Non-SMC condensin I complex subunit H promotes the malignant progression and cisplatin resistance of breast cancer MCF-7 cells

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Abstract. Breast cancer is one of the most frequently diagnosed types of cancer worldwide. The present study aimed to investigate the role and underlying regulatory mechanism of non-structural maintenance of chromosome condensin I complex subunit H (NCAPH) in the malignant progression and cisplatin (DDP) resistance of breast cancer cells. Therefore, the mRNA and protein expression levels of NCAPH were first determined in breast cancer cells via reverse transcription-quantitative PCR and western blotting. Furthermore, following transfection of NCAPH interference plasmids, the effect of NCAPH knockdown on cell proliferation, migration, invasion were also assessed using CCK-8, wound healing and Transwell assays. Apoptosis was evaluated using TUNEL assay, and western blotting was performed in breast cancer cells and DDP-resistant breast cancer cells. The association between NCAPH and its downstream target, aurora kinase B (AURKB), was verified using bioinformatic analysis and the co-immunoprecipitation assay. Furthermore, the effect of AURKB overexpression on the aforementioned processes and the Akt/mTOR signaling pathway were also assessed. The results demonstrated that NCAPH mRNA and protein expression levels were significantly upregulated in breast cancer cells, whereas NCAPH knockdown significantly attenuated the proliferation, migration and invasion of breast cancer cells. NCAPH silencing also exacerbated the apoptosis of DDP-resistant breast cancer cells. AURKB mRNA and protein expression levels were also significantly upregulated in MCF-7 cells, whereas its overexpression significantly reversed the effects of NCAPH knockdown on breast cancer cells and the Akt/mTOR signaling pathway. Overall, NCAPH knockdown significantly downregulated AURKB mRNA and protein expression levels to block the Akt/mTOR signaling pathway and inhibited breast cancer cell proliferation, migration, invasion, and aggravate DDP-resistant breast cancer cell apoptosis, indicating that NCAPH may serve as a promising therapeutic target for breast cancer.

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Introduction

Breast cancer is the most commonly diagnosed type of cancer, surpassing lung cancer, and therefore presents a risk to patients worldwide (1). Breast cancer accounts for 11.7% of all new cancer cases and 6.9% of all cancer-related deaths in women worldwide (1). Although the overall survival and prognosis of patients with breast cancer have significantly improved in recent years (2), the analysis of data from the U.S. National Center for Health Statistics highlights that further investigations are required; these data indicated that after 2010, the breast cancer mortality rate continued to decline by 1.2-2.2% per year in women aged between 40-79 years. However, the breast cancer mortality rate stopped decreasing in women aged <40 years, whereas the mortality rate for women aged between 20-29 years increased by 2.8% per year (3). Therefore, improving the health awareness of young women, identifying novel therapeutic targets and developing effective biomarkers to prevent the progression of breast cancer are of importance.

Cisplatin (DDP) is a non-specific, first-generation platinum drug, which affects the cell cycle and can be used to treat several types of cancer, including breast, testicular, ovarian and lung cancer (4). The major targets of DDP are nucleophilic DNA, proteins and RNA (5). It has previously been reported that patients commonly exhibit a good initial response to DDP chemotherapy (6). However, drug resistance is an intractable problem in breast cancer treatment (7). Moreover, the specific mechanism underlying DDP drug resistance remains largely unknown. The clinical use of DDP is limited due to its significant cytotoxicity to normal tissues and increased potential for drug resistance in cancer cells (8). Therefore, investigating the mechanism underlying the sensitivity of breast cancer cells to DDP is of great importance.

Non-structural maintenance of chromosome (SMC) condensin I complex subunit H (NCAPH) is a structural component of chromosomes during mitosis (9). NCAPH and other subunits (NCAPD2 and NCAPG) that form the condensin I complex cooperate with SMC to regulate the structure of chromosomes (10). Moreover, it has been reported that NCAPH is involved in the progression of several types of cancer. A recent study reported that NCAPH was significantly upregulated in endometrial cancer (EC), thus acting as an oncogene to promote the development of EC (11). Another study indicated that NCAPH knockdown could inhibit cell proliferation, migration and invasion and induce cell cycle arrest in non-small cell lung cancer (NSCLC) (12). Furthermore, a bioinformatic analysis study using the ONCOMINE, University of Alabama at Birmingham Cancer Data Analysis Portal and Gene Expression-Based Outcome for Breast Cancer Online databases, determined that NCAPH is upregulated in breast cancer, which therefore indicates that NCAPH may be a promising biomarker in breast cancer (13).

The present study aimed to explore the role and regulatory mechanism of NCAPH in the malignant phenotype and DDP resistance of breast cancer cells. Overall, the results of the present study have provided novel insights into the progression and resistance of breast cancer cells to cancer therapeutics, which may be used to prevent and treat breast cancer in the future.

Materials and methods

Bioinformatic analysis. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; string-db.org; organism=Homo sapiens) (14); HumanBase (hb.flatironinstitute.org) (15) using the default data types 'Co-expression' AND 'Interaction' AND 'TF binding' AND 'GSEA microRNAs targets' AND 'GSEA perturbations', with the criteria value of mimimum interaction confidence as 0.86 and the maximum number of genes as 9; and GeneMANIA (genemania.org) (16) databases using the default networks 'Physical Interactions' AND 'Co-expression' AND 'Predicted' AND 'Co-localization' AND 'Genetic Interactions' AND 'Pathway' AND 'Shared protein domains', were used to evaluate the interaction between NCAPH and aurora kinase (AURK)B. The Gene Expression Profiling Interactive Analysis (GEPIA) database (gepia.cancer-pku.cn) (17) was used to analyze the positive or negative association between the mRNA expression levels of NCAPH and AURKB. $P \leq 0.05$ indicates that the result of the model is reliable; $R>0$ indicates a positive correlation and the closer R^2 is to 1, the more relevant the correlation between NCAPH and AURKB is.

Cell culture. The human mammary epithelial MCF-10A cell line (cat. no. MCF-10A), the breast cancer cell lines MDA-MB-231 (cat. no. TCHu227), SUM190PT (cat. no. CVCL_3423), SK-BR-3 (cat. no. TCHu225) and MCF-7 (cat. no. ACC 115) and the DDP-resistant cell line MCF-7/DDP (cat. no. MCF-7/DDP) were purchased from Beijing Protein Biotechnology Co., Ltd. MCF-10A, SUM190PT, MCF-7 and MCF-7/DDP cells were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.), MDA-MB-231 cells in Leibovitz's L-15 medium (Gibco; Thermo Fisher Scientific, Inc.) and SK-BR-3 cells in McCoy's 5A medium (Gibco; Thermo Fisher Scientific, Inc.). The media were supplemented with 10% FBS (Merck KGaA) and 1% penicillin/streptomycin solution (Gibco; Thermo Fisher Scientific, Inc.). All cell lines were cultured at 37˚C with 5% CO_2, except MDA-MB-231 cells, which were cultured without CO_2.

