Tropical Journal of Pharmaceutical Research October 2022; 21 (10): 2099-2105
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v21i10.9

Original Research Article

NUCKS1 promotes breast cancer cell proliferation and metastasis via PI3K/ AKT pathway

Song Li[1], Daoliang Wang[2]*
[1]Department of Head and Neck Oncology, Edong Healthcare Huangshi Central Hospital, Huangshi, Hubei Province 435000, [2]Department of Breast Surgery, Jingzhou First People's Hospital, Jingzhou, Hubei Province 434000, China

For correspondence:* ***Email:* *dlwang8972@163.com;* ***Tel:*** *+86-18163138972*

Sent for review: 7 May 2022 *Revised accepted: 26 September 2022*

Abstract

Purpose: *To investigate the role of nuclear casein kinase and cyclin-dependent kinase substrate 1 (NUCKS1) in breast cancer.*
Methods: *Breast cancer cells were maintained in RPMI-1640 medium containing 10 % fetal bovine serum in a incubator at 37 °C. Cell proliferation was determined by CCK8 and colony formation assays. Flow cytometry and Transwell assays were used to determine cell cycle and metastasis, respectively.*
Results: *Expression of NUCKS1 was significantly elevated in breast cancer ($p < 0.01$). Overexpression of NUCKS1 significantly increased cell viability ($p < 0.01$), and promoted proliferation of breast cancer cells. Knockdown of NUCKS1 inhibited cell proliferation, and induced cell cycle arrest at G1 phase. However, overexpression of NUCKS1 promoted cell cycle progression via down-regulation of p21 and up-regulation of cyclin D1 and CDK1. Cell migration and invasion were induced by overexpression of NUCKS1, and suppressed by silencing of NUCKS1. Overexpression of NUCKS1 enhanced p-AKT and p-PI3K expression, while knockdown of NUCKS1 reduced the expression of p-AKT and p-PI3K in breast cancer cells.*
Conclusion: *NUCKS1 promotes breast cancer cell proliferation and metastasis via activation of PI3K/AKT signaling. The silencing of NUCKS1 can be used as a strategy to develop therapies for the management of breast cancer.*

Keywords: *NUCKS1, Breast cancer, Proliferation, Cycle, Migration, Invasion*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Breast cancer is the leading cause of death in women [1]. Although advanced strategies have improved the prognosis of breast cancer, the mortality of breast cancer is increasing and the overall survival is unsatisfactory [2]. Therefore, therapeutic targets are urgently needed for breast cancer treatment.

Nuclear casein kinase and cyclin-dependent kinase substrate 1 (NUCKS1) is a member of the high mobility group family and mediates gene transcription and chromatin remodeling [3]. It functions as a paralog of RAD51-associated protein 1 and is involved in genome stability and homologous recombination during DNA damage response and metabolism [4]. Moreover,

NUCKS1 is also regarded as a transcriptional regulator for components of insulin signaling and has been implicated in the pathogenesis of metabolic and inflammatory diseases [3]. Increasing evidence has shown that NUCKS1 was related to poor prognoses of several cancers, and that it modulated tumor progression. For example, high levels of NUCKS1 in cervical squamous cell carcinoma have been associated with tumor recurrence [5] and predicted poor prognoses of colorectal cancer [6]. It binds to CDK1 and promotes non-small cell lung cancer cell proliferation and metastasis [7]. Knockdown of NUCKS1 reduces the viability of hepatocellular carcinoma cells, and inhibits tumor growth [8]. In breast cancer, the expressions of NUCKS1 in low-grade nonspecific invasive tumors were lower than those in high-grade invasive cancers [9]. Moreover, NUCKS1 is up-regulated in breast cancer tissues and might be involved in inflammation and cell cycling [10]. However, the specific role of NUCKS1 in breast cancer has not yet been clearly elucidated.

