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CKAP2 overexpression correlates with worse overall survival in patients with lung adenocarcinoma

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Abstract

Background: Adenocarcinoma is a non–small-cell lung cancer that is common cancer in both genders, and has poor clinical outcomes. We aimed to evaluate the role of cytoskeleton-associated protein 2 (CKAP2), its prognostic significance, and the relationship between CKAP2 expression and lung adenocarcinoma driver genes.

Methods: The expression of CKAP2 was studied by immunohistochemical staining of specimens from 88 patients with lung adenocarcinoma. The correlation between clinicopathological features and CKAP2 expression was analyzed. Kaplan-Meier analysis and Cox proportional hazard models were used to examine the prognostic value of CKAP2 in terms of overall survival (OS). The correlation between epidermal growth factor receptor (EGFR) mutation, anaplastic lymphoma kinase (ALK) rearrangement, and CKAP2 expression was analyzed. All histological samples were detected by fluorescence in situ hybridization for EGFR mutations and ALK rearrangements.

Results: Eighty-eight patients with positive CKAP2 expression were observed in this study. Patients with high levels of CKAP2 expression were associated with OS (P = .021). Multivariate Cox regression analysis disclosed that positive CKAP2 expression (P = .043) could independently predict unfavorable OS. In addition, CKAP2 expression was not associated with EGFR mutation (P = .219) and ALK rearrangement (P = .389) in lung adenocarcinoma patients.

Conclusion: High expression of CKAP2 may serve as a marker of poor prognosis in lung adenocarcinoma.

Abbreviations: ALK = anaplastic lymphoma kinase, CI = confidence interval, CKAP2 = cytoskeleton-associated protein 2, df = degrees of freedom, EGFR = epidermal growth factor receptor, FISH = fluorescence in situ hybridization, IHC = immunohistochemistry, LAC = lung adenocarcinoma, OS = overall survival, SE = standard error.

Keywords: cell cycle, cytoskeletal-associated protein 2, lung adenocarcinoma, prognosis

1. Introduction

Non–small-cell lung cancer is the leading cause of cancer incidence and cancer-related mortality worldwide,[1] Non–small-cell lung cancer is histologically divided into 3 subtypes, with lung adenocarcinoma (LAC) and lung squamous cell carcinoma accounting for approximately 50% and approximately 40% of the cases, respectively.[2] Therefore, adenocarcinoma represents the most common histological subtype.[3] LAC continues to have poor clinical outcomes, with a 5-year survival of only 18%.[4] TNM staging is the most important prognostic factor for predicting the recurrence rates and survival times of LAC patients.[5] Therefore, a better understanding of the mechanisms of LAC tumor progression is needed and useful prognostic molecular markers for accurately predicting the clinical outcomes of LAC are of great clinical significance.

Cytoskeleton-associated protein 2 (CKAP2) is related to the assembly and/or maintenance of microtubules and mitotic spindles.[6–8] The expression level increases in stomach cancers, diffuse B-cell lymphoma, and cutaneous T-cell lymphoma.[9–11] In addition, the expression of CKAP2 protein changes in a cell cycle-dependent manner. Its expression is relatively low during G1, starts to incline during G1-S transition, and peaks at G2/M phases of the cell cycle.[12] CKAP2 expression appears most during the mitotic phase and was well correlated with proliferation activity.[13] CKAP2 expression has been studied in breast, gastric, and ovarian cancers, but less so in LAC.

Therefore, in the present study, we investigated the protein expression status of CKAP2 in resected LAC tumors. Moreover, we assessed the prognostic significance of CKAP2 expression in LAC using our Lung Cancer Tissue Microarrays. CKAP2 may be a target gene for targeted lung cancer therapy.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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2. Materials and methods

