Pathology - Research and Practice 215 (2019) 152621

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

# Shp2 expression is upregulated in cervical cancer, and Shp2 contributes to cell growth and migration and reduces sensitivity to cisplatin in cervical cancer cells

Meng Cao[a,b,*], Dan Gao[c], Nana Zhang[b], Yixin Duan[b], Ying Wang[b], Hasan Mujtaba[b], Yili Wang[b]

[a] The Key Laboratory of Biomedical Information Engineering, Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, Shaanxi, 710049, China

[b] Institute for Cancer Research, School of Basic Medical Science, Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, China

[c] Department of Respiratory and Critical Care Medicine, the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, China

ARTICLE INFO

*Keywords:*
Cervical cancer
Shp2
HPV
Biological behavior
Cisplatin

ABSTRACT

Src homology phosphotyrosine phosphatase 2 (Shp2) has been found to be overexpressed in cervical cancer tissues. However, the influence of Shp2 on the biological behavior and sensitivity to cisplatin of cervical cancer cells remains unclear. We aimed to assess Shp2 expression in cervical tissues and cell lines and to detect the influence of Shp2 knockdown and overexpression on the biological behavior and sensitivity to cisplatin in cervical cancer cells. We found that Shp2 expression was significantly upregulated in cervical cancer tissues and cell lines, and Shp2 overexpression was associated with lymph node metastasis and a high human papillomavirus (HPV) DNA load. Shp2 knockdown inhibited cell growth and migration and enhanced sensitivity to cisplatin in the HeLa and SiHa cervical cancer cell lines. In contrast, Shp2 overexpression had the opposite effects. These tumor-promoting effects of Shp2 may be partly related to Akt signaling. In conclusion, Shp2 is involved in the occurrence and development of cervical cancer and may confer cisplatin resistance in cervical cancer. Shp2 blockade may be a new strategy for cervical cancer treatment.

## 1. Introduction

Cervical cancer is one of the leading malignant cancers that cause death in women worldwide, with an estimated 569,847 new cases and 311,365 deaths in 2018 [1]. Human papillomavirus (HPV) plays a causal role in cervical cancer tumorigenesis [2]. In recent years, with the development of treatment technologies and the application of related vaccines, the morbidity and mortality of cervical cancer have decreased in many developed countries [3]. However, in some developing countries, most cervical cancer cases are locally advanced at diagnosis because the early symptoms are not obvious and screening programs have not been popularized [4]. Thus, the incidence and mortality rates in these regions are still increasing.

Chemotherapy is an important treatment strategy for cervical cancer and is widely used to treat locally advanced cervical cancer. Cisplatin, a nonspecific cellular drug that can induce cell death, is the first-line choice in chemotherapy for cervical cancer [5]. However, primary or acquired resistance to cisplatin is the main challenge in treating cervical cancer [6]. Therefore, the identification of key molecules involved in cervical cancer and new strategies that increase the sensitivity of cervical cancer cells to cisplatin are urgently needed.

Accumulating studies have suggested that Src homology phosphotyrosine phosphatase 2 (Shp2), a nonreceptor protein tyrosine phosphatase encoded by the *PTPN11* gene, is implicated in the carcinogenesis and development of various cancers. Shp2 was first reported to be overexpressed in human leukemia and to potentially contribute to leukemogenesis [7]. Subsequently, researchers observed upregulation of Shp2 expression in gastric cancer [8,9], breast cancer [10], thyroid cancer [11], non-small cell lung cancer [12], and oral squamous cell cancer [13]. To some extent, Shp2 overexpression contributes to the initiation and/or progression of these tumors. Conversely, a few studies revealed that Shp2 was down-regulated in hepatocellular cancer [14] and colon cancer [15], suggesting that Shp2 acts as a tumor suppressor due to its down-regulation in these two tumor types. Additionally, mutations in the *PTPN11* gene can change the function of Shp2 and therefore affect tumorigenesis and progression in different cancers

* Corresponding author at: The Key Laboratory of Biomedical Information Engineering, Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, No.28 Xianning West Road, Xi'an, 710049, China.

*E-mail address:* caomeng@stu.xjtu.edu.cn (M. Cao).

https://doi.org/10.1016/j.prp.2019.152621
Received 19 April 2019; Received in revised form 20 August 2019; Accepted 1 September 2019

[16,17].

Previous studies found that Shp2 expression levels were significantly higher in HPV-infected cervical cancer tissues than in normal cervical tissues [18,19]. However, little is known about the correlation between Shp2 expression and clinical parameters. Furthermore, the influence of Shp2 on cervical cancer cell biological behavior and cisplatin treatment remains unknown. Thus, the present study aimed to examine Shp2 expression in cervical tissues and cell lines, the relationship between Shp2 expression and clinical parameters, and the influence of Shp2 knockdown and overexpression on the biological behavior and sensitivity to cisplatin of cervical cancer cells.

## 2. Materials and methods

### 2.1. Tissue specimens

A total of 128 formalin-fixed and paraffin-embedded cervical tissue samples with clinical data from tissue microarrays (TMAs), including 85 cervical cancer (CC) samples, 21 cervical intraepithelial neoplasia (CIN) samples and 22 normal cervix (Normal) samples, were purchased from US Biomax, Inc. Patients' age ranged from 15 to 76 years, with a mean age of 44.73 ± 10.74 years. The company states that all human tissues are collected from informed donors with their consent. This study was approved by the Ethics Committee of the School of Basic Medical Science, Xi'an Jiaotong University.

### 2.2. Cell culture

The human cervical cancer cell lines HeLa, SiHa, CaSki and C33A and the human normal cervical epithelial cell line HcerEpic were preserved at the Institute for Cancer Research, School of Basic Medical Science of Xi'an Jiaotong University. The cancer cells were maintained in 89% Dulbecco's modified Eagle's medium (DMEM) (HyClone, USA) supplemented with 10% fetal bovine serum (FBS) (Biological Industries, Israel) and 1% penicillin-streptomycin solution (Beyotime, China). HcerEpic cells were maintained in 89% Eagle's minimal essential medium (EMEM, MEM medium supplemented with 1% nonessential amino acid solution) (HyClone, USA) supplemented with 10% FBS and 1% penicillin-streptomycin solution. All cells were routinely cultured in a cell culture incubator at 37 °C with 5% $CO_2$ and saturating humidity.

### 2.3. Immunohistochemistry

Immunohistochemistry (IHC) was performed on TMAs and cells growing on coverslips. After treatment with xylene and alcohol, the slices were placed in citrate buffer (0.01 M, pH 6.0) and heated for antigen retrieval (cells growing on coverslips did not require this step). Then, 3% hydrogen peroxide and 5% goat serum were successively added to the slices. After washing with TBS buffer, the slices were incubated with anti-Shp2 primary antibody (1:200, Santa Cruz, USA) at 4 °C overnight. The slices were then incubated with HRP-conjugated goat anti-rabbit IgG (H + L) secondary antibody (1:200, ABclonal, USA) for 1 h at room temperature, visualized with a DAB substrate kit (Maxim, China), and counterstained with hematoxylin (Maxim, China). TBS was used as a negative control instead of the primary antibody for IHC of the cells.