The PI3K signaling pathway is essential for cell proliferation and growth, and is involved in the progression of tumors [11]. The PI3K/AKT is abnormally activated in breast cancer, and inhibition of PI3K/AKT is currently in a clinical trial for breast cancer treatment [12]. Interestingly, NUCKS1 has been shown to be involved in miR-137-mediated inhibition of PI3K/AKT in lung cancer [13], and promoting the activation of PI3K/AKT signaling to induce cell aggressiveness of gastric cancer [14]. Therefore, NUCKS1/PI3K/AKT might be involved in breast cancer tumorigenesis.

The aim of this study was to investigate the effects of NUCKS1 on cell proliferation and cell metastasis of breast cancer, and the underlying mechanisms.

EXPERIMENTAL

Cell culture

Breast cancer cells (MCF-7, MDA-MB-231, and MCF10A) were acquired from the Cell Center of the Institute of Basic Medical Sciences (Beijing, China). The cells were identified as mycoplasma-free by short tandem repeat profiling. The cells were maintained in RPMI-1640 medium containing 10 % fetal bovine serum (Gibco, Grand Island, NY, USA) in a 37 °C incubator.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Cells were lysed in TRIzol (Invitrogen, Carlsbad, CA, USA) to isolate RNAs. The RNAs were then reverse transcribed into cDNAs, and a PreTaq II kit (Takara, Dalian, Liaoning, China) was used to determine the mRNA expression of NUCKS1. The relative expression of NUCKS1 was calculated using the $2^{-\Delta\Delta Cq}$ method through normalization to GAPDH. The primers used are shown in Table 1.

Cell transfection and viability assays

The pcDNA3.1-NUCKS1 (pc-NUCKS1), pcDNA3.1 vector (NC), siRNA targeting NUCKS1 (siNUCKS1), and negative control (siNC) were synthesized by Invitrogen. The pcDNA vectors and siRNAs were transfected into MCF-7 and MDA-MB-231 using Lipofectamine 2000. Cells were then incubated with 10 µL CCK8 solution (Beyotime) for another 2 h. The absorbance at 450 nm was measured using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Cell proliferation and cell cycle assays

The MCF-7 and MDA-MB-231 cells were seeded in 6-well plates for 10 days and then fixed in methanol. The cells were stained with crystal violet and photographed using a light microscope (Olympus, Tokyo, Japan). For flow cytometry, MCF-7 and MDA-MB-231 cells were harvested 48 h post-transfections and then resuspended in the binding buffer from the BD Cycletest™ Plus DNA Reagent Kit (BD Biosciences, San Jose, CA, USA). The cells were stained with propidium iodide (BD Biosciences) and analyzed in a FACS flow cytometer (Life Technologies, Darmstadt, Germany).

Transwell assay

The MCF-7 and MDA-MB-231 cells were harvested and suspended in serum-free RPMI-1640 medium.

Table 1: Primers for qRT-PCR

Gene	Forward (5´-3´)	Reverse (5´-3´)
NUCKS1	TGCCCAAACCCAGACTAAAG	GACCCTTCATCCCCAGATTT
GAPDH	TCAACGACCACTTTGTCAAGCAGAGT	GCTGGTGGTCCAGGGGTCTTACT

The cells were seeded into the upper Matrigel-coated Transwell insert chambers (Corning, NY, USA), while the lower chamber was plated with RPMI-1640 medium with 15 % fetal bovine serum. The cells in the upper chamber were removed 24 h later, and the invasive cells in the lower chamber were stained with crystal violet. The cells were photographed using a light microscope (Olympus). For the cell migration assay, cells were also plated into a chamber without Matrigel coating, and the same above mentioned experiments were performed.

Western blot assay

The cells were lysed in RIPA buffer (Beyotime), and the protein samples were separated by 10 % SDS-PAGE. Proteins were then transferred onto nitrocellulose membranes and blocked with 5 % bovine serum albumin. The membranes were probed with specific antibodies: anti-CDK1, anti-NUCKS1, and anti-β-actin (1:2,000), anti-cyclin D1 and anti-p21 (1:2,500), anti-p-AKT and anti-AKT (1:3,000), and anti-p-PI3K and anti-PI3K (1:3,500). The membranes were washed using PBST buffer and incubated with horseradish peroxidase-conjugated secondary antibody (1:4,000). Immunoreactivities were visualized using enhanced chemiluminescence (Sigma-Aldrich, St. Louis, MO, USA). All antibodies were acquired from Abcam (Cambridge, MA, USA). Antibody to β-actin was used as an internal control.

