SPP1 Might Be a Novel Prognostic Biomarker for Patients With Malignancy: a Meta-analysis and Sequential Verification Based on Bioinformatic Analysis

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Research article

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Abstract

Background: Several studies have investigated the relationship between secreted phosphoprotein 1 (SPP1) expression level and prognosis of various tumors, but the results are far from conclusive. Therefore, we performed the present meta-analysis to investigate the prognostic value of SPP1 in pan-cancer. Furthermore, a followed confirmation based on The Cancer Genome Atlas (TCGA) database was also performed to verify our results.

Methods: We performed a systematic search from PubMed, Embase, Web of Science, and Cochrane Library databases and 19 articles, including 3403 patients and 9 types of tumors, were pooled in our meta-analysis. Overall survival (OS) and disease-free survival (DFS), which correlated with SPP1 expression, were considered as the primary outcome. Subgroup analyses, sensitivity analysis, and publication bias were used to investigate heterogeneity and reliability of the results. Furthermore, we also explored the relationship between SPP1 expression and clinical parameters of tumor patients. Finally, the results were verified with TCGA database and we further explored the relationship between SPP1 expression and tumor immuno-microenvironment (TIME), DNA methylation, and enriched gene pathway.

Results: Our meta-analysis showed that high-expressed SPP1 was significantly related to poor OS and DFS in various cancers, especially in liver hepatocellular carcinoma (LIHC). Furthermore, we also identified that the high expression level of SPP1 was significantly correlated with tumor grade. The expression level of SPP1 in the majority of tumor types were much higher than the corresponding normal tissues analyzed from databases. Besides, we also observed that high-expressed SPP1 was related to poor OS and DFS in LIHC, which supported the conclusion of meta-analysis. In addition, high-expressed SPP1 is related to 6 immune cells in TIME and DNA methylation regulatory genes. Ultimately, the results of Gene Set Enrichment Analysis (GSEA) suggested that tumor-related gene sets, such as hypoxia and lipid metabolism, were significantly enriched in high-expressed SPP1 group.

Conclusions: SPP1 is high-expressed in various tumor tissues and correlated with poor prognosis. SPP1 might promote cancer invasion and metastasis by affecting tumor grade, TIME, DNA methylation, hypoxia, and lipid metabolism. SPP1 is expected to become a new clinical indicator for tumor detection and prognosis, and provide a new idea for tumor targeted therapy.

Background

Nowadays, the incidence and mortality of tumor are increasing year by year, which has become a major public health problem all over the world. According to incomplete statistics, about 18 million people were newly diagnosed with cancer and about 9 million people died from cancer each year around the world [1]. In recent decades, although significant advances have been made in diagnosis, treatment, and precise management of oncological patients, their prognosis remains bleak. On the one hand, the majority of patients were diagnosed at an advanced stage. On the other hand, early diagnosis of cancer is lack of more accurate markers. Therefore, it is pivotal to find biomarkers that can early diagnose and evaluate the prognosis of cancer.

Secreted phosphoprotein 1 (SPP1), also known as osteopontin (OPN), is encoded by the *SPP1* gene located on the long arm of chromosome 4 region 22 (4q22.1) of human [2]. SPP1 is mainly secreted by osteoblasts, vascular smooth muscle cells, endothelial and epithelial cells and it makes SPP1 widely detectable in body fluids such as blood and bile [3]. In normal tissues, SPP1 expression is found in the bone matrix, gallbladder, bile and pancreatic ducts, gastrointestinal (GI) tract, respiratory, urinary and reproductive tracts [4, 5]. SPP1 is primarily located in the extracellular matrix and involved in many physiologic processes, including bone matrix remodeling, biomineralization, immune regulation, and anti-apoptosis [6–8].

Recently, a growing number of studies have elucidated that SPP1 is related to cancer invasion, metastasis, and poor prognosis. SPP1 promotes the growth of tumor cells by regulating cell-matrix interaction and cellular signaling through binding with CD44 receptors and integrin [9]. SPP1 can also promote tumorigenesis and metastasis by stimulating angiogenesis and inhibiting tumor cell apoptosis [10, 11]. Numerous studies also suggested that SPP1 expression level in tumor tissues is increased compared with normal tissues and it is associated with poor prognosis of some cancers, such as lung cancer, glioblastoma, hepatocellular carcinoma, gastric cancer, colorectal cancer, and so on [12–16]. Hence, we conducted the present meta-analysis to explore the relationship between the expression level of SPP1 and the prognosis of pan-cancer. In addition, we further verified the results of meta-analysis through The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov) database.

Methods

Search strategy

Our meta-analysis was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [17]. We performed an overall literature retrieval for PubMed, Embase, Web of Science, and Cochrane Library database published up to October 1, 2020, using both MeSH and free terms searching for Title/Abstract. We used the following terms for literature selection: ("cancer" OR "tumor" OR "neoplasm" OR "carcinoma") AND ("SPP1" OR "Secreted phosphoprotein 1") AND ("prognosis" OR "prognostic" OR "outcome"). Besides, reference lists and other relevant studies were reviewed to find more potential articles. The search was conducted independently by two investigators (AM Jiang and N Liu).

Inclusion and exclusion criteria

The selection process of eligible articles was done by two investigators (QQ Ding and FM Zhao). Inclusion criteria were as follows: (1) the object of the study is human and the type of cancer is the solid tumor; (2) the study involved the correlation between the expression level of SPP1 and survival data of tumor patients; (3) the study provided the relevant clinicopathological parameters. Literature that satisfied the following criteria was excluded: (1) conference, reviews, patents, case reports, or meta-analysis without original data; (2) articles that were not described in the English language; (3) studies that were not based on human; (4) overlapping or duplicate data; (5) insufficient Hazard ratios (HRs) or other data.

Data extraction and quality assessment

Two investigators (HR Zheng and QQ Ding) extracted the data independently, using a standardized method. In the study selection and data extraction phases, any disagreement was resolved by discussing with the third investigator (AM Jiang). The following data information was retrieved from each article: (1) the first author's name; (2) year of publication; (3) country; (4) number of patients; (5) tumor type; (6) detection method of SPP1; (7) cut-off criteria; (8) data of overall survival (OS) or disease-free survival (DFS); (9) antibody type of immunohistochemistry (IHC); (10) clinical parameters. The Engauge Digitizer 4.1 software was used to extract data from the Kaplan-Meier (K-M) plot, when there was no HRs and its 95% confidence inters (CIs) offered directly [18]. Newcastle Ottawa Quality Assessment Scale (NOS) was used to assess the quality of selected studies in our research [19].

