



SPIB Knockdown Inhibits the Immune Escape of Ovarian Cancer Cells by Reducing PD-L1 (CD274) Expression and Inactivating the JAK/STAT Pathway

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ABSTRACT

Background: Spi-B transcription factor (SPIB) is an E-twenty-six (ETS) transcription factor associated with tumor immunity.

Objective: To investigate the functions and mechanisms of SPIB in ovarian cancer (OC) cells.

Methods: Cell proliferation, apoptosis, migration, and invasion were determined using colony formation, EdU, flow cytometry, and transwell assays, respectively. The binding sites of programmed death-ligand 1 (PD-L1) and SPIB were predicted using the JASPAR database and verified using the ChIP and luciferase reporter assays.

Results: SPIB knockdown inhibited OC cell proliferation, migration, and invasion, and significantly boosted apoptosis ($p < 0.05$). SPIB directly enhanced PD-L1 transcription in OVCAR-3 and SKOV3 cells ($p < 0.05$). Importantly, the JAK/STAT pathway was markedly inactivated in OC cells upon SPIB knockdown. SPIB knockdown markedly decreased JAK2 and STAT1 phosphorylation in OVCAR-3 and SKOV3 cells ($p < 0.05$).

Conclusion: These data indicate that SPIB knockdown inhibits OC cell progression by downregulating PD-L1 and inactivating the JAK/STAT pathway.

Keywords: JAK/STAT Pathway, Ovarian Cancer, PD-L1 (CD274), SPIB

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INTRODUCTION

Ovarian cancer (OC) is a heavily lethal disease in women (1). The 5-year survival rate for OC patients is 30-40% (2). High-grade serous ovarian cancer (HGSOC) is a malignant subtype of OC (3). The survival rate of HGSOC is poor because HGSOC lacks specific symptoms, often accompanied by extensive pelvic and abdominal metastases, and even distant metastases (4). Hence, the elucidation of mechanisms underlying OC development is crucial.

Spi-B transcription factor (SPIB) is an E-twenty-six (ETS) transcription factor associated with tumor immunity (5, 6). SPIB expression is significantly enhanced in colon, hepatocellular carcinoma, and gastric cancer (7). Colon cancer is strongly connected to a poor prognosis in SPIB-positive patients (8). SPIB abates apoptosis via the phosphoinositide 3-kinase (PI3K)/AKT pathway in diffuse large B-cell lymphoma (DLBCL) (9). SPIB is reduced in colorectal cancer cells, and SPIB restrains tumor progression by activating the NF- κ B and c-Jun N-terminal kinase (JNK) pathways via enhancing MAP kinase kinase kinase 1 (MAP4K1) expression (10). According to Montes-Moreno et al., SPIB expression could be used to diagnose blastic plasmacytoid dendritic cell neoplasms (11). Uppal et al. have reported that SPIB in blood mononuclear cells could serve as a discriminator for the diagnosis of HGSOC (12). Thus, there is an urgent need to find the role of SPIB in the cell biological function of OC.

Programmed death-ligand 1 (PD-L1, CD274) is an immune co-signaling molecule that suppresses T cell activation, leading to tumor progression (13). High PD-L1 level is profoundly linked to poor survival in OC (14). Manifold transcriptional factors control the expression of PD-L1 (15-17). Signal transducer and activator of transcription 3 (STAT3) binds to the PD-L1 promoter and adjusts the PD-L1 level (18). PD-L1 expression is elevated due to the modulation of the Janus kinase (JAK) and

STAT in cervical squamous cell carcinoma cells (19).

Here, we determined the function of SPIB in OC cells and explored the underlying mechanisms. SPIB knockdown was found to suppress OC progression by downregulating PD-L1 expression and inactivating the JAK/STAT pathway.

MATERIALS AND METHODS

Bioinformatics Analysis

SPIB expression was analyzed using The Cancer Genome Atlas (TCGA) dataset. The relationship between SPIB expression and survival time was analyzed using Kmpplot (<http://kmpplot.com/analysis/>). The SPIB-related pathways were determined using KEGG and GSEA. A possible SPIB and PD-L1 binding site was found using JASPAR.

Tumor Samples

Sixteen HGSOC and six normal uterine tube tissues were collected from our hospital between May 2019 and July 2021. Patients with HGSOC did not undergo neoadjuvant chemotherapy before surgery. The tissues were cut into sections for immunohistochemical staining. The slices were incubated with SPIB (ab241570, 1:500, Abcam, UK), followed by a secondary antibody (ab6789, 1:2000, Abcam, UK) and DAB complex (Solarbio, China). Brown and yellow granules indicated positive staining. Staining intensity score: no staining (0 points), light yellow (1 point), brown yellow (2 points), and brown (3 points). The proportion of stained cells: <5% (0 points), 5-25% (1 point), 26-50% (2 points), 51-75% (3 points), and >75% (4 points). The result of multiplying the two scores: 0-2 (-), 3-4 (+), 5-8 (+ +), and 9-12 (+ + +). Each sample was scored by two independent observers, and disagreements were resolved by a third observer. The protocol of this research has been approved by the Ethics Committee of Binzhou Medical University Hospital. All patients have signed written informed consent.

Cell Culture

IOSE80 (Procell, Wuhan, China) was maintained in a complete medium for human ovarian epithelial cells (CM-H055, Procell, China). A2780 and OVCAR-3 cells (Cobioer, China) were maintained in RPMI-1640 medium (Gibco, USA). SKOV3 (Cobioer, China) was cultured in McCoy's 5A medium (Gibco, USA). The cells were grown in a medium containing 10% fetal bovine serum (FBS; HyClone, USA) at 37°C 5% CO₂. SPIB expression plasmid (pcDNA3.1), small interference RNA (siRNA) targeting SPIB, and control sequences (GenePharma, China) were transfected to cells using Lipofectamine 3000 (Invitrogen, USA). The final transfection concentration was 50 nM. The siRNA sequences are listed in Table 1.

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

We extracted total RNA from tissues and cells using the RNA isolator Total RNA Extraction Reagent (Vazyme, China). Thereafter, HiScript II Q Select RT SuperMix (Vazyme, China) was used to synthesise cDNA. PCR analysis was conducted using ChamQ SYBR qPCR Master Mix (Vazyme, China)

on an ABI 7900 system (Applied BioSystems, USA). The primers are listed in Table 2.

Soft Agar Colony Formation Assay

The bottom layer of 6-well plates was bespread 1.2% agarose gel (Beyotime, Shanghai, China) containing medium. 0.6% agarose gel containing OVCAR-3 and SKOV3 cells (5×10^3 cells/well) was added into the top layer of 6-well plates. After incubation for 14 days, the colonies were stained with 0.1% crystal violet (Sigma-Aldrich, USA), and counted under a microscope.

