# Expression of cancerous inhibitor of protein phosphatase 2A in human triple negative breast cancer correlates with tumor survival, invasion and autophagy

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**Abstract.** Cancerous inhibitor of protein phosphatase 2A (CIP2A) is a recently characterized oncoprotein which is involved in the progression of several human malignancies. The present study aimed to investigate its biological function in human triple negative breast cancer (TNBC). The expression of CIP2A in TNBC cells was examined and it was observed that CIP2A was elevated in the TNBC cell line compared with poorly invasive breast cancer cells. CIP2A depletion in TNBC cell lines inhibited proliferation, and induced apoptosis and autophagy. In addition, CIP2A depletion inhibited invasion and migration of TNBC cells. Furthermore, CIP2A depletion downregulated Akt/mTOR/P70S6K phosphorylation. These results validate the role of CIP2A as a invasion-associated oncoprotein and established CIP2A as a promising therapeutic target of TNBC.

## Introduction

Breast cancer is one of the most prevalent carcinomas and is the second leading cause of mortality in women worldwide, with more than one million cases occurring and more than 400,000 cases of associated mortality annually worldwide (1). Breast cancer is a heterogeneous disease, which can be further divided into subtypes via their histopatological and gene expression profiles. In particular, triple-negative breast cancers (TNBCs) are among the most aggressive and treatment-resistant breast subtypes. TNBC comprises ~15-20% of all breast cancer cases (2), and is defined as breast carcinoma that does not express the estrogen receptor (ER), progesterone receptor (PR) or human epidermal growth factor receptor type 2 (HER2). TNBC is characterized by invasive potential, aggressive behaviors with a high recurrence rate and poor prognosis. However, the driving factors underlying TNBC invasion remain poorly defined and a better understanding of TNBC invasion mechanisms is required for the development of rational strategies for the prevention and treatment of TNBC recurrence.

Cancerous inhibitor of protein phosphatase 2A (CIP2A), a recently identified human oncoprotein that stabilizes c-Myc by inhibiting protein phosphatase 2A-mediated dephosphorylation of Myc at serine 62 (3). Previous studies have shown that CIP2A serves a critical role in the progression of several cancer types, including head and neck squamous cell carcinoma, oral squamous cell carcinoma, oesophageal squamous cell carcinoma, colon, gastric, breast, prostate, tongue, lung, cervical cancer and acute myeloid leukaemia (3-9). In 2009, Côme *et al* (10) demonstrated that CIP2A is associated with clinical aggressivity in human breast cancer and promotes the malignant growth of breast cancer cells (10). Then in 2012, Tseng *et al* (11) found that CIP2A is a target of the proteasome inhibitor bortezomib in human TNSC cells. However, the expression and the role that CIP2A serves in pathogenesis of human TNBC requires further investigation.

In the present study, the expression and the functional role of CIP2A in TNSC cells is examined. The results show that CIP2A is overexpressed in TNBC cell lines. CIP2A depletion led to proliferation and clonogenic activity inhibition of TNBC cell lines MDA-MB-231 and MDA-MB-468. Interestingly, CIP2A depletion in TNBC cells induced autophagy and apoptosis. In addition, the invasive behavior of MDA-MB-231 cells was examined by CIP2A small interfering (si)siRNA, and found that CIP2A depletion inhibits the invasion and migration of MDA-MB-231. Previously, CIP2A has been shown to be an oncoprotein capable of modulating phosphorylated-Akt (pAkt) (9,12). Results of the present study demonstrated that CIP2A depletion inhibits phosphorylation of Akt and its downstream molecules, mechanistic target of rapamycin (mTOR) and p70 ribosomal protein S6 kinase (P70S6K).

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The protein kinase mTOR is an Akt signaling protein and a critical regulator of cellular metabolism, growth and proliferation, with p70S6K1 and 4E-BP1 (eIF4E binding protein 1) as two important effectors (13). These results suggest that CIP2A promotes invasion and migration of TNSCs through Akt/mTOR/P70S6K signaling pathways, therefore the function of CIP2A in TNSC warrants further investigation.