Cell transfection. NCAPH knockdown was achieved by transfecting breast cancer cells with 50 nM short hairpin RNAs (shRNAs/sh) targeting NCAPH (sh-NCAPH-1/2). Cells transfected with scrambled shRNAs served as the negative control (NC; sh-NC) group. For AURKB overexpression (oe; oe-AURKB), MCF-7 cells were transfected with the pcDNA3.1 plasmid (2 μg) encoding AURKB complementary DNA (cDNA), whereas empty vectors served as the oe-NC group. All plasmids were synthesized by Shanghai GenePharma Co., Ltd. and the cells seeded in 6-well plates at a density of $1x10^6$ were transfected with the aforementioned plasmids/shRNAs using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h at 37˚C according to the manufacturer's instructions. The cells were collected for subsequent experiments 48 h after transfection. The targeting sequences used were as follows: sh-NCAPH-1, 5'-CCCAAG GATTAGACATCACAA-3'; sh-NCAPH-2, 5'-ACACGCAGA TTACGGAACATT-3'; and sh-NC, 5'-GCACTACCAGAG CTAACTCAG-3'.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from untransfected or transfected cells in 6-well plates at a density of $2x10^5$ cells/well using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and reverse transcribed into cDNA using the PrimeScript RT Reagent Kit (Takara Biotechnology Co., Ltd.) according to the manufacturer's protocol. qPCR was performed using SYBR Premix Ex Taq™ II (Takara Bio, Inc.) with Thermal Cycler Dice™ Real Time System III (Takara Bio, Inc.). The PCR conditions were as follows: 95˚C for 10 min for initial denaturation, followed by 40 cycles of denaturation for 15 sec at 95˚C, annealing for 30 sec at 60˚C, elongation for 30 sec at 72˚C and a final extension for 5 min at 72˚C. The relative mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (18) following normalization with GAPDH. The primer sequences used were as follows: NCAPH forward (F), 5'-AAACAACCTCAATGT CTCCGAAG-3' and reverse (R), 5'-ACAACCTAACTCTGG CAACTCG-3'; AURKB F, 5'-TCACCCCATCTGCACTTG TC-3' and R, 5'-TGTGAAGTGCCGCGTTAAGA-3'; and GAPDH F, 5'-GACTCATGACCACAGTCCATGC-3' and R, 5'-AGAGGCAGGGATGATGTTCTG-3'.

Western blotting. Transfected or untransfected cells ($2x10^6$) were lysed using RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd.) and the protein concentration was assessed using the BCA method (Beijing Solarbio Science & Technology Co., Ltd.). Subsequently, 30 μg protein/lane was separated via SDS-PAGE on a 10% gel and then transferred onto PVDF membranes (Beijing Solarbio Science & Technology Co., Ltd.). Subsequently the membranes were blocked with 5% skimmed milk for 2 h at room temperature followed by incubation with specific primary antibodies at 4˚C overnight. After being washed in a TBST solution, the membranes were incubated with

Table I. Antibodies used for western blotting.

Antibody	Dilution	Catalog no.	Host	Company
NCAPH	1:1,000	PA5-80842	Rabbit	Invitrogen; Thermo Fisher Scientific, Inc.
Ki67	1:500	Orb389335	Rabbit	Biorbyt Ltd.
PCNA	1:1,000	Orb48485	Rabbit	Biorbyt Ltd.
MMP2	1:2,000	GTX59880	Rabbit	GeneTex, Inc.
MMP9	1:1,000	GTX100458	Rabbit	GeneTex, Inc.
Bcl-2	1:1,000	AB112	Rabbit	Beyotime Institute of Biotechnology
Bax	1:2,000	AF1270	Rabbit	Beyotime Institute of Biotechnology
Cleaved caspase-3	1:500	ab32042	Rabbit	Abcam
Cleaved caspase-9	1:1,000	GTX132331	Rabbit	GeneTex, Inc.
Caspase-3	1:5,000	GTX110543	Rabbit	GeneTex, Inc.
Caspase-9	1:1,000	GTX112888	Rabbit	GeneTex, Inc.
AURKB	1:20,000	ab45145	Rabbit	Abcam
GAPDH	1:50,000	GTX100118	Rabbit	GeneTex, Inc.
Anti-rabbit IgG (HRP)	1:1,000	A0208	Goat	Beyotime Institute of Biotechnology

AURKB, aurora kinase B; NCAPH, non-structural maintenance of chromosome condensin I complex subunit H; PCNA, proliferating cell nuclear antigen.

HRP-conjugated secondary antibodies for 1 h at room temperature. The protein bands were developed with MilliporeSigma™ Luminata™ Western HRP Chemiluminescence Substrates (MilliporeSigma) and all data were analyzed using ImageJ software (version 1.52; National Institutes of Health). The antibodies used in the present study are presented in Table I.

Determination of cell proliferation. The Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Inc.) and colony formation assays were performed to assess cell proliferation. For the CCK-8 assay, untreated or transfected MCF-7 cells were seeded into a 96-well plate at a density of $3x10^3$ cells/well and incubated at 37˚C for 24, 48 and 72 h following transfection. Following incubation for the indicated time points, 10 μl CCK-8 solution was added into each well and the cells were then incubated for an additional 3 h. Finally, the absorbance at a wavelength of 450 nm was assessed in each well using a microplate reader (Bio-Rad Laboratories, Inc.). Cell viability was calculated as follows: (OD value-OD value at 0 h)/(OD value at 0 h) x100. The results are presented with the proliferation rate of the control group at 24 h set as 100%.

For the colony formation assay, the untreated and transfected MCF-7 cells were seeded into culture dishes at a density of 500 cells/dish. Subsequently, cells were cultured for two weeks at 37˚C and the medium was changed every three days. Following incubation, cells were washed twice with PBS, fixed with 4% paraformaldehyde (Merck KGaA) at room temperature for 15 min and stained with 0.5% crystal violet (Shanghai Yeasen Biotechnology Co., Ltd.) at room temperature for 30 min. Colonies containing >50 cells were imaged and counted manually using an inverted light microscope (magnification, x10; Olympus Corporation).

Determination of cell viability. The effect of different concentrations of DDP (0.1, 1, 5, 10, 25, 50 and 100 μM; Shanghai Yuanye Bio-Technology, Co., Ltd.) on MCF-7 and MCF-7/DDP cell viability was assessed using the CCK-8 assay. Cells at a density of $5x10^3$ cells/well were seeded into 96-well plates and cultured with medium containing DDP for 48 h at 37˚C. The other experimental steps were performed as aforementioned when describing the procedures of determination of proliferation of untreated or transfected MCF-7 cells.

Wound healing assay. Transfected cells at a density of $5x10^5$ cells/well were inoculated into a 6-well plate and incubated at 37˚C for 24 h until a cell monolayer was created when cells were at 70-80% confluence. Subsequently, a 200-μl pipette tip was used to introduce a straight scratch in the middle of the cell monolayer. Following culturing with serum-free medium for 24 h at 37˚C, images of the migrated cells at 0 and 24 h were captured using an inverted light microscope (magnification, x100; Olympus Corporation) and quantified using ImageJ software (version 1.52; National Institutes of Health).

Transwell assay. The upper chamber of the Transwell insert (8-μm pore; Corning Inc.) was first pre-coated with Matrigel (MilliporeSigma) for 1 h at room temperature and was then supplemented with 0.1 ml cell suspension ($3x10^3$ cells in FBS-free DMEM). The lower chamber was filled with DMEM supplemented with 20% FBS. Following incubation for 24 h at 37˚C, the cells on the lower surface of the membrane were fixed and stained using the aforementioned methods from the colony formation assay. The invasive cells were quantified using an inverted light microscope (magnification, x100; Olympus Corporation) and quantified using ImageJ software (version 1.52; National Institutes of Health).

