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Research Article

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SPP1 is a biomarker of cervical cancer prognosis and involved in immune infiltration

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Abstract

***Background**: Cervical cancer is the fourth commonly occurred cancer in women around the world. However, it still lacks effective approaches to improve current prognosis of cervical cancer and prevent metastasis. **Objective**: We aim to discover a promising biomarker for cervical cancer prognosis by utilizing bioinformatics analysis. **Methods**: Gene expression was analyzed by the datasets from The Cancer Genome Atlas Program-Cervical squamous cell carcinoma and endocervical adenocarcinoma (TCGA-CESC) dataset and three independent patient cohort datasets. Biological process and pathway enrichment were performed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis. Immune infiltration was analyzed through TISIDB tool. **Results**: SPP1 gene was highly expressed in cervical cancer tissues. In addition, SPP1 was positively correlated to advanced CESC stages and nodal metastasis status. SPP1 co-expressed genes are mainly enriched in immunological processes. Furthermore, SPP1 expression is involved in immune infiltration level, in which several tumour infiltrating lymphocytes are correlated with SPP1. SPP1 overexpression promotes a wide spectrum of chemokines and immunoinhibiors which contribute to CESC progression. **Conclusions**: SPP1 is a promising biomarker and a prognostic factor of CESC. Tumour infiltrating lymphocytes are also possibly regulated by SPP1. Our study suggests that investigation on SPP1 is a new direction for CESC therapy.*

***Keywords**: bioinformatics analysis, SPP1, tumour infiltrating lymphocytes, cervical cancer*

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Introduction

Cervical cancer is the fourth commonly occurring cancer in women around the world. There are more than 500,000 new cases and around 200,000 deaths each year (1). Human papillomavirus (HPV) causes invasive cervical cancer, in which the normal cervical epithelium is transformed into a preneoplastic cervical type (2). Recently, the standard radiotherapy and chemotherapy are applied to the treatment of cervical cancer patients. However, metastasis still occurs even when cervical cancer is treated by multiple therapies at early stage. Therefore, new therapeutical methods and screening approaches are

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urgently needed to lower the occurence rate of therapy failures in cervical cancer.

Currently, tumour infiltrating lymphocytes (TILs) are reported to relate the progression and prognosis of various cancers such as colorectal cancer, breast cancer, and endometrial cancer (3-5). Previous studies have identified that the development and prognosis of cervical cancer are affected by immune infiltrating cells (6, 7). Nevertheless, the detailed landscape of TILs associated with the specific gene expression profiles is still largely unknown in cervical cancer. Thus, investigation of potential biomarkers with TILs profile is possibly beneficial for generating a prognosis model of cervical cancer.

In this study, we investigated the *secreted phosphoprotein 1* (*SPP1*) gene as a prognosis biomarker in cervical cancer. According to previous research, *SPP1* codes secreted protein in different cells including osteoclasts and macrophages (8). In addition, *SPP1* is positively correlated with metastasis in numerous cancers such as colorectal cancer, lung cancer, ovarian cancer, and breast cancer (9-12). However, the function and expression profile of *SPP1* in cervical cancer is still elusive. Here we performed bioinformatics analysis on *SPP1* in cervical cancer patient data, which provides the evidence of *SPP1* as a biomarker in cervical cancer.

Materials and methods

Gene expression data acquisition and processing

The RNA expression profiles and clinical data of CESC patients were downloaded from TCGA database (https://portal.gdc.cancer.gov/) and UCSC Xena database (https://xenabrowser.net/datapages/). In TCGA-CESC datasets, 13 samples are included in the normal group and 305 samples were included in the tumour group. *SPP1* expression profile in human body was obtained from GEPIA database (http://gepia.cancer-pku.cn/index.html). In comparison of *SPP1* expression in cancer stages and nodal metastasis status, the charts were obtained from The University of Alabama at Birmingham Cancer (UALCAN, http://ualcan.path.uab.edu/index.html) data analysis Portal and TCGA-CESC dataset. Only groups with clinical information and normal control were kept for analysis. The exact number of cases in each stage is indicated in Figure 2A and 2B.

The gene expression profiles GSE67522, GSE63514, and GSE9750 in CESC were obtained and downloaded from GEO database (https://www.ncbi.nlm.nih.gov/geo/). GSE67522 comprised 11 groups of non-malignant tissues and 31 groups of cervical cancer from GPL10558 Illumina Human HT-12 V4.0 expression beadchip. GSE63514 comprised 24 groups of normal tissues and 104 groups of cervical cancer from GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. GSE9750 comprised 24 groups of normal tissues and 33 groups of cervical cancer from GPL96 [HG-U133A] Affymetrix Human Genome U133A Array. The differentially expressed genes were screened with the criteria of $|\log2(FC)| > 1$ and $q < 0.05$. The Wilcoxon test was used to assess the significance of differential SPP1 expression in CESC.