Statistical analysis

All data with at least triple replicates are expressed as the mean ± standard error of the mean (SEM), and analyzed by Student's *t*-test or one-way analysis of variance using SPSS statistical software for Windows (SPSS, Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Elevation of NUCKS1 in breast cancer

The expression level of NUCKS1 was identified in breast cancers. Results based on The Cancer Genome Atlas database indicated up-regulation of NUCKS1 in breast cancer tissues (Figure 1 A and B). Moreover, breast cancer cells (MCF-7 and MDA-MB-231) showed higher expression of NUCKS1 than MCF10A cells (Figure 1 C and D), indicating a possible correlation between NUCKS1 and breast cancer.

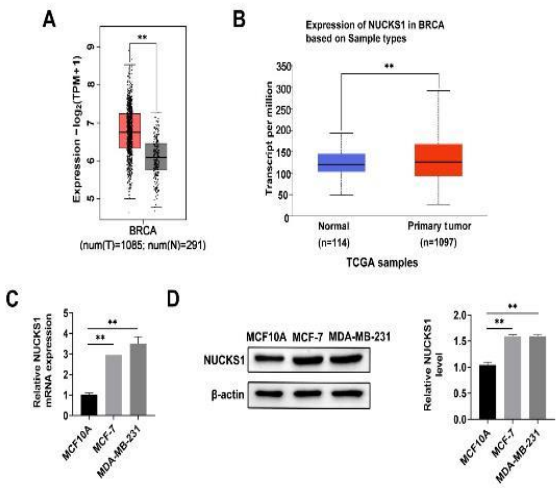

Figure 1: Elevation of NUCKS1 in breast cancer. (A) GEPIA analysis showing that NUCKS1 was up-regulated relative to normal tissues in breast cancer tissues. (B) Ualcan analysis showing that NUCKS1 was up-regulated in breast cancer tissues relative to normal tissues. (C) NUCKS1 mRNA was up-regulated in breast cancer cells (MCF-7 and MDA-MB-231) relative to a non-tumorigenic epithelial cell line (MCF10A). (D) NUCKS1 protein was up-regulated in breast cancer cells (MCF-7 and MDA-MB-231) relative to a non-tumorigenic epithelial cell line (MCF10A). ***P* < 0.01 vs. MCF10A

NUCKS1 contributed to breast cancer cell survival

MCF-7 and MDA-MB-231 cells were transfected with siNUCKS1 or pcDNA-NUCKS1 for the down-regulation or up-regulation of NUCKS1 expression, respectively (Figure 2 A). NUCKS1 overexpression increased cell viability (Figure 2 B) and enhanced the number of colonies (Figure 2 C). Moreover, the cell viability (Figure 2 B) and proliferation (Figure 2 C) were reduced by silencing of NUCKS1, revealing the antiproliferative effect of NUCKS1 silencing in breast cancer cells.

NUCKS1 promoted breast cancer cell cycle progression

The ratios of G1 phases in MCF-7 and MDA-MB-231 cells were decreased by overexpression of NUCKS1 (Figure 3 A and B). However, knockdown of NUCKS1 increased the ratio of cells in the G1 phase and promoted cell cycle arrest (Figure 3 A and B). Overexpression of NUCKS1 down-regulated protein expression of p21, while it up-regulated cyclin D1 and CDK1 expressions (Figure 3 C). Silencing of NUCKS1 enhanced p21 expression and reduced cyclin D1 and CDK1 expressions (Figure 3 C).

