

SFRP2 Promotes Peritoneal Metastasis of Ovarian Cancer within Phosphorylation of GSK3 β

Tengfei Zhang

Shaoxing University Affiliated Hospital

Shimin Chen Qin

Shaoxing University

Jianrong Yuan

Shaoxing University

Jianlong Zhao

Shaoxing University

Yutong Wang

Jiangnan University

Jianqiang Jin (✉ 1281916576@qq.com)

Affiliated hospital of Jiangnan University

Xiaping Guo

Zhuji Hospital of Traditional Chinese Medicine

Research

Keywords: Ovarian cancer, Peritoneal metastasis, SFRP2, β -Catenin/Wnt, Poor prognosis, Biomarkers

Posted Date: January 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-141176/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

The aim was to study further the molecular mechanism of ovarian cancer peritoneal metastasis and the corresponding prognostic markers.

Materials

We analysed the sequencing data of 10 pairs of primary ovarian cancer and peritoneal metastasis from GSE98281 to determine SFRP2 through GO, KEGG and GSEA analysis. Western blot, invasion and migration experiments were used to detect the biological effects of SFRP2 on ovarian cancer cells. Immunohistochemistry was used to detect ovarian cancer in tissue samples from a TCGA-Ovarian Cancer cohort, and the survival analysis of SFRP2 was then performed.

Results

SFRP2 affected the phosphorylation level of GSK3 β and upregulated the expression level of β -catenin. The high expression of SFRP2 in ovarian cancer cells improves cell invasion and migration capabilities. In histology studies, high expression of SFRP2 has increased the positive expression of β -catenin in the nucleus, and patients with high expression of SFRP2 had a worse prognosis. Similar results also appeared in ovarian cancer cases with high expression of SFRP2 mRNA in the TCGA data set.

Conclusion

SFRP2 activates the β -Catenin/Wnt signalling pathway through phosphorylation of GSK3 β , which promotes the metastasis of ovarian cancer that leads to poor prognosis.

Introduction

Ovarian cancer is one of the most common female types of tumour, with high morbidity and mortality rates [1]. In the malignant progression of ovarian cancer, peritoneal metastasis of ovarian cancer cells is a common type of malignancy. Secreted frizzled related protein 2 (SFRP2) is an important protein involved in tissue repair and biosynthesis [2, 3]. Histologically, there are various types of ovarian cancer, among which ovarian epithelial tumors are the most common type, accounting for nearly 90% of all reported cases.

The Wnt/ β -catenin signalling pathway is related to the occurrence of various cancers [4]. When there are no Wnt ligands, no receptors or the receptors are blocked, the pathway is "closed". In previous studies, SFRP2 participates in the biological role of the Wnt signalling pathway and has an essential impact on tissue development, damage repair and tumorigenesis [5].

In this study, we conducted a bioinformatics analysis of ovarian cancer peritoneal metastasis by sequencing samples. We found that SFRP2 was highly expressed in peritoneal metastasis tissues. Moreover, through cytological experiments, we found that SFRP2 affects the expression of β -Catenin by phosphorylation of GSK3 β . Through histological samples and external cohort studies, it was shown that the relationship between the expression of SFRP2 and the metastasis of ovarian cancer correlated with the poor prognosis of patients with ovarian cancer.

Materials And Methods

Bioinformatics analysis

The high-throughput gene expression profiles of ovarian cancer primary tissues and peritoneal metastasis tissues were extracted from the GEO database (GSE98281) [6]. The DEGs between primary tissues and peritoneal metastasis tissues were analysed by R 3.4 (edgeR and LIMMA package). The cut-off criterion was limited to $|\logFC| \geq 1$ to be statistically significant. In order to characterise the functional roles of the DEGs, we used the ClusterProfiler package for Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), pathways analysis and Gene Set Enrichment Analysis (GSEA). Survival and RNA expression were analysed to see if there was a relationship between gene expression and the immune cell abundance from ovarian cancer survivors using TIMER (Tumor Immune Estimation Resource) [7]. The transcriptome data from The Cancer Genome Atlas (TCGA) P-value <0.05 were used and considered statistically significant to the survival of patients.

Cell culture

Human serous ovarian cancer cell line ES-2 and SKOV3 were purchased from American Type Culture Collection (ATCC). A2780 and OVCAR8 were purchased from the Basic Medical Research Center of Chinese Academy of Medical Sciences (Beijing, China). SKOV3 was cultured in McCoy's 5A medium with 10% fetal bovine serum (FBS), 100 IU/mL penicillin and 100 μ g/mL streptomycin in 5% CO₂ at 37 °C. The ES-2, A2780 were cultured in RPMI-1640 medium containing 10% FBS, 100 IU/mL penicillin and 100 μ g/mL streptomycin in 5% CO₂ at 37 °C. The OVCAR8 was cultured in DMEM medium with 10% FBS, 100 IU/mL penicillin and 100 μ g/mL streptomycin in 5% CO₂ at 37 °C. The culture media, FBS, penicillin and streptomycin were purchased from Clark Bioscience (Virginia, US).