Linked Omics database

The correlated genes of *SPP1* in CESC were analysed and obtained from the Linked Omics database (http://www.linkedomics.org/login.php). The expression of genes was selected from the dataset of RNAseq performed on HiSeq RNA platform by University of North Carolina (UNC) from the database. Gene correlations were calculated and evaluated by Pearson correlation coefficient heatmaps. For gene function analysis, the modules of Gene Ontology (GO) of biological process and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis were applied.

Immune infiltration analysis

The Spearman correlation analysis of *SPP1* expression and tumour infiltrating lymphocytes was performed by using the dataset of mRNA-seq data from TCGA-CESC. The correlation map was output by applying the R software package ggstatsplot. We used Spearman's correlation analysis to describe the correlation between quantitative variables without a normal distribution. A p-value of less than 0.05 was considered statistically significant.

TISIDB database and TIMER database

The association of *SPP1* expression for lymphocytes, immunoinhibitors and chemokines was analysed through using the TISIDB database (http://cis.hku.hk/TISIDB). High-throughput screened immune-tumor related genes are stored and updated. The heatmap was generated via searching *SPP1* in TISIDB database and selecting related modules of lymphocytes, immunomodulators, and chemokines. The results obtained from TISIDB database were confirmed in TIMER2.0 database as described previously (13).

Results

SPP1 is highly expressed in CESC

SPP1 expression was firstly identified in TCGA-CESC dataset. In the CESC tumour group, *SPP1* was confirmed highly expressed compared to the normal group (Figure 1A). The body interactive map also showed *SPP1* was enriched in cervical tissues with cancer (Figure 1B). Next, three independent patient cohorts (GSE67522, GSE63514 and GSE9750) were analysed. Indeed, *SPP1* was observed having higher expression levels in these cervical cancer patients (Figure 1C). Above results indicate that *SPP1* is highly expressed in CESC and potentially promotes CESC progression.

SPP1 is correlated with clinical and pathological parameters in CESC

Since the function of *SPP1* is unclear in CESC progression, here we performed correlation analysis of *SPP1* with clinical and pathological parameters. By using UALCAN database, results revealed that *SPP1* expression was positively correlated with the advanced CESC stages (Figure 2A). The expression of *SPP1* in stage 1-4 is significantly higher than in normal samples ($p < 0.05$). In addition, higher *SPP1* expression was observed in the advanced nodal metastasis, which suggests *SPP1* is possibly involved in CESC metastasis regulation (Figure 2B). By analysing TCGA-CESC datasets, high *SPP1* expression indicated a poor survival probability in CESC (Figure 2C). Furthermore, the higher *SPP1* expression was specifically correlated with the advanced CESC TNM stages (Figure 2D).

Characterization of SPP1 co-expression genes in CESC

To clarify the function of *SPP1* in CESC, we applied LinkedOmics portal to check the co-expressed genes of *SPP1*. For both positively and negatively correlated genes, the top 50 genes were presented in the heatmaps in Figure 3A. GO analysis showed that the *SPP1* co-expressed genes were mainly enriched in immunological processes, such as granulocyte activation, neutrophil mediated immunity, interleukin-1 production, phagocytosis, macrophage activation and interleukin 2 activation (Figure 3B, left). KEGG pathway analysis indicated that the co-expressed genes were involved in immunology pathways such as Toll-like receptor signaling pathway, chemokine signaling pathway, and complement and coagulation cascades. Of note, several inflammatory diseases including rheumatoid arthritis and inflammatory bowel disease (Figure 3B, right) were also linked to the enriched *SPP1* correlated genes. Furthermore,