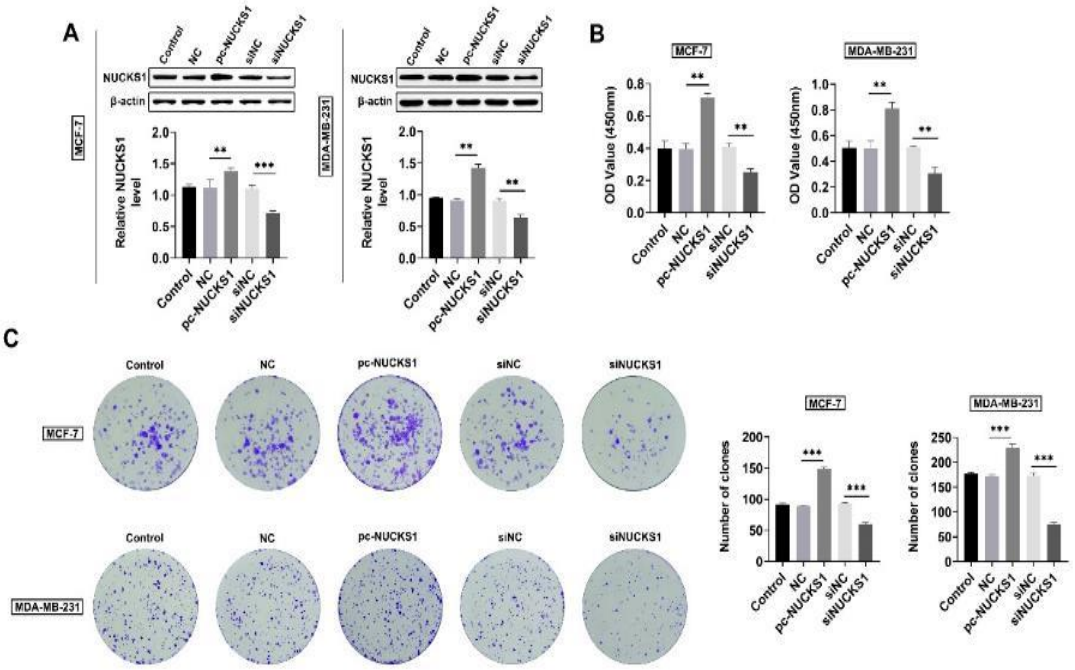

Figure 2: NUCKS1 promoted breast cancer cell survival. (A) MCF-7 and MDA-MB-231 cells were transfected with siNUCKS1 or pcDNA-NUCKS1 for the down-regulation or up-regulation of NUCKS1 expressions. (B) Overexpression of NUCKS1 increased the cell viabilities of MCF-7 and MDA-MB-231 cells, while silencing of NUCKS1 reduced the cell viabilities of MCF-7 and MDA-MB-231 cells. (C) Overexpression of NUCKS1 enhanced the number of colonies of MCF-7 and MDA-MB-231 cells, while silencing of NUCKS1 reduced the number of colonies of MCF-7 and MDA-MB-231 cells. ***P* < 0.01, ****p* < 0.001 vs. NC or siNC

