Upregulation of centromere protein K is crucial for lung adenocarcinoma cell viability and invasion

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Abstract

Background. Identification of functional genes or biomarkers may be helpful for developing new treatment strategies in lung adenocarcinoma (LUAD). The centromere protein K (*CENPK*) gene has been discovered to be overexpressed and could influence tumor progression in several tumor types. However, its role in LUAD has never been revealed.

Objectives. The purpose of the current study was to detect the effects of *CENPK* and its mechanisms in the progression of LUAD.

Materials and methods. Data from The Cancer Genome Atlas (TCGA) and Oncomine databases was used to analyze the expression of *CENPK*. The relationship between *CENPK* expression and the prognosis of LUAD was investigated using Kaplan–Meier and Cox regression analyses. The cell viability was monitored with Cell Counting Kit-8 (CCK-8) and colony forming assays, while migration and invasion were analyzed with a transwell assay. The effect of *CENPK* on the expression of epithelial–mesenchymal transition (EMT) markers were estimated using western blotting.

Results. *CENPK* was significantly overexpressed in LUAD tissues and cells ($p < 0.01$). The overall survival rate in the low *CENPK* expression group was significantly higher than in the high *CENPK* expression group ($p = 0.003$). Furthermore, the overexpression of *CENPK* facilitated cell viability, migration and invasion of tumor cells, while knockdown of *CENPK* prevented these behaviors ($p < 0.01$). Moreover, upregulation of *CENPK* decreased the expression of E-cadherin and enhanced the expression of N-cadherin, vimentin and Snail in LUAD cells ($p < 0.01$). Conversely, knockdown of *CENPK* resulted in the opposite trend ($p < 0.01$).

Conclusions. *CENPK* was upregulated in LUAD tissues and cells, and the enhancement of *CENPK* promoted the viability, migration, invasion, and EMT of LUAD cells.

Key words: *CENPK*, viability, migration, epithelial–mesenchymal transition, lung adenocarcinoma

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Background

Lung cancer is one of the most common cancers and the leading cause of cancer-related death worldwide,[1–3] with the five-year overall survival (OS) of less than 20%.[4] Lung adenocarcinoma (LUAD) accounts for approx. 40% of all lung cancers, and is frequently diagnosed at an advanced stage.[5–7] Currently, the most effective methods for the treatment of LUAD are surgical, radiation and targeted, and immunotherapies directed at the molecular or immunological traits of tumors. Nevertheless, the survival rate of patients with LUAD that have received curative resection is only about 50%.[8] Therefore, further research into the mechanisms of LUAD and identification of effective biomarkers will contribute to early diagnosis, precise prognosis and development of effective treatments for LUAD.

The kinetochore is a protein structure on chromatids and plays a crucial role in the segregation of chromosomes, the dysfunction or dysregulation of which may lead to aneuploidy and promote carcinogenesis.[9] Centromere protein K (*CENPK*), also named AF5alpha, FKSG14, P33, or Solt5,[10] is a subunit of CENPH-CENPI-associated centromeric complex, which targets centromere protein A (CENPA) to centromeres and is critical for proper kinetochore function and mitotic progression.[11] In many malignant tumors, *CENPK* has been reported to be dysregulated and related to tumor progression.[12–14] For example, *CENPK* is specifically upregulated in ovarian and hepatocellular cancer tissues, and its overexpression is associated with worse overall survival.[12] More importantly, Wang et al. has previously demonstrated that depletion of *CENPK* could repress the proliferation, migration, invasion, and epithelial–mesenchymal transition (EMT) of hepatocellular carcinoma cells.[13] Furthermore, through genome-wide gene expression profiling analysis, *CENPK* was screened out as one of the novel therapeutic targets for triple negative breast cancer and knockdown of *CENPK* attenuated cell viability.[14] However, it remains unclear as to whether *CENPK* plays a role in LUAD.

Objectives

In the current study, we analyzed the expression of *CENPK* in LUAD cells and evaluated its prognostic value in patients with LUAD through Kaplan–Meier and Cox regression analyses. Then, the effects on viability, invasion and migration of LUAD cells were explored. Finally, we investigated the influence of *CENPK* on the expression of the key markers of EMT, including E-cadherin, N-cadherin, vimentin, and Snail, in LUAD cells.

Materials and methods

Bioinformatics analysis

The gene expression profile and clinical data of 535 LUAD tumor samples and 59 normal samples were downloaded from The Cancer Genome Atlas (TCGA) (https://cancergenome.nih.gov/). Patients diagnosed as LUAD were divided into high and low *CENPK* expression groups based on the median expression of *CENPK*. Then, Kaplan–Meier analysis and univariate and multivariate Cox regression analyses were performed to assess the prognostic value of *CENPK*. In addition, the expression profiles of 45 LUAD samples and 65 normal samples in the Oncomine database (www.oncomine.org) were downloaded to analyze the mRNA expression of *CENPK*.