Data collection and analysis from TCGA database

TCGA database includes more than twenty thousand tumor samples from 33 types of tumor and their corresponding normal samples. The RNA sequencing data of SPP1 gene expression for tumor and adjacent non-carcinoma tissues and their clinicopathological parameters were extracted from TCGA database. Because of normal samples in TCGA database are too little, we integrated the data of normal tissues in the Genotype-Tissue Expression (GTEx, https://gtexportal.org) database to analyze the difference of SPP1 expression level between tumor and normal tissues. All patients were divided into two groups according to the median value of SPP1 expression level. Subsequently, the K-M survival curves and log-rank tests were exploited to compare the survival difference between these two groups. DNA methylation is associated with tumorigenesis and cancer metastasis. As DNMT1, DNMT2, DNMT3A, and DNMT3B are the major enzymes of DNA methylation [20], we further analyzed the relationship between their expression levels and those of SPP1 in tumor tissues from TCGA database.

Relationship between SPP1 and tumor immuno-microenvironment

We explored the correlation between SPP1 expression level and tumor immuno-microenvironment (TIME) by the Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer) database. TIMER is a database designed to analyze immune cell infiltration in pan-cancer. The database used statistical methods confirmed by pathological examination to estimate the infiltration of neutrophils, macrophages, dendritic cells, B cells, and $CD4^+$/$CD8^+$ T cells in tumor tissues [21]. Using the TIMER database, we examined the associations between SPP1 expression level and 6 immune cells infiltration.

The ESTIMATE algorithm in the estimate package of R software was used to estimate the ratio of the immune-stromal component in TIME, which was shown in the form of three kinds of scores: ImmuneScore, StromalScore and ESTIMATEScore. It's related to the ratio of immune, stromal, and the sum of both, which means the higher the respective score, the larger the ratio of the corresponding component in TIME [22]. We analysed the correlation between SPP1 expression and the three kinds of scores. Spearman's test was used for correlation analysis.

Gene Set Enrichment Analysis

We downloaded the Hallmark dataset from the MolecularSignatures (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp) database as the target set for Gene Set Enrichment Analysis (GSEA) [23]. The whole transcriptome of all tumor samples was used for GSEA and only gene sets with NOM $p < 0.05$ and FDR $q < 0.25$ were considered as significant.

Statistical analysis

The prognostic value of SPP1 on OS and DFS was calculated by pooled HRs with 95% CIs. Odds ratios (ORs) with 95% CIs were used to evaluate the relationship between SPP1 expression and clinicopathological features. Heterogeneity was assessed using Cochran's Q test and the I^2 statistic test. When the heterogeneity was statistically significant ($P < 0.05$ and $I^2 > 50\%$), a random-effects would be adopted; otherwise, a fix-effect model would be performed. Subgroup analyses were conducted to explore the sources of heterogeneity. We also performed a sensitivity analysis to evaluate the quality and stability of results by omitting one study in each turn. Begg's test and Egger's test were used to assess the publication bias. All analyses were achieved using Stata V.14.0 (Stata Corporation, College Station, TX) and R V3.6.0 software (R Foundation, Vienna, Austria). $P < 0.05$ was considered statistically significant in all statistical methods.

Results

Study characteristics and quality assessment

In total, 753 studies were identified and 378 duplicates were excluded. After excluding irrelevant researches by reading titles and abstracts, 78 articles were eligible in our study. Of these, 59 studies don't have HRs or other data. Finally, 19 eligible studies were included in our meta-analysis [24–42]. The detailed flow chart of the study selection process was presented in Fig. 1. These eligible studies contained 3403 patients, involved 9 types of cancers, including the breast cancer (BRCA) (n = 5), intrahepatic cholangiocarcinoma (n = 3), non-small cell lung cancer (NSCLC) (n = 2), soft tissue cancer (n = 1), oesophageal squamous cell carcinoma (n = 1), renal clear cell carcinoma (n = 2), hepatocellular carcinoma (n = 3), colorectal cancer (n = 1), and nasopharyngeal carcinoma (n = 1). The characteristics of the eligible studies were listed in Table 1. NOS scores for all studies were more than 5 points. **Table S1** showed the results of the quality assessment.

Table 1
Characteristics of the studies in this meta-analysis.

Author	Year	Country	Number of patients	Gender (male/female)	Age	Tumor type	Method	Cut-off	Outcome	Analysis	Antib
Kim YW [28]	1998	Korea	253	0/253	21–77	Breast cancer	IHC	IHC score > 0	OS	K-M Curve	M
Tuck AB [39]	1998	Canada	154	0/154	26–83	Breast cancer	IHC	IHC score > 4	DFS, OS	K-M Curve	M
Rudland PS [34]	2002	U.K.	333	0/333	31–89	Breast cancer	IHC	IHC ≥ 5%	OS	Multivariate	M + P
Terashi T [38]	2004	Japan	73	43/30	33–79	Intrahepatic cholangiocarcinoma	IHC	IHC score > 0	OS	K-M Curve	M
Boldrini L [24]	2005	Italy	136	126/10	45–80	Non-small cell lung cancer	IHC	IHC ≥ 20%	DFS, OS	K-M Curve	P
Bramwell VH [25]	2005	Canada	33	20/13	18–90	Soft tissue cancer	IHC	IHC score ≥ 3	OS	Multivariate	M + P
Kita Y [29]	2006	Japan	175	160/15	36–83	Oesophageal squamous cell carcinoma	IHC	IHC ≥ 10%	OS	Multivariate	M
Matusan K [31]	2006	Croatia	171	103/68	NA	Renal clear cell carcinoma	IHC	IHC ≥ 5%	OS	Multivariate	M
Rudland PS [34]	2006	U.K.	312	0/312	17–89	Breast cancer	IHC	IHC ≥ 1%	OS	K-M Curve	P
Chen RX [26]	2010	China	151	125/26	26–74	Hepatocellular carcinoma	IHC	IHC ≥ 20%	DFS, OS	Multivariate	M
Sulpice L [37]	2013	France	40	NA	NA	Intrahepatic cholangiocarcinoma	IHC	IHC score ≥ 1	DFS, OS	Multivariate	NA
Seo KJ [36]	2015	Korea	174	103/71	30–86	Colorectal cancer	IHC	IHC ≥ 10%	DFS, OS	K-M Curve	M
Dong Q [27]	2016	China	374	306/68	NA	Hepatocellular carcinoma	IHC	IHC ≥ Median	DFS, OS	Multivariate	M
Rabjerg M [33]	2017	Denmark	97	54/43	NA	Renal clear cell carcinoma	IHC	IHC ≥ 10%	DFS, PFS, OS	Multivariate	NA
Li S [30]	2018	China	149	72/77	NA	Non-small cell lung cancer	IHC	NA	OS	Multivariate	NA
Qin H [32]	2018	China	68	36/32	NA	Nasopharyngeal carcinoma	IHC	IHC score ≥ 6	OS	Multivariate	NA
Walaszek K [40]	2018	USA	434	0/434	NA	Breast cancer	IHC	IHC score > 1	DFS	Multivariate	P
Zheng Y [41]	2018	China	180	108/72	NA	Intrahepatic cholangiocarcinoma	IHC	IHC ≥ Median	DFS, OS	Multivariate	M
Zhu Y [42]	2018	China	96	84/12	NA	Hepatocellular carcinoma	IHC	IHC score > 3.5	DFS, OS	Multivariate	M