2.1. Lung cancer tissue microarray and immunohistochemistry staining

A lung cancer tissue microarray was purchased from Shanghai Outdo Biotech (Shanghai, China), which contained 88 carcinoma tissues and paired para-carcinoma tissues. The tissue samples on the tissue microarrays that we used in this study were collected from Tai Zhou hospital of Zhejiang province, China. Informed consent was obtained from all patients, and the collection of tissue samples for the study was approved by the ethics committee of Tai Zhou Hospital. All patients were pathologically diagnosed with adenocarcinoma of the lung after surgery. The immunohistochemistry (IHC) analysis was performed as follows according to a simple description. The tissue sections were blocked with goat serum and then incubated with anti-CKAP2 antibody (Abnova, Taipei City,Taiwan) at 4°C overnight. The sections were stained with 3,3-diaminobenzidine and counterstained with hematoxylin after incubation with a secondary antibody. phosphate buffer saline was used as a negative control. Both staining intensity and positive rate were used to examine the expression of CKAP2 in lung cancer tissue. The IHC staining score (values 0–12) was calculated by multiplying the scores for intensity of positive staining (negative = 0, weak = 1, moderate = 2, or strong = 3) and the percentage of positive-stained cells (0%–25% = 1, 26%–50% = 2, 51%–75% = 3, >75% = 4). An IHC score ≥3 defined high expression, whereas a score <3 was defined as low expression.

2.2. Detection of epidermal growth factor receptor mutation and anaplastic lymphoma kinase rearrangement by fluorescence in situ hybridization

All the histological samples were subjected to epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) assays by fluorescence in situ hybridization (FISH); the LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen prob and the Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular, Des Plaines, IL) were used for detection. According to a published protocol, a 4-μm thick tissue section was prepared for FISH staining; the procedure and interpretation of the test were performed according to the manufacturer's instructions. Positive cases were defined as those split signals when the separation distance between the 4′,6′-diamidino-2-phenylindole or the 5′-part (green fluorescence) and 3′-part(red fluorescence) signals were greater than the diameter of 2 fluorescence signals. The interpretations of FISH staining for EGFR mutations and ALK rearrangements were made by 2 biologists.

2.3. Statistical analysis

The χ^2 test was used to determine the correlation between CKAP2 expression and clinicopathologic variables. The Kaplan-Meier method was used to evaluate the overall survival. All statistical analyses were performed using the SPSS V.17.0 statistical software (SPSS, Chicago, IL, USA). All statistical tests were 2-sided, and *P* values <.05 were considered statistically significant.

3. Results

3.1. CKAP2 expression in LAC

To investigate the role of CKAP2 in the clinical outcome of LAC, the expression of CKAP2 was assessed by IHC in tumor tissues and paired adjacent lung tissues of 88 LAC patients. Figure 1 demonstrates that the expression of CKAP2 was significantly increased in the tumor tissues, and the staining was located in the cytoplasm of tumor cells. Among all patients, the percentage of tumor tissues exhibiting high CKAP2 expression was 80.68% (71/88). In contrast, only 0.045% (4/88) of the adjacent lung tissues exhibited high CKAP2 expression, and the LAC tissues showed significantly higher expression than the paired adjacent lung tissues (Table 1). In conclusion, these results suggest that the protein expression level of CKAP2 is elevated in LAC tissues.

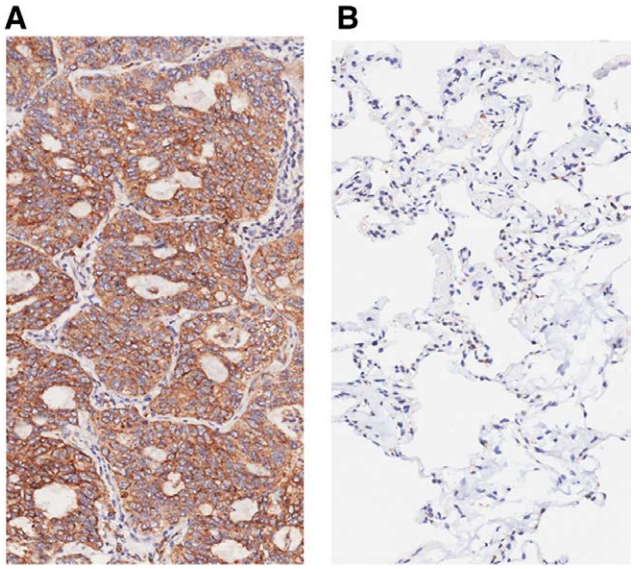

Figure 1. The expression of CKAP2 was assessed by IHC in tumor tissues (A) and paired adjacent lung tissues (B) from lung adenocarcinoma patients. CKAP2 = cytoskeleton-associated protein 2, IHC = immunohistochemistry.

3.2. Relationship between CKAP2 expression and clinical factors in patients with LAC

The associations between CKAP2 expression levels and clinical factors of LAC patients (including gender, age, histological type, degree of differentiation, TNM stage, T stage, N stage) were analyzed (Table 2). The results demonstrated that no significant association was found between CKAP2 protein expression and gender (*P* = .056), age (*P* = .085), tumor size (*P* = .436), histological type (*P* = .771), T stage (*P* = .771) or N stage (*P* = .574), and TNM stage (*P* = .516).