Cell culture

The LUAD cell lines, including HA109, A549 and Calu-3, were purchased from the Cell Bank of the Shanghai Institute of Cell Biology, Chinese Academy of Medical Sciences (Shanghai, China). The normal human lung epithelial cell line BEAS2B was purchased from American Type Culture Collection (ATCC, Manassas, USA). All the cell lines were serially cultured in Roswell Park Memorial Institute (RPMI)-1640 medium mixed with 10% fetal bovine serum (FBS; Gibco, Rockville, USA), 100 U/mL of penicillin and 100 μg/mL of streptomycin (Life Technologies, Gaithersburg, USA) at 37°C with 5% CO_2. Tumor cells between passages 4 and 6 were utilized in the experiments.

Cell transfection

Cell transfection was conducted when the cell confluence reached 80% in a six-well plate. Calu-3 cells were transfected with small interfering RNA (siRNA) to downregulate the expression of *CENPK*. The siRNAs used include si-CENPK#1 (5'-TGAGTACCTTGGGCGAGTTTC-3') and si-CENPK#2 (5'-ATATCTGAGGTGGCATAATTT-3'), together with Si-con (5'-CGAACUCACUGGUCUGACC-3') that was transfected as the negative control. Meanwhile, pcDNA3.1-CENPK vector was constructed and transfected into HA109 cells. An empty vector (pcDNA3.1; Genepharma, Shanghai, China) was used as the negative control. Cell transfection was conducted using the Lipofectamine 2000 reagent kit (Invitrogen, Carlsbad, USA). The expression of *CENPK* in LUAD cell lines was detected using quantitative real-time polymerase chain reaction (qRT-PCR) and western blotting 48 h following transfection.

RNA extraction and quantitative real-time PCR

RNA trizol reagent (Invitrogen) was used to extract the total RNA from conventionally cultured cells or cells transfected

Table 1. The sequences of *CENPK* and *GAPDH* primers in qRT-PCR

Genes	Sequences
CENPK	F:5′-ACCGCTGAACTCAGTCAATGGC-3′ R:5′-TTGACTCCTTAGTGGACAGTACC-3′
GAPDH	F:5′-TGTGTCCGTCGTGGATCTGA-3′ R:5′-CCTGCTTCACCACCTTCTTGA-3′

48 h prior. Subsequently, the extracted RNA was converted to cDNA using a MiRcute miRNA First-strand cDNA synthesis kit (Tiangen Biotech, Beijing, China). Then, the mRNA expression of *CENPK* was measured using quantitative real-time polymerase chain reaction (qRT-PCR) analysis. The relative *CENPK* level was calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to *GAPDH*. The sequences of the primers for *CENPK* and *GAPDH* are shown in Table 1.

Western blotting

Protein was isolated from the cells 48 h following transfection with ice-cold IPA lysis buffer and the concentration was measured using a BCA protein assay kit (Beyotime, Shanghai, China). Then, 20 μg of protein was put into each well of a vertical electrophoresis tank and separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The protein was transferred onto polyvinylidene fluoride (PVDF) membranes, blocked with 5% skim milk powder diluted in TBST buffer (Tris-buffered saline, 0.1% Tween 20) for 1 h then incubated with primary antibodies at 4°C overnight. The rabbit-anti-human primary antibodies against *CENPK* (ab236739, 1:1000; Abcam, Cambridge, UK), E-cadherin (#3195, 1:1000; Cell Signaling Technology, Danvers, USA), N-cadherin (#4061, 1:1000; Cell Signaling Technology), Vimentin (#5741, 1:1000; Cell Signaling Technology), Snail (#3879, 1:1000; Cell Signaling Technology), and *GAPDH* (#5174, 1:1000; Cell Signaling Technology) were used. Next, samples were washed 3 times (5 mins each) with TBST, before the goat-anti-rabbit secondary antibody (#7074, 1:3000; Cell Signaling Technology) was added and incubated at room temperature for 1 h. The blots were visualized using the ECL-Plus western blotting detection system (Thermo Fisher Scientific, Waltham, USA) after washing. The relative protein expression levels of *CENPK*, E-cadherin, N-cadherin, vimentin, and Snail were separately measured through scanning the gray value with QUANTITY ONE software (Bio-Rad, Hercules, USA) and *GAPDH* was used as the internal reference.

Cell Counting Kit-8 assay

The cells were removed from culture and counted 48 h after transfection. A cell suspension was prepared and plated into 96-well plates at a concentration of 1×10^3 cells/well. Then the plate was kept in an incubator at 37°C with 5% CO_2. The cell viability was tested every 24 h using Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) reagent according to manufacturer's instruction. After being incubated for 1.5 h at 37°C, the optical density (OD) value of each well was measured at 450 nm using a microplate reader to reflect cell viability.

Colony formation assay

Following transfection, cells were suspended and seeded into a 60 mm plate (400 cells/plate) with 5 mL of pre-warmed medium at 37°C. Then the cells were cultured at 37°C with 5% CO_2 for 1–2 weeks until colonies were visible. After that, 4% paraformaldehyde and 0.1% crystal violet were used to fix and stain the colonies, respectively, and the number of colonies with a diameter greater than 0.8 mm was counted.