## Materials and methods

*Cell culture*. Human breast cancer cell lines MCF-7 (ER+/PR+/HER2-), MDA-MB-231 (ER-/PR-/HER2-), MDA-MB-468(ER-/PR-/HER2-)andBT549(ER-/PR-/HER2-) were obtained from American Type Culture Collection (Manassas, VA, USA). MCF-7 and BT549 cells were maintained in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; HyClone; GE Healthcare Life Sciences, Chalfont, UK) and antibiotics, and incubated in a humidified atmosphere with 5% $CO_2$ at 37˚C. MDA-MB-231 and MDA-MB-468 cells were maintained in Leibovitz's-15 (L-15) (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS and antibiotics and incubated in a humidified atmosphere without $CO_2$ at 37˚C.

*Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)*. Expression of the *CIP2A* gene was examined by quantitative polymerase chain reaction (PCR) normalized to the expression of GAPDH. Total RNA was extracted from cell lines or patients' cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. qPCR was performed using SYBR *Premix Ex Taq* (Perfect Real Time; Takara Bio, Inc., Otsu, Japan) according to the manufacturer's protocol (14). RT-qPCR analysis of *CIP2A* was performed with 2 $\mu$g of total RNA and ReverTra Ace qPCR RT kit (Toyobo Co., Ltd., Osaka, Japan). The reverse transcription conditions were as follows: 37˚C for 15 min and 98˚C for 5 min, followed by storage at -20˚C. For qPCR, the following primers were used: *CIP2A* forward, 5'-5'-TGC GGCACTTGGAGGTAATTTC-3' and reverse, 5'-AGCTCT ACAAGGCAACTCAAGC-3'; and *GAPDH* forward, 5'-TGT TGCCATCAATGACCCCTT-3' and reverse 5'-CTCCAC GACGTACTCAGCG-3'. RT-qPCR was performed in an ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The reaction mix contained: 10 $\mu$l SYBR Green PCR Master mix, 200 nm forward and reverse primers, 100 ng cDNA template and $ddH_2O$ up to 20 $\mu$l volume. The PCR cycling conditions consisted of the following: 94˚C for 3 min for denaturation, 94˚C for 30 sec for annealing and 58˚C for 40 sec for extension, for a total of 40 cycles. The threshold cycle for each sample was selected from the linear range and converted to a starting quantity by interpolation from a standard curve generated on the same plate for each set of primers. The CIP2A mRNA levels were normalized for each well to GAPDH mRNA levels using the $2^{-\Delta\Delta Cq}$ method (15). Each experiment was repeated three times.

*Western blot*. Cell pellets were lysed in radioimmunoprecipitation assay buffer containing 50 mM Tris (pH 8.0), 150 mM NaCl, 0.1% SDS, 0.5% deoxycholate, 1% NP-40, 1 mM DTT, 1 mM NaF, 1 mM sodium vanadate, 1 mM PMSF (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) and 1% protease inhibitors cocktail (Merck Millipore). Protein extracts were quantified and loaded (25 $\mu$g) on 8-12% sodium dodecyl sulfate polyacrylamide gel, electrophoresed, and transferred to a polyvinylidene difluoride membrane (Merck Millipore). The membrane was blocked with 5% skimmed milk at room temperature for 1 h. The membrane was incubated with primary antibodies overnight at 4˚C, followed by washing with TBST. Subsequently, membranes were incubated with goat anti-rabbit or anti-mouse horseradish peroxidase-conjugated secondary antibody (1:10,000; cat. no., E030120-01 and E030110-01; EarthOx Life Sciences, Millbrae, CA, USA) at room temperature for 1.5 h. Detection was performed by using a SuperSignal® West Pico Trial kit (Pierce Biotechnology, Inc., Rockford, IL, USA) (16). The defined sections of the film were scanned for image capture and quantification using Adobe Photoshop software (CS4; Adobe Systems, Inc., San Jose, CA, USA) and ImageJ software (National Institutes of Health, Bethesda, MD, USA). The primary antibodies used were anti-CIP2A (1:1,000; cat. no. sc-80662; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), anti-phospho-Akt (1:500; cat. no. sc-7985; Santa Cruz Biotechnology, Inc.), anti-Akt (1:500; cat no. sc-8312; Santa Cruz Biotechnology, Inc.), anti-c-Myc (1:500; cat. no. sc-788; Santa Cruz Biotechnology, Inc.) anti-caspase-3 (1:1,000; cat. no. 9662; Cell Signaling Technology, Inc., Danvers, MA, USA), anti-poly ADP ribose polymerase (PARP) (1:1,000; cat. no. 9542; Cell Signaling Technology, Inc.), anti-LC-3 (1:1,000; cat no. 2775; Cell Signaling Technology, Inc.), anti-phospho-P70S6K (1:1,000; cat. no. 2983; Cell Signaling Technology, Inc.), anti-P70S6K (1:1,000; cat. no. 2708; Cell Signaling Technology, Inc.), anti-phospho-mTOR (1:1,000; cat. no. 5536), anti-mTOR (1:1,000; cat. no. 2973; Cell Signaling Technology, Inc.) and anti-GAPDH (1:5,000; cat. no. AB10016; Sangon Biotech Co., Ltd., Shanghai, China).

