SPTBN2 regulates endometroid ovarian cancer cell proliferation, invasion and migration via ITGB4-mediated focal adhesion and ECM receptor signalling pathway

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Abstract. Ovarian cancer is as a major contributor to gynaecologic death globally. The present study aimed to investigate the regulatory role of spectrin β non-erythrocytic 2 gene (SPTBN2) in endometroid ovarian cancer and its mechanism of action. According to the Gene Expression Profiling Interactive Analysis (GEPIA) database, SPTBN2 expression is elevated in ovarian cancer tissues and higher SPTBN2 expression indicated a worse prognosis. The present study assessed SPTBN2 mRNA and protein expression levels by reverse transcription-quantitative PCR and western blotting, respectively. Cell viability, proliferation, migration and invasion were assessed with Cell Counting Kit-8, 5-ethynyl-2'-deoxyuridine incorporation, wound healing and Transwell assays, respectively. SPTBN2 expression was notably enhanced in ovarian cancer cell lines, especially in A2780 cells compared with HOSEPiC cells (P<0.001). Following transfection with small interfering (si)RNA targeting SPTBN2, the viability, proliferation, migration and invasion of A2780 cells were decreased compared with those of A2780 cells transfected with siRNA-NC (P<0.001). Gene Set Enrichment Analysis database revealed that SPTBN2 was primarily enriched in 'focal adhesion' and 'extracellular matrix (ECM)-receptor interaction', whereas SPTBN2 was significantly associated with integrin β4 (ITGB4) in the GEPIA database. In addition, rescue experiments were performed to determine the mechanism of SPTBN2 in endometroid ovarian cancer. ITGB4 overexpression reversed the inhibitory effects of the SPTBN2 knockdown on viability, proliferation, migration and invasion of A2780 cells (P<0.05). The impacts of SPTBN2 on the expression of focal adhesion and downstream ECM receptor signalling-related proteins, including Src and p-FAK/FAK, were significantly reversed by ITGB4 overexpression (P<0.01). Collectively, SPTBN2 may regulate endometroid ovarian cancer cell proliferation, invasion and migration through the ITGB4-mediated focal adhesion and ECM receptor signalling pathway.

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Introduction

As a key contributor to cancer-related deaths among women, ovarian cancer has the highest mortality rate among gynaecological malignancies (1). It is estimated that there are ~14,000 mortalities due to this condition every year in the USA (2). Risk factors can result in ovarian cancer, such as BRCA mutation, smoking, obesity, nulliparity, early-onset menarche and late-onset menopause (3,4). The 5-year survival rate of patients with ovarian cancer is low and remained at 48% in the USA during 2009 through 2015, as most patients are diagnosed at advanced stages owing to the unspecific clinical manifestations of ovarian cancer (2,5,6). At present, the methods for the treatment of ovarian cancer include surgery, chemotherapy, immunotherapy and targeted therapy (7). Although great progress has been made in treatment methods, the 5-year survival rate of patients with ovarian cancer remains unsatisfactory (8).

Spectrin β non-erythrocytic 2 (SPTBN2) encodes β-III spectrin; mutation in this gene is the cause of spinocerebellar ataxia type 5 (9). SPTBN2 is expressed in body cells including kidney, pancreatic and liver cells, especially in Purkinje cells (10). Studies have demonstrated that SPTBN2 is increased in endometrioid, endometrial and colorectal cancer, as well as lung adenocarcinoma; it is associated with several tumorigenesis-related biological processes, including proliferation, migration and invasion (11-13). Additionally, SPTBN2 has been recognized as a marker gene and serves as a crucial factor in the pathogenetic process of several types of cancer (14). Notably, SPTBN2 is shown to be correlated with integrin β4 (ITGB4) in Gene Expression Profiling Interactive Analysis (GEPIA) database.

As a heterodimeric transmembrane receptor, ITGB4 is located at the basal surface of airway epithelial cells in hemidesmosomal structures (15). Previous studies have reported that ITGB4 is aberrantly expressed in numerous types of cancer. For example, ITGB4 is upregulated in patients with colorectal cancer (CRC) and may be a potential serum biomarker for CRC (16). In addition, downregulation of ITGB4 may be a

target for treatment of gastric cancer (17). ITGB4 is aberrantly expressed in ovarian cancer cells and ITGB4-mediated focal adhesion kinase (FAK) has been reported to regulate the metastatic potential of ovarian cancer cells by disrupting the basement membrane barrier and promoting cell motility (18).

The present study aimed to investigate the role of SPTBN2 in endometroid ovarian cancer as well as its mechanism of action to lay the foundation for the future exploration of targeted therapy for endometroid ovarian cancer.

Materials and methods

Cell culture. Human ovarian surface epithelial cell line HOSEPiC (cat. no. YS2089C) was purchased by Shanghai YaJi Biological Co., Ltd. Ovarian cancer cell lines SKOV3 (cat. no. BNCC310551) and A2780 (cat. no. BNCC351906) were from BeNa Culture Collection (Beijing Beina Chunglian Institute of Biotechnology). In addition, the ovarian cancer cell line HEY A8 (cat. no. CL-0671) was purchased from Procell Life Science & Technology Co., Ltd. All cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Procell Life Science & Technology Co., Ltd.) supplemented with 10% foetal bovine serum (FBS; Beijing Solarbio Science & Technology Co., Ltd.) and 1% penicillin-streptomycin at 37˚C with 5% CO_2.

Cell transfection. Two small interfering (si)RNAs specific to SPTBN2 (siRNA-SPTBN2-1, 5'-ACGTCAATGTACACA ACTTCACC-3'; and siRNA-SPTBN2-2, 5'-ACCATTACT TCTCCAAGATGAAG-3'), a pcDNA3.1 expression vector carrying ITGB4 (Ov-ITGB4) as well as their corresponding negative controls (NC) si-NC (5'-AAGACAUUGUGUGUC CGCCTT-3') and Ov-NC (empty vector) were purchased from GeneChem, Inc. Using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.), 100 nM siRNA-SPTBN2-1/2, siRNA-NC and 4 μg Ov-ITGB4 and Ov-NC were transfected into A2780 cells at 37˚C for 24 h, according to the manufacturer's protocol. After 48 h, cells were washed with PBS and incubated in DMEM and reverse transcription-quantitative PCR (RT-qPCR) and western blotting were performed to test transfection efficacy.

RT-qPCR. Total RNA was extracted from ovarian cancer cells and human ovarian surface epithelial cell line ($6x10^4$ cells/well) with TRIzol® (Thermo Fisher Scientific, Inc.) and reverse-transcribed into cDNA using the PrimeScript™ RT Reagent kit (cat. no. RR047A; Takara Bio, Inc.) according to the manufacturer's instructions. qPCR was performed using SYBR Green Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. The thermocycling conditions were as follows: Initial denaturation at 95˚C for 8 min; 40 cycles of denaturation at 95˚C for 25 sec; annealing at 60˚C for 30 sec; extension at 72˚C for 30 sec and final extension at 72˚C for 10 min. The mRNA levels were quantified using the $2^{-\Delta\Delta Cq}$ method and normalized to the internal reference gene GAPDH (19). The following primer pairs were used: SPTBN2 forward, 5'-GAGGTCTCGCATTAAGGCTCT-3' and reverse, 5'-CTTTGGCAGTATCTCTCCCGA-3'; ITGB4 forward, 5'-GCAGCTTCCAAATCACAGAGG-3' and reverse, 5'-CCA GATCATCGGACATGGAGTT-3' and GAPDH forward, 5'-TGTGGGCATCAATGGATTTGG-3' and reverse, 5'-ACA CCATGTATTCCGGGTCAAT-3'.

