

SAPCD2 promotes invasiveness and migration ability of breast cancer cells *via* YAP/TAZ

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Abstract. – OBJECTIVE: The aim of this study was to explore whether SAPCD2 can affect the proliferation of breast cancer cells through YAP/TAZ, thus promoting the development of breast cancer (BCa).

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed to examine SAPCD2 expression level in BCa tissues collected from patients in different tumor TNM-stage. The correlation between SAPCD2 expression and clinicopathological features of patients were analyzed, and the Kaplan-Meier test was used for survival analysis. In addition, after knocking down SAPCD2 in cells, qRT-PCR and Western blot were applied to analyze the expression of the related genes and proteins, respectively. Moreover, Cell Counting Kit-8 (CCK-8) and transwell experiments were conducted to detect cell viability, migration, and invasion abilities. Furthermore, the changes in cell viability and migration and invasion abilities were examined after the simultaneous overexpression of YAP.

RESULTS: It was found that SAPCD2 expression levels in BCa tissues were remarkably higher than those in the normal control samples; meanwhile, patients with tumor size >3 cm or in the T3+T4 stage had a relatively higher expression of SAPCD2 than those with tumor size <3 cm or in T1+T2 stage. At the same time, the overall survival rate of BCa patients with highly expressed SAPCD2 was remarkably lower than that of patients in the low expression group. Moreover, it was found that the SAPCD2 level was correlated to the tumor size, TNM stage, and lymph node metastasis. After knocking down SAPCD2 in cells, cell viability, migration, and invasion abilities, as well as the YAP/TAZ protein expression levels were all found to be remarkably attenuated, which, however, were reversed after simultaneous overexpression of YAP in cells.

CONCLUSIONS: SAPCD2 may be able to enhance the proliferation ability of BCa cells *via* modulating the expression of YAP/TAZ, thereby prompting the progression of BCa.

Key Words:

Breast cancer, SAPCD2, YAP/TAZ.

Introduction

Breast cancer (BCa) is the most common malignant tumor in women¹. According to the 2015 China Cancer Statistics, the incidence of BCa ranks first among female malignant tumors in China, and the mortality rate ranks fourth, showing an increasing trend year by year². With the advancement of the treatment level of surgery, radiotherapy, and chemotherapy, the overall survival rate of BCa patients has been greatly improved. However, the onset of BCa is concealed and the degree of malignancy is high, so patients have been in the advanced stage when typical symptoms occur, so the treatment efficiency of BCa is not satisfactory, seriously affecting the prognosis of the patient^{3,4}. Therefore, it is urgent to find a convenient and high-efficiency diagnostic marker for BCa⁵.

Suppressor anaphase-promoting complex domain containing 2 (SAPCD2), also known as p42.3 or C9orf140, is a newly discovered highly conserved gene in mammals, which affects the cell cycle and participates in chromosome segregation^{6,7}. Related studies⁸⁻¹¹ have shown that the SAPCD2 gene is highly expressed in various tumor tissues, such as gastric cancer, glioma, and liver cancer, and is associated with tumor malignancy, cell proliferation, and invasiveness. However, there have been no reports on the relationship between SAPCD2 and BCa.

Yes-associated protein (YAP), the transcriptional CO-activator with PDZ-binding motif (TAZ), is an important member of the Hippo signaling pathway and is mainly involved in the

regulation of organ development and tumors¹². Zheng et al¹³ and Liu et al¹⁴ have found that elevated levels of YAP and TAZ or nuclear translocation can be found in various human tumors, which are more common in breast and ovarian cancers. The expression levels of YAP and TAZ can be used as independent prognostic indicators for some malignant tumors¹⁵. Huang et al¹⁶ have demonstrated that inhibition of the Hippo-YAP/TAZ signaling pathway is able to suppress the development of BCa; however, there is no research on the relationship between SAPCD2, YAP/TAZ, and BCa so far. Therefore, in this study, we explored whether SAPCD2 can affect the proliferation of BCa cells through the regulation of YAP/TAZ, so as to provide potential application value of SAPCD2 for the preparation of drugs.

Patients and Methods

Clinical Sample

BCa patients admitted to the Chui Yang Liu Hospital Affiliated to Tsing Hua University from January 2017 to January 2019 were enrolled in the study. The surgically resected BCa tissue specimens were assigned to the cancer group, while the para-carcinoma normal breast tissues were assigned to the control group. All patients were not treated with chemoradiotherapy. The study was informed to the patient and approved by the Hospital Ethics Committee. All patients provided written informed consent. This study was conducted in accordance with the Declaration of Helsinki.

Cell Culture

The cell lines were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and routinely cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 u/ml penicillin and 100 µg/ml streptomycin in an incubator with 5% CO₂ at 37°C.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Detection

The total RNA in tissues and cells was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) reagent, and the expression level of the related genes was detected using a PCR detection kit (TaKaRa, Tokyo, Japan). The primer sequences were

as follows: SAPCD2: forward: 5'-TGG CAT CTT TAC TGG ACT GG-3', reverse: 5'-TGG CAC CTC GTG GAT AGA GC-3'; β-Actin forward: 5'-TCA CCC ACA CTG TGC CCA TCT ACGA-3', reverse: 5'-CAG CGG AAC CGC TCATTG CCA ATG G-3'.

