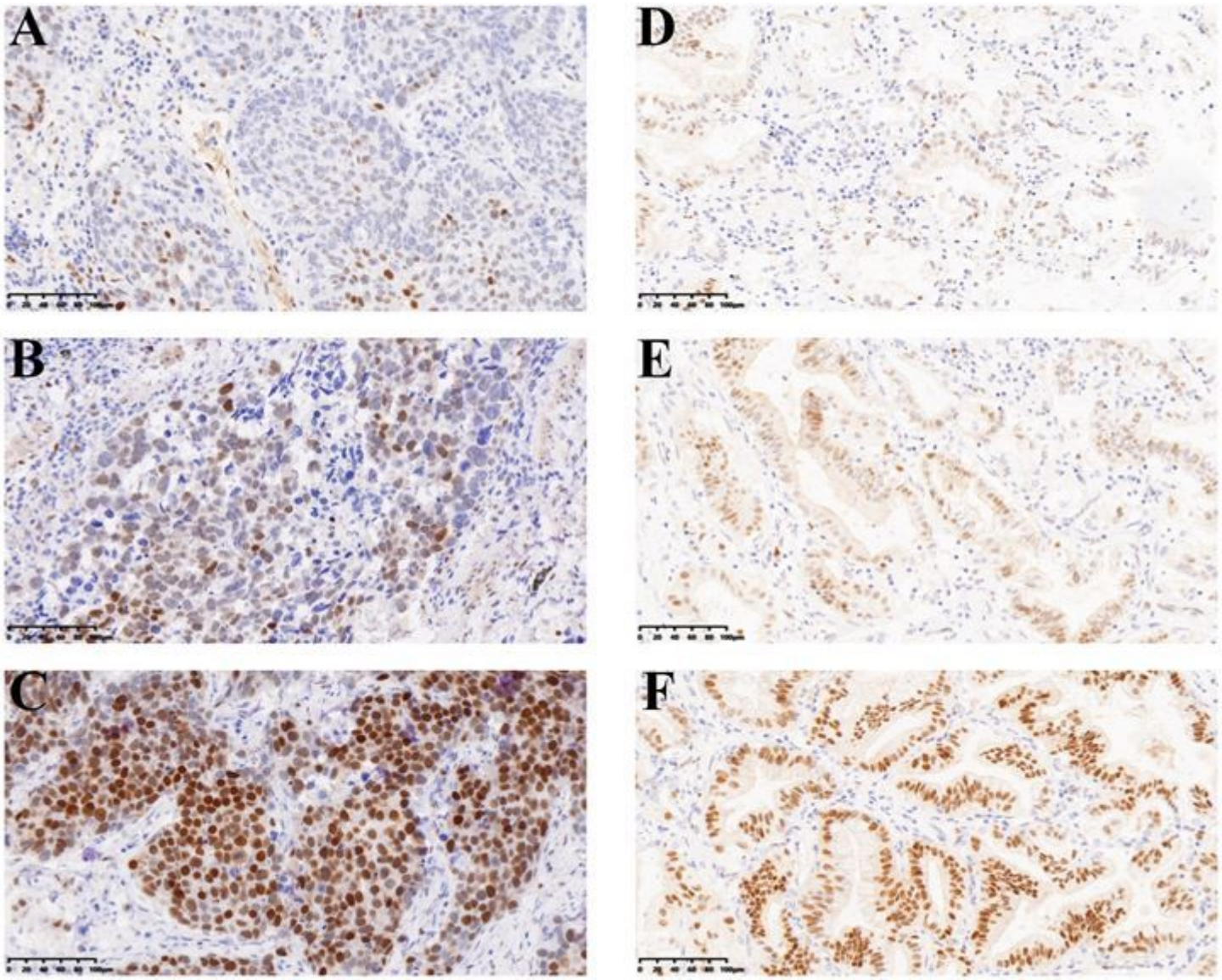

Figure 1

Immunohistochemical analysis of KLF4 expression in adenocarcinoma and squamous cell carcinoma. (A)(B)(C) KLF4 positive staining in squamous cell carcinoma; (D)(E)(F) KLF4 positive staining in adenocarcinoma.

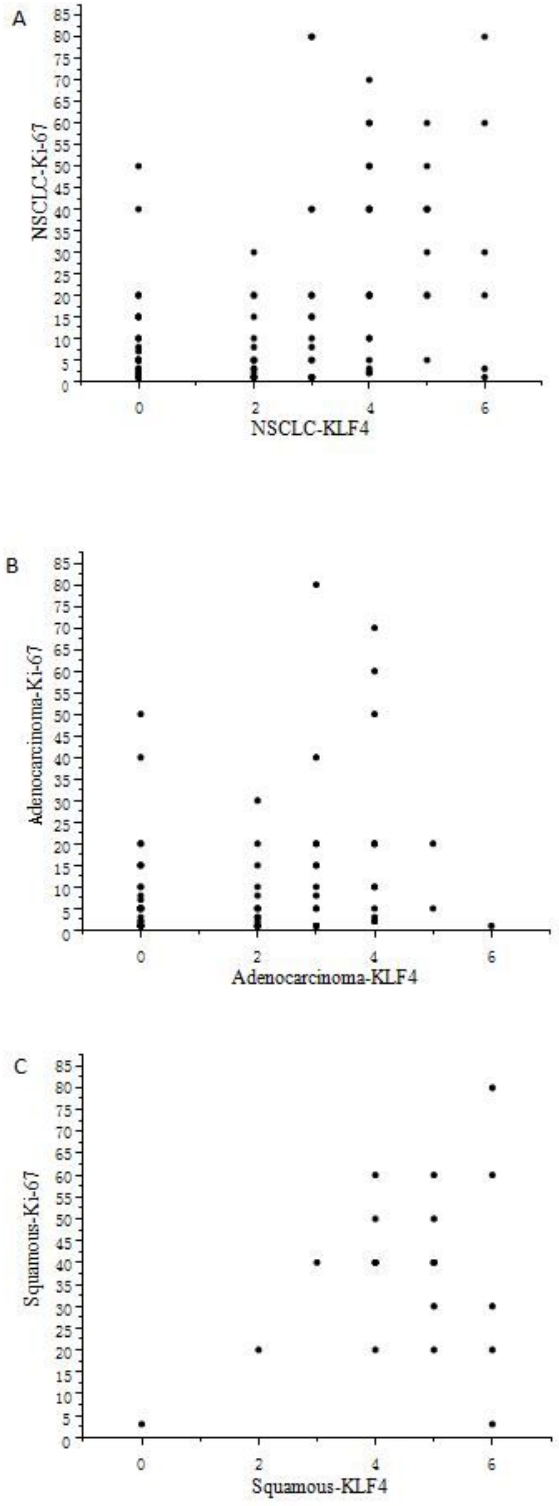

Figure 2

(A) Scatter map of KLF4 and Ki-67 of NSCLC. (B) Scatter map of KLF4 and Ki-67 of lung adenocarcinoma. (C) Scatter map of KLF4 and Ki-67 of lung squamous cell carcinoma.