Cell invasion and migration

To analyze cell invasion, 100 μL of Matrigel was prepared in advance by incubating in serum-free medium overnight and then added into the upper transwell chamber. After being shaken well, the chamber was maintained at 37°C and maintained for 4–6 h. Then, 500 μL of serum-free medium was put into the lower chamber and incubated for ½ h. Next, 100 μL of cell suspension (1×10^5 cells) was prepared using serum-free medium after 48 h of transfection and inoculated into the upper chamber, while 500 μL of complete culture solution was put into the lower chamber. Twenty four hours later, the chamber was removed and washed with phosphate-buffered saline (PBS). It was then fixed with 4% paraformaldehyde for 30 min and stained using 0.1% crystal violet for 20 min. The number of invading cells were quantified. For the cell migration assay, chamber was not coated with Matrigel and the remaining procedures were the same as the invasion assay.

Statistical analyses

Data analyses were performed using SPSS v. 22.0 software (IBM Corp., Armonk, USA) and the figures were plotted in GraphPad Prism software v. 5.0 (GraphPad Software, San Diego, USA). The difference between ranked data as well as the association between *CENPK* expression and clinical factors were analyzed with a χ^2 test. In addition, Kaplan–Meier analysis was performed to estimate the relationship between *CENPK* expression and the overall survival of patients with LUAD. The difference between these groups was evaluated with log-rank test. The prognostic significance of numerous variables was analyzed with univariate and multivariate Cox regression analyses. For the experimental data, the statistical evaluation between 2 groups was performed with Student's t-test, while the statistical evaluation among 3 groups was conducted using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. A value of $p < 0.05$ was used to determine statistical significance.

Results

The prognostic value of *CENPK* mRNA expression in LUAD

We analyzed genes that were differentially expressed between LUAD and normal tissue samples using TCGA. As a result, 3430 differentially expressed genes were found, including 2094 upregulated genes and 1336 downregulated genes. Among them, *CENPK* was upregulated in tumor tissues when compared to normal tissues (Fig. 1A, $p < 0.001$). To confirm this result, we analyzed the expression of *CENPK* in LUAD and normal lung tissues with the data from the Oncomine database, finding higher expression of *CENPK* in LUAD tumor tissues than in normal lung tissues (Fig. 1B, $p < 0.001$). To further investigate the expression of *CENPK* in LUAD, we detected its expression in the LUAD cell lines HA109, A549 and Calu-3, and in normal lung cell line BEAS2B. These results showed that *CENPK* was significantly overexpressed in LUAD cells compared with normal BEAS2B cells (Fig. 1C, $p < 0.001$).

As *CENPK* was upregulated in LUAD, we decided to test whether *CENPK* was related to patient prognosis. After analyzing the relationship between clinical factors and *CENPK* expression in LUAD samples, we found that the expression of *CENPK* was statistically associated with gender ($p = 0.048$), tumor stage ($p = 0.028$) and pathologic-N (lymph node) stage ($p = 0.007$) of patients with LUAD (Table 2). Subsequently, we evaluated the influence of *CENPK* on the overall survival of patients with LUAD using Kaplan–Meier analysis, observing the overall survival time of patients with higher *CENPK* expression was shorter than those with lower expression (Fig. 1D, log-rank test, $p = 0.003$). Finally, univariate and multivariate Cox