Western blotting. Total protein was extracted from ovarian cancer cells and human ovarian surface epithelial cell line in six-well plates at $1x10^5$ cells/well using RIPA lysis buffer (Beyotime Institute of Biotechnology). Total protein was quantified with a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology) and separated by SDS-PAGE on an 8% gel (30 μg protein/lane). The separated proteins were transferred onto a PVDF membrane (Beijing Solarbio Science & Technology Co., Ltd.) and blocked with 5% skimmed milk or 5% BSA (MilliporeSigma) for 2 h at room temperature. Subsequently, membranes were incubated at 4˚C overnight with primary antibodies (all from Abcam) against SPTBN2 (cat. no. ab264178; 1:2,000), ITGB4 (cat. no. ab182120; 1:1,000), MMP2 (cat. no. ab92536; 1:1,000), MMP7 (cat. no. ab207299; 1:1,000), MMP9 (cat. no. ab76003; 1:1,000), proto-oncogene tyrosine-protein kinase Src (Src; cat. no. ab133283; 1:1,000), phosphorylated (p)-FAK (cat. no. ab81298; 1:1,000), FAK (cat. no. ab40794; 1:2,000) and GAPDH (cat. no. ab9485; 1:2,500). Membranes were subsequently incubated with HRP-labelled goat anti-rabbit secondary antibody (cat. no. ab6721; 1:2,000; Abcam) for 2 h at room temperature. Protein bands were visualized with Enhanced ECL Chemiluminescent Substrate Kit (Shanghai Yeasen Biotechnology Co., Ltd.) on a Bio-Rad Image Lab system (Shanghai Aiyan Biotechnology Co., Ltd.). Densitometric analysis was performed using Image J software (version 1.46; National Institutes of Health) with GAPDH as the loading control.

Cell Counting Kit-8 (CCK-8) assay. A2780 cells were plated into 96-well plates at a density of $5x10^3$ cells/well and incubated at 37˚C for 24 h. Subsequently, 10 μl CCK-8 reagent (Beyotime Institute of Biotechnology) was added to each well and cells were incubated for another 3 h. Finally, the absorbance at 450 nm was measured with a microplate reader (Thermo Fisher Scientific, Inc.).

5-ethynyl-2'-deoxyuridine (EdU) incorporation cell proliferation assay. A2780 cells were plated into 6-well plates at a density of $4x10^5$ cells/well and incubated overnight at room temperature. Subsequently, each well was filled with 50 μM EdU solution (Beyotime Institute of Biotechnology) and further incubated for 4 h at 37˚C. After removing the working solution, cells were fixed with 4% paraformaldehyde for 15 min at room temperature and permeabilized with 0.5% Triton X-100 for 10 min at room temperature. Following addition of 100 μl Apollo® Reaction Cocktail (Guangzhou RiboBio Co., Ltd.), cells were incubated in the dark for 30 min at room temperature. The cells were observed under a fluorescence microscope (Olympus Corporation).

Wound healing. The migration of A2780 cells was assessed by wound healing assay. Cells were plated into 6-well plates ($2x10^5$ cells/well) and incubated at 37˚C until 80-90% confluency was reached. A 10-μl pipette tip was used for creation of a wound in the cell monolayer. The cells were rinsed with PBS then cultured in serum-free DMEM medium at 37˚C with 5% CO_2. Images were captured at 0 and 24 h under a light microscope (Olympus Corporation) and tracked with Image-J software (version 1.46; National Institutes of Health).

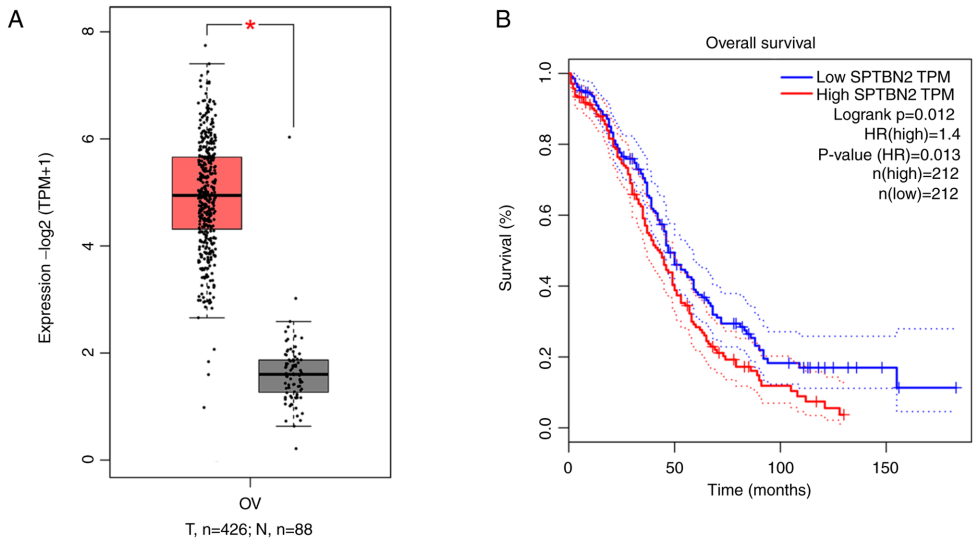

Figure 1. SPTBN2 is upregulated in ovarian cancer tissue and is associated with poor prognosis. According to GEPIA database, (A) SPTBN2 is upregulated in ovarian cancer tissue, *P<0.05, and (B) high expression of SPTBN2 is associated with poor prognosis. Cutoff-High, 50% and Cutoff-Low, 50%. Solid lines indicate survival curves; dotted lines indicate 95% confidence interval. GEPIA, Gene Expression Profiling Interactive Analysis; HR, hazard ratio; N, normal; SPTBN2, spectrin β non-erythrocytic 2; T, tumour; TPM, transcripts per million.

Transwell assay. The invasive ability of A2780 cells was assessed by Transwell assay. Cells ($1x10^4$ cells/well) were plated into the upper compartment of a Transwell plate (8 μm) that was pre-coated with Matrigel (BD Biosciences) at 37˚C for 30 min. The serum-free DMEM medium was placed in the upper chamber. In the lower chamber, the DMEM medium was supplemented with 10% FBS. Following 24 h incubation at 37˚C, the invaded cells were subjected to 4% paraformaldehyde fixation at room temperature for 25 min and 0.1% crystal violet staining at room temperature for 10 min. Finally, the invaded cells was observed under a light microscope (magnification, x100; Olympus Corporation) and the percentage invaded area was calculated with Image-J software (version 1.46; National Institutes of Health).