The IHC scores of the samples were evaluated by two pathologists based on the following system: for staining intensity, negative results were scored as 0, a light yellow color was scored as 1, a moderate yellow color was scored as 2, and a strong yellow color was scored as 3 [20]; for the positive rate of stained cells, < 5% was scored as 0, 5 ~ 25% was scored as 1, 26 ~ 50% was scored as 2, 51 ~ 75% was scored as 3, and > 75% was scored as 4. The final score of each case was calculated by multiplying the scores of the staining intensity and positive rate [21]. The Shp2 expression level was graded as follows: cases with IHC scores < 10%, 10 ~ 50%, and > 50% of the maximum IHC score were graded as no expression, low expression, and high expression, respectively.

### 2.4. In situ hybridization

In situ hybridization (ISH) for a mixture of HPV 16, 18 and 58 probes was carried out on TMAs. The sequences of the probes were: HPV 16: 5′-TATGAGCAATTAAATGACAGCTCA GAGGAGGAGGATGAA ATAGATGGTCCA-3′, HPV 18: 5′-TGAAATAGATGGAGTTAATC ATCAA CATTTACCAGCCCGACGAGCCGAACC-3′, and HPV 58: 5′-GCCAGATG GACAAG CACAACCGGCCACAGCTAATTACTACATTGTAACTTG-3′ [22]. The probes were labeled with a DIG Oligonucleotide Tailing Kit (Roche, Germany) and detected according to the manufacturer's instructions using a DIG Nucleic Acid Detection Kit (Roche, Germany). The specificity of the probes was confirmed by NCBI BLAST and by hybridization with plasmids harboring the HPV 16, 18 or 58 genomes.

After treatment with xylene and alcohol, the TMAs were incubated with 0.4% pepsin solution and fixed with 4% paraformaldehyde solution. The TMAs were then treated with 0.2 M HCl and increasing concentrations of alcohol. After prehybridization, the probes were added to the TMAs and hybridized at 42 °C overnight. The TMAs were washed with decreasing concentrations of SSC buffer and treated with anti-digoxin-AP conjugate. NBT/BCIP was used to visualize the ISH signal, and nuclear fast red (Maxim, China) was used to counterstain the tissues.

The semi-quantified HPV DNA load was measured by integrated optical density (IOD) scores of the ISH results using Image-Pro Plus 6.0 software [22] and graded as follows: cases with IOD scores < 10%, 10 ~ 50%, and > 50% of the maximum IOD score were graded as no damage, low viral load, and high viral load, respectively.

### 2.5. Lentivirus transduction

Lentiviral products carrying control shRNA (shControl), Shp2 shRNA (shShp2), GFP (Control) or Shp2 (Shp2) were obtained from Shanghai Genechem Co., Ltd. (China). The cells were cultured at a density of $2 \times 10^5$ cells per well in 6-well plates, and lentivirus suspension (titer $1 \times 10^9$ TU/mL) was added to HeLa cells (4 μL) or SiHa cells (2 μL). 12 h after transduction, the medium containing lentivirus was replaced with normal medium. 48 h later, the cells were selected with medium containing puromycin (3 μg/mL for HeLa cells, 6 μg/mL for SiHa cells) for at least 10 days.

### 2.6. RNA extraction and qRT-PCR

Total RNA was extracted from the different cells using the TRIzol reagent (Invitrogen, USA) and then was reverse transcribed into cDNA using the PrimeScript™ RT reagent Kit (Takara, Japan). qRT-PCR assays were performed using the SYBR® Premix Ex Taq™ II (Tli RNase H Plus) Kit (Takara, Japan) by a StepOne Real-Time PCR System (Applied Biosystems, USA), and the results were analyzed by StepOne Software version 2.3. The primers were synthesized by GenScript Biotech (China) with the following sequences: β-actin F: 5′-CATCATGAAGTGTGACG TGG-3′, β-actin R: 5′-GATCCACACGGAGTACTTGC-3′; and Shp2 F: 5′-GACTTTTGGCGGATGGTGTTCC-3′, Shp2 R: 5′-CGGCGCTTTCTTTG ACGTTCCT-3′ [13]. The PCR parameters consisted of an initial incubation for 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 1 min at 60 °C, and a final cycle of 15 s at 95 °C and 1 min at 60 °C. PCRs for each group were performed in triplicate, and the average threshold cycle value was calculated. The levels of Shp2 mRNA relative to control (β-actin) mRNA were analyzed using the $2^{-\Delta\Delta Ct}$ method.

### 2.7. Protein extraction and western blot analysis

Total cellular protein was extracted from the different cells using RIPA lysis buffer with PMSF and phosphatase inhibitor solution. The protein concentration was determined by a BCA protein quantification

kit (Beyotime, China) using a Synergy 2 Multi-Mode Microplate Reader (BioTek, USA). The same amount of protein from each sample (40~60 μg/lane) was separated by SDS-PAGE and transferred onto activated PVDF membranes (Millipore, USA). After blocking with 5% skim milk at room temperature for 1 h, the membranes were incubated with primary antibodies at 4 °C overnight. The primary antibodies and dilutions were as follows: anti-Shp2 (1:500, Santa Cruz Biotechnology, USA); anti-β-actin and anti-Bax (1:800, ABclonal, USA); and anti-Bcl-2, anti-Akt, anti-p-Akt, anti-Erk, anti-p-Erk, and anti-β-catenin (1:1000, Cell Signaling Technology, USA). After washing with TBST, the membranes were incubated with HRP-conjugated goat anti-rabbit IgG (H + L) secondary antibody (1:2000, ABclonal, USA) at room temperature for 1 h. The protein bands were visualized by an Immobilon Western Chemiluminescent HRP Substrate kit (Millipore, USA), and images were captured by a GeneGnome Bio Imaging System (SYNGENE, UK). Signals were semi-quantitatively analyzed and normalized to β-actin using Image-Pro Plus 6.0 software. The expression of each protein in the experimental group relative to the corresponding control group is showed in the figures.

### 2.8. MTT assay

MTT assays were used to determine the half-maximal inhibitory concentration ($IC_{50}$) value of cisplatin and cell viability in different groups. For $IC_{50}$ assessments, the cells from different groups were inoculated at a density of $1 \times 10^4$ cells/well in a 96-well plate, with triplicate wells per group. After cell attachment, cisplatin (Solarbio, China) (final concentrations: 0, 5, 10, 15, and 20 μmol/L) was added to the cells in the different groups, and the cells were incubated for another 24 h. For cell viability assessments, the cells from different groups were inoculated at a density of $4 \times 10^3$ cells/well in a 96-well plate with triplicate wells per group and conventionally cultured in a cell incubator for 24, 48, 72, 96 or 120 h. After culture for the indicated time, the cells were harvested. Then, 20 μL of MTT solution (5 mg/mL) (Sigma, USA) was added to each well, and the plate was incubated at 37 °C for 4 h. After removing the supernatant, 100 μL of DMSO was added to each well to dissolve the blue formazan precipitate. The absorbance of each well was measured by a Synergy 2 Multi-Mode Microplate Reader (BioTek, USA) at 490 nm. The experiments were repeated three times.