3.3. Analysis of the relationship between CKAP2 expression and survival in LAC

Kaplan-Meier method was used to determine the relationship between CKAP2 expression and the survival curves of LAC patients. The results showed that high expression of CKAP2 was significantly associated with shorter overall survival (OS) time in patients with LAC (*P* = .021, Fig. 2). Univariate analysis demonstrated that CKAP2 expression status, TNM stage, and T classification were associated with the prognosis of patients with LAC. Multivariate analysis revealed that CKAP2 expression was a prognostic marker for OS in LAC patients (*P* = .043; Table 3).

Table 1

Differential expression of CKAP2 in lung cancer tissues and adjacent tissues.

CKAP2 expression	Cases	Median	Za	Pa
Cancer tissue	88	4		–7.634
Para-carcinoma tissue	88	1		–
Paired nonparametric test	–	–		–

CKAP2 = cytoskeleton-associated protein 2.

Table 2

Relations between CKAP2 expression and clinical factor of lung cancer patients.

Clinical factor	Low expression (16)	High expression (72)	All cases (88)	χ^2	*P*
Gender				3.663	.056
Male	12	35	47		
Female	4	37	41		
Age				2.975	.085
≤60	10	28	38		
>60	6	44	50		
Tumor size				0.607	.436
<5 cm	8	49	57		
≥5 cm	5	15	20		
Pathological grade				0.52	.771
Grade I	1	5	6		
Grade II	10	38	48		
Grade III	5	29	34		
T stage				1.127	.771
T1	4	18	22		
T2	5	31	36		
T3	3	8	11		
T4	1	7	8		
N stage				0.316	.574
N0	8	28	31		
N1–3	7	28	35		
M stage				0.064	.8
M0	15	71	86		
M1	1	1	2		
cTNM				2.279	.516
Stage I	6	18	24		
Stage II	2	16	18		
Stage III	4	17	21		
Stage IV	1	1	2		

CKAP2 = cytoskeleton-associated protein 2.

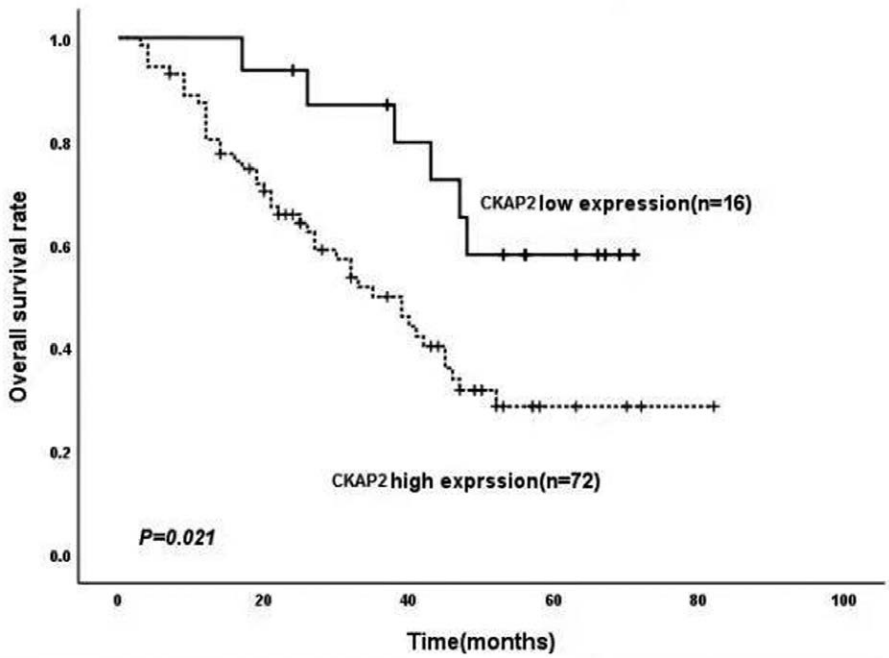

Figure 2. Log rank (Mantel-Cox) actuarial analysis of CKAP2 expression with respect to overall survival in all patients. CKAP2 = cytoskeleton-associated protein 2.