5-Ethynyl-2'-Deoxyuridine (EdU) Staining Assay

The Cell-Light EdU Apollo567 In Vitro Kit (Ribbio, China) was utilized to measure cell proliferation. Cells were cultured with EdU for 2 h, fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton X-100, and cultured in an ApolloR reaction cocktail. After 30 min, the samples were incubated with DAPI and observed using a microscope (Olympus, Japan).

Apoptosis Analysis

The cells were stained at room temperature

Table 1. The siRNA sequences

siRNA	Sequences
si-SPIB-1	Sense:5'-GCUGCAAGCAUCCAGCUACC-3'
	Antisense:5'-UAGCUGGAAUGCUUGCAGCUG-3'
si-SPIB-2	Sense:5'-AGUUCUCCUCCAAGCACAAGG-3'
	Antisense:5'-UUGUGCUUGGAGGAGAACUGG-3'
si-SPIB-3	Sense:5'-GCGAGAUCCGCAAGGUCAAGC-3'
	Antisense:5'-UUGACCUUGCGGAUCUCGCCG-3'
si-NC	Sense:5'-UUCUCCGAACGUGUCACGUTT-3'
	Antisense:5'-ACGUGACACGUUCGGAGAATT-3'

siRNA: small interference RNA, SPIB: Spi-B transcription factor, NC: negative control

Table 2. The primer sequences

Gene	Sequences
SPIB	CCCTATGAAGCCTTCGACCC
	GGGAACCAGGGTCTGGCTA
β-actin	CCCTATGAAGCCTTCGACCC
	AGGTCTTTGCGGATGTCCACGT

SPIB: Spi-B transcription factor

using an Annexin V-FITC/PI kit (Beyotime, China). Apoptosis was then assessed using a flow cytometer (Accuri C6 Plus, BD Biosciences, USA).

Transwell Assay

OVCAR-3 and SKOV3 cells (1×10^5) in serum-free media were seeded into the upper chamber of Matrigel-coated 24-well Transwell (Corning, USA) and a medium containing 10% FBS was added to the lower chamber. After 24 h, the cells on the inner side of the upper chamber were eliminated using cotton swabs. Migratory or invasive cells were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, and counted.

Immunofluorescence

OVCAR-3 and SKOV3 cells were blocked with 5% FBS in PBS and incubated with anti-PD-L1 (ab205921, 1:1000, Abcam, UK) for 1 h, followed by incubating with anti-rabbit IgG (ab150079, Abcam, UK) for 1 h at 37°C. The nuclei were stained using DAPI (Beyotime, China).

Chromatin Immunoprecipitation (ChIP)

The ChIP assay was conducted using a ChIP Assay Kit (Beyotime, China). Briefly, after crosslinking with 1% formaldehyde at 37°C for 10 min, followed by keeping for 5 min with glycine, the cells were lysed and sonicated to obtain DNA fragments. The chromatin was precipitated using anti-SPIB [14337, 1:1000, cell signaling technology (CST), USA] and rabbit monoclonal to human IgG (ab109489, Abcam, UK) at 4°C overnight. The complexes were washed, eluted, and purified. qPCR was performed to detect the enrichment.

Luciferase Reporter Assay

To analyze PD-L1 promoter activity, PD-L1 promoter wild-type (WT) and mutant-type (MUT) luciferase reporter vectors were synthesized by RiboBio (Guangzhou, China). Promoter luciferase reporter vectors and SPIB expression or empty plasmids were co-transfected into the cells for 48 h. Luciferase

activity was determined using the Dual-Luciferase Assay System (Promega, USA).

Western Blot Analysis

The cells were lysed in RIPA (Beyotime, China). Proteins were separated using 10% SDS-PAGE and transferred onto PVDF membranes (Millipore, USA). After blocking, the membranes were cultured with the primary antibodies SPIB (14337, 1:1000, CST, USA), Bcl-2 (3498, 1:1000, CST, USA), Bax (5023, 1:1000, CST, USA), MMP2 (40994, 1:1000, CST, USA), MMP9 (3852, 1:1000, CST, USA), PD-L1 (ab205921, 1:1000, Abcam, UK), p-JAK2 (ab195055, 1:1000, Abcam, UK), JAK2 (ab108596, 1:1000, Abcam, UK), p-STAT1 (ab109461, 1:1000, Abcam, UK), STAT1 (10144-2-AP, 1:1000, Proteintech, China), and β -actin (81115-1-RR, 1:10000, Proteintech, China) at 4°C overnight. Thereafter, the membranes were incubated with a secondary antibody (SA00001-2, 1:10000, Proteintech, China) for 2 h. Bands were detected using enhanced chemiluminescence (Vazyme, China).

Statistical Analysis

The data are expressed as the mean $\pm$ standard deviation. Statistical analysis was performed using unpaired Student's t-test and one-way ANOVA, followed by Tukey's post-hoc test (GraphPad Prism 7, USA). Statistical significance was considered as $p < 0.05$.

RESULTS

SPIB is Highly Expressed in OC

TCGA dataset of OC was analyzed using GEPIA. SPIB levels were found to increase in OC tissues (Fig. 1A). In Kmpplot analysis, patients with low SPIB levels had better survival rate than those with high SPIB levels (Figs. 1B, 1C). Using IHC and qRT-PCR, SPIB expression was found to be elevated in the HGSOC tissues (Figs. 1D, 1E). Indeed, SPIB expression was enhanced in OC cells (Fig. 1F).