Western blot

Ovarian cancer cells lysed in RIPA Lysis Reagent (Sangon) supplemented with phosphatase and protease inhibitors mixture (NCMbio). The protein samples were separated on a 6–15% SDS-polyacrylamide gel (Epizyme). After transfer to PVDF membrane, incubate primary antibody with anti-SFRP2 (1:2000; CST), anti- β -Catenin (1:1000; BBI Lifesciences), anti-pGSK3 β (1:1000; Cell Signaling Technology), anti-GAPDH (1:5 000; Beyotime). After the reaction overnight at 4 °C, secondary antibodies were incubated for 2 hours. Membranes were exposed using the ECL reagent (Beyotime) as the manufacturer's instructions.

Quantitative real time-qPCR

Total RNA was isolated following the manufacturer's instructions using RNA isolater Total RNA Extraction Reagent (Vazyme). cDNA was prepared using an HiScript II 1st Strand cDNA Synthesis Kit (Vazyme). The qPCR assay was performed with 7500 Fast Real-Time System using a PrimeScript™ RT reagent Kit, and SYBR Green Master Mix (Solarbio) was used to synthesise cDNA and quantify the expression of SFRP2. The primers of SFRP2 were: sense 5'-CAACGACATAATGGAAACGCTTTGT-3', antisense 5'-TCAGCTTGTAATGGTCTTGCTCTT-3'. The expression was normalized to the expression of GAPDH: sense 5'-TGCACCACCAACTGCTTAGC-3', and antisense 5'-GGCATGGACTGTGGTCATGAG-3'.

Small interference RNA (siRNA) and plasmid transfection

Transfect cells with Lipofectamine RNAiMAX at a working concentration of 50 nM siRNA (Invitrogen) according to the manufacturer's instructions using the following siRNAs: siControl (Invitrogen), siSFRP2 and siNC (Invitrogen). A plasmid of SFRP2 and a vector were purchased from Gemma Gene, using Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's instructions. Regular mycoplasma testing was also carried out to exclude any possible cell culture contamination.

Cell invasion and migration assays

A2780 cells were cultured in 6-well plates and treated with siSFRP2 for 24 hours. OVCAR8 cells were grown in 6-well plates and treated with plasmids for 12 hours. After stabilizing for 24 hours, digest the cell monolayer and prepare a cell concentration of 1×10^5 /ml in serum-free medium. Transfer the cell suspension to a Transwell chamber with a membrane pore size of $0.8 \mu\text{m}$ (Corning) and placed on a 24 well cell culture plate. A 10% fetal bovine serum solution was added as a chemo-attractant at the bottom of the insert, and the plate was incubated at 37°C for 24 h. Remove non-migrated cells by gently wiping inside each insert. The cells were fixed and stained with 4% crystal violet solution for 5 minutes. Before observation with a microscope, the insert was thoroughly washed with phosphate buffered saline and completely air-dried. The cells were counted at $\times 200$ magnification. This experiment was repeated 3 times, statistical analysis was performed with 4 fields of view, and the average of the number of cells passing through the chamber was taken as the number of migrating cells.

Specimen and immunohistochemistry (IHC)

We collected 115 FFPE specimens of ovarian cancer from June 2013 to March 2015 from the Zhuji Affiliated Hospital of Shaoxing University and the Affiliated Hospital of Jiangnan University. The Ethics Committee of the First People's Hospital of Shaoxing City and the Affiliated Hospital of Jiangnan University approved the collection. IHC was performed to detect SFRP2 in the cohort with SFRP2 antibody (Cell Signaling Technology). Antigen retrieval was performed in sodium citrate buffer (pH = 6.0) in a water bath at 100°C for 10 min. After sealing with 5% BSA solution for 1 hour, primary antibodies were incubated at 4°C overnight. And secondary antibody (Beyotime) was incubated at room temperature for 45 min, followed by visualisation with horseradish peroxidase substrate DAB (Beyotime) for 1 min at

room temperature. Finally, the stained sections were counterstained with hematoxylin. Two pathologists assessed and scored the degree of immunostaining according to the intensity of staining: 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). Strong and moderate stainings were determined as SFRP2-positive, while weak and no stainings were determined as negative [8].