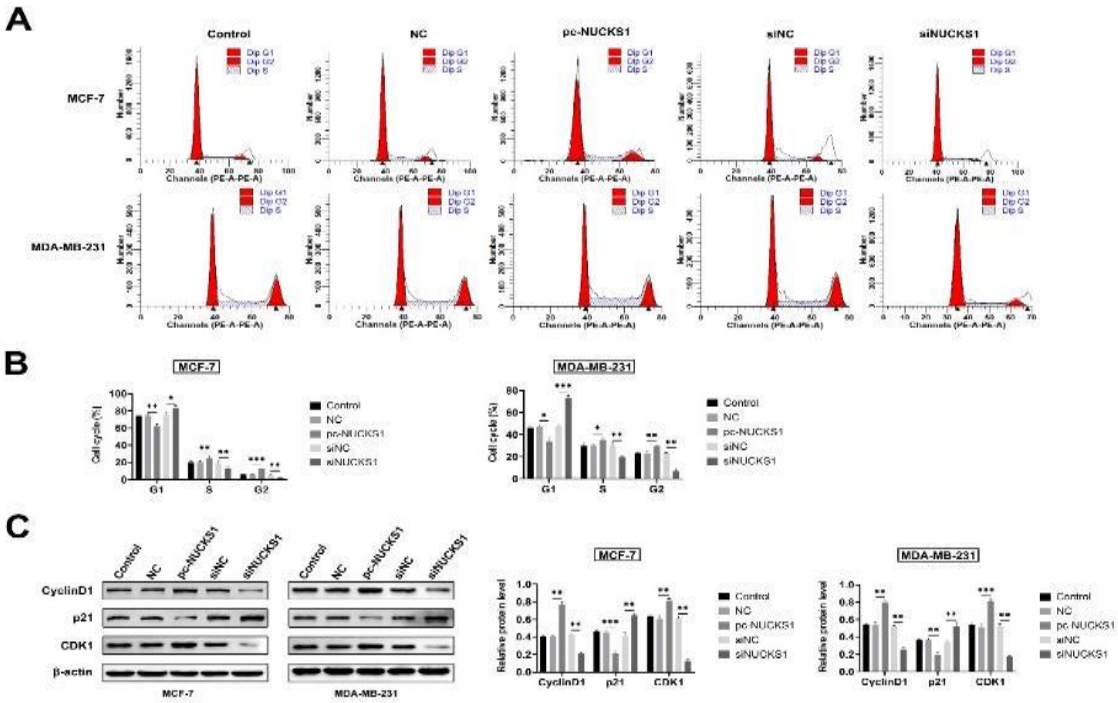

Figure 3: NUCKS1 promoted breast cancer cell cycle progression. (A) Overexpression of NUCKS1 decreased the ratios of cells in G1 phase in MCF-7 and MDA-MB-231 cells, while silencing of NUCKS1 increased the ratios of cells in G1 phase. (B) The relative ratios of cells in G1, S, and G2 phases in MCF-7 and MDA-MB-231 cells transfected with siNUCKS1 or pcDNA-NUCKS1. (C) Overexpression of NUCKS1 down-regulated protein expressions of p21 in MCF-7 and MDA-MB-231 cells, while it up-regulated expressions of cyclin D1 and CDK1. Silencing of NUCKS1 enhanced p21 expression, but reduced expressions of cyclin D1 and CDK1. **P* < 0.05, ***p* < 0.01, ****p* < 0.001 vs. NC or siNC

NUCKS1 contributed to breast cancer cell metastasis

Cell migration (Figure 4 A) and invasion (Figure 4 B) in MCF-7 and MDA-MB-231 were enhanced by NUCKS1 overexpression, and reduced by knockdown of NUCKS1. These results indicated the anti-metastatic effect of NUCKS1 silencing on breast cancer cells.

NUCKS1 contributed to activation of PI3K/AKT

Overexpression or knockdown of NUCKS1 did not affect protein expressions of PI3K and AKT (Figure 5 A and B). However, NUCKS1 overexpression increased expressions of p-PI3K and p-AKT. The p-PI3K and p-AKT were reduced by knockdown of NUCKS1 (Figure 5 A and B), indicating the suppressive effect of NUCKS1 silencing on PI3K/AKT signaling in breast cancer cells.

DISCUSSION

Immunohistochemical analysis showed that NUCKS was overexpressed in breast cancerous tissues when compared to non-cancerous tissues [15]. NUCKS functions as an oncogene to suppress autophagy and promote cell proliferation of gastric cancer [16]. This study, therefore, identified the oncogenic role of NUCKS1 in breast cancer.

This NUCKS1 was up-regulated in breast cancer tissues, and high expression of NUCKS1 was related to distant metastasis and lymph node involvement and predicted poor prognoses in patients with invasive breast carcinomas [9]. This study also showed increased expression of NUCKS1 in breast cancer. Overexpression of NUCKS1 promoted cell proliferation and metastasis of breast cancer; however, silencing of NUCKS1 suppressed the proliferation and metastasis.

