European Review for Medical and Pharmacological Sciences 2018; 22: 7768-7777

Centromere protein U promotes cell proliferation, migration and invasion involving Wnt/β-catenin signaling pathway in non-small cell lung cancer

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Abstract. – OBJECTIVE: The purpose of this study was to examine centromere protein U (CENPU) expression in non-small cell lung cancer (NSCLC) and identify the clinical values of CENPU, as well as investigate the potential molecular mechanisms in NSCLC.

PATIENTS AND METHODS: The expression levels and clinical significance of CENPU were systematically evaluated in human protein atlas datasets and TCGA datasets. CENPU protein expression was studied by Western blotting. CENPU mRNA expression was studied by Real Time-Polymerase Chain Reaction (RT-PCR). Proliferation, migration, and invasion capacities of CENPU cells were assessed after silencing CENPU. Apoptosis was determined using flow cytometry. Western blotting was performed to assess the protein expression levels.

RESULTS: We found that the expression of CENPU at mRNA and protein levels was significantly up-regulated in both NSCLC tissues and cell lines. Overexpression of CENPU was significantly associated with poor prognosis of NSCLC patients. Knockdown of CENPU significantly suppressed proliferation, migration, and invasion, and caused apoptosis of NSCLC cells in vitro. In addition, knockdown of CENPU suppressed epithelial-mesenchymal transition (EMT). Furthermore, our results revealed that the abnormal expression of CENPU could influence the Wnt/β-catenin signaling pathway.

CONCLUSIONS: CENPU was highly expressed in NSCLC tissues and its knockdown of CENPU strongly suppressed NSCLC cell proliferation and metastasis through modulating Wnt/β-catenin signaling. Targeting CENPU could be a promising therapeutic strategy for patients with CENPU.

Key Words:
CENPU, NSCLC, Wnt/β-catenin pathway, Proliferation, Metastasis.

Introduction

Lung cancer remains the most prevalent and lethal cancer among males and has been the leading cause of cancer death among females in more developed countries[1,2]. Non-small cell lung cancer (NSCLC) accounts for 75-80% of all lung cancer[3]. The 5-year survival rate after diagnosis is 15.6%, which is lower than the survival rate for breast, colon, or prostate cancer[4,5]. Despite recent advances in the diagnosis and management of NSCLC, the overall 5-year survival rate for lung cancer is only 17.1%, and it declines to 3.6% for those with stage IV disease[6,7]. Lack of adequate tumor biomarkers for early diagnosis and metastasis identification remains one of the main reasons for such a high mortality[8]. Thus, novel findings on prognosis biomarkers that are correlated with NSCLC development and metastasis would be of great clinical relevance.

CENPU (centromere protein U, also called PBIP1/KLIP1/CENP-50/MLF1IP) gene encodes a 47-kDa protein and maps to chromosome 4q35.1. It has been confirmed[9,10] that CENPU is required for stable kinetochore-microtubule attachment, proper chromosome segregation, and recovery from spindle damage during mitosis. Recently, mounting evidence[11-13] indicates that CENPU is dysregulated in a variety of cancers, such as ovarian cancer, luminal breast cancer, and prostate cancer, indicating that CENPU may play an important role in progression and development of tumors. Of note, Wang et al[14] reported that CENPU was highly expressed in bladder cancer and its knockdown could suppress bladder cancer cells proliferation via

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modulating HMGB1 signaling pathway. Nevertheless, few investigations were performed on the specific effect of CENPU on tumor genesis and development.

In this study, we firstly reported that CENPU was highly expressed in NSCLC tissues and cells compared with normal lung tissue and lung cells. Then we analyze the clinical significance of CENPU in the TCGA datasets and the Human Protein Atlas, finding that both CENPU proteins and mRNAs were associated with prognosis of NSCLC patients. We also investigated the biological functions of CENPU in NSCLC cell proliferation, apoptosis, and metastasis and found that CENPU promotes NSCLC cell proliferation and metastasis, and suppressed apoptosis through Wnt/β-catenin signaling.

Materials and Methods

Cell Lines and Cell Culture

Human lung adenocarcinoma cell lines (A549, H1299, SPC-A1, and H358) and BEAS-2B (human immortalized normal epithelial cell) were obtained from the Cell Bank of the Chinese Academy of Science (Xuhui, Shanghai, China), cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, Utah, USA), 100 U/ml penicillin (Solarbio, Haidian, Beijing, China), and 100 μg/ml streptomycin (Solarbio, Haidian, Beijing, China). All the cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Cell Transfection

Small interfering RNAs (siRNAs) targeting CENPU (si-CENPU-1 and siCENPU-2) and negative control siRNAs (si-NC) were synthesized by RiboBio Co. (Guandong, Guangzhou, China). Cell transfection was performed using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's manuals. In brief, A549 or H1299 cells were maintained 6-well plates (Corning Incorporated, Corning, NY, USA) at appropriate concentrations (approximately 2×10^5 cells per well in this case). When the cells reached approximately 60-80% of confluence, the cells (per well) were co-transfected with a mixture of 12 μl siRNA duplex (20 μM) and 10 μl Lipofectamine 2000 regents. Subsequently, the plates were shaken gently, and the cells were cultured at 37°C in a 5% CO_2 incubator for 24 h before the experiments were performed.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNAs from lung cancer tissues and cell lines were extracted using TRIzol reagents (Sangong, Xuhui, Shanghai, China) according to manufacturer's protocols. Firstly, the RNA was reverse transcribed into cDNA using Primescript 1st Strand cDNA Synthesis kit (TaKaRa, Otsu, Shiga, Japan). Subsequently, the expression levels of CENPU in lung adenocarcinoma tissues and cell lines were determined by qRT-PCR using the SYBR-Green method (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's protocol and normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The Applied Biosystems 7500 Fluorescent Quantitative PCR system (Applied Biosystems Life Technologies, Foster City, CA, USA) was utilized to run the reactions. All the primers were listed in Table I. The relative mRNA fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method. Each experiment was repeated three times.