3.4. Correlation of CKAP2 expression with driving genes of LAC

EGFR mutations and ALK gene fusion are important driver genes of LAC. We analyzed the relationship between CKAP2 and EGFR and ALK by FISH. The results showed that CKAP2 expression in LAC patients may not be associated with EGFR ($P = .219$) and ALK ($P = .389$); however, a large number of samples are needed to further confirm.

4. Discussion

Tumor growth is a complex, multistep process; in which cell cycle, mitosis, and cell proliferation all have crucial roles and are

Table 3

Analysis of independent prognostic factor in lung cancer patients by Cox multivariate analysis variables.

							95% CI for exp (*B*)	
Factors	***B***	**SE**	**Wald**	**df**	***P* value**	**Exp (*B*)**	**Lower**	**Upper**
CKAP2 expression	1.494	0.498	9.022	1	.003	4.457	1.681	11.817
Gender	−0.811	0.409	3.922	1	.048	0.445	0.199	0.992
Age	−0.204	0.391	0.273	1	.601	0.815	0.379	1.754
Pathological grade	0.549	0.319	2.969	1	.085	1.732	0.927	3.234
Tumor size	−0.442	0.42	1.104	1	.293	0.643	0.282	1.466
T stage	0.053	0.273	0.037	1	.847	1.054	0.618	1.798
N stage	−1.448	0.69	4.409	1	.036	4.26	1.101	16.393
M stage	−1.636	1.28	1.632	1	.201	0.195	0.016	2.396
cTNM	1.149	0.45	6.526	1	.011	3.156	1.307	7.62

CI = confidence interval, CKAP2 = cytoskeleton-associated protein 2, df = degrees of freedom, SE = standard error.

influenced by numerous regulators. Because cell proliferation is one of the most vital biological mechanisms in tumorigenesis,[14] cell proliferation activity has been recognized as a promising prognostic marker. CKAP2 serves a vital role in cell mitosis and cell death in a P53-dependent manner[15]; therefore, we used our lung cancer tissue microarray to assess the prognostic significance of CKAP2 expression in LAC.

In the present study, the results demonstrated that CKAP2 expression was increased at the protein level in human LAC tissues; the expression of CKAP2 was independent of age, sex, tumor size, tumor differentiation, TNM stage; in addition, it was found that patients with LAC with high CKAP2 expression had a significantly shorter OS time compared with those with low CKAP2 expression. Multivariate analysis revealed that CKAP2 may be a prognostic factor for OS in LAC patients. Since EGFR mutation and ALK gene fusion are important driver genes in LAC, we analyzed the relationship between CKAP2 and EGFR, ALK by FISH; the results showed that CKAP2 in LAC patients was not associated with EGFR and ALK. These results suggest the potential value of CKAP2 as a novel biomarker for predicting the prognosis of LAC.

Several studies have revealed that CKAP2 is associated with prognosis in other different types of cancer. Wang et al[16]demonstrated that CKAP2 expression is associated with glioma tumor growth and served as a prognostic factor in high-grade glioma. Similar results were obtained in HER2-negative luminal type breast cancer, gastric, and ovarian cancer.[16–19] Some studies have shown that chromatin CKAP2-positive cell count was correlated with OS, but our study shows it is cytoplasmic expression, the reason is the absence of cytosolic expression of CKAP2 referring to different antibody clones or in lung cancer tissues. We analyzed our data based on the intensity of cytoplasmic expression; these results also show the potential value of CKAP2 as a novel biomarker for predicting LAC prognosis. Several studies suggest that CKAP2 gene silencing may inhibit the proliferation, migration, and invasion of cancer cells through the JAK2/STAT3 signaling pathway or FAK-ERK2 pathway as a possible mechanism for the poor prognosis of LAC.[20–23]

There are some limitations to this study; the use of tissue arrays is not representative of the whole tumor, and duplicated arrays were not investigated in this study. The limited sample size weakens the impact of our findings; therefore, more complete studies with a large sample size are still needed in the future. In addition, only 1 clone of antibody was used, and the results should be further validated with a different antibody clone.

In conclusion, the present study showed that CKAP2 is expressed in LAC and that high CKAP2 protein expression is associated with OS. These results indicated that CKAP2 serves as a novel prognostic indicator for LAC. However, further studies are needed to determine the clear role of CKAP2 in LAC.

Author contributions

Data curation: Sen Zhang
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Software: Sen Zhang, Na Li
Writing – original draft: Sen Zhang
Writing – review & editing: Jun Cai, Na Li, Yonghua Yang, Yan Li

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