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Original Article

CENPH overexpression promotes the progression, cisplatin resistance, and poor prognosis of lung adenocarcinoma via the AKT and ERK/P38 pathways

Qiao Li[1*], Xin Huang[2*], Qian-Yun Li[3], Yang Tang[1], Lu-Biao Liang[1], Qian Luo[1], Ming Gong[1], Yong-Xiang Song[1], Qiang Guo[4], Cheng Chen[1]

[1]Department of Thoracic Surgery, Affiliated Hospital of Zunyi Medical University, Zunyi 563000, Guizhou, China; [2]Department of Thoracic Surgery, People's Hospital of Dongxihu District, Wuhan 430040, Hubei, China; [3]The Fourth Affiliated Hospital, Zhejiang University School of Medicine, Yiwu 310030, Zhejiang, China; [4]Department of Cardiothoracic Surgery, Taihe Hospital, Hubei University of Medicine, Shiyan 442012, Hubei, China. [*]Equal contributors.

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Abstract: Overexpression of centromere protein H (CENPH) promotes cancer growth and progression. However, the roles and underlying mechanisms have not been elucidated. Therefore, we aim to explore the roles and mechanisms of CENPH in lung adenocarcinoma (LUAD) progression by using comprehensive data analysis and cell experiments. In this study, the relationship between CENPH expression, which was obtained from the TCGA, and GTEx databases, and the prognosis and clinical characteristics of LUAD patients was analyzed, and the diagnostic values of CENPH was evaluated. CENPH-related risk models and nomograms were constructed to evaluate the prognosis of LUAD via Cox and LASSO regression analysis. The roles and mechanisms of CENPH in LUAD cells were studied using CCK-8 assay, wound healing and migration tests, and western blotting. The relationship between CENPH expression and immune microenvironment and RNA modifications was explored through correlation analysis. We found that CENPH was overexpressed in LUAD tissues, and tumors with diameter >3 cm, lymph node metastasis, distant metastasis, late stage, men, and dead cancer patients. Increased expression of CENPH was related to the diagnosis, poor survival rate, disease specific survival rate, and progression of LUAD. CENPH-related nomograms and risk models could predict the survival rate of LUAD patients. Inhibiting the expression of CENPH in LUAD cells decreased their migration, proliferation, and invasion, and promoted their sensitivity to cisplatin, which was related to the downregulation of p-AKT, p-ERK, and p-P38. However, there was no effect on AKT, ERK, and P38. Enhanced expression of CENPH was significantly correlated with immune score, immune cells, cell markers, and RNA modifications. In conclusion, CENPH was strongly expressed in LUAD tissues and was associated with poor prognosis, immune microenvironment, and RNA modifications. CENPH overexpression could enhance cell growth and metastasis and promote resistance to cisplatin via the AKT and ERK/P38 pathways, indicating its potential as a biomarker for the prognosis of LUAD.

Keywords: LUAD, CENPH, immune microenvironment, risk model, TCGA, RNA modification

Introduction

Targeted therapy could improve the patient outcome for lung adenocarcinoma (LUAD) [1-3]. SP101, a newly synthesized gefitinib compound, inhibited Survivin expression and tumor growth in EGFR wild-type lung cancer, blocked EGFR kinase activity and induced apoptosis in EGFR wild-type and EGFR-T790M lung cancer cells, and inhibited tumor growth in nude mice. It could be used to treat gefitinib-resistant patients with EGFR wild-type and EGFR mutations and improve the survival rate [3]. Novel molecules such as GPX2 were recently found to be aberrantly expressed in LUAD and were associated with its prognosis and increased A549 cell growth and migration and decreased apoptosis [4-6]. Inhibition of GPX2 expression

resulted in the opposite trend owing to increased protein expression of Bcl-2, MMP2, and MMP9 and decreased protein expression of Bax and E-cadherin [5].

Centromere protein H (CENPH), a component of the centromere complex, was associated with the prognosis and progression of colorectal, renal cell, endometrial, and other cancers [7-13]. CENPH was overexpressed in colorectal cancer and could activate the GOLPH3-dependent mTOR pathway and suppress rapamycin sensitivity [7]. Similarly, upregulated CENPH expression was positively correlated with Fuhrman grade, M stage, and clinical stage, and was an independent prognostic factor in renal cell carcinoma (RCC) patients. Inhibition of CENPH expression decreased cancer cell proliferation and increased the rate of apoptosis [8], indicating that CENPH could function as an oncogene. The expression level of CENPH in non-small cell lung cancer (NSCLC) tissues was higher than that in normal tissues and was associated with clinical stage, tumor class, Ki-67 expression, and poor prognosis [14, 15]. However, the roles and mechanisms of CENPH on cell proliferation, migration, and drug sensitivity in LUAD are not fully elucidated. Therefore, the expression profile of CENPH in LUAD, and the potential relationship of CENPH expression with patient survival rate, clinical characteristics and immune microenvironment were analyzed using bioinformatics. The effects and mechanisms of inhibiting CENPH expression on LUAD cell growth, migration, and drug sensitivity were validated at the cellular level, highlighting a new candidate marker for LUAD patient treatment.

Materials and methods

Identification of CENPH expression in LUAD

The TPM type data were obtained for 59 normal tissues and 535 cancer tissues from the Cancer Genome Atlas (TCGA) database, and were obtained for 288 normal lung tissues from the Genotype-Tissue Expression (GTEx) database. The data from TCGA database were matched using expression analysis. The expression levels of CENPH in unpaired and 57 paired tissues of LUAD patients were determined. In 535 LUAD tissues, the expression levels of CENPH were analyzed after grouping for T stage, N stage, M stage, pathological stage, sex, age, overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI).

CENPH diagnostic and prognostic values

CENPH expression data from the TCGA and GTEx databases were utilized and the diagnostic values of CENPH in normal and cancer tissues were experimentally verified by receiver operating characteristic (ROC) analysis. In addition, the median value of CENPH was divided into high and low CENPH groups to obtain OS, DSS and PFI via Kaplan Meier (K-M) survival analysis.

Lung cancer explorer (LCE) database

In the LCE database, an intelligent online tool that covers data from lung cancer studies of multicenter origin, CENPH gene was entered through the meta-analysis interface with the cancer type set as LUAD, and finally, the relationship between CENPH expression and the prognosis was displayed through forest plots.

Construction of a CENPH-related nomograms

The potential relationship between clinical characteristics and prognostic indicators in LUAD patients by univariate Cox method, which was commonly used to explore the relationship between the target factors and prognosis [16, 17]. Subsequently CENPH-related nomograms were constructed.

Construction of CENPH-associated risk models

Long noncoding RNAs (lncRNAs) associated with CENPH were determined by correlation analysis with correlation coefficient of 0.4. The lncRNAs and prognostic factors associated with OS, DSS, and PFI in LUAD patients were determined by LASSO regression analysis, and risk models were constructed by calculating the risk scores of LUAD patients. Finally, the prognosis of high-risk and low-risk LUAD patients was evaluated by K-M survival analysis, and $P<0.05$ was used as the criterion of significant difference.

Relationship between CENPH expression and the immune microenvironment in LUAD

Immune cells and immune score proportion in 535 LUAD tissues were calculated by estimate

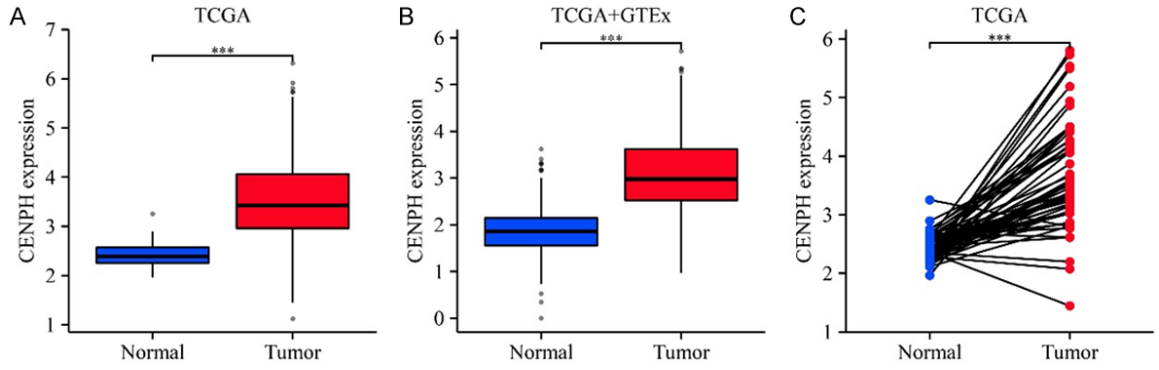

Figure 1. CENPH overexpression in LUAD tissues. (A) Unpaired data from the TCGA database and (B) TCGA and GTEx databases; (C) Paired data from the TCGA database. Note: LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression; ***, P<0.001.