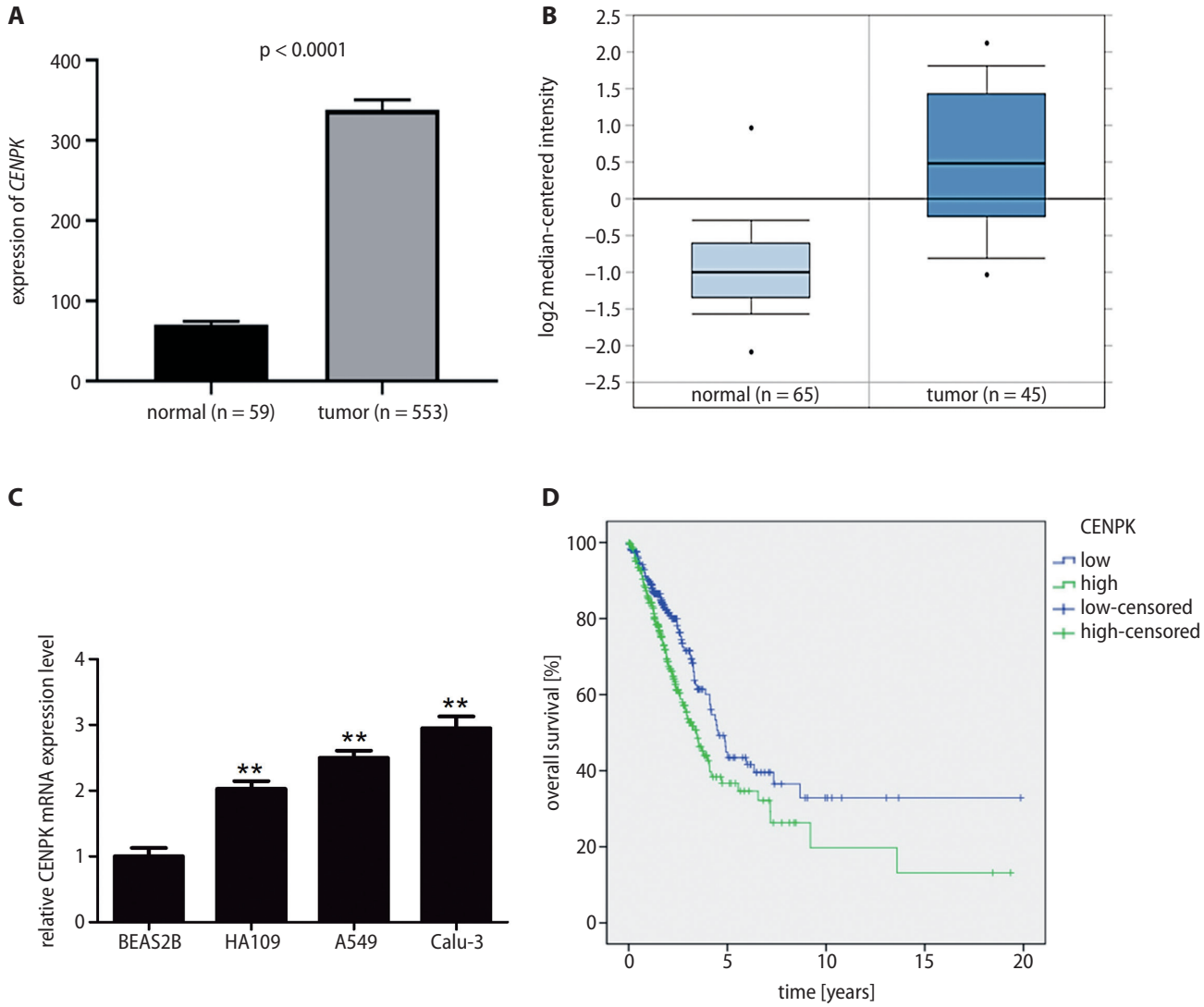

Fig. 1. The overexpression of *CENPK* and its association with cancer prognosis in LUAD. A and B. The mRNA expression of *CENPK* in tumor tissues was higher than in normal tissues according to TCGA (A) and Oncomine (B) databases; C. *CENPK* was upregulated in LUAD cell lines in comparison to normal lung cell lines. The data were analyzed using the $2^{-\Delta\Delta ct}$ method and presented as the mean ±SD; D. Kaplan–Meier analysis of *CENPK* expression in samples from TCGA showed that patients with higher *CENPK* level had a much shorter survival time

*$p < 0.05$; **$p < 0.01$.

Table 2. The relationship between clinical characteristics and the expression of *CENPK*

Characteristics	Expression of *CENPK*		p-value
	low	high	
Age			
<60	62	74	0.205
≥60	184	170	
Gender			
female	146	124	0.048*
male	104	126	
Clinical stage			
I+II	201	184	0.028*
III+IV	43	64	
Pathologic-T			
T1+T2	215	218	0.776
T3+T4	33	31	
Pathologic-N			
N0	174	149	0.007*
N1	68	98	
Pathologic-M			
M0	162	170	0.143
M1	8	16	

p < 0.05 indicated statistical significance.

regression analyses were used to determine the prognostic significance of *CENPK* in LUAD. This data revealed that *CENPK* expression (p = 0.004), clinical stage (p < 0.001), pathologic-T (tumor) (p < 0.001), pathologic-M (metastasis) (p = 0.006), and pathologic-N (p = 0.000) were all unfavorable LUAD prognostic factors. Furthermore, multivariate Cox regression analysis suggested that pathologic-T (hazard ratio (HR) = 1.687, 96% confidence interval (95% CI) = 1.049–2.713, p = 0.031) and pathologic-N (HR = 2.031, 95% CI = 1.363–3.026, p < 0.001) can serve as independent prognostic markers, but *CENPK* (HR = 1.399, 95% CI = 0.898–1.979, p = 0.058) cannot be a significant predictor of disease progression in patients with LUAD (Table 3).

CENPK promotes the viability of LUAD cells

Calu-3 and HA109 cells were used to conduct the loss and gain of function of *CENPK* assays, respectively. Calu-3 cells with downregulated *CENPK* (p < 0.001) and HA109 cells with overexpressed *CENPK* (p = 0.001) were successfully constructed (Fig. 2A–D).