NUCKS1 has been regarded as a transcription factor, and it up-regulates expression of S phase kinase-associated protein 2, resulting in degradation of p21 and p27 during cell cycle progression [17]. Moreover, knockdown of CDK1 attenuates NUCKS1 overexpression-induced non-small cell lung cancer cell proliferation and metastasis [7]. This study also found that NUCKS1 functioned as a cell cycle regulator, and shRNA-dependent NUCKS1 enhanced expression of p21, and reduced expression of cyclin D1 and CDK1 to induce cell cycle arrest in breast cancer. NUCKS1 is involved in homologous recombination during DNA damage response and metabolism [4], and DNA damage induces p53-dependent suppression of NUCKS1 and inhibits cell cycle progression [17]. Therefore, NUCKS1 might also regulate DNA damage repair during the progression of breast cancer.

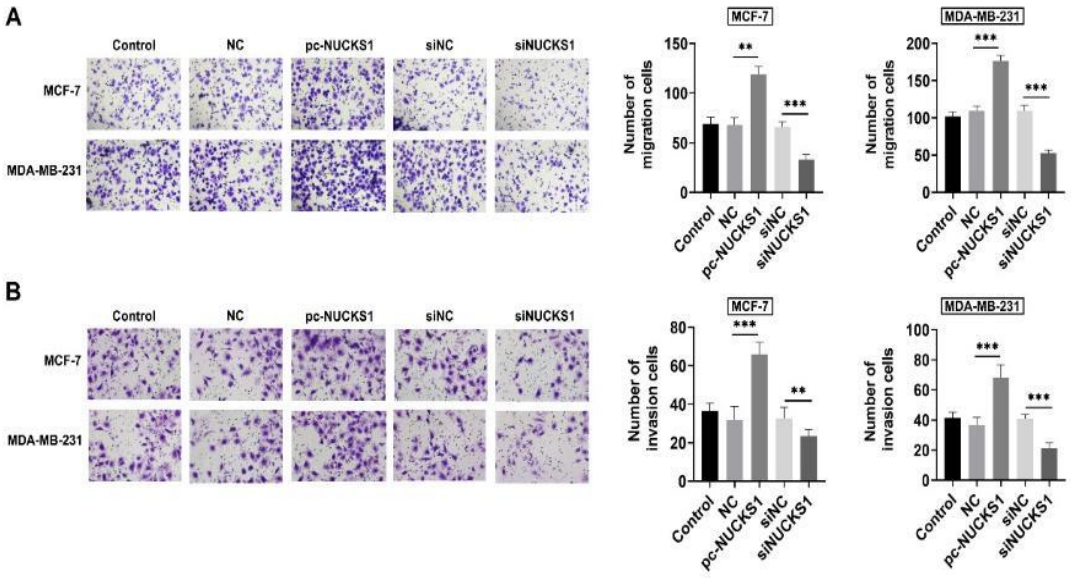

Figure 4: NUCKS1 contributed to breast cancer cell metastasis. (A) Overexpression of NUCKS1 promoted migrations of MCF-7 and MDA-MB-231 cells, while silencing of NUCKS1 suppressed migrations of MCF-7 and MDA-MB-231 cells. (B) Overexpression of NUCKS1 promoted invasion in MCF-7 and MDA-MB-231 cells, while silencing of NUCKS1 suppressed invasions of MCF-7 and MDA-MB-231 cells. $^{**}P < 0.01$, $^{***}p < 0.001$ vs. NC or siNC