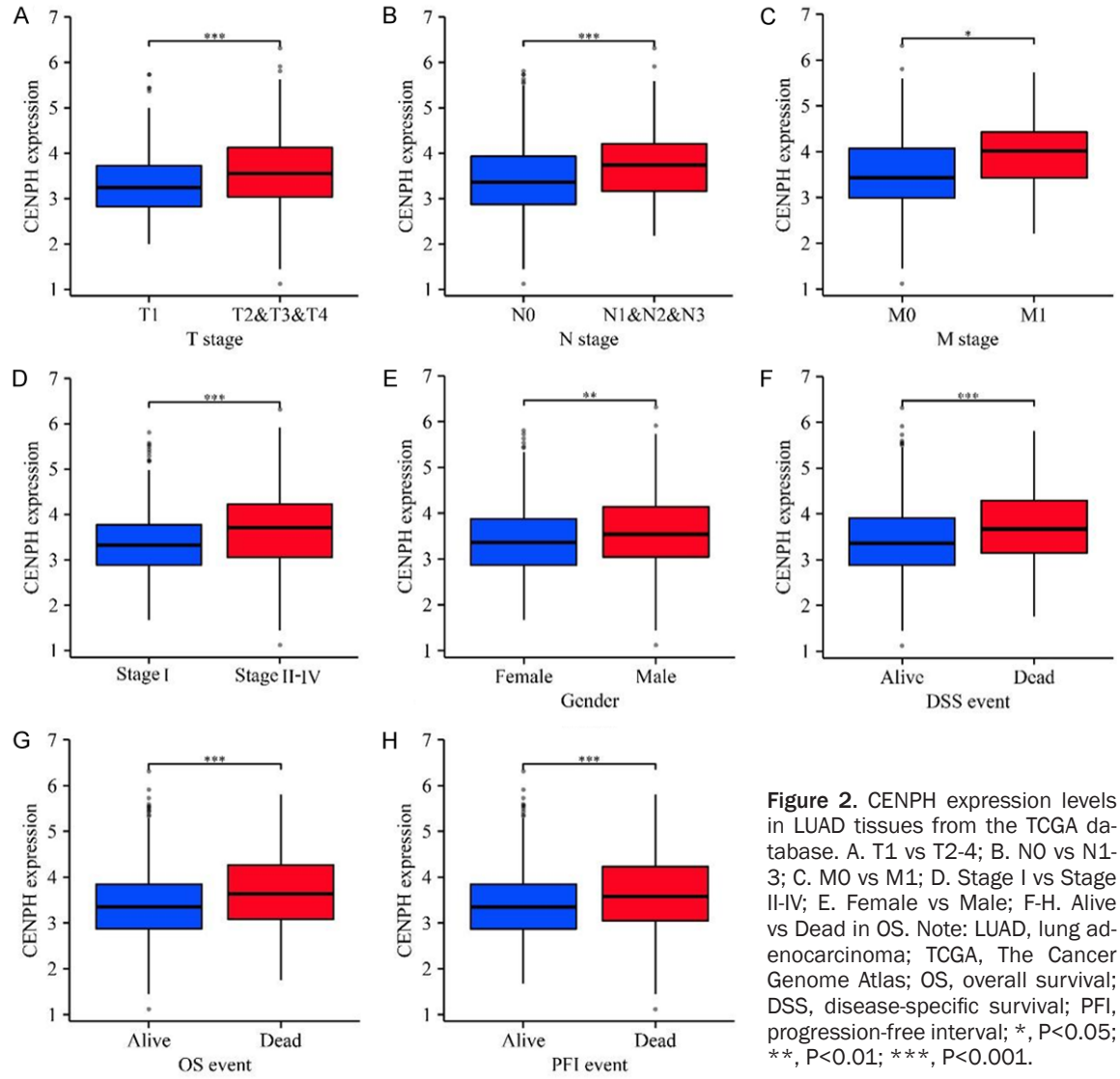

Figure 2. CENPH expression levels in LUAD tissues from the TCGA database. A. T1 vs T2-4; B. N0 vs N1-3; C. M0 vs M1; D. Stage I vs Stage II-IV; E. Female vs Male; F-H. Alive vs Dead in OS. Note: LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval; *, P<0.05; **, P<0.01; ***, P<0.001.

and ssGSEA methods, and the relationship of CENPH expression with immune scores and immune cells was studied. In addition, the expression levels of immune cell marker genes

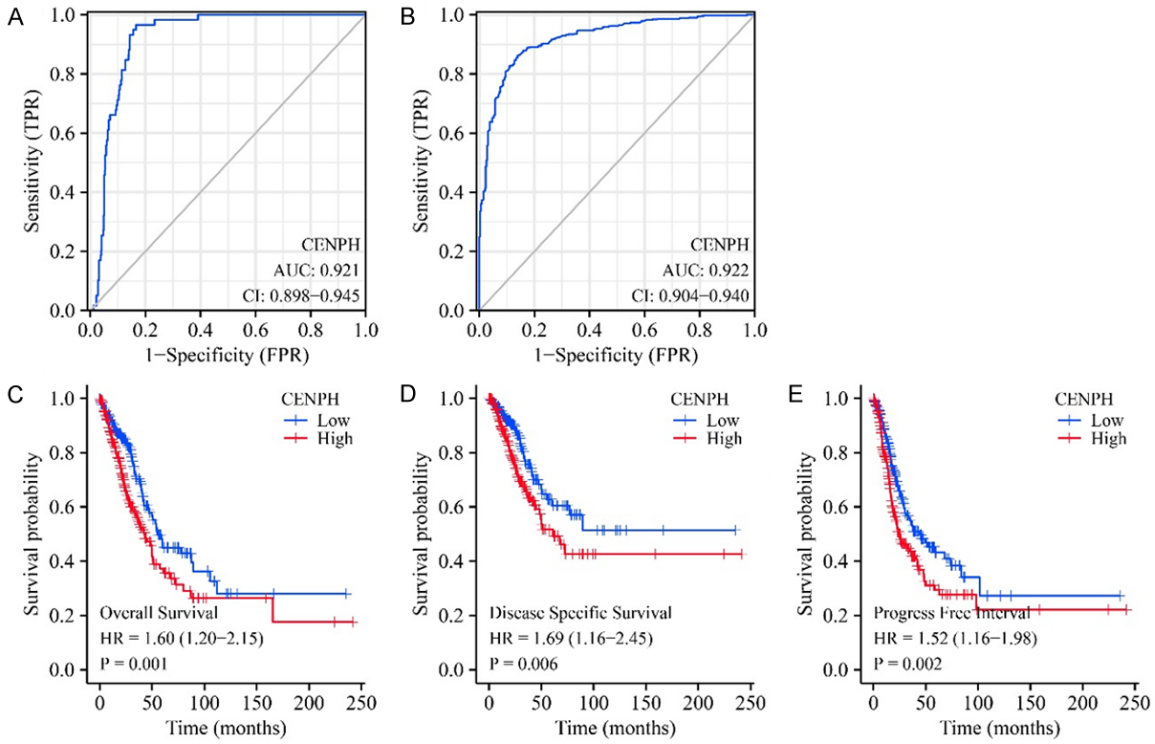

Figure 3. CENPH overexpression was associated with LUAD diagnosis and poor prognosis. (A) The diagnostic values of data from the TCGA database and (B) from the TCGA and GTEx databases using ROC analysis; (C-E) The prognostic values of data from the TCGA database using survival analysis. Note: LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression; ROC, receiver operating characteristic.