Abbreviations: NA: not available; IHC: immunohistochemistry; OS: overall survival; DFS: disease-free survival; PFS: progression-free survival; M: monoclonal a polyclonal antibody; M + P: monoclonal antibody and polyclonal antibody; NOS: Newcastle Ottawa Quality Assessment Scale.

Meta-analysis of SPP1 expression levels on OS and DFS

A total of 18 studies, including 2969 patients, were recruited to evaluate the expression level of SPP1 on OS. The pooled HR and 95%CI showed that high expression of SPP1 was significantly correlated with poor OS in tumor patients (HR = 1.85, 95%CI = 1.50–2.27, $P < 0.001$) with significant heterogeneity across these studies by using the random-effect model ($I^2 = 59.7\%$, $P = 0.001$) (Fig. 2A). Additionally, 9 articles, including 1836 patients, were recruited to evaluate the expression level of SPP1 on DFS. The pooled HR and 95%CI showed that high-expressed SPP1 was significantly correlated with poor DFS in tumor patients (HR = 1.60, 95%CI = 1.18–2.18, $P = 0.002$) with significant between-study heterogeneity, also by using the random-effect model ($I^2 = 57.6\%$, $P = 0.016$) (Fig. 2B).

Subgroup analysis of OS and DFS

Subgroup analysis of OS and DFS were performed to find the source of heterogeneity (Table 2). Patients were classified based on tumor type, analysis, antibody type, region, sample size, and NOS score. Soft tissue cancer, renal clear cell carcinoma and nasopharyngeal carcinoma were defined as “other cancers” subgroup.

Table 2
Subgroup analysis of pooled HR for OS and DFS.

	OS					DFS				
	Test of association			Test of heterogeneity		Test of association			Test of heterogeneity	
Variables	No. of studies	Pooled-HR (95% CI)	P-value	I^2	P-value	No. of studies	Pooled-HR (95% CI)	P-value	I^2	P-value
Total	18	1.85 (1.50–2.27)	< 0.001	59.7%	0.001	9	1.60 (1.18–2.28)	0.002	57.6%	0.016
Tumor type										
NSCLC	2	1.60 (1.07–2.40)	0.022	0.0%	0.398	1	1.46 (0.79–2.69)	0.225		
Hepatobiliary cancers [a]	6	1.81 (1.38–2.37)	< 0.001	30.6%	0.206	4	2.07 (1.36–3.16)	0.001	59.6%	0.060
BRCA	4	3.40 (1.08–10.68)	0.036	85.7%	< 0.001	2	0.96 (0.72–1.28)	0.798	0.0%	0.589
GI tract cancers [b]	2	1.24 (0.83–1.83)	0.291	0.0%	0.783	1	1.24 (0.47–3.26)	0.663		
Others [c]	4	1.68 (1.30–2.16)	< 0.001	31.5%	0.223	1	1.59 (0.51–4.97)	0.425		
Analysis										
K-M Curve	6	1.49 (1.02–2.16)	0.039	21.6%	0.271	3	1.39 (0.86–2.26)	0.178	0.0%	0.962
Multivariate	12	1.99 (1.55–2.55)	< 0.001	68.8%	< 0.001	6	1.72 (1.15–2.57)	0.009	73.4%	0.002
Antibody Type										
Monoclonal antibody	10	1.59 (1.31–1.95)	< 0.001	22.0%	0.240	5	1.83 (1.26–2.67)	0.002	45.8%	0.117
Polyclonal antibody	2	1.59 (1.07–2.37)	0.020	0.0%	0.418	2	1.08 (0.73–1.59)	0.702	36.2%	0.211
NA	4	2.22 (1.64–3.01)	< 0.001	0.0%	0.690	2	2.12 (1.05–4.29)	0.036	0.0%	0.527
M + P	2	4.11 (0.47–35.90)	0.202	95.9%	< 0.001					
Region										
Europe	6	2.43 (1.38–4.29)	0.002	78.4%	< 0.001	3	1.72 (1.08–2.72)	0.022	0.0%	0.599
America	2	2.54 (0.60-10.69)	0.203	77.0%	0.037	2	0.96 (0.72–1.28)	0.798	0.0%	0.589
Asia	10	1.67 (1.38–2.01)	< 0.001	19.9%	0.260	4	1.88 (1.23–2.87)	0.004	58.9%	0.063
Sample size										
< 150	8	1.80 (1.40–2.30)	< 0.001	29.9%	0.189	4	2.17 (1.43–3.30)	< 0.001	20.8%	0.285
≥ 150	10	1.90 (1.37–2.64)	< 0.001	72.1%	< 0.001	5	1.32 (0.96–1.82)	0.086	49.1%	0.097
NOS score										
≥ 7	14	1.92 (1.48–2.48)	< 0.001	66.9%	< 0.001	6	1.46 (1.15–1.87)	0.002	0.0%	0.888
< 7	4	1.67 (1.27–2.18)	< 0.001	0.0%	0.405	3	1.81 (0.83–3.93)	0.134	88.2%	< 0.001

OS	DFS

Abbreviations: NCSLC: non-small cell lung cancer; BRCA: breast cancer; NA: not available; M + P: monoclonal antibody and polyclonal antibody; OS: overall survival; DFS: disease-free survival. HR: Hazard ratio; CI: Confidence inter.