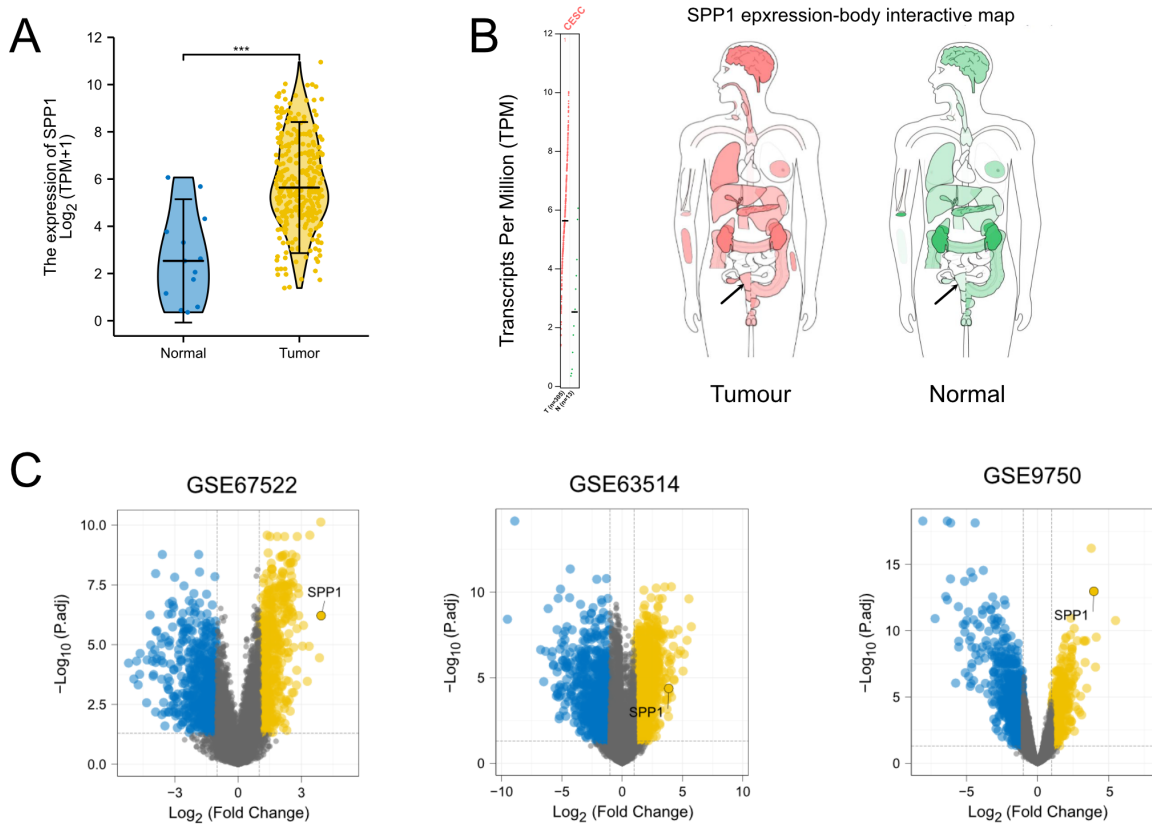


Fig. 1. *SPP1* expression in CESC. (A) *SPP1* expression was verified in TCGA-CESC dataset. (B) The median expression of CESC tumor and normal samples in the body map. Tumor was indicated in red and normal sample was indicated in green. (C) Validation of *SPP1* expression in three independent CESC patient cohorts namely GSE67522, GSE63514, and GSE9750. Highly expressed genes were marked with yellow and down-regulated genes were marked with blue. The position of *SPP1* was indicated.

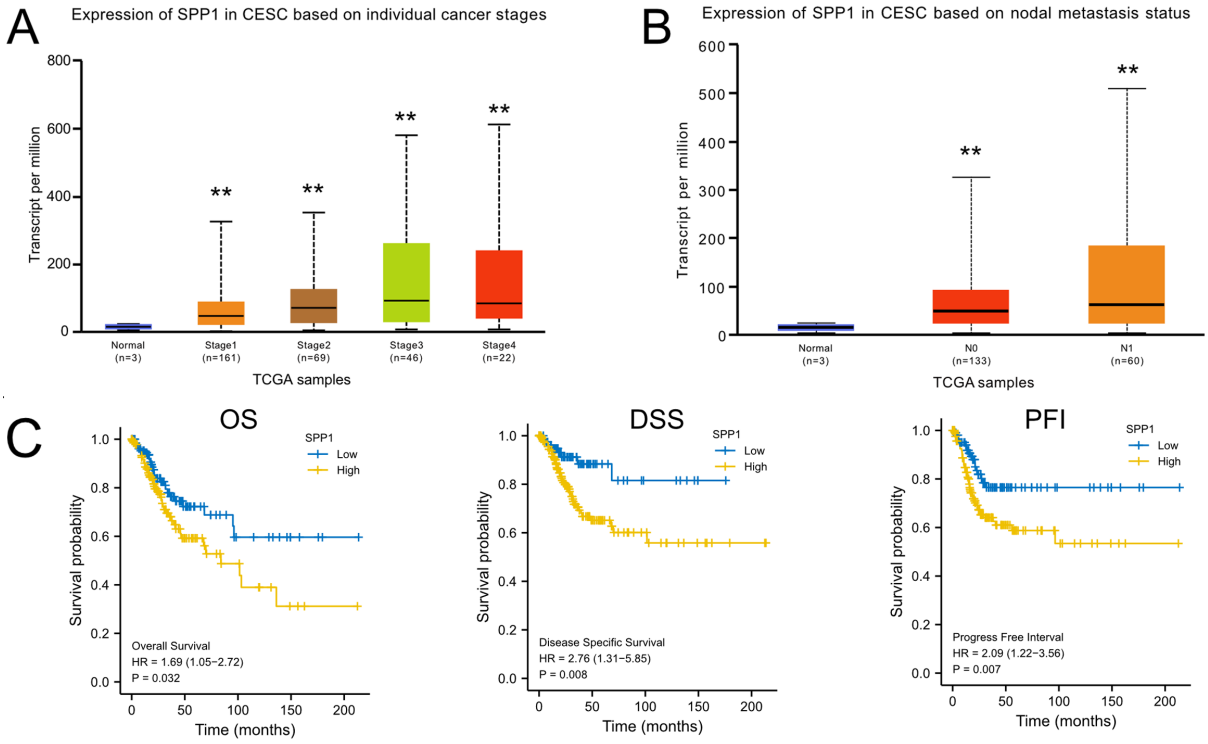
GSEA analysis revealed that the *SPP1* positively correlated genes were enriched in lymphocyte activation, cytokine production, and lymphocyte mediated immunity (Figure 3C).