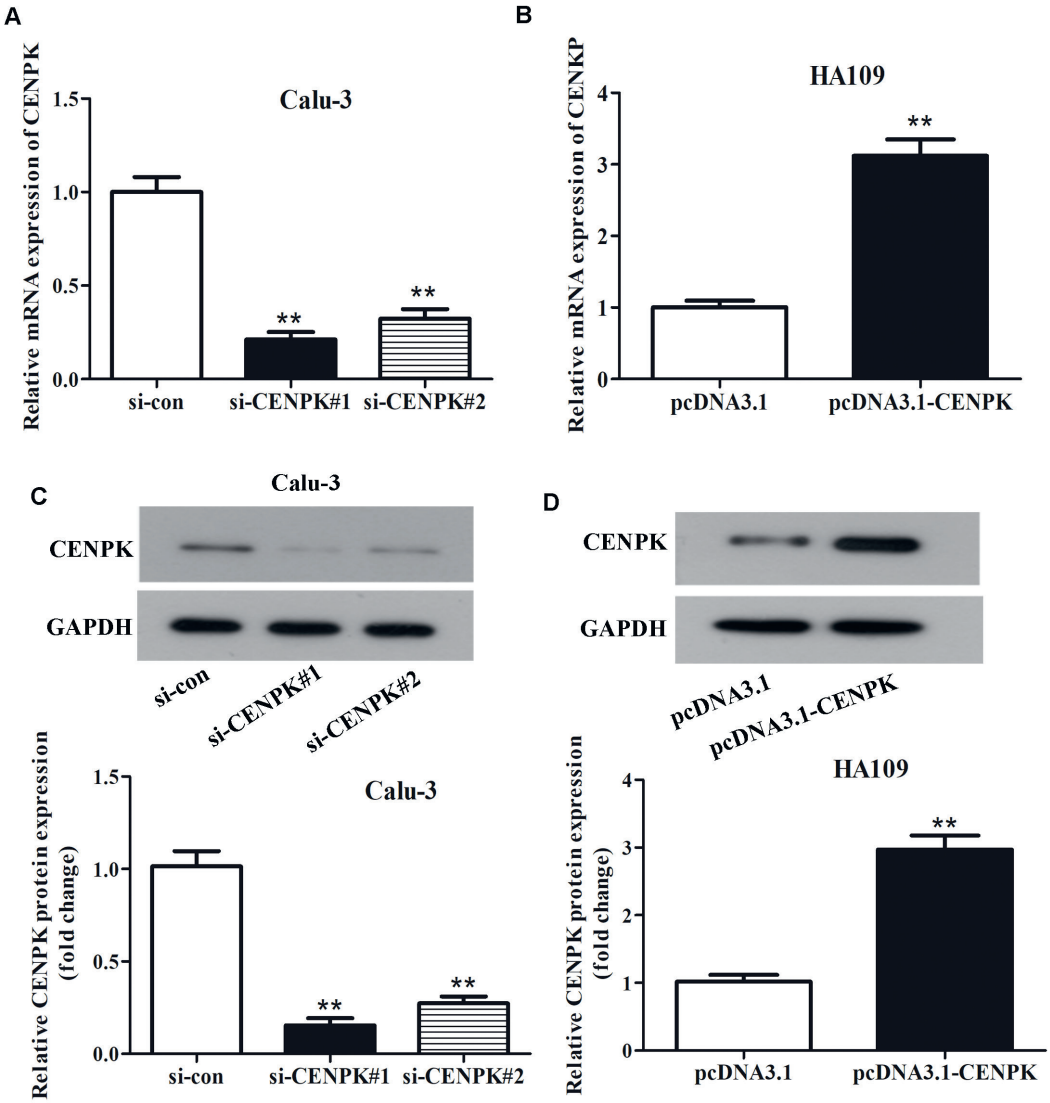

Fig. 2. The expression of *CENPK* in LUAD cells was examined with qRT-PCR and western blotting. A and C. *CENPK* was knocked down in Calu-3 cells by si-CENPK; B and D. *CENPK* was overexpressed in HA109 cells after transfection with pcDNA3.1-CENPK. The data are shown as mean ±SE

*p < 0.05; **p < 0.01.

Table 3. Univariate and multivariate Cox regression analysis

Variables	Univariate analysis			Multivariate analysis		
	p-value	HR	95% CI	p-value	HR	95% CI
CENPK expression (high/low)	0.004*	1.551	1.154–2.084	0.058	1.399	0.898–1.979
Clinical stage (I+II/III+IV)	<0.001*	2.466	1.810–3.360	0.276	1.317	0.803–2.160
Pathologic-T (T1+T2/T3+T4)	<0.001*	2.147	1.467–3.140	0.031*	1.687	1.049–2.713
Pathologic-M (M0/M1)	0.006*	2.132	1.244–3.653	0.354	1.356	0.712–2.582
Pathologic-N (N0/N1+N2+N3)	<0.001*	2.507	1.864–3.370	<0.001*	2.031	1.363–3.026
Age (<60/≥60)	0.746	1.056	0.760–1.468	–	–	–
Gender (female/male)	0.616	1.077	0.805–1.442	–	–	–

HR – hazard ratio; 95% CI – 95% confidence interval; p < 0.05 indicated statistical significance.