TUNEL assay. TUNEL staining was performed to assess cell apoptosis using the One Step TUNEL Apoptosis Assay Kit (cat. no. C1086; Beyotime Institute of Biotechnology). Briefly,

transfected or untransfected MCF-7(/DDP) cells at a density of $5x10^5$ cells/well were fixed with 4% paraformaldehyde for 15 min at room temperature, permeated in PBS supplemented with 0.3% Triton X-100 for 10 min at 4˚C and blocked with 3% H_2O_2 for 15 min at room temperature. Before the cell nuclei were mounted with Vectashield® mounting medium containing 1 mg/ml DAPI for 10 min at room temperature, cells were incubated with 50 μl TUNEL detection solution for 1 h at 37˚C in the dark. Dehydrated transparent neutral gum was used to mount the sections. TUNEL-positive cells in six randomly selected fields of view were imaged using a fluorescence microscope (magnification, x200; Olympus Corporation).

Co-immunoprecipitation (Co-IP) assay. The interaction between NCAPH and AURKB was assessed using a Co-IP assay kit (cat. no. P2179; Beyotime Institute of Biotechnology) according to the manufacturer's protocol. First, MCF-7 cells were lysed in lysis buffer supplemented with protease inhibitors. Subsequently, 250 μl of cell lysates containing 100 μg Protein A + G magnetic beads were supplemented with antibodies against NCAPH (1/20), AURKB (1/20) or IgG (cat. no. ab172730; 1/60; Abcam) as the negative control, and a certain proportion of supernatant without any antibody (Input) was used as the positive control, followed by incubation for 2 h, with tumbling, at room temperature. The magnetic beads were separated via magnetic force followed by washing with ice-cold lysis buffer. Subsequently, the magnetic beads were immersed in SDS-PAGE sample loading buffer and boiled for 5 min. Following magnetic separation for 10 sec, the supernatant was collected for western blotting, using the aforementioned method.

Statistical analysis. All experiments were repeated independently three times. The results are presented as the mean ± SD. All statistical analysis was performed using GraphPad Prism 8.0 software (GraphPad Software, Inc.). Unpaired Student's t-test and one-way ANOVA followed by Tukey's post-hoc test were used to compare the differences between two and three or more groups, respectively. $P<0.05$ was considered to indicate a statistically significant difference.

Results

NCAPH knockdown attenuates breast cancer cell proliferation, migration and invasion. The mRNA and protein expression levels of NCAPH in the MCF-10A, MDA-MB-231, SUM190PT, SK-BR-3 and MCF-7 cell lines were determined using RT-qPCR and western blotting, respectively (Fig. 1A and B). The results demonstrated that the mRNA expression levels of NCAPH were significantly increased in all of the breast cancer cell lines compared with MCF-10A cells and the protein expression levels of NCAPH were significantly increased in all of the breast cancer cell lines except SK-BR-3 compared with MCF-10A cells. To highlight the potential role of NCAPH in breast cancer, the widely used MCF-7 cell line was selected for use in the subsequent assays. These cells demonstrated the highest NCAPH expression levels and were therefore transfected with sh-NCAPH-1/2 and sh-NC as a control. The transfection efficiency was assessed using RT-qPCR and western blotting (Fig. 1C and D). The results demonstrated that the mRNA and protein expression levels of NCAPH were markedly reduced in the sh-NCAPH-2 group and sh-NCAPH-1 group compared with the sh-NC group. The interference efficiency of sh-NCAPH-2 was more efficient than that of sh-NCAPH-1. Therefore, the sh-NCAPH-2 construct was selected for use in the subsequent assays. The effect of NCAPH knockdown on cell proliferation was determined via CCK-8 and colony formation assays (Fig. 1E and F). These assays demonstrated that NCAPH knockdown significantly reduced cell proliferation and significantly reduced the colony formation ability of breast cancer cells compared with sh-NC. Furthermore, the wound healing and Transwell assays demonstrated that NCAPH knockdown significantly reduced cell migration and invasion compared with the sh-NC group (Fig. 2A and B). Moreover, western blotting was performed to detect the protein expression levels of proliferation- and migration-related proteins (Fig. 2C). The results demonstrated that the protein expression levels of Ki67, proliferating cell nuclear antigen (PCNA), MMP2 and MMP9 were all significantly reduced in the sh-NCAPH-2 group compared with the sh-NC group. In summary, NCAPH knockdown suppressed breast cancer cell proliferation, migration and invasion.

NCAPH knockdown attenuates the resistance of breast cancer cells to DDP. The mRNA and protein expression levels of NCAPH in the MCF-7 and MCF-7/DDP cells were determined using RT-qPCR and western blotting, respectively. NCAPH mRNA and protein expression levels in the MCF-7/DDP cells were significantly higher compared with the MCF-7 cells (Fig. 3A and B). The effect of DDP (0.1, 1, 5, 10, 25, 50 and 100 μM) on MCF-7 and MCF-7/DDP cell viability was assessed using the CCK-8 assay (Fig. 3C). Treatment of MCF-7 and MCF-7/DDP cells with different concentrations of DDP markedly decreased cell viability in a dose-dependent manner. The decrease in cell viability was significantly less in the MCF-7/DDP cell line compared with the MCF-7 cell line. The IC_{50} values for DDP were 10.90 and 50.11 μM in MCF-7 and MCF-7/DDP cells, respectively (Fig. 3D). Furthermore, MCF-7, MCF-7/DDP and transfected MCF-7/DDP cells were treated with 10 μM DDP and cell apoptosis was detected using the TUNEL assay (Fig. 3E) and western blotting (Fig. 3F). The apoptotic rate of cells in the MCF-7/DDP group was significantly lower compared with the MCF-7 group. Furthermore, NCAPH knockdown significantly increased the apoptotic rate of MCF-7/DDP cells compared with the MCF-7/DDP + sh-NC group. The protein expression levels of Bax, cleaved caspase-3 and cleaved caspase-9 were significantly decreased, whereas those of Bcl-2 were significantly increased in MCF-7/DDP cells compared with MCF-7 cells. Furthermore, NCAPH knockdown partially restored their levels compared with MCF-7/DDP + sh-NC cells. Overall, NCAPH knockdown reduced the resistance of breast cancer cells to DDP.

NCAPH knockdown downregulates AURKB expression levels. The mRNA and protein expression levels of AURKB in MCF-10A and MCF-7 cells were detected using RT-qPCR and western blotting, respectively (Fig. 4A and B). mRNA and protein expression levels of AURKB were demonstrated to be significantly upregulated in MCF-7 cells compared with MCF-10A cells. Furthermore, the STRING, HumanBase and