Immunofluorescence (IF) assay. Following transfection, A2780 cells were plated into 24-well plates ($5x10^4$ cells/well) and cultivated at 37˚C until 100% confluence was reached. Subsequently, A2780 cells were fixed with 4% paraformaldehyde for 20 min at room temperature and 0.5% Triton X-100 (MP Biomedicals, LLC) permeation for 20 min at room temperature. To block non-specific staining, A2780 cells were incubated with 5% BSA (MilliporeSigma) in PBS at room temperature for 2 h. Subsequently, cells were incubated overnight with anti-paxillin antibody (1:50; cat. no. ab32084; Abcam) at 4˚C. Cells were incubated with a FITC-conjugated anti-rabbit IgG secondary antibody (1:5,000; cat. no. 150077; Abcam) and phalloidin (1:10,000; Abcam) for 60 min at room temperature. DAPI (Beyotime Institute of Biotechnology) was used to counterstain the nuclei at room temperature for 5 min. Coverslips were placed on a microscope slide with a drop of anti-fading mounting medium. Finally, cells were observed under a fluorescence microscope (magnification, x400; Olympus Corporation).

Bioinformatics analysis. GEPIA database (gepia.cancer-pku.cn) (20) was used to determine the expression of SPTBN2 (accession no. ENSG00000173898) in 426 ovarian cancer (OV dataset) and 88 normal tissue samples obtained from The Cancer Genome Atlas and Genotype-Tissue Expression projects. GEPIA database also analysed the association of SPTBN2 expression with prognosis of patients with ovarian cancer (Cutoff-High, 50% and Cutoff-Low, 50%). Moreover, GEPIA database was used to explore the relationship between SPTBN2 and ITGB4 (accession no. ENSG00000132470) in the focal adhesion and ECM receptor signalling pathway. Gene Set Enrichment Analysis (GSEA; linkedomics.org/login.php) (21) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway database (kegg.jp/kegg/pathway.html) (22) detected the enrichment of SPTBN2 in 'focal adhesion' and 'ECM-receptor interaction'. The false discovery rate (FDR) was calculated using the Benjamini-Hochberg method (23).

Statistical analysis. All experimental data are presented as the mean ± standard deviation of three replicates and were analysed using GraphPad Prism 8.0 software (GraphPad Software, Inc.; Dotmatics). One-way analysis of variance followed by Tukey's post hoc test was utilized for comparisons between multiple groups. P<0.05 was considered to indicate a statistically significant difference.

Results

SPTBN2 is upregulated in ovarian cancer tissue and is associated with a poor prognosis. The GEPIA database was used to examine SPTBN2 expression in ovarian cancer tissues as well as its association with prognosis of patients with ovarian cancer. Expression of SPTBN2 was increased in ovarian cancer compared with that in normal ovarian tissue (Fig. 1A). In addition, a higher SPTBN2 expression was associated with poorer prognosis (Fig. 1B).

SPTBN2 is upregulated in ovarian cancer cell lines and SPTBN2 silencing inhibits proliferation. RT-qPCR and western blotting results showed that mRNA and protein expression levels, respectively, of SPTBN2 were increased in ovarian cancer cell

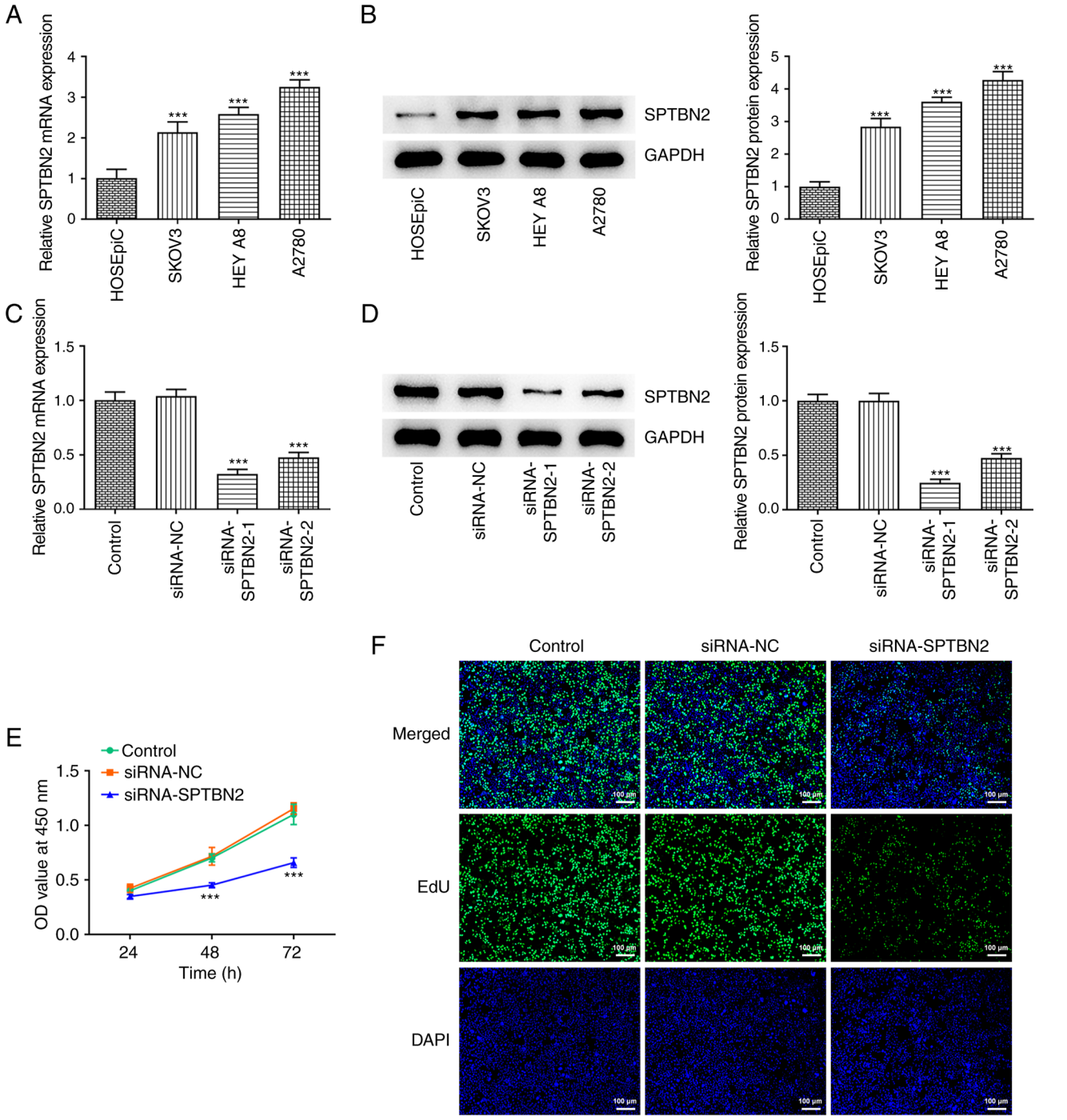

Figure 2. SPTBN2 is upregulated in ovarian cancer cell lines and SPTBN2 knockdown inhibits proliferation of ovarian cancer cells. Expression of SPTBN2 in ovarian cancer cell lines was detected using (A) RT-qPCR and (B) western blotting. ***P<0.001 vs. HOSEpiC. The siRNA transfection efficacy was confirmed using (C) RT-qPCR and (D) western blotting. (E) Cell viability was measured using Cell Counting Kit-8 assay. (F) Cell proliferation was analysed using EdU incorporation assay (magnification, x100). ***P<0.001 vs. Control. EdU, 5-ethynyl-2'-deoxyuridine; NC, negative control; OD, optical density; RT-qPCR, reverse transcription-quantitative PCR; siRNA, small interfering RNA; SPTBN2, spectrin β non-erythrocytic 2.