[a] Intrahepatic cholangiocarcinoma and hepatocellular carcinoma; [b] Oesophageal squamous cell carcinoma and colorectal cancer; [c] Soft tissue cancer, renal clear cell carcinoma and nasopharyngeal carcinoma.

The subgroup analysis of OS found that, high-expressed SPP1 was linked with poor OS in NSCLC (HR = 1.60, 95%CI = 1.07–2.40, *P* = 0.022), hepatobiliary cancers (HR = 1.81, 95%CI = 1.38–2.37, *P* < 0.001), BRCA (HR = 3.40, 95%CI = 1.08–10.68, *P* = 0.036), and other cancers (HR = 1.68, 95%CI = 1.30–2.16, *P* < 0.001), except for GI tract cancers (HR = 1.24, 95%CI = 0.83–1.83, *P* = 0.291). In the subgroups based on analysis, antibody type, region, sample size and NOS score, we also found that the relationship between high-expressed SPP1 and poor OS, except for patients from America and the antibody type of monoclonal antibody and polyclonal antibody. We didn't find the source of heterogeneity. Due to the small number of studies, we didn't conduct the regression analysis to further look for the source of heterogeneity.

The subgroup analysis of DFS found that, high expression of SPP1 was linked with poor DFS in hepatobiliary cancers (HR = 2.07, 95%CI = 1.36–3.16, *P* = 0.001), but didn't correlate with NSCLC (HR = 1.46, 95%CI = 0.79–2.69, *P* = 0.225), BRCA (HR = 0.96, 95%CI = 0.72–1.28, *P* = 0.798), GI tract cancers (HR = 1.24, 95%CI = 0.47–3.26, *P* = 0.663) and other cancers (HR = 1.59, 95%CI = 0.51–4.97, *P* = 0.425). Other subgroups also found the relationship between high expression of SPP1 and poor DFS, except for the analysis of K-M Curve, Polyclonal antibody for IHC, patients from America, sample size ≥ 150 and NOS score < 7. The antibody type and sample size might be the potential sources of heterogeneity.

The relationship between SPP1 and clinical parameters

We explored the relationship between SPP1 expression and clinical parameters to find more clinical values of SPP1 (Table 3). High-expressed SPP1 was related with the tumor grade (HR = 2.03, 95%CI = 1.19–3.47, *P* = 0.009, random effects) in our study. Whereas, there were no significant correlations between high expression of SPP1 and age (HR = 0.88, 95%CI = 0.69–1.13, *P* = 0.331, fixed effects), gender (HR = 0.90, 95%CI = 0.68–1.19, *P* = 0.453, fixed effects), tumor size (HR = 0.92, 95%CI = 0.71–1.20, *P* = 0.534, fixed effects), TNM stage (HR = 1.66, 95%CI = 0.83–3.32, *P* = 0.152, random effects), tumor differentiation (HR = 1.27, 95%CI = 0.75–2.17, *P* = 0.379, fixed effects), distant metastasis (HR = 1.06, 95%CI = 0.19–6.05, *P* = 0.948, random effects), lymph node metastasis (HR = 1.29, 95%CI = 0.76–2.19, *P* = 0.347, random effects), and vascular invasion (HR = 1.03, 95%CI = 0.69–1.54, *P* = 0.901, random effects). In conclusion, high-expressed SPP1 might affect tumor grade, which in turn causes poor clinical prognosis.

Table 3
Clinicopathological parameters of the enrolled studies with high-expressed SPP1 in tumor patients.

Clinicopathological parameters	Studies	No. of patients	Risk of high SPP1 OR (95% CI)	Significant Z	*P*-value	Heterogeneity I^2 (%)	*P*-value	Model
Age (≤ 60 vs. > 60)	8	1350	0.88 (0.69, 1.13)	0.97	0.331	13.1	0.328	Fixed effects
Gender (male vs. female)	8	1292	0.90 (0.68, 1.19)	0.75	0.453	0.0	0.435	Fixed effects
Tumor size (< 5 cm vs. ≥ 5 cm)	7	1302	0.92 (0.71, 1.20)	0.62	0.534	41.0	0.118	Fixed effects
TNM stage (III-IV vs. I-II)	5	671	1.66 (0.83, 3.32)	1.43	0.152	71.8	0.007	Random effects
Tumor grade (3–4 vs. 1–2)	7	1720	2.03 (1.19, 3.47)	2.61	0.009	76.6	< 0.001	Random effects
Tumor differentiation (moderate/well vs. poor)	4	490	1.27 (0.75, 2.17)	0.88	0.379	51.1	0.105	Fixed effects
Distant metastasis (positive vs. negative)	3	340	1.06 (0.19, 6.05)	0.07	0.948	87.4	< 0.001	Random effects
Lymph node metastasis (positive vs. negative)	8	1568	1.29 (0.76, 2.19)	0.94	0.347	73.3	< 0.001	Random effects
Vascular invasion (positive vs. negative)	6	1265	1.03 (0.69, 1.54)	0.12	0.901	53.1	0.059	Random effects

OR: Odds ratios; CI: Confidence inter.

Sensitivity analysis and publication bias

The results of sensitivity analysis showed that no individual studies influenced the overall results (Fig. 3A and 3B). Begg's test (Fig. 3C and 3D) and Egger's test showed that no publication bias existed in studies on associations between high-expressed SPP1 and OS (P = 0.173 for Begg's test; P = 0.083 for Egger's test) and DFS (P = 0.917 for Begg's test; P = 0.184 for Egger's test).

The expression level of SPP1 extracted from TCGA and GTEx databases.