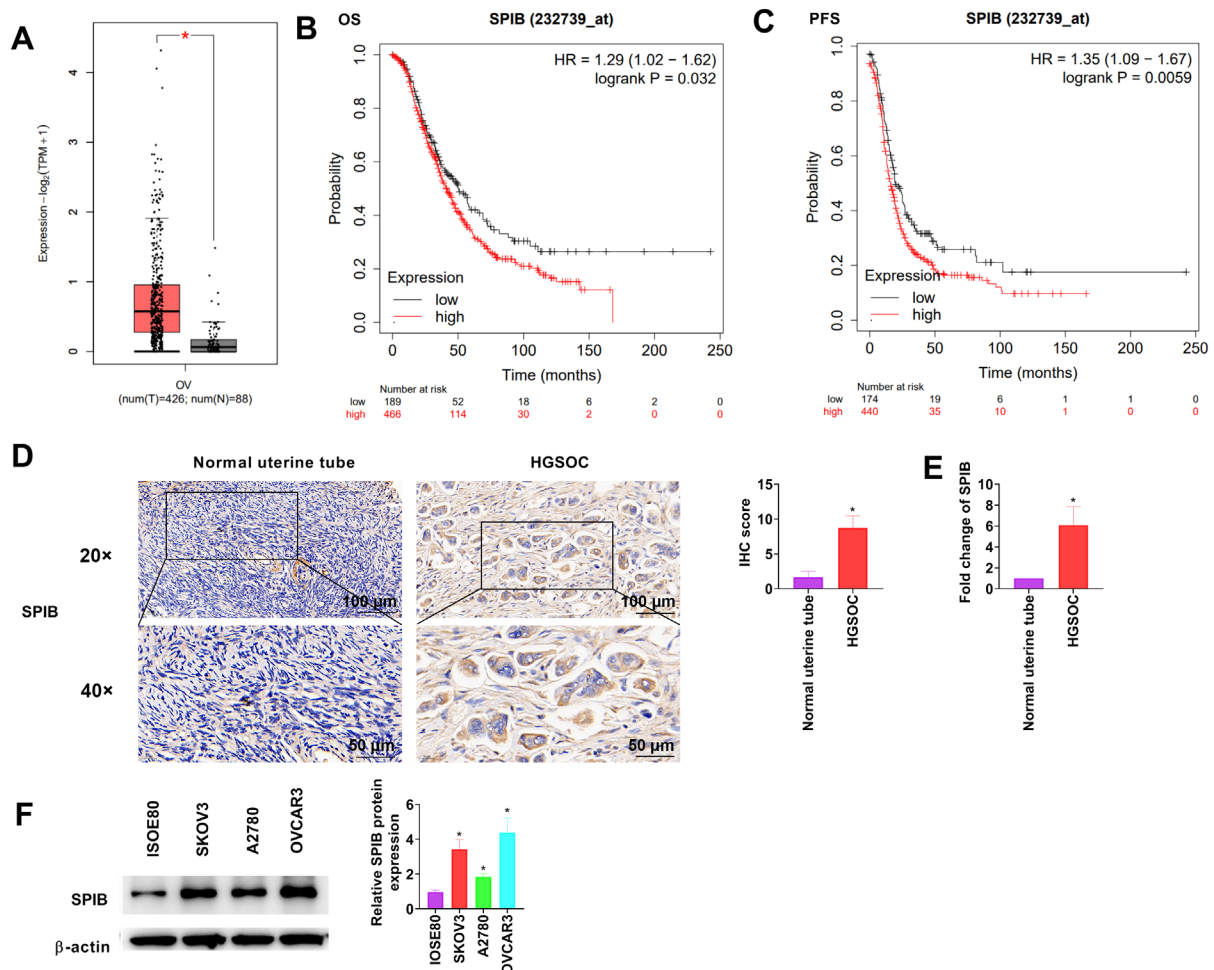


Fig. 1. SPIB expression is high in OC. (A) The expression of SPIB in OC tissues from TCGA. (B and C) Survival curves were plotted using Kmpot analysis. (D and E) Using IHC and qRT-PCR, SPIB expression was identified in tissues. (F) SPIB expression in OC cells was determined by western blotting. Bands were quantified using ImageJ software. * $p < 0.05$, vs normal tissues group, IOSE80 cells group. spi-B transcription factor (SPIB), ovarian cancer (OC), The Cancer Genome Atlas (TCGA), immunohistochemistry (IHC)

SPIB Knockdown Inhibits OC Cell Proliferation and Induces Apoptosis

The si-SPIB knockdown efficiency was assessed using western blotting (Fig. 2A). Cell proliferation reduced when cells were transfected with si-SPIB (Figs. 2B, 2C). Furthermore, SPIB knockdown induced the apoptosis of OC cells (Fig. 2D). SPIB knockdown enhanced Bax expression and declined Bcl-2 expression in both OVCAR-3 and SKOV3 cells (Fig. 2E).

SPIB Knockdown Hampers OC Cell Migration and Invasion

si-SPIB hampered OC cell migration and

invasion (Figs. 3A, 3B). Metalloproteinases (MMPs) are considered potent molecular targets for restraining cell migration and invasion (20). Silencing SPIB markedly reduced MMP2 and MMP9 protein levels (Fig. 3C).

SPIB Knockdown Suppresses PD-L1 Expression

We assessed the link between SPIB and PD-L1 in TISIDB. SPIB expression was positively linked to PD-L1 expression (Fig. 4A). SPIB knockdown markedly decreased PD-L1 expression (Fig. 4B). Similar results were obtained using immunofluorescence assay (Fig. 4C).