Cell Transfection

MCF-7 and BT549 cells were seeded at a density of 2 x 10⁵ cells/well in 6-well plates, cultured overnight, and transfected with plasmid using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 24 hours, the cells were passaged to fresh growth medium at a ratio of 1:10. The next day, 400 mg/mL G418 (Gibco, Rockville, MD, USA) was added in the culture medium. After 21 days, cell population with antibiotics resistance was selected, and the stable transfectants were maintained in DMEM supplemented with 10% calf serum and 200 mg/mL G418. (si-SAPCD2 1# forward 5'-TCCCACTCCCAGTTTACAGCTTGATCAAGAGTCAAGCTGTAAACTGGGAGTTT-3', reverse 5'-CAAAAAACTCCAGTTTACAGCTTGACTCTTGATCAAGCTGTAAACTGGGAGT-3', 2# forward 5'-TCCCACTGTGATGACACCCGGAAATCAAGAGTTTCCGGGTGTCATCACAGTTT-3', reverse 5'-CAAAAAACTGTGATGACACCCGGAAACTCTTGATTTCGGGTGTCATCACAGT-3').

Cell Counting Kit-8 (CCK-8) Assay

The cells in log phase growth were seeded in 96-well plates at 2×10³ cells/well. After 6, 24, 48, 72, and 96 h, 10 µl of CCK-8 (Dojindo, Kumamoto, Japan) was added to each well, and the cells continued to be cultured in the incubator for other 2-4 h. After that, the optical density (OD) of each well was measured at 450 nm with a microplate reader.

Transwell Cell Migration Assay

The transwell chamber was placed in a 24-well plate, 600 µL of the culture medium, and 200 µL of the previously prepared cell suspension was added to the lower chamber. The cell suspension was prepared in serum-free Roswell Park Memorial Institute-1640 (RPMI-1640; HyClone, South Logan, UT, USA) medium containing various concentrations (0, 0.25, 0.5, and 1 mmol/L) of sinomenine. Three replicate wells were set for each concentration, and the cells were cultured for 12 h under normal conditions. After the chamber was

taken out, the cells in the chamber were wiped with a cotton swab, washed twice with phosphate-buffered saline (PBS), fixed for 30 min with formaldehyde, stained with crystal violet for 20 min, and washed twice with PBS. The migrating cells attached to the lower surface of the chamber were observed under a microscope and photographed.

Transwell Cell Invasion Assay

The method was basically the same as the transwell migration experiment, except that Matrigel and RPMI-1640 medium were diluted at 1:6 in advance. 50 μ L was evenly spread to the bottom of the transwell chamber; then, the transwell chamber was placed in a 24-well plate and incubated at 37°C. At this time, the invading cells were observed on the lower surface of the chamber.

Western Blot

The total protein was extracted by radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China) lysing cells, and the protein concentration was determined by ultraviolet spectrophotometry. 25 μ g of protein sample was taken for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), blocked with blocking solution (5% BSA/TBST) for 1 h, and incubated with anti-SAPCD2, anti-YAP, anti-TAZ (1:200) antibodies at 4°C overnight. After the membrane was washed with TBST 3 times, the protein was incubated again with secondary antibodies for 1 h at room temperature. After washing the membrane 3 times with TBST, enhanced chemiluminescence (ECL; Pierce, Rockford, IL, USA) was added for luminescence reaction. The protein in the membrane was developed and photographed in the darkroom, and grayscale analysis was performed using Image J 1.45s software (NIH, Bethesda, MD, USA).

Statistical Analysis

SPSS 22.0 (SPSS IBM Corp., Armonk, NY, USA) statistical software was used to process the data. The measurement data were analyzed by an independent sample *t*-test; the count data was tested by χ^2 . Survival curves were plotted using the Kaplan-Meier method, and the difference in patient survival was compared using the Log-rank test. The difference was statistically significant at $p < 0.05$. * $p < 0.05$.

Results

High Expression of SAPCD2 in BCa

qRT-PCR revealed that SAPCD2 level in 48 BCa tissues was remarkably higher than that in the normal control group (Figure 1A); the expression in patients with tumor size > 3 cm was remarkably higher than in patients with tumor size < 3 cm (Figure 1B); the expression in patients in T3+T4 stage was remarkably higher than in patients in T1+T2 stage (Figure 1C). According to the qRT-PCR results of SAPCD2 expression, the BCa tissue samples were divided into high and low expression groups. Survival analysis revealed that the overall survival rate of BCa patients with high expression of SAPCD2 was remarkably lower than that of low expression group, HR=2.276, $p=0.0348$ (Figure 1D). The above observations indicated that high expression of SAPCD2 in BCa tissues can promote the progression of BCa and may lead to a poor prognosis of BCa patients.

