

Original Research Article

Synaptopodin 2 represses cervical cancer cell growth and enhances the sensitivity of cervical cancer cells to cisplatin via Hippo pathway

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Abstract

Purpose: To explore the functions and regulatory mechanisms of synaptopodin-2 (SYNPO2) in cervical cancer progression.

Methods: Normal cervical cell lines (Ect1/E6E7) and cervical cancer cell lines (HeLa, SiHa, C-33A, and CaSki) were cultured. The pcDNA3.1 vector overexpressing SYNPO2, a negative control (vector), and blank control (control) were transfected into HeLa and SiHa cells. Protein expression from normal and cervical cancer cell lines was examined by western blot. Cell viability and proliferation were evaluated in HeLa and SiHa cells using Cell Counting Kit-8 and colony formation assays, while cell migration and invasion were assessed by wound healing and Transwell assays, respectively. Cell apoptosis was determined by flow cytometry.

Results: SYNPO2 expression was decreased in cervical cancer based on the Gene Expression Profiling Interactive Analysis website ($p < 0.05$). Additionally, Kaplan–Meier Plotter website showed that cervical cancer patients with low SYNPO2 expression showed worse prognoses than patients with high SYNPO2 expression ($p < 0.05$). Subsequent investigations revealed that SYNPO2 overexpression repressed cell proliferation, migration, and invasion in cervical cancer ($p < 0.01$). Furthermore, SYNPO2 overexpression enhanced cervical cancer cell apoptosis ($p < 0.001$) and increased the sensitivity of cervical cancer cells to cisplatin ($p < 0.01$). The regulatory function of SYNPO2 on Hippo pathway in cervical cancer indicate that SYNPO2 inactivated Hippo pathway ($p < 0.05$).

Conclusion: Synaptopodin 2 represses cervical cancer cell growth and enhances the sensitivity of cervical cancer cells to cisplatin via Hippo pathway, thus indicating its potentials for development for the treatment of cervical cancer.

Keywords: Synaptopodin-2 (SYNPO2), Cervical cancer, Hippo pathway, HeLa and SiHa cells, pcDNA3.1 vector

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INTRODUCTION

Cervical cancer is a common pernicious tumor, and the fourth-leading cause of cancer-related

death in women [1]. Human papillomavirus (HPV) infection is a key factor for cervical cancer, but other factors play a role as well [2]. Treatments for cervical cancer include surgery,

chemotherapy, and radiotherapy. However, treatments for this disease are difficult, and treatments have only limited effectiveness. Treatment outcomes for cervical cancer patients remain poor due to the high recurrence rate. The underlying pathogenesis of cervical cancer is also vague. With the evolution of molecular biology, useful molecular targets have been identified, thus enabling breakthroughs in cancer treatment [3]. It is, therefore, crucially important to examine these novel molecular targets for cervical cancer treatment.

Synaptopodin-2 (SYNPO2) was the second member of the podin family to be identified, and its encoding gene is located in chromosome 4q26. Previous reports have revealed that SYNPO2 is expressed in diverse cancers, and it acts as a tumor repressor. For instance, SYNPO2 targets the yes-associated protein 1 (YAP)–Krueppel-like factor 5 (KLF5) axis in colorectal cancer, and reduces hypoxia-stimulated cell proliferation and migration [4]. Moreover, in glioma, vitamin C enhances SYNPO2 expression, which then inhibits cell proliferation and migration [5]. Additionally, SYNPO2 affects the PI3K/Akt/mTOR pathway and suppresses breast cancer progression [6]. In triple-negative breast cancer, vitamin C modulates YAP1 and SYNPO2 so as to inhibit metastasis [7]. However, the functions and regulatory mechanisms of SYNPO2 in cervical cancer remain unclear.

The Hippo pathway was shown to be involved in cell apoptosis and proliferation. More importantly, it has been shown that SYNPO2 reduces YAP/TAZ activity in order to inhibit triple-negative breast cancer metastasis [8]. Nevertheless, it is unclear whether SYNPO2 affects the Hippo pathway in cervical cancer.

EXPERIMENTAL

Cell lines and cultures

Normal cervical cell line (Ect1/E6E7) and cervical cancer cell lines (HeLa, SiHa, C-33A, and CaSki) were obtained from the American Tissue Culture Collection (ATCC, USA). Cell cultivation was performed in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) and 10 % fetal bovine serum (FBS, Gibco) in a drippy incubator at 37 °C containing 5 % CO₂.

Cell transfection

The pcDNA3.1 vector overexpressing SYNPO2 (SYNPO2), a negative control (vector), and blank control (control) were obtained from

GenePharma (Shanghai, China). Transfection into HeLa and SiHa cells was done using Lipofectamine 2000 (Invitrogen, USA).