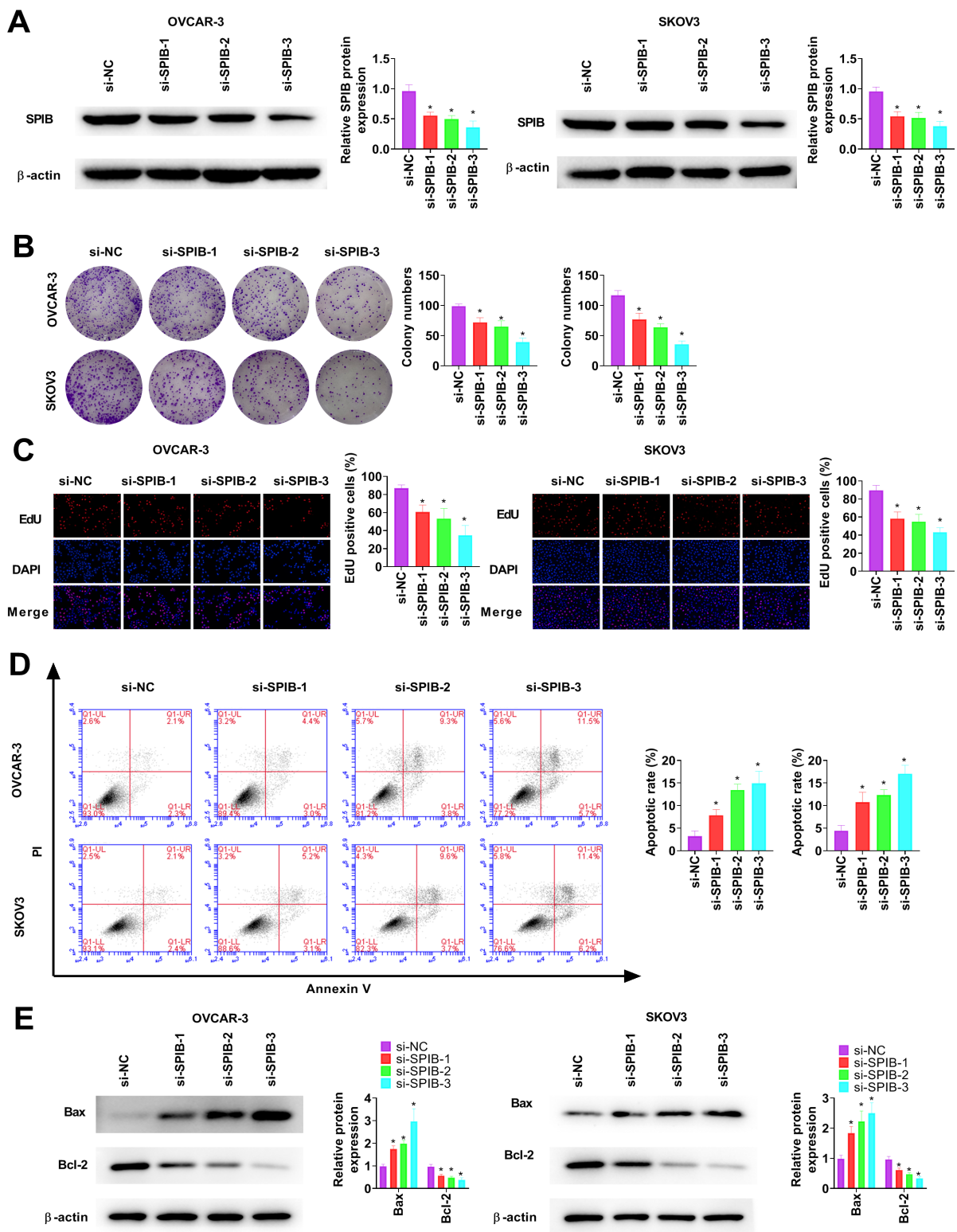


Fig. 2. SPIB knockdown inhibits OC cell proliferation. (A) The SPIB protein expression was measured in OVCAR-3 and SKOV3 cells transfected with si-NC, si-SPIB-1, si-SPIB-2, and si-SPIB-3. Bands were quantified using ImageJ software. (B) The colony numbers of cells. (C) EdU assay was employed to detect cell proliferation. (D) Cell apoptosis was detected by flow cytometry. (E) Bcl-2 and Bax expression was detected using western blotting. Bands were quantified using ImageJ software. * $p < 0.05$ vs si-NC group. spi-B transcription factor (SPIB), ovarian cancer (OC), siRNA-negative control (si-NC), 5-Ethynyl-2'-deoxyuridine (EdU)

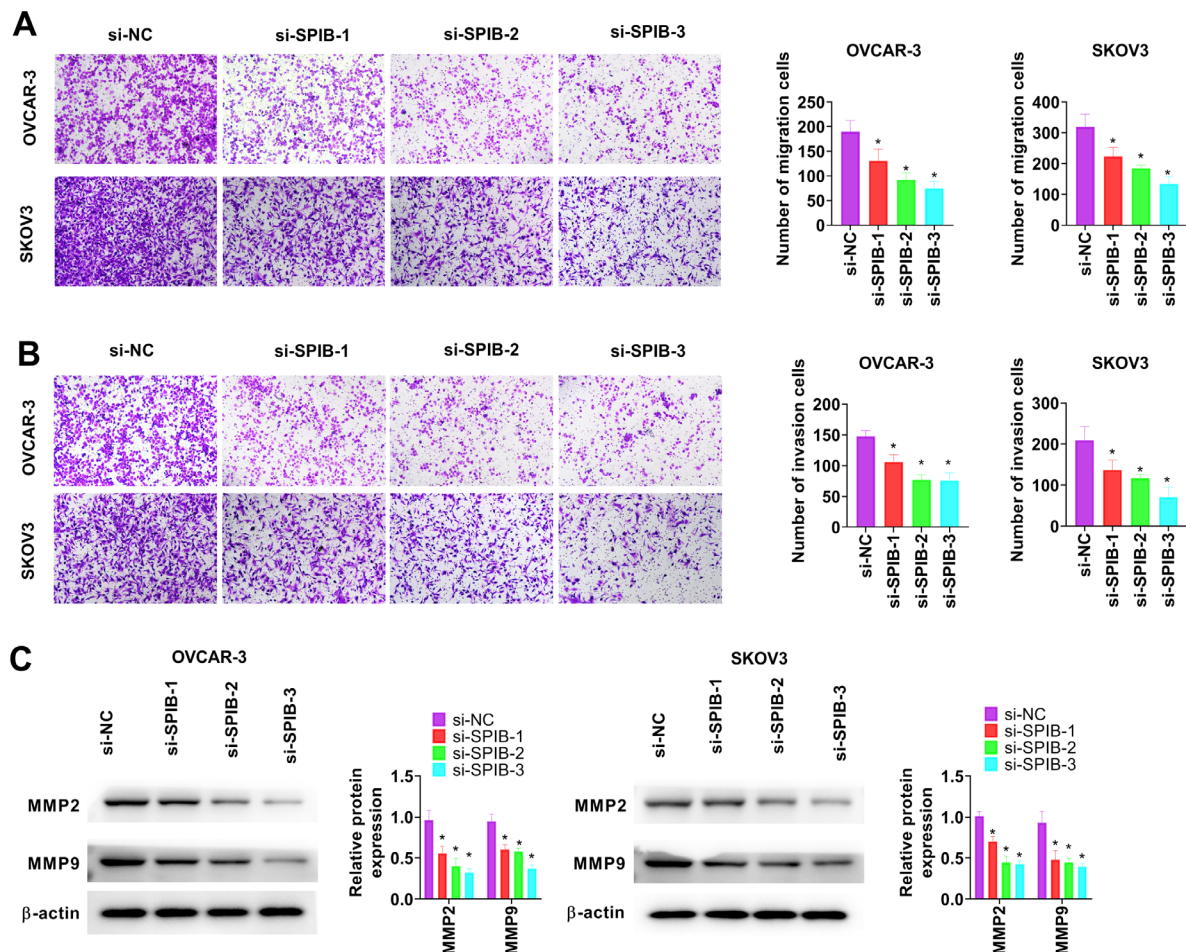


Fig. 3. SPIB knockdown inhibits OC cell migration and invasion. (A and B) Transwell assay was employed to determine cell migration and invasion. (C) MMP2 and MMP9 expressions were examined by western blotting. Bands were quantified using ImageJ software. * $p < 0.05$ vs si-NC group. spi-B transcription factor (SPIB), ovarian cancer (OC), matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 9 (MMP9)

SPIB Directly Promotes PD-L1 Transcription

JASPAR analysis predicted that SPIB binds to PD-L1 promoter (Fig. 5A). Sequence logo of potential SPIB binding site is shown in Fig. 5B. Based on the ChIP assay, anti-SPIB upregulated PD-L1 expression, verifying that SPIB binds tightly to this transcriptional area of PD-L1 (Fig. 5C). Compared with the cells transfected with WT PD-L1+SPIB vector, the cells transfected with MUT PD-L1+SPIB vector showed markedly lower relative luciferase activity (Fig. 5D). Additionally, the levels of SPIB and PD-L1 elevated in SPIB-transfected cells (Fig. 5E).

SPIB Knockdown Inactivates the JAK/STAT Pathway in OC Cells

GSEA revealed that SPIB activated the “JAK/STAT pathway” (Figs. 6A, 6B).

As shown in Fig. 6C, JAK2, and STAT1 phosphorylation levels decreased after SPIB knockdown. To clarify whether SPIB inhibited OC cell progression via the JAK2/STAT1 pathway, the cells were treated with the JAK2 activator, coumermycin A1 (CA1). CA1 blocked the effects of SPIB knockdown on cells (Figs. 6D, 6E).