*Transfection of siRNA*. Two siRNA targeting CIP2A were designed and synthesized by Shanghai GenePharma Co. (Shanghai, China), referred to as siRNA1 and siRNA2. The siRNA sequences were as follows: 5'-CUGUGGUUGUGU UUGCACUTT-3' (CIP2A siRNA1), 5'-ACCAUUGAUAUC CUUAGAATT-3' (CIP2A siRNA2) and 5'-UUCUCCGAA CGUGUCACGUTT-3' [negative control (NC) siRNA].

Using lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, MDA-MB-231 and MDA-MB-468 cells were transfected with 100 nM siRNA. And 48 h transfection, the cells were harvested for western blot, cell viability, soft-agar colony formation assay, invasion assay and wound healing assay, as previously described (5,17).

*Cell viability*. Cell viability was estimated by trypan blue dye exclusion, as previously described (18). Briefly, a 0.4% solution of trypan blue was prepared in phosphate-buffered saline (PBS; pH 7.2-7.3). Then, 0.1 ml trypan blue stock solution was added to 1 ml cell suspension. A hemacytometer was then loaded with the samples and they were examined immediately under a microscope at low magnification (IX70; Olympus Corporation, Tokyo, Japan). The number of blue stained cells

and the number of total cells were counted. Cell viability should be ≥95% for healthy log-phase cultures. Percentage viable cells = [1.00 - (Number blue cells / number total cells)] x 100. To calculate the number of viable cells per ml of culture, the following formula was used: Number of viable cells x ($10^4$ x 1.1) = cells/ml culture.

*Soft agar colony formation assay*. Cells were suspended in 1 ml L-15 containing 0.3% low-melting-point agarose (Amresco Inc., Farmingham, MA, USA) and 10% FBS, and plated on a bottom layer containing 0.6% agarose and 10% FBS in 6-well plate in triplicate. After 2 weeks, plates were stained with 0.2% gentian violet and the colonies were counted under light microscope (5).

*Invasion assay*. An invasion assay was performed using a 24-well plate (Corning). A polyvinyl-pyrrolidone-free polycarbonate filter (8 $\mu$m pore size) (Corning, Inc., Corning, NY, USA) was coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). The lower chamber was filled with medium containing 20% FBS as a chemoattractant agent. The coated filter and upper chamber were laid over the lower chamber. Cell suspension (1x$10^4$ cells/well) was seeded onto the upper chamber wells. After incubation for 20 h at 37˚C, the filter was fixed and stained with 2% ethanol containing 0.2% crystal violet (15 min). After drying, the stained cells were enumerated under a light microscope at 10x objective. For quantification, the invaded stained cells on the other side of the membrane were extracted with 33% acetic acid. The absorbance of the eluted stain was determined at 570 nm.

*Wound healing assay*. Cells (4x$10^5$ cells/2 ml) were seeded in a 6-well plate and incubated at 37˚C until 90 to 100% confluent. After the confluent cells were scratched with a 200 $\mu$l pipet tip, followed by washing with PBS, they were then re-suspended in complete medium. After 24 h incubation, the cells were fixed and stained with 2% ethanol containing 0.2% crystal violet powder (15 min), and randomly chosen fields were photographed under a light microscope at 4x objective. The number of cells migrated into the scratched area was calculated.