### 2.9. Colony formation assay

The cells from different groups were inoculated at a density of $2 \times 10^3$ cells/well into a 6-well plate. After attaching to the plate, the cells were treated with medium containing PBS (blank) or cisplatin (final concentration determined according to the $IC_{50}$ value) for 24 h. The cells were then conventionally cultured for 10 days in routine medium. The colonies were fixed with 4% paraformaldehyde solution for 15 min and stained with 0.1% crystal violet solution (Sigma, USA) for 30 min at room temperature. Then, the plates were washed with PBS and left to dry, and the colonies in each well were counted and analyzed. The experiments were repeated three times with triplicate wells in each replicate.

### 2.10. Wound-healing assay

A total of $1 \times 10^6$ cells from each group were inoculated into a 6-well plate and cultured overnight to form confluent monolayers. A 200 μL sterile pipette tip was used to scratch a wound in the monolayer of each group. Subsequently, the cells were washed with PBS three times to remove floating cells and cultured in DMEM with 2% FBS for 48 h. Photographs of the wounds were taken under an inverted microscope at 0 h and 48 h. The wound area was analyzed by ToupView software (ToupTek, China). The experiments were repeated three times with triplicate wells in each replicate.

### 2.11. Cell apoptosis analysis

Cell apoptosis in different groups was analyzed by flow cytometry using an Annexin V-PE/7-AAD Apoptosis Detection Kit (KeyGen, China). Briefly, a total of $1 \times 10^6$ cells from each group were inoculated into a 6-well plate and cultured overnight. PBS (blank) or cisplatin (final concentration determined according to the $IC_{50}$ value) was then added to the cells. 24 h later, the cells were digested with trypsin without EDTA (Genview, USA), harvested and washed with PBS twice. The cells were gently resuspended in 50 μL of Binding Buffer containing 5 μL of 7-AAD staining solution and incubated for 15 min at room temperature in the dark. The cells were then mixed with 450 μL of Binding Buffer containing 1 μL of Annexin V-PE staining solution and incubated for 15 min at room temperature in the dark. The stained cells were finally examined by a FACSCalibur flow cytometer (BD, USA) within 1 h. CellQuest Pro software (BD, USA) was used to analyze the results. The experiments were repeated three times.

### 2.12. Statistical analysis

SPSS 18.0 software was used for statistical analysis. Data are shown as the mean ± SD. Pearson's chi-square test was used to analyze the rate of positive Shp2 expression in the cervical cancer, CIN and normal groups. Pearson's chi-square test or Fisher's exact test was used to analyze correlations between Shp2 expression and clinical parameters. Groups were compared by Student's t test or one-way ANOVA. All statistical analyses were two-tailed, and $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Upregulation of Shp2 expression in cervical cancer tissues and cell lines

The IHC results showed that the positive expression rates of Shp2 were 96.47% in the cervical cancer group, 90.48% in the CIN group, and 36.36% in the normal group (Fig. 1A and C). Compared with the normal group, Shp2 expression was obviously increased in the CIN and cervical cancer groups, and the high expression level rate was slightly higher in the cervical cancer group. We also assessed Shp2 expression in the human cervical cell lines by IHC and western blot (Fig. 1B, D, E and F). Similar to the results of tissues, although Shp2 could be detected in all 5 cell lines, Shp2 expression was weak in HcerEpic but significantly strong in the cervical cancer cell lines.

### 3.2. The association of Shp2 expression with clinical parameters and the HPV DNA load in cervical cancer patients

According to the IHC results, we divided the 85 cervical cancer cases into a high Shp2 expression group and a low Shp2 expression group (cases with no expression were included in the low expression group). The chi-square test and Fisher's exact test showed no significant correlations between Shp2 expression and patient age ($P = 0.892$), FIGO stage ($P = 0.191$), grade ($P = 0.542$), pathologic type ($P = 1.000$) and T stage ($P = 0.407$). However, Shp2 expression and N stage were significantly correlated, and cases with a high Shp2 expression level were more likely to have an advanced N stage ($P = 0.048$*) (Table 1).

We performed ISH of a mixture of HPV 16, 18 and 58 probes on the same batch of tissues (Fig. 1A). The ISH results showed that the positive rate of HPV was 94.12% in the cervical cancer group. According to the ISH results, we divided the cervical cancer cases into a high HPV DNA load group and a low HPV DNA load group (cases with no damage were included in the low HPV DNA load group). The chi-square test showed a significant association of Shp2 expression with the HPV DNA load ($P = 0.004$*) (Table 1). Cases with high HPV DNA loads expressed higher levels of Shp2.