Statistical analysis

Appropriate cut-off values for SFRP2 were obtained from the Receiver Operating Characteristic curve (ROC). Pearson's Chi-square tests and Fisher's exact tests were used to evaluate the association between SFRP2 expression and the clinicopathological parameters. Kaplan-Meier method is used to analyze the overall survival rate of patients. Survival data were analysed using log-rank tests. Univariate and multivariate survival analysis was performed using the Cox proportional hazard model and overall survival (OS) model. All statistical analyses were carried out using SPSS for Windows 22.0 software, P-value less than 0.05 were considered statistically significant.

Results

Identification of SFRP2 in ovarian cancer peritoneal metastasis

In order to study the biological function differences between primary ovarian cancer and peritoneal metastases, we analysed the high-throughput RNAseq data set GSE98281 of primary ovarian cancer and metastases of the greater omentum. In 10 omental metastatic tissues and 10 matched primary tumour tissues, we found that genes were upregulated and genes down-regulated in peritoneal metastases. In Figure 1A and 1B, we found that the SFRP2 gene was highly expressed in tissues that were metastasised to the greater omentum, and we performed a paired test analysis on the expression of SFRP2. In Figure 1C, the expression of SFRP2 in the metastatic tissues of the greater omentum was significantly higher than that of the primary tissue ($P=0.0166$). We then performed GO (Figure 1D) and KEGG (Figure 1E) analysis on the highly expressed genes in the tissues of the omentum metastasis. We found that the gene enrichment analysis of omentum transfer shows that the Wnt signalling pathway was significantly activated. Moreover, we conducted a GSEA (Gene Set Enrichment Analysis) analysis of the Wnt signalling pathway and found that omental metastasis was positively correlated with the Wnt signalling pathway (Figure 1F).

SFRP2 activates the wnt signalling pathway through phosphorylation of GSK3 β

Ovarian cancer cell lines were selected for the molecular experiments in order to study the mechanism of SFRP2 upregulating the Wnt signalling pathway. The expression level of SFRP2 was analysed in four typical ovarian cancer cell lines. Figure 2A shows that the OVCAR8 cell line expression level of SFRP2 was the lowest; and in A2780, it was the highest. In subsequent experiments, we chose OVCAR8 and A2780 cell lines for corresponding molecular biology experiments.

We constructed siRNA for A2780 to interfere with expression and transferred the SFRP2 vector to the OVCAR8 cell line with low baseline expression, to see if it increased. siNC and empty vectors were treated as controls, respectively. Based on the results of the previous KEGG analysis, we detected protein expression levels of GSK3 β , pGSK3 β and β -Catenin in ovarian cell lines. We found that after knocking down SFRP2 in A2780 when the expression level of GSK3 β was stable, the level of pGSK3 β increased, while the expression of β -Catenin protein decreased ($p < 0.05$). Conversely, after upregulating SFRP2 in OVCAR8, when the expression level of GSK3 β was stable, the level of pGSK3 β decreased, while the expression of β -Catenin protein increased ($p < 0.05$).

SFRP2 affects the invasion and migration of ovarian cancer cells

Based on the results of this study, we aim to verify that SFRP2 affects the metastasis of ovarian cancer through the β -Catenin/Wnt signalling pathway. We tested the invasion and migration ability of A2780 and OVCAR8 cells. In Figure 2C, the invasion ability of A2780-siSFRP2 was lower than that of A2780-siNC ($p < 0.01$). After OVCAR8 was transfected with SFRP2, the invasion ability was significantly enhanced ($p < 0.01$), Figure 2D. In terms of migration ability, the migration ability of A2780-siSFRP2 was lower than that of A2780-siNC ($p < 0.05$), as shown in Figure 2E. The migration ability of OVCAR8 transfected with SFRP2 was significantly enhanced ($p < 0.05$); this can be seen in Figure 2F.

Analysis of SFRP2 protein expression and survival in tumour tissues

We used immunohistochemistry to detect the expression of SFRP2 and β -Catenin protein in 115 cases of ovarian cancer tissues. As shown in Figure 3A, in the primary samples of ovarian cancer, SFRP2 was mainly expressed in the cytoplasm, and the intensity was low. β -Catenin was mainly expressed in the nucleus. In samples with metastatic ovarian cancer, SFRP2 was expressed more strongly in the cytoplasm, while β -Catenin was more strongly expressed in the nucleus than in the primary samples.

Through the follow-up of these 115 patients with ovarian cancer, we found that the prognosis of patients with high SFRP2 expression was worse than that of patients with low SFRP2 expression, in Figure 3B, log-rank P value = 0.0063.

