Physiology International 109 (2022) 3, 334–347
DOI: 10.1556/2060.2022.00028

AKADÉMIAI KIADÓ

NCAPH promotes proliferation as well as motility of breast cancer cells by activating the PI3K/AKT pathway

TING ZHANG, PENG LI, WANYING GUO, QIPENG LIU, WEIQIANG QIAO and MIAO DENG*

Department of Breast Surgery, The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology, Luoyang, Henan Province, 471003, China

Received: January 10, 2022 • Revised manuscript received: April 11, 2022 • Accepted: May 16, 2022
Published online: September 5, 2022

ABSTRACT

Objective: This study aimed to assess the expression of NCAPH in human breast cancer, and to investigate its effects on breast cancer cells. *Methods:* Bioinformation analysis was performed to analyze the expression of NCAPH in human breast cancer tissues and normal tissues in TCGA database. qPCR and Immunoblot assays were performed to clarify the expression of NCAPH in breast cancer tissues and cell lines, respectively. CCK-8, colony formation, FCM, transwell, and immunoblot assays were performed to reveal the effects of NCAPH on breast cancer proliferation, cell cycle, motility and EMT of breast cancer cells. Additionally, immunoblot assays were performed to investigate the effects of NCAPH on the PI3K/AKT pathway in breast cancer. *Results:* We found that NCAPH was highly expressed in human breast cancer cell lines. The depletion of NCAPH suppressed the viability of breast cancer cells. Further, we noticed that its downregulation restrained breast cancer cell migration as well as invasion, and the EMT process. Mechanically, we noticed that NCAPH mediated the PI3K/AKT pathway, and therefore contributed to breast cancer progression. *Conclusion:* In summary, NCAPH has the potential to serve as a breast cancer target.

KEYWORDS

NCAPH, breast cancer, viability, motility, EMT, PI3K/AKT pathway

* Corresponding author. Department of Breast Surgery, The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology, No. 24 Jinghua Road, Jianxi District, Luoyang, Henan Province, China. Tel: +86-0379-64541520. E-mail: Dengmiao_666@163.com

INTRODUCTION

Breast cancer is one of the three most common cancers worldwide [1]. In recent years, the incidence of breast cancer (BC) has been increasing, and the onset age is getting younger, posing a serious threat to women's health [1, 2]. The etiology of breast cancer is not completely clear [3, 4]. With the development of medical science, the prognosis of breast cancer has improved, but the incidence of breast cancer is still increasing [5]. In view of the lack of obvious symptoms of early breast cancer, some breast cancer patients are often in the advanced stage when they seek treatment, and breast cancer often develops metastases [6]. Conventional treatments, such as surgical excision and radiation and chemotherapy, are ineffective in treating advanced breast cancer [7]. To further improve the prognosis of patients, research on the pathogenesis of BC is urgently needed to develop more effective therapeutic drugs by means of targeted therapy, and to identify the key therapeutic targets [8]. In addition, epithelial-mesenchymal transformation (EMT) is a biological process in which epithelial cells are transformed into mesenchymal phenotypes through a specific procedure, which plays an important role in breast cancer progression and metastasis.

NCAPH, also known as BRRN1, maintains the stability of the agglutinin protein complex and ensures accurate separation of the sister chromatids during the mitosis process [9]. The role of NCAPH in the progression and metastasis of cancers has been widely reviewed [9, 10]. The earliest study found that NCAPH expression was significantly increased in advanced melanoma [11]. High expression of NCAPH was also associated with poor prognosis in patients with lung cancer, prostate cancer, and ovarian cancer [12, 13].

In addition, down-regulation of NCAPH significantly suppressed the proliferation and motility of colon cancer and liver cancer, and affected cervical cancer progression via regulating the activation of the PI3K/AKT/SGK pathway [14, 15]. Notably, upregulation of NCAPH in breast cancer indicated poor prognosis [16]. However, the function of NCAPH and the underlying molecular mechanisms in breast cancer remain unclear.

In this study, we investigated the possible effect of NCAPH on the progression of breast cancer and explored its mechanism. Our data confirmed the high NCAPH expression in human BC cells, and indicated that its depletion suppressed the proliferation and motility of BC cells via targeting the PI3K/AKT pathway. Therefore, it has the potential to be explored as a BC target.

MATERIALS AND METHODS

Antibodies and plasmids

NCAPH antibody (1:500 dilution, ab154105, Abcam), E-cadherin antibody (1:500 dilution, ab212059, Abcam), N-cadherin antibody (1:1000 dilution, ab76011, Abcam), Vimentin (1:500 dilution, ab92547, Abcam), ZO-1 antibody (1:500 dilution, ab221547, Abcam), p-PI3K antibody (1:1000 dilution, ab278691, Abcam), PI3K antibody (1:500 dilution, ab32089, Abcam), p-AKT antibody (1:500 dilution, ab38449, Abcam), AKT antibody (1:500 dilution, ab8805, Abcam), and beta-actin antibody (1:2000 dilution, ab8226, Abcam) were obtained from the indicated company.

The NC (negative control) siRNAs and two NCAPH siRNAs were bought from Riobio. The sequence of NCAPH siRNA was #1: 5′-GAGUUCAGGAGCUGGAAGG-3′. #2: 5′-ACCA-CAGGGAAGCUGGAAA-3′.

The plasmids including pcDNA3.1-vector and pcDNA3.1-control were constructed in our lab. LY294002 was bought from Sigma.

Cell culture and transfection

The human breast cell line MCF-10A and breast cancer cell line MCF-7 and T47D were purchased from ATCC. The cell lines were maintained in Dulbecco's modified Eagle medium (DMEM; Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and maintained in a 5% CO_2 incubator at 37 °C.

siRNAs and plasmids were purchased from RioBio (Guangzhou, China) and addgene (USA), respectively, and transfected into cells using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.). A total of 1×10^5 cells were seeded into six-well plates and 5 μL siRNAs or 1 ug plasmid were used for transfection. The cells were transfected using 10 μL Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) in each well. Cells were cultured for 4 h with Lipofectamine®/plasmid mix at 37 °C and the transfection was completed. Subsequent assays were performed after 24 h. LY294002 (10 μM) was used and the cells were treated for 24 h.