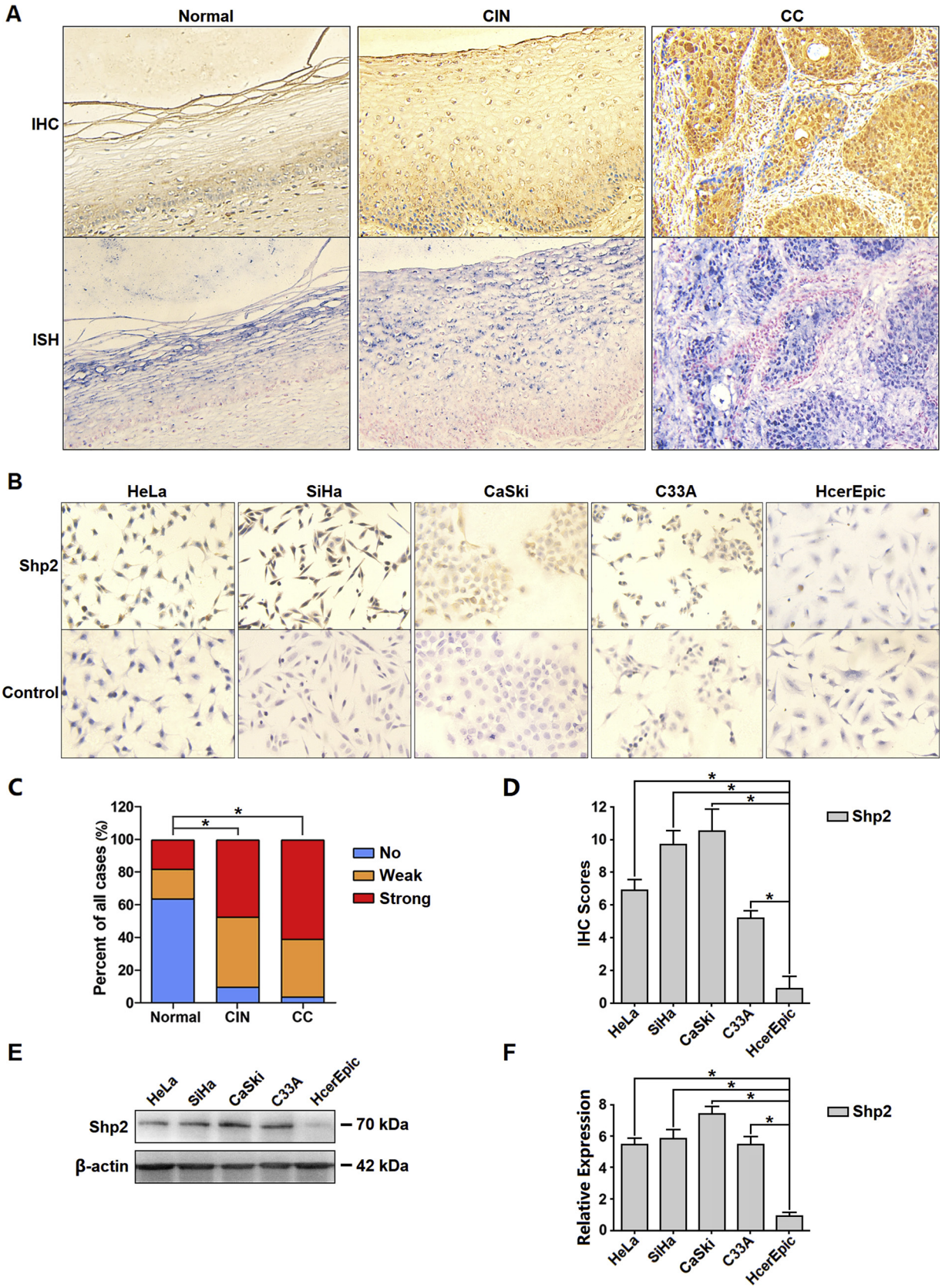


**Fig. 1.** Shp2 expression is upregulated in cervical cancer tissues and cell lines. (A) IHC showing Shp2 expression and ISH showing the HPV DNA load in Normal, CIN and CC tissues (200× magnification). (B) IHC showing Shp2 expression in cervical cell lines (200× magnification). (C) Statistical analysis of Shp2 expression in Normal, CIN and CC tissues in (A). (D) Shp2 IHC scores of cervical cell lines in (B). (E) Western blot analysis of Shp2 expression in cervical cell lines. (F) Quantification of relative Shp2 expression in (E). *$P < 0.05$.

**Table 1**
Correlations between Shp2 expression and clinical parameters or the HPV DNA load in 85 cervical cancer patients.

| | Shp2 expression (%) | | $\chi^2$ | *P* value |
|---|---|---|---|---|
| | High | Low | | |
| Age (years) | | | 0.019 | 0.892 |
| ≤ 44 | 26 (50.00) | 17 (51.52) | | |
| > 44 | 26 (50.00) | 16 (48.48) | | |
| FIGO stage | | | 3.314 | 0.191 |
| I | 17 (32.69) | 12 (36.36) | | |
| II | 13 (25.00) | 13 (39.40) | | |
| III | 22 (42.31) | 8 (24.24) | | |
| Grade | | | 1.224 | 0.542 |
| 1 | 7 (13.46) | 6 (18.18) | | |
| 2 | 30 (57.69) | 15 (45.46) | | |
| 3 | 15 (28.85) | 12 (36.36) | | |
| Pathologic type | | | 0.000[a] | 1.000 |
| SCC | 49 (94.23) | 31 (93.94) | | |
| ADC | 3 (5.77) | 2 (6.06) | | |
| T stage | | | 1.842[a] | 0.407 |
| T1 | 27 (51.92) | 13 (39.39) | | |
| T2 | 19 (36.54) | 17 (51.52) | | |
| T3 | 6 (11.54) | 3 (9.09) | | |
| N stage | | | 3.911 | 0.048* |
| N0 | 32 (61.54) | 27 (81.82) | | |
| N1 | 20 (38.46) | 6 (18.18) | | |
| HPV DNA load | | | 8.163 | 0.004* |
| High | 29 (55.77) | 8 (24.24) | | |
| Low | 23 (44.23) | 25 (75.76) | | |

SCC: squamous cell carcinoma; ADC: adenocarcinoma.
[a]: $\chi^2$ value from Fisher's Exact Test.
* : $P < 0.05$ was considered statistically significant.

### 3.3. Validation of Shp2 knockdown and overexpression in the HeLa and SiHa cell lines

Shp2-knockdown cell lines (HeLa-shShp2 and SiHa-shShp2) and Shp2-overexpressing cell lines (HeLa-Shp2 and SiHa-Shp2), as well as corresponding control cell lines (HeLa-shControl, SiHa-shControl, HeLa-Control and SiHa-Control), were established by lentivirus transduction. We detected Shp2 mRNA and protein expression levels to validate the efficiency of Shp2 knockdown or overexpression in the HeLa and SiHa cell lines. The qRT-PCR results indicated that, compared to the shControl groups, the shShp2 groups showed reductions in Shp2 mRNA levels by about 61% in HeLa cells and 76% in SiHa cells; compared to the Control groups, the Shp2 groups showed increases in Shp2 mRNA levels by about 79 fold in HeLa cells and 47 fold in SiHa cells (Fig. 2A). Western blot analysis also confirmed that Shp2 protein expression levels were significantly reduced in the shShp2 groups and increased in the Shp2 groups in both cell lines (Fig. 2B and C).

### 3.4. Shp2 reduced sensitivity to cisplatin in cervical cancer cells

We calculated cisplatin $IC_{50}$ values and detected cisplatin-induced apoptosis in the cells from different groups to determine their sensitivity to cisplatin. As shown in Fig. 3A, the $IC_{50}$ values were 24.14 ± 1.81 μg/mL and 19.08 ± 2.28 μg/mL ($P = 0.040$*) in the shControl and shShp2 groups of HeLa cells, respectively, and 42.11 ± 3.57 μg/mL and 33.13 ± 2.58 μg/mL ($P = 0.024$*) in the shControl and shShp2 groups of SiHa cells, respectively. For comparison, the $IC_{50}$ values were 28.28 ± 3.03 μg/mL and 35.16 ± 1.97 μg/mL ($P = 0.030$*) in the Control and Shp2 groups of HeLa cells and 41.47 ± 3.07 μg/mL and 60.41 ± 4.63 μg/mL ($P = 0.004$*) in the Control and Shp2 groups of SiHa cells. The $IC_{50}$ value of cisplatin was significantly lower in Shp2-knockdown cells and higher in Shp2-overexpressing cells.

Cell apoptosis was examined by flow cytometry, and the results indicated that Shp2 knockdown and overexpression did not significantly influence cell death directly. Nevertheless, when cells were treated with cisplatin (5 μg/mL and 10 μg/mL for Shp2-knockdown HeLa and SiHa cells, respectively; 8 μg/mL and 12 μg/mL for Shp2-overexpressing HeLa and SiHa cells, respectively) to induce apoptosis, the cell apoptosis rates were twice as high in the shShp2 groups than in the shControl groups for both HeLa and SiHa cells, and Shp2 overexpression reduced the apoptosis rates by approximately 10% in both cells (Fig. 3B and C).

We then detected the expression of the apoptosis-related proteins Bcl-2 and Bax. We found that cisplatin mainly reduced Bcl-2 expression, and Shp2 knockdown further enhanced this decrease in Bcl-2 expression, but Shp2 overexpression increased Bcl-2 expression, thereby affecting the cisplatin-induced apoptosis of cervical cancer cells (Fig. 3D and E). These results indicate that Shp2 reduces the sensitivity of cervical cancer cells to cisplatin.