*Statistical analysis*. All experiments were repeated at least three times and the data are presented as the mean ± standard deviation unless noted otherwise. Data were analyzed using SPSS version 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). All experiments were repeated at least 3 times and the data are presented as the mean ± standard deviation unless noted otherwise. Differences between data groups were evaluated for significance using Student's t-test of unpaired data of one way analysis of variance, followed by the Bonferroni post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

## Results

*CIP2A is overexpressed in TNSC cell lines*. CIP2A expression was studied by RT-qPCR and western blot analysis in TNSC cell lines MDA-MB-231, MDA-MB-468, and BT-549 cells. The poorly invasive ER+ breast cancer line MCF-7 was used as a positive control. The results show that CIP2A is expressed in BNSC cell lines at mRNA level. Interestingly, highly invasive TNSC breast cancer cells have increased CIP2A expression compared with poorly invasive ER+ MCF-7 cells (Fig. 1A). Consistent with mRNA expression, western blot analysis of the protein level of CIP2A revealed that highly invasive TNSC cells have increased CIP2A expression compared with poorly invasive ER+ MCF-7 cells (Fig. 1B). The results show that CIP2A is overexpressed in TNSC cells, and that higher expression of CIP2A in highly invasive TNSC cells may contribute towards invasion.

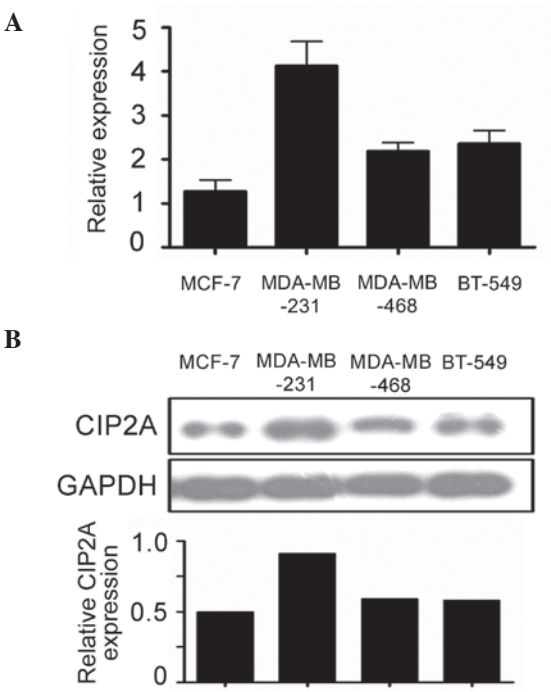


Figure 1. CIP2A mRNA and protein expression in triple negative breast cancer cell lines. (A) *CIP2A* relative protein expression determined by reverse transcription-quantitative polymerase chain reaction, displayed as relative to GAPDH. (B) Western blot analysis showing CIP2A expression in different cell lines. MCF-7 (estrogen receptor +/progesterone receptor +/herceptin 2 receptor -) was used as positive control. CIP2A, cancerous inhibitor of protein phosphatase 2A.

*CIP2A depletion in TNSC cell lines inhibits cell proliferation and clonogenic activity*. To evaluate the role that CIP2A overexpression serves in TNSC cells, MDA-MB-231 and MDA-MB-468 cells were transfected with siRNA1 or siRNA2 targeting CIP2A (Fig. 2A). CIP2A silencing significantly inhibited proliferation of MDA-MB-231 and MDA-MB-468 cells (Fig. 2B; P<0.01). The proliferation rate was determined by trypan blue dye exclusion assay. Consistent with the trypan blue dye exclusion results, colony formation assay showed that CIP2A knockdown in MDA-MB-231 cells led to a significant decrease in focus numbers (Fig. 2C; P<0.01). These data demonstrate that CIP2A serves a critical role in TNSC cell proliferation.

*CIP2A depletion in TNBC cells induces apoptosis and autophagy*. To determine which cell death pathway was induced by CIP2A depletion, MDA-MB-231 and MDA-MB-468 cells were treated with CIP2A siRNA for