Quantitative PCR assays

The study was approved by the ethical committee of the First Affiliated Hospital, and the College of Clinical Medicine of Henan University of Science and Technology, and informed consent was provided by all patients. Total RNA was extracted from cells using Trizol reagent (15596-018, Invitrogen). Quantitative PCR was subsequently conducted via SYBR mixture (RR420A, Takara). Total RNA was reverse transcribed into cDNA at 42 °C for 1 h using M-MLV reverse transcriptase (cat. no. M1701; Promega Corporation, includes M-MLV 5X Reaction Buffer 5 μL, dNTP, 10 mM 1.25 μL, Recombinant RNasin® Ribonuclease Inhibitor 25 units, M-MLV RT 200 units, and Nuclease-Free Water to final volume 25 μL). NCAPH mRNA levels were normalized to GAPDH. PCR primer sequences of NCAPH were: forward, 5′-AAA-CAACCTCAATGTCTCCGAAG-3′ and reverse, 5′-ACAACCTAACTCT GGCAACTCG -3′. The following thermocycling conditions were used: Initial denaturation at 95 °C for 3 min; followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s. The 2-ΔΔCq method was used to quantify the results.

Immunoblot assay

Samples were lysed by the use of RIPA buffer (Beyotime, China). The BCA kit was used for protein concentration determination, after which proteins were separated (20 μg per lane) by 10% SDS-PAGE, then transferred onto PVDF membranes. Then membranes were blocked with 5% fat-free milk. All membranes were subsequently incubated with the indicated primary antibodies for 1.5 h. Subsequently the membranes were treated with secondary antibodies for 1 h. Signals were visualized using an ECL kit (Beyotime Institute of Biotechnology). ImageJ (version 1.8.0; National Institutes of Health) was used for densitometry.

CCK-8 assay

Breast cancer cells were plated into the 96-well plates (1000 cell/well) and maintained for 48 h. Cells were then incubated with CCK-8 for 4 h and the OD 450 value was measured.

Colony formation assay

Breast cancer cells were re-seeded into 6-well plates with a density of 1000 cells in each well and maintained for 14 d. Colonies were considered to consist of >100 cells. Then colonies were fixed with PFA for 10 min and stained with 0.1% crystal violet for 30 min. Colonies were then photographed.

Cell cycle assay

The cells were fixed using 70% ethyl alcohol for 24 h at −20 °C and incubated with 100 μg mL^{-1} PI at 37 °C for 20 min; subsequently the assays were conducted by the flow cytometer. The percentages of cells at different phases were compared.

Transwell assays

Breast cancer cells transfected with the indicated plasmids were plated into the upper chamber with 20% matrigel (Transwell-invasion) or without matrigel (Transwell-migration). Then, medium (10% FBS) was added into the bottom chambers to stimulate invasion or migration. After 24 h, the remaining cells were stained with 0.1% crystal violet for 20 min.

Statistics

GraphPad 5.0 software was used for the statistical analysis. Data were represented as mean ± SEM. The results in this study were analyzed by the use of one-way ANOVA followed by Turkey's post hoc test when multiple groups were compared, and $P < 0.05$ was considered as significant.

RESULTS

NCAPH was expressed at high level in breast cancer

To uncover the effects of NCAPH on BC progression, we first analyzed the expression of NCAPH in human breast cancer tissues through the TCGA database. We noticed that the TPM of NCAPH in tumor tissues ($n = 1085$) was higher than normal ($n = 291$), suggesting high expression (Fig. 1A). qPCR assays were conducted to confirm the mRNA levels of NCAPH in BC tissues and adjacent tissues collected. Consistently, increased levels of NCAPH mRNA were determined in breast cancer tissues (Fig. 1B).

We further detected NCAPH in the normal cell line MCF10A and in breast cancer cell lines MCF-7 and T47D. Through immunoblot assays, we found high NCAPH levels in BC cell lines MCF-7 and T47D, compared to the normal breast cell line MCF10A (Fig. 1C). Taken together, we detected high expression of NCAPH in BC.

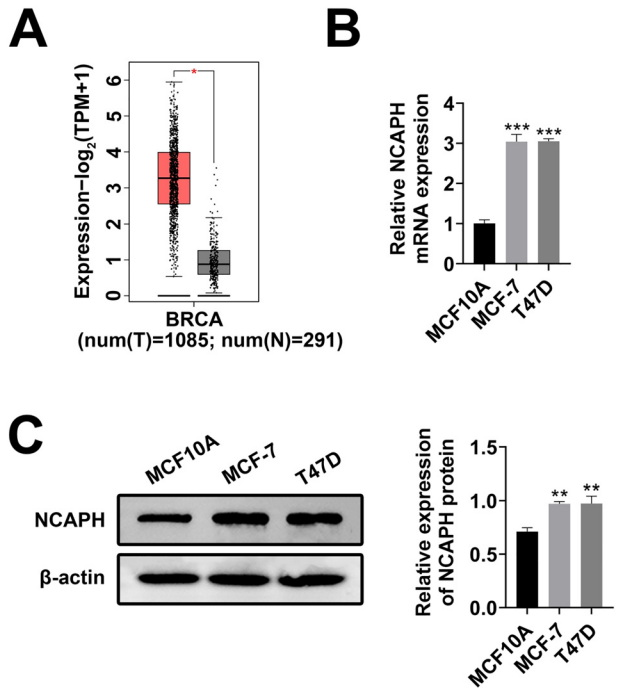

Fig. 1. NCAPH was expressed at high level in breast cancer tissues and cell lines (A). GEPIA was used to analyze the data in the TCGA database, which showed the levels of transcript per million (TPM) in 1085 tumor tissues compared to 291 normal tissues. (B) qPCR assays showed the mRNA levels of NCAPH in breast cancer tissues and normal tissues. (C). Immunoblot assays showed the mRNA levels of NCAPH in normal cell line MCF-10A, and 2 breast cancer cell lines, MCF-7 and T47D. Data are presented as mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$. Each experiment was performed in 3 replicates.