Western blot

The proteins from cervical cancer cells were extracted using RIPA lysis buffer (Beyotime, Shanghai, China). The separation of extracted proteins was carried out with 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and were subsequently transferred to polyvinylidene fluoride (PVDF) membranes (Beyotime, Shanghai, China). After blocking, the membranes and primary antibodies were incubated overnight at 4 °C. The primary antibodies were SYNPO2 (1 µg/mL; ab105421; Abcam), B cell lymphoma 2 (Bcl-2; 1: 1,000; ab196495; Abcam), Bcl-2-associated X protein (Bax; 1 µg/ml; ab104156; Abcam), phosphorylated (p)-LAST1 (1: 1,000, Cell Signaling Technology, USA), LAST1 (1: 1,000, Cell Signaling Technology), p-YAP (1 µg/ml; ab62751; Abcam), YAP (1: 5,000; ab52771; Abcam), histone H3 (1: 1,000; ab1791; Abcam), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1: 10,000; ab128915; Abcam). Next, the membranes were incubated with the appropriate secondary antibodies (1: 2,000; ab7090; Abcam) at room temperature for 1 h. The western blot was subsequently analyzed with a chemiluminescence determination kit (Thermo Fisher Scientific, Inc., USA).

Cell Counting Kit-8 (CCK-8) assay

The transfected cervical cancer cells were seeded in 96-well plates. The CCK-8 solution (10 µL; Dojindo Laboratories, Kumamoto, Japan) was then added to the plates at 0, 1, 2, and 3 days. The optical density (OD) value was measured with a microplate reader (BioTek Instruments, USA) at 450 nm. For IC₅₀ value determination, Cisplatin (DDP) was added in 96-well plates, and then CCK-8 solution was added.

Colony formation assay

Cervical cancer cells (1,000 cells/well) were cultured for 14 days in 6-well plates. The cells were then fixed with 4 % paraformaldehyde and stained with 0.1 % crystal violet so as to assess colony formations. The images of the colonies were obtained with a microscope.

Wound-healing assay

Cervical cancer cells were grown to 80 – 90 % confluency in the 6-well plates. A scratch-wound was made with a sterile pipette tip (10 µL). Cell

migration distance was measured at the beginning and after 24 h under a microscope.

Transwell assay

Cell invasion assays were performed using Transwell Matrigel inserts (BD Biosciences, MD, USA). Serum-free DMEM medium was added into the upper chamber. The DMEM medium with FBS (20 %) was added into the lower chamber. Crystal violet was used to stain the invading cells, and they were observed with a microscope.

Flow cytometry

The Annexin V-FITC Apoptosis Determination Kit (Beyotime) and a BD FACS Calibur (BD Bioscience, USA) were utilized for flow cytometry. After washing, the cells were stained with Annexin V-FITC and propidium iodide in the dark. Cell apoptosis was evaluated by flow cytometry.

Statistical analysis

Data are reported as mean ± standard deviation (SD). Each experiment was carried out three times. SPSS 22.0 statistical software (IBM Corp, USA) was used for statistical analysis. Comparisons were performed with Student's *t*-test (for two groups) or one-way analysis of variance (ANOVA) for multiple groups. Statistical significance was set at *p* < 0.05.

RESULTS

SYNPO2 was associated with a lower expression in cervical cancer

Using the Gene Expression Profiling Interactive Analysis (GEPIA) website, the expression of SYNPO2 was confirmed to be decreased in cervical cancer cells (Figure 1 A). Moreover, using the Kaplan–Meier Plotter website, a worse prognosis was given for patients with low SYNPO2 expression than those with high SYNPO2 expression (Figure 1 B). Compared with normal cervical cell line (Ect1/E6E7), the cervical cancer cell lines (HeLa, SiHa, C-33A, and CaSki) had lower SYNPO2 protein expression (Figure 1 C). These results revealed that SYNPO2 displayed a lower expression in cervical cancer.

SYNPO2 suppressed cell proliferation

The overexpression efficiency of SYNPO2 was shown in Figure 2 A. Moreover, it was found that the OD value was reduced after overexpressing SYNPO2 (Figure 2 B), and the number of colony-

forming cells was decreased after SYNPO2 overexpression (Figure 2 C). In general, the data illustrated that SYNPO2 suppressed cell proliferation in cervical cancer.