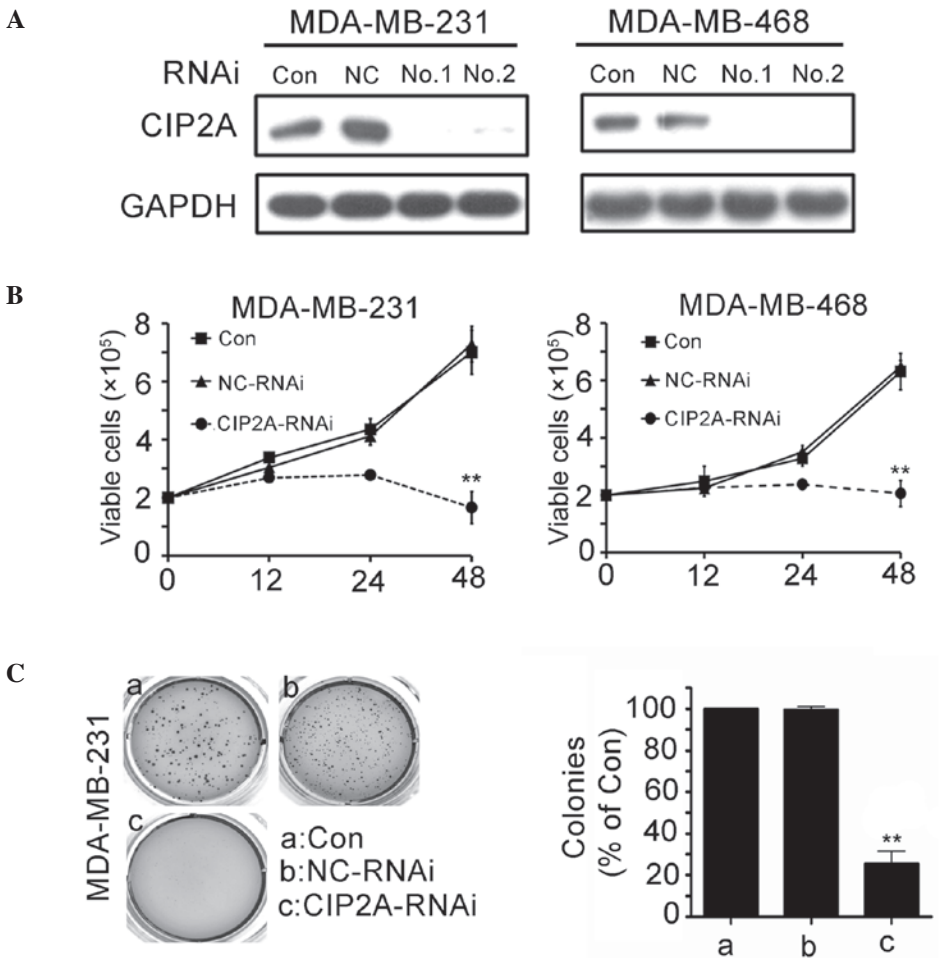


Figure 2. CIP2A depletion in triple negative breast cancer cell lines inhibits cell proliferation and clonogenic activity. (A) MDA-MB-231 and MDA-MB-468 cells were transfected with 100 nM CIP2A-specific siRNA1, siRNA2 or NC siRNA for 48 h, then cells were harvested for western blot analyses. (B) MDA-MB-231 and MDA-MB-468 cells were transfected with 100 nM CIP2A-specific siRNA or NC siRNA for 48 h. To evaluate cell growth, the cells were analyzed at indicated time points by trypan blue exclusion assay. (C) MDA-MB-231 cells were transfected with 100 nM CIP2A-specific siRNA or NC siRNA for 48 h. To evaluate cell colony formation, the cells were analyzed for 2 weeks by Soft-agar colony formation assay. $^{**}$P<0.01. CIP2A, cancerous inhibitor of protein phosphatase 2A; Con, control; NC, negative control; RNAi, RNA interference.