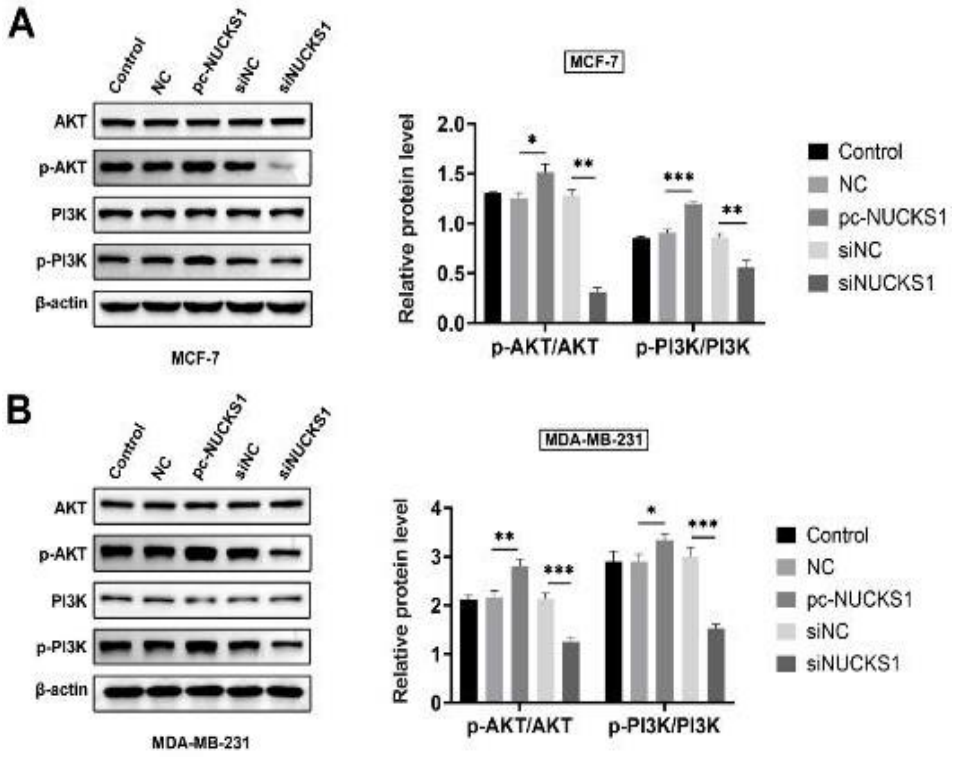

Figure 5: NUCKS1 promoted activation of PI3K/AKT. (A) Overexpression of NUCKS1 increased p-PI3K and p-AKT expressions in MCF-7 cells, while silencing of NUCKS1 decreased the protein expressions of p-PI3K and p-AKT. (B) Overexpression of NUCKS1 increased expressions of p-PI3K and p-AKT in MDA-MB-231 cells, while silencing of NUCKS1 decreased protein expressions of p-PI3K and p-AKT. *P < 0.05, **p < 0.01, ***p < 0.001 vs. NC or siNC

PI3K/AKT signaling is essential for the metastasis, progression, and drug resistance of breast cancer [18]. Inhibitors of PI3K and AKT have been used in clinical trials to treat breast cancer [11]. NUCKS1 stimulated the up-regulation of p-PI3K, p-AKT, and p-mTOR in gastric cancer, and promoted the aggressiveness of gastric cancer [14]. The results of the present study showed that knockdown of NUCKS1 reduced the expressions of p-PI3K and p-AKT in breast cancer

CONCLUSION

The NUCKS1 function as an oncogene in breast cancer. Silencing of NUCKS1 reduces cell proliferation and metastasis of breast cancer cells, while it induces cell cycle arrest via inactivation of PI3K/AKT signaling, suggesting that NUCKS1 silencing can be further investigated as a strategy to treat breast cancer.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

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

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Song Li designed and conducted the experiments. Daoliang Wang analyzed and interpreted the data, and wrote the manuscript with input from all co-authors.