TCGA database explores SFRP2 and prognostic analysis

In order to verify the results of our study with an external cohort, we used the ovarian cancer data set of TCGA-OV to analyse the survival of patients. We have obtained similar results. Patients with high SFRP2 expression have a worse prognosis than patients with low SFRP2 expression, log-rank P value = 0.019 (Figure 3C).

The relationship between SFRP2 and ovarian cancer metastasis also indicated a poor prognosis. We analysed the infiltration of SFRP2 and various immune cells in the microenvironment of ovarian cancer. We found that SFRP2 expression was negatively correlated with B cell infiltration ($P = 0.0117$, Figure 3D), suggesting that SFRP2 was related to immunosuppression. SFRP2 correlated positively with tumour-associated macrophages ($P = 0.220$, Figure 3E), and also with neutrophils ($P = 0.440$, Figure 3F).

Discussion

In previous studies, it was found that the expression of SFRP2 was related to the classic activation of the Wnt signaling pathway [9], and affects the formation of scar tissue. Some studies have reported that activated Wnt/ β -Catenin signalling can promote the metastasis of epithelial tumour cells [10], and is related to tumour growth, E-cadherin production and epithelial-mesenchymal transition in a variety of tumour developments [11, 12]. In addition, it was found that Wnt-related signals are dysregulated in gastrointestinal cancers. In particular, the Wnt/ β -Catenin pathway is highly activated in the occurrence and development of ovarian cancer, thereby regulating cell invasion, migration and proliferation [13, 14].

In this study, the expression of SFRP2 and β -Catenin in ovarian cancer was the focus along with the effects on metastasis in ovarian cancer. Our selected models were ovarian cancer cell lines ES-2, A2780, OVCAR8 and SKOV3. These models give a good representation of the tissue types involved in ovarian cancer. The expression of SFRP2 was assessed. Both A2780 and ES-2 have high expression levels. Considering the cell type of ES-2 and the strong mesenchymal characteristics [15], we confirm that the A2780 cell line can be used as a high SFRP2 expression model.

Among the cases of ovarian epithelial cell carcinoma, metastasis, peritoneal metastasis and omental metastasis are the main types. In our study, the transcriptome data set GSE98281 was selected through bioinformatics analysis. It was also confirmed that SFRP2 was highly expressed in cases of omental metastasis. Through our intervention on SFRP2, we found that SFRP2 has a significant increase in the invasion and migration ability of ovarian cancer cell lines, suggesting that it affects the metastasis ability of ovarian cancer cells [16]. We also found that the high expression of SFRP2 and β -Catenin in the nucleus were significantly enhanced in samples with ovarian cancer metastasis.

In this study, we found the effect of SFRP2 on the phosphorylation level of GSK3 β . However, we lack in-depth research on the pathway and the site of phosphorylation. We validated SFRP2 for GSK3 β , an important molecule in the Wnt/ β -Catenin signalling pathway. We confirmed that the high expression of SFRP2 could be down-regulated. The expression level of phosphorylated GSK3 β and the low expression of pGSK3 β suggest that the nuclear level of β -Catenin increases and may affect the malignant progression of ovarian cancer. We have also analysed the relationships between SFRP2, the poor prognosis of ovarian cancer and the infiltration of immune cells, including B cells, TAM and Neutrophils [17]. However, the mechanism of action between the immune microenvironment and SFRP2 is still unknown and is included in our future research plan.

Conclusions

In ovarian cancer, SFRP2 activates the β -Catenin/Wnt signalling pathway through phosphorylation of GSK3 β , which promotes the metastasis of ovarian cancer and leads to poor prognosis.

Declarations

Acknowledgements

The authors thank Zhigang Yin the Department of Pathology, Affiliated Hospital of Jiangnan University for their help in this study.

Authors' Contributions

X. Guo and J. Jin designed the research. T. Zhang performed the research. S. Chen and J. Yuan reviewed the surgical pathology. J. Zhao and Y. Wang analyzed the data. T. Zhang and J. Jin wrote the paper. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request. Ovarian cancer sequencing data is available at NCBI GEO database, GSE98281.