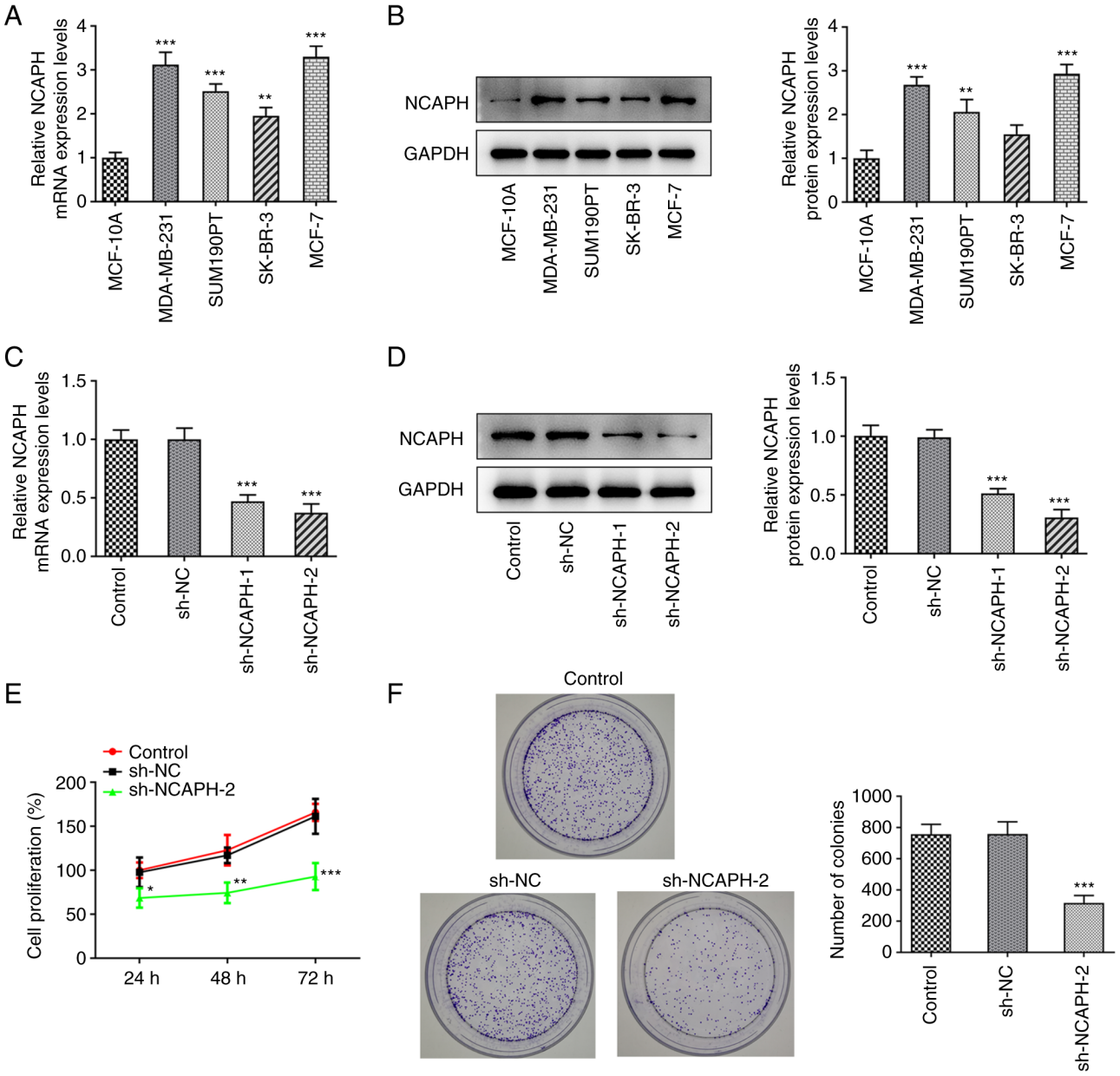

Figure 1. NCAPH knockdown attenuates breast cancer cell proliferation. mRNA and protein expression levels of NCAPH in MCF-10A cells and four breast cancer cell lines were determined via (A) RT-qPCR and (B) western blotting; respectively. MCF-7 cells were transfected with sh-NCAPH-1/2 and the transfection efficiency was assessed using (C) RT-qPCR and (D) western blotting; respectively. Effect of NCAPH knockdown on cell proliferation was determined using (E) the Cell Counting Kit-8 and (F) colony formation assays. Magnification, x10. *P<0.05, **P<0.01 and ***P<0.001 vs. MCF-10A or sh-NC groups. NCAPH, non-structural maintenance of chromosome condensin I complex subunit H; RT-qPCR, reverse transcription-quantitative PCR; sh, short hairpin RNA; NC, negative control.

GeneMANIA databases were used to assess the association between NCAPH and AURKB (Fig. 4C-E). Bioinformatic analysis using the GEPIA database predicted that NCAPH was positively associated with AURKB mRNA expression (Fig. 4F). The co-IP assay was used to verify the association between NCAPH and AURKB, which demonstrated that NCAPH and AURKB could interact as they coprecipitated (Fig. 4G). Finally, RT-qPCR and western blotting demonstrated that the mRNA and protein expression levels of AURKB in MCF-7 cells transfected with sh-NCAPH-2 were significantly reduced compared with those in the sh-NC group (Fig. 4H and I). In conclusion, NCAPH bound to AUKRB and NCAPH silencing downregulated AURKB expression levels.

AURKB overexpression partially reverses the effects of NCAPH knockdown on cell proliferation, migration and invasion. To assess the role of AURKB in the regulation of NCAPH, MCF-7 cells were transfected with oe-AURKB. The transfection efficiency was assessed via RT-qPCR and western blotting, and the results revealed that AURKB expression was significantly elevated in the oe-AURKB compared with the oe-NC group (Fig. 5A and B). Furthermore, MCF-7 cells were co-transfected with sh-NCAPH-2 and oe-AURKB and the AURKB mRNA and protein expression levels were assessed. The mRNA and protein expression levels of AURKB were significantly elevated in the sh-NACPH-2 + oe-AURKB group compared with the