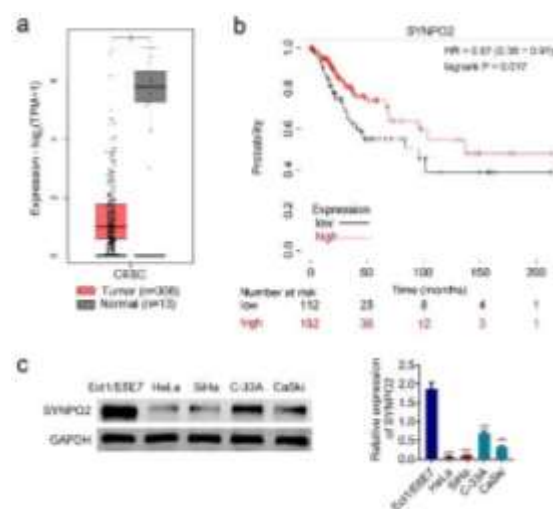


Figure 1: SYNPO2 had a lower expression in cervical cancer. (a) Expression of SYNPO2 confirmed using the Gene Expression Profiling Interactive Analysis (GEPIA) website; (b) The prognoses of patients with low or high SYNPO2 expression assessed from the Kaplan–Meier online website; (c) The protein expression of SYNPO2 measured in normal cervical cell line (Ect1/E6E7) and cervical cancer cell lines (HeLa, SiHa, C-33A, and CaSki) through western blot. **P* < 0.05 vs. normal group; ^{^^}*p* < 0.001 vs. the Ect1/E6E7 group

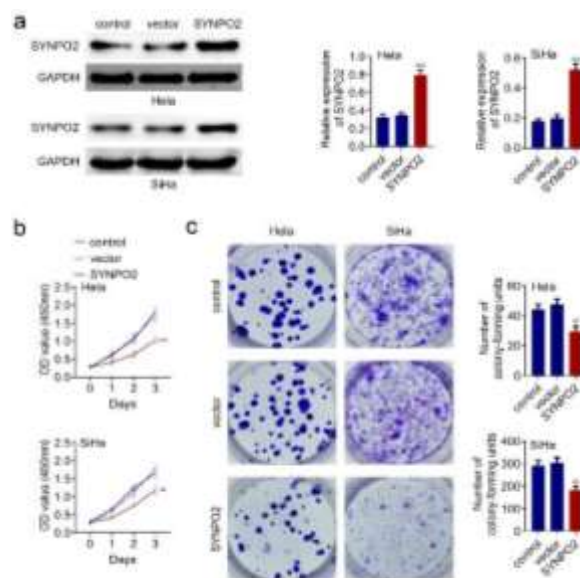


Figure 2: SYNPO2 suppressed cell proliferation in cervical cancer. (a) The overexpression efficiency of SYNPO2 confirmed in HeLa and SiHa cells through western blot; (b, c) Cell proliferation of HeLa and SiHa cells measured after overexpressing SYNPO2 with the Cell Counting Kit-8 (CCK-8) and colony formation assays. [^]*P* < 0.01, ^{^^}*p* < 0.001 vs the vector group

SYNPO2 inhibited cell migration and invasion

It was demonstrated that cell migration ability was weakened after SYNPO2 up-regulation (Figure 3 A). Additionally, cell invasion was reduced after SYNPO2 overexpression (Figure 3 B). These findings suggest that SYNPO2 inhibited cell migration and invasion in cervical cancer.

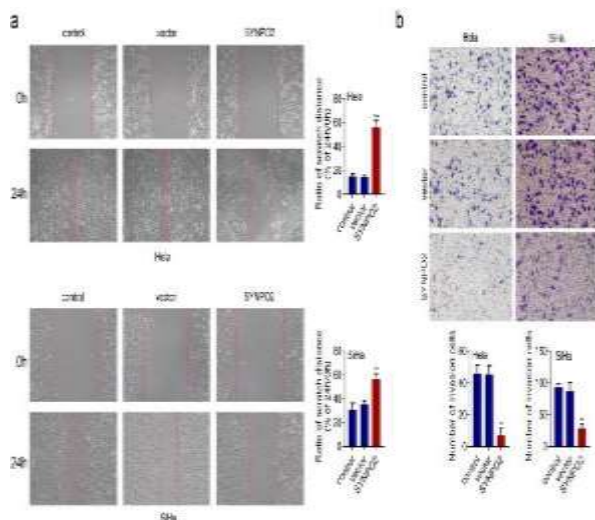


Figure 3: SYNPO2 inhibited cell migration and invasion in cervical cancer. (a) Cell migration of HeLa and SiHa cells measured after overexpressing SYNPO2 through wound-healing assay; (b) Cell invasion of HeLa and SiHa cells measured after overexpressing SYNPO2 through Transwell assay. $^{**}P < 0.01$, $^{***}p < 0.001$ vs the vector group

SYNPO2 enhanced cell apoptosis

Cell apoptosis was increased after SYNPO2 overexpression (Figure 4 A). Furthermore, Bax protein expression increased, while Bcl-2 protein expression decreased after SYNPO2 overexpression (Figure 4 B). Thus, these results show that SYNPO2 enhanced cell apoptosis in cervical cancer.