NCAPH promotes the proliferation of breast cancer cells

In view of the high expression of NCAPH in BC, we then investigated the role of NCAPH in breast cancer cell proliferation. Two NCAPH siRNAs and pcDNA3.1-NCAPH plasmids, respectively, were used and transfected into MCF-7 and T47D cells to alter the expression of NCAPH in breast cancer cells. We detected decreased expression of NCAPH after transfection with its siRNA, and found the obvious high expression after the transfection of pcDNA3.1-NCAPH plasmids in MCF-7 and T47D cells (Fig. 2A, S1A). Through CCK-8 assays, we further found that downregulation of NCAPH suppressed the proliferation of MCF-7 and T47D cells, with decreased OD value at 450 nm, and its overexpression promoted cell proliferation (Fig. 2B, S1B). Similarly, we found that knockdown of NCAPH restrained MCF-7 and T47D cell proliferation, whereas its overexpression promoted proliferation (Fig. 2C, S1C). Through FCM assays, we further found that downregulation of NCAPH suppressed the cell cycle in both MCF-7 and T47D cells (Fig. 2D, S1D). Therefore, NCAPH promoted BC proliferation.

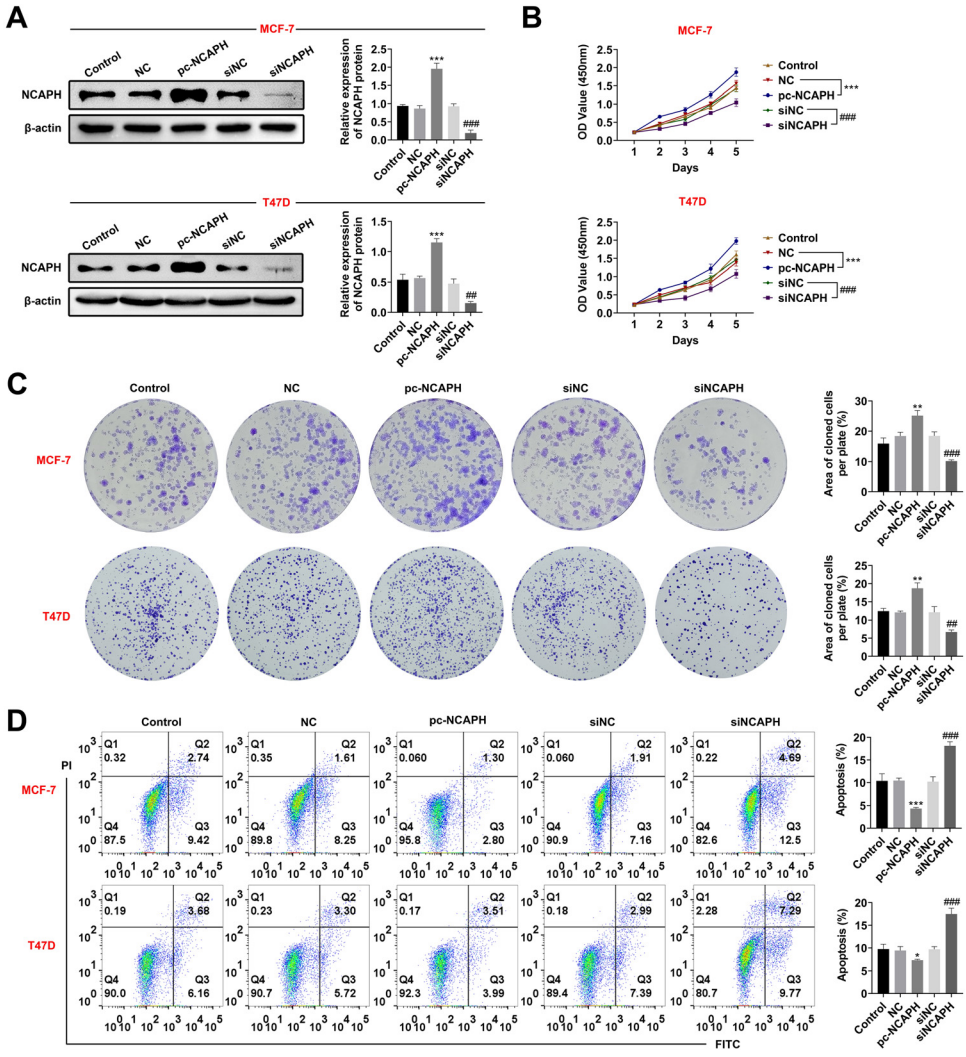

Fig. 2. NCAPH ablation suppressed the viability of breast cancer cells and stimulated apoptosis (A). Immunoblot showed the expression of NCAPH in MCF-7 cells upon transfection with control or NCAPH siRNA (#2) or plasmids. (B). CCK-8 assays showed the OD value at 450 nm wavelength of MCF-7 cells upon transfection with control or NCAPH siRNAs or plasmids. (C). Colony formation assays showed the colony number of MCF-7 cells upon transfection with control or NCAPH siRNAs or plasmids. (D). Flow cytometry (FCM) assays showed the apoptosis percentage of MCF-7 cells upon transfection with control or NCAPH siRNAs or plasmids. Data are presented as mean ± SEM, siNCAPH vs siNC, ** $P < 0.01$, *** $P < 0.001$. pcDNA3.1-NCAPH vs pcDNA3.1, ## $P < 0.01$, ### $P < 0.001$. NC, negative control. Each experiment was performed in 3 replicates.