Correlation Between SAPCD2 Expression and Clinicopathological Features in Patients with BCa

In order to clarify the interplay between SAPCD2 and clinicopathological features of BCa patients, we collected and processed the related data, and the results of χ^2 -test revealed that SAPCD2 level was statistically different in tumors with different tumor size and in different TNM stage; in addition, whether lymph node metastasis occurred was also related to the SAPCD2 expression in tumors ($p < 0.05$). However, no significant difference was found in the age and gender of the patients ($p > 0.05$) (Table I). These results indicated that the SAPCD2 level was associated with tumor size, TNM stage, and lymph node metastasis.

Inhibition of Invasiveness and Migration Ability of MCF-7 and BT549 Cells After Knockdown of SAPCD2

To explore the mechanism of action of SAPCD2, SAPCD2 was knocked down in MCF-7 and BT549 cells, after which qRT-PCR and Western blot revealed a significant decrease in SAPCD2 gene and protein expression (Figure 2A, 2B), suggesting that the transfection effect was good and the cells could be used in subsequent experimental studies. Furthermore, it was found by CCK-8 assay that the cell viability was remarkably decreased after the knockdown of SAPCD2 (Figure 2C); meanwhile, transwell assay revealed a decrease in cell migration and invasion ability

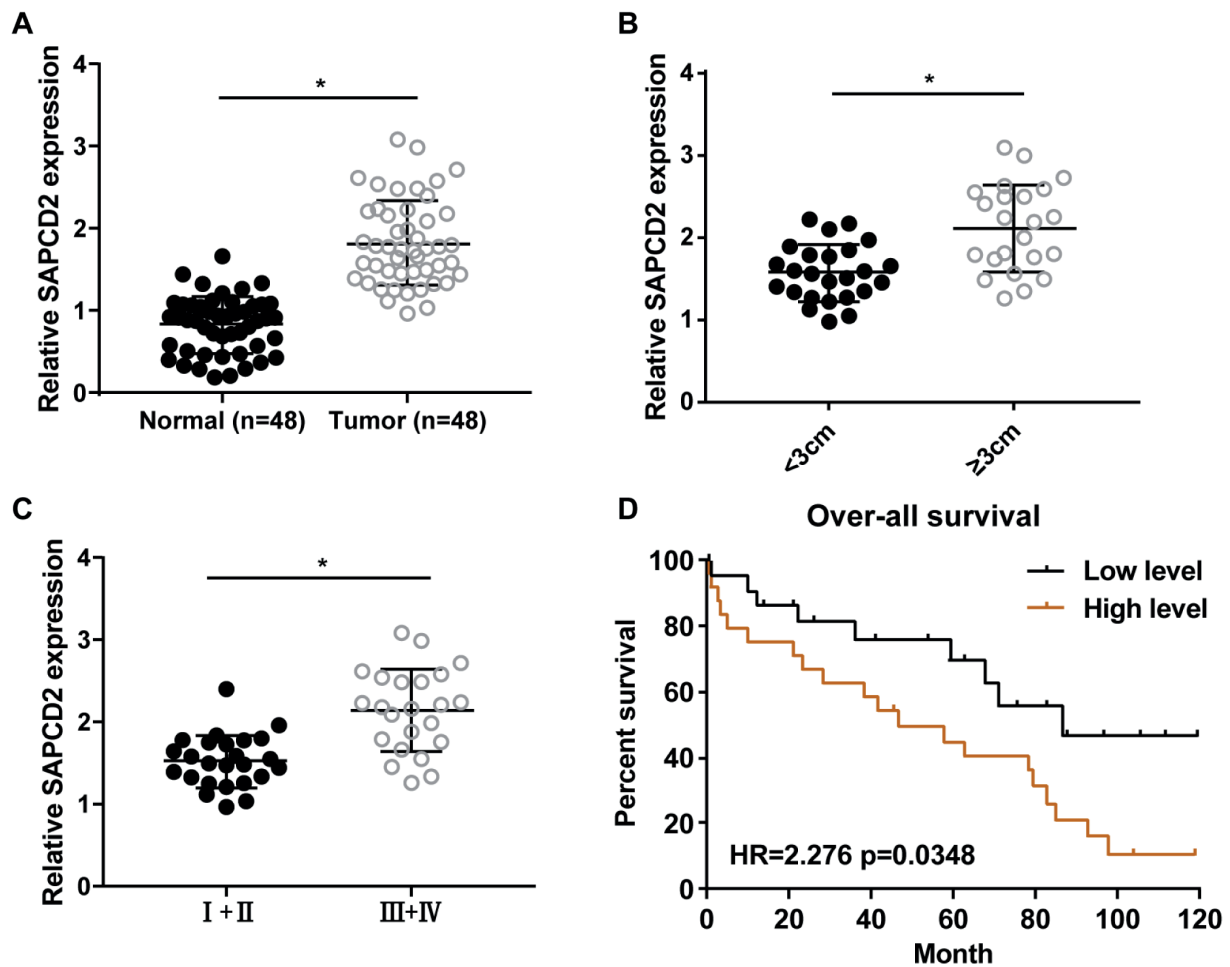


Figure 1. SAPCD2 is highly expressed in breast cancer. **A**, qRT-PCR showed that the expression of SAPCD2 in 48 breast cancer tissues was significantly higher than that in the normal control group. **B**, qRT-PCR showed that the expression of SAPCD2 in patients with tumor size of ≥ 3 cm was significantly higher than in patients with tumors < 3 cm. **C**, qRT-PCR showed that the expression of SAPCD2 in patients with T3+T4 tumors was significantly higher than that in patients with T1+T2 tumors. **D**, The overall survival rate of breast cancer patients with high expression of SAPCD2 was significantly lower than that of SAPCD2 low expression group.

Table I. Correlation between SAPCD2 expression and clinicopathological features in breast cancer patients (n = 48).

Clinicopathologic features	Number of cases	SAPCD2 expression		p-value
		Low (n=24)	High (n=24)	
Age (years)				0.5623
≤50	26	12	14	
>50	22	12	10	
Gender				0.3861
Male	23	10	13	
Female	25	14	11	
Tumor size				0.0088*
≤2 cm	27	18	9	
>2 cm	21	6	15	
TNM stage				0.0325*
I-II	26	17	9	
III-IV	23	8	15	
Lymph node metastasis				0.0431*
Absent	25	16	9	
Present	23	8	15	

(Figure 2D), indicating that SAPCD2 can affect cell viability, migration, and invasion capacities, thus participating in the progression of BCa.

Inhibition of YAP/TAZ Expression After Knockdown of SAPCD2

To clarify the relationship between SAPCD2 and YAP/TAZ, we knocked down SAPCD2 in MCF-7

cells, and the Western blot result confirmed that the protein expression level of YAP/TAZ was remarkably decreased (Figure 3A), indicating that SAPCD2 can affect the expression of YAP/TAZ. After knockdown of SAPCD2 in BT549 cells, the protein expression level of YAP/TAZ was also remarkably decreased (Figure 3B), which further demonstrated that SAPCD2 expression is able to modulate YAP/TAZ level.