Funding

This research was not funded by scientific research projects.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Zhuji Affiliated Hospital of Shaoxing University and the Affiliated Hospital of Jiangnan University and was performed in accordance with the ethical standards of the Declaration of Helsinki and its later amendments.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Obstetrics and Gynecology, Zhuji Affiliated Hospital of Shaoxing University, Shaoxing, China. ²Department of Basic Medical Sciences, Jiangnan University Wuxi Medical College, Wuxi, China. ³Department of Pathology, Affiliated Hospital of Jiangnan University, Wuxi, China. ⁴Department of Gynecology, Traditional Chinese Medical Hospital of Zhuji, Shaoxing, China;

References

1. Domchek Susan M, Postel-Vinay Sophie, Im Seock-Ah. et al. Olaparib and durvalumab in patients with germline BRCA-mutated metastatic breast cancer (MEDIOLA): an open-label, multicentre, phase

- 1/2, basket study. [J]Lancet Oncol. 2020;21:1155–64.
2. Han Mingzhi,Wang Shuai,Fritah Sabrina. et al. Interfering with long non-coding RNA MIR22HG processing inhibits glioblastoma progression through suppression of Wnt/ β -catenin signalling. [J]Brain. 2020;143:512–30.
 3. Cheng Chi Keung,Li Libby,Cheng Suk Hang. et al. Secreted-frizzled related protein 1 is a transcriptional repression target of the t(8;21) fusion protein in acute myeloid leukemia. [J]Blood. 2011;118:6638–48.
 4. Schaefer Kristina N,Peifer Mark,Wnt/Beta-Catenin Signaling Regulation and a Role for Biomolecular Condensates.[J].Dev Cell, 2019, 48: 429–444.
 5. Veeck, Jürgen,Noetzel Erik,Bektas Nuran et al. Promoter hypermethylation of the SFRP2 gene is a high-frequent alteration and tumor-specific epigenetic marker in human breast cancer.[J].Mol Cancer, 2008, 7: 83.
 6. Sallinen H, Janhonen S,Pölonen P, et al. Comparative transcriptome analysis of matched primary and distant metastatic ovarian carcinoma.[J]BMC Cancer. 2019;19:1121.
 7. Song Danjun,Wang Yining,Zhu. Kai, et al. DCK is a promising prognostic biomarker and correlated with immune infiltrates in hepatocellular carcinoma.[J].World J Surg Oncol, 2020, 18: 176.
 8. Wang Fang,WuJ, Wang Y, et al. Gut Microbiota Functional Biomolecules With Immune-Lipid Metabolism for a Prognostic Compound Score in Epstein-Barr Virus-Associated Gastric Adenocarcinoma: A Pilot Study.[J].Clin Transl Gastroenterol, 2019, 10: e00074.
 9. Lin Mingzhuo,Liu Xinyue,Zheng Haoxiao. et al. IGF-1 enhances BMSC viability, migration, and anti-apoptosis in myocardial infarction via secreted frizzled-related protein 2 pathway.[J]Stem Cell Res Ther. 2020;11:22.
 10. Xi Ying,Kim Thomas,Brumwell. Alexis N, et al. Local lung hypoxia determines epithelial fate decisions during alveolar regeneration.[J]Nat Cell Biol, 2017, 19: 904–14.
 11. Kimura-Yoshida Chiharu,Mochida Kyoko,Nakaya Masa-Aki et al. Cytoplasmic localization of GRHL3 upon epidermal differentiation triggers cell shape change for epithelial morphogenesis.[J].Nat Commun, 2018, 9: 4059.
 12. So Juhoon,Khaliq Mehwish,Evason Kimberley. et al. Wnt/ β -catenin signaling controls intrahepatic biliary network formation in zebrafish by regulating notch activity.[J].Hepatology, 2018, 67: 2352–2366.
 13. Liu S,Liu Ying,Lu Qiang et al. TUG1The lncRNA promotes epithelial ovarian cancer cell proliferation and invasion via the WNT/ β -catenin pathway.[J].Onco Targets Ther, 2018, 11: 6845–6851.
 14. Cao Penglong,Zhao Shuai,Sun. Zhigang, et al. BRMS1L suppresses ovarian cancer metastasis via inhibition of the β -catenin-wnt pathway.[J]Exp Cell Res, 2018, 371: 214–21.
 15. Duran George E, Wang Yan C, Moisan, François, et al. Decreased levels of baseline and drug-induced tubulin polymerisation are hallmarks of resistance to taxanes in ovarian cancer cells and are associated with epithelial-to-mesenchymal transition.[J]. Br J Cancer. 2017;116:1318–28.

16. Li Peng,Zhao, Shu. Hu Yi,SFRP2 modulates non–small cell lung cancer A549 cell apoptosis and metastasis by regulating mitochondrial fission via Wnt pathways.[J]. Mol Med Rep. 2019;20:1925–32.
17. MacGregor Heather L, Sayad Azin,Elia Andrew. et al. High expression of B7-H3 on stromal cells defines tumor and stromal compartments in epithelial ovarian cancer and is associated with limited immune activation.[. J]J Immunother Cancer. 2019;7:357.