NCAPH stimulates the motility of breast cancer cells *in vitro*

Since NCAPH could regulate the proliferation of breast cancer cells, we further investigated its effects on the motility of breast cancer cells through transwell assays. We observed that knockdown of NCAPH dramatically restrained the migration of BC cells, resulting in decreased stained cell number, whereas NCAPH overexpression increased cell number, suggesting that NCAPH stimulated the migration of breast cancer cells (Fig. 3A). Subsequently we performed

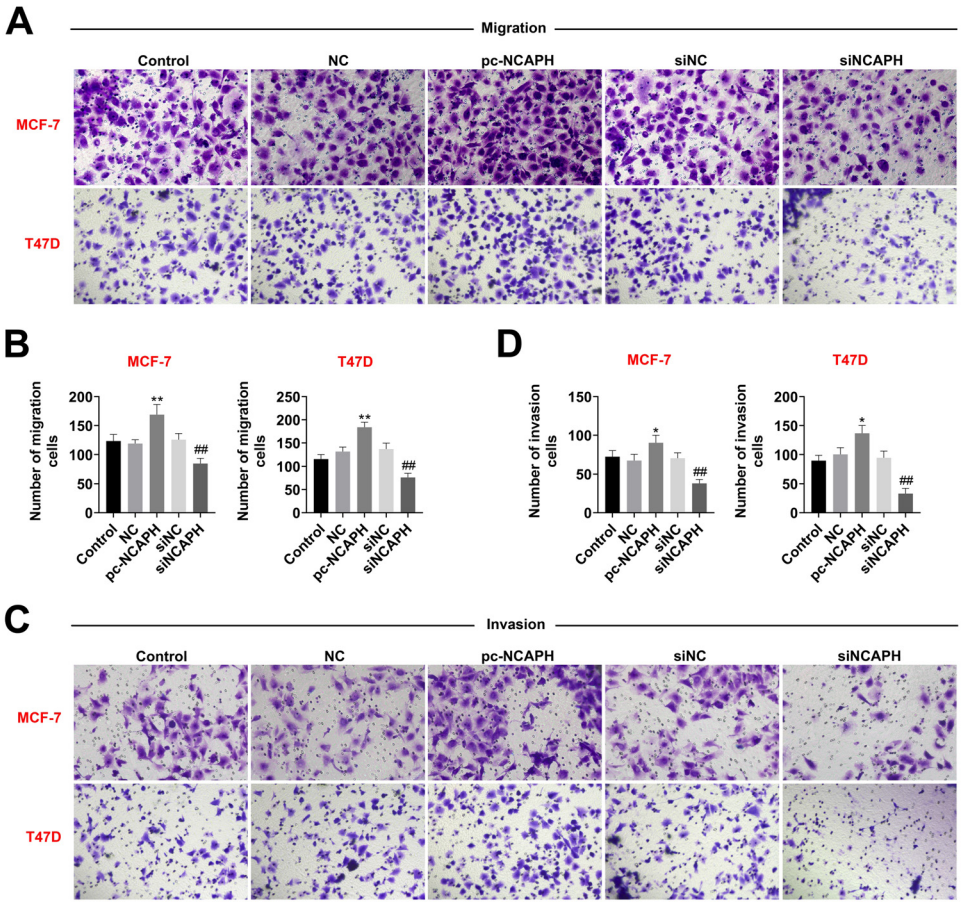

Fig. 3. NCAPH knockdown inhibited the migration as well as invasion of breast cancer cells (A, B). Transwell-migration assays showed the wound healing degree of MCF-7 and T47D cells upon transfection with control or NCAPH siRNAs or plasmids. Representative images are shown in (A). Cell number is analyzed in (B). (C, D). Transwell-invasion assays showed the invasive MCF-7 and T47D cells upon transfection with control or NCAPH siRNAs or plasmids. Representative images are shown in (C). I Invasive cell number is shown in (D). Data are presented as mean ± SEM, siNCAPH vi siNC, * $P < 0.05$, ** $P < 0.01$. pcDNA3.1-NCAPH vs pcDNA3.1, ## $P < 0.01$. NC, negative control. Each experiment was performed in for 3 replicates.

transwell-invasion assays, and the results revealed that NCAPH knockdown suppressed breast cancer cell invasion, whereas overexpression of NCAPH promoted the invasion (Fig. 3B–D). We therefore concluded that NCAPH stimulated the motility of BC cells *in vitro*.

Knockdown of NCAPH suppressed EMT in breast cancer cells

Having shown the effects of NCAPH on BC cell viability and migration, we then investigated its role in the EMT process of breast cancer cells. We detected the expression of several EMT markers. Through immunoblot assays, we found that NCAPH knockdown increased the protein levels of E-cadherin and ZO-1, and downregulated the expression of N-cadherin and Vimentin in BC cells, whereas its overexpression decreased the protein levels of E-cadherin and ZO-1, and upregulated those of N-cadherin and Vimentin (Fig. 4). Therefore, downregulation of NCAPH suppressed the EMT process in BC cells.

NCAPH activates the PI3K/AKT pathway in breast cancer cells

Several studies have shown that the PI3K/AKT signaling pathway was widely involved in the regulation of cancer cell proliferation and migration. We next tested whether NCAPH promoted BC via this pathway.

Through immunoblot assays, NCAPH overexpression was shown to increase the phosphorylation levels of PI3K and AKT in BC cells (Fig. 5A). Additionally, downregulation of NCAPH suppressed the levels of PI3K and AKT phosphorylation in both MCF-7 and T47D cells (Fig. 5B), further confirming the regulation of NCAPH by this pathway. Interestingly, our data revealed that treatment with LY294002, an inhibitor of PI3K/AKT pathway could rescue activation of the PI3K/AKT pathway in breast cancer cells caused by NCAPH overexpression (Fig. 6A), confirmed by immunoblot assays. CCK-8 and colony formation assays further confirmed that LY294002 treatment rescued the promotion of cell proliferation caused by NCAPH overexpression in BC cells (Fig. 6B and C). In addition, FCM assays also revealed that LY294002 reversed the suppression of BC cell apoptosis caused by NCAPH overexpression (Fig. 6D). Therefore, NCAPH activates the PI3K/AKT signaling pathway in BC cells.