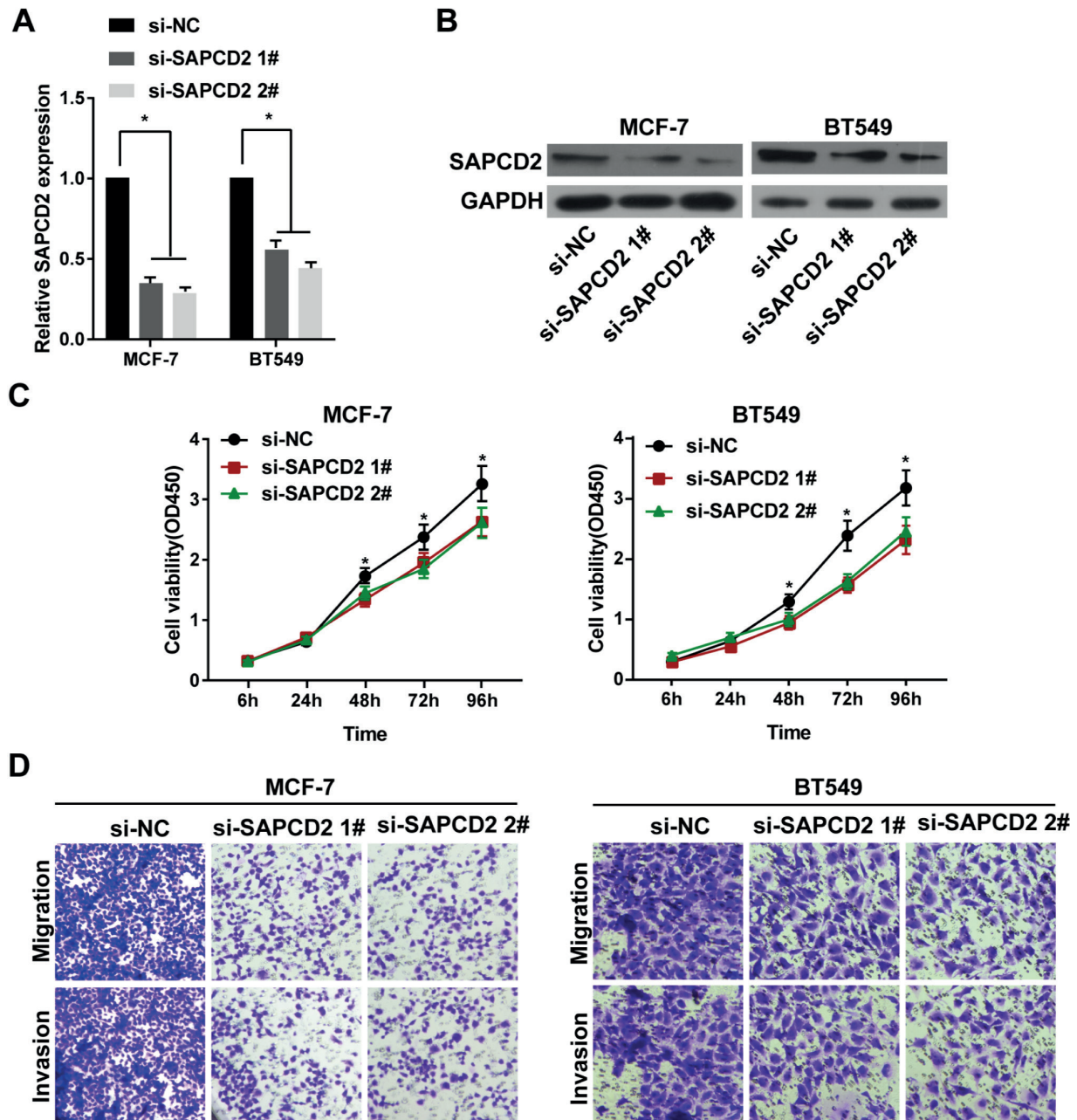


Figure 2. Inhibition of invasion and migration of MCF-7 and BT549 cells after knockdown of SAPCD2. After knockdown of SAPCD2 in MCF-7 and BT549 cells, (A) qRT-PCR detected a significant decrease in SAPCD2 expression; (B) Western blot detected a significant decrease in SAPCD2 protein expression; (C) CCK-8 detected a significant decrease in cell viability; (D) the transwell assay detected a decrease in cell migration and invasion ability (magnification: 40x).

