CDK14 expression is elevated in patients with non-small cell lung cancer and correlated with poor prognosis

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Abstract

Objective: To investigate the clinical significance of cyclin-dependent kinase 14 (CDK14) expression in patients with non-small cell lung cancer (NSCLC).

Methods: The present prospective observational study included 193 patients diagnosed with NSCLC between January 2010 and December 2014. NSCLC tumor tissues and paired paracancerous normal tissues were obtained from all patients. CDK14, thyroid transcription factor 1 (TTF-1), cytokeratin 5/6 (CK5/6), and Ki67 expression was measured via immunohistochemistry (IHC).

Results: CDK14 staining was strong (>3) in 129 patients (66.49%) and weak (≤3) in 64 patients (33.16%). The mean IHC scores were markedly higher in tumor tissues than in paracancerous tissues. Pearson’s analysis demonstrated that the IHC scores of CDK14 expression were positively correlated with TTF-1, CK5/6, and Ki67 scores. Kaplan–Meier analysis illustrated that 5-year overall survival was markedly longer in patients with weak CDK14 staining. TNM stage, pleural invasion, lymph node metastasis, CDK14 expression, and Ki67 expression were risk factors for 5-year overall survival in patients with NSCLC.

Conclusion: CDK14 overexpression portended poor outcomes in patients with NSCLC, and CDK14 expression was correlated with TTF-1, CK5/6, and Ki67 expression.

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Introduction
Non-small cell lung cancer (NSCLC) is the most common lung cancer, accounting for almost 85% of lung cancer cases.\(^1,2\) Currently, several cancer biomarkers are used to diagnose NSCLC, such as thyroid transcription factor 1 (TTF-1), napsin A, cytokeratin 7 (CK7), CK5/6, and Ki67.\(^3–5\) However, because of its mild symptoms, NSCLC is generally not diagnosed in early stages, leading to a poor prognosis and low 5-year survival rate.\(^6\)

Cyclin-dependent kinase 14 (CDK14) is a member of CDK family. Currently, most CDKs are considered cancer promoters, including CDK2, CDK4, CDK6, CDK9, CDK12, CDK14, and CDK16.\(^7–9\) In lung cancer, several studies also found that CDK family members facilitate oncogenesis and cancer development. Wang et al. demonstrated that CDK inhibitors could be used to treat solid tumors, including lung cancer, by regulating the cell cycle and gene transcription.\(^10\) Danilov et al.\(^11\) revealed that dinaciclib could induce anaphase catastrophe in lung cancer cells by suppressing CDK1 and CDK2. Other studies also found that inhibitors of CDKs, such as CDK4/6 dual inhibitors, have the potential to be used in lung cancer treatment.\(^12,13\) However, few studies focused on the role of CDK14 in lung cancer.

We conducted observational research to investigate the clinical significance of CDK14 in patients with NSCLC. We observed that patients with strong CDK14 expression had worse prognosis and shorter 5-year overall survival (OS). This research might provide additional clinical evidence of the role of CDK14 in NSCLC.

Materials and methods
Subjects and tissue samples
The present prospective observational study included patients diagnosed with NSCLC for the first time as confirmed via histological analysis between January 2010 and December 2014. No patient had received chemotherapy or radiotherapy prior to enrollment. Patients’ pathological types were identified. The exclusion criteria were diagnoses of other primary cancers, cancer metastasis to the lungs, the presence of severe infections such as pneumonia or pulmonary tuberculosis, and the presence of severe renal, liver, or cardiovascular diseases. All patients provided written informed consent. The present study was approved by the Ethic Committee of Qujing No. 1 Hospital (approval number: QJH-2009-4-13).

Immunohistochemistry (IHC) for CDK14
NSCLC tumor tissues and paired paracancerous normal tissues were obtained from all patients. For IHC analysis, the tissues were fixed, embedded, sectioned, and stained with hematoxylin and eosin. After immersion in 3% H\(_2\)O\(_2\), samples were incubated with anti-CDK14 primary antibody (PFTK1, Abcam, Cambridge, UK) overnight at 4°C, followed by incubation with Goat Anti-Rabbit IgG H&L (HRP) (ab205718, Abcam) at 37°C for 45 minutes. Samples were then stained with
diaminobenzidine, and staining was quantified using the Allred scoring system. The total IHC score was calculated as the sum of the scores for staining intensity (0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining) and the percent area of staining (0, 0%–10%; 1, 11%–25%; 2, 26%–50%; 3, 51%–75%; 4, 76%–100%). IHC scores of >3 were defined as strong staining and high expression, and scores of ≤3 were defined as weak staining and low expression.