DISCUSSION

Breast cancer ranks first in the incidence of female malignant tumor [17]. The incidence of BC is increasing, with more than 300,000 women diagnosed with BC each year [18]. The pathogenesis of BC is still unclear, and a variety of high-risk factors have been proved to promote the occurrence and development of breast cancer [18]. Although commonly used treatments, such as surgical resection and chemoradiotherapy improve patients' prognosis to some extent, they are still ineffective for patients with advanced BC [18]. Recently, targeted therapy has attracted extensive attention, and many targeted therapy drugs have been successfully used in the clinical treatment of breast cancer, achieving a series of positive effects [19]. Our data confirmed that NCPAH contributed to the viability, motility and EMT of breast cancer cells.

Through a series of *in vitro* assays, such as CCK-8, colony formation, FCM, immunoblot, and transwell, we found that NCPAH promoted the cellular processes of breast cancer cells, such as proliferation, motility, and EMT. Our data confirmed the promoting effects of NCPAH on

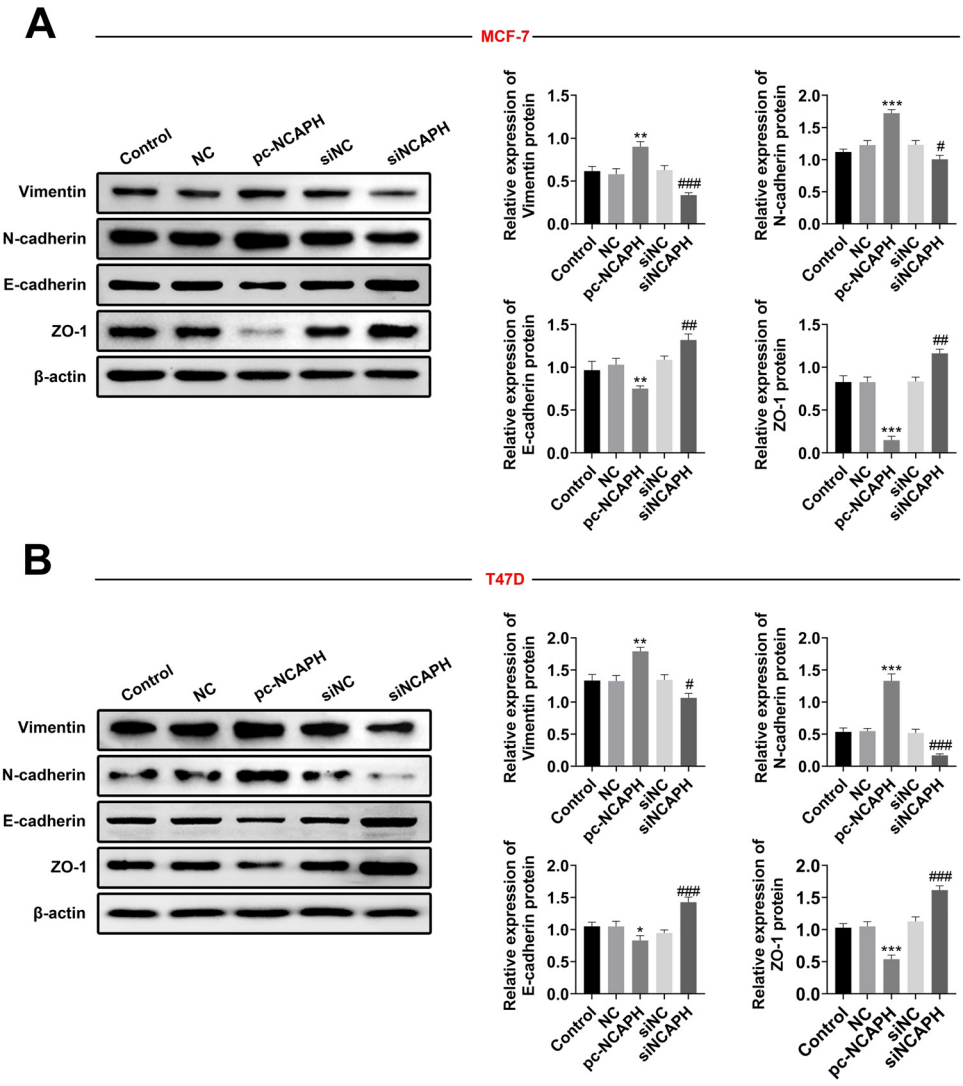

Fig. 4. NCAPH promoted the EMT process in breast cancer cells

Immunoblot assays showed the expression of E-cadherin, N-cadherin, Vimentin, and ZO-1 in MCF-7 and T47D cells upon transfection with control or NCAPH siRNAs or plasmids. Data are presented as mean ± SEM, siNCAPH vi siNC, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. pcDNA3.1-NCAPH vs pcDNA3.1, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$. NC, negative control. Each experiment was performed in 3 replicates.

breast cancer progression. Similarly, the findings of the paper by T. Ogura et al. (2021) indicated the effects of NCAPH on the proliferation and cell cycle of breast cancer cells, suggesting that NCAPH could serve as a promising target of breast cancer. Since there are several subtypes of breast cancer, we should next clarify whether NCAPH affects all types of breast cancer. In addition, the effects of NCAPH on different types of tumors have been widely reported. For