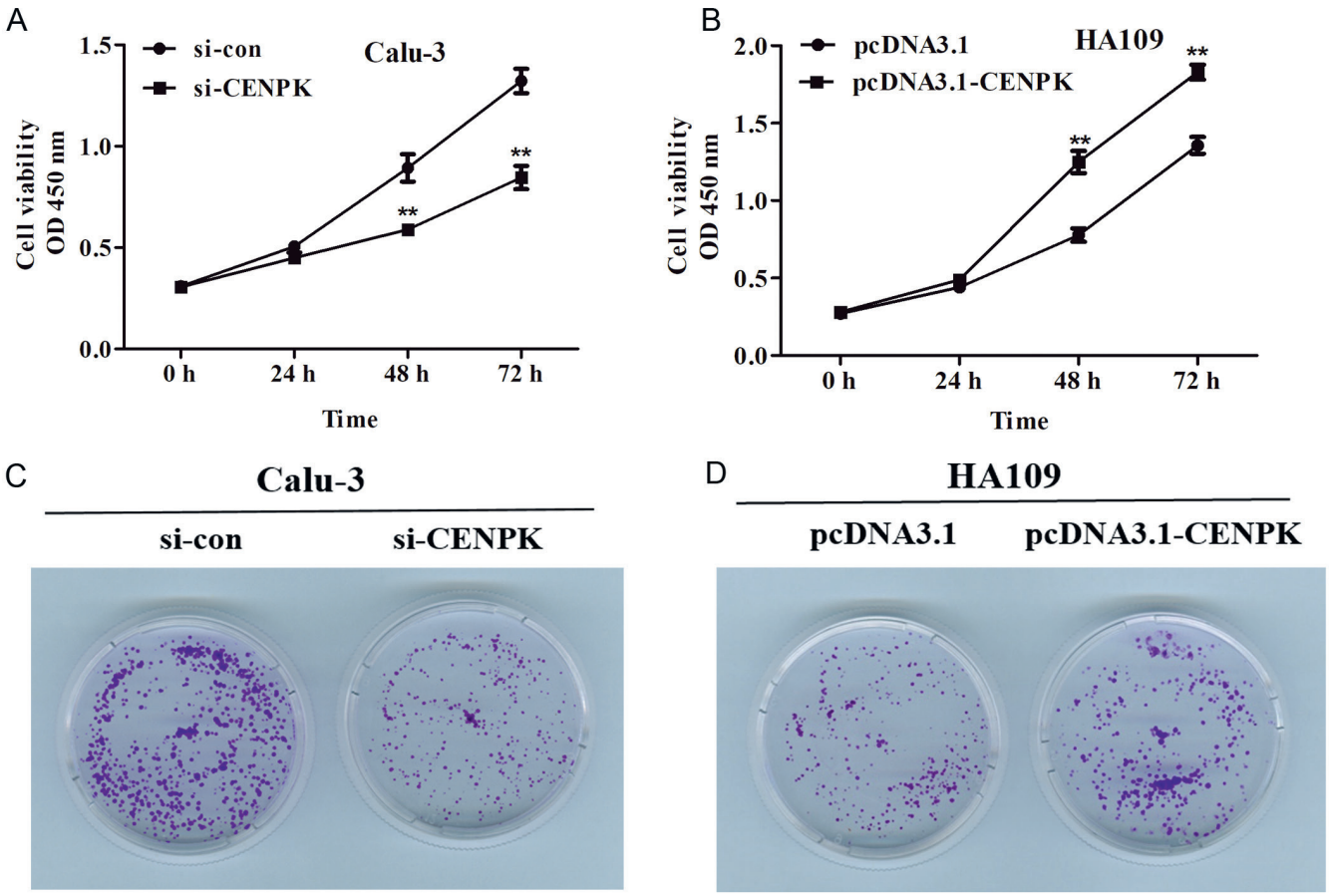

Fig. 3. The role of *CENPK* in the viability of LUAD cells. A and B. The cell viability of LUAD cells was detected using a CCK-8 assay after *CENPK* was knocked down in Calu-3 cell line (A) and overexpressed in HA109 cell line (B). The data was shown as mean ±SD; C and D. The colonies formed by Calu-3 cell line with depleted *CENPK* (C) and HA109 cell line with overexpressed *CENPK* (D)

*p < 0.05; **p < 0.01.

To study the role of *CENPK* in the viability of LUAD cells, the CCK-8 assay and colony forming assay were performed. The CCK-8 results demonstrated that knockdown of *CENPK* could inhibit the viability of Calu-3 cells (p = 0.002; p = 0.001), while the overexpression of *CENPK* accelerated the growth of HA109 cells (p = 0.001; p < 0.001) at 48 h and 72 h (Fig. 3A,B; p < 0.001). To verify this conclusion, we implemented colony forming assays. The data indicated that the number of the colonies of Calu-3 cells was decreased (p = 0.002), while the number of the colonies of HA109 cells was increased (p = 0.014) compared with their corresponding control groups (Fig. 3C,D).

CENPK enhances cell migration and invasion, and promotes EMT of LUAD cells

We next investigated the function of *CENPK* on the migration and invasion of LUAD cells by employing a transwell