### 3.5. Shp2 enhanced the proliferation and colony formation of cervical cancer cells

The proliferation of Shp2-knockdown cells, Shp2-overexpressing cells and corresponding control cells was detected by MTT assays. The results showed that cell proliferation was obviously decreased by Shp2 knockdown and increased by Shp2 overexpression (Fig. 4A).

In the colony formation assay without cisplatin, the shShp2 groups formed substantially fewer and smaller colonies than the shControl groups. In contrast, compared with the Control groups, the Shp2 groups formed many more colonies. In the presence of cisplatin (same concentration as in the apoptosis assay), the colony formation assay results showed the same trends (Fig. 4B and C).

Taken together, the data indicate that Shp2 enhances the proliferation and colony formation of cervical cancer cells, suggesting that Shp2 is required to promote the growth of cervical cancer cells.

### 3.6. Shp2 enhanced the migration of cervical cancer cells

Wound-healing assays were used to assess the influence of Shp2 knockdown and overexpression on cervical cancer cell migration. Fig. 5 shows that Shp2 knockdown inhibited cell migration by about 25% in HeLa cells and by 21% in SiHa cells after 48 h. Shp2 overexpression increased migration by about 52% in HeLa cells and by 46% in SiHa cells after 48 h. Thus, Shp2 considerably enhanced cervical cancer cell migration in cervical cancer cells.

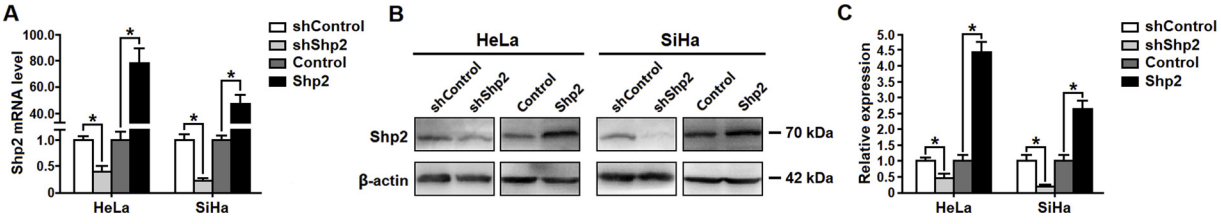


**Fig. 2.** Validation of Shp2 knockdown and overexpression in HeLa and SiHa cell lines. (A) Shp2 mRNA levels in the cell lines. (B) Western blot analysis of Shp2 expression in HeLa and SiHa cell lines. (C) Quantification of relative Shp2 expression in (B). *$P < 0.05$.

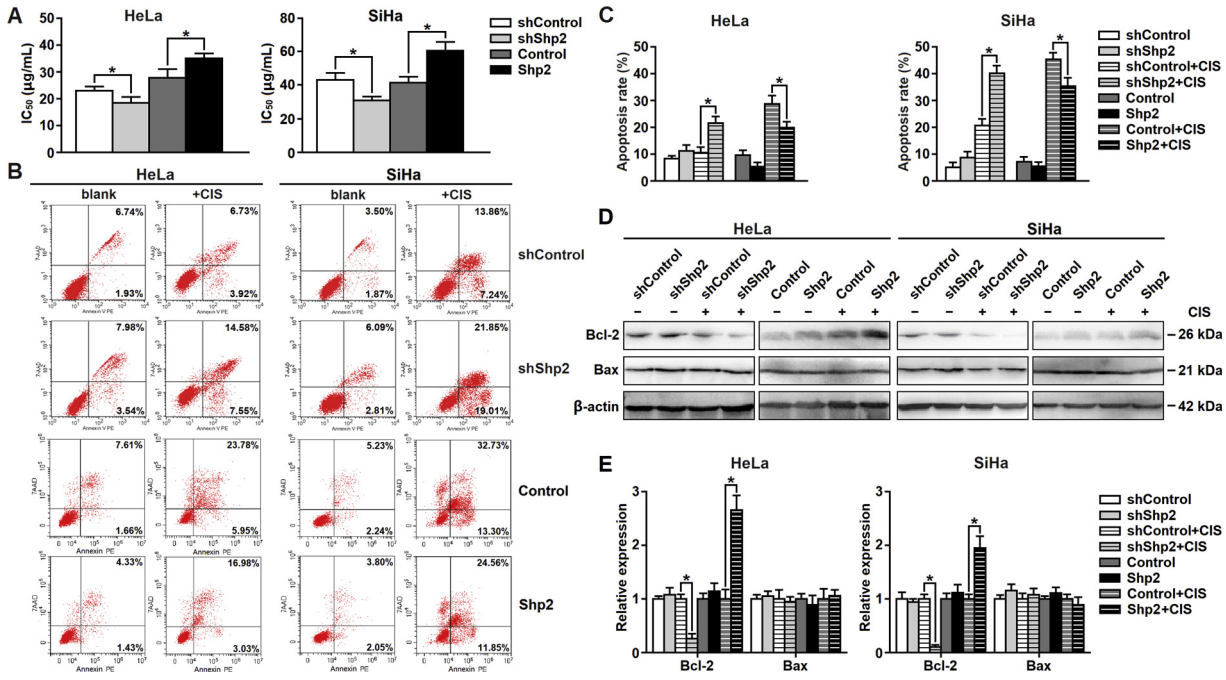


**Fig. 3.** Shp2 reduced sensitivity to cisplatin in HeLa and SiHa cell lines. (A) Cisplatin $IC_{50}$ values in shControl, shShp2, Control and Shp2 cells. (B) Apoptosis analysis of shControl, shShp2, Control and Shp2 cells with or without cisplatin. (C) Quantification of apoptosis rates in (B). (D) Western blot was used to analyze apoptosis-related proteins Bcl-2 and Bax expression in shControl, shShp2 Control and Shp2 cells. β-actin was used as an internal control. (E) Quantification of the protein bands in (D). *$P < 0.05$.

### 3.7. Shp2 influenced Akt signaling

To further understand the molecular mechanisms underlying the effects of Shp2 knockdown and overexpression in cervical cancer cells, we detected several key proteins that were previously identified in Shp2-related pathways in other cancers [10,23]. The results showed that phospho-Akt levels were decreased by Shp2 knockdown and increased by Shp2 overexpression, with no effect on total Akt levels. The expression levels of Erk, phospho-Erk and β-catenin showed no significant differences among the groups (Fig. 6). These results suggested that Shp2 mainly influenced Akt signaling, which may be one of the reasons for the altered biological behaviors of the cervical cancer cells.