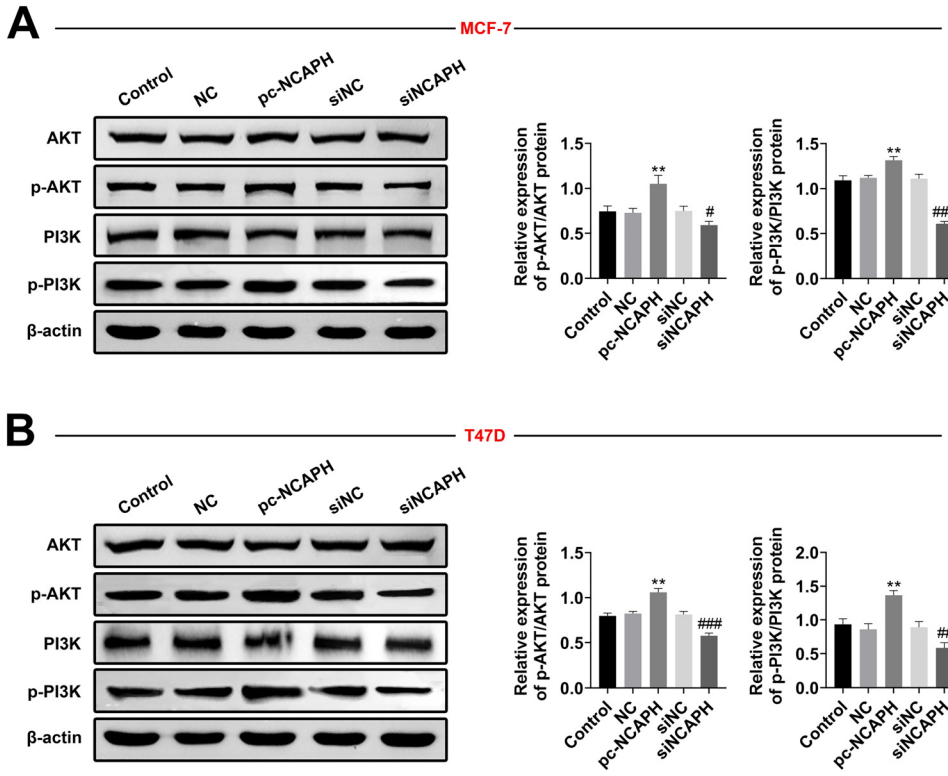

Fig. 5. NCAPH mediated the AKT/PI3K pathway in breast cancer cells
Immunoblot assays showed the expression of p-AKT, AKT, p-PI3K, and PI3K in BC cells upon the indicated treatment. Data are presented as mean ± SEM, NCAPH vi Control, ** $P < 0.01$. siNCAPH vs siControl, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$. NC, negative control. Each experiment was performed in 3 replicates.

example, NCAPH contributed to the proliferation of bladder cancer cells via the MEK/ERK pathway, which was similar to our finding [9]. However, these authors also described the effects of NCAPH on the apoptosis of bladder cancer cells. The effects of NCAPH on breast cancer apoptosis need further study [9]. Another study indicated that NCAPH mediated the progression of gastric cancer via affecting DNA damage response [10]. NCAPH was negatively correlated with Mcl-1 expression in lung cancer [20]. Importantly, another study showed that NCAPH was required for the viability and motility of lung cancer cells, similar to our study [12]. In addition, NCAPH was also correlated with lymphangiogenesis and drug resistance in oral cancer, and mediated mature chromosome condensation as well as DNA damage in pancreatic cancer (PDAC) cells [21, 22]. All these studies confirmed that NCAPH could affect the progression of multiple types of tumors.

Notably, ectopic expression of NCAPH mediated the progression of cervical carcinoma via the PI3K/AKT axis. Importantly, we also found that NCAPH mediated this pathway in breast cancer cells. The PI3K/AKT pathway can promote the progression of multiple cancer types [23].

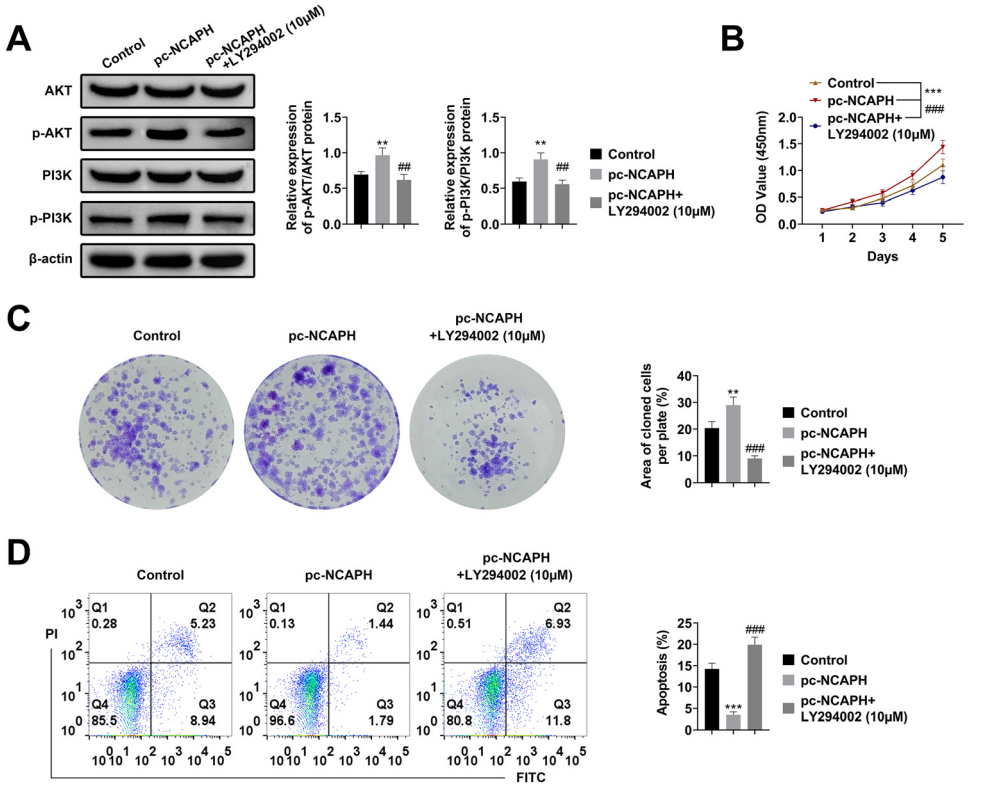

Fig. 6. AKT/PI3K pathway inhibition reversed the effects of NCAPH overexpression on breast cancer progression

(A) Immunoblot assays showed the expression of p-AKT, AKT, p-PI3K, and PI3K in breast cancer cells upon the indicated treatment. (B). CCK-8 assays showed the OD value at 450 nm wavelength of breast cancer cells upon the indicated treatment. (C). Colony formation assays showed the colony number of breast cancer cells upon the indicated treatment. (D). Flow cytometry (FCM) assays showed the percentage of apoptosis upon the indicated treatment. Data are presented as mean ± SEM, NCAPH vi Control, ** $P < 0.01$, *** $P < 0.001$. NCAPH+LY294002 (10 μM) vs NCAPH, ## $P < 0.01$, ### $P < 0.001$. NC, negative control. Each experiment was performed in 3 replicates.