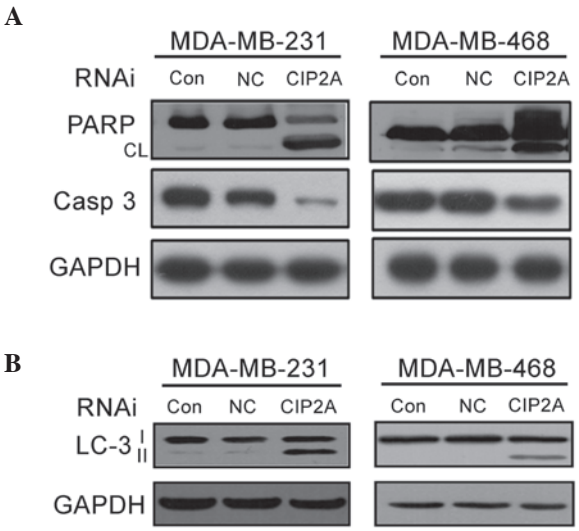


Figure 3. CIP2A depletion in triple negative breast cancer cells induces apoptosis and autophagy. MDA-MB-231 cells were transfected with 100 nM CIP2A-specific siRNA or NC siRNA for 48 h, and (A) PARP and Casp 3, and (B) LC-3 were detected. Casp 3, caspase-3; CIP2A, cancerous inhibitor of protein phosphatase 2A; Con, control; NC, negative control; PARP, poly ADP ribose polymerase; RNAi, RNA interference; CL, cleaved.

48 h and apoptosis proteins were measured using western blot analysis. As presented in Fig. 3A, the results showed that PARP cleavage, as well as cleaved-caspases-3, were detected in CIP2A depleted MDA-MB-231 and MDA-MB-468 cells. These results indicate that the apoptosis pathway is primarily activated along with caspase-dependent apoptosis in BNSC cells following CIP2A depletion. To assess whether autophagy is also involved in CIP2A siRNA-induced cell death, the expression level of LC-3 II in cells treated with CIP2A siRNA was subsequently analyzed. Interestingly, a marked increase in LC-3 II was observed following CIP2A siRNA treatment for 48 h (Fig. 4B). These findings suggest that CIP2A is associated with proliferation, apoptosis and autophagy.

*CIP2A depletion in TNSC cells inhibits cell invasive behavior and Akt/mTOR/P70S6K phosphorylation*. Considering that highly invasive TNSC cells have higher CIP2A expression than poorly invasive MCF-7 cells, it was determined whether CIP2A depletion inhibited the invasive behavior of breast cancer cells. An invasion assay was performed in highly invasive MDA-MB-231 cells using Matrigel-coated 24-well microchemotaxis chambers. As shown in Fig. 3A, MDA-MB-231 cells