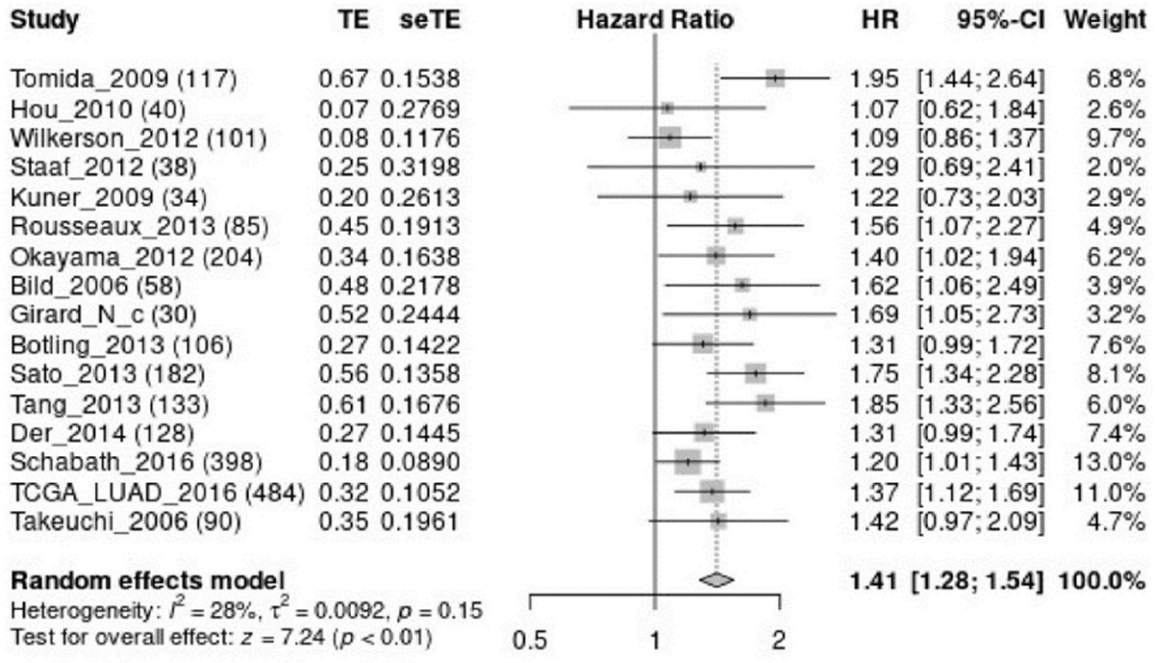

Figure 4. CENPH overexpression was associated with poor prognosis in LUAD patients using meta-analysis in LCE database. Note: LUAD, lung adenocarcinoma; LCE, lung cancer explorer.

Table 1. Analysis of risk factors for poor OS in LUAD patients using the Cox method

Characteristics	Total (N)	HR (95% CI)	*P* value
T stage	523		
T1	175	Reference	
T2	282	1.521 (1.068-2.166)	0.020
T3	47	2.937 (1.746-4.941)	<0.001
T4	19	3.326 (1.751-6.316)	<0.001
N stage	510		
N0	343	Reference	
N1	94	2.381 (1.695-3.346)	<0.001
N2	71	3.108 (2.136-4.521)	<0.001
N3	2	0.000 (0.000-Inf)	0.994
M stage	377		
M0	352	Reference	
M1	25	2.136 (1.248-3.653)	0.006
Gender	526		
Female	280	Reference	
Male	246	1.070 (0.803-1.426)	0.642
Age	516		
≤65	255	Reference	
>65	261	1.223 (0.916-1.635)	0.172
CENPH	526		
Low	263	Reference	
High	263	1.604 (1.199-2.146)	0.001

Note: LUAD, lung adenocarcinoma; OS, overall survival.

and CENPH were determined by Perl language, and the correlation between them was subsequently investigated by a correlation study.

Relationship between CENPH and RNA modifications

The genes affected by RNA modifications such as m6A, m5C, and m1A were accessed, and associated data were surveyed [18, 19]. Correlation studies were performed to investigate the possible relationship between CENPH expression and RNA modifications.

Construction of LUAD cell models with decreased CENPH expression

A549 and A549/DDP cells were grown in RPMI-1640 medium with 10% fetal bovine serum. Appropriate concentration of cisplatin was added to maintain the drug resistance of cells. At good confluency, the A549 and A549/DDP cells were counted and plated in 6-well plates, followed by the addition of siRNA solution according to standard procedures [20]. The A549 and A549/DDP cells were collected at 24 h of transfection, and the expression of CENPH was detected by western blotting. The siRNA interference sequences of CENPH were 5'-GUGUUGCUGAUGUAACAUTT-3', 5'-CUGCUUGAUAUUAGAAAGATT-3', and 5'-3'CAGAGAGGAUAAAGAUCAUTT-3'.

Western blotting

The protein concentrations of the control group (si-NC) and suppressed CENPH expression group (si-CENPH) were determined by using a BCA kit. Electrophoresis, electroblotting onto polyvinylidene fluoride (PVDF) membranes, and washing of the membranes were performed after protein quantification with 1:5000 CENPH (Proteintech, China), 1:1000 AKT (Wanleibio, China), 1:1000 ERK (Wanleibio, China), 1:1000 P38 (Wanleibio, China), 1:1000 p-AKT (Wanleibio, China), 1:1000 p-ERK (Wanleibio, China), 1:1000 p-P38 (Wanleibio, China), and 1:50000 β-actin (Proteintech, China). Protein exposure was performed after secondary antibody (Bioss, China) incubation and washing of the membrane, and CENPH protein expression levels in the si-NC and si-CENPH groups were determined [21, 22]. The experiments were repeated thrice.

CCK-8 assay

A549 and A549/DDP cell concentrations were determined by cell counting, followed by seeding in a 96-well plate at a density of 3000 cells/well. CCK-8 solution (10 μL) was added at 0, 1, 2, and 3 days after cell attachment, and the plates were incubated at 37°C to detect the cell activity of control group and intervention group by using microplate reader. In addition, a proportional dilution of cisplatin was added to a 96-well plate, and cell activity was detected by a microplate reader after incubation with 10 μL CCK-8 solution. The experiments were repeated thrice.

Wound healing test

After confluency was attained, cells were seeded in 6-well plates. A straight line was scratched at the bottom of the plate using a 200 μL sterile pipette tip when the cell density reached 90%, and floating dead A549 and A549/DDP

Table 2. Analysis of risk factors for poor DSS in LUAD patients using the Cox method

Characteristics	Total (N)	HR (95% CI)	*P* value
T stage	488		
T1	168	Reference	
T2	262	1.701 (1.085-2.668)	0.021
T3	43	2.846 (1.453-5.572)	0.002
T4	15	2.770 (1.061-7.230)	0.037
N stage	475		
N0	327	Reference	
N1	83	2.751 (1.808-4.185)	<0.001
N2	63	2.762 (1.698-4.493)	<0.001
N3	2	0.000 (0.000-Inf)	0.995
M stage	344		
M0	323	Reference	
M1	21	2.455 (1.269-4.749)	0.008
Gender	491		
Female	262	Reference	
Male	229	0.989 (0.687-1.424)	0.954
Age	481		
≤65	243	Reference	
>65	238	1.013 (0.701-1.464)	0.944
CENPH	491		
Low	247	Reference	
High	244	1.689 (1.165-2.448)	0.006

Note: LUAD, lung adenocarcinoma; DSS, disease specific survival.

Table 3. Analysis of risk factors for poor PFI in LUAD patients using the Cox method

Characteristics	Total (N)	HR (95% CI)	*P* value
T stage	523		
T1	175	Reference	
T2	282	1.758 (1.276-2.422)	<0.001
T3	47	3.495 (2.199-5.556)	<0.001
T4	19	1.113 (0.444-2.791)	0.819
N stage	510		
N0	343	Reference	
N1	94	1.540 (1.118-2.122)	0.008
N2	71	1.498 (1.018-2.205)	0.040
N3	2	0.906 (0.127-6.485)	0.922
M stage	377		
M0	352	Reference	
M1	25	1.513 (0.855-2.676)	0.155
Gender	526		
Female	280	Reference	
Male	246	1.172 (0.901-1.526)	0.236
Age	516		
≤65	255	Reference	
>65	261	1.023 (0.784-1.335)	0.867
CENPH	526		
Low	263	Reference	
High	263	1.516 (1.163-1.977)	0.002

Note: LUAD, lung adenocarcinoma; PFI, progression-free interval.

cells were washed using phosphate buffered saline (PBS). The migration distances of LUAD cells in the control and intervention groups were measured after changing RPMI-1640 medium without serum, and the percentage migration was calculated. The experiments were repeated thrice.

Transwell assay

Transwell assay could be used to detect the migration and invasion ability of cancer cells [20]. After achieving a good mixing concentration, A549 and A549/DDP cells were plated in 24-well plates. Transwell chambers were pre-cooled. The Matrigel diluted with serum-free medium was added to the upper chamber, and the plate was placed in the incubator at 37°C. The cells were diluted in serum-free media and 200 μL was added to the upper chamber of the Transwell chamber. The lower chamber was supplemented with 600 mL of culture medium containing 10% serum, which was placed at 37°C for incubation. Staining was performed using 4% paraformaldehyde fixation for 15 min and 0.2% crystal violet dye for 20 min after washing the upper and lower chambers. Photographs were taken after the plates were cleaned. The experiments were repeated thrice.