DISCUSSION

Of all types of OC, HGSOC remains the most aggressive owing to its immune escape and the lack of efficient specific therapies (21). In the present study, SPIB deficiency inhibited the progression of OVCAR-3 and SKOV3 cells via downregulating PD-L1 expression and the JAK/STAT pathway.

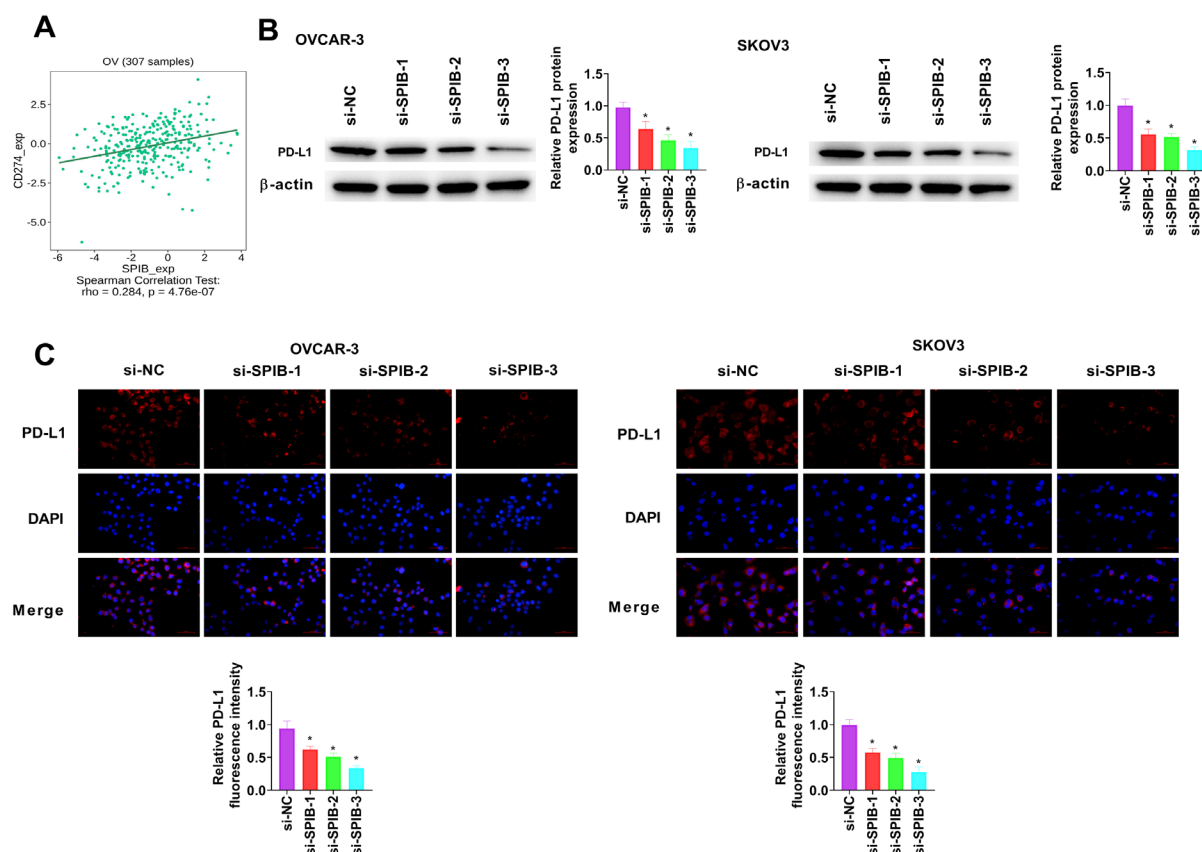


Fig. 4. SPIB knockdown suppresses PD-L1 expression. A. The correlations between SPIB and PD-L1 expression in TISIDB. B. Western blotting of PD-L1 expression. Bands were quantified using ImageJ software. C. Immunofluorescence of PD-L1 expression. * $p < 0.05$ vs si-NC group. spi-B transcription factor (SPIB), programmed cell death ligand-1 (PD-L1, also known as CD274)

SPIB is an ETS transcription factor that regulates the tumor immune environment and cancer progression (22). SPIB/SPI1 depletion suppresses aerobic glycolysis and tumorigenesis (8). SPIB inhibits cervical cancer cell progression and prevents macrophage infiltration (23). In the present study, we analyzed SPIB levels in 426 OC and 88 normal samples from TCGA and detected SPIB levels in 16 HGSOC and 6 normal uterine tube issues from our hospital. SPIB expression enhanced in OC samples. Silencing SPIB inhibited proliferation and induced apoptosis. SPIB also inhibits apoptosis in DLBCL (9). Overall, these results indicate that SPIB promoted OC cell proliferation by inhibiting apoptosis.

Previous studies have highlighted immune checkpoint blockade (ICB) therapy as a new therapy for OC (24). However, most patients do not benefit from ICB therapy (25). PD-L1

is a critical immune checkpoint molecule that conduces to the tumorigenesis (26). Increased PD-L1 expression in OC predicts poor survival and is associated with decreased cytotoxic T lymphocyte infiltration (27). Higher PD-L1 staining in HGSOC is associated with lower survival, indicating that PD-L1 is a biomarker of poor prognosis (28). Recent studies have attempted to maximize the curative effect of PD-1/PD-L1 for OC (29). Platelets enhance OC cell growth and downregulate antitumor immunity by increasing PD-L1 level (30). SETDB1 knockout increases the antitumor response of ICB with anti-PD-L1 in OC (31). In cisplatin-resistant OC cells, miR-34a-5p improves the sensitivity to cisplatin by targeting PD-L1 (32). SPIB binds to promoter regions and regulates gene expression, cell proliferation, and migration (33). SPIB has been found to enhance SNAP47 transcription and promote anoikis resistance in the lung