SPP1 is associated with immune infiltration level

Next, we performed analysis on immune infiltration though TISIDB and TIMER databases. Results showed the *SPP1* was correlated with tumour infiltrating lymphocytes (TILs). The abundances of macrophage, immature dendritic cells, neutrophils, T reg cells, NK CD56 (dim) cells were positively correlated with *SPP1* expression. However, NK CD56 (bright) cells, plasmacytoid dendritic cells, mast cells, CD8 T cells, and B cells were negatively correlated with *SPP1* expression (Figure 4A and 4B). We also performed analysis on six specific TILs abundance. High *SPP1* expression indicated fewer B cells, CD4+ T cells and CD8+ T cells in CESC, whereas neutrophils, macrophages and myeloid dendritic cells were positively correlated with *SPP1* expression (Figure 4C).

***SPP1* is associated with immune molecules**

Since *SPP1* expression had been identified correlated with TILs, here we utilized TISIDB da-



D

Characteristics	Total(N)	HR(95% CI) Univariate analysis	P value
T stage	243		
T1	140		
T2	72	1.140 (0.557−2.333)	0.72
T3&T4	31	4.019 (2.072−7.797)	<0.001
N stage	193		
N0	133		
N1	60	2.844 (1.446−5.593)	0.002
M stage	127		
M0	116		
M1	11	3.555 (1.187−10.641)	0.023
Clinical stage	298		
Stage I	161		
Stage II	69	0.813 (0.413−1.600)	0.548
Stage III	46	1.390 (0.707−2.734)	0.34
Stage IV	22	4.376 (2.354−8.137)	<0.001
SPP1	306		
Low	153		
High	153	1.686 (1.046−2.719)	0.032

Fig. 2. The correlation between expression of *SPP1* and clinical/ pathological parameters in CESC. (A) The expression profile of *SPP1* in individual CESC stages. Stage 1 to stage 4 were compared to normal samples. **p < 0.05. (B) The expression of *SPP1* in different nodal metastasis status. (C) The overall survival, disease specific survival and progress free interval survival of CESC with high *SPP1* expression. (D) The clinical relations of CESC TMN stages, clinical stages, and *SPP1* expressions presented by forest plot.