lines SKOV3, HEY A8 and A2780 relative to HOSEPiC normal ovarian surface epithelial cell line cells (Fig. 2A and B). The highest SPTBN2 expression was observed in A2780 cells, thus, this line was selected for further experiments. To knock down the SPTBN2 expression, two siRNAs specific for SPTBN2 were transfected into the A2780 cells; RT-qPCR and western blotting were used to test the transfection efficacy. Compared with the siRNA-NC group, siRNAs targeting SPTBN2 significantly decreased the expression of SPTBN2 (Fig. 2C and D). In particular, siRNA-SPTBN2-1 exhibited notably higher transfection efficacy compared with the siRNA-SPTBN2-2 group; therefore, siRNA-SPTBN2-1 was selected for subsequent experiments. CCK-8 and EdU incorporation assays were performed to assess the impact of SPTBN2 knockdown on the viability and proliferation of A2780 cells, respectively. The viability and proliferation of A2780 cells were significantly decreased after the transfection with siRNA-SPTBN2 compared with the siRNA-NC group (Fig. 2E and F).

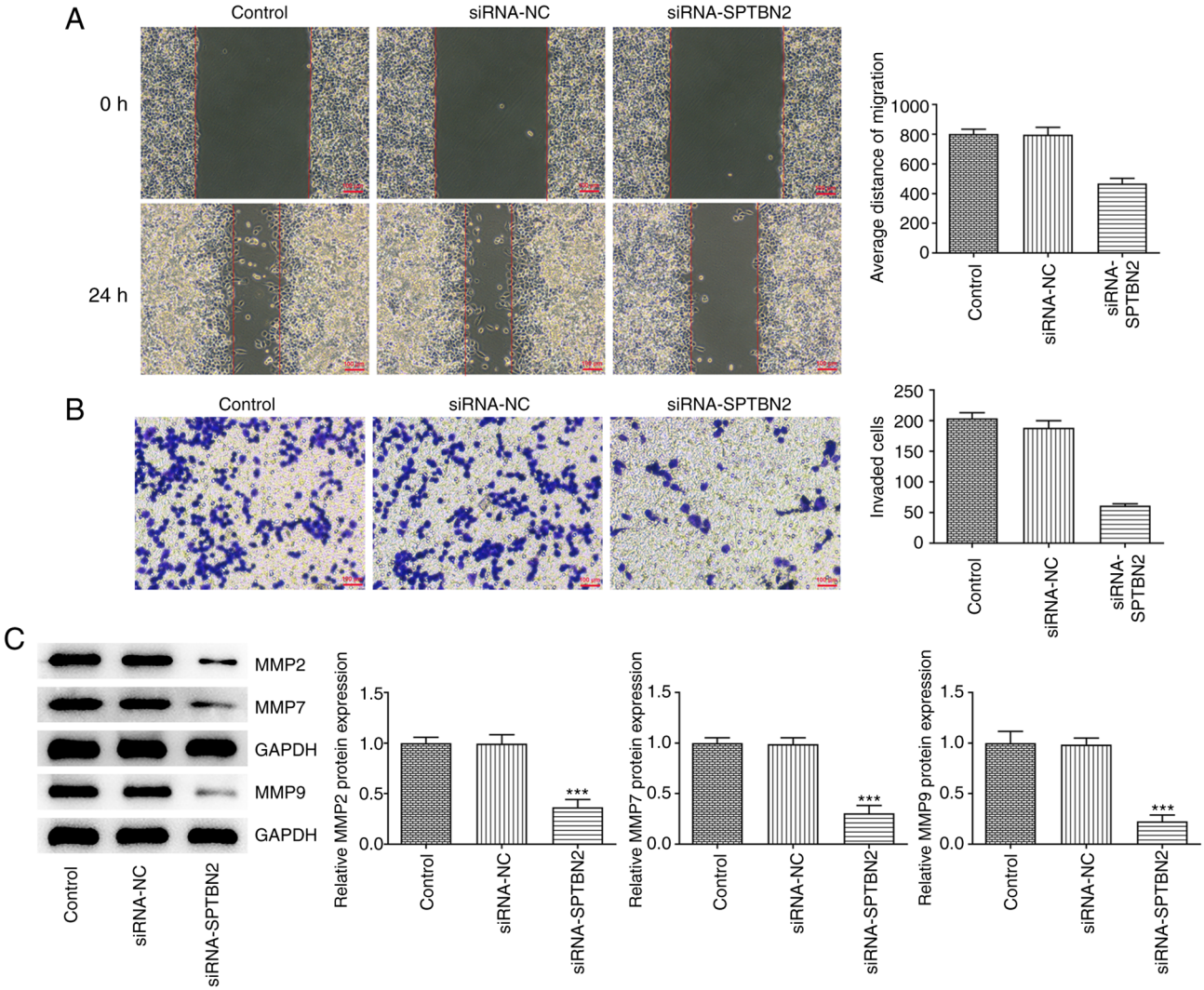

Figure 3. SPTBN2 knockdown inhibits migration and invasion of ovarian cancer cells. (A) Cell migration was analysed using wound healing assay (magnification, x100). (B) Cell invasion was detected by Transwell assay (magnification, x100). (C) Expression levels of migration-associated proteins were detected by western blotting. ***P<0.001 vs. Control. NC, negative control; siRNA, small interfering RNA; SPTBN2, spectrin β non-erythrocytic 2.

SPTBN2 silencing inhibits the migration and invasion of ovarian cancer cells. The migration and invasion of A2780 cells, which were assessed by wound healing and Transwell assays, respectively, were decreased after SPTBN2 knockdown (Fig. 3A and B). In addition, SPTBN2 knockdown decreased the protein levels of MMP2, MMP7 and MMP9 compared with the siRNA-NC group (Fig. 3C).