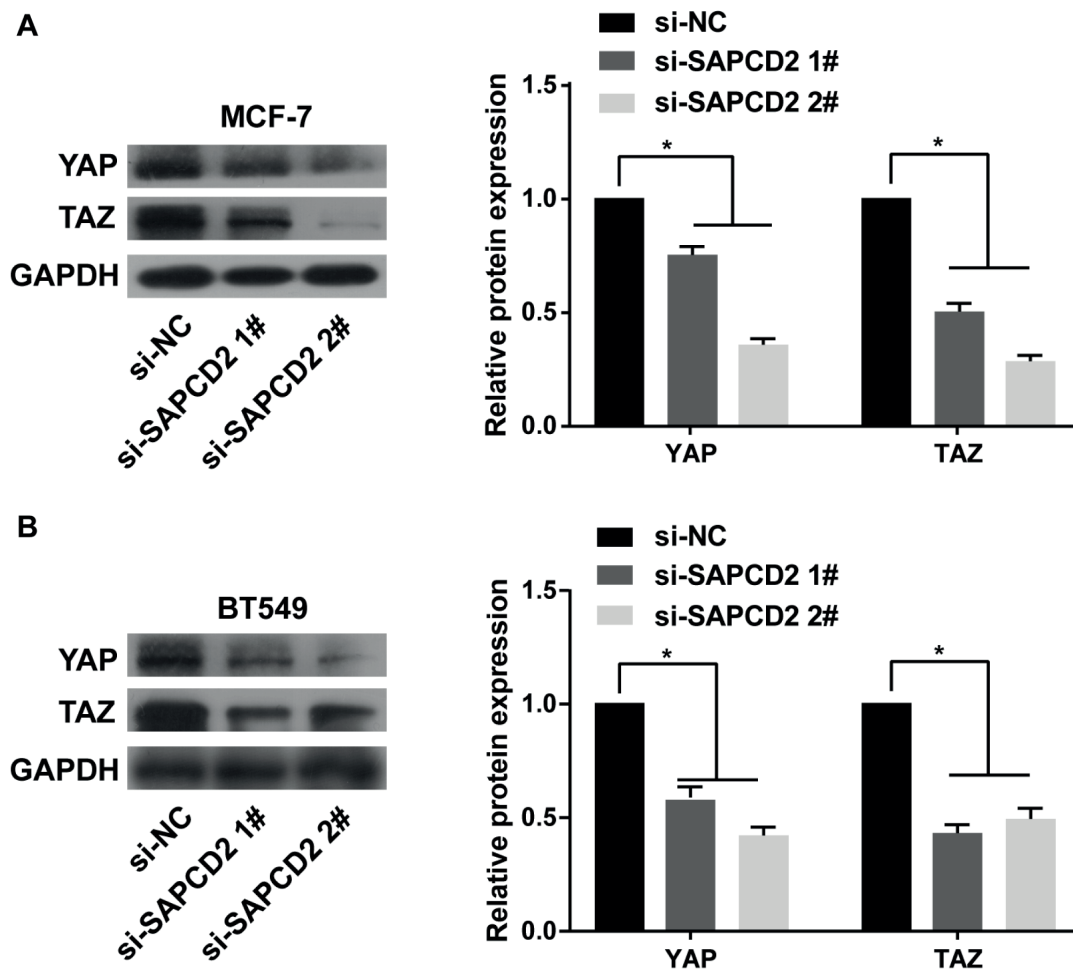


Figure 3. Inhibition of YAP/TAZ expression after knockdown of SAPCD2. **A**, Western blot result showed that after knocking down SAPCD2 in MCF-7 cells, the protein expression level of YAP/TAZ was significantly decreased. **B**, Western blot result showed that after knocking down SAPCD2 in BT549 cells, the protein expression level of YAP/TAZ was significantly decreased.