SYNPO2 increased the sensitivity of cervical cancer cells to cisplatin (DDP)

The inhibitory concentration 50 (IC_{50}) value increased ($4.15 \mu\text{g/mL}$ to $22.82 \mu\text{g/mL}$) in HeLa/DDP (or SiHa/DDP) cells, compared with HeLa (or SiHa) cells (Figure 5 A). Moreover, in HeLa/DDP (or SiHa/DDP) cells, the IC_{50} value decreased after SYNPO2 overexpression (Figure 5 B). These findings show that SYNPO2 overexpression increased the sensitivity of cervical cancer cells to DDP.

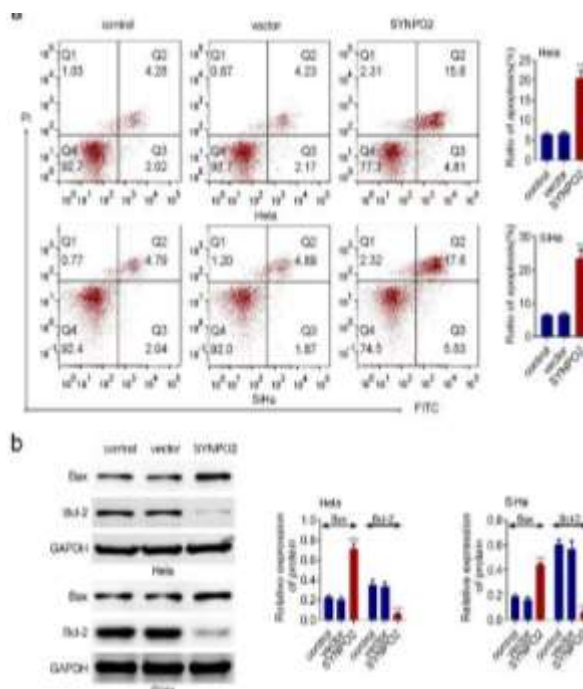


Figure 4: SYNPO2 enhanced cell apoptosis in cervical cancer. (a) Cell apoptosis determined after overexpressing SYNPO2 in HeLa and SiHa cells through flow cytometry; (b) Expression of the apoptosis-related proteins Bcl-2-associated X protein (Bax) and B cell lymphoma 2 (Bcl-2) measured after overexpressing SYNPO2 in HeLa and SiHa cells by western blot. $^{***}P < 0.001$ vs the vector group

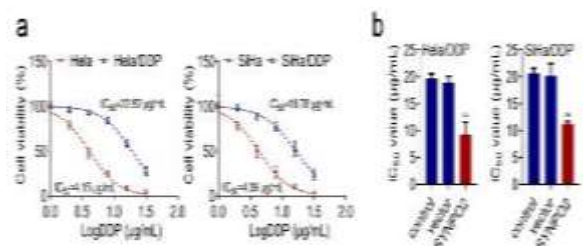


Figure 5: SYNPO2 increased the sensitivity of cervical cancer cells to cisplatin. (a) Cell viability (IC_{50} value) in HeLa (or SiHa) cells and HeLa/DDP (or SiHa/DDP) cells measured through using the CCK-8 assay; (b) IC_{50} values in HeLa/DDP and SiHa/DDP cells confirmed after overexpressing SYNPO2. $^{**}P < 0.01$

SYNPO2 inactivated the Hippo pathway in cervical cancer

It was found that the p-LAST1/LAST1 and p-YAP/YAP levels were elevated after SYNPO2 overexpression (Figure 6 A). In addition, YAP and TAZ levels in the nucleus reduced after SYNPO2 overexpression (Figure 6 B). Thus, in summary, the data suggested that SYNPO2 retarded the Hippo pathway and reduced cervical cancer progression.