Table I. The primer for RT-PCR in this study.

Name		Sequence (5′-3′)
CENPU	Forward	ACCCACCTAGAGCATCAACAA
	Reverse	ACTTCAATCATACGCTGCCTTT
β-catenin	Forward	AAAATGGCAGTGCGTTTAG
	Reverse	TTTGAAGGCAGTCTGTCGTA
cyclin D1	Forward	CGGGATCCCCAGCCATGGAACACCAGC
	Reverse	CGGAATTCGCGCCCTCAGATGTCCACG
c-myc	Forward	TCAAGAGGTGCCACGTCTCC
	Reverse	TCTTGGCAGCAGGATAGTCCTT
GAPDH	Forward	TCACCAGGGCTGCTTTTAAC
	Reverse	GACAAGCTTCCCGTTCTCAG

Western Blot Analysis

At the designated time point, the cells were harvested and subjected for total protein extraction. The cells were lysed in ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (Solarbio, Haidian, Beijing, China) containing protease inhibitor cocktail (Roche, Pudong, Shanghai). The protein concentrations were determined by BCA kit (Beyotime, Xuhui, Shanghai, China). Then, equal amounts of proteins were loaded and separated by 8%-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane (Millipore Corporation, Billerica, MA, USA) followed by 3% BSA blocking. Target proteins were probed using specific antibodies as follows: Anti-Vimentin antibody (#10366-1-AP, Protein Tech, Wuhan, Hubei, China), Anti-E-Cadherin antibody (#BM0476, Protein Tech, Wuhan, Hubei, China), Anti-N-Cadherin antibody (#BM1573, BOSTER, Wuhan, Hubei, China), Anti-β-catenin antibody (#8480, Cell Signaling Technology, Xuhui Shanghai, China), Anti-Cyclin D1 antibody (#2922, Cell Signaling Technology, Xuhui Shanghai, China), Anti-c-Myc antibody (#13987, Cell Signaling Technology, Xuhui Shanghai, China), Anti-GAPDH antibody (#5174, Cell Signaling Technology, Xuhui Shanghai, China). Following three times washing, membranes were incubated with secondary antibodies for 60 min at 37°C in TBST buffer (Sangon, Xuhui, Shanghai, China). The luminescent assay was carried out using an ECL chromogenic substrate (Sangon, Xuhui, Shanghai, China) and imaged by a BioSpectrum Gel Imaging System (Bio-Rad, Hercules, CA, USA).

Cell Proliferation Assay

Cell proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, the A549 or H1299 cells (1×10^3 cells per well) of different groups were cultured in 96-well plates. After culturing for 24 h, 20 μl MTT solution (0.5 mg/ml) were added into each well and continued to culture for 4 h. After removing the supernatant, 100 μl of dimethyl sulfoxide (DMSO; Sangon, Pudong, Shanghai, China) was added to the cells to dissolve the formazan. Subsequently, the absorbance of the cells was determined by a Microplate Reader (BioTek, Winooski, VT, USA) at a wavelength of 490 nm.

Colony Formation Assay

The CENPU knockdown and negative control A549 or H1299 cells were plated into 6-well plates (500 cells per well) and incubated in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% FBS (HyClone, Logan, Utah, USA) at 37°C in a humidified atmosphere containing 5% CO_2. The cells were further cultured for about two weeks. Then, the cell colonies were fixed with 70% ethanol (Sangon, Xuhui, Shanghai, China) and stained with 0.3% crystal violet solution (Beyotime, Pudong, Shanghai, China) for 15 min at room temperature. After washed with PBS for three times, the number of colonies was counted using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Flow Cytometry Analysis of Apoptosis

For apoptosis analysis, an Annexin V-fluorescein Isothiocyanate Apoptosis Detection Kit (Cell Signaling Technology, Beverly, MA, USA) was utilized to perform the flow cytometry. In short, cells were collected and centrifuged after siRNAs transfection. After washing twice with ice-cold PBS, the cells were resuspended in 500 μL of binding buffer and incubated with 5 μl Annexin V-fluorescein isothiocyanate (FITC) as well as 5 μl propidium iodide (PI) for 15 min in the dark at 4°C. Subsequently, the cell apoptotic rate was detected by a Beckman-Coulter CyAN ADP Analyzer (Beckman Coulter, Fullerton, CA, USA).

Wound-Healing Migration Assay

To evaluate the effects of CENPU in cell migration of A549 or H1299 cells, the wound healing assay was carried out using a 35 mm μ-dish with culture insert (Ibidi, Martinsried, Germany). Briefly, cell suspensions (70 μl, 5×10^5 cells/ml) of A549 or H1299 cells were added into each reservoir of the μ-dish. After appropriate cell attachment for 24 h, the insert was removed and fresh culture medium was added to start the migration process. At 0 h and 24 h, cells were washed twice with PBS before photographs of the gap area and pictures were taken by use of a Nikon Eclipse TE2000-S microscope (Nikon, Chiyoda-Ku, Tokyo, Japan).