The differences of SPP1 RNA expression between various tumor tissues and corresponding normal tissues were obtained from TCGA and GTEx databases (Fig. 4). The results showed that SPP1 expression level was much higher than the corresponding normal tissues in 25 types of cancers, such as BRCA, cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), liver hepatocellular carcinoma (LIHC), NSCLC, and so on. On the contrary, SPP1 expression was lower than normal tissues in kidney chromophobe (KICH) and kidney renal clear cell carcinoma (KIRC).

Correlation between SPP1 expression and survival from TCGA database.

To validate the clinical prognosis indication value of SPP1, we explored the relationship between SPP1 expression level and the OS and DFS of tumor patients from TCGA database. The results showed that high-expressed SPP1 was related to poor OS in LIHC, bladder urothelial carcinoma (BLCA), glioblastoma multiforme (GBM), brain lower-grade glioma (LGG), ovarian serous cystadenocarcinoma (OV), and thyroid carcinoma (THCA). Also, the high expression of SPP1 was linked with poor DFS in LIHC and esophageal carcinoma (ESCA). In conclusion, high-expressed SPP1 was correlated with poor OS and DFS in LIHC (Fig. 5) and it is consistent with our results of the meta-analysis. Based on this result, we further explored the effect of SPP1 expression on the TIME and methylation in LIHC.

Relationship between SPP1 and the TIME of LIHC

To explore the correlation between SPP1 expression level and the TIME of LIHC, we examined the associations between SPP1 expression level and 6 immune cells infiltration by the TIMER database (Fig. 6A). There were positive correlations between high-expressed SPP1 and infiltration of B cells (R = 0.311, P < 0.001), $CD4^+$ T cells (R = 0.264, P < 0.001), $CD8^+$ T cells (R = 0.242, P < 0.001), neutrophils (R = 0.328, P < 0.001), macrophages (R = 0.391, P < 0.001), and dendritic cells (R = 0.392, P < 0.001). ImmuneScore, StromalScore and ESTIMATEScore for LIHC were calculated using the ESTIMATE algorithm. Then we assessed the associations between these scores and SPP1 expression (Fig. 6B) in LIHC. The results showed that SPP1 expression level was significantly positive correlated with ImmuneScore (R = 0.222, P < 0.001), StromalScore (R = 0.152, P = 0.003) and ESTIMATEScore (R = 0.258, P < 0.001). Based on these results, SPP1 might have a certain effect on the TIME of LIHC.

Correlation between SPP1 expression and DNA methylation

We explored the correlations between the expression of DNA methylation regulatory genes (DNMT1, DNMT2, DNMT3A and DNMT3B) and SPP1 expression level (Fig. 7). The results showed that SPP1 affected the expression of DNA methylation regulatory genes in 14 types of cancers, such as LIHC, BRCA, COAD, and so on. Not surprisingly, we observed that there were positive correlations between high-expressed SPP1 and DNMT2 (R = 0.20, P < 0.001), DNMT3A (R = 0.15, P = 0.010), and DNMT3B (R = 0.20, P < 0.001) in LIHC.

Gene Set Enrichment Analysis

GSEA was used to assess the biological significance of SPP1 expression in cancers (Fig. 8). Three pathways, including Pathogenic Escherichia COL1 infection, pentose phosphate pathway and proteasome, were significantly enriched in high-expressed SPP1 group of KEGG, and three pathways, including ABC transporters, ether lipid metabolism and linolenic acid metabolism were significantly enriched in low-expressed SPP1 group of KEGG. mTORC1 signaling, hypoxia and glycolysis were significantly enriched in high-expressed SPP1 group of HALLMARK collection. Hedgehog signaling, WNT beta catenin signaling, bile acid metabolism and KRAS signaling were significantly enriched in low-expressed SPP1 group of HALLMARK collection.

Discussion

SPP1 plays an important role in many physiologic processes, such as bone matrix remodeling, immune regulation, anti-apoptosis, wound healing, and so on [6–8, 43]. However, more and more researches have shown that SPP1 was correlated with tumor microenvironment and poor prognosis in various cancers. A single research is limited because of the insufficient data and single experimental model, so that a meta-analysis of pooling researches is necessary to explore the potential clinical value of SPP1.

Our meta-analysis showed that high-expressed SPP1 was significantly correlated with poor OS and DFS in various cancers, especially in LIHC. The analysis of clinical parameters found that high-expressed SPP1 might affect tumor grade, which in turn caused poor clinical prognosis. Then we used TCGA database to validate the results of our meta-analysis. SPP1 expression level was much higher than the corresponding normal tissues in 25 types of cancers from TCGA and GTEx database. Survival analysis from TCGA database showed that high-expressed SPP1 was related to poor OS and DFS in LIHC, which supported the conclusion of our meta-analysis. Besides, our research further found that SPP1 affected TIME and DNA methylation of LIHC. High-expressed SPP1 was correlated with many biological pathways of tumor prognosis, such as hypoxia, lipid metabolism, and mTOR signaling from GSEA.

Although, SPP1 had been proved that it was highly expressed in various tumor tissues and was related to tumorigenesis and metastasis, the exact mechanisms of which role did SPP1 play was not clear. The research from Bramwell et al. found that high-expressed SPP1 mRNA was correlated with the higher grade of soft tissue tumors [25] and it is consistent with our result. But our research didn't find the relationship between SPP1 expression level and other

advanced features of cancer, such as tumor size, distant metastasis, lymph node metastasis and vascular invasion. The reason might be that we accepted more resectable tumor samples in the early stage, while the advanced tumor samples were too few.

We found the positive correlations between high-expressed SPP1 and infiltration of B cells, T cells, neutrophils, macrophages, and dendritic cells in LIHC. Besides osteoblasts, vascular smooth muscle cells, endothelial and epithelial cells, SPP1 can also be synthesized by activated immune cells as a multifunctional cytokine, including T cells, natural killer cells, and macrophages [3, 44]. In addition, SPP1 promotes cell-mediated immune responses and plays an important part in chronic inflammatory [45]. During local inflammation, SPP1 promotes immune cells' chemotaxis, B cells' multiplication, immunoglobulin production, and mast cell degranulation [46]. Furthermore, SPP1 improves the activity of Th1 cells by stimulating the production of IL-12 and IFN-γ and inhibiting the production of Th2-dependent IL-10 [47–49]. SPP1 also affected the maturation, migration, and polarization of dendritic cells [50]. All in all, SPP1 might have a certain effect on the TIME and the specific mechanisms need to be further verified by more vitro and vivo experiments.