SPTBN2 is mainly enriched in focal adhesion and ECM receptor signalling pathway. As displayed in Fig. 4A, GSEA analysed that SPTBN2 was mainly enriched in 'focal adhesion' and 'ECM receptor interaction' pathways. Additionally, KEGG pathway database revealed that SPTBN2 was enriched in 'focal adhesion' and 'ECM-receptor interaction' (Fig. 4B and C). In addition, IF demonstrated that SPTBN2 interference decreased the expression of focal adhesion adaptor protein paxillin compared with that in the siRNA-NC group (Fig. 4D).

SPTBN2 knockdown inhibits expression of ITGB4 and related proteins in focal adhesion and ECM receptor signalling pathway. As reported, ITGA and ITGB superfamily members can serve as prognostic markers for serous ovarian cancer (24). In the present study, KEGG pathway database showed the involvement of ITGA and ITGB receptors in 'focal adhesion' and 'ECM-receptor interaction' (Fig. 5). According to GEPIA, SPTBN2 was significantly correlated with ITGB4 in ovarian cancer tissues (Fig. 6A). RT-qPCR and western blotting were used to evaluate mRNA and protein levels, respectively, of ITGB4 in siRNA-transfected A2780 cells. Compared with that in the siRNA-NC group, SPTBN2 knockdown decreased expression of ITGB4 (Fig. 6B and C). Additionally, western blotting analysis showed that SPTBN2 knockdown decreased the protein expression levels of Src and p-FAK but had no notable effect on FAK compared with the siRNA-NC group (Fig. 6D). Collectively, SPTBN2 knockdown inhibited the expression of ITGB4 and focal adhesion and ECM receptor signalling pathway-associated proteins.

SPTBN2 inhibits proliferation, migration and invasion of ovarian cancer cells through ITGB4. To investigate the mechanism of SPTBN2 in ovarian cancer, rescue experiments

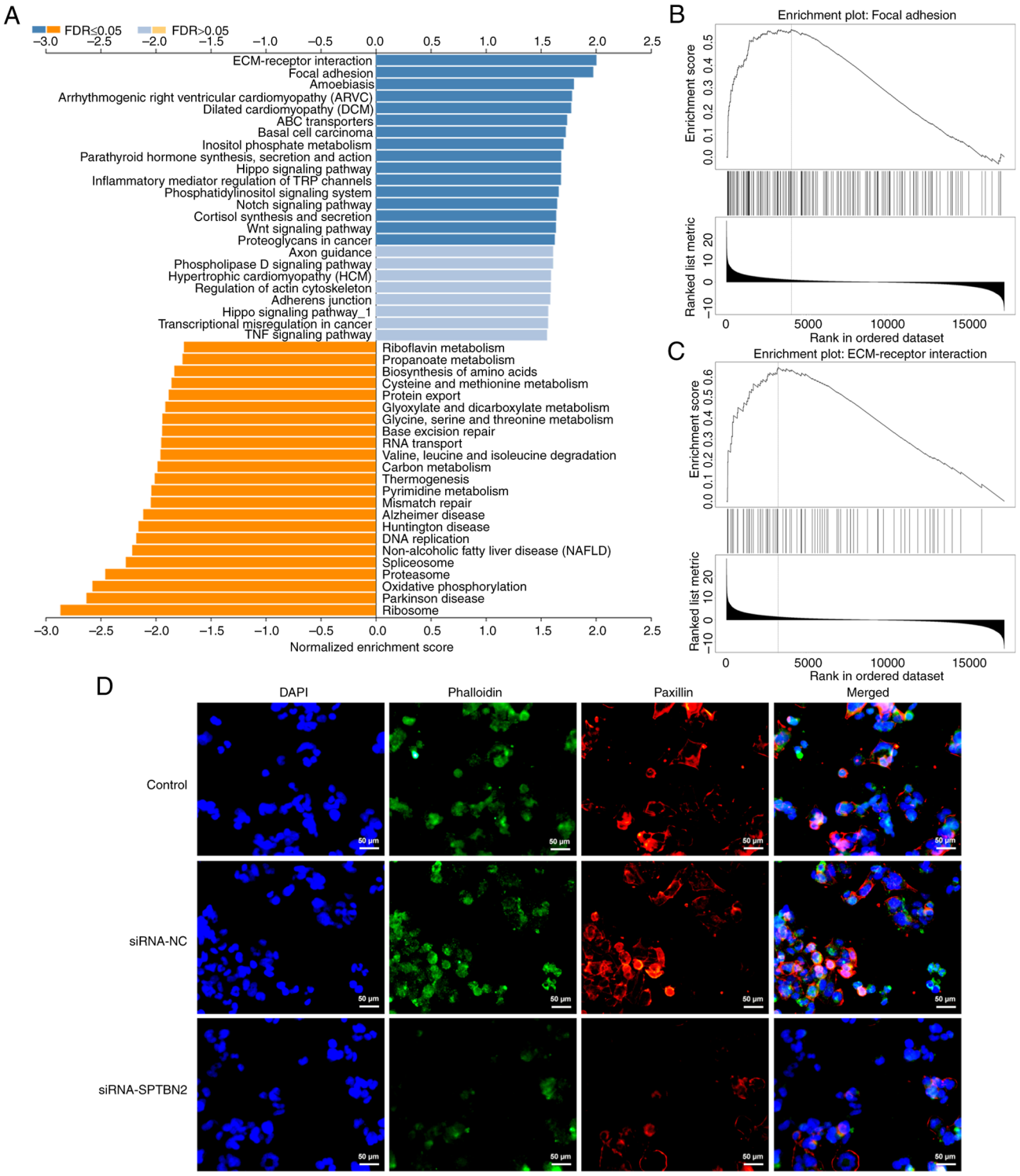

Figure 4. SPTBN2 is enriched in focal adhesion and ECM receptor signalling pathway. (A) 'Focal adhesion' and 'ECM-receptor interaction' in Gene Set Enrichment Analysis. Enrichment of SPTBN2 in (B) focal adhesion and (C) ECM-receptor interaction. (D) Paxillin and Phalloidin expression was detected by immunofluorescence (magnification, x200). ECM, extracellular matrix; FDR, false discovery rate; NC, negative control; siRNA, small interfering RNA; SPTBN2, spectrin β non-erythrocytic 2.

were performed. Plasmids carrying ITGB4 were transfected into A2780 cells to overexpress ITGB4. RT-qPCR and western blotting analyses showed that, compared with that in the Ov-NC group, the mRNA and protein expression levels of ITGB4 were significantly increased following ITGB4 overexpression (Fig. 7A and B). Co-transfection experiments demonstrated that the decreased cell viability and proliferation caused by SPTBN2-knockdown A2780 cells were significantly reversed by ITGB4 overexpression (Fig. 7C and D). Similarly, the decreased migration and invasion of A2780 cells caused by SPTBN2 knockdown were rescued after ITGB4 overexpression compared with those in the siRNA-SPTBN2 + Ov-NC group (Fig. 7E and F). Moreover, the decreased levels of MMP2, MMP7 and MMP9 were reversed by ITGB4 overexpression