Transwell Assay

To evaluate cell invasive ability, 8 mm of pore 24-well Matrigel invasion chambers (Millipore, Billerica, MA, USA) was used according to the instructions provided by the manufacturer. After digested routinely, cells were washed twice using PBS and re-suspended with RPMI-1640 medium.

The cell density was then adjusted to 2.5×10^5/ml. A total of 200 µl cell suspension was added in the upper chamber without serum while the medium in the lower chamber containing 10% FBS. Subsequently, the cells on the upper chamber were removed with cotton wool after incubation for 24 h and the cells on the lower surface of the chamber were fixed in 4% formaldehyde (Sangon, Xuhui, Shanghai, China) and stained with 0.1% crystal violet (Beyotime, Xuhui, Shanghai, China). Images were taken under an inverted microscope (Nikon, Chiyoda-Ku, Tokyo, Japan).

TOP-Flash Luciferase Assay

The activities of the WNT pathway were determined using a TOP-Flash luciferase reporter system (Biovector, Haidian, Beijing, China). Indicated cells were seeded in 96-well plates (Nunc, Roskilde, Denmark), and transfected with TCF/b-catenin reporter plasmids and Renilla TK-luciferase vector (Promega, Madison, WI, USA). Then, the luciferase activity was evaluated using the Dual Luciferase-reporter 1000 assay system (Promega, Madison, WI, USA).

Statistical Analysis

Statistical analyses were performed using the SPSS 18.0 software package (SPSS Inc., Chicago, USA). Measurement data are presented as mean ± SEM from at least three independent experiments. Differences between two groups were evaluated by the two-tailed Student's *t*-test. A value of $p < 0.05$ was considered statistically significant.

Results

CENPU is Highly Expressed in NSCLC and Associated with Unfavorable Prognosis

To explore the role of CENPU in the progression of NSCLC, we analyzed the protein expression of CENPU proteins in clinical specimens from the human protein atlas (www.proteinatlas.org). We found that CENPU proteins were overexpressed in NSCLC tissues (Figure 1A). Then, we also explore the expression of CENPU mRNA in NSCLC using TCGA RNA sequencing data and

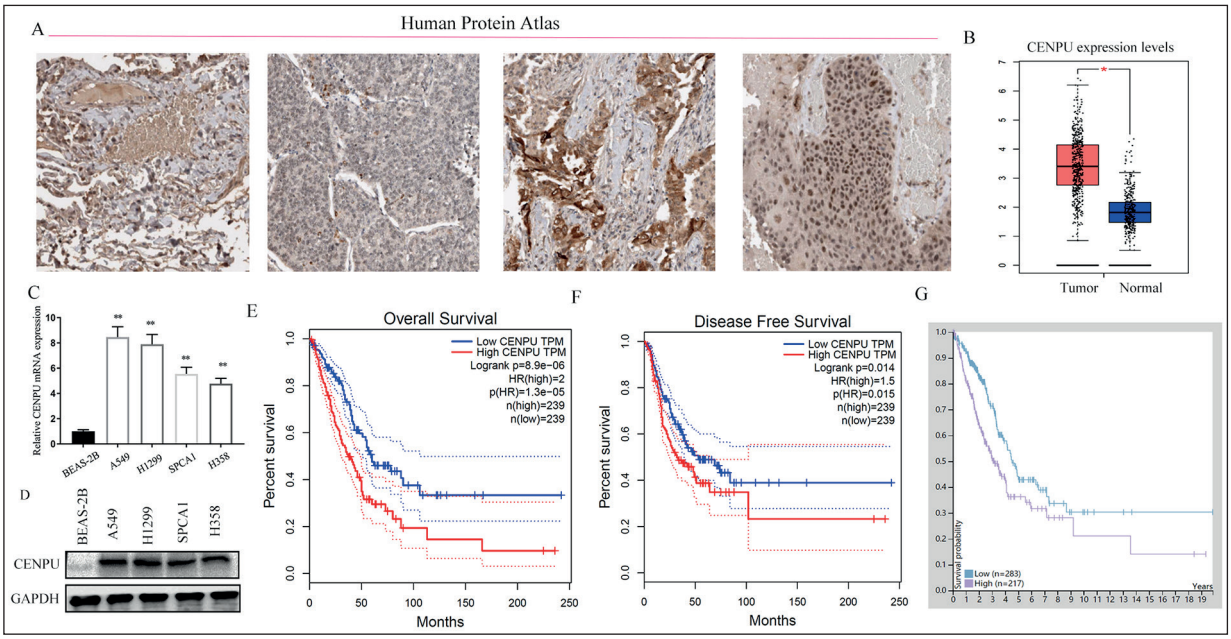

Figure 1. Relative CENPU expression in NSCLC and its clinical significance. **A,** The expression of CENPU proteins in NSCLC specimens and normal lung tissue. Images were taken from the Human Protein Atlas (http://www.proteinatlas.org) online database. **B,** Tissue expression of CENPU mRNA in cancer and normal tissues of NSCLC patients from TCGA datasets. **C,** The relative expression of CENPU mRNA in four NSCLC cell lines and normal lung cells (BEAS-2B) by RT-PCR. **D,** The relative expression of CENPU proteins in four NSCLC cell lines and normal lung cells (BEAS-2B) by Western blot. **E-F,** Kaplan-Meier survival plots demonstrated that higher CENPU mRNA expression correlated with poorer overall survival and disease-free survival, using TCGA datasets from 478 lung cancer patients. **G,** Kaplan-Meier survival plots demonstrated that higher CENPU proteins expression correlated with poorer overall survival and disease-free survival, using Human Protein Atlas datasets from 500 lung cancer patients. *$p < 0.05$, **$p < 0.01$.