Data collection and follow-up

Patient characteristics, including age, sex, body mass index (BMI), tumor-nodes-metastasis (TNM) stage, pathological types, and smoking habits, were recorded. TTF-1, CK5/6, and Ki67 expression in tumor tissues was measured using IHC. All patients were followed up for 5 years. For survival analysis, OS was defined as the time from admission to death or the last follow-up.

Statistical analysis

Data were presented as the mean ± standard deviation. The chi-square test was used to analyze odds ratios. Student’s t-test was used for comparisons between two groups. Pearson’s correlation analysis was used to assess correlations between factors. Kaplan–Meier curves were generated for survival analysis. P < 0.05 indicated statistical significance. Logistic regression was performed to identify risk factors for mortality. All statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA).

Results

Patient characteristics

As presented in Table 1, the study cohort included 193 patients (119 men, 74 women) with a mean age of 53.01 ± 11.56 years. The mean BMI of the patients was 22.75 ± 3.02 kg/m². Seventy-nine patients (40.93%) had TNM stage I or II lesions, whereas 114 patients (59.07%) had TNM stage III or IV lesions. Squamous cell carcinoma was found in 106 patients (54.92%), and adenocarcinoma was found in 87 patients (45.08%).

| Table 1. Characteristics of all patients with non-small cell lung cancer. |
|-----------------|-----------------|
| Variables       | Values          |
| Age, years      | 53.01 ± 11.56   |
| BMI, kg/m²      | 22.75 ± 3.02    |
| Sex, male:female| 119:74          |
| Current smoker, n (%) | 107 (55.44) |
| TNM stage, n (%) |                 |
| I–II            | 79 (40.93)      |
| III–IV          | 114 (59.07)     |
| Pathological type, n (%) |        |
| Squamous cell carcinoma | 106 (54.92) |
| Adenocarcinoma  | 87 (45.08)      |
| Lymph node metastasis, n (%) | 128 (65.98) |
| Pleural invasion, n (%) | 103 (53.57)    |
| CDK14 staining, n (%) |        |
| Strong          | 129 (66.49)     |
| Weak            | 64 (33.16)      |

BMI, body mass index; CDK14, cyclin-dependent kinase 14.

CDK14 expression was elevated in NSCLC tumor tissues

The IHC results illustrated that CDK14 expression was strong in 129 patients (66.49%) and weak in 64 patients (33.16%, Table 1). Meanwhile, the mean IHC score was markedly higher in tumor tissues than in paracancerous normal tissues (P < 0.05, Figure 1). No significant difference in the mean IHC score was detected between squamous cell carcinoma and adenocarcinoma lesions. This result suggested that CDK14 expression was elevated in NSCLC tumors.
Correlations among CDK14, TTF-1, CK5/6, and Ki67 expression

We next investigated the relationships among the NSCLC biomarkers TTF-1, CK5/6, Ki67, and CDK14. Patients with strong CDK14 staining were significantly more likely to have strong TTF-1, CK5/6, and Ki67 staining than those with weak CDK14 staining (all \( P < 0.05 \), Table 2). The mean IHC score for TTF-1 was significantly higher in patients with squamous cell carcinoma than in those with adenocarcinoma, whereas the score for CK5/6 was markedly higher in patients with adenocarcinoma (both \( P < 0.05 \)). Pearson’s correlation analysis demonstrated that the IHC score for CDK14 expression was positively correlated with those for TTF-1, CK5/6, and Ki67 (all \( P < 0.05 \), Table 3).

High CDK14 expression was associated with the clinical characteristics of patients with NSCLC

To further reveal the clinical significance of CDK14 in NSCLC, the relationships between CDK14 expression and clinical characteristics were analyzed. As presented in Table 4, patients with strong CDK14 staining were more likely to have TNM stage III–IV tumors, pleural invasion, and lymph node metastasis (all \( P < 0.05 \)). The 5-year OS rate was also markedly higher in patients with weak CDK14 staining (17.19%) than in those with strong staining.