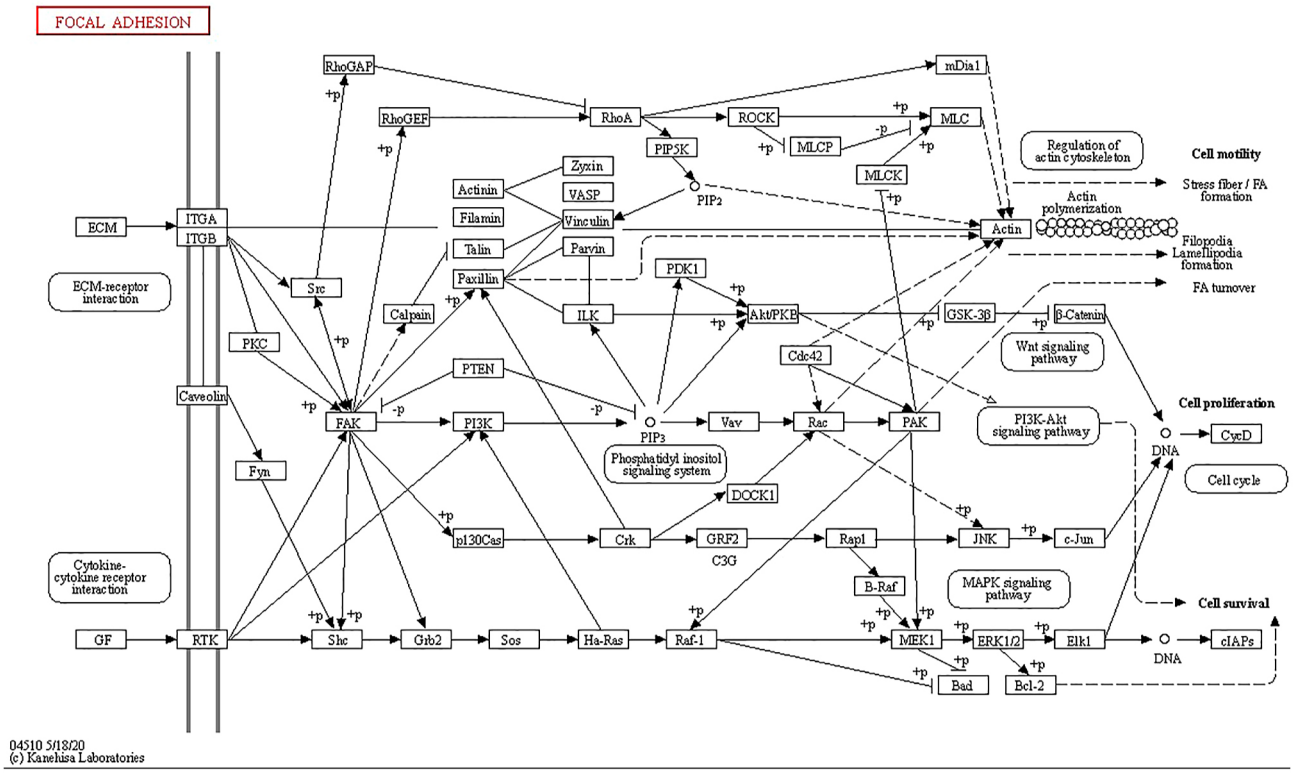

Figure 5. Involvement of ITGA and ITGB receptors in 'focal adhesion' and 'ECM receptor interaction' from the Kyoto Encyclopaedia of Genes and Genomes pathway database. ECM, extracellular matrix. The figure was obtained from Kanehisa Laboratories.

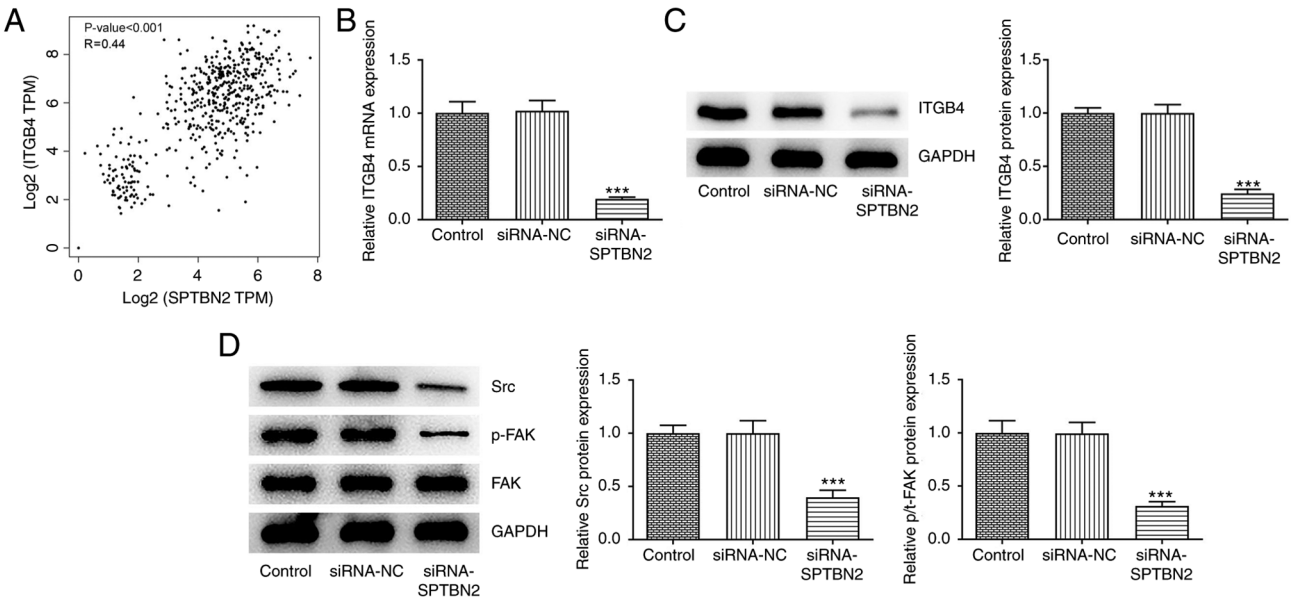

Figure 6. SPTBN2 knockdown inhibits expression of ITGB4 and associated proteins in focal adhesion and extracellular matrix receptor signalling pathway. (A) According to the Gene Expression Profiling Interactive Analysis database, SPTBN2 expression is correlated with ITGB4. (B and C) mRNA and protein expression levels of ITGB4 in transfected cells were detected using reverse transcription-quantitative PCR and western blotting, respectively. (D) Protein expression levels of Src, p-FAK and FAK were detected using western blot. ***P<0.001 vs. Control. FAK, focal adhesion kinase 1; ITGB4, integrin β4; NC, negative control; p-, phosphorylated; si, small interfering RNA; SPTBN2, spectrin β non-erythrocytic 2; Src, proto-oncogene tyrosine-protein kinase; TPM, transcripts per million.

compared with the siRNA-SPTBN2 + Ov-NC group (Fig. 7G). In conclusion, the data suggested that SPTBN2 may inhibit proliferation, migration and invasion of ovarian cancer cells through ITGB4.