found that CENPU mRNA was also significantly up-regulated in NSCLC tissue samples (Figure 1B). To validate these results, we performed RT-PCT and Western blot to detect the expression of CENPU in NSCLC cell lines, finding that CENPU at both mRNA and proteins was significantly up-regulated in for NSCLC cell lines (Figure 1C and 1D). Thus, CENPU up-regulation may be involved in the progression of NSCLC. Then, analysis using GEPIA online database indicated that patients with high expression of CENPU mRNA had poorer overall survival and disease-free survival than patients with low expression (Figure 1E and 1F). Also, we used human protein atlas to further analyze the clinical significance of CENPU proteins in NSCLC patients, also confirming the prognostic value of CENPU proteins in 500 lung cancer patients (Figure 1G). From what has been discussed above, we concluded that upregulated CENPU might play an important role in the development of NSCLC.

Inhibition of CENPU Decreased NSCLC Cells Proliferation and Promoted Apoptosis

As the above data found that CENPU was upregulated in NSCLC, we next asked whether it played important roles in modulating the proliferation and apoptosis of NSCLC cells. The results showed that the expression levels of CPNE1 mRNA were significantly reduced after transfection of A549 and H1299 cells with two siRNAs against CENPU (Figure 2A). Moreover, MTT assays revealed that down-regulation of CENPU dramatically decreased the growth rates of both A549 and H1299 cells (Figure 2B and 2C). By colony formation assays, we further found that clonogenic abilities were markedly suppressed in CENPU siRNA transfected cells compared with NC cells (Figure 2D). Additionally, flow cytometry assays were performed to evaluate the apoptotic rates of A549 and H1299 cells. The data demonstrated that the proportion of apoptotic cells after CENPU silencing was dramatically increased in A549 and H1299 cells (Figure 2E and F). These results suggested that knockdown of CENPU suppressed A549 and H1299 cells proliferation and induced cells apoptosis.

Depression of CENPU Impaired Lung Cancer Cells Migration and Invasion

To investigate the roles of CENPU in NSCLC cell migration and invasion, we conducted wound healing assays and transwell invasion assays in A549 and H1299 cells. As shown in Figure 3A and 3B, wound healing assays suggested that depletion of CENPU significantly suppressed the migration of A549 and H1299 cells. In addition, transwell invasion assays indicated that the cell invasive potential of A549 and H1299 cells showed a trend toward a substantial decrease following knockdown of CENPU (Figure 3C). Next, we performed Western blotting assays to determine the effects of CENPU on epithelial to mesenchymal (EMT) related proteins in A549 and H1299 cells. The results demonstrated that knockdown of CENPU markedly increased the protein levels of E-cadherin while the protein levels of N-cadherin and vimentin decreased significantly (Figure 3D and 3E). Taken together, our results indicated that knockdown of CENPU inhibited the migration and invasion abilities of lung cancer cells via regulating EMT signal pathway.

Depression of CENPU Modulated the Activity of Wnt/β-Catenin Signaling in Lung Adenocarcinoma

To identify key downstream signaling pathways that were regulated by CENPU involved in the development and progression of NSCLC, the expression levels of Wnt/β-catenin signaling-related proteins were investigated in A549 and H1299 cells. Firstly, TOP/FOP flash reporter assays were carried out to explore the impacts of CENPU on Wnt/β-catenin signaling pathway in A549 cells. The results revealed that, after transfecting with CENPU siRNAs, the luciferase activities of A549 cells were significantly decreased compared with the control group suggested that silence of CENPU impaired the activation of the Wnt/β-catenin signaling (Figure 4A). Furthermore, qRT-PCR assays proved that reduced expression of CENPU significantly inhibited the expression of classic downstream genes of the Wnt/β-catenin signaling pathway such as β-catenin, cyclin D1, and c-myc (Figure 4B and 4C). Similarly, the protein levels of β-catenin, cyclin D1, and c-myc were also decreased in A 549 cells as well as H1299 cells transfected with CENPU siRNAs (Figure 4D and E). Therefore, our data suggested that the CENPU knockdown could attenuate the activation of the Wnt/β-catenin signaling pathway. In addition, these results further indicated that CENPU served as critical roles in regulating the biological functions of NSCLC by affecting Wnt/β-catenin signaling pathway.