Statistical analysis

The Wilcoxon rank sum test was applied to identify CENPH expression levels in LUAD tissues. ROC, K-M survival, and meta-analyses were used to identify the diagnostic and prognostic values of CENPH. Correlation analysis was performed to understand the association between CENPH expression levels and the immune microenvironment and RNA modifications. The t-test was performed to determine whether LUAD cell growth, migration, and drug sensitivity were statistically significant when

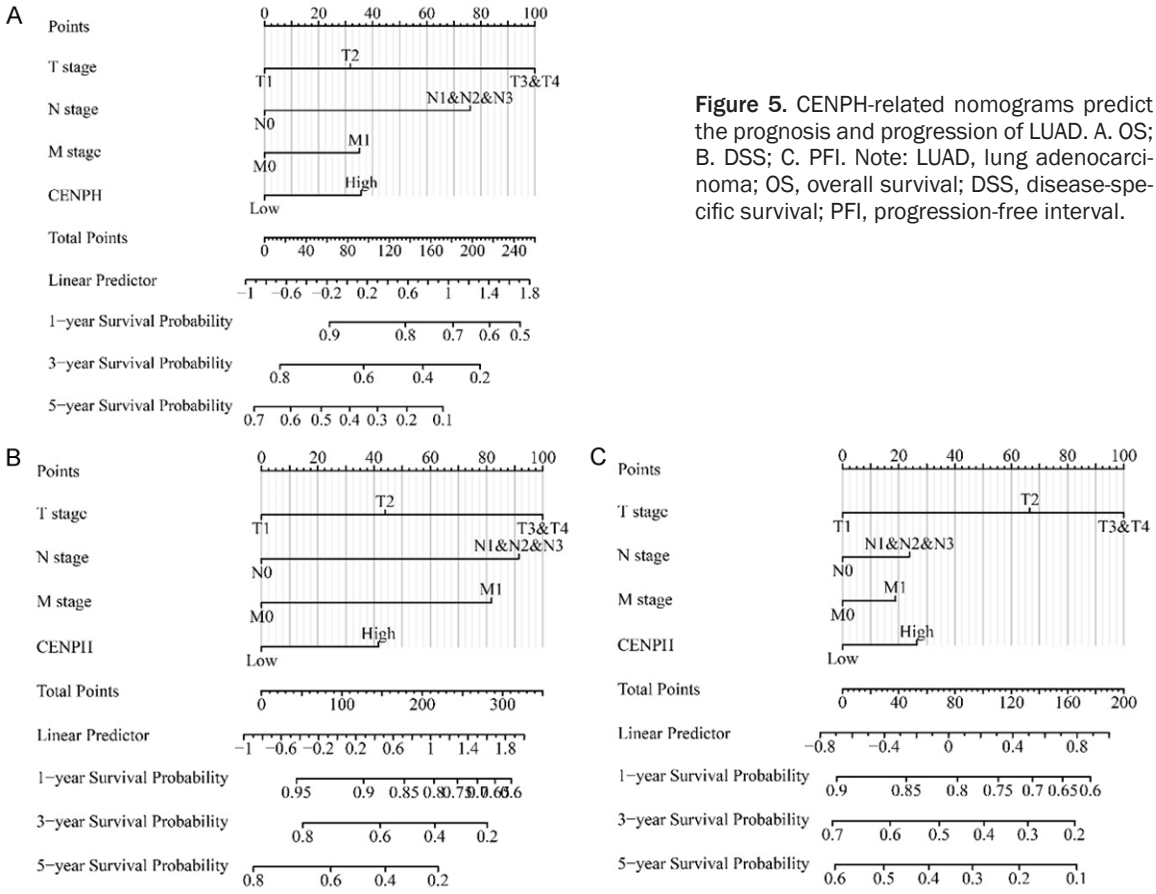

Figure 5. CENPH-related nomograms predict the prognosis and progression of LUAD. A. OS; B. DSS; C. PFI. Note: LUAD, lung adenocarcinoma; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval.

CENPH expression was inhibited. P<0.05 was considered statistically significant.

Results

CENPH was overexpressed in LUAD

CENPH expression levels were significantly higher in unpaired LUAD tissues than in normal lung tissues (**Figure 1A** and **1B**) and were also higher in paired LUAD tissues (**Figure 1C**). In addition, CENPH expression levels were significantly higher in tissues from patients with stage T2-4 LUAD (vs T1 LUAD; **Figure 2A**), lymph node positive cancer patient tissues (vs lymph node negative cancer patient tissues; **Figure 2B**), tissues of LUAD patients with M1 stage (vs LUAD patients with M0 stage; **Figure 2C**), stage II-IV cancer patient tissues (vs stage I cancer patient tissues; **Figure 2D**), male LUAD patient tissues (vs female LUAD patient tissues; **Figure 2E**), and tissues from dead LUAD patients (vs tissues from surviving LUAD patients; **Figure 2F-H**).

Elevated CENPH expression levels have diagnostic and prognostic values in LUAD

The TPM type data from TCGA database and from GTEx database showed that the area under the curve (AUC) of CENPH in normal and cancerous tissues was 0.921 (**Figure 3A**) and 0.922 (**Figure 3B**), respectively, indicating that CENPH overexpression had diagnostic values. K-M survival analysis showed that LUAD patients with strong expression of CENPH had poor OS, DSS, and PFI (**Figure 3C-E**). Furthermore, meta-analysis revealed that increased CENPH expression levels were significantly associated with poor prognosis (**Figure 4**).

CENPH overexpression was a risk factor for poor prognosis in LUAD patients

Cox regression analysis revealed tumor size, lymph node metastasis, distant metastasis, and CENPH expression to be the risk factors for poor prognosis of LUAD (**Tables 1-3**). It was well known that tumor size, lymph node metastasis and distant metastasis were important factors

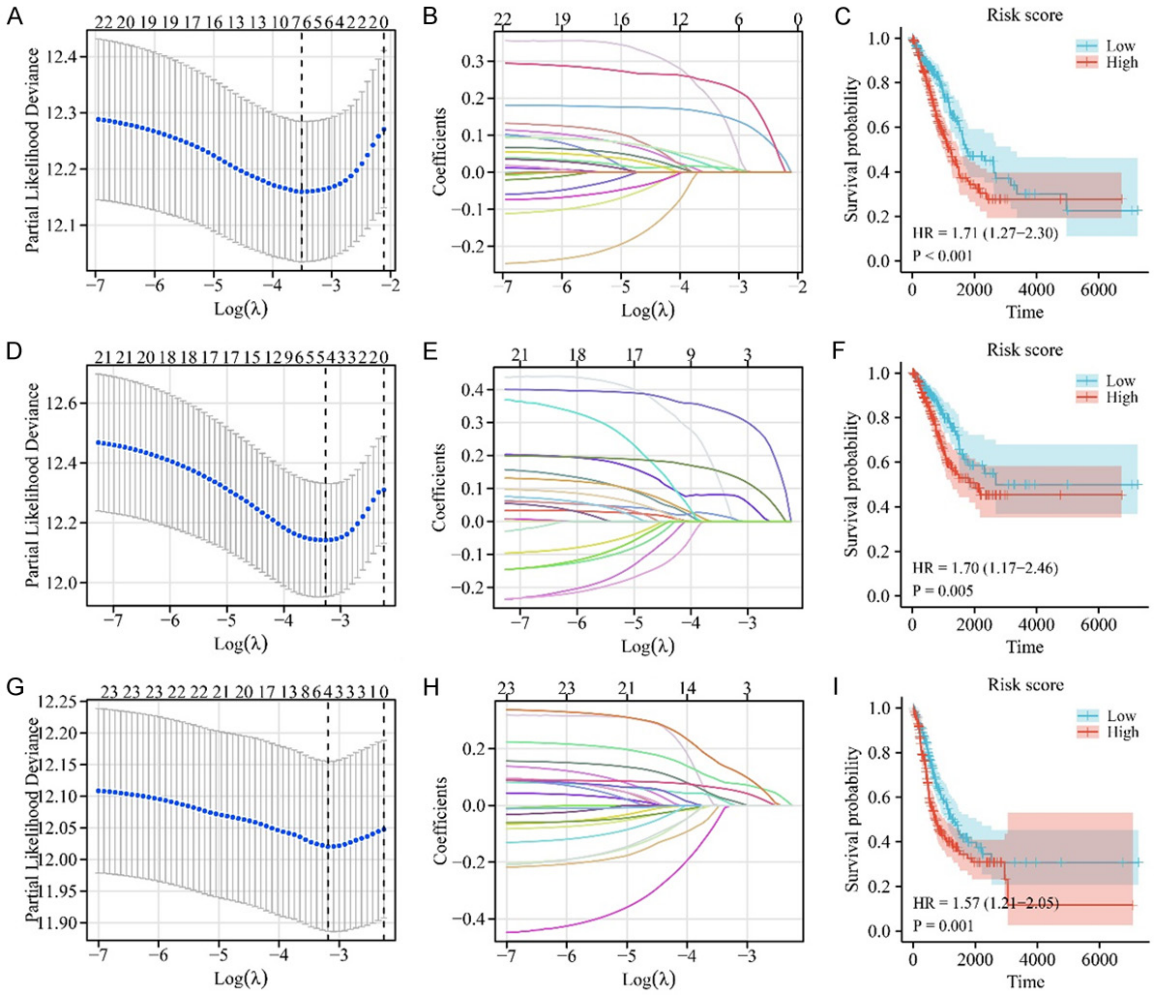

Figure 6. The high-risk model of CENPH-related lncRNAs predicts the poor prognosis of LUAD via LASSO method. (A-C) OS; (D-F) DSS; (G-I) PFI. Note: LUAD, lung adenocarcinoma; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval.

for evaluating the poor prognosis of LUAD patients. Therefore, we showed the predictive relationships of tumor size, lymph node metastasis, distant metastasis, and CENPH expression with 1-, 3- and 5-year OS, DSS, and PFI of LUAD patients by using nomograms (**Figure 5**).