SPTBN2 inhibits focal adhesion and the expression of downstream signalling pathway-related proteins in ovarian cancer cells through ITGB4. Compared with the siRNA-SPTBN2 + Ov-NC group, the decreased

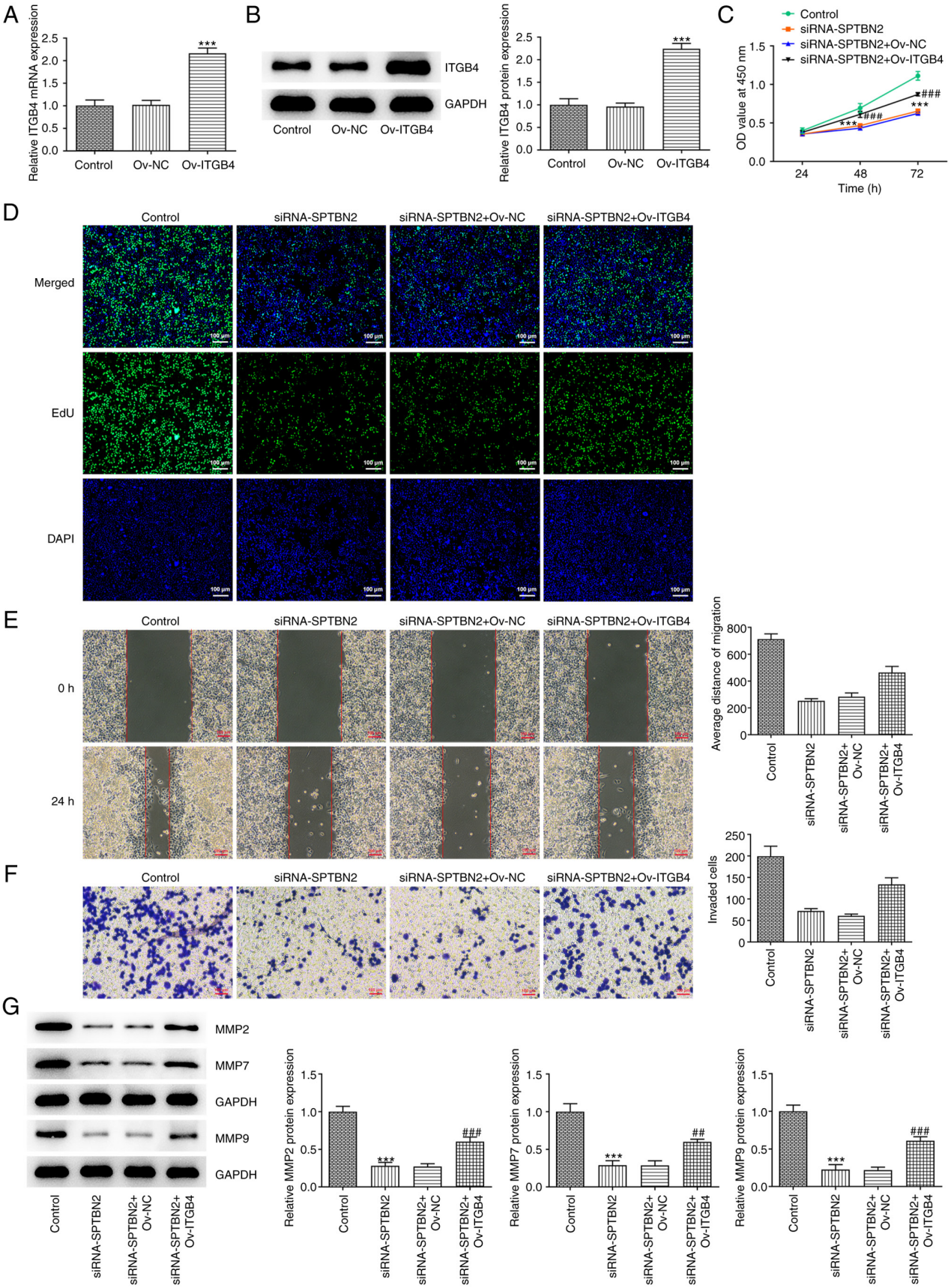

Figure 7. SPTBN2 inhibits proliferation, migration and invasion of ovarian cancer cells through ITGB4. mRNA and protein expression levels of ITGB4 were analysed following Ov-ITGB4 transfection using (A) reverse transcription-quantitative PCR and (B) western blotting, respectively. ***P<0.001 vs. Control. (C) Cell viability was detected using Cell Counting Kit-8 assay. (D) Cell proliferation was detected using EdU incorporation assay (magnification, x100). (E) Cell migration was analysed using a wound healing assay. (F) Cell invasion was analysed by Transwell assay. (G) Expression levels of migration-related proteins were measured by western blotting. ***P<0.001 vs. Control; ##P<0.01 and ###P<0.001 vs. siRNA-SPTBN2 + Ov-NC. EdU, 5-ethynyl-2'-deoxyuridine; ITGB4, integrin β4; SPTBN2, spectrin β non-erythrocytic 2; NC, overexpression negative control; OD, optical density; Ov, overexpression; si, small interfering RNA.