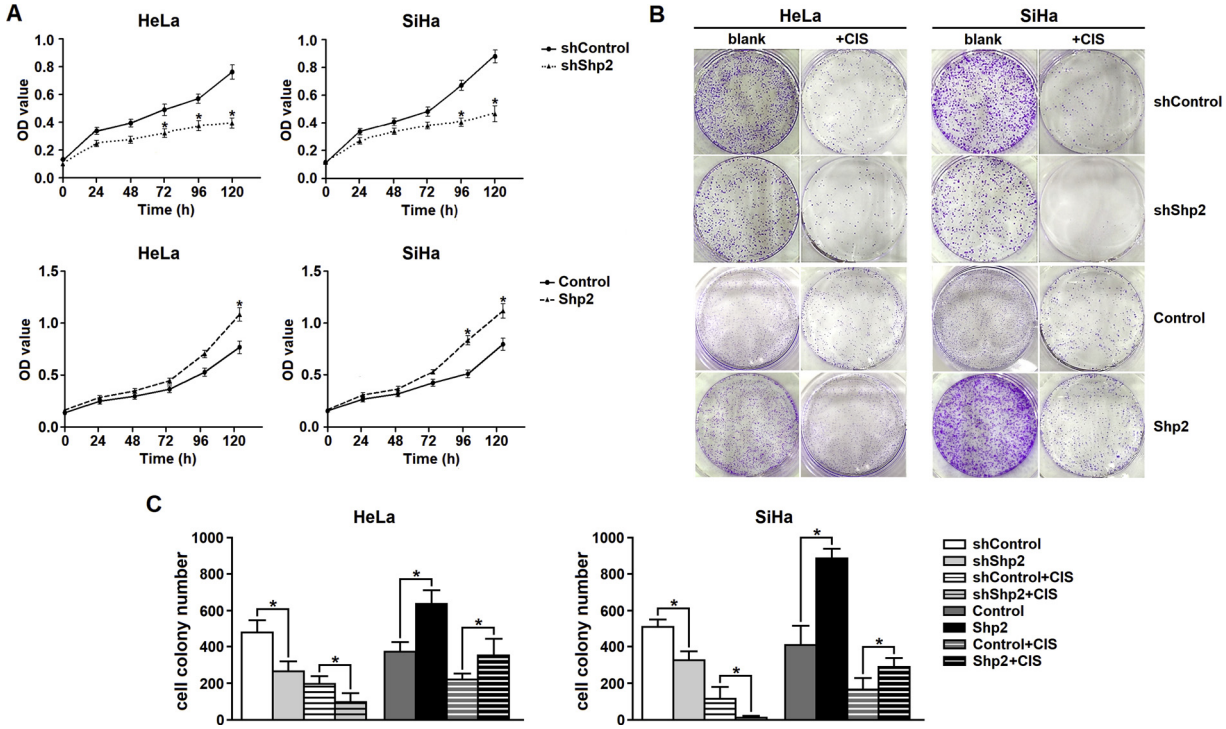


**Fig. 4.** Shp2 enhanced the proliferation and colony formation of HeLa and SiHa cells. (A) MTT assays of shControl, shShp2, Control and Shp2 cells. (B) Colony formation assays of shControl, shShp2, Control and Shp2 cells treated with or without cisplatin. (C) Quantification of colony number in (B). *$P < 0.05$.

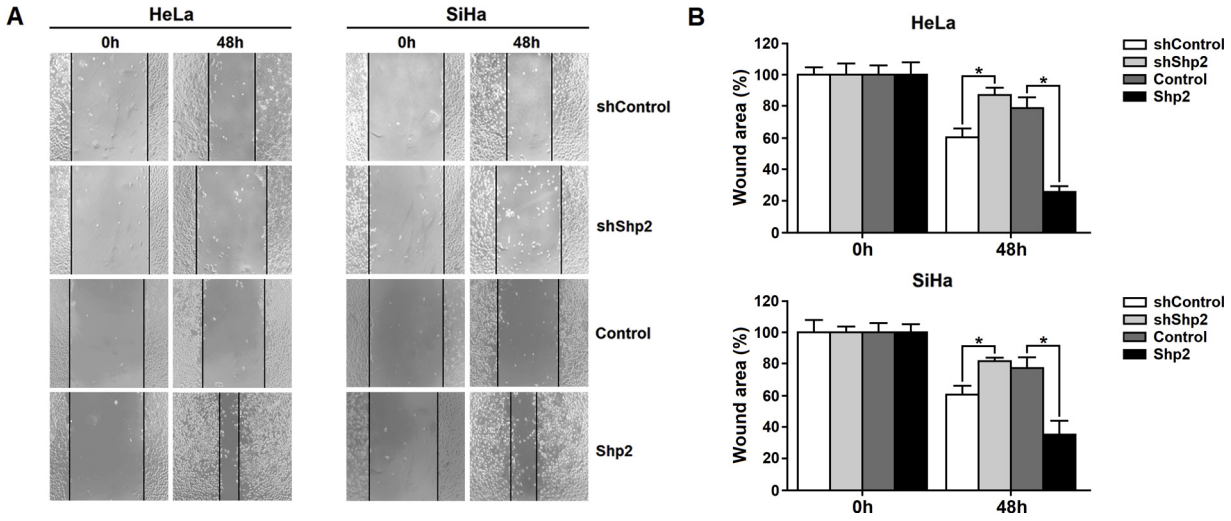


**Fig. 5.** Shp2 enhanced the migration of HeLa and SiHa cells. (A) Images of wound healing by shControl, shShp2, Control and Shp2 cells (40× magnification). (B) Quantification of wound area in (A). *$P < 0.05$.

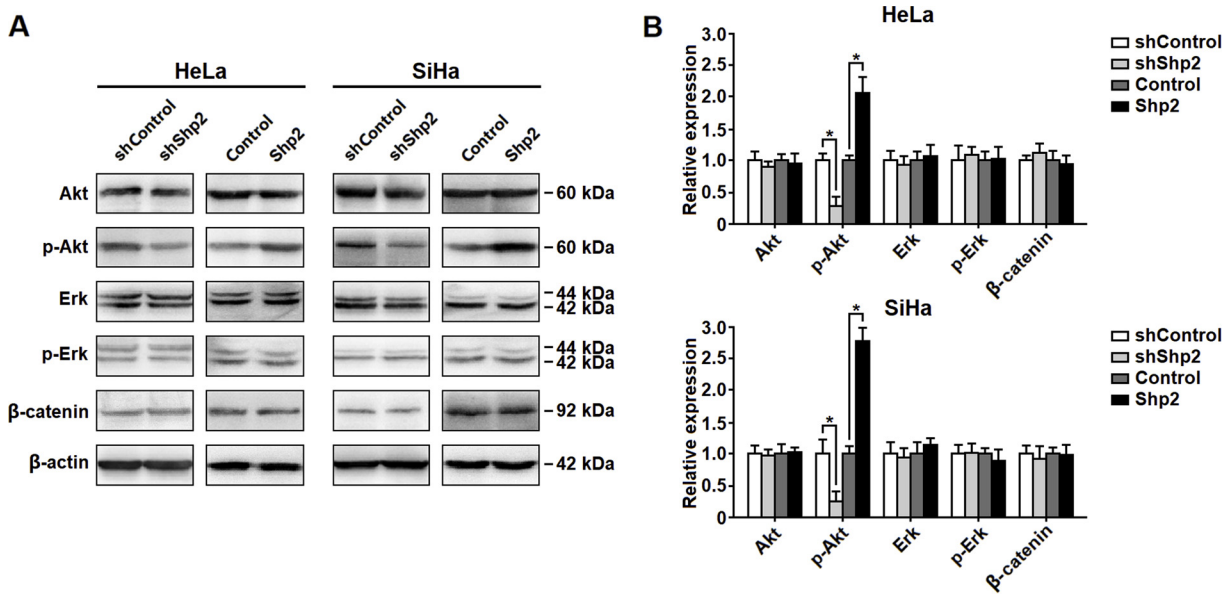


**Fig. 6.** Effect of Shp2 knockdown and overexpression on the regulation of several key proteins in HeLa and SiHa cells. (A) Western blot was used to analyze the expression of Akt, p-Akt, Erk, p-Erk and β-catenin in shControl, shShp2, Control and Shp2 cells. β-actin was used as an internal control. (B) Quantification of the protein bands in (A). *$P < 0.05$.