Inhibition of Invasiveness and Migration Ability of MCF-7 and BT549 Cells by Overexpression of YAP Reverse Knockdown of SAPCD2

To further investigate the interplay between SAPCD2, YAP/TAZ, and BCa, we overexpressed YAP in cells and found that it reversed the inhibitory effect of SAPCD2 knockdown on cell viability, as well as invasion and migration abilities (Figure 4A, 4B), indicating that SAPCD2 can promote cell viability, invasiveness, and migration abilities through YAP/TAZ.

Discussion

As a highly heterogeneous solid tumor¹⁷, BCa has become a great threat to the health of contem-

porary women. Although various endocrine therapy drugs, chemotherapy drugs, targeted therapeutic drugs, and immunotherapy drugs have improved the prognosis of various molecular BCa patients to some extent, the 5 year-survival rate and prognosis of patients are still unsatisfactory¹⁸. Therefore, the diagnosis markers are significant for further treating BCa and improving the survival rate of BCa patients to a greater extent¹⁹.

SAPCD2, a newly discovered gene associated with cell cycle regulation, is expressed only in tumors but not in normal adult tissues, which is likely to be an important potential marker in the progression of colorectal cancer²⁰. To the role of oncogenes, Weng et al²¹ have shown that SAPCD2 level in colorectal cancer is related to the patient's age, gender, tumor location, tumor differentiation, and lymph node metastasis. According

to the analysis in this experiment, we found that SAPCD2 is associated with tumor size, clinical stage, and lymph node metastasis of BCa, but not with the age and gender of the patient. In addition, the results of this study also revealed that compared with that in normal tissue, SAPCD2 level in BCa tissues was remarkably increased, which is consistent with the relevant research results^{20,21}. In addition, SAPCD2 level is higher in patients with larger tumors and higher clinical stage, indicating that SAPCD2 expression has a certain relationship with the occurrence of BCa, and it is likely to be a new potential marker for the early diagnosis of BCa.

In mammals, YAP and TAZ are downstream effector molecules of the Hippo pathway. The

YAP gene is localized on human chromosome 11q22 and widely expressed in body tissues under normal physiological conditions. TAZ, also known as WWTR1, is homologous to YAP, with 46% identical amino acid sequence²². It was found by Hiemer et al²³ that abnormal nuclear YAP accumulation in malignant oral tissues is associated with the spread of cancer cells, and abnormal activation of nuclear YAP, and TAZ promotes cell proliferation. It is speculated that YAP and TAZ play a significant role in the development of oral squamous cell carcinoma. YAP promotes BCa metastasis *via* inhibiting growth differentiation factor-15²⁴. Another study also revealed that TAZ is highly expressed in about 20% of BCa samples, and its expression level