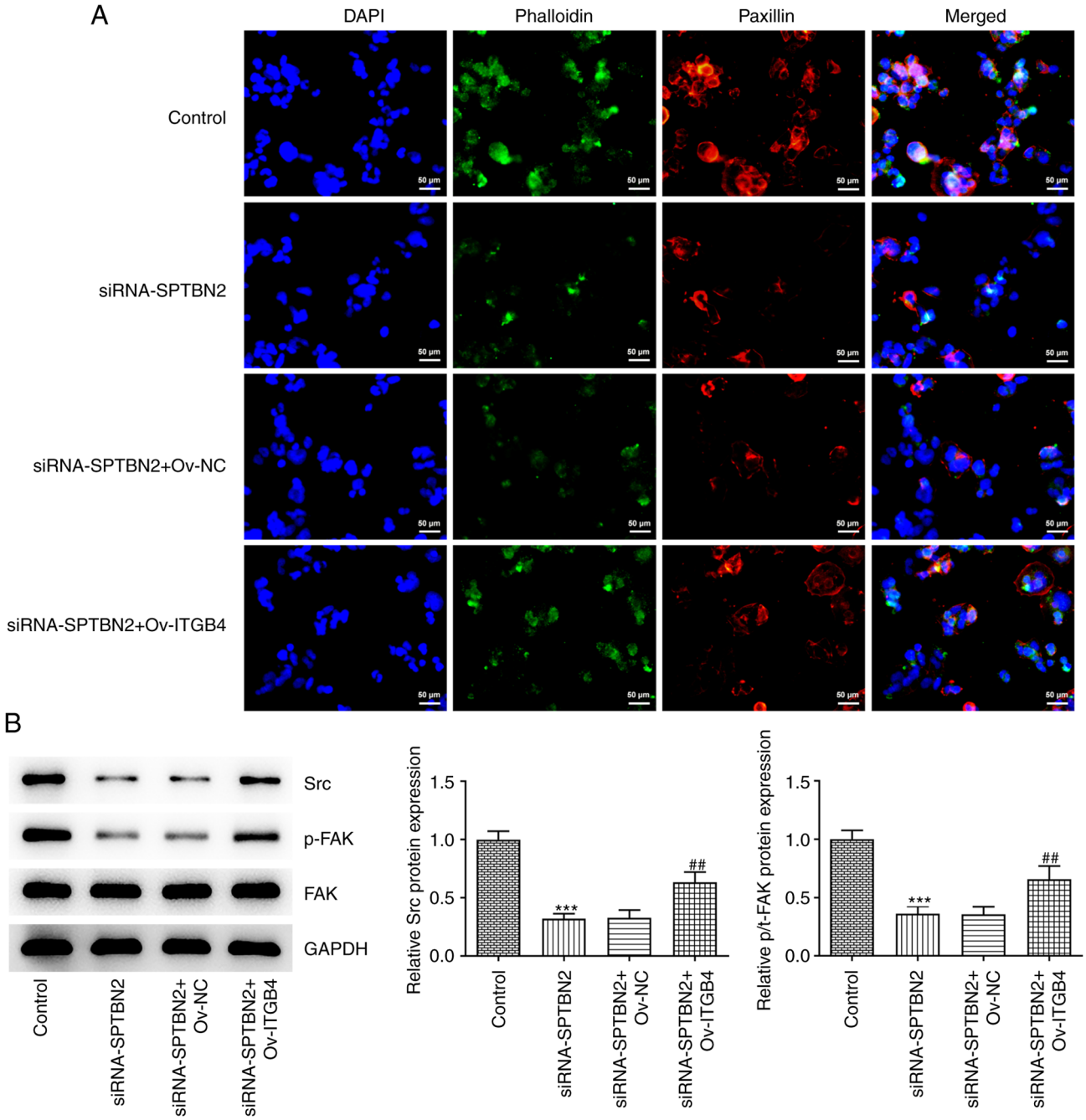

Figure 8. SPTBN2 inhibits focal adhesion and the expression of downstream signalling pathway proteins in ovarian cancer cells through ITGB4. (A) Paxillin and Phalloidin expression was detected by immunofluorescence microscopy (magnification, x200). (B) Protein expression levels of Src, p-FAK and FAK were detected using western blot. ***P<0.001 vs. Control; $^{\#\#}$P<0.01 vs. siRNA-SPTBN2 + Ov-NC. FAK, focal adhesion kinase 1; ITGB4, integrin β4; si, small interfering RNA; NC, negative control; Ov, overexpression; p-, phosphorylated; SPTBN2, spectrin β non-erythrocytic 2; Src, proto-oncogene tyrosine-protein kinase.

expression of focal adhesion adaptor protein paxillin caused by SPTBN2-knockdown A2780 cells was increased by ITGB4 overexpression (Fig. 8A). In addition, the SPTBN2-knockdown-induced decrease of Src and p-FAK expression levels were reversed in the siRNA-SPTBN2 + ITGB4 overexpression group (Fig. 8B). Notably, SPTBN2 knockdown combined with ITGB4 overexpression had no effect on FAK protein expression. In summary, SPTBN2 may inhibit focal adhesion and the expression of downstream signalling pathway-associated proteins in ovarian cancer cells through ITGB4.

Discussion

Ovarian cancer is one of the most common lethal epithelial malignancies and has poor outcome owing to late diagnosis (25). SPTBN2 is associated with tumorigenesis-associated biological activity (12). Previous studies have shown that SPTBN2 is involved with numerous types of malignant tumour. For example, SPTBN2 has been proposed as a potential driver gene in breast cancer (26). Huang *et al* (27) showed that SPTBN2 significantly affects the prognosis of patients with bladder cancer. Additionally, SPTBN2 is elevated in patients

with colorectal cancer, and increased SPTBN2 expression is associated with worse prognosis (28). According to the GEPIA database, SPTBN2 is increased in ovarian cancer tissue, and its upregulation is associated with poor prognosis of patients with ovarian cancer (29). The aforementioned results revealed that SPTBN2 may influence the progression of ovarian cancer. Consistently, the present study used GEPIA to assess expression of SPTBN2 in clinical samples and it was also noticed that SPTBN2 was overexpressed in ovarian cancer tissues. In line with findings from the GEPIA database, mRNA and protein expression levels of SPTBN2 were increased in ovarian cancer cell lines (SKOV3, HEY A8 and A2780), particularly in A2780 cells.

A recent study demonstrated that SPTBN2 is highly expressed in lung adenocarcinoma and its downregulation is associated with suppressive effects on proliferative, migratory and invasive capabilities of lung cancer cells (11). In addition, Wang *et al* (12) suggested that SPTBN2 inhibition is implicated in halting proliferation, migration and invasion of endometrioid endometrial cancer cells. SPTBN2 upregulation promotes the proliferation, migration and invasion of colorectal cancer cells (30). Consistent with these findings, the present study demonstrated that SPTBN2 knockdown suppressed the proliferation, migration and invasion of A2780 cells, suggesting the oncogenic role of SPTBN2 in ovarian cancer. Furthermore, according to GSEA, SPTBN2 was primarily enriched in focal adhesion and ECM receptor signalling pathway, implying that SPTBN2 might contribute to ovarian cancer partially via modulation of focal adhesion and ECM receptor signalling pathway.

As a structural adhesion molecule, ITGB4 forms heterodimers with ITGA6 to achieve its biological functions (31). A previous study has demonstrated that ITGB4 expression is elevated in ovarian cancer tissue (24). In the present study, KEGG pathway analysis showed the involvement of ITGA and ITGB receptors in focal adhesion and ECM receptor signalling pathway. A member of ITGB, ITGB4 was associated with SPTBN2 in the GEPIA database. Additionally, further experiments elucidated that ITGB4 expression was decreased by SPTBN2 knockdown. Moreover, the expression levels of downstream signalling pathway-associated proteins Src and p-FAK were decreased by SPTBN2 knockdown. To assess the regulatory mechanism of SPTBN2 in ovarian cancer, rescue experiments were performed; results showed that the inhibitory effects of SPTBN2 knockdown on proliferation, migration and invasion, as well as the levels of Src and p-FAK, were rescued by ITGB4 overexpression, indicating that SPTBN2 knockdown suppressed the proliferation, migration and invasion, and inactivated focal adhesion and downstream signalling in ovarian cancer cells by reducing ITGB4 expression.

The present study demonstrated that SPTBN2 showed a protective effect against ovarian cancer through ITGB4, revealing that SPTBN2 may be a potential target for treatment of endometroid ovarian cancer. Nevertheless, the regulatory role of SPTBN2 in other ovarian cancer cell lines and animal models of ovarian cancer was not investigated by the present study. Therefore, SPTBN2 expression in ovarian cancer tissue and the association between SPTBN2 expression and prognosis should be confirmed in future studies.

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Availability of data and material

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

Authors' contributions

YL designed and conceived the study and wrote the manuscript. YL and GY conducted the experiments and analysed the data. YL and GY confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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