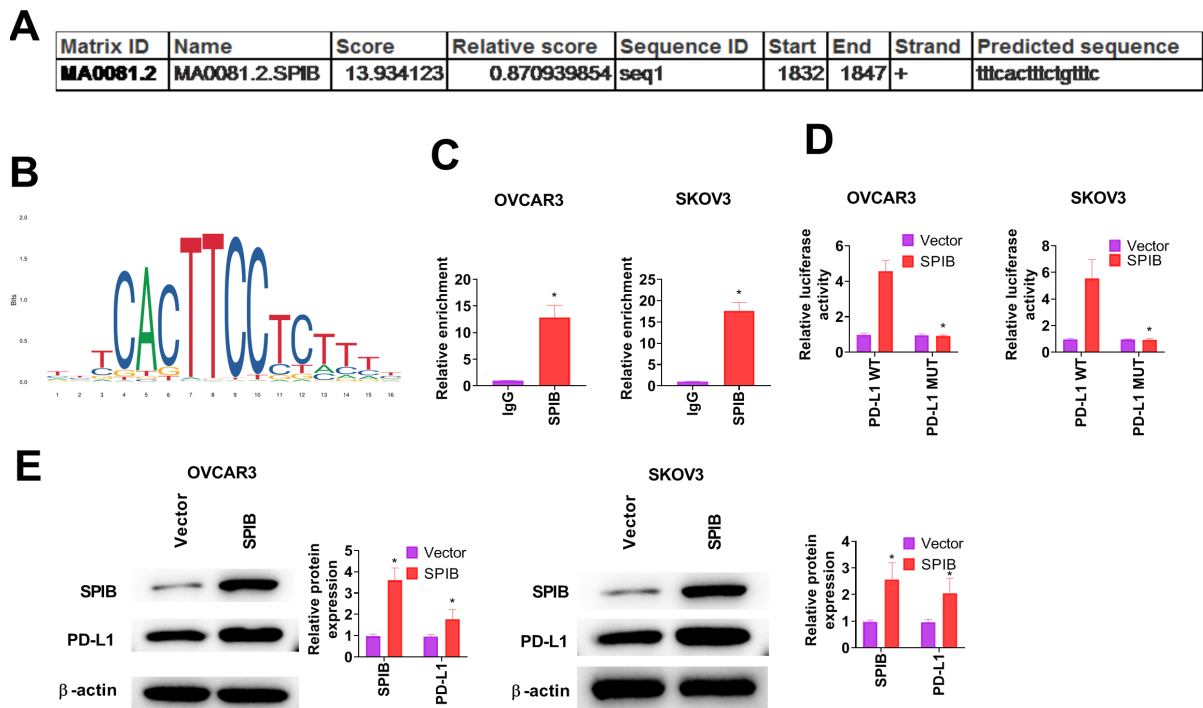


Fig. 5. SPIB directly promotes PD-L1 transcription in OC cells. (A) The data of JASPAR database indicating PD-L1 might be transcriptionally regulated by SPIB. (B) The JASPAR sequence logo of SPIB binding. (C) The bind between SPIB and PD-L1 promoter was verified by ChIP. (D) The bind between SPIB and PD-L1 promoter was verified by luciferase reporter assay. (E) Western blotting of SPIB and PD-L1 expression. Bands were quantified using ImageJ software. * $p < 0.05$ vs IgG, PD-L1 WT+SPIB, and vector group. spi-B transcription factor (SPIB), programmed cell death ligand-1 (PD-L1, also known as CD274), ovarian cancer (OC), chromatin immunoprecipitation (ChIP), wild-type (WT)

cancer (34). In our study, SPIB knockdown blunted PD-L1 expression in OC cells. ChIP and luciferase reporter assays verified this prediction from the JASPAR. Overall, SPIB regulated the immune escape of OC cells by influencing PD-L1 transcription.

MMPs assumed important responsibility in the OC metastases (35). MMPs regulate the proteolytic cleavage of PD-L1 (36). PD-L1 knockdown weakens migration and invasion of colorectal cancer cells via mitigating MMP1, MMP2, and MMP9 expression (37). MiR-124-3p reduces PD-L1 and MMP-9 expression and obstructs the progression of colorectal cancer cells via the STAT3 pathway (38). In advanced bladder cancer, a positive correlation exists between serum PD-L1 and MMP-7 levels (39). SB-3CT is an MMP2/MMP9 inhibitor that regulates tumor immunity by modulating PD-L1 (40). In the current study, SPIB deficiency decreased PD-L1, MMP2, and MMP9 expression.

PD-L1 is principally regulated by interferon- γ (IFN- γ), related to the JAK/STAT pathway (41). IFN- γ -stimulated PD-L1 level is regulated via JAK1, STAT1, and interferon regulatory factor-1 (IRF1) (42). Chen et al. have found that silencing DENR hampers JAK2 translation and reduces PD-L1 expression for the tumor immune evasion (43). Xu et al. have demonstrated that HDAC2 promotes PD-L1 expression by increasing JAK1, JAK2, and STAT1 phosphorylation, highlighting an approach to improve the antitumor immunity of triple-negative breast cancer (44). In OC, STAT1 deficiency reduces the PD-L1 expression (45). Suppressor of cytokine signaling 1 (SOCS-1), an endogenous inhibitor of the JAK/STAT pathway, suppresses PD-L1 level and induces T cell tumor-infiltrating (46). In this study, SPIB knockdown decreased JAK2 and STAT1 phosphorylation in OVCAR-3 and SKOV3 cells. The JAK2 activator, CA1, was used to confirm this possibility.