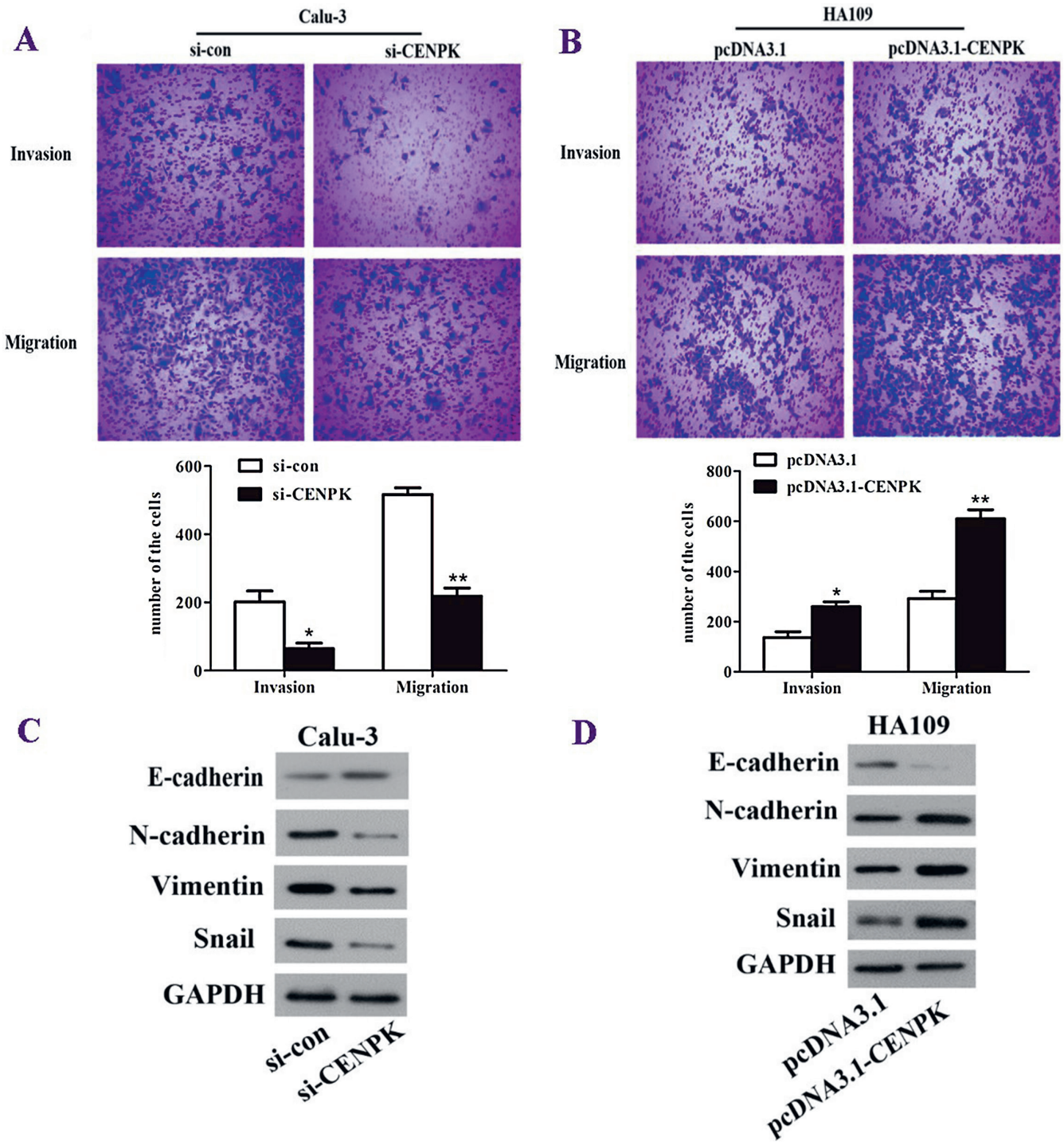

Fig. 4. The function of *CENPK* on cell migration and invasion. A. Knockdown of *CENPK* inhibited the migration and invasion of Calu-3 cells. The data are shown as mean ±SE; B. Overexpressed *CENPK* promoted cell migration and invasion of HA109 cells. The data is shown as mean ±SE; C. Knockdown of *CENPK* increased the expression of E-cadherin and decreased the expression of N-cadherin, vimentin and Snail in Calu-3 cells; D. Overexpression of *CENPK* decreased the expression of E-cadherin and increased the expression of N-cadherin, vimentin and Snail in HA109 cells

*p < 0.05; **p < 0.01.

assay system. Knockdown of *CENPK* alleviated the migration ($p < 0.001$) and invasion ($p = 0.018$) of Calu-3 cell (Fig. 4A), while in contrast, both the migratory ($p = 0.002$) and invasive ($p = 0.014$) abilities of HA109 cells were strengthened when *CENPK* was overexpressed (Fig. 4B).

The EMT participates in the progression of many tumor types and it is thought to regulate the function of some genes in LUAD.[15,16] The main characteristic of EMT is the loss of cell–cell adhesion markers, such as E-cadherin, along with an increased rate of cell migration and invasiveness.[17] Therefore, we hypothesized that the function of *CENPK* might be connected with EMT. To explore this hypothesis, we detected the expression of the EMT markers E-cadherin, N-cadherin, vimentin, and Snail. As expected,

CENPK could mediate the expression of E-cadherin, N-cadherin, vimentin, and Snail. Knockdown of *CENPK* increased the expression of E-cadherin but reduced the levels of N-cadherin, vimentin and Snail in Calu-3 cells (Fig. 4C). Conversely, the overexpression of *CENPK* was found to increase the levels of N-cadherin, vimentin and Snail and decrease the expression of E-cadherin in HA109 cells (Fig. 4D). These results suggested that *CENPK* facilitates the EMT process of LUAD cells.

Discussion

In the present study, we demonstrated that *CENPK* was upregulated in LUAD tissues and cells, and higher expression of *CENPK* was correlated with shorter survival time of patients. Biological experiments then revealed that enhancement of *CENPK* expression promoted the viability, migration, invasion, and the EMT process of LUAD cells, suggesting the participation of *CENPK* in LUAD progression.