Figure 1. CDK14 expression in tumor and paracancerous normal tissues from patients with NSCLC. (a) CDK14 expression in NSCLC tumor and paracancerous normal tissues in patients with squamous cell carcinoma and adenocarcinoma. (b) Mean immunohistochemistry scores in different tissue samples from patients with squamous cell carcinoma and adenocarcinoma. CDK14, cyclin-dependent kinase 14; NSCLC, non-small cell lung cancer.
(6.98%), indicating that high CDK14 expression predicted poorer clinical characteristics and higher mortality rates.

**Elevated CDK14 expression predicted shorter 5-year OS in patients with NSCLC**

Finally, the association between CDK14 expression and 5-year OS was analyzed using Kaplan–Meier analysis. The median survival time was significantly shorter in patients with strong CDK14 staining than in those with weak CDK14 staining ($P < 0.05$). The Kaplan–Meier curve demonstrated that 5-year OS was markedly longer in patients with weak CDK14 staining than in those with strong CDK14 staining ($P < 0.05$ [log rank test], Figure 2). Logistic regression analysis identified TNM stage, pleural invasion, lymph node metastasis, CDK14 expression, and Ki67 expression as risk factors for 5-year OS in patients with NSCLC (Table 5).

**Table 2.** Correlations of CDK14 staining with TTF-1, CK5/6, and Ki67 staining in patients with non-small cell lung cancer.

| Variables                  | Strong CDK14 staining (n = 129) | Weak CDK14 staining (n = 64) | $P$  |
|---------------------------|---------------------------------|-------------------------------|------|
| TTF-1                     |                                 |                               | <0.001|
| Strong                    | 113 (87.60)                     | 13 (20.31)                    |      |
| Weak                      | 16 (12.40)                      | 51 (79.69)                    |      |
| CK5/6                     |                                 |                               | <0.001|
| Strong                    | 91 (70.54)                      | 22 (34.38)                    |      |
| Weak                      | 38 (29.46)                      | 42 (65.63)                    |      |
| Ki67                      |                                 |                               | <0.001|
| Strong                    | 86 (66.67)                      | 20 (31.25)                    |      |
| Weak                      | 43 (33.33)                      | 44 (68.75)                    |      |

CDK14, cyclin-dependent kinase 14; TTF-1, thyroid transcription factor 1; CK5/6, cytokeratin 5/6.

**Discussion**

Currently, lung cancer remains a leading cause of cancer-related death, and its 5-year survival rate remains extremely low, especially among patients with advanced cancer. Most members of the CDK family are regarded as cancer promoters, including CDK14. However, the clinical significance of CDK14 in lung cancer has not been illuminated. In the present study, we demonstrated that CDK14 was overexpressed in NSCLC tumor tissues, and CDK14 overexpression portended poor prognosis.

**Table 3.** Pearson's correlation analysis of CDK14, TTF-1, CK5/6, and Ki67 expression in patients with non-small cell lung cancer.

| CDK14 | TTF-1 | CK5/6 | Ki67 |
|-------|-------|-------|------|
| CDK14 |       |       |      |
| Pearson's correlation coefficient | 1     | 0.431 | 0.255 | 0.190 |
| $P$   | –     | <0.001| <0.001| <0.001|
| TTF-1 |       |       |      |
| Pearson's correlation coefficient | 0.431 | 1     | 0.203 | –0.005 |
| $P$   | <0.001| –     | 0.005 | 0.943 |
| CK5/6 |       |       |      |
| Pearson's correlation coefficient | 0.255 | 0.203 | 1     | 0.150 |
| $P$   | <0.001| 0.005 | –     | 0.037 |
| Ki67  |       |       |      |
| Pearson's correlation coefficient | 0.190 | –0.005| 0.150 | 1     |
| $P$   | <0.001| 0.943 | 0.037 | –     |

CDK14, cytokeratin-dependent kinase 14; TTF-1, thyroid transcription factor 1; CK5/6, cytokeratin 5/6.
First, this study demonstrated that CDK14 expression was elevated in tumor tissue in patients with NSCLC. CDKs inhibitors have already been used as anti-cancer agents in the clinic. In breast cancer, both CDK4 and CDK6 inhibitors were used as therapies, including the CDK 4/6 inhibitor palbociclib (PD0332991), which has been examined in phase II clinical trials.\textsuperscript{14} In gastric cancer, CDK2 promoted aerobic glycolysis and cell proliferation in gastric cancer cells by inhibiting SIRT5.\textsuperscript{15} Meanwhile, recent studies revealed that CDK15 was upregulated in lung cancer and that it suppressed cell apoptosis in breast cancer.\textsuperscript{16,17} In our research, we also found that CDK14 was overexpressed in NSCLC and associated with poor prognosis.