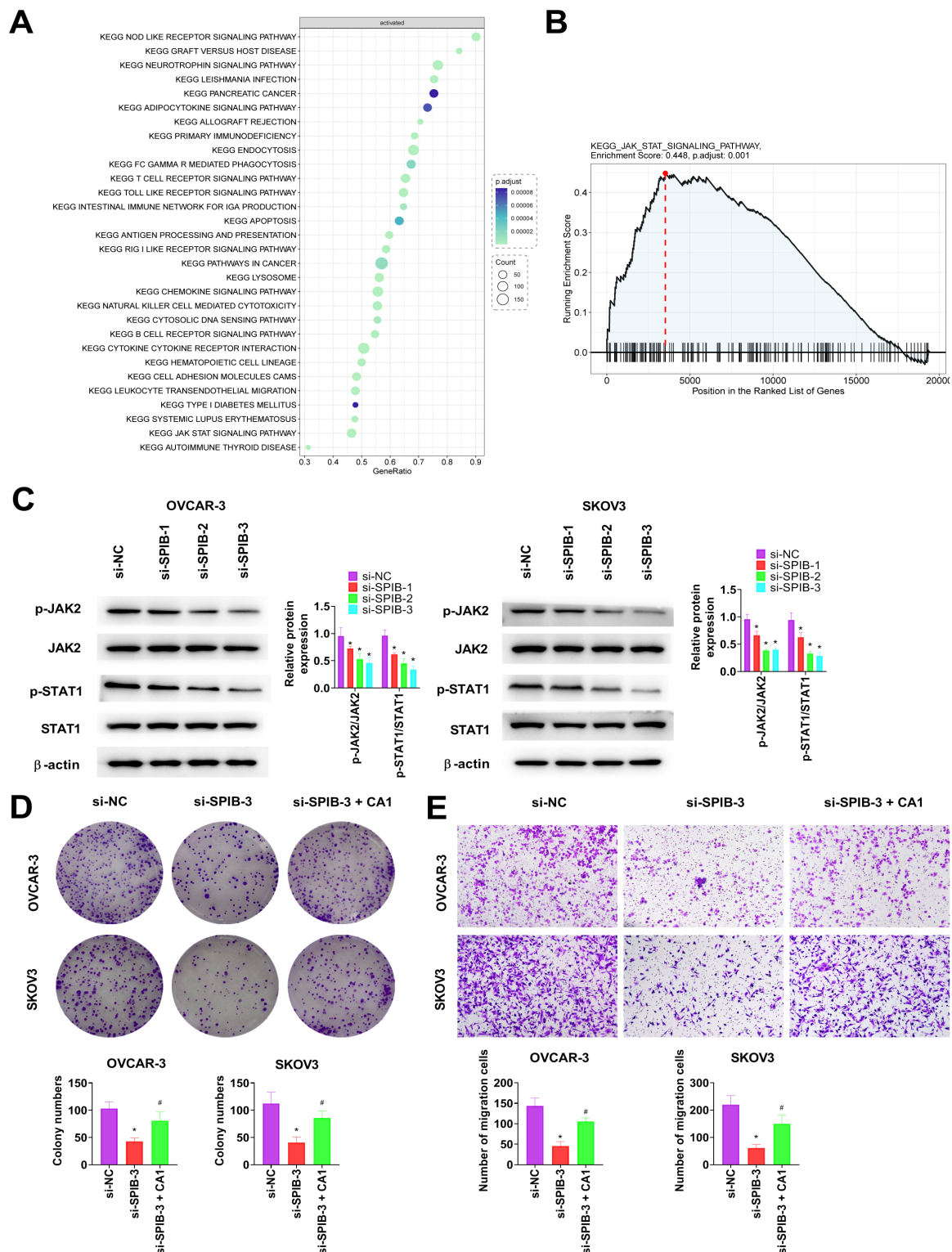


Fig. 6. SPIB knockdown inactivates the JAK/STAT pathway in OC cell. (A) Bubble plots show the top 30 significantly enriched pathways using KEGG-pathway enrichment analysis. (B) GSEA plots based on the TCGA data using KEGG gene sets. GSEA analysis showed that SPIB could activate the JAK/STAT pathway. (C) The levels of the JAK/STAT pathway related proteins. Bands were quantified using ImageJ software. (D) Cell proliferation was determined by colony formation assay. (E) Cell migration was detected by Transwell assay. * $p < 0.05$ vs si-NC group, # $p < 0.05$ vs si-SPIB-3 group. spi-B transcription factor (SPIB), Janus kinase-signal transducer and activator of transcription (JAK/STAT), ovarian cancer (OC), Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Set Enrichment Analysis (GSEA), coumermycin A1 (CA1)

CONCLUSION

In summary, SPIB knockdown inhibits the malignant progression of OC cells by regulating PD-L1 transcription and the JAK2/STAT1 pathway.

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Declared none.

AUTHORS' CONTRIBUTION

Wenfei Gu performed the experiments. Wenfei Gu, GuangTao Qi and Li Chen provided technical support and analyzed the data. Li Chen wrote the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018; 68:394-424
- Allemani C, Weir HK, Carreira H, Harewood R, Spika D, Wang XS et al. Global surveillance of cancer survival 1995-2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). *Lancet.* 2015; 385:977-1010
- Lisio MA, Fu L, Goyeneche A, Gao ZH, Telleria C High-Grade Serous Ovarian Cancer: Basic Sciences, Clinical and Therapeutic Standpoints. *International journal of molecular sciences.* 2019; 20
- Nero C, Vizzielli G, Lorusso D, Cesari E, Daniele G, Loverro M et al. Patient-derived organoids and high grade serous ovarian cancer: from disease modeling to personalized medicine. *Journal of experimental & clinical cancer research : CR.* 2021; 40:116
- Costa RM, Soto X, Chen Y, Zorn AM, Amaya E spib is required for primitive myeloid development in *Xenopus*. *Blood.* 2008; 112:2287-2296
- Ding M, Li Q, Tan X, Zhang L, Tan J, Zheng L Comprehensive pan-cancer analysis reveals the prognostic value and immunological role of SPIB. *Aging.* 2022; 14:6338-6357
- Ho YJ, Lin YM, Huang YC, Yeh KT, Lin LI, Lu JW Tissue microarray-based study of hepatocellular carcinoma validating SPIB as potential clinical prognostic marker. *Acta histochemica.* 2016; 118:38-45
- Wang J, Wang X, Guo Y, Ye L, Li D, Hu A et al. Therapeutic targeting of SPIB/SPI1-facilitated interplay of cancer cells and neutrophils inhibits aerobic glycolysis and cancer progression. *Clinical and translational medicine.* 2021; 11:e588
- Takagi Y, Shimada K, Shimada S, Sakamoto A, Naoe T, Nakamura S et al. SPIB is a novel prognostic factor in diffuse large B-cell lymphoma that mediates apoptosis via the PI3K-AKT pathway. *Cancer science.* 2016; 107:1270-1280
- Zhao X, Li L, Yuan S, Zhang Q, Jiang X, Luo T SPIB acts as a tumor suppressor by activating the NFkB and JNK signaling pathways through MAP4K1 in colorectal cancer cells. *Cell Signal.* 2021; 88:110148
- Montes-Moreno S, Ramos-Medina R, Martínez-López A, Barrionuevo Cornejo C, Parra Cubillos A, Quintana-Truyenque S et al. SPIB, a novel immunohistochemical marker for human blastic plasmacytoid dendritic cell neoplasms: characterization of its expression in major hematolymphoid neoplasms. *Blood.* 2013; 121:643-647
- Uppal S, Medlin E, Felder M, Kapur A, Sterner RC, Vazquez J et al. Identifying novel ovarian tumor biomarkers through mining of the transcriptome of circulating immune cells: A proof-of-concept study. *American journal of reproductive immunology (New York, NY : 1989).* 2021; 86:e13469
- Wang X, Teng F, Kong L, Yu J PD-L1 expression in human cancers and its association with clinical outcomes. *Onco Targets Ther.* 2016; 9:5023-5039
- Shi X, Yun L, Chaoqun Liu L, Ding H, Liang D, Geng F et al. PD-L1 and the Clinical Outcomes of Ovarian Cancer: Meta-Analysis and Bioinformatical Analysis. *Asian Pac J Cancer Prev.* 2022; 23:2285-2290
- Casey SC, Tong L, Li Y, Do R, Walz S, Fitzgerald KN et al. MYC regulates the antitumor immune response through CD47 and PD-L1. *Science.* 2016; 352:227-231
- Wu B, Song M, Dong Q, Xiang G, Li J, Ma X et al. UBR5 promotes tumor immune evasion through enhancing IFN-γ-induced PDL1 transcription in triple negative breast cancer. *Theranostics.* 2022; 12:5086-5102