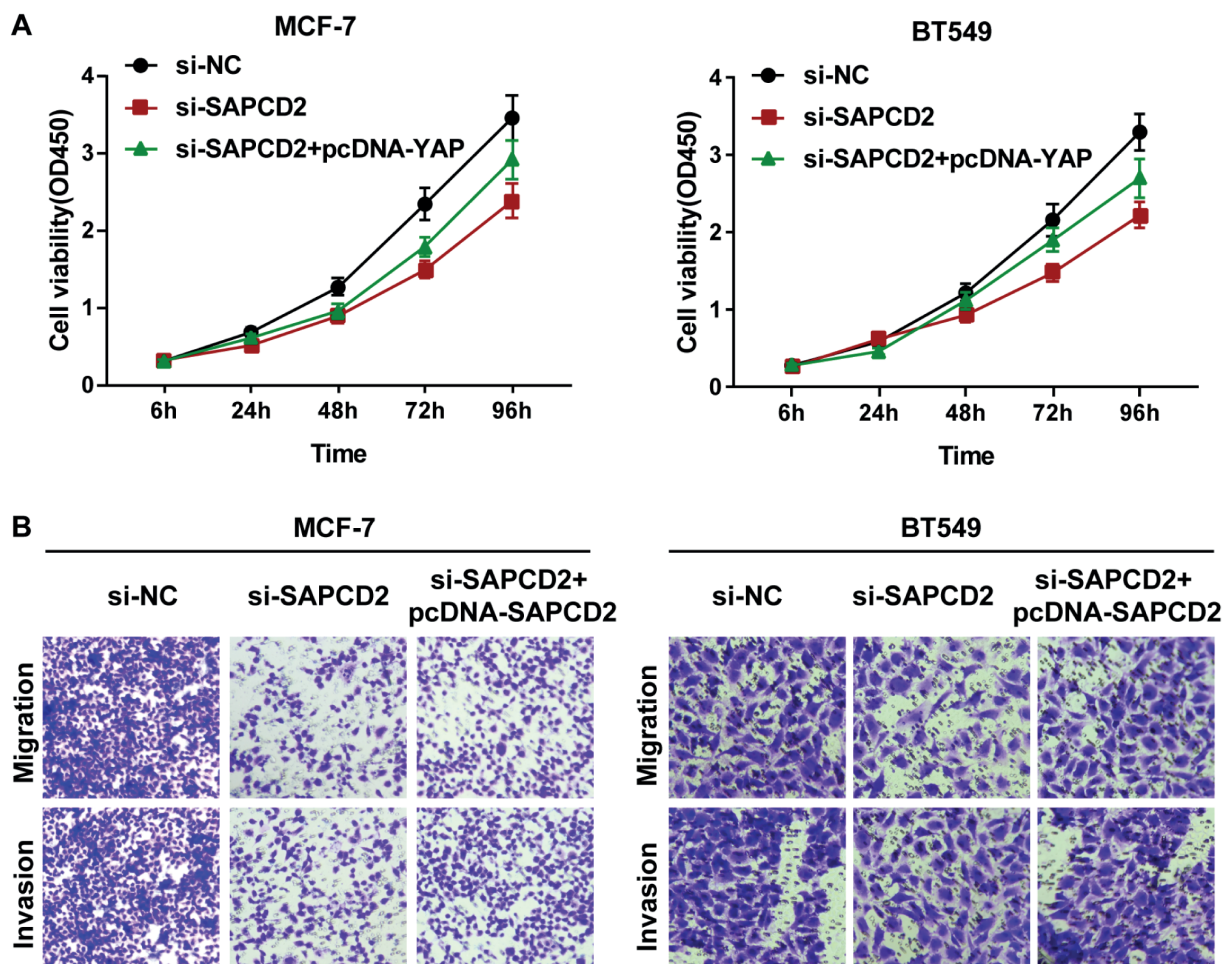


Figure 4. Overexpression of YAP reversed the inhibitory effect of knockdown of SAPCD2 on the invasion and migration of MCF-7 and BT549 cells. **A**, CCK-8 assay detected the effect of knockdown of SAPCD2 and simultaneous overexpression of YAP on cell proliferation. **B**, The transwell assay detected the effect of knockdown of SAPCD2 and simultaneous overexpression of YAP on cell invasion and migration (magnification: 40x).

is related to the aggressiveness of cancer cells. High expression of TAZ enhances the migration and invasion ability of BCa cells²⁵. It was found in this study that the downregulation of SAPCD2 inhibited the expression of YAP/TAZ, while simultaneous overexpression of YAP could reverse the inhibitory effect of SAPCD2 on the invasiveness and migration ability of MCF-7 and BT549 cells.

In summary, SAPCD2 is highly expressed in BCa tissues. SAPCD2 can upregulate the expression of YAP/TAZ and promote the viability, invasiveness, and migration ability of BCa cells, which has opened up new ideas for the research on the pathogenesis of BCa.

Conclusions

We found that SAPCD2 can enhance cell viability, as well as invasiveness and migration ability, by modulating YAP/TAZ, thus affecting the development of BCa.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