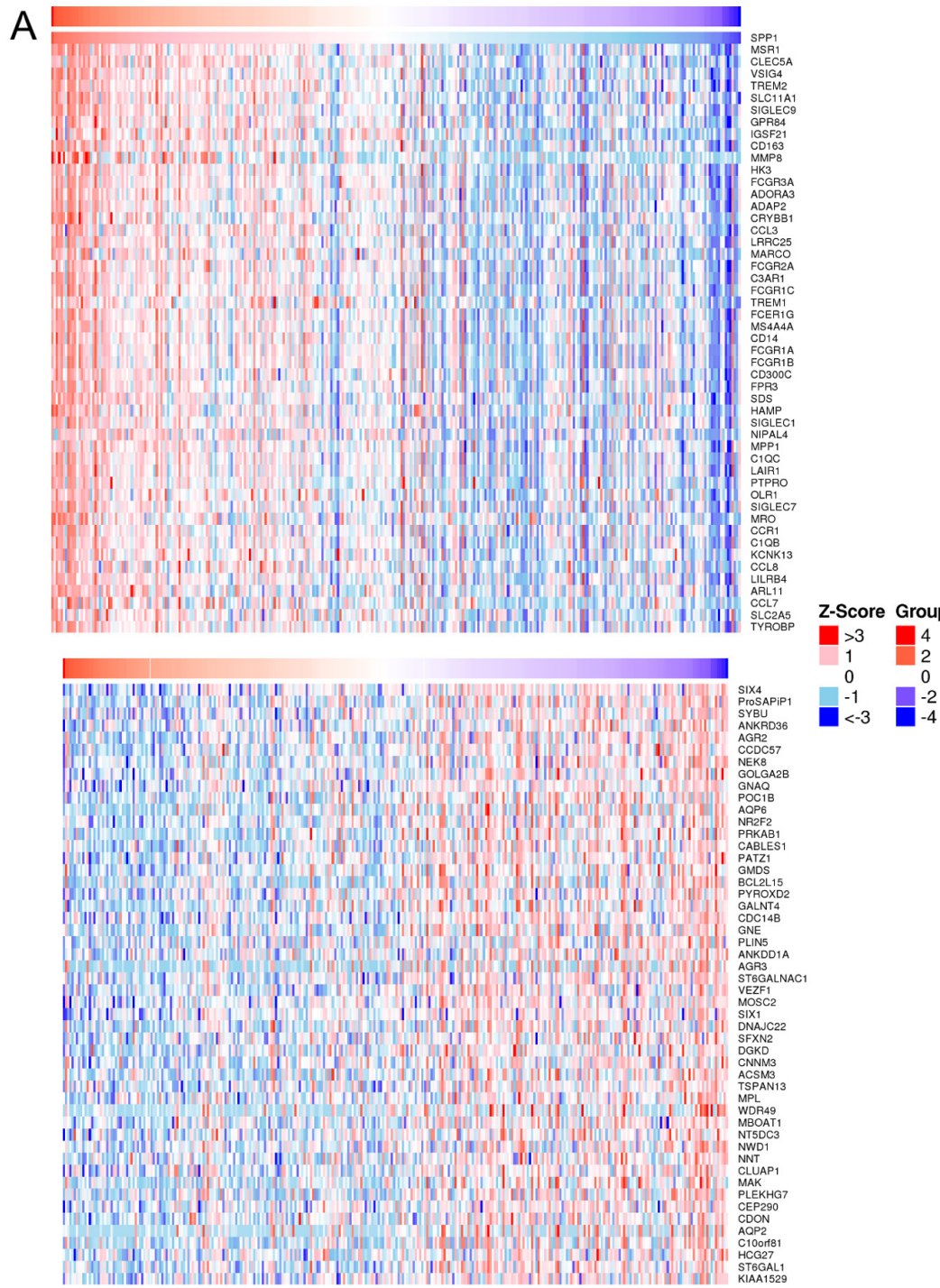


Fig. 3A. Characterization of *SPP1* co-expression genes in CESC. Heat map of co-expression genes in CESC, including top 50 positively (upper panel) and 50 negatively (lower panel) correlated genes respectively.