Construction of risk models for CENPH-associated lncRNAs

A total of 23 CENPH-associated lncRNAs (AL138724.1, DEPDC1-AS1, TMPO-AS1, AP000251.1, AL133215.2, AL138789.1, MIR924HG, DDX11-AS1, AC002116.2, AC092718.4, AC099850.3, TYMSOS, AC012073.1, RNASEH1-AS1, AC026401.3, AC091057.1, ALMS1IT1, LINC01775, SNHG1, SFTA1P, LINC01116, GASAL1, and LINC02709) were acquired ($P<0.05$). LASSO regression analysis showed that the risk model based on DEPDC1-AS1, AL138789.1, AC092718.4, AC099850.3, LINC01116, and GASAL1 was significantly associated with the OS of LUAD patients (**Figure 6A-C**). The risk model based on TMPO-AS1, AC099850.3, LINC01116 and GASAL1 was significantly associated with the DSS in LUAD patients (**Figure 6D-F**). The risk model based on AC026401.3, AC099850.3, LINC01116, and GASAL1 was significantly associated with the PFI in LUAD patients (**Figure 6G-I**). Cox method showed that CENPH and these lncRNAs were associated with patient prognosis with. Therefore, the risk model based on DEPDC1-AS1, AL138789.1, AC092718.4, CENPH, LINC01116, and GASAL1 was significantly associated with the OS of LUAD patients (**Figure 7A-C**). The risk model based on AC099850.3, LINC01116, GASAL1, and CENPH was significantly associated with PFI in LUAD patients (**Figure 7D-F**).

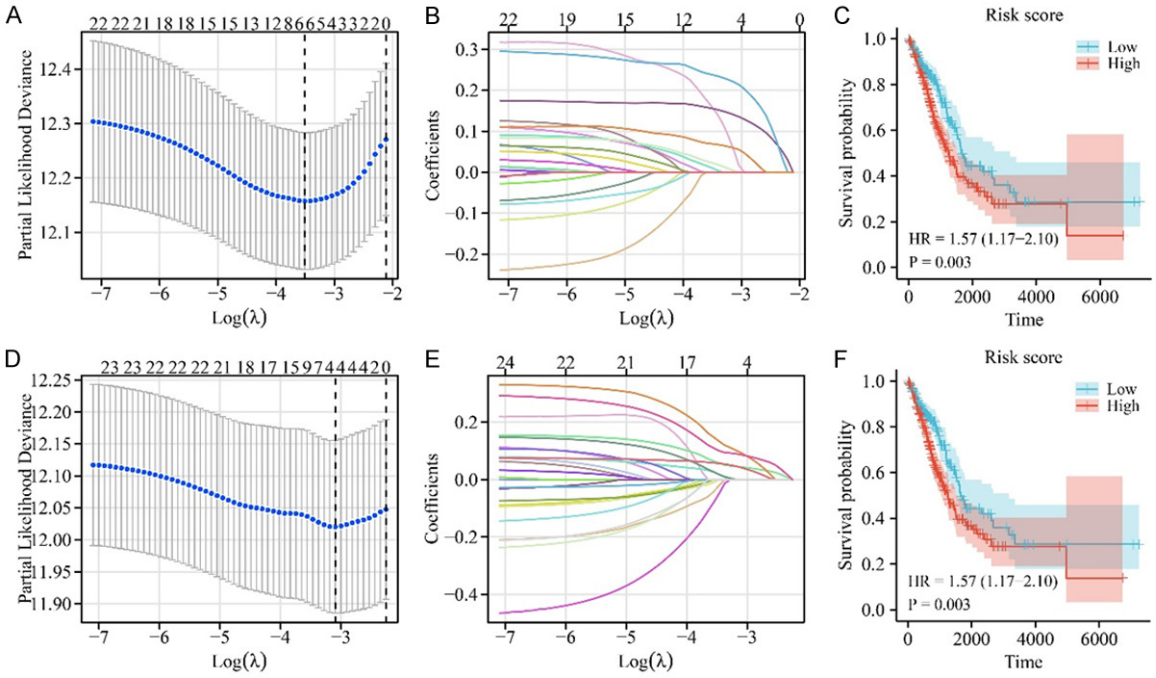

Figure 7. The high-risk model of CENPH predicts the poor prognosis of LUAD via LASSO method. (A-C) OS; (D-F) PFI. Note: LUAD, lung adenocarcinoma; OS, overall survival; PFI, progression-free interval.

Decreased CENPH expression inhibited A549 cell progression and promoted cell sensitivity to cisplatin via the AKT and ERK/P38 pathways

A549 cell model with suppressed CENPH expression level was studied using western blotting technique (**Figure 8A**, **8B**). CCK-8 assay revealed that inhibition of CENPH expression significantly inhibited A549 cell proliferation, migration, and invasion (**Figure 8C-G**). Similarly, suppression of CENPH expression inhibited A549/DDP cell proliferation, migration and invasion (**Figure 9A**, **9B**). The stability of A549/DDP cells with suppressed CENPH expression decreased significantly with increasing cisplatin concentration and was worse than that of control A549/DDP cells (**Figure 9C-H**). In addition, inhibition of the expression of CENPH downregulated p-AKT, p-ERK, and p-P38 proteins, but had no effect on AKT, ERK, and P38 proteins (**Figure 10**).

CENPH overexpression was associated with RNA modifications

CENPH overexpression was related to the RNA modification regulatory genes ALYREF (r=0.604), HNRNPC (r=0.526), YBX1 (r=0.499), TRMT10C (r=0.497), TRMT6 (r=0.492), LRPPRC (r=0.487), RBMX (r=0.483), HNRNPA2B1 (r=0.470), DNMT1 (r=0.460), DNMT3B (r=0.444), IGF2BP3 (r=0.440), NOP2 (r=0.437), NSUN2 (r=0.432), TRMT61B (r=0.407), NSUN5 (r=0.356), DNMT3A (r=0.348), WTAP (r=0.347), IGF2BP2 (r=0.340), IGF2BP1 (r=0.303), YTHDF1 (r=0.265), RBM15B (r=0.264), ALKBH1 (r=0.260), RBM15 (r=0.229), FMR1 (r=0.217), YTHDF2 (r=0.215), TRMT61A (r=0.204), YTHDF3 (r=0.176), YTHDC1 (r=0.173), ALKBH5 (r=0.167), ZC3H13 (r=0.149), METTL14 (r=0.139), NSUN3 (r=0.126), BMT2 (r=0.124), METTL3 (r=0.106) and NSUN4 (r=0.095) (**Figure 11**).

CENPH expression was associated with the LUAD immune microenvironment

CENPH overexpression was significantly correlated with stromal score (r=-0.184), immune score (r=-0.134), and estimate score (r=-0.170) (**Figure 12A-C**), and with Th2 cells (r=0.650), mast cells (r=-0.362), eosinophils (r=-0.251), iDC (r=-0.242), Th17 cells (r=-0.23), TFH (r=-0.189), DC (r=-0.166), Tgd (r=0.161), pDC (r=-0.159), T helper cells (r=0.153), B cells (r=-0.147), NK CD56dim cells (r=0.143), CD8 T cells (r=-0.128), macrophages (r=-0.124), aDC