- 1) TORRE LA, BRAY F, SIEGEL RL, FERLAY J, LORTET-TIEULENT J, JEMAL A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; 65: 87-108.
- 2) CHEN W, ZHENG R, BAADE PD, ZHANG S, ZENG H, BRAY F, JEMAL A, YU XO, HE J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; 66: 115-132.
- 3) FAN L, STRASSER-WEIPPL K, LI JJ, ST LJ, FINKELSTEIN DM, YU KD, CHEN WO, SHAO ZM, GOSS PE. Breast cancer in China. *Lancet Oncol* 2014; 15: e279-e289.
- 4) LIU XM, YANG B, HAN J. Increased long noncoding RNA LINP1 expression and its prognostic significance in human breast cancer. *Eur Rev Med Pharmacol Sci* 2018; 22: 8749-8754.
- 5) DOS APB, DA LF, DE FARIA PR, OLIVEIRA AP, DE ARAUJO RA, SILVA MJ. Far beyond the usual biomarkers in breast cancer: a review. *J Cancer* 2014; 5: 559-571.
- 6) CAO WJ, DU WQ, MAO LL, ZHENG JN, PEI DS. Overexpression of p42.3 promotes cell proliferation, migration, and invasion in human gastric cancer cells. *Tumour Biol* 2016; 37: 12805-12812.
- 7) MAO L, SUN W, LI W, CUI J, ZHANG J, XING R, LU Y. Cell cycle-dependent expression of p42.3 promotes mitotic progression in malignant transformed cells. *Mol Carcinog* 2014; 53: 337-348.
- 8) WAN W, XU X, JIA G, LI W, WANG J, REN T, WU Z, ZHANG J, ZHANG L, LU Y. Differential expression of p42.3 in low- and high-grade gliomas. *World J Surg Oncol* 2014; 12: 185.
- 9) SUN W, DONG WW, MAO LL, LI WM, CUI JT, XING R, LU YY. Overexpression of p42.3 promotes cell growth and tumorigenicity in hepatocellular carcinoma. *World J Gastroenterol* 2013; 19: 2913-2920.
- 10) ZHANG J, LU C, SHANG Z, XING R, SHI L, LV Y. p42.3 gene expression in gastric cancer cell and its protein regulatory network analysis. *Theor Biol Med Model* 2012; 9: 53.
- 11) XU X, LI W, FAN X, LIANG Y, ZHAO M, ZHANG J, LIANG Y, TONG W, WANG J, YANG W, LU Y. Identification and characterization of a novel p42.3 gene as tumor-specific and mitosis phase-dependent expression in gastric cancer. *Oncogene* 2007; 26: 7371-7379.
- 12) ZHANG Y, DEL RE DP. A growing role for the Hippo signaling pathway in the heart. *J Mol Med (Berl)* 2017; 95: 465-472.
- 13) ZHENG L, XIANG C, LI X, GUO Q, GAO L, NI H, XIA Y, XI T. STARD13-correlated ceRNA network-directed inhibition on YAP/TAZ activity suppresses stemness of breast cancer via co-regulating Hippo and Rho-GTPase/F-actin signaling. *J Hematol Oncol* 2018; 11: 72.
- 14) LIU J, YE L, LI Q, WU X, WANG B, OUYANG Y, YUAN Z, LI J, LIN C. Synaptopodin-2 suppresses metastasis of triple-negative breast cancer via inhibition of YAP/TAZ activity. *J Pathol* 2018; 244: 71-83.
- 15) BOUVIER C, MACAGNO N, NGUYEN Q, LOUNDOU A, JIGUET-JIGLAIRE C, GENTET JC, JOUVE JL, ROCHWERGER A, MATTEI JC, BOUVARD D, SALAS S. Prognostic value of the Hippo pathway transcriptional coactivators YAP/TAZ and β 1-integrin in conventional osteosarcoma. *Oncotarget* 2016; 7: 64702-64710.
- 16) HUANG X, TANG F, WENG Z, ZHOU M, ZHANG Q. MiR-591 functions as tumor suppressor in breast cancer by targeting TCF4 and inhibits Hippo-YAP/TAZ signaling pathway. *Cancer Cell Int* 2019; 19: 108.
- 17) CLARK BZ, ONISKO A, ASSYLBEKOVA B, LI X, BHARGAVA R, DABBS DJ. Breast cancer global tumor biomarkers: a quality assurance study of intratumoral heterogeneity. *Mod Pathol* 2019; 32: 354-366.
- 18) LIU M, TSANG JYS, LEE M, NI YB, CHAN SK, CHEUNG SY, HU J, HU H, TSE GMK. CD147 expression is associated with poor overall survival in chemotherapy treated triple-negative breast cancer. *J Clin Pathol* 2018; 71: 1007-1014.
- 19) RESENDE U, CABELLO C, OLIVEIRA BRS, ZEGERINO LC. Predictors of pathological complete response in women with clinical complete response to neoadjuvant chemotherapy in breast carcinoma. *Oncology* 2018; 95: 229-238.
- 20) YUAN XS, ZHANG Y, GUAN XY, DONG B, ZHAO M, MAO LL, LU YY, TIAN XY, HAO CY. P42.3: a promising biomarker for the progression and prognosis of human colorectal cancer. *J Cancer Res Clin Oncol* 2013; 139: 1211-1220.

- 21) WENG YR, YU YN, REN LL, CUI Y, LU YY, CHEN HY, MA X, QIN WX, CAO W, HONG J, FANG JY. Role of C9orf140 in the promotion of colorectal cancer progression and mechanisms of its upregulation via activation of STAT5, β -catenin and EZH2. *Carcinogenesis* 2014; 35: 1389-1398.
- 22) MENG Z, MOROISHI T, GUAN KL. Mechanisms of Hippo pathway regulation. *Genes Dev* 2016; 30: 1-17.
- 23) HIEMER SE, ZHANG L, KARTHA VK, PACKER TS, ALMERSHED M, NOONAN V, KUKURUZINSKA M, BAIS MV, MONTI S, VARELAS X. A YAP/TAZ-regulated molecular signature is associated with oral squamous cell carcinoma. *Mol Cancer Res* 2015; 13: 957-968.
- 24) WANG T, MAO B, CHENG C, ZOU Z, GAO J, YANG Y, LEI T, QI X, YUAN Z, XU W, LU Z. YAP promotes breast cancer metastasis by repressing growth differentiation factor-15. *Biochim Biophys Acta Mol Basis Dis* 2018; 1864: 1744-1753.
- 25) CHAN SW, LIM CJ, GUO K, NG CP, LEE I, HUNZIKER W, ZENG Q, HONG W. A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells. *Cancer Res* 2008; 68: 2592-2598.