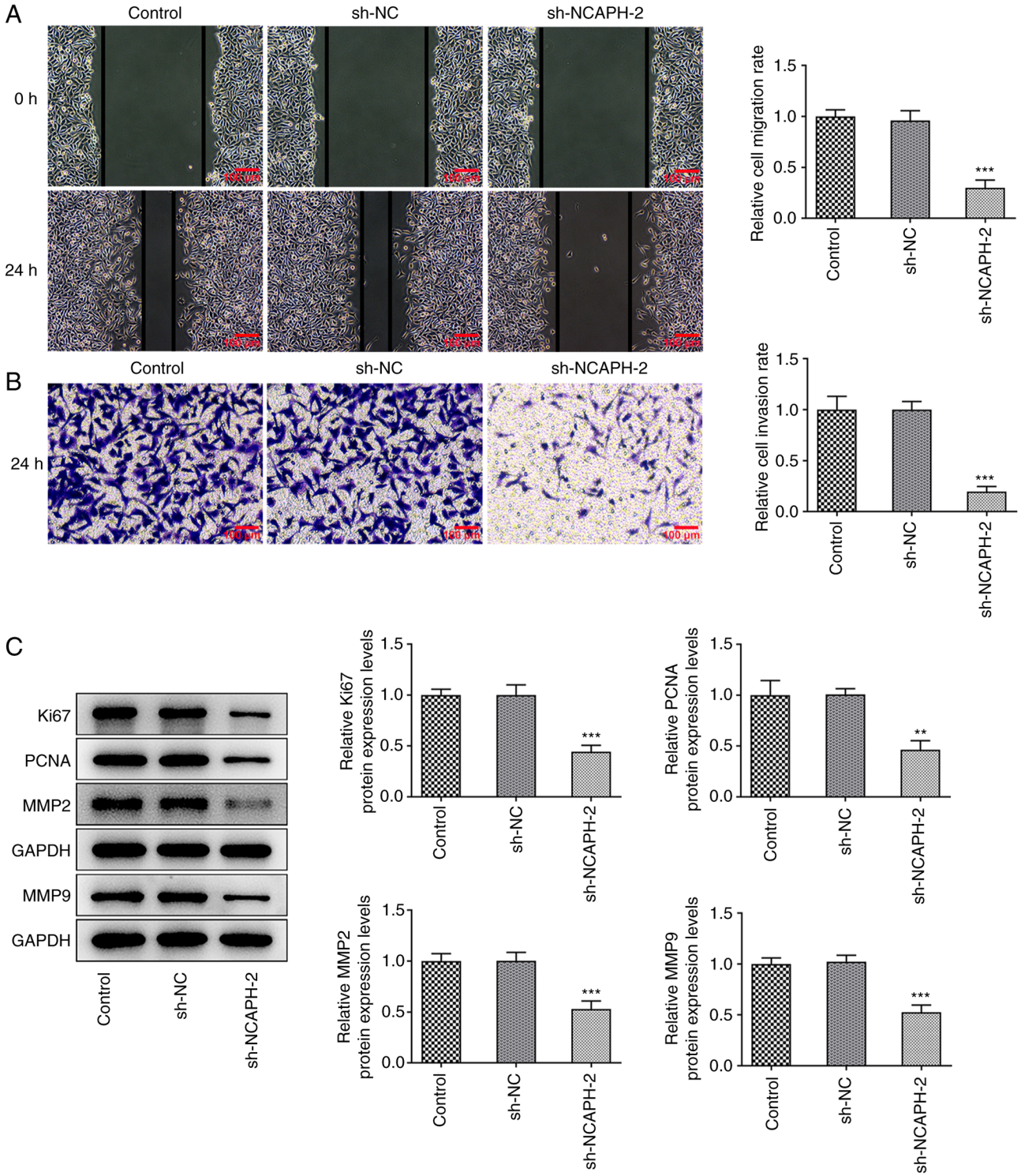

Figure 2. NCAPH knockdown suppresses the migration and invasion abilities of breast cancer cells. (A) Effect of NCAPH silencing on cell migration was determined via the wound healing assay. Magnification, x100. (B) Effect of NCAPH knockdown on cell invasion was assessed using the Transwell assay. Magnification, x100. (C) Western blotting was performed to detect the expression levels of proliferation- and migration-related proteins. **P<0.01 and ***P<0.001 vs. sh-NC. NCAPH, non-structural maintenance of chromosome condensin I complex subunit H; sh, short hairpin RNA; NC, negative control; PCNA, proliferating cell nuclear antigen.

sh-NACPH-2 + oe-NC group (Fig. 5C and D). The CCK-8 and colony formation assays demonstrated that AURKB overexpression significantly increased cell proliferation compared with the sh-NACPH-2 + oe-NC group, which suggested that AURKB potentially reduced the effect of NCAPH knockdown on cell proliferation (Fig. 5E and F). Furthermore, the wound healing and Transwell assays demonstrated that AURKB overexpression significantly increased cell migration and invasion compared with the sh-NCAPH-2 + oe-NC group (Fig. 5G-J). Moreover, the western blotting results demonstrated that AURKB overexpression significantly increased the protein expression levels of Ki67, PCNA, MMP2 and

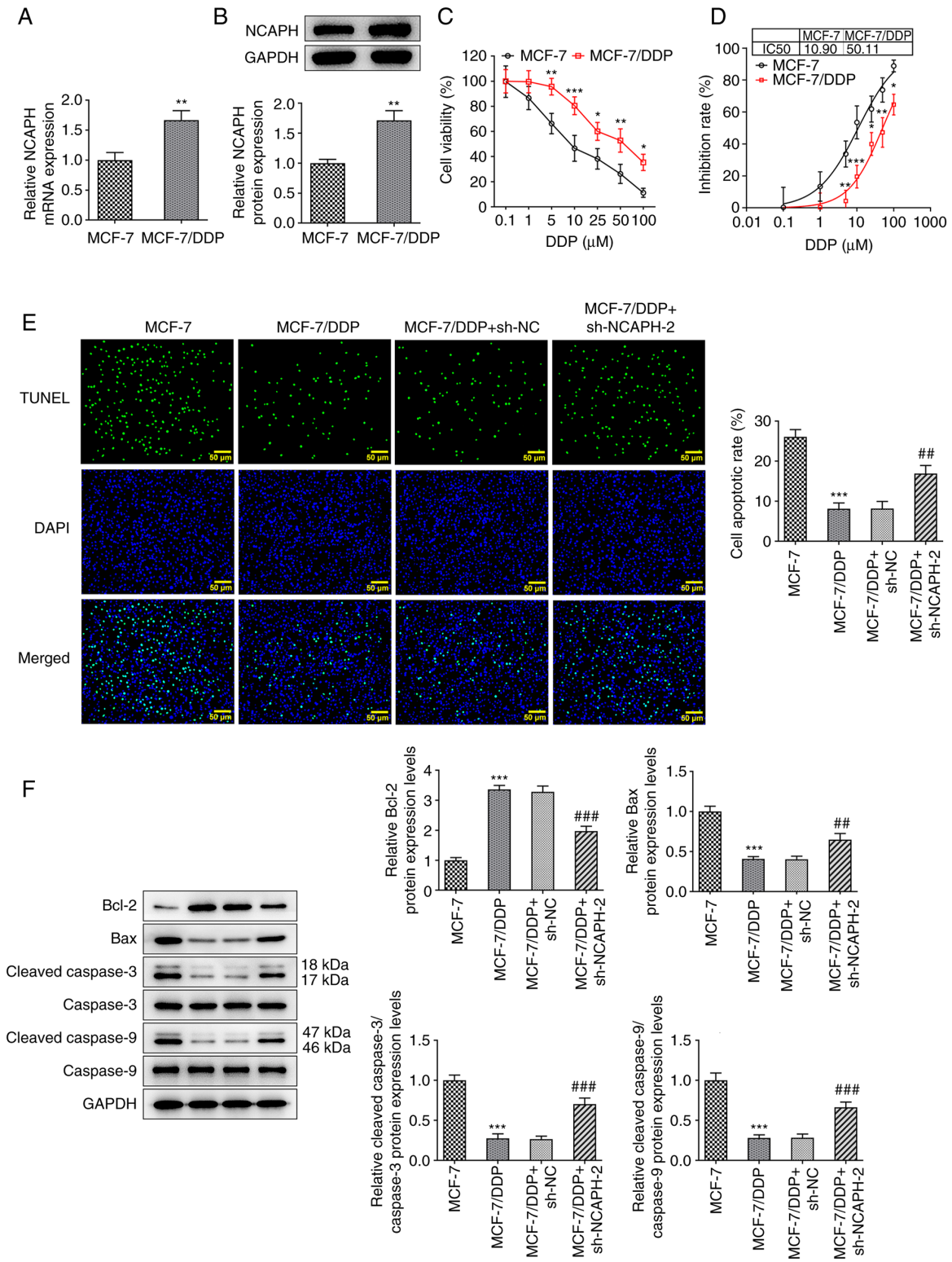

Figure 3. NCAPH silencing alleviates the resistance of breast cancer cells to DDP. mRNA and protein expression levels of NCAPH in MCF-7 and MCF-7/DDP cells were determined via (A) RT-qPCR and (B) western blotting respectively. (C) Effect of different concentrations of DDP on MCF-7 and MCF-7/DDP cell viability was assessed using the Cell Counting Kit-8 assay. (D) IC_{50} values of MCF-7 and MCF-7/DDP cells. (E) Cell apoptotic rate in each group was assessed using the TUNEL assay. Magnification, x200. (F) Protein expression levels of apoptosis-related proteins were detected using western blotting. *P<0.05, **P<0.01 and ***P<0.001 vs. MCF-7; $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. MCF-7/DDP + sh-NC. NCAPH, non-structural maintenance of chromosome condensin I complex subunit H; DDP, cisplatin; RT-qPCR, reverse transcription-quantitative PCR; sh, short hairpin RNA; NC, negative control.