17. Hays E, Bonavida B YY1 regulates cancer cell immune resistance by modulating PD-L1 expression. *Drug Resist Updat.* 2019; 43:10-28
18. Marzec M, Zhang Q, Goradia A, Raghunath PN, Liu X, Paessler M et al. Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-L1, B7-H1). *Proceedings of the National Academy of Sciences of the United States of America.* 2008; 105:20852-20857
19. Chen X, He H, Xiao Y, Hasim A, Yuan J, Ye M et al. CXCL10 Produced by HPV-Positive Cervical Cancer Cells Stimulates Exosomal PDL1 Expression by Fibroblasts via CXCR3 and JAK-STAT Pathways. *Frontiers in oncology.* 2021; 11
20. Nabeshima K, Inoue T, Shimao Y, Sameshima T Matrix metalloproteinases in tumor invasion: role for cell migration. *Pathol Int.* 2002; 52:255-264
21. James NE, Miller K, LaFranzo N, Lips E, Woodman M, Ou J et al. Immune Modeling Analysis Reveals Immunologic Signatures Associated With Improved Outcomes in High Grade Serous Ovarian Cancer. *Frontiers in oncology.* 2021; 11
22. Huang Q, Liu J, Wu S, Zhang X, Xiao Z, Liu Z et al. Spi-B Promotes the Recruitment of Tumor-Associated Macrophages via Enhancing CCL4 Expression in Lung Cancer. *Frontiers in oncology.* 2021; 11:659131
23. Jin J, Du X, Zhou L, Yao D, Zou Q SPI1-related protein inhibits cervical cancer cell progression and prevents macrophage cell migration. *The journal of obstetrics and gynaecology research.* 2022; 48:2419-2430
24. Cojocaru E, Parkinson CA, Brenton JD Personalising Treatment for High-Grade Serous Ovarian Carcinoma. *Clinical oncology (Royal College of Radiologists (Great Britain)).* 2018; 30:515-524
25. Peng H, He X, Wang Q Immune checkpoint blockades in gynecological cancers: A review of clinical trials. *Acta obstetrica et gynecologica Scandinavica.* 2022; 101:941-951
26. Dermani FK, Samadi P, Rahmani G, Kohlan AK, Najafi R PD-1/PD-L1 immune checkpoint: Potential target for cancer therapy. *Journal of cellular physiology.* 2019; 234:1313-1325
27. Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8⁺ T lymphocytes are prognostic factors of human ovarian cancer. *Proceedings of the National Academy of Sciences of the United States of America.* 2007; 104:3360-3365
28. Baş Y, Koç N, Helvacı K, Koçak C, Akdeniz R, Şahin HHK Clinical and pathological significance of programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) expression in high grade serous ovarian cancer. *Translational oncology.* 2021; 14:100994
29. Dumitru A, Dobrica EC, Croitoru A, Cretoiu SM, Gaspar BS Focus on PD-1/PD-L1 as a Therapeutic Target in Ovarian Cancer. *International journal of molecular sciences.* 2022; 23
30. Cho MS, Lee H, Gonzalez-Delgado R, Li D, Sasano T, Carlos-Alcalde W et al. Platelets Increase the Expression of PD-L1 in Ovarian Cancer. *Cancers.* 2022; 14
31. Lin J, Guo D, Liu H, Zhou W, Wang C, Müller I et al. The SETDB1-TRIM28 Complex Suppresses Antitumor Immunity. *Cancer immunology research.* 2021; 9:1413-1424
32. Zuo Y, Zheng W, Liu J, Tang Q, Wang SS, Yang XS MiR-34a-5p/PD-L1 axis regulates cisplatin chemoresistance of ovarian cancer cells. *Neoplasma.* 2020; 67:93-101
33. Wu F, Tian F, Qin C, Qin X, Zeng W, Liu X et al. Peroxiredoxin2 regulates trophoblast proliferation and migration through SPIB-HDAC2 pathway. *Experimental cell research.* 2023; 422:113428
34. Zhang H, Wang G, Zhou R, Li X, Sun Y, Li Y et al. SPIB promotes anoikis resistance via elevated autolysosomal process in lung cancer cells. *The FEBS journal.* 2020; 287:4696-4709
35. Carey P, Low E, Harper E, Stack MS Metalloproteinases in Ovarian Cancer. *International journal of molecular sciences.* 2021; 22
36. Hira-Miyazawa M, Nakamura H, Hirai M, Kobayashi Y, Kitahara H, Bou-Gharios G et al. Regulation of programmed-death ligand in the human head and neck squamous cell carcinoma microenvironment is mediated through matrix metalloproteinase-mediated proteolytic cleavage. *International journal of oncology.* 2018; 52:379-388
37. Chen YL, Wang GX, Lin BA, Huang JS MicroRNA-93-5p expression in tumor tissue and its tumor suppressor function via targeting programmed death ligand-1 in colorectal cancer. *Cell Biol Int.* 2020; 44:1224-1236
38. Roshani Asl E, Rasmi Y, Baradaran B MicroRNA-124-3p suppresses PD-L1 expression and inhibits tumorigenesis of colorectal cancer cells via modulating STAT3 signaling. *Journal of cellular physiology.* 2021; 236:7071-7087
39. Krafft U, Olah C, Reis H, Kesch C, Darr C, Grünwald V et al. High Serum PD-L1 Levels Are Associated with Poor Survival in Urothelial Cancer Patients Treated with Chemotherapy and Immune Checkpoint Inhibitor Therapy. *Cancers.* 2021; 13
40. Ye Y, Kuang X, Xie Z, Liang L, Zhang Z, Zhang Y et al. Small-molecule MMP2/MMP9 inhibitor

- SB-3CT modulates tumor immune surveillance by regulating PD-L1. *Genome medicine*. 2020; 12:83
41. Mimura K, Teh JL, Okayama H, Shiraishi K, Kua LF, Koh V et al. PD-L1 expression is mainly regulated by interferon gamma associated with JAK-STAT pathway in gastric cancer. *Cancer science*. 2018; 109:43-53
 42. Padmanabhan S, Gaire B, Zou Y, Uddin MM, Vancurova I IFN γ -induced PD-L1 expression in ovarian cancer cells is regulated by JAK1, STAT1 and IRF1 signaling. *Cell Signal*. 2022; 97:9
 43. Chen B, Hu J, Hu X, Chen H, Bao R, Zhou Y et al. DENR controls JAK2 translation to induce PD-L1 expression for tumor immune evasion. *Nat Commun*. 2022; 13:022-29754
 44. Xu P, Xiong W, Lin Y, Fan L, Pan H, Li Y Histone deacetylase 2 knockout suppresses immune escape of triple-negative breast cancer cells via downregulating PD-L1 expression. *Cell Death Dis*. 2021; 12:021-04047
 45. Liu F, Liu J, Zhang J, Shi J, Gui L, Xu G Expression of STAT1 is positively correlated with PD-L1 in human ovarian cancer. *Cancer Biol Ther*. 2020; 21:963-971
 46. Nakagawa S, Serada S, Kakubari R, Hiramatsu K, Sugase T, Matsuzaki S et al. Intratumoral Delivery of an Adenoviral Vector Carrying the SOCS-1 Gene Enhances T-Cell-Mediated Antitumor Immunity By Suppressing PD-L1. *Mol Cancer Ther*. 2018; 17:1941-1950