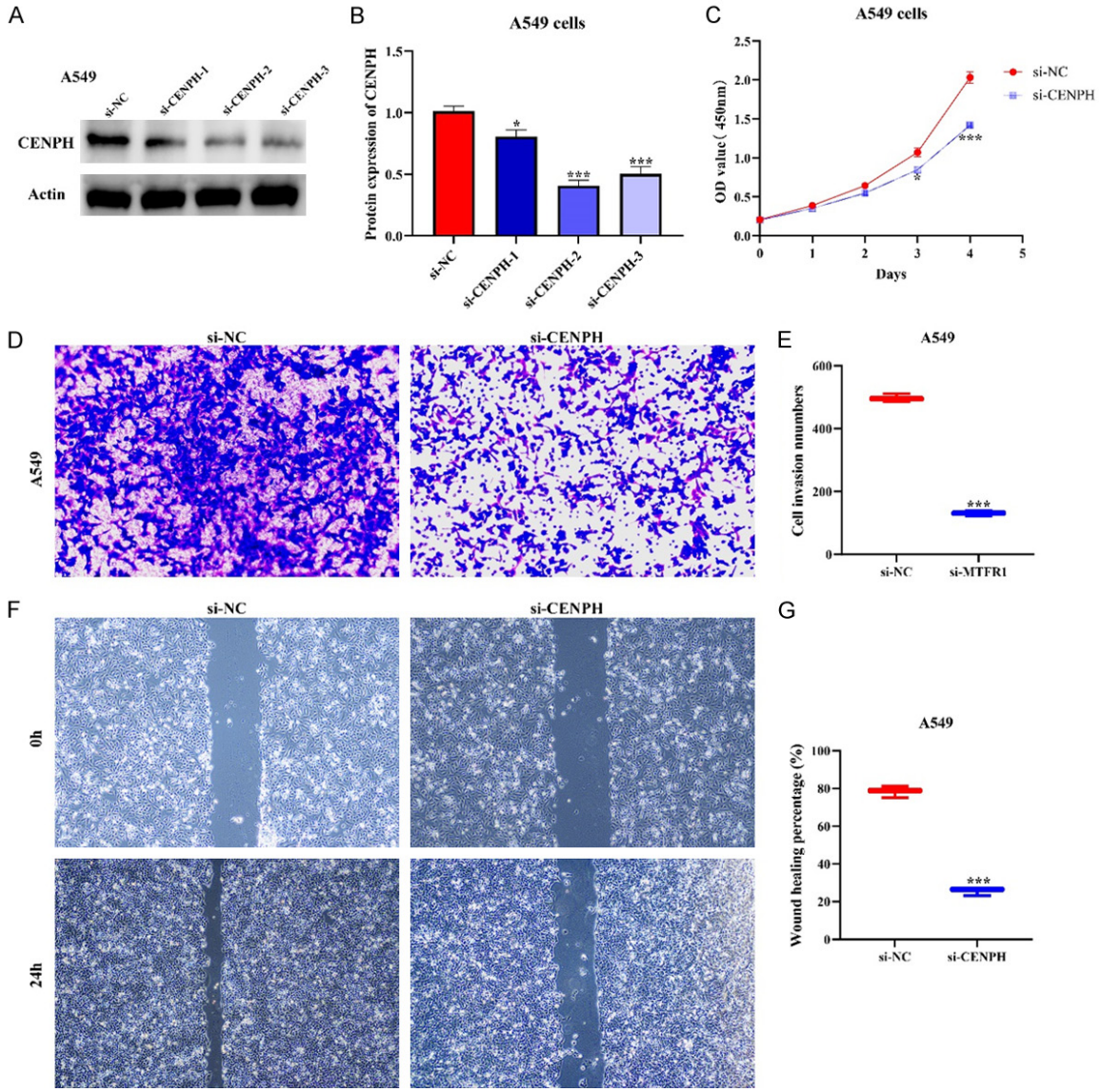

Figure 8. Inhibition of CENPH expression inhibits the proliferation, invasion, and migration of A549 cells. A, B. Construction of A549 cell model; C. Cell proliferation analysis using CCK-8; D, E. Cell invasion analysis using Transwell; F, G. Cell migration analysis using wound healing test. Note: *, P<0.05; ***, P<0.001.

(r=0.124), NK CD56bright cells (r=-0.118), TReg (r=0.117), Tem (r=-0.107), and neutrophils (r=-0.101) (**Figure 13**). In addition, CENPH overexpression was correlated with LUAD immune cell markers, such as STAT1, GZMB, CD1C, KIR2DL4, LAG3, IFNG, CEACAM8, STAT6, HLA-DPB1, CD8B, GATA3, and PDCD1 (**Table 4**).

Discussion

CENPH expression was associated with the progression of several cancers [7-15]. CENPH was overexpressed in tongue carcinoma and was correlated with clinical stage, T stage, and poor prognosis. Inhibition of CENPH expression inhibited tongue cancer cell proliferation via downregulation of Survivin [9]. Similarly, suppression of CENPH expression inhibited cell proliferation and colony forming ability and promoted cell apoptosis in hepatocellular carcinoma; this was associated with increased Bax expression and decreased Bcl-2 expression [12]. In addition, CENPH overexpression was correlated with tumor size, lymph node metastasis, depth of invasion, distant metastasis, and cancer stage, and was an independent

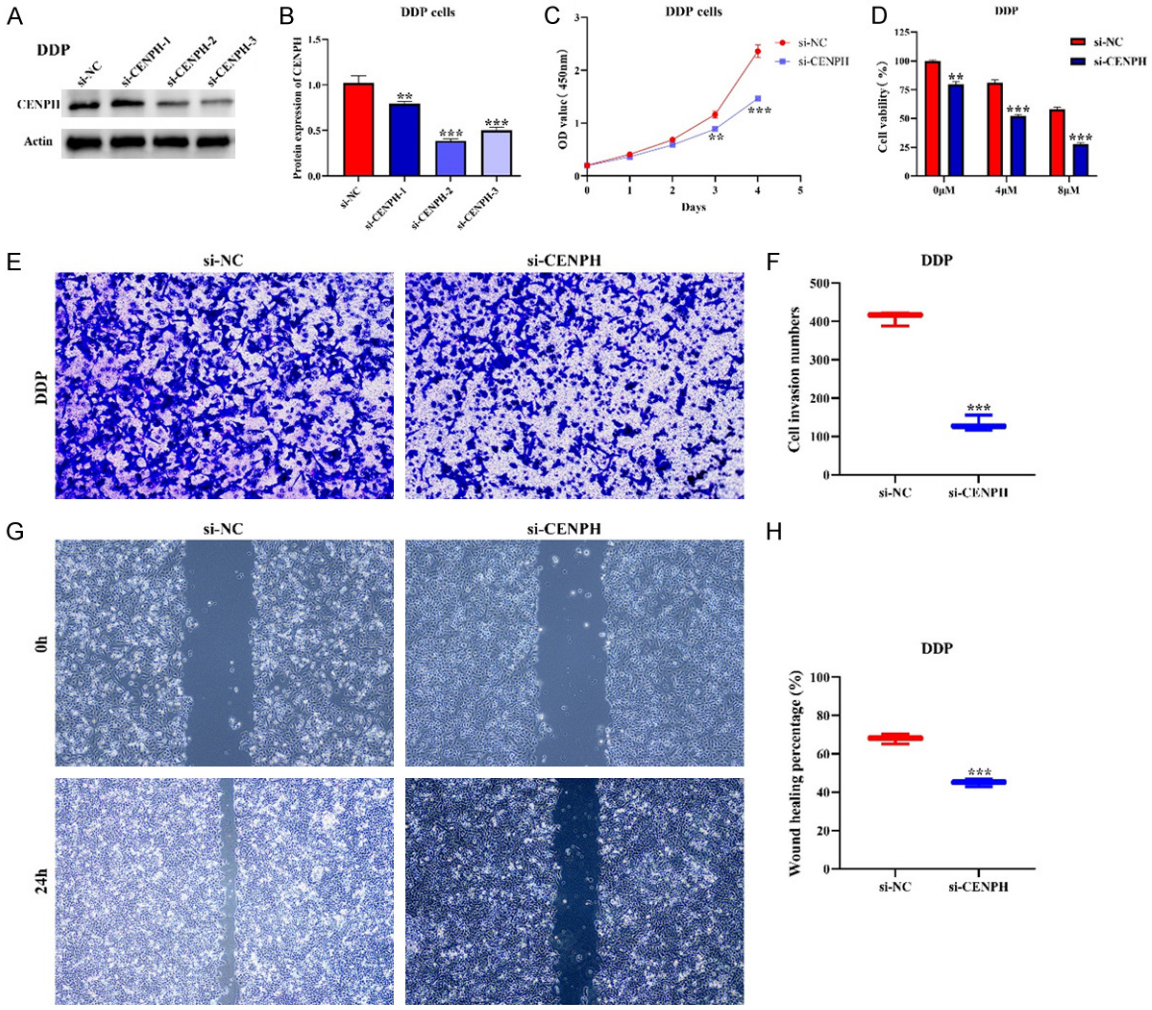

Figure 9. Inhibition of CENPH expression inhibits the proliferation, invasion, and migration of A549/DDP cells and promotes their sensitivity to cisplatin. A, B. Construction of A549/DDP cell model; C, D. Cell proliferation and sensitivity analysis using CCK-8; E, F. Cell invasion analysis using Transwell; G, H. Cell migration analysis using wound healing test. Note: **, P<0.01; ***, P<0.001.

prognostic predictor of survival in gastric cancer (GC). Inhibition of CENPH expression inhibited GC cell growth [13]. However, the roles and mechanisms of CENPH in LUAD and its relationship with drug sensitivity were not elucidated. In the present study, CENPH was found to be significantly upregulated in unpaired and paired LUAD tissues. CENPH overexpression correlated with T stage, lymph node metastasis, distant metastasis, TNM stage, sex, diagnosis, and poor prognosis. In addition, the data from the TCGA and LCE databases showed that CENPH overexpression was related to OS and was a risk factor for poor prognosis. Our results were consistent with previous findings, suggesting that CENPH was an oncogene and a poor prognosis biomarker for LUAD.