MMP9 compared with the sh-NACPH-2 + oe-NC group (Fig. 5K). Collectively, AURKB overexpression partially reversed the effects of NCAPH knockdown on breast cancer cell proliferation, migration and invasion.

AURKB overexpression partially reverses the effects of NCAPH knockdown on DDP resistance and the Akt/mTOR signaling pathway. Subsequently, the effect of AURKB on the resistance of breast cancer cells to DDP was

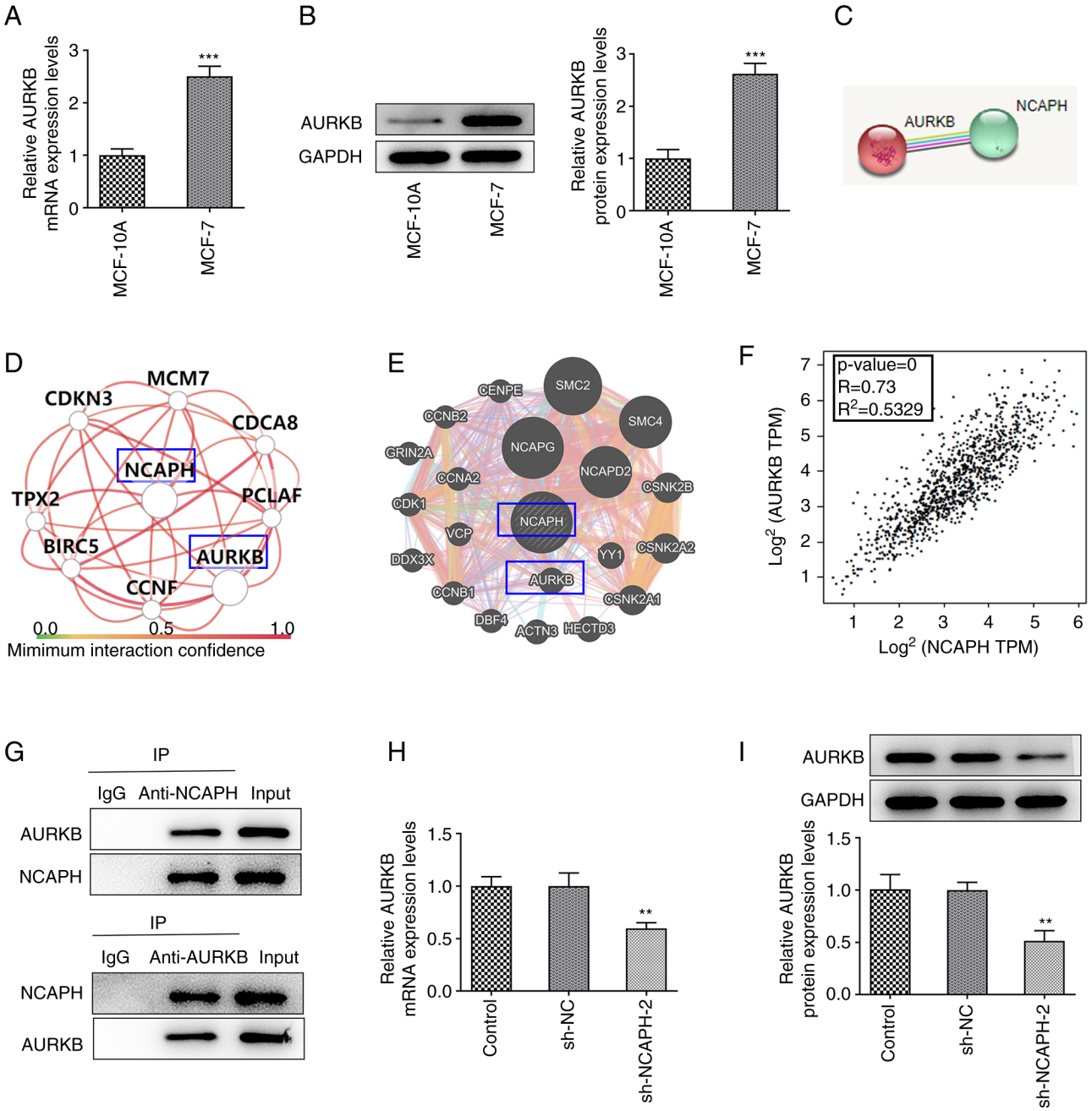

Figure 4. NCAPH knockdown downregulates AURKB. mRNA and protein expression levels of AURKB in MCF-10A and MCF-7 cells were detected via (A) RT-qPCR and (B) western blotting, respectively. (C) Search Tool for the Retrieval of Interacting Genes/Proteins, (D) HumanBase and (E) GeneMANIA databases were used to predict the association between NCAPH and AURKB. (F) Bioinformatics analysis in the Gene Expression Profiling Interactive Analysis database predicted that NCAPH was positively associated with AURKB. P≤0.05 indicates that the result of the model is reliable; R>0 indicates a positive correlation and the closer R^2 is to 1, the more relevant the correlation between NCAPH and AURKB is. (G) Co-immunoprecipitation assay was performed to verify the association between NCAPH and AURKB. mRNA and protein expression levels of AURKB in transfected MCF-7 cells were determined via (H) RT-qPCR and (I) western blotting respectively. **P<0.01 and ***P<0.001 vs. MCF-10A or sh-NC. NCAPH, non-SMC condensin I complex subunit H; AURKB, aurora kinase B; RT-qPCR, reverse transcription quantitative PCR; sh, short hairpin RNA; NC, negative control; TPM, transcript per million; CDKN3, cyclin dependent kinase inhibitor 3; MCM7, mini-chromosome maintenance complex component 7; CDCA8, cell division cycle-associated 8; TPX2, targeting protein for *Xenopus* plus end-directed kinesin-like protein 2; BIRC5, Baculoviral inhibitor of apoptosis domain repeat containing 5; PCLAF, proliferating cell nuclear antigen clamp associated factor; CCN, cyclin; SMC, structural maintenance of chromosome; CENPE, centromere-associated protein E precursor; GRIN2A, glutamate ionotropic receptor N-methyl-D-aspartate type subunit 2A; NCAPG, non-SMC condensin I complex; CSNK, casein kinase; VCP, valosin-containing protein; DDX3X, DEAD-box helicase 3 X-linked; YY1, YY1 transcription factor; DBF4, dumbbell former 4 protein ACTN3, α-actinin-3; HECTD2, HECT domain E3 ubiquitin protein ligase 2; IP, immunoprecipitation.

further assessed. MCF-7/DDP cells were co-transfected with sh-NCAPH and oe-AURKB and were then treated with 10 μM DDP. The results demonstrated that the apoptotic rate in the MCF-7/DDP + sh-NCAPH-2 + oe-AURKB group was significantly reduced compared with the MCF-7/DDP + sh-NCAPH-2 + oe-NC group (Fig. 6A). Furthermore the reduced apoptotic rate in the MCF-7/DDP + sh-NCAPH-2 + oe-AURKB group was further