Figures

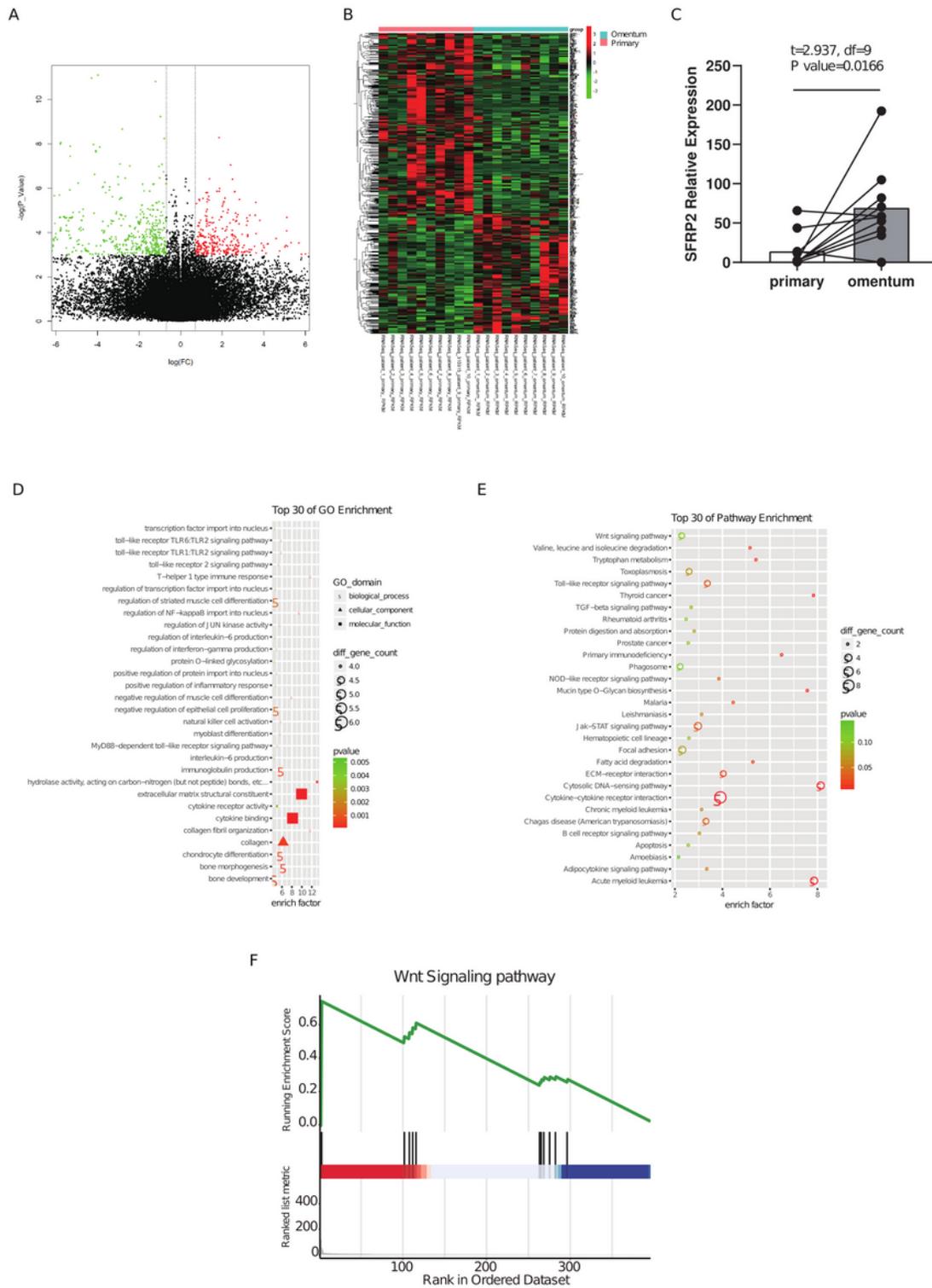


Figure 1

A, Volcano plot, In the GSE98281 data set, the difference between peritoneal metastasis and primary tumor of ovarian cancer was analyzed; B, Heat map analysis of differentially expressed genes between peritoneal metastasis and primary tumor; C, Difference analysis of SFRP2 expression between peritoneal metastasis and primary tumor, ($p < 0.05$); D, GO (Gene Ontology) analysis of differentially expressed genes between peritoneal metastasis and primary tumor; E, KEGG (Kyoto Encyclopedia of Genes and Genomes)

analysis of differentially expressed genes between peritoneal metastasis and primary tumor. F, GSEA analysis of Wnt signaling pathway between peritoneal metastasis and primary tumor gene expression.