The finding that CDK14 may serve as a cancer promoter in NSCLC is consistent with prior findings. In previous research, CDK14 activation led to the promotion of cell viability and metastasis in NSCLC cell lines.\textsuperscript{18} In addition, several studies have reported the role of CDK14 in cancer development. Ji et al.\textsuperscript{19} found that inhibition of CDK14 by miR-216 suppressed the proliferation, migration, and invasion of osteosarcoma cells. Another study demonstrated that suppression of CDK14 resulted in decreased breast cancer cell

\begin{table}[h]
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Variables & Strong CDK14 staining (n = 129) & Weak CDK14 staining (n = 64) & P \\
\hline
Age, years & 53.27 ± 11.93 & 52.46 ± 10.82 & 0.648 \\
BMI, kg/m\textsuperscript{2} & 22.85 ± 3.03 & 22.54 ± 3.02 & 0.509 \\
Sex, male:female (%) & 78 (60.47):51 (39.53) & 41 (64.06):23 (35.94) & 0.600 \\
Current smoker, n (%) & 69 (53.49) & 38 (59.38) & 0.401 \\
TNM stage, n (%) & <0.001 \\
I–II & 48 (37.21) & 41 (64.06) & \\
III–IV & 81 (62.79) & 23 (35.94) & \\
Pathological type, n (%) & 0.018 \\
Squamous cell carcinoma & 78 (60.47) & 28 (43.75) & \\
Adenocarcinoma & 51 (39.53) & 36 (56.25) & \\
Pleural invasion, n (%) & 79 (76.70) & 24 (37.50) & <0.001 \\
Lymph node metastasis, n (%) & 97 (75.19) & 31 (48.44) & <0.001 \\
5-year survival, n (%) & 9 (6.98) & 11 (17.19) & 0.027 \\
\hline
\end{tabular}
\caption{Relationships between CDK14 and the clinical characteristics of patients with non-small cell lung cancer.}
\end{table}

CDK14, cyclin-dependent kinase 14; BMI, body mass index.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{chart.png}
\caption{Kaplan–Meier curve of 5-year OS in patients with strong or weak CDK14 staining expression. OS, overall survival; CDK14, cyclin-dependent kinase.}
\end{figure}
proliferation.20 All of these studies highlighted that CDK14 is a promoter of various cancers, including lung cancer. In this paper, we also demonstrated the elevated CDK14 expression in NSCLC predicted advanced disease stage, lymphatic metastasis, and shorter 5-year survival. Additionally, CDK14 expression was positively correlated with the expression of the tumor biomarkers TTF-1, CK5/6, and Ki67. In addition to the aforementioned studies, another study found that cigarette smoke downregulated CDK14 expression in testicular and lung cells, suggesting that CDK14 has different roles in normal lung tissue and lung cancer tissue.21 Thus, further studies are needed to reveal the underlying molecular mechanisms of CDK14 in NSCLC.

The present study had some limitations. First, the sample size was limited and all patients were recruited from a single center. Second, the underlying molecular mechanism of the effects of CDK14 in lung cancer is unclear. Thus, additional research is required for confirmation.

**Conclusion**

This observational study illustrated that CDK14 was overexpressed in patients with NSCLC, and CDK14 overexpression predicted poor prognoses and 5-year OS. The present study might provide new insights into the role of CDK14 in NSCLC development.

**Declaration of conflicting interest**

The authors declare that there is no conflict of interest.

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**References**

1. Herbst RS, Morgensztern D and Boshoff C. The biology and management of non-small cell lung cancer. *Nature* 2018; 553: 446–454.

2. Remark R, Becker C, Gomez JE, et al. The non-small cell lung cancer immune
contexture. A major determinant of tumor characteristics and patient outcome. Am J Respir Crit Care Med 2015; 191: 377–390.