LUAD occurrence and development and cisplatin resistance were correlated with molecular expression changes [9, 20, 23, 24]. The expression level of WDHD1 in A549/DDP cells was higher than that in A549 cells. Downregulation of WDHD1 expression increased the sensitivity of A549/DPP cells to cisplatin. WDHD1 overexpression showed a negative correlation with the OS of LUAD patients. Ubiquitin ligase WDHD1 induced resistance to cisplatin by promoting MAPRE2 expression [23]. miR-612 was significantly under-expressed in endometrial cancer tissues and could act as a tumor suppressor to inhibit cell proliferation, migration, and invasion, and promote apoptosis. CENPH was overexpressed in endometrial cancer tissues and was associated with its diagnosis and

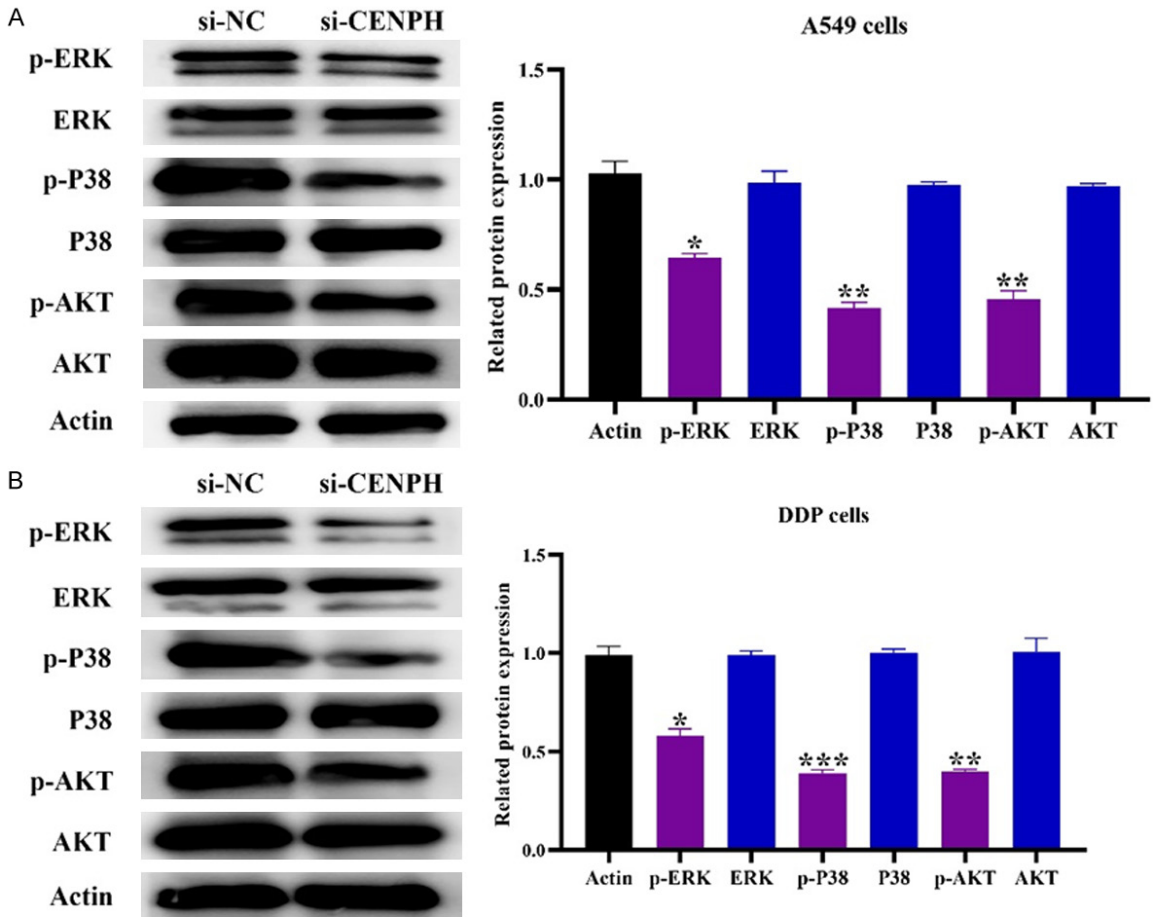

Figure 10. Signaling mechanisms involved in inhibiting the expression of CENPH in LUAD cells. Note: LUAD, lung adenocarcinoma; *, P<0.05; **, P<0.01; ***, P<0.001.

prognosis. It enhanced cancer cell proliferation, migration and invasion, inhibited cancer cell apoptosis, and prevented the antitumor effect of miR-612. The antitumor effect of downregulation of PVT1 was also inhibited by downregulation of miR-612. Inhibition of PVT1 and upregulation of miR-612 had potent antitumor effects in nude mice tumorigenesis assays and were associated with the CDK1/AKT/mTOR signaling pathway [9]. Inhibition of CENPH expression in A549 and A549/DDP cells inhibited proliferation, migration and invasion, and promoted sensitivity to cisplatin. In addition, CENPH knockdown induced downregulation of p-AKT, p-ERK, and p-P38 proteins, but had no effect on AKT, ERK, and P38 proteins in A549 and A549/DDP cells. Therefore, CENPH has an important biological role in LUAD progression.

Immune microenvironment and RNA modifications were found to have important biological roles and clinical values in LUAD progression [25-27]. PD-L1 expression was negatively correlated with lymph node metastasis. PD-1-negative effector T lymphocytes had a positive effect on survival and immunotherapy response in resected cancer patients with advanced NSCLC [25]. The expression levels of KIAA1429 significantly increased in LUAD cells and tissues, which correlated with tumor diameter, lymph node metastasis, distant metastasis, and poor prognosis. Downregulation of KIAA1429 significantly abrogated LUAD cell proliferation, migration, invasion, and cell cycle arrest and reversed the effects of decreased MUC3A expression by modifying m6A regulation [27]. CENPH expression levels were significantly correlated with immune microenvironment, immune cells, and cell markers. CENPH expression levels were significantly correlated with the levels of the genes affected by RNA modifications, ALYREF, HNRNPC, and YBX1. However, this requires further investigation.