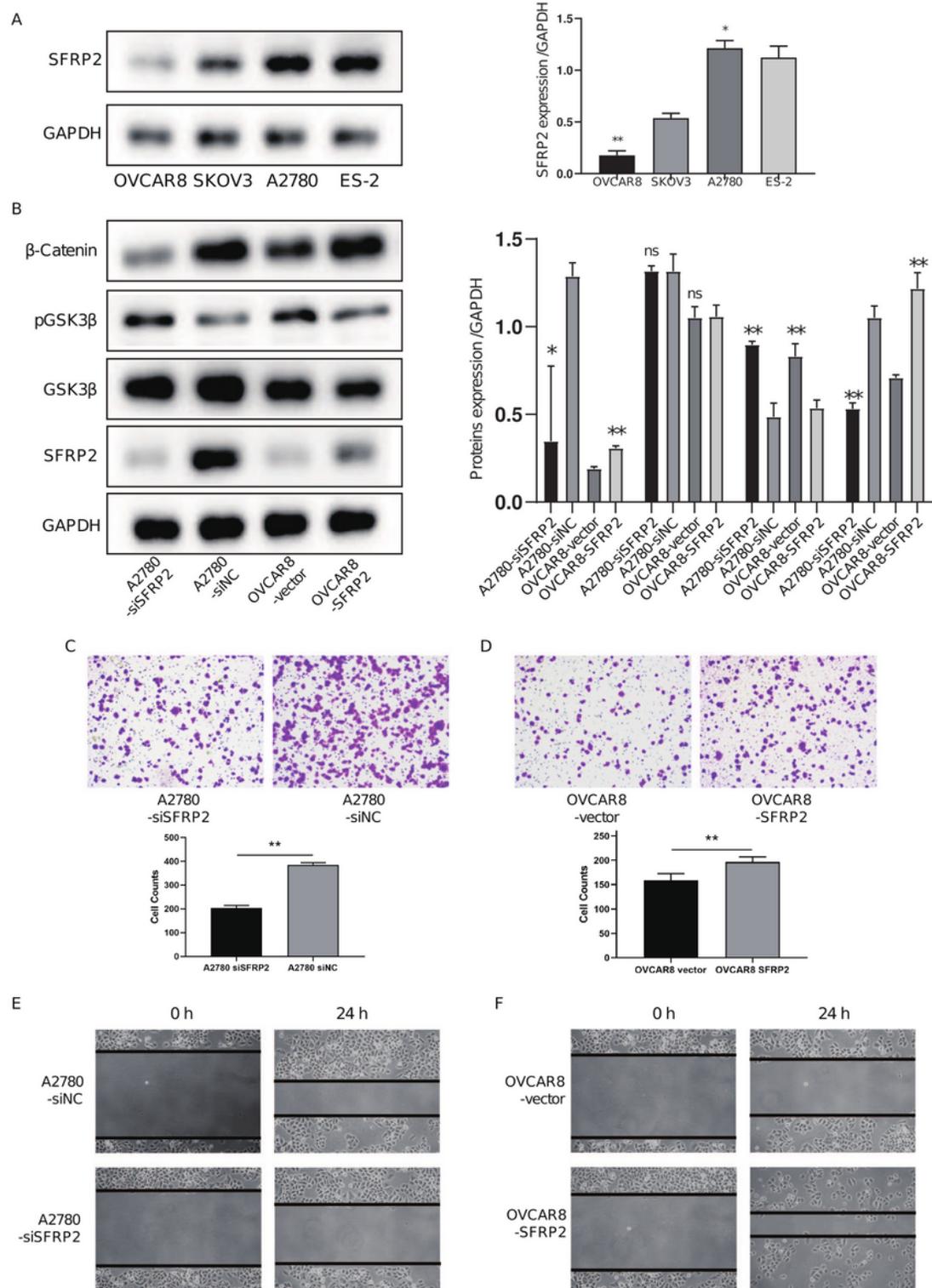


Figure 2

A, The expression of SFRP2 in A2780, ES-2, SKOV3 and OVCAR8 cell lines; B, The change of SFRP2 expression affects the expression of pGSK3 β and β -Catenin, ($p < 0.05$); C, Changes in invasion ability of A2780 cell line after knocking down SFRP2; D, Changes in invasion ability of OVCAR8 cell line after up-

regulating SFRP2; E, Changes in migration ability of A2780 cell line after knocking down SFRP2; F, Changes in migration ability of OVCAR8 cell line after up-regulating SFRP2;(p<0.05).