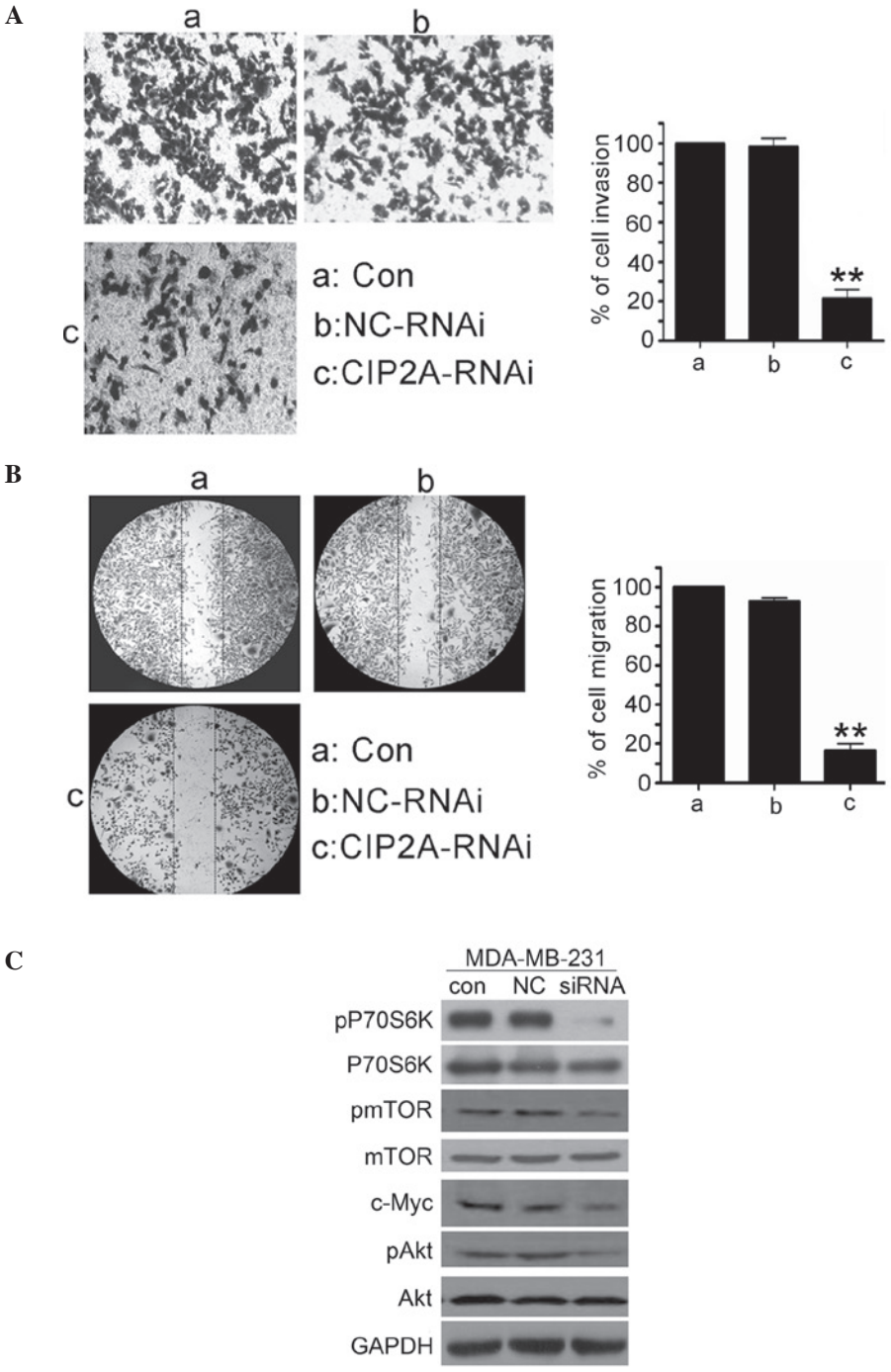


Figure 4. CIP2A depletion in triple negative breast cancer cells inhibits cell invasive behavior and Akt/mTOR/P70S6K phosphorylation. (A) MDA-MB-231 cells were transfected with 100 nM CIP2A-specific siRNA or NC siRNA for 48 h. To evaluate cell invasion, the cells were analyzed for 20 h by invasion assay. (B) MDA-MB-231 cells were transfected with 100 nM CIP2A-specific siRNA or NC siRNA for 48 h. To evaluate cell migration, the cells were analyzed for 24 h by wound healing assay. (C) MDA-MB-231 cells were transfected with 100 nM CIP2A-specific siRNA or NC siRNA for 48 h, then harvested for western blot analysis. con, control; CIP2A, cancerous inhibitor of protein phosphatase 2A; NC, negative control; RNAi, interfering RNA; siRNA, small interfering RNA; P70S6K, p70 ribosomal protein S6 kinase; pP70S6K, phosphorylated P70S6K; mTOR, mechanistic target of rapamycin; pmTOR, phosphorylated mTOR; pAkt, phosphorylated Akt.

were treated with CIP2A siRNA (100 nM), and cell invasion was determined after 20 h. CIP2A depletion markedly suppressed the invasion of MDA-MB-231 cells. In addition, the effect of CIP2A depletion on migration was explored. MDA-MB-231 cells were treated with CIP2A siRNA (100 nM), and cell migration was determined after 48 h. As shown in Fig. 3B, CIP2A depletion significantly decreased MDA-MB-231 cell migration. To investigate the mechanism underlying invasion and migration inhibition, the effect of CIP2A knockdown on c-Myc, Akt, mTOR and P70S6K levels was explored. The results indicated that depletion of CIP2A by siRNA resulted in inhibition of c-Myc protein expression, Akt, mTOR and P70S6K phosphorylation in MDA-MB-231 cells (Fig. 4C). These results indicated that CIP2A promotes TNSC cell invasion through the c-Myc upregulatory effect on c-Myc expression, and through the activation of the Akt/mTOR/P70S6K signaling pathway.

## Discussion

As one of the most common human malignancies, breast cancer remains a challenging disease. TNBC are characterized by occurrence in younger women, aggressive behaviors with a metastasis potential, high recurrence rate and poor prognosis (11). Because of a lack of targeted therapies (such as anti-HER2 therapy or hormone therapy), chemotherapy is currently the primary treatment of TNBC. Therefore, the development of novel diagnostic markers, understanding of the molecular pathways implicated in TNBC pathogenesis, and targeted therapies, remain an urgent and unmet need. One of the primary purposes of this study was to address the above issues.