## 4. Discussion

An increasing number of studies have focused on Shp2, and the results of these studies have shown that Shp2 has dual functions in the occurrence and development of different cancers [24]. Researchers found a carcinogenic effect of Shp2 in various cancers, such as gastric cancer [8], breast cancer [10] and non-small cell lung cancer [12]. Conversely, a few studies revealed a tumor suppressive effect of Shp2 in hepatocellular carcinoma [14] and colon cancer [15]. Little is known about the role of Shp2 in cervical cancer, and the influence and mechanisms of Shp2 on the biological behavior of cervical cancer cells have not been explored.

Studies by Tao et al. and Meng et al. showed that the positive expression rates of Shp2 were 90% and 88.8% in cervical cancer tissues, respectively, which are significantly higher than that in normal cervix tissues [18,19]. Consistently, our IHC results showed that the positive Shp2 expression rates were 96.47% in the cervical cancer group and 36.36% in the normal group ($P < 0.05$). The results in cell lines also showed that the Shp2 signal was weak in the normal cervical epithelial cell line but considerably stronger in the cervical cancer cell lines. Correlation analysis showed that high Shp2 expression levels correlated with an advanced N stage, suggesting that Shp2 was associated with tumor lymph node metastasis in cervical cancer. All of these results support the hypothesis that Shp2 contributes to the occurrence and development of cervical cancer.

High-risk HPV infections play a causal role in the occurrence and development of cervical cancer [2], and the HPV DNA load is the main distinguishing factor among HPV-positive cervical cancer cases [25]. Whether the HPV DNA load affects Shp2 expression remains unknown. Therefore, we examined the relationship between the HPV DNA load and Shp2 expression. Correlation analysis showed a significant association between the HPV DNA load and Shp2 expression ($P = 0.004$*), indicating that the HPV DNA load is an important factor affecting the Shp2 expression level, and Shp2 expression increased with increasing HPV DNA load.

Previous studies have shown that Shp2 plays a regulatory role in

various tumor cells. Hu et al. revealed that Shp2 overexpression enhanced the proliferation, clone formation, and migration inabilities of breast cancer cells and could increase Erk and Akt signaling activity [10]. Xiang et al. showed that Shp2 was also involved the β-catenin pathway [23] in hepatocellular carcinomas.

In the present study, the results of MTT and colony formation assay showed that Shp2 enhanced the proliferation and colony formation of cervical cancer cells. Using a wound-healing assay, we found that Shp2 enhanced cervical cancer cell migration in vitro, which may be an evidence for the correlation between Shp2 and lymph node metastasis in cervical cancer. We also detected Erk, Akt and β-catenin signaling in the cell lines and found that phospho-Akt expression levels were decreased by Shp2 knockdown and increased by Shp2 overexpression in HeLa and SiHa cells, but no significant variations in Erk or β-catenin signaling were observed. The slight differences in the effects of Shp2 on biological behavior and signaling pathways among different tumors might be due to cellular contexts. These results suggested that Shp2 is essential for the maintenance of malignant behavior in cervical cancer cells, and Akt signaling is involved in this process.

Since cisplatin is the first-line chemotherapeutic for advanced cervical cancer and the influence of Shp2 on cisplatin resistance in cervical cancer is still unclear, we determined the $IC_{50}$ values of cisplatin in Shp2-knockdown cells, Shp2-overexpressing cells and corresponding control cells. We found that the cisplatin $IC_{50}$ value in cervical cancer cell lines was significantly decreased by Shp2 knockdown and increased by Shp2 overexpression. Furthermore, Shp2 knockdown significantly increased cervical cancer cell death induced by cisplatin due to downregulation of Bcl-2 expression. The opposite results were found in the Shp2 overexpression groups. Therefore, Shp2 could reduce the sensitivity of cervical cancer cells to cisplatin.

In conclusion, our study demonstrated that Shp2 expression was upregulated in cervical cancer and closely correlated with lymph node metastasis and the HPV DNA load. Shp2 knockdown suppressed cervical cancer cell growth and migration and enhanced the sensitivity of cervical cancer cells to cisplatin in HeLa and SiHa cell lines. In contrast, Shp2 overexpression enhanced cell growth and migration and reduced cisplatin sensitivity in the two cell lines. Further studies showed that the tumor-promoting effects of Shp2 were related to Akt signaling. Therefore, Shp2 is involved in the occurrence and development of cervical cancer and may confer cisplatin resistance in cervical cancer, and Shp2 blockade may serve as a new strategy for cervical cancer treatment.

## Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

We are grateful to Haiyan Ding, Ni Liu, Peitao Cao, Ruixiang Ni, Bo Wang and Yitong Wang for their assistance throughout this research.

## References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, Ca-Cancer J. Clin. 68 (2018) 394–424, https://doi.org/10.3322/caac.21492.

[2] F.X. Bosch, A. Lorincz, N. Munoz, C. Meijer, K.V. Shah, The causal relation between human papillomavirus and cervical cancer, J. Clin. Pathol. 55 (2002) 244–265, https://doi.org/10.1136/jcp.55.4.244.

[3] M. Vu, J. Yu, O.A. Awolude, L. Chuang, Cervical cancer worldwide, Curr. Probl. Cancer 42 (2018) 457–465, https://doi.org/10.1016/j.currproblcancer.2018.06.003.

[4] P.A. Cohen, A. Jhingran, A. Oaknin, L. Denny, Cervical cancer, Lancet 393 (2019) 169–182, https://doi.org/10.1016/s0140-6736(18)32470-x.

[5] S. Li, B. Ren, Y. Shi, H. Gao, J. Wang, Y. Xin, B. Huang, S. Liao, Y. Yang, Z. Xu, Y. Li, Q. Zeng, Notch1 inhibition enhances DNA damage induced by cisplatin in cervical cancer, Exp. Cell Res. 376 (2019) 27–38, https://doi.org/10.1016/j.yexcr.2019.01.014.

[6] S. Karageorgopoulou, I.D. Kostakis, M. Gazouli, S. Markaki, M. Papadimitriou, E. Bournakis, M.A. Dimopoulos, C.A. Papadimitriou, Prognostic and predictive factors in patients with metastatic or recurrent cervical cancer treated with platinum-based chemotherapy, BMC Cancer 17 (2017) 451, https://doi.org/10.1186/s12885-017-3435-x.