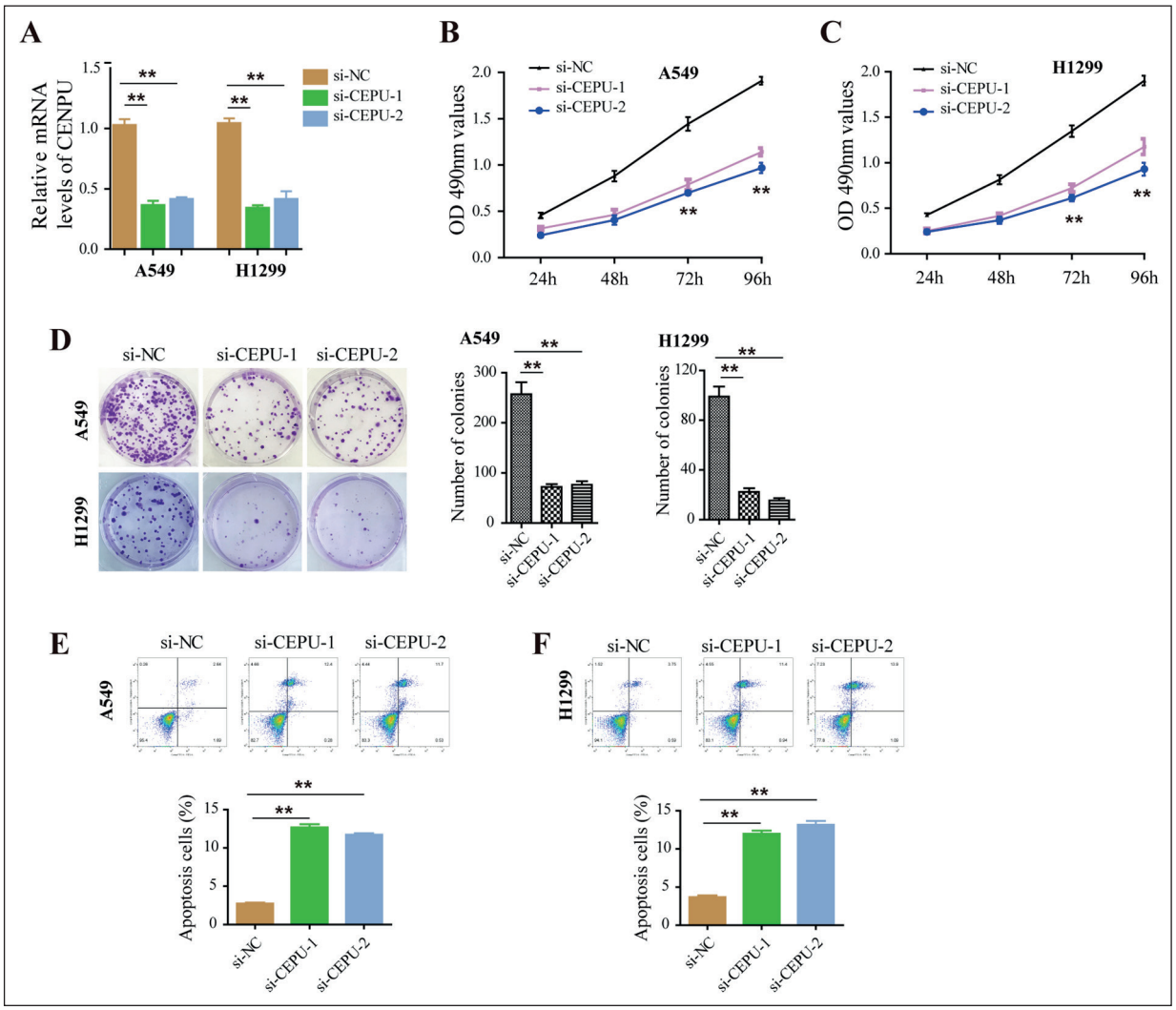

Figure 2. CENPU knockdown suppressed the proliferation and promoted the apoptosis of A549 and H1299 cells. **A,** CENPU mRNA levels detected by qRT-PCR assays in A549 and H1299 cells when transfected with si-CENPU-1, si-CENPU-2 or si-NC. **B** and **C,** MTT assay was performed to assess the influences of CENPU siRNAs on cell viability. **D,** Effects of CENPU alteration on the colony formation of A549 and H1299 cells. **E** and **F,** Cell apoptosis of A549 and H1299 cells after CENPU siRNAs transfection was analyzed by flow cytometry. *$p < 0.05$, **$p < 0.01$.

Discussion

NSCLC is a type of malignant tumor threatening human health and had an increasing incidence in the past few decades[15]. It is very hard to diagnose NSCLC at an early stage because of lacking noticeable symptoms in early NSCLC patients[16]. Although the early diagnosis cannot guarantee recovery, appropriate treatment can be administered to prevent the advancement of this malignancy[17]. Therefore, the molecular mechanisms underlying NSCLC migration and metastasis need to be elucidated to explore sensitive biomarkers and therapeutic targets for diagnosis and prognosis prediction of NSCLC.

In this study, we first detect the expression of CENPU in NSCLC cell lines, finding that CENPU proteins and mRNAs were significantly overexpressed in NSCLC cell lines when compared to matched normal lung cell lines. Then, the results of human protein atlas confirmed our findings. In addition, we used TCGA data to explore whether CENPU mRNA expression was dysregulated in NSCLC tissues. Then, we further analyzed the clinical significance of CENPU in