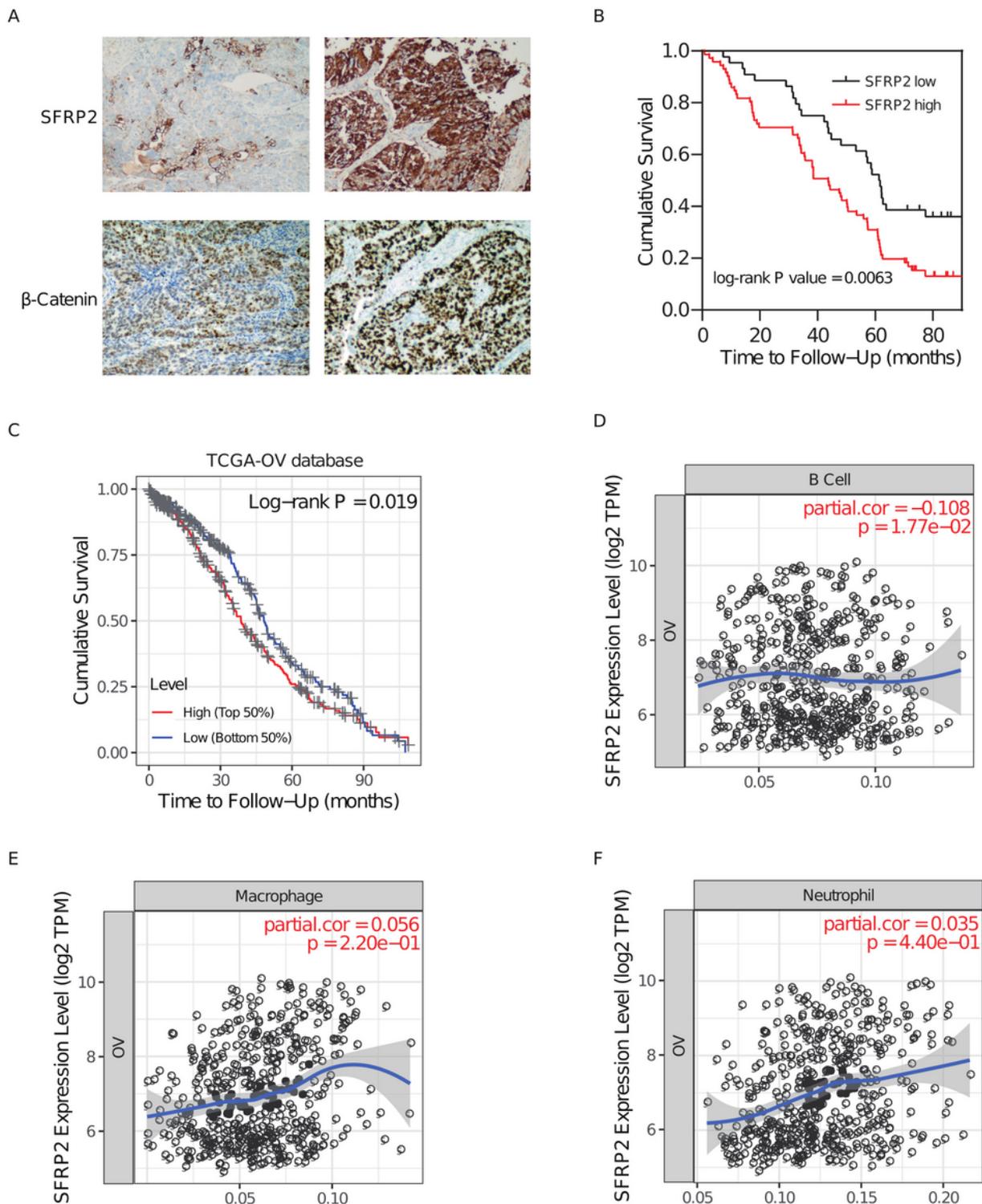


Figure 3

A, Immunohistochemistry (IHC) detection of SFRP2 and β -Catenin protein in samples of non-metastatic tumors and samples of metastatic primary tumors; B, Among 115 cases of ovarian cancer, patients with high SFRP2 expression (n=71) had significantly worse survival than patients with low SFRP2 expression

(n=44); C, In the TCGA data set of patients with ovarian cancer, patients with high SFRP2 expression (51-100%) have significantly worse survival than patients with low SFRP2 expression (0-50%); D, Analysis of the correlation between SFRP2 expression and B cells infiltration in the tumor microenvironment in patients with ovarian cancer in the TCGA data set; E, Analysis of the correlation between SFRP2 expression and Macrophages infiltration in the tumor microenvironment in patients with ovarian cancer in the TCGA data set; F, Analysis of the correlation between SFRP2 expression and Neutrophils infiltration in the tumor microenvironment in patients with ovarian cancer in the TCGA data set.