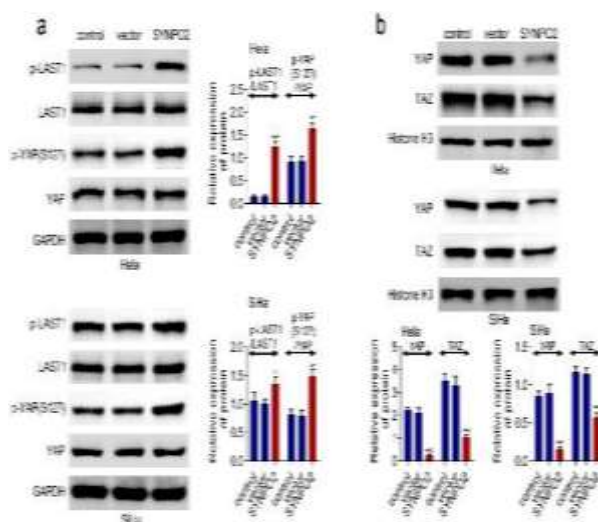


Figure 6: SYNPO2 inactivated the Hippo pathway in cervical cancer. (a) The protein expression levels of LAST1, phosphorylated (p)-LAST1, yes-associated protein 1 (YAP), and p-YAP(S127) in HeLa and SiHa cells evaluated by western blot after overexpressing SYNPO2; (b) The protein expression levels of YAP and TAZ in the nucleus determined after overexpressing SYNPO2 in HeLa and SiHa cells through western blot. $^*P < 0.01$, $^{***}p < 0.001$

DISCUSSION

SYNPO2 has been shown to play a repressive role in many types of cancers [4-8]. However, the detailed impact of SYNPO2 in cervical cancer has not been clarified. In this study, through use of the GEPIA website, it was demonstrated that the expression of SYNPO2 decreased in cervical cancer. Additionally, using the Kaplan–Meier Plotter website, it was found that cervical cancer patients with low SYNPO2 expression had worse prognoses than patients with high SYNPO2 expression. Results from western blot analyses revealed lower levels of SYNPO2 protein expression in cervical cancer cells compared with control cells. Further experiments showed that SYNPO2 overexpression repressed cervical cancer cell proliferation, migration and invasion, and enhanced cervical cancer cell apoptosis. Cisplatin affects cellular processes to regulate cervical cancer progression [9]. In this study, it was observed that SYNPO2 increased the sensitivity of cervical cancer cells to cisplatin.

The Hippo pathway is known to be involved in cell apoptosis and proliferation. The core portion of the Hippo pathway is comprised of coactivators, scaffold proteins, and a kinase cascade [10]. In addition, YAP and TAZ are two downstream members of the Hippo pathway that act as coactivators of TEA domain (TEAD) transcription factors, thereby stimulating cell proliferation and tumorigenesis [11]. Upon Hippo

pathway activation, phosphorylated YAP/TAZ is targeted for proteasomal degradation and maintained in the cytoplasm. Conversely, with the inactivation of the Hippo pathway, YAP/TAZ undergoes nuclear translocation and combines with TEAD to form the YAP/TAZ-TEAD complex. This complex modulates pro-survival genes to strengthen cell survival and growth [12].

The Hippo pathway has been observed to participate in multiple cancers. For example, fatty acid-binding protein 5 (FABP5) regulates the Hippo pathway so as to promote gastric cancer cell proliferation and attenuate apoptosis [13]. Additionally, S100A14 modulates the Hippo pathway to aid the suppression of prostate cancer growth and metastasis [14]. Furthermore, NIMA-regulated kinase 2 (NEK2) cooperated with STRIPAK to inactivate the Hippo pathway, thus accelerating the progression of cervical cancer [15]. In addition, ubiquitin-specific peptidase 21 (USP21) de-ubiquitinates forkhead box protein M1 (FOXO1), which modulates the Hippo pathway and enhances cervical cancer radio-resistance [16]. Importantly, YAP/TAZ has been shown to be enhanced in cervical cancer tissues [17], and SYNPO2 inhibits the YAP/TAZ activity in triple-negative breast cancer to reduce tumor metastasis [8]. In this study, it was observed that SYNPO2 inhibits the Hippo pathway in cervical cancer.

Limitations of this study

This study also has the limitations of not using human samples and mouse models.

CONCLUSION

To the best of our knowledge, this study is the first to unravel the roles of SYNPO2 in cervical cancer. The findings indicate that SYNPO2 overexpression reduces cell proliferation, migration, and invasion, and enhances cell apoptosis in cervical cancer by modulating Hippo pathway. Therefore, SYNPO2 as a useful bio-target for drug development for cervical cancer therapy.

DECLARATIONS

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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. Yuanhang Chen, Lang He, Yi Xu, and Qianqian Feng designed the study and carried out the experiments, supervised the data collection, analyzed and interpreted the data, prepared the manuscript for publication, and reviewed a draft of the manuscript. All authors have read and approved the manuscript.

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