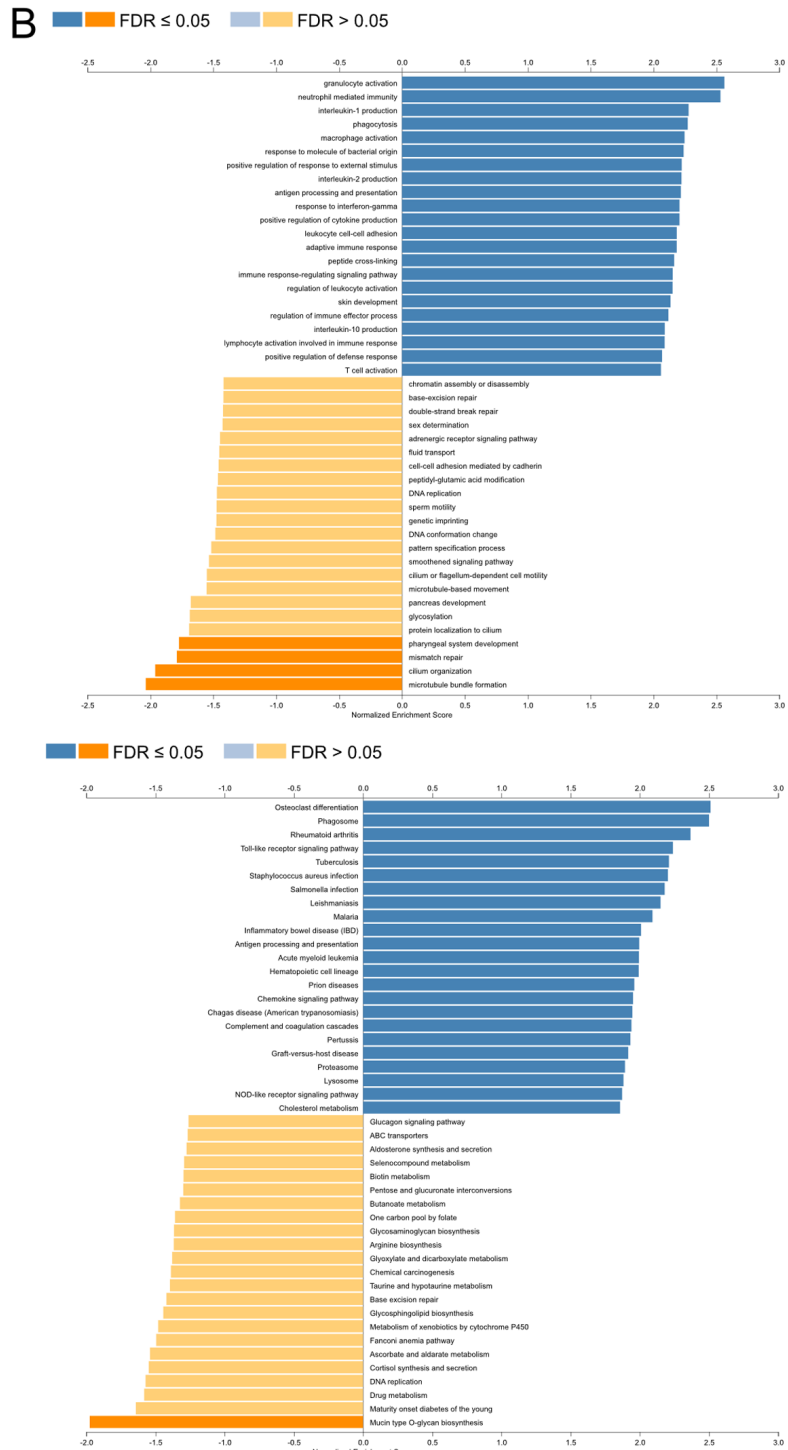


Fig. 3B. Characterization of *SPP1* co-expression genes in CESC. GO analysis (upper panel) and KEGG pathway analysis (lower panel) on co-expression genes of *SPP1*.

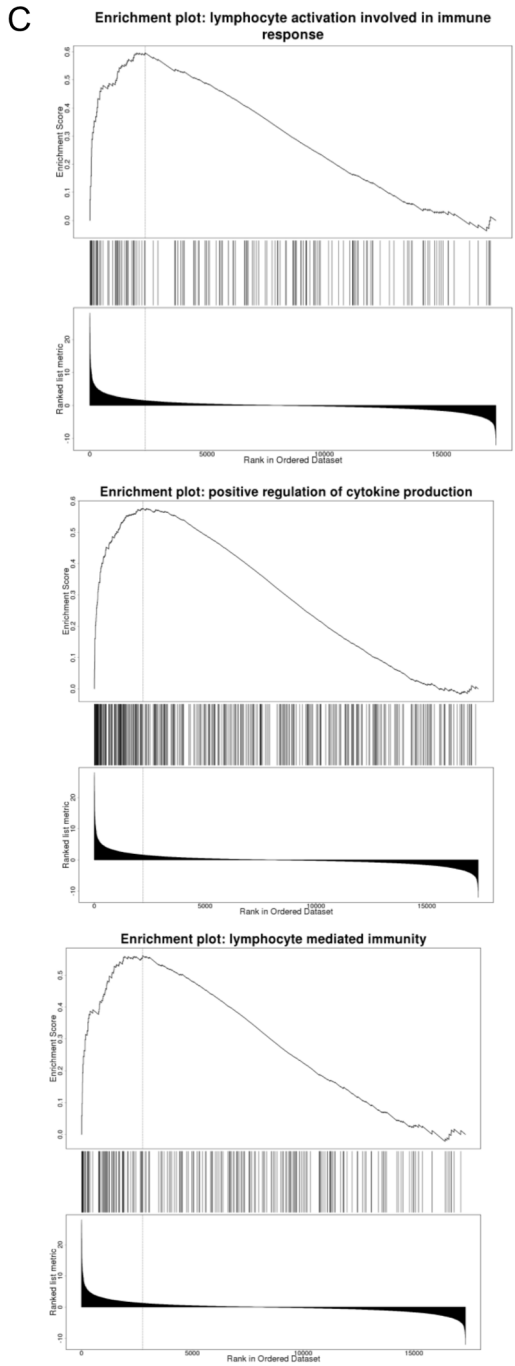


Fig. 3C. Characterization of *SPP1* co-expression genes in CESC. GSEA enrichment analysis on three specific biological processes of immunity regulation were presented. From top to bottom: lymphocyte activation, cytokine production, lymphocyte mediated immunity.

tabase to characterize the association of *SPP1* and the immune molecules including chemokines and immunoinhibitors in pan-cancer. Most chemokines were positively correlated with *SPP1* expression. In CESC, *CCL2*, *CCL3*, *CCL4*, *CCL7*, *CCL8*, *CCL11*, *CCL13*, *CXCL9*, *CXCL10*, *CXCL11*, *CXCL13*, and *CXCL14* were positively correlated with *SPP1* expression (Figure 5A). Similarly, most immunoinhibitors were increased together with high *SPP1* expression in different cancer types. In CESC, *CSF1R*, *LAG3*, *TGFB1*, and *TFGBR1* were positively correlated with high *SPP1* expression (Figure 5B). Therefore, *SPP1* overexpression promotes a wide spectrum of chemokines and immunoinhibiors which contribute to CESC progression.