3. Liu D and Zhang Z. [Issues Relevant to Surgical Intervention in ‘Tiny’ Non-small Cell Lung Cancer Detected by ‘Lung Screening’–Orientation, Lung Resection and Lymph Node Resection]. Zhongguo Fei Ai Za Zhi 2016;19:347–350.

4. Argon A, Nart D, Veral A. The Value of Cytokeratin 5/6, p63 and Thyroid Transcription Factor-1 in Adenocarcinoma, Squamous Cell Carcinoma and Non-Small-Cell Lung Cancer of the Lung. Turk Patoloji Derg 2015; 31: 81–88.

5. Muhammad FR, Pity IS and Safo AS. Cytokeratin 5/6, p63 and ttf-1 immuno marker use in tiny non-small cell lung cancer. J Cancer Prev Curr Res 2018; 9: 191–195.

6. Bracht JWP, Mayo-de-Las-Casas C, Berenguer J, et al. The Present and Future of Liquid Biopsies in Non-Small Cell Lung Cancer: Combining Four Biosources for Diagnosis, Prognosis, Prediction, and Disease Monitoring. Curr Oncol Rep 2018;20:70.

7. Whittaker SR, Mallinger A, Workman P, et al. Inhibitors of cyclin-dependent kinases as cancer therapeutics. Pharmacol Ther 2017; 173: 83–105.

8. Sonawane YA, Taylor MA, Napoleon JY, et al. Cyclin Dependent Kinase 9 Inhibitors for Cancer Therapy: Miniperspective. J Med Chem 2016; 59: 8667–8684.

9. Giovanni CD, Novellino E, Chilin A, et al. Investigational drugs targeting cyclin-dependent kinases for the treatment of cancer: An update on recent findings (2013-2016). Expert Opin Investig Drugs 2016; 25:1215–1230.

10. Wang X, Deng K, Wang C, et al. Novel CDKs inhibitors for the treatment of solid tumour by simultaneously regulating the cell cycle and transcription control. J Enzyme Inhib Med Chem 2020; 35: 414–423.

11. Danilov AV, Hu S, Orr B, et al. Dinaciclib Induces Anaphase Catastrophe in Lung Cancer Cells via Inhibition of Cyclin Dependent Kinases 1 and 2. Mol Cancer Ther 2016; 15:2758–2766.

12. Niu Y, Xu J and Sun T. Cyclin-Dependent Kinases 4/6 Inhibitors in Lung cancer: Current Status, Resistance, and Combination Strategies. J Cancer 2019; 10: 5504–5517.

13. Qin A, Reddy HG, Weinberg FD, et al. Cyclin-dependent kinase inhibitors for the treatment of lung cancer. Expert Opin Pharmacother 2020; 21: 941–952.

14. DeMichele A, Clark AS, Tan KS, et al. CDK 4/6 inhibitor palbociclib (PD0332991) in Rb+ advanced breast cancer: phase II activity, safety, and predictive biomarker assessment. Clin Cancer Res 2015; 21: 995–1001.

15. Tang Z, Li L, Tang Y, et al. CDK 2 positively regulates aerobic glycolysis by suppressing SIRT 5 in gastric cancer. Cancer Sci 2018; 109: 2590–2598.

16. Park MH, Kim SY, Kim YJ, et al. ALS2CR7 (CDK15) attenuates TRAIL induced apoptosis by inducing phosphorylation of survivin Thr34. Biochem Biophys Res Commun 2014; 450: 129–134.

17. Vastrad C and Vastrad B. Investigation into the underlying molecular mechanisms of non-small cell lung cancer using bioinformatics analysis. Gene Rep 2019; 15: 100394.

18. Jin B, Jin H, Wu HB, et al. Long non-coding RNA SNHG15 promotes CDK14 expression via miR-486 to accelerate non-small cell lung cancer cells progression and metastasis. J Cell Physiol 2018; 233: 7164–7172.

19. Ji Q, Xu X, Li L, et al. miR-216a inhibits osteosarcoma cell proliferation, invasion and metastasis by targeting CDK14. Cell Death Dis 2017; 8: e3103.

20. Wang B, Zou A, Ma L, et al. miR-455 inhibits breast cancer cell proliferation through targeting CDK14. Eur J Pharmacol 2017; 807: 138–143.

21. Pollack D, Xiao Y, Shrivastava V, et al. CDK14 expression is down-regulated by cigarette smoke in vivo and in vitro. Toxicol Lett 2015; 234: 120–130.