SPP1 is correlated with histone H3 lysine 4 trimethylation (H3K4me3) as a key regulator [51]. H3K4me3 often leads to transcriptional activation of tumor-related genes [52] and is linked to tumor progression and poor prognosis in hepatocellular carcinoma, lung cancer, prostate cancer and pancreatic cancer [53–56]. Additionally, SPP1 expression is stimulated by hypoxia and SPP1 signaling also transcriptionally upregulates the expression of hypoxia markers, enhancing tumor angiogenesis and promoting cancer progression and invasion in breast and melanoma cancer [57, 58]. SPP1 is involved in lipid metabolism and related to the prognosis of breast cancer [59]. These researches are consistent with our results.

At present, a large number of studies have shown that the high expression level of SPP1 in body fluids and tumor tissues is correlated with the occurrence and development of many kinds of tumors. SPP1 can easily be detected in body fluids, such as blood, urine, pleural and peritoneal ascites as a secreted protein [3]. SPP1 may be a convenient clinical test biomarker to diagnosis cancer and evaluate the prognosis. However, SPP1 splice variants (OPN-a, OPN-b and OPN-c) in tumors are cell/tissue-type specific and might have functional heterogeneity [60]. It challenges the detection methods and predictive value of SPP1.

However, our research still has some limitations. To begin with, many unavoidable factors, such as different tumor types, analysis methods, antibody types, sample size, and so on, contributed to the heterogeneity. Although, we did subgroup analysis, we didn't find the source of heterogeneity in OS. Because of the small number of studies, we didn't conduct regression analysis to further look for the source of heterogeneity. Secondly, we used Engauge Digitizer software to extract HRs and its 95% CIs from K-M plots, when the data could not be obtained from the article directly. It might affect the accuracy of the results. Thirdly, we properly unified the names of some subgroups in clinicopathological features, such as age and tumor size, in order to facilitate comparison. Last but not least, our study explored the relationship between SPP1 expression and tumor immuno-microenvironment, DNA methylation and enrichment analysis based on the database. The specific mechanisms still need to be further verified by basic and clinical research.

Conclusion

SPP1 is high-expressed in various tumor tissues and correlated with poor prognosis, especially in LIHC. SPP1 might promote cancer invasion and metastasis by affecting tumor grade, TIME, DNA methylation, hypoxia, lipid metabolism, and so on. SPP1 is expected to become a new clinical indicator for tumor detection and prognosis, and provide a new idea for tumor targeted therapy.

Abbreviations

ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast cancer; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; CI: Confidence inter; COAD: Colon adenocarcinoma; DFS: Disease-free survival; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; GI: Gastrointestinal; GSEA: Gene Set Enrichment Analysis; GTEx: Genotype-Tissue Expression; HNSC: Head and neck squamous cell carcinoma; HR: Hazard ratio; IHC: Immunohistochemistry; K-M: Kaplan-Meier; KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute myeloid leukemia; LGG: Brain lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; NOS: Newcastle Ottawa Quality Assessment Scale; NSCLC: Non-small cell lung cancer; OPN: Osteopontin; OR: Odds ratio; OS: Overall survival; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PRAD: Prostate adenocarcinoma; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; READ: Rectum adenocarcinoma; SKCM: Skin cutaneous melanoma; SPP1: Secreted phosphoprotein 1; STAD: Stomach adenocarcinoma; TCGA: The Cancer Genome Atlas; TGCT: Testicular germ cell tumors; THCA: Thyroid carcinoma; TIME: Tumor immuno-microenvironment; TIMER: Tumor Immune Estimation Resource; TPM: Transcripts Per Million; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine carcinosarcoma.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

ZHR, JAM, YY, FX, TT, LX, and RZP conceived the study. JAM and LN searched the databases. ZHR, DQQ, and ZFM extracted the data. ZHR, JAM, and LN analyzed the data. ZHR wrote the draft of the paper. JAM and LN reviewed the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article and referenced articles are listed in the References section.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

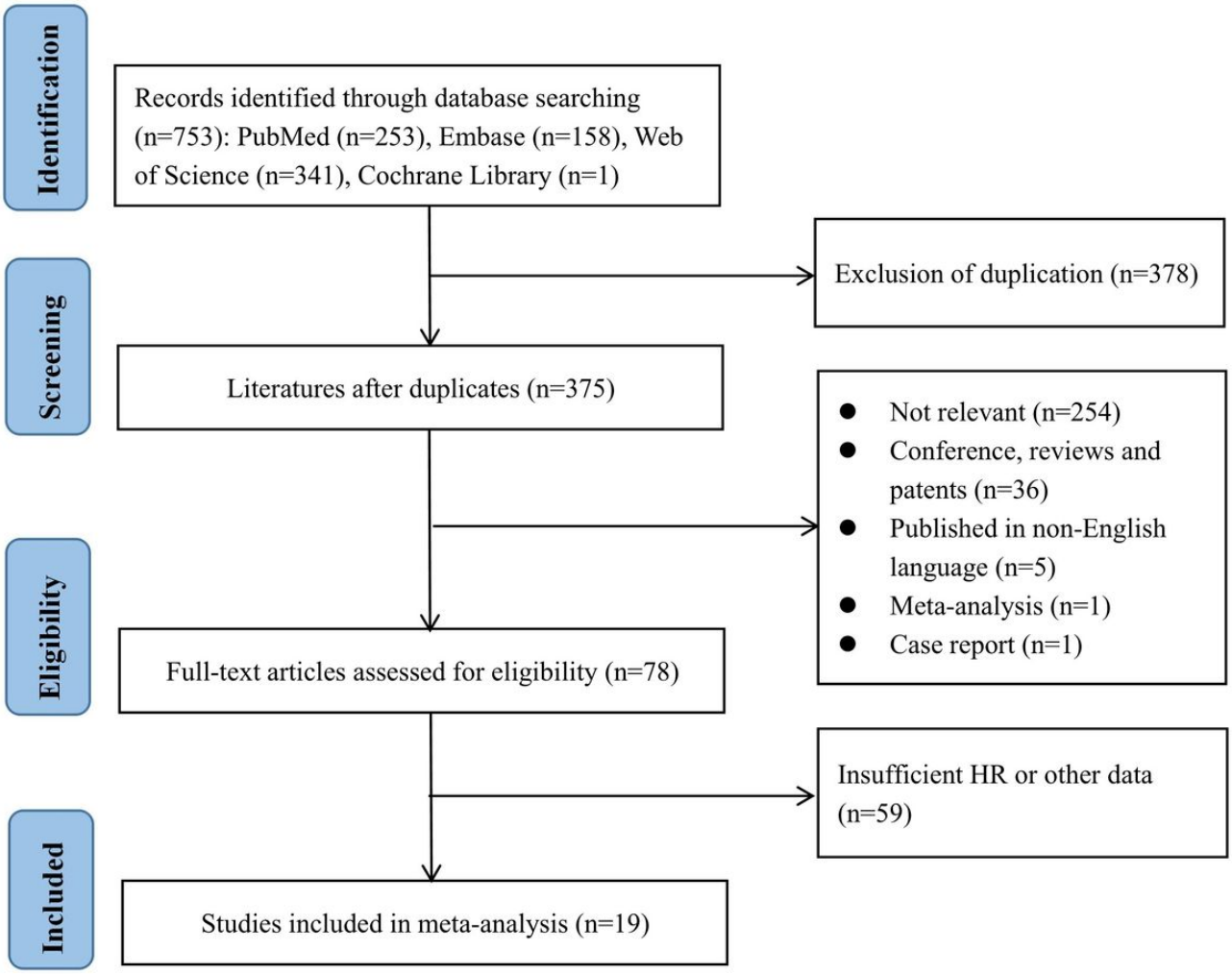

Figure 1

Flow chart of the eligible studies for meta-analysis.

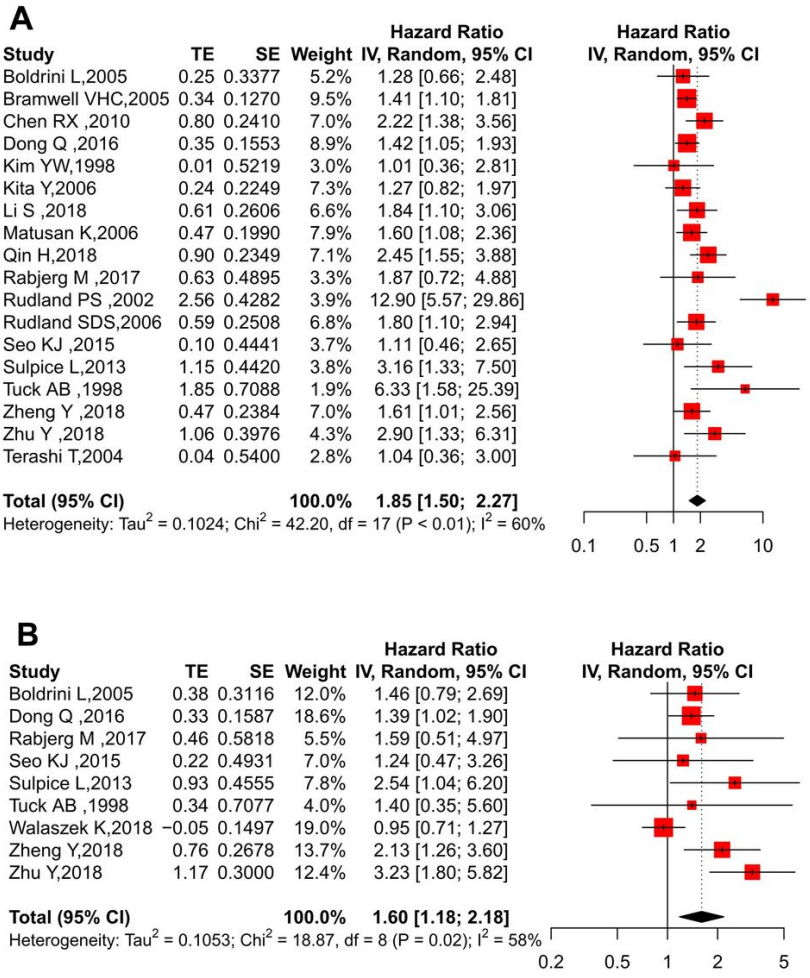

Figure 2

Forest plots of studies evaluating HRs of SPP1 expression level and the prognosis of cancer patients. (A) High expressed SPP1 and the OS; (B) High expressed SPP1 and the DFS. OS: Overall survival; DFS: Disease-free survival; HR: Hazard ratio; CI: Confidence inter.

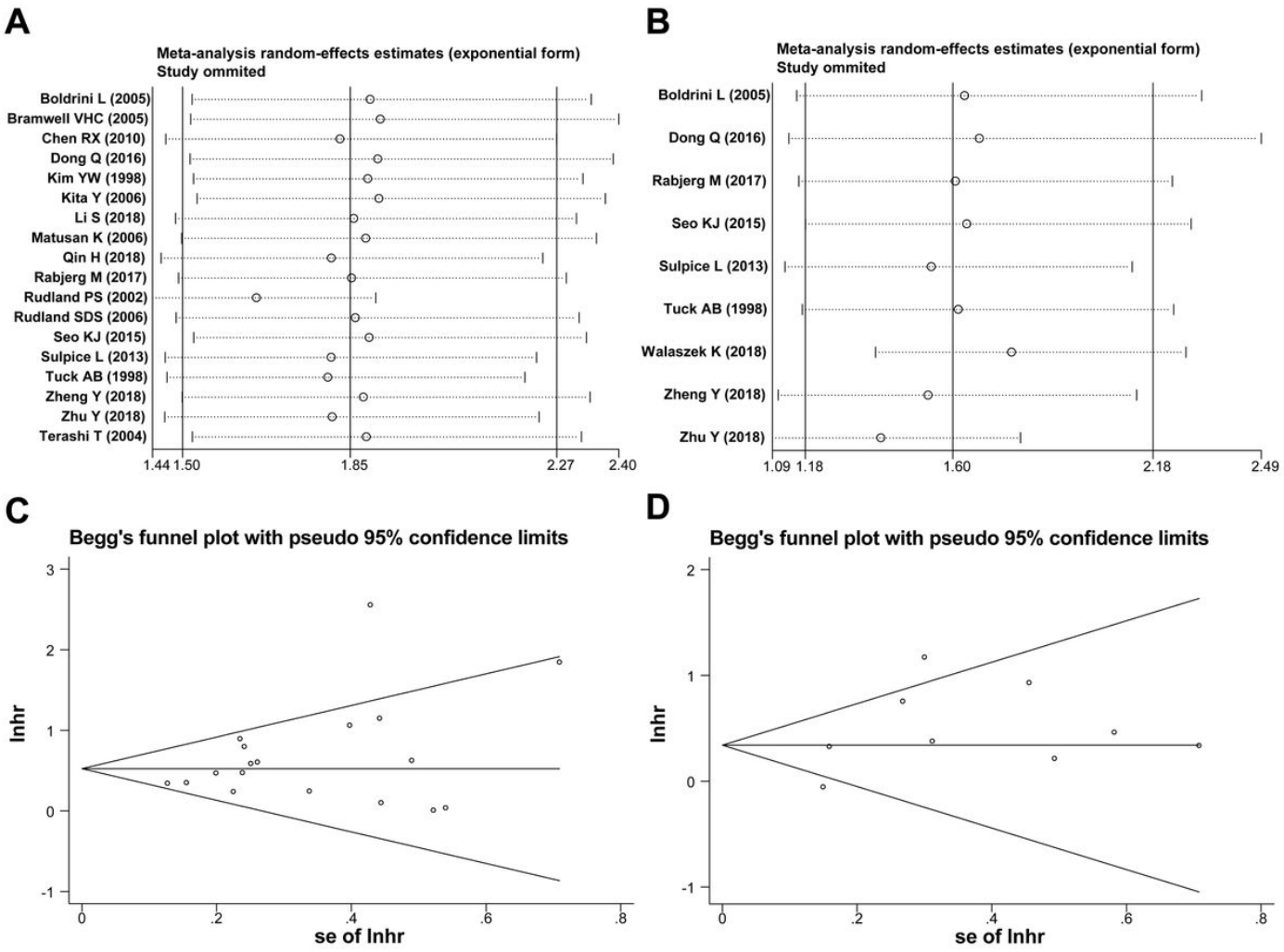

Figure 3

Sensitivity analysis and Begg's funnel plots for the studies involved in the meta-analysis. (A) Sensitivity analysis of OS; (B) Sensitivity analysis of DFS; (C) Begg's funnel plot of OS; (D) Begg's funnel plot of DFS. OS: Overall survival; DFS: Disease-free survival.

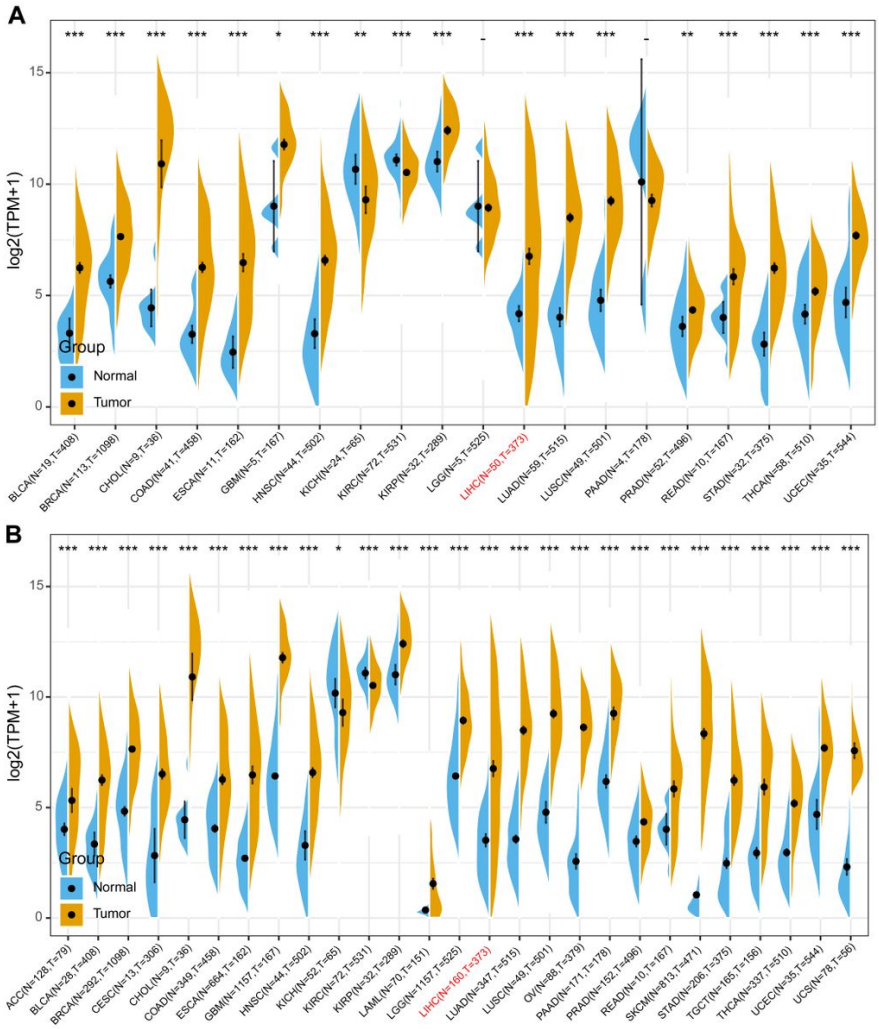

Figure 4

SPP1 expression in tumor tissues and corresponding normal tissues. (A) SPP1 expression from TCGA database; (B) SPP1 expression from TCGA and GTEx databases. * P< 0.05, ** P< 0.01, *** P< 0.001. TCGA: The Cancer Genome Atlas; GTEx: Genotype-Tissue Expression; TPM: Transcripts Per Million.