REFERENCES

1. *Zhang Q, Yang H, Tang C, Wang Q, Ren L, Jia C, Zou H. FMNL1 promotes growth and metastasis of breast cancer by inhibiting BRCA1 via upregulation of HMGA1. Trop J Pharm Res 2021; 20(8): 1559-1564.*
2. *DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, Jemal A, Siegel RL. Breast cancer statistics, 2019. CA-Cancer J Clin 2019; 69(6): 438-451.*
3. *Huang P, Cai Y, Zhao B, Cui L. Roles of NUCKS1 in diseases: susceptibility, potential biomarker, and regulatory mechanisms. Biomed Res Int 2018; 2018: 7969068.*
4. *Parplys AC, Zhao W, Sharma N, Groesser T, Liang F, Maranon DG, Leung SG, Grundt K, Dray E, Idate R et al. NUCKS1 is a novel RAD51AP1 paralog important for homologous recombination and genome stability. Nucleic Acid Res 2015; 43(20): 9817-9834.*
5. *Gu L, Xia B, Zhong L, Ma Y, Liu L, Yang L, Lou G. NUCKS1 overexpression is a novel biomarker for recurrence-free survival in cervical squamous cell carcinoma. Tumor Biol 2014; 35(8): 7831-7836.*
6. *Kikuchi A, Ishikawa T, Mogushi K, Ishiguro M, Iida S, Mizushima H, Uetake H, Tanaka H, Sugihara K. Identification of NUCKS1 as a colorectal cancer prognostic marker through integrated expression and copy number analysis. Int J Cancer 2013; 132(10): 2295-2302.*
7. *Zhao S, Wang B, Ma Y, Kuang J, Liang J, Yuan Y. NUCKS1 promotes proliferation, invasion and migration of non-small cell lung cancer by upregulating CDK1 expression. Cancer Manag Res 2020; 12: 13311-13323.*
8. *Cheong J, Kim Y, Woo J, Kim D, Yeo M, Yang SJ, Yang KS, Soon S, Wang H, Kim B et al. Identification of NUCKS1 as a putative oncogene and immunodiagnostic marker of hepatocellular carcinoma. Gene 2016; 584(1): 47-53.*
9. *Symonowicz K, Duś-Szachniewicz K, Woźniak M, Murawski M, Kołodziej P, Osiecka B, Jurczyszyn K, Ziółkowski P. Immunohistochemical study of nuclear ubiquitous casein and cyclin-dependent kinase substrate 1 in invasive breast carcinoma of no special type. Exp Ther Med 2014; 8(4): 1039-1046.*
10. *Soliman N, Zineldeen D, Elkhadrawy O. Effect of NUCKS-1 overexpression on cytokine profiling in obese women with breast cancer. Asian Pac J Cancer P 2014; 15: 837-845.*
11. *Yang J, Nie J, Ma X, Wei Y, Peng Y, Wei X. Targeting PI3K in cancer: mechanisms and advances in clinical trials. Mol Cancer 2019; 18(1): 26-26.*
12. *Paplomata E, O'Regan R. The PI3K/AKT/mTOR pathway in breast cancer: targets, trials and biomarkers. Ther Adv Med Oncol 2014; 6(4): 154-166.*
13. *Shen H, Wang L, Ge X, Jiang C-F, Shi Z-M, Li D-M, Liu W-T, Yu X, Shu Y-Q. MicroRNA-137 inhibits tumor growth and sensitizes chemosensitivity to paclitaxel and cisplatin in lung cancer. Oncotarget 2016; 7(15): 20728-20742.*
14. *Huang Y-K, Kang W-M, Ma Z-Q, Liu Y-Q, Zhou L, Yu J-C. NUCKS1 promotes gastric cancer cell aggressiveness by upregulating IGF-1R and subsequently activating the PI3K/Akt/mTOR signaling pathway. Carcinogenesis 2019; 40(2): 370-379.*
15. *Drosos Y, Kouloukoussa M, Østvold AC, Grundt K, Goutas N, Vlachodimitropoulos D, Havaki S, Kollia P, Kittas C, Marinos E et al. NUCKS overexpression in breast cancer. Cancer Cell Int 2009; 9: 19-19.*
16. *Zhao E, Feng L, Bai L, Cui H. NUCKS promotes cell proliferation and suppresses autophagy through the mTOR-Beclin1 pathway in gastric cancer. J Exp Clin Canc Res 2020; 39(1): 194-194.*
17. *Hume S, Grou CP, Lascaux P, D'Angiolella V, Legrand AJ, Ramadan K, Dianov GL. The NUCKS1-SKP2-p21/p27 axis controls S phase entry. Nat Commun 2021; 12(1): 6959-6959.*
18. *Guerrero-Zotano A, Mayer IA, Arteaga CL. PI3K/AKT/mTOR: role in breast cancer progression, drug resistance, and treatment. Cancer Metast Rev 2016; 35(4): 515-524.*