Discussion

In this study, we performed multiple bioinformatics analysis approaches on *SPP1* gene in cervical cancer. Based on the clinical data obtained from TCGA-CESC dataset, *SPP1* was confirmed highly expressed in cervical cancer patients. In addition, the high expression of *SPP1* was validated in three independent cervical cancer patient cohorts. The above results suggest that *SPP1* is positively correlated with cervical cancer and it is supposed to be involved in CESC progression. In line with our results, previous studies also identified that *SPP1* is highly expressed and promotes metastasis in other cancers including breast cancer, lung cancer, and colorectal cancer (9, 10, 12). Therefore, *SPP1* is an oncogene in different cancers. Furthermore, high expression of *SPP1* indicates advanced tumour stages and nodal metastasis status, suggesting that *SPP1* is a promising biomarker of CESC progression and metastasis.

By performing analysis on correlated gene expression and GO/KEGG pathway, the positively correlated genes of *SPP1* were enriched in several immunity related pathways and biological processes, including neutrophil mediated immunity process, macrophage activation, interleukin

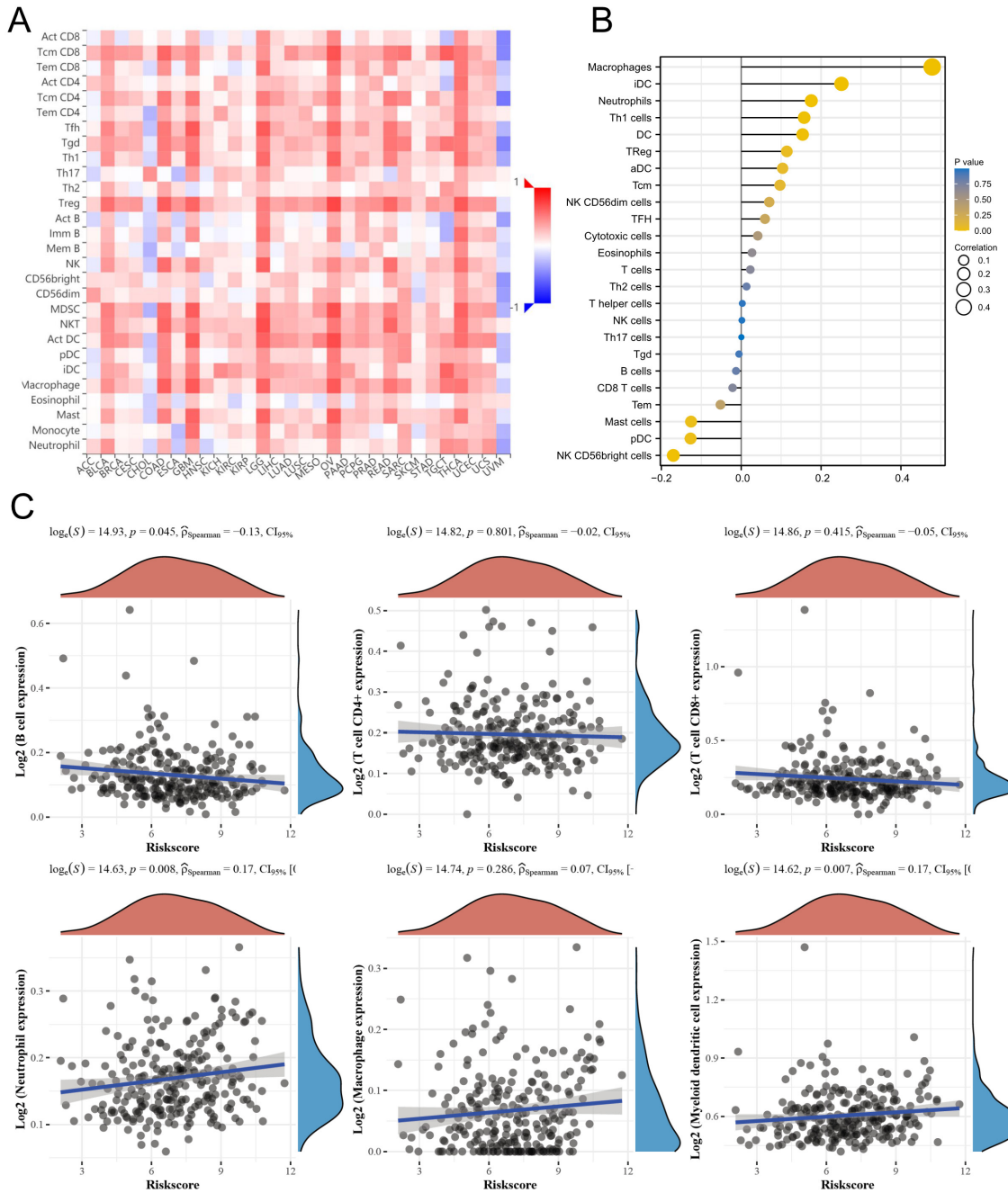


Fig. 4. The association between *SPP1* expression and immune infiltration level. (A) Heatmap of association between *SPP1* expression level and different lymphocytes. (B) The Pearson correlation analysis on *SPP1* expression and different lymphocytes in CESC. (C) The Spearman correlation analysis on *SPP1* expression and 6 typical lymphocytes in CESCE: B cell, CD4+ T cell, CD8+ T cell, neutrophil, macrophage, and myeloid dendritic cell. The horizontal axis in the figure represents the expression distribution of the gene, and the ordinate is the expression distribution of the score.