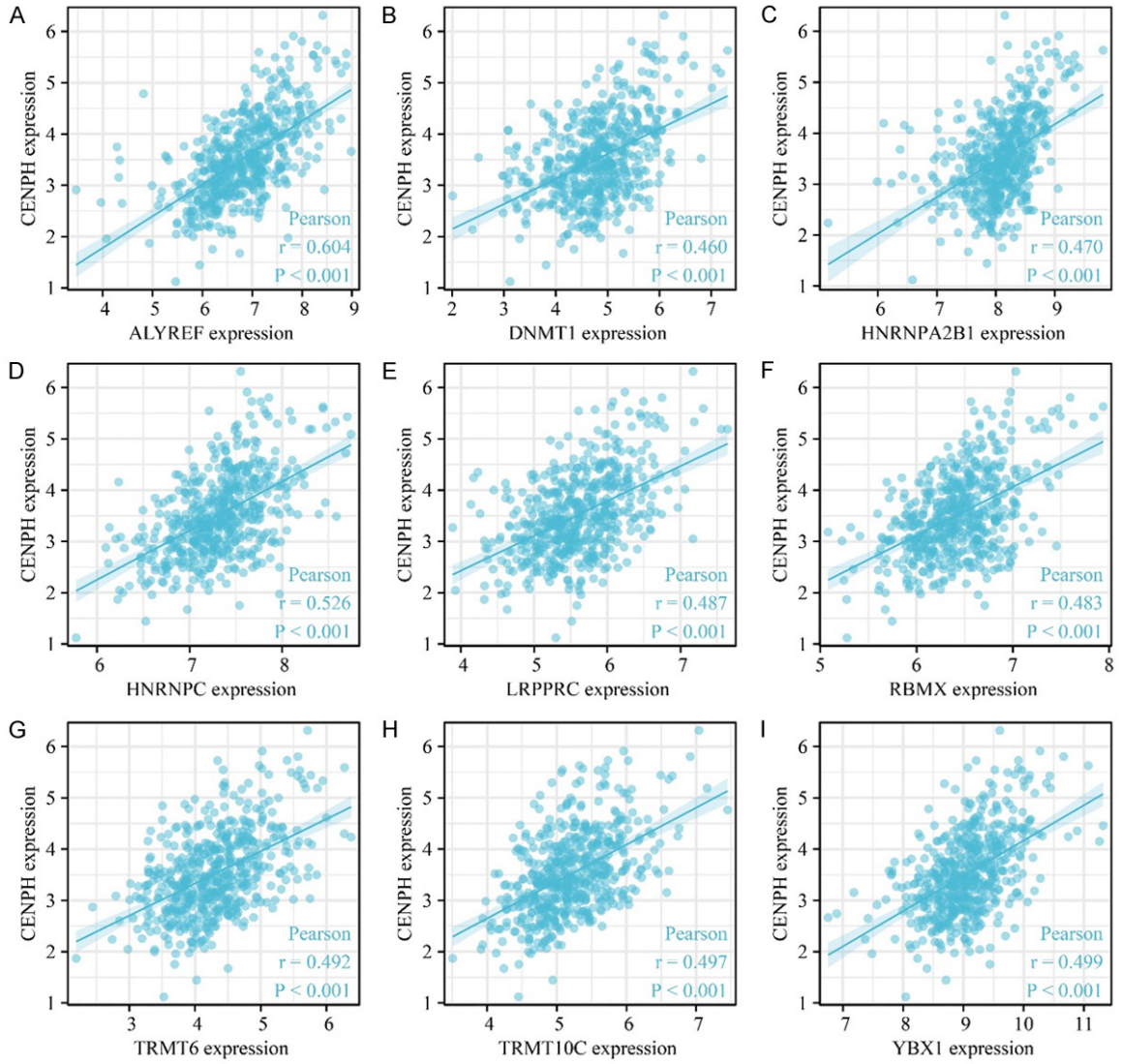

Figure 11. Expression of CENPH is related to the RNA modifications in LUAD. A. ALYREF; B. DNMT1; C. HNRNPA2B1; D. HNRNPC; E. LRPPRC; F. RBMX; G. TRMT6; H. TRMT10C; I. YBX1. Note: LUAD, lung adenocarcinoma.

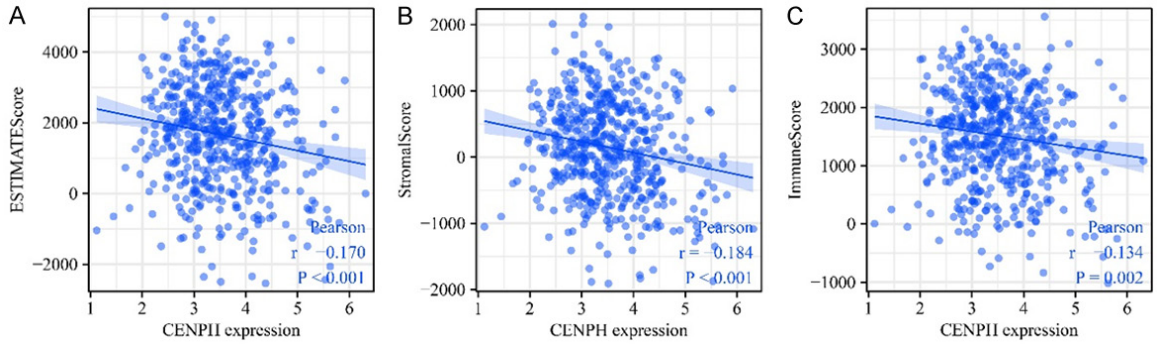

Figure 12. Expression of CENPH is related to LUAD immune microenvironment scores. A. ESTIMATE score; B. Stromal score; C. Immune score. Note: LUAD, lung adenocarcinoma.

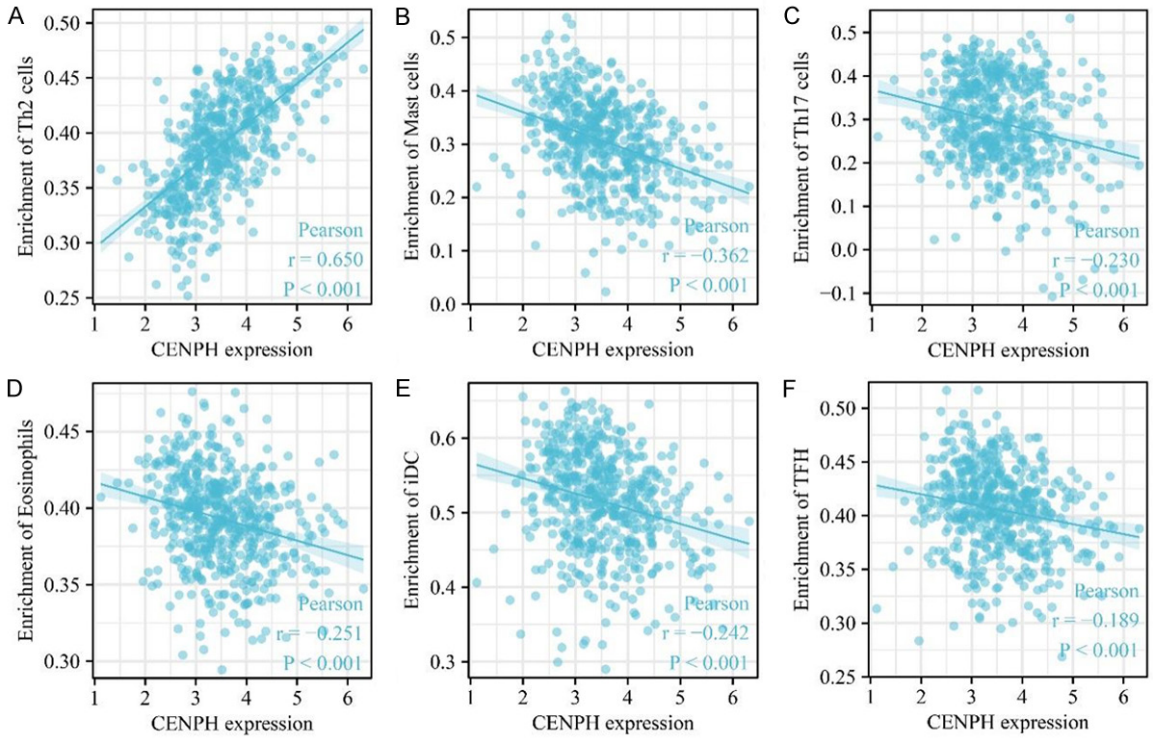

Figure 13. Expression of CENPH is related to LUAD immune cells. A. Th2 cells; B. Mast cells; C. Th17 cells; D. Eosinophils; E. iDC; F. TFH. Note: LUAD, lung adenocarcinoma.

Table 4. Relationship between expression of CENPH and immune cell markers in LUAD

Gene	Cor	*P* value	Gene	Cor	*P* value
STAT1	0.357	<0.001	CD8A	0.137	0.002
GZMB	0.323	<0.001	FOXP3	0.136	0.002
CD1C	-0.291	<0.001	KIR2DL3	0.135	0.002
KIR2DL4	0.281	<0.001	KIR2DS4	0.133	0.002
LAG3	0.224	<0.001	CCR7	-0.129	0.003
IFNG	0.219	<0.001	CD19	-0.114	0.008
CEACAM8	-0.210	<0.001	BCL6	-0.114	0.008
STAT6	-0.204	<0.001	IL21	0.114	0.008
HLA-DPB1	-0.184	<0.001	CD79A	-0.112	0.010
CD8B	0.162	<0.001	KIR2DL1	0.110	0.011
GATA3	0.160	<0.001	CTLA4	0.106	0.015
PDCD1	0.155	<0.001	CCR8	0.105	0.015
HLA-DQB1	-0.146	<0.001	KIR3DL2	0.104	0.016
HLA-DPA1	-0.143	<0.001	KIR3DL3	0.086	0.047
HLA-DRA	-0.139	0.001			

Note: LUAD, lung adenocarcinoma.

Bioinformatics analysis could provide the research targets for scientific researchers, and the verification of the roles of research targets through basic research was worth advocating. The roles and the mechanisms involved of CENPH by bioinformatics analysis and basic research were investigated in this study, and the TCGA, GTEx and LCE databases utilized had a large sample size, and reliable data quality. However, this study has some limitations. First,

the CENPH overexpression cells were constructed to verify the roles and mechanisms of CENPH in the progression of LUAD. We should also verify the effects of CENPH on the tumorigenicity of nude mice *in vivo*. Overall, CENPH expression was significantly enhanced in LUAD, which was associated with poor prognosis, immune microenvironment, and RNA modifications. CENPH overexpression was implicated in cancer cell growth metastasis and cisplatin resistance, suggesting its potential as a biomarker for LUAD prognosis.

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Disclosure of conflict of interest

None.

Address correspondence to: Yong-Xiang Song and Cheng Chen, Department of Thoracic Surgery, Affiliated Hospital of Zunyi Medical University, Zunyi 563000, Guizhou, China. E-mail: songtang2004@163.com (YXS); 375647557@qq.com (CC); Qiang Guo, Department of Cardiothoracic Surgery, Taihe Hospital, Hubei University of Medicine, Shiyan 442012, Hubei, China. E-mail: guoqianglidan@163.com

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