CIP2A is a widespread oncogenic factor in human neoplasms (4-9,3). Similar to the Ras oncogene, CIP2A is required for anchorage-independent cell growth and malignant transformation of human cells (4). More recently, CIP2A expression has been found to be associated with the invasive function of fibroblast-like synoviocytes and synovial hyperplasia in rheumatoid arthritis (19). In addition, it has been reported that CIP2A immunostaining level positively correlated with metastasis in renal cell carcinomas (6). More recently, In addition, Zhai *et al* (20) found that CIP2A was overexpressed in osteosarcoma tissues, and that CIP2A depletion attenuated cell proliferation and invasion. In addition, CIP2A depletion inhibited matrix metalloproteinases-9 (MMP-9) mRNA expression. MMP-9 is the enzyme most crucial to tumor invasion owing to their ability to degrade extracellular matrix and basement membrane. In conclusion, increasing evidence suggests that CIP2A is an oncoprotein promoting proliferation and cell invasion. However, the expression pattern of CIP2A in TNBC and its involvement in aggressiveness of TNBC cells is less reported. The present study provides evidence that CIP2A overexpression widely occurs in TNSC cells and positively correlates with proliferation and metastasis.

In the present study, it was identified that the rate of CIP2A overexpression was high in TNBCs, which is known to show relatively strong metastasis potential, aggressive behaviors and thus poor prognosis compared with ER+ breast cancer cells of MCF-7 (Fig. 1). To gain insight into the potential mechanism of CIP2A overexpression in TNBCs, CIP2A expression was knocked down in MDA-MB-231 and MDA-MB-468 cell lines. Consistent with clinical findings by Côme (10), the experiments in the present study demonstrated that CIP2A depletion significantly inhibits cell proliferation and colony formation, suggesting that CIP2A depletion inhibited the anchorage-dependent (cell proliferation) and anchorage-independent (colony formation) growth of highly invasive TNBCs.

Autophagy and apoptosis are two important self-destructive processes that serve a pivotal role in the maintenance of human health, as well as in the pathogenesis of several tumors (21,22). In the present study, CIP2A depletion significantly induced caspase-3 activation, followed by PARP cleavage in two TNBC cell lines, suggesting that CIP2A depletion induces caspase-dependent apoptosis in TNBC cells (Fig. 3A). Western blot analysis evaluated the expression of LC-3 II, and it was identified that CIP2A depletion induces autophagy of TNBC cells.

Migration and invasion are two important prerequisites of TNBC cancer progression and metastasis. Therefore, therapeutic strategies for preventing or suppressing cancer invasion and metastasis can significantly improve the survival of TNBC patients. The present data showed that CIP2A depletion markedly inhibited the invasive and migratory abilities of MDA-MB-231 cells. Recently, CIP2A expression has been found to be associated with the invasive function of breast cancer (10). Based on the role of CIP2A in stabilizing and upregulating the c-Myc oncoprotein, previous reports found that c-Myc is involved in CIP2A-stimulated invasiveness of tumor cells (3,9,10). The expression of c-Myc was analyzed by western blot, and it was observed that c-Myc is downregulated by CIP2A depletion. Myc oncoprotein confers a selective advantage on cancer cells by promoting cell survival, proliferation, differentiation blockade, angiogenesis and genetic instability, all of which may contribute towards invasion and metastasis (23,24). Recently, Myc has been reported to regulate the epithelial-to-mesenchymal transition, a critical cellular programme for migration and invasion (25,26). Above reports of Myc may contribute to cancer cell invasion driven by CIP2A, and further study is required to investigate the function of Myc in CIP2A-mediated invasion and migration.

In conclusion, the present study reports that CIP2A depletion induces autophagy of TNBC cancer cells. Studies show that the CIP2A downstream molecule Akt serves an important role in autophagy. The current study reports that while pAkt (Ser473) is downregulated in cells upon CIP2A knockdown, p-mTOR (Ser2448) and the mTOR effector protein P70S6K (Thr412/389) are decreased. A previous study reported that CIP2A controls cell growth and autophagy through mTORC1 activation in MCF-7 cells (22). The results of the present study indicate that the downregulation of the Akt/mTOR/ P70S6K signaling pathway may contribute to autophagy induced by CIP2A depletion. Collectively, these results indicate a critical role of CIP2A in driving disease progression and the spread of TNSC cells.

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