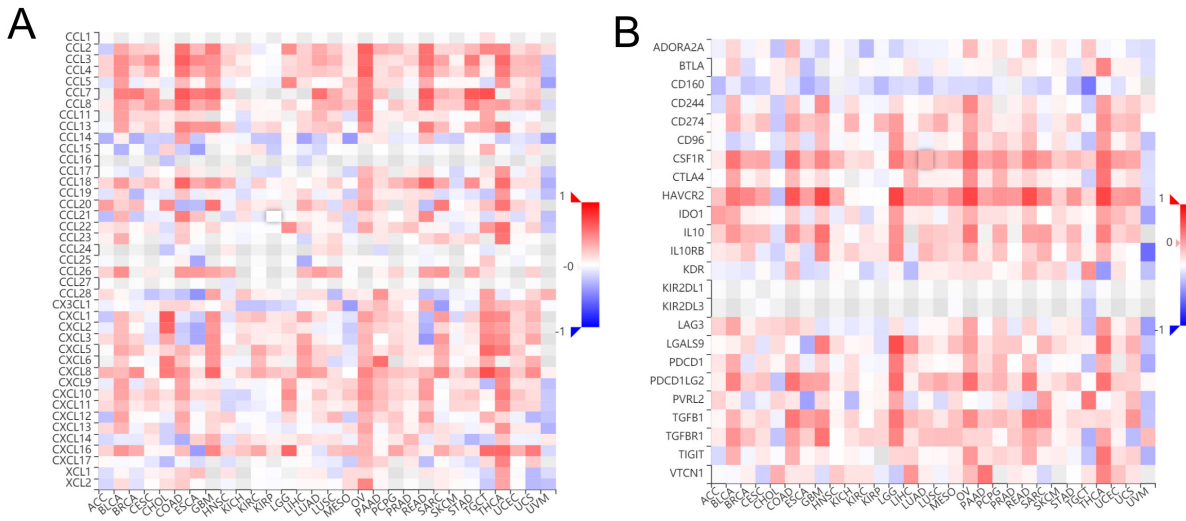


Fig. 5. The association between *SPP1* expression and immune molecules. (A) Heatmap of association between *SPP1* expression level and different chemokines including CCLs and CXCLs. (B) Heatmap of association between *SPP1* expression level and different immunoinhibitors.

production, antigen processing and presentation, interferon response, and adaptive immune response. Hence, it is supposed that *SPP1* is critical and involved in immunity and TILs regulations in CESC. Interestingly, *SPP1* protein is secreted by various cells including macrophage (8). Therefore, it is likely that *SPP1* stimulates lymphocytes and can itself be regulated by TILs. Immune cells, stromal cells, and extracellular matrix form a complex tumour microenvironment (14) which is crucial for tumour progression. The microenvironment influences clinical treatment strategies in cancer. The abundance of T cells and B cells is beneficial for tumour supression by restoring the immunity against tumor cells (15, 16). M2 macrophage was identified promoting tumour progression (17). Tumor infiltrating dendritic cells tend to drive tumour progression through elevating the immunosuppression and tolerance (18). Infiltrating neutrophils play pro-tumoral functions by N2-subtype polarization (19). Here, high *SPP1* indicated less CD8+ T cell and B cell tumor infiltration, whereas more infiltrated neutrophils, macrophages, and myeloid dendritic cells were correlated with high *SPP1* expression. The above results demonstrate that *SPP1* promotes CESC through stimulating TILs, which provides a possibility of personalized therapeutic strategy based on *SPP1* expression and TILs.

Furthermore, a variety of chemokines are positively correlated with high *SPP*1 expression in pan-cancer analysis. Both CCL family and CXC chemokines are associated with tumor metastasis and progression (20). Previous studies characterized specific CCLs and CXCs serve as biomarkers or prognostic factors for tumours. Therefore, *SPP1* is a potential regulator on chemokine secretion, which results in promoted CESC progression. Since the study was performed based on the bioinformatics analysis according to the data of patient cohorts, the validation of our findings are warranted in further experiments.

In conclusion, *SPP1* is a promising biomarker and prognostic factor of CESC. Tumor infiltrated lymphocytes are also possibly regulated by *SPP1*. Our study suggests that investigation on *SPP1* is a new direction for CESC therapy.

Authors' contributions

QG and PZ designed the study. QG, WH and DN performed bioinformatics analysis in TCGA datasets and GEO datasets. WL performed GO analysis and KEGG analysis. QG performed bioinformatics analysis of immune infiltration. QG and PZ wrote the paper. All authors read and approved the final manuscript.

Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

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