CENPK is located on chromosome 5q12.3 and has previously been verified to play a crucial role in several types of cancers.[18] In the study by Lee et al., *CENPK* was shown to be upregulated and could serve as a novel tumor marker for ovarian cancer.[12] Furthermore, Wang et al. discovered that the expression of *CENPK* was increased in hepatocellular carcinoma and it promoted its aggressive progression by regulating YAP1.[13] Finally, Komatsu et al. confirmed that *CENPK* was upregulated in triple-negative breast cancer and could act as a novel molecular therapeutic target.[14] All these published reports hint at the critical role of *CENPK* in tumor progression. In the current study, we also found for the first time that *CENPK* was overexpressed in both LUAD tissue samples and cell lines which suggested the oncogenic role of *CENPK* in LUAD.

As the prognosis of LUAD is poor,[19] it is meaningful to seek some functional genes or biomarkers to predict the prognosis of LUAD. For that reason, we estimated the prognostic value of *CENPK* in LUAD. Firstly, we assessed the connection between *CENPK* expression and clinical features which showed that *CENPK* expression was related to gender, pathologic tumor stage and pathologic-N (lymph node involvement). Then, we performed the Kaplan–Meier analysis, and demonstrated that patients with high expression of *CENPK* often had much shorter survival times than those with low expression of *CENPK*, indicating that *CENPK* expression was connected with the prognosis of LUAD. However, *CENPK* was not found to be an independent prognostic predictor, and should be utilized with other markers.

As mentioned above, *CENPK* can promote the malignant progression of some tumors such as hepatocellular carcinoma, ovarian cancer and triple-negative breast cancer. However, the role of *CENPK* in LUAD has never been evaluated. Of note, CENPA, CENPE and CENPH, which are also members of CENP family of proteins, have been discovered to be abnormally expressed in cancer and are correlated with tumor growth in lung cancer.[20–24] Wu et al. reported that enhanced CENPA expression is correlated with shorter overall survival of LUAD patients and could be an independent marker for LUAD.[23] Centromere protein A was also screened as a member of potential diagnostic biomarkers in LUAD by an integrated microarray analysis.[22] Through bioinformatics, Qi et al. uncovered that CENPA was a pivotal gene that was closely related to the overall survival of patients with lung squamous cell carcinoma.[20] CENPE, which highly expressed in the G2/M phase of the cell cycle, was also found to be upregulated in LUAD tissues, and could facilitate the proliferation of LUAD cells by directly mediating by FOXM1.[21] Similarly, CENPH presented at higher levels in non-small cell lung cancer cells and tissues than the surrounding normal tissue as well, and this high expression was associated with poor outcome in patients.[24] This data suggested the vital role of CENP family proteins in the progression of lung cancer. Hence, we analyzed the effects of *CENPK* on cell viability, cell migration and invasion of LUAD cells in our study. We discovered that overexpressed *CENPK* could enhance the cell viability, cell migration and invasion of LUAD cells, hinting the involvement of *CENPK* in the aggressive progression of LUAD tumors.

The EMT is a reversible progression during which polarized epithelial cells can transform into a mesenchymal phenotype according to the interaction with the surrounding mesenchyme and the basement membrane.[25] It has been reported that EMT is related to embryonic development and organ formation, wound healing and fibrosis, and cancer progression.[26] Previous studies have shown that the initiation of EMT in tumor cells may regulate cancer metastasis, recurrence and therapeutic resistance, and has been shown to play an important role in the tumorigenesis of lung cancer.[27] Meng et al. found that the overexpression of Williams syndrome transcription factor (WSTF) promoted the proliferation and invasiveness of lung cancer cells by upregulating the expression of EMT-related genes.[28] The EMT is also considered to be associated with the prognosis of lung cancers.[29] With respect to LUAD, there is a large volume of literature regarding the function of EMT in its development and progression. In the study by Yuanhua et al., the overexpression of KRT16 was induced by TFAP2A and it promoted tumorigenicity via regulating EMT in LUAD.[19] Pang et al. revealed that RCC2 promoted intrapulmonary metastases, and cell migration, invasion and proliferation via inducing EMT and stimulating the expression of matrix metalloproteinase (MMP)-2 and MMP-9.[30] In addition, the activation of EMT was also shown to influence the malignant behavior or prognosis of LUAD via regulating different genes.[31–33] Finally, *CENPK* could accelerate cell proliferation, migration and invasion, as well as EMT progression in hepatocellular carcinoma

cells.[13] Herein, we speculated that the expression of *CENPK* may have effects on EMT-related genes in LUAD cells, so we detected the expression of EMT markers including E-cadherin, N-cadherin, vimentin, and Snail. It was shown that N-cadherin, vimentin and Snail were all decreased while E-cadherin was raised in LUAD cells when *CENPK* was knocked down, and that this was reversed when *CENPK* was overexpressed. This might indicate that *CENPK* affects tumor progression of LUAD by activating EMT. However, we have only detected the relative expression of genes connecting with EMT, and the detailed mechanism of how *CENPK* communicates with these EMT markers deserves further investigation.

Conclusions

The enhanced expression of *CENPK* functioned as a promoter of the viability, migration, invasion, and EMT of LUAD cells in vitro, which suggests the oncogenic role of *CENPK* in LUAD. However, larger case-control studies containing clinical data and the corresponding experiments in vivo are lacking herein. Furthermore, the detailed and accurate mechanisms still need further research.

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