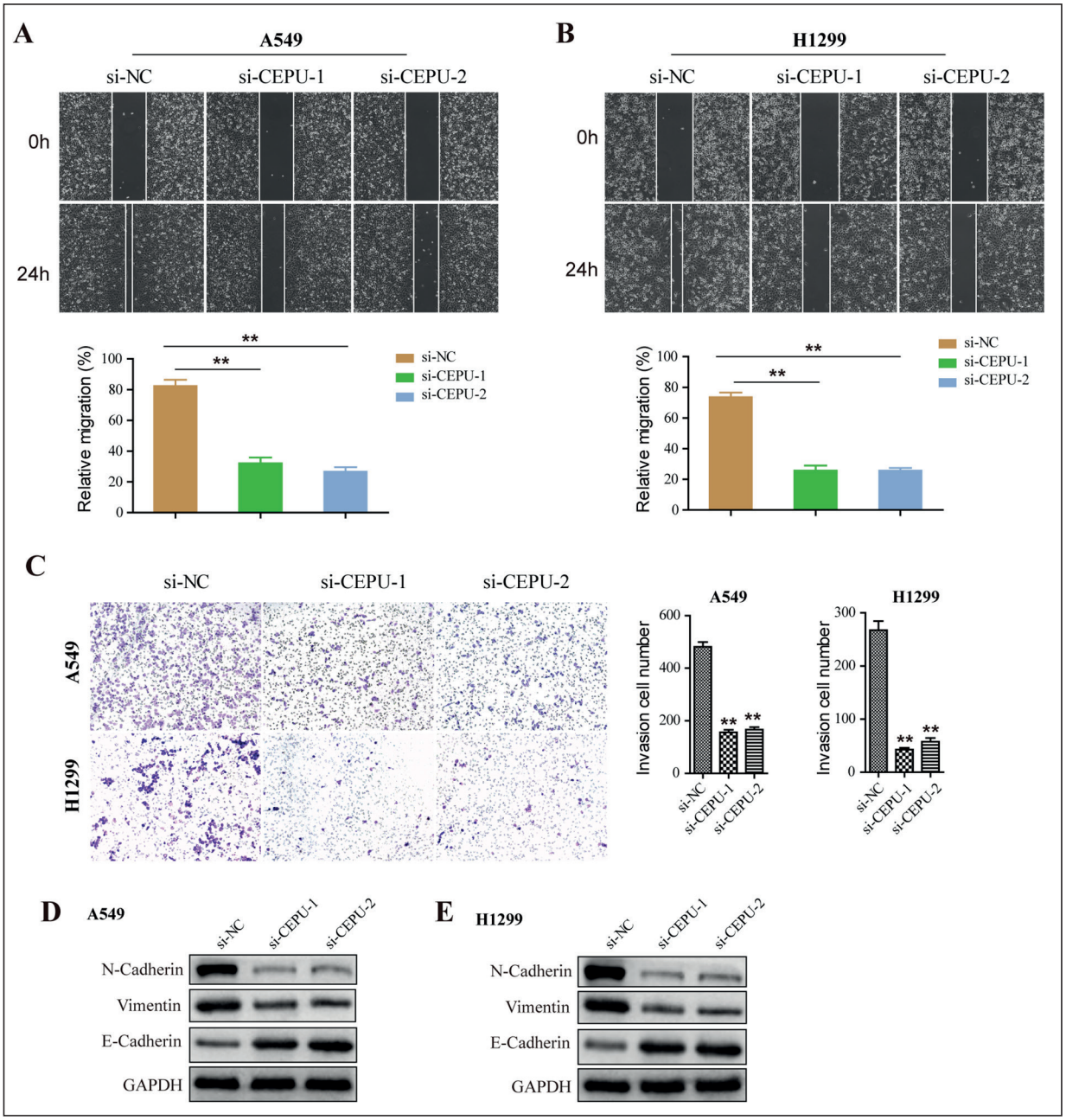

Figure 3. Inhibition of lung adenocarcinoma migration and invasion by silencing of CENPU. **A** and **B,** The wound healing assays showed that the speed with which cells migrated towards the scratch was lower in CENPU-silenced cells than in control cells. **C,** Transwell invasion assays showed the invasive cell number in A549 and H1299 cells transfected with CENPU siRNAs and negative control siRNA. **D** and **E,** Western-blot assays determined the protein levels of N-cadherin, vimentin, and E-cadherin in A549 and H1299 cells. *$p < 0.05$, **$p < 0.01$.

NSCLC patients using TCGA dataset and human protein atlas dataset. We found that high CENPU proteins and mRNAs expression were significantly associated with poor prognosis of NSCLC patients, suggesting that CENPU may be used as a potential prognostic biomarker.

An increasing number of researches have verified that CENPU was highly expressed in various cancers and promoted cancer progression. For instance, Li et al[11] reported that Centromere protein U was highly expressed in ovarian cancer patients and facilitates metastasis of ovarian