The PI3K/AKT pathway has been revealed to affect the proliferation and motility of tumors as well as EMT progression [24]. Multiple proteins or drugs affect breast cancer progression via this pathway [24]. Andrographolide could suppress proliferation in bladder cancer cells by interfering with PI3K/AKT signaling [24]. Amorphophalli rhizoma could restrain breast cancer cell proliferation and motility via this pathway [23]. All these studies suggest that the PI3K/AKT pathway could serve as a BC target, and until now, several drugs targeting this pathway have been successfully developed.

NCAPH is a protein which maintains the stability of the agglutinin protein complex and ensures accurate separation of the sister chromatids during mitosis. The role of NCAPH in the progression of cancers has also been revealed. In this study, we found that NCAPH stimulated

the PI3K/AKT pathway in breast cancer cells, and therefore affected the proliferation, motility, and EMT of breast cancer cells. In fact, NCAPH is not a transcription factor, and no data demonstrate any interactions between NCAPH and other proteins. We therefore need further studies to show the more precise mechanism.

In summary, we found high NCAPH expression in human breast cancer cells and tissues. NCAPH knockdown restrained the viability, motility, and EMT of BC cells. Mechanistic studies showed that NCAPH could mediate the PI3K/AKT pathway, thus affecting BC progression. Therefore, our study suggests that NCAPH has the potential to serve as a BC target.

Funding: Not applicable.

Competing interests: The authors state that there are no conflicts of interest to disclose.

Ethics approval: Not applicable.

Statement of human and animal rights: Not applicable.

Statement of informed consent: Not applicable.

Authors' contributions: Ting Zhang and Peng Li designed the study and carried out the experiments, Wanying Guo and Qipeng Liu supervised the data collection, analyzed the data, interpreted the data, Weiqiang Qiao and Miao Deng prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ACKNOWLEDGMENTS

Not applicable.

REFERENCES

1. Romero-Cordoba S, Tagliabue E, Triulzi T, Bianchi F. Deep into breast cancer heterogeneity to increase immunotherapeutic effectiveness. JCO Precis Oncol 2020; 4: 1267–8. https://doi.org/10.1200/PO.20.00313.
2. Park JH, Han HS, Lim SD, Kim WY, Park KS, Yoo YB, et al. Fatty acid synthetase expression in triple-negative breast cancer. J Pathol Transl Med 2022; 56(2): 73–80. https://doi.org/10.4132/jptm.2021.10.27.
3. Ahearn TU, Zhang H, Michailidou K, Milne RL, Bolla MK, Dennis J, et al. Common variants in breast cancer risk loci predispose to distinct tumor subtypes. Breast Cancer Res 2022; 24(1): 2. https://doi.org/10.1186/s13058-021-01484-x.
4. Camilloni A, Nati G, Maggiolini P, Romanelli A, Carbone G, Giannarelli D, et al. Chronic non-cancer pain in primary care: An Italian cross-sectional study. Signa Vitae 2021; 17(2): 54–62. https://doi.org/10.22514/sv.2020.16.0111.
5. Yang ZY, Yin SP, Ren Q, Lu DW, Tang T, Li Y, et al. BAHD1 serves as a critical regulator of breast cancer cell proliferation and invasion. Breast Cancer 2022; 29(3): 516–30. https://doi.org/10.1007/s12282-022-01333-5.