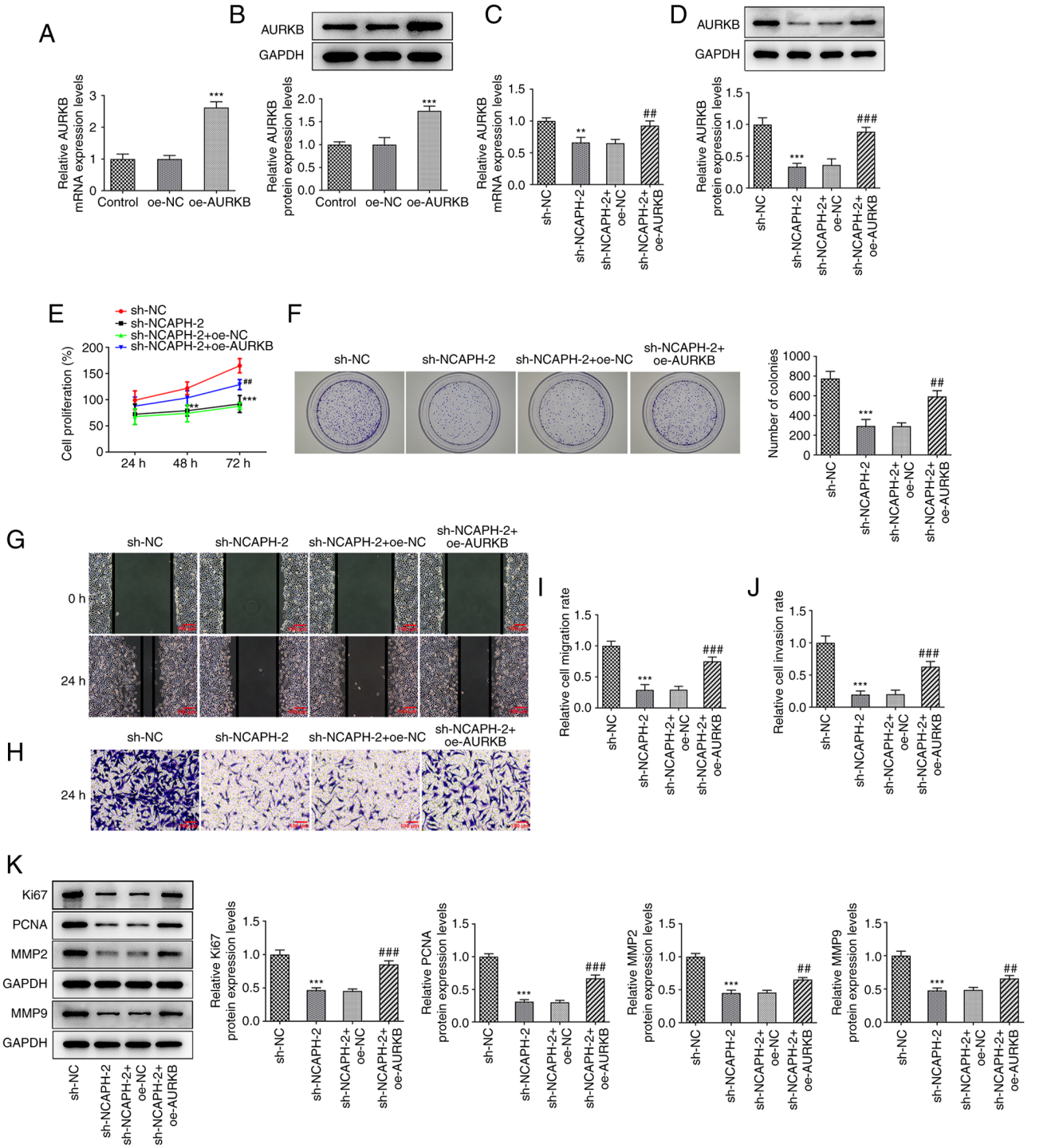

Figure 5. AURKB overexpression partially reverses the effects of NCAPH silencing on cell proliferation, migration and invasion. Transfection efficiency of MCF-7 cells transfected with oe-AURKB was assessed via (A) RT-qPCR and (B) western blotting; respectively. MCF-7 cells were co-transfected with sh-NCAPH and oe-AURKB and the AURKB mRNA and protein expression levels were assessed via (C) RT-qPCR and (D) western blotting respectively. Cell proliferation was assessed using the (E) Cell Counting Kit-8 and (F) colony formation assays. Magnification, x10. (G) Cell migration was determined via wound healing assays. Magnification, x100. (H) Cell invasion was assessed via Transwell assay. Magnification, x100. Quantitative histograms of (I) wound healing and (J) Transwell assays. (K) Western blotting was performed to determine the expression levels of proliferation- and migration-related proteins. **P<0.01 and ***P<0.001 vs. oe-NC or sh-NC; $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. sh-NCAPH-2 + oe-NC. AURKB, aurora kinase B; NCAPH, non-structural maintenance of chromosome condensin I complex subunit H; oe, overexpression; NC, negative control; RT-qPCR, reverse transcription-quantitative PCR; sh, short hairpin RNA; PCNA, proliferating cell nuclear antigen.

supported by the significantly decreased protein expression levels of Bax, cleaved caspase-3 and cleaved caspase-9 levels and the significantly increased protein expression levels of Bcl-2, compared with the MCF-7/DDP + sh-NCAPH-2 + oe-NC group (Fig. 6B). The protein expression levels of Akt/mTOR signaling pathway-related proteins were detected using western blotting. NCAPH knockdown significantly down-regulated the protein expression levels of both phosphorylated