[7] X. Rongzhen, Y. Yingzi, Z. Shu, Z. Xiaoying, D. Qinghua, H. Zhiwen, L. Yun, L. Qinghua, F. Yongmin, G. Xiaoxian, Overexpression of Shp2 tyrosine phosphatase is implicated in leukemogenesis in adult human leukemia, Blood 106 (2005) 3142–3149, https://doi.org/10.1182/blood-2004-10-4057.

[8] S. Dong, F.Q. Li, Q. Zhang, K.Z. Lv, H.L. Yang, Y. Gao, J.R. Yu, Expression and clinical significance of SHP2 in gastric Cancer, J. Int. Med. Res. 40 (2012) 2083–2089, https://doi.org/10.1177/030006051204000605.

[9] J. Jiang, M.S. Jin, F. Kong, Y.P. Wang, Z.F. Jia, D.H. Cao, H.X. Ma, J. Suo, X.Y. Cao, Increased expression of tyrosine phosphatase SHP-2 in Helicobacter pylori-infected gastric cancer, World J. Gastroentero. 19 (2013) 575–580, https://doi.org/10.3748/wjg.v19.i4.575.

[10] H. Zhongqian, F. Haoshu, W. Xinyi, C. Danlei, C. Zhuo, W. Siying, Overexpression of SHP2 tyrosine phosphatase promotes the tumorigenesis of breast carcinoma, Oncol. Rep. 32 (2014) 205–212, https://doi.org/10.3892/or.2014.3201.

[11] Z.Q. Hu, R. Ma, C.M. Zhang, J. Li, L. Li, Z.T. Hu, Q. Gao, W.M. Li, Expression and clinical significance of tyrosine phosphatase SHP2 in thyroid carcinoma, Onco. Lett. 10 (2015) 1507–1512, https://doi.org/10.3892/ol.2015.3479.

[12] C.L. Tang, D. Luo, H.P. Yang, Q.L. Wang, R. Zhang, G.X. Liu, X.L. Zhou, Expression of SHP2 and related markers in non-small cell lung Cancer: a tissue microarray study of 80 cases, Appl. Immunohistochem. Mol. Morphol. 21 (2013) 386–394, https://doi.org/10.1097/PAI.0b013e31827da3f9.

[13] H.J. Xie, S.Y. Huang, W.G. Li, H.B. Zhao, T.Q. Zhang, D.S. Zhang, Upregulation of Src homology phosphotyrosyl phosphatase 2 (Shp2) expression in oral cancer and knockdown of Shp2 expression inhibit tumor cell viability and invasion in vitro, Or. Surg. Or. Med. Or. Pa. 117 (2014) 234–242, https://doi.org/10.1016/j.oooo.2013.10.018.

[14] C.Y. Jiang, F.K. Hu, Y.H. Tai, J.L. Du, B.B. Mao, Z.Q. Yuan, Y. Wang, L.X. Wei, The tumor suppressor role of Src homology phosphotyrosine phosphatase 2 in hepatocellular carcinoma, J. Cancer Res. Clin. Oncol. 138 (2012) 637–646, https://doi.org/10.1007/s00432-011-1143-5.

[15] P.F. Cai, W.J. Guo, H.Q. Yuan, Q. Li, W.C. Wang, Y. Sun, X.M. Li, Y.H. Gu, Expression and clinical significance of tyrosine phosphatase SHP-2 in colon cancer, Biomed. Pharmacother. 68 (2014) 285–290, https://doi.org/10.1016/j.biopha.2013.10.012.

[16] Z.Q. Hu, X.Y. Wang, H.S. Fang, Y.K. Liu, D.L. Chen, Q. Zhang, X. Liu, D.Y. Wei, C.K. Qu, S.Y. Wang, A tyrosine phosphatase SHP2 gain-of-function mutation enhances malignancy of breast carcinoma, Oncotarget 7 (2016) 5664–5676, https://doi.org/10.18632/oncotarget.6561.

[17] M.L. Loh, S. Vattikuti, S. Schubbert, M.G. Reynolds, E. Carlson, K.H. Lieuw, J.W. Cheng, C.M. Lee, D. Stokoe, J.M. Bonifas, N.P. Curtiss, J. Gotlib, S. Meshinchi, M.M. Le Beau, P.D. Emanuel, K.M. Shannon, Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis, Blood 103 (2004) 2325–2331, https://doi.org/10.1182/blood-2003-09-3287.

[18] T. Xiao-Hua, S. Jian-Gen, P. Wei-Li, D. Yu-E, M. Qun, K.V. Honn, J. Rongxian, Significance of SHP-1 and SHP-2 expression in human papillomavirus infected Condyloma acuminatum and cervical cancer, Pathol. Oncol. Res. 14 (2008) 365–371, https://doi.org/10.1007/s12253-008-9065-5.

[19] F. Meng, X.Y. Zhao, S.L. Zhang, Expression and significance of SHP-2 in human papillomavirus infected cervical cancer, J. Huazhong U. Sci-Med. 32 (2012) 247–251, https://doi.org/10.1007/s11596-012-0044-4.

[20] D. Xu, B. Zhang, C. Liao, W. Zhang, W. Wang, Y. Chang, Y. Shao, Human beta-defensin 3 contributes to the carcinogenesis of cervical cancer via activation of NF-κB signaling, Oncotarget 7 (2016) 75902–75913, https://doi.org/10.18632/oncotarget.12426.

[21] X.P. Hao, T.G. Pretlow, J.S. Rao, T.P. Pretlow, Beta-catenin expression is altered in human colonic aberrant crypt foci, Cancer Res. 61 (2001) 8085–8088, https://doi.org/10.1002/1097-0142(20011115)92:10<2727::AID-CNCR1627>3.0.CO;2-B.

[22] M. Cao, W. Shah, J. Qi, Y. Zhou, Y. Wang, H. Chen, Prognostic significance of human papillomavirus viral load in correlation with different therapeutic modalities in cervical cancer patients, Pathol. Res. Pract. 212 (2016) 804–810, https://doi.org/10.1016/j.prp.2016.06.011.

[23] D. Xiang, Z. Cheng, H. Liu, X. Wang, T. Han, W. Sun, X. Li, W. Yang, C. Chen, M. Xia, Shp2 promotes liver cancer stem cell expansion by augmenting β-catenin signaling and predicts chemotherapeutic response of patients, Hepatology 65 (2016) 1566–1580, https://doi.org/10.1002/hep.28919.

[24] S. Li, D.D. Hsu, H. Wang, G.-S. Feng, Dual faces of SH2-containing protein-tyrosine phosphatase Shp2/PTPN11 in tumorigenesis, Front. Med. (Lausanne) 6 (2012) 275–279, https://doi.org/10.1007/s11684-012-0216-4.

[25] D.M. Hernandez-Hernandez, L. Ornelas-Bernal, M. Guido-Jimenez, T. Apresa-Garcia, I. Alvarado-Cabrero, M. Salcedo-Vargas, A. Mohar-Betancourt, A. Garcia-Carranca, Association between high-risk human papillomavirus DNA load and precursor lesions of cervical cancer in Mexican women, Gynecol. Oncol. 90 (2003) 310–317, https://doi.org/10.1016/s0090-8258(03)00320-2.