6. Dai R, Peng X, Lin B, Xu D, Lv R. NIR II Luminescence imaging for sentinel lymph node and enhanced chemo-/photothermal therapy for breast cancer. Bioconjug Chem 2021; 32(9): 2117–27. https://doi.org/10.1021/acs.bioconjchem.1c00393.
7. Hickey BE, Lehman M. Partial breast irradiation versus whole breast radiotherapy for early breast cancer. Cochrane Database Syst Rev 2021; 8: CD007077. https://doi.org/10.1002/14651858.CD007077.pub4.
8. Karahaliloglu Z, Kilicay E, Hazer B. Herceptin-conjugated magnetic polystyrene-Agsbox nanoparticles as a theranostic agent for breast cancer. J Biomater Appl 2022; 36(9): 1599–616. https://doi.org/10.1177/08853282211065085.
9. Li B, Xiao Q, Shan L, Song Y. NCAPH promotes cell proliferation and inhibits cell apoptosis of bladder cancer cells through MEK/ERK signaling pathway. Cell Cycle 2022; 21(4): 427–38. https://doi.org/10.1080/15384101.2021.2021050.
10. Wang Y, Li JQ, Yang ZL, Wang L, Zhang JC, Sun YF, et al. NCAPH regulates gastric cancer progression through DNA damage response. Neoplasma 2022; 69(2): 283–91. https://doi.org/10.4149/neo_2021_210607N761.
11. Ryu B, Kim DS, Deluca AM, Alani RM. Comprehensive expression profiling of tumor cell lines identifies molecular signatures of melanoma progression. PLoS One 2007; 2(7): e594. https://doi.org/10.1371/journal.pone.0000594.
12. Kim B, Kim SW, Lim JY, Park SJ. NCAPH is required for proliferation, migration and invasion of non-small-cell lung cancer cells. Anticancer Res 2020; 40(6): 3239–46. https://doi.org/10.21873/anticanres.14305.
13. Cui F, Hu J, Xu Z, Tan J, Tang H. Overexpression of NCAPH is upregulated and predicts a poor prognosis in prostate cancer. Oncol Lett 2019; 17(6): 5768–76. https://doi.org/10.3892/ol.2019.10260.
14. Yin L, Jiang LP, Shen QS, Xiong QX, Zhuo X, Zhang LL, et al. NCAPH plays important roles in human colon cancer. Cell Death Dis 2017; 8(3):e2680. https://doi.org/10.1038/cddis.2017.88.
15. Wang M, Qiao X, Cooper T, Pan W, Liu L, Hayball J, et al. HPV E7-mediated NCAPH ectopic expression regulates the carcinogenesis of cervical carcinoma via PI3K/AKT/SGK pathway. Cell Death Dis 2020; 11(12): 1049. https://doi.org/10.1038/s41419-020-03244-9.
16. Lu H, Shi C, Wang S, Yang C, Wan X, Luo Y, et al. Identification of NCAPH as a biomarker for prognosis of breast cancer. Mol Biol Rep 2020; 47(10): 7831–42. https://doi.org/10.1007/s11033-020-05859-9.
17. Bilgilier C, Fuereder T, Kastner MT, Vass Z, Brandl I, Sahbegovic H, et al. Oral abundance of Actinomyces spp. in breast cancer patients. Oncology 2022; 100(4): 221–7. https://doi.org/10.1159/000522070.
18. Tang L, Jiang W, Wu L, Yu X, He Z, Shan W, et al. TPGS2000-DOX prodrug micelles for improving breast cancer therapy. Int J Nanomedicine 2021; 16: 7875–90. https://doi.org/10.2147/IJN.S335405.
19. Qi Y, Zhao T, Han M. The application of radiomics in predicting gene mutations in cancer. Eur Radiol 2022; 32(6): 4014–24. https://doi.org/10.1007/s00330-021-08520-6.
20. Xiong Q, Fan S, Duan L, Liu B, Jiang X, Chen X, et al. NCAPH is negatively associated with Mcl1 in nonsmall cell lung cancer. Mol Med Rep 2020; 22(4): 2916–24. https://doi.org/10.3892/mmr.2020.11359.
21. Shimomura H, Sasahira T, Nakashima C, Kurihara-Shimomura M, Kirita T. Non-SMC condensin I complex subunit H (NCAPH) is associated with lymphangiogenesis and drug resistance in oral squamous cell carcinoma. J Clin Med 2019; 9(1): 72. https://doi.org/10.3390/jcm9010072.
22. Kim JH, Youn Y, Kim KT, Jang G, Hwang JH. Non-SMC condensin I complex subunit H mediates mature chromosome condensation and DNA damage in pancreatic cancer cells. Sci Rep 2019; 9(1): 17889. https://doi.org/10.1038/s41598-019-54478-3.
23. Li H, Chen M, Yang Z, Xu C, Yu Q, Song J, et al. Amorphophalli Rhizoma inhibits breast cancer growth, proliferation, migration, and invasion via the PI3K/AKT pathway. J Ethnopharmacol 2022; 286: 114926. https://doi.org/10.1016/j.jep.2021.114926.
24. Li J, Zhang C, Jiang H, Cheng J. Andrographolide inhibits hypoxia-inducible factor-1 through phosphatidylinositol 3-kinase/AKT pathway and suppresses breast cancer growth. Onco Targets Ther 2015; 8: 427–35. https://doi.org/10.2147/OTT.S76116.

Appendix

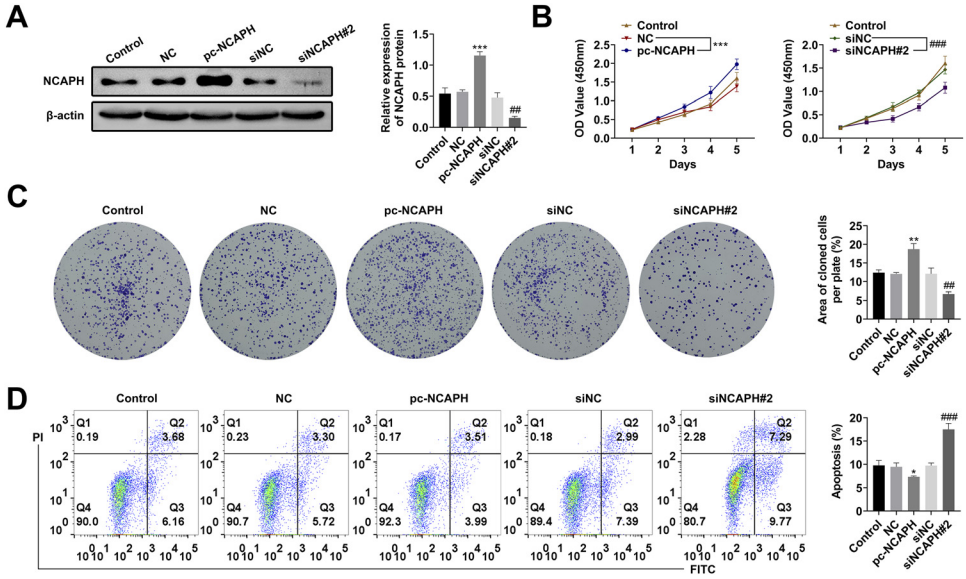

Fig. S1. NCAPH ablation suppressed the viability of T47D cells and stimulated apoptosis (A). Immunoblots showed the expression of NCAPH in T47D cells upon transfection with control or two NCAPH siRNAs (#1 and #2) or plasmids. (B). CCK-8 assays showed the OD value at 450 nm wavelength of T47D cells upon transfection with control or NCAPH siRNAs or plasmids. (C). Colony formation assays showed the colony number of T47D cells upon transfection with control or NCAPH siRNAs or plasmids. (D). Flow cytometry (FCM) assays showed the apoptosis percentage of T47D cells upon transfection with control or NCAPH siRNAs or plasmids. Data are presented as mean ± SEM, siNCAPH vi siNC, ** $P < 0.01$, *** $P < 0.001$. pcDNA3.1-NCAPH vs pcDNA3.1, ## $P < 0.01$, ### $P < 0.001$. NC, negative control. Each experiment was performed in 3 replicates.