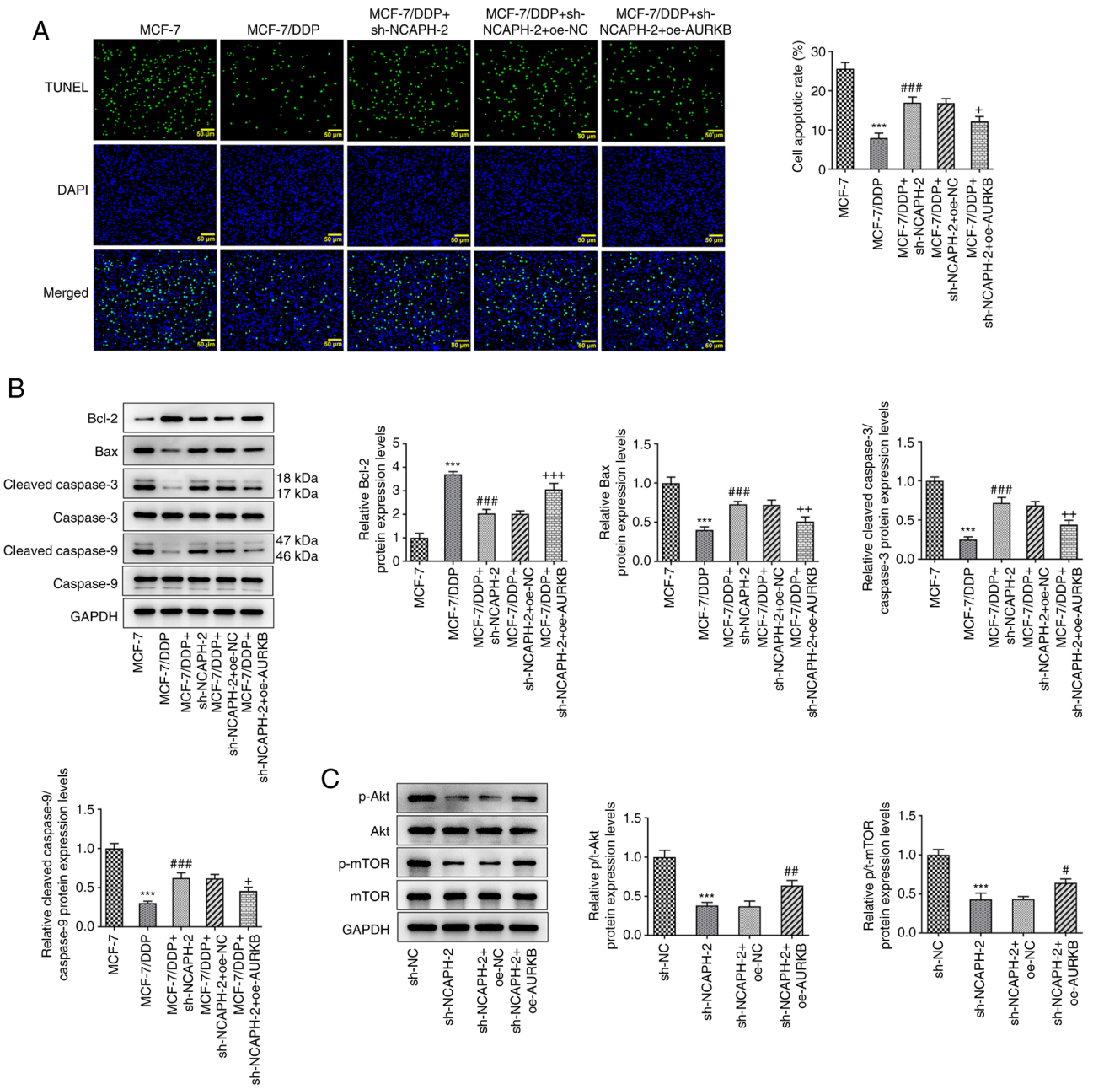

Figure 6. AURKB overexpression partially reverses the effects of NCAPH knockdown on DDP resistance and the Akt/mTOR signaling pathway. (A) Cell apoptotic rate in each group was assessed using the TUNEL assay. Magnification, x200. (B) Protein expression levels of apoptosis-related proteins were detected using western blotting. (C) Protein expression levels of the Akt/mTOR signaling pathway-related proteins were detected via western blotting. ***P<0.001 vs. MCF-7 or sh-NC; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. MCF-7/DDP or sh-NCAPH-2 + oe-NC; $^{+}$P<0.05, $^{++}$P<0.01 and $^{+++}$P<0.001 vs. MCF-7/DDP + sh-NCAPH-2 + oe-NC. AURKB, aurora kinase B; NCAPH, non-structural maintenance of chromosome condensin I complex subunit H; sh, short hairpin RNA; DDP, cisplatin; oe, overexpression; NC, negative control; p, phosphorylated; t, total.

(p)-Akt and p-mTOR compared with the sh-NC group, which were significantly restored following AURKB overexpression compared with the sh-NACPH-2 + oe-NC group (Fig. 6C). Taken together, AURKB overexpression partially reversed the effects of NCAPH knockdown on breast cancer cell DDP resistance and the Akt/mTOR signaling pathway.

Discussion

Breast cancer is the leading cause of cancer-related deaths in women and is principally classified into three subtypes based on the presence of estrogen (ER) and progesterone (PR) receptors and human epidermal growth factor receptor 2 (HER2), in the breast cancer tissue (19). In total, ~90% of all breast cancer cases are not metastatic at the time of diagnosis (20). Therefore, the goal for non-metastatic breast cancer treatment is to eradicate the tumor and prevent recurrence (21). Endocrine therapy, chemotherapy and combined chemotherapy accompanied by tumor resection, can be performed depending on the breast cancer subtype (22). Triple-negative breast cancer is most likely to recur and is characterized by a 5-year survival rate of 85% for stage I cancer compared with >94% for PR-positive and HER2-positive breast cancer (21). Instead of preventing recurrence, the aim of metastatic

breast cancer treatment is to prolong life and relieve symptoms. Therefore, chemotherapy and radiotherapy are most commonly adopted (23). According to a previously published study, ER-positive/PR-positive/HER2-negative cancer accounts for the majority of all breast cancer cases (24) and is clinically known as luminal A breast cancer. It has previously been reported that MCF-7 cells, mimic luminal breast cancer cells (25). In the present study, the results demonstrated that NCAPH mRNA and protein expression levels were significantly upregulated in breast cancer cells. Furthermore, NCAPH knockdown significantly decreased the proliferation, migration and invasion abilities of MCF-7 cells and reduced DDP resistance in MCF-7/DDP cells.

Bioinformatic analysis indicated that AURKB was a downstream regulatory target of NCAPH. AURKB belongs to a group of highly conserved AURK isoforms that together with AURKA and AURKC regulate chromosome arrangement and segregation during mitosis and meiosis in mammals (26). Previous studies have reported that during the chromosome segregation stage, abnormal AURKB expression can promote the formation of abnormal binucleate daughter cells via cytoplasmic bridges, which results in tumorigenesis (27,28). The results of the present study demonstrated that the mRNA and protein expression levels of AURKB were significantly upregulated in MCF-7 cells. Furthermore, AURKB overexpression could potentially reverse the inhibitory effect of NCAPH knockdown on the progression of breast cancer and on the resistance of breast cancer cells to DDP. The aforementioned results suggested that AURKB could potentially trigger the tumor-promoting and regulatory effects of NCAPH. It can therefore be hypothesized that this process may be caused via AURKB regulation of the phosphorylation of NCAPH-containing condensin and histone ligation, suggesting a role for AURKB in histone phosphorylation (29). Abnormal NCAPH expression may lead to the unregulated expression of AURKB, which may cause the dysregulation of mitosis. However, the specific regulatory mechanism of this merits further research. Furthermore, a previous study reported that AURKB is upregulated in gastric cancer, whereas AURKB silencing can inhibit the invasion and migration of gastric cancer cells (30). Moreover, AURKB is associated with the resistance of NSCLC cells to DDP, which could be associated with a poor prognosis in patients with breast cancer (31).

Mechanistically, mTOR serves an integral role in signal transduction pathways involved in the regulation of cell proliferation, protein synthesis and survival. mTOR is also involved in several cellular processes that may lead to the uncontrolled proliferation of cancer cells (32,33). In the present study, the protein expression levels of mTOR and those of its upstream target Akt were detected and the results demonstrated that NCAPH knockdown significantly inhibited the activation of the Akt/mTOR signaling pathway. However, AURKB overexpression significantly activated Akt/mTOR signaling. These findings suggested that AURKB could potentially mediate the regulation of the Akt/mTOR signaling pathway via NCAPH. A previous study reported that AURKB cooperates with histone deacetylases to regulate the Akt signaling pathway (34); however, the specific underlying regulatory mechanism requires future exploration. To the best of our knowledge the present study was the first to present the mechanism of NCAPH in breast cancer cells, as well as its role in DDP resistance. However, the present study was limited to *in vitro* experiments and therefore further *in vivo* experiments are required to confirm the aforementioned findings.

In conclusion, the present study demonstrated that NCAPH knockdown significantly downregulated AURKB and significantly inhibited breast cancer cell proliferation, migration, invasion, DDP resistance and the Akt/mTOR signaling pathway. These findings have provided novel insights into the identification of novel therapeutic targets in breast cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LL and HC designed the study and performed the experiments. SL performed the experiments and data analysis, and made considerable contributions to the drafting of the manuscript. All authors read and approved the final manuscript. LL and SL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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