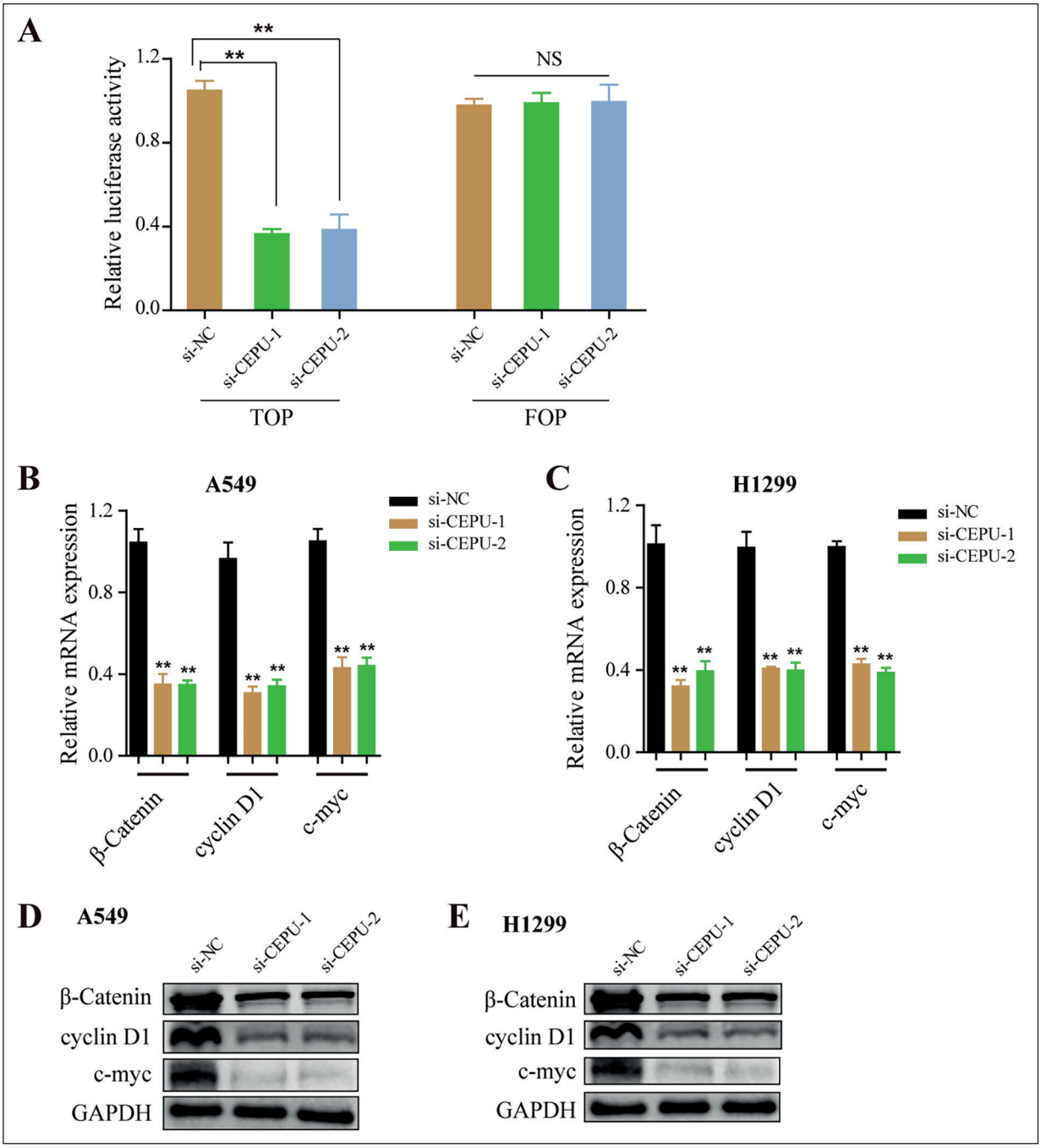

Figure 4. The effects of CENPU on the activities of Wnt/β-catenin signaling pathway in A549 cells. **A,** β-catenin transcription factor/lymphoid enhancer binding factor (TCF/LEF) promoter activity in A549 cells was assessed using TOP-Flash luciferase reporter assays. **B-C,** qRT-PCR was applied to detect the mRNA levels of β-catenin, cyclin D1, and c-myc in both A549 and H1299 cells. **D-E,** The protein levels of β-catenin, cyclin D1, and c-myc were determined by Western-blot assay. *$p < 0.05$, **$p < 0.01$.

cancer cells by targeting HMGB2. Huang et al[12] found that CENPU was highly expressed in luminal breast cancer and associated with poor prognosis of breast cancer patients. Therefore, CENPU may regulate the progression of tumors. However, the biological function of CENPU in NSCLC remains largely unclear. In this work, we firstly performed *in vitro* assay by suppressing the expression of CENPU to explore the effect of CENPU on tumor progression. Our results

revealed that knockdown of CENPU significantly suppressed NSCLC cells proliferation and promoted apoptosis, suggesting that CENPU may contribute to the progression of NSCLC. In addition, we also performed wound-healing migration assay and transwell assay to explore the effect of CENPU on metastasis of NSCLC cells, finding that suppression of CENPU significantly inhibited the migration and invasion of NSCLC cells. On the other hand, the findings of Western blot revealed that CENPU exhibited its carcinogenic role by modulating the EMT pathway. Thus, our function assay confirmed that CENPU acted as an oncogene in the progression of NSCLC.

Although previous researches have clarified the role of CENPU in different cancer types, the potential mechanism by which CENPU modulates the proliferation, apoptosis, migration, and invasion remains elusive. Recently, advances in molecular biology have led to an increased knowledge of the mechanisms underlying NSCLC progression[18,19]. The Wnt/β-catenin signaling pathway is very important to many physiological and pathological conditions, such as cell proliferation, angiogenesis, metabolism, and differentiation[20,21]. In addition, activation of Wnt/β-catenin signaling pathway signaling is one of the most frequent events in cancer[22,23]. In this study, in order to explore whether CENPU promoted NSCLC cells proliferation, migration, and invasion, we performed Western blot to detect the expression of the key molecular factors of Wnt/β-catenin signaling pathway in NSCLC cells transfected with si-CENPU. We found that CENPU knockdown significantly reduced the expression of β-catenin, Cyclin D1, and c-Myc. Thus, our findings strongly suggest that the tumor-promotive effect of CENPU is attributed to its ability to promote the activation of Wnt/β-catenin signaling pathway in NSCLC.

Conclusions

We firstly reported that CENPU expression was up-regulated in NSCLC and associated with the prognosis of NSCLC patients. Knockdown of CENPU expression in NSCLC cells could suppress cell proliferation, migration, and invasion, and promote apoptosis *in vitro*. Furthermore, the expression of CENPU could regulate the Wnt/β-catenin signaling pathway in NSCLC cells. CENPU might be a potential biomarker and therapeutic target in NSCLC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.
2) Dubey AK, Gupta U, Jain S. Epidemiology of lung cancer and approaches for its prediction: a systematic review and analysis. Chin J Cancer 2016; 35: 71.
3) Reck M, Heigener DF, Mok T, Soria JC, Rabe KF. Management of non-small-cell lung cancer: recent developments. Lancet 2013; 382: 709-719.
4) Parums DV. Current status of targeted therapy in non-small cell lung cancer. Drugs Today (Barc) 2014; 50: 503-525.
5) Blanco R, Maestu I, de la Torre MG, Cassinello A, Nunez I. A review of the management of elderly patients with non-small-cell lung cancer. Ann Oncol 2015; 26: 451-463.
6) Dawe DE, Christiansen D, Swaminath A, Ellis PM, Rothney J, Rabbani R, Abou-Setta AM, Zarychanski R, Mahmud SM. Chemoradiotherapy versus radiotherapy alone in elderly patients with stage III non-small cell lung cancer: A systematic review and meta-analysis. Lung Cancer 2016; 99: 180-185.
7) Kuribayashi K, Funaguchi N, Nakano T. Chemotherapy for advanced non-small cell lung cancer with a focus on squamous cell carcinoma. J Cancer Res Ther 2016; 12: 528-534.
8) De Wever W, Verschakelen J, Coolen J. Role of imaging in diagnosis, staging and follow-up of lung cancer. Curr Opin Pulm Med 2014; 20: 385-392.
9) Walter RB, Walter DJ, Boswell WT, Caballero KL, Boswell M, Lu Y, Chang J, Savage MG. Exposure to fluorescent light triggers down regulation of genes involved with mitotic progression in Xiphophorus skin. Comp Biochem Physiol C Toxicol Pharmacol 2015; 178: 93-103.
10) Kagawa N, Hori T, Hoki Y, Hosoya O, Tsutsui K, Saga Y, Sado T, Fukagawa T. The CENP-O complex requirement varies among different cell types. Chromosome Res 2014; 22: 293-303.
11) Li H, Zhang H, Wang Y. Centromere protein U facilitates metastasis of ovarian cancer cells by targeting high mobility group box 2 expression. Am J Cancer Res 2018; 8: 835-851.
12) Huang DP, Luo RC. MLF1IP is correlated with progression and prognosis in luminal breast cancer. Biochem Biophys Res Commun 2016; 477: 923-926.
13) Zhang L, Ji G, Shao Y, Qiao S, Jing Y, Qin R, Sun H, Shao C. MLF1 interacting protein: a potential gene therapy target for human prostate cancer? Med Oncol 2015; 32: 454.

14) Wang S, Liu B, Zhang J, Sun W, Dai C, Sun W, Li Q. Centromere protein U is a potential target for gene therapy of human bladder cancer. Oncol Rep 2017; 38: 735-744.

15) Hasan N, Kumar R, Kavuru MS. Lung cancer screening beyond low-dose computed tomography: the role of novel biomarkers. Lung 2014; 192: 639-648.

16) Liam CK, Andarini S, Lee P, Ho JC, Chau NQ, Tscheikuna J. Lung cancer staging now and in the future. Respirology 2015; 20: 526-534.

17) Zhao LX, Gao Q, Liu YJ, Ma QL, Li DY, Jiang YZ. Study on 256-slice spiral CT bronchial artery imaging of common pathological types of central-type lung cancer. Eur Rev Med Pharmacol Sci 2016; 20: 3211-3216.

18) Zheng H, Liu JF. Studies on the relationship between P13K/AKT signal pathway-mediated MMP-9 gene and lung cancer. Eur Rev Med Pharmacol Sci 2017; 21: 753-759.

19) Kenific CM, Debnath J. Cellular and metabolic functions for autophagy in cancer cells. Trends Cell Biol 2015; 25: 37-45.

20) Xie W, Zhou L, Li S, Hui T, Chen D. Wnt/beta-catenin signaling plays a key role in the development of spondyloarthritis. Ann N Y Acad Sci 2016; 1364: 25-31.

21) Libro R, Bramanti P, Mazzon E. The role of the Wnt canonical signaling in neurodegenerative diseases. Life Sci 2016; 158: 78-88.

22) Xiao C, Wu CH, Hu HZ. LncRNA UCA1 promotes epithelial-mesenchymal transition (EMT) of breast cancer cells via enhancing Wnt/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2016; 20: 2819-2824.

23) Yang Y, Liu L, Cai J, Wu J, Guan H, Zhu X, Yuan J, Li M. DEPDC1B enhances migration and invasion of non-small cell lung cancer cells via activating Wnt/beta-catenin signaling. Biochem Biophys Res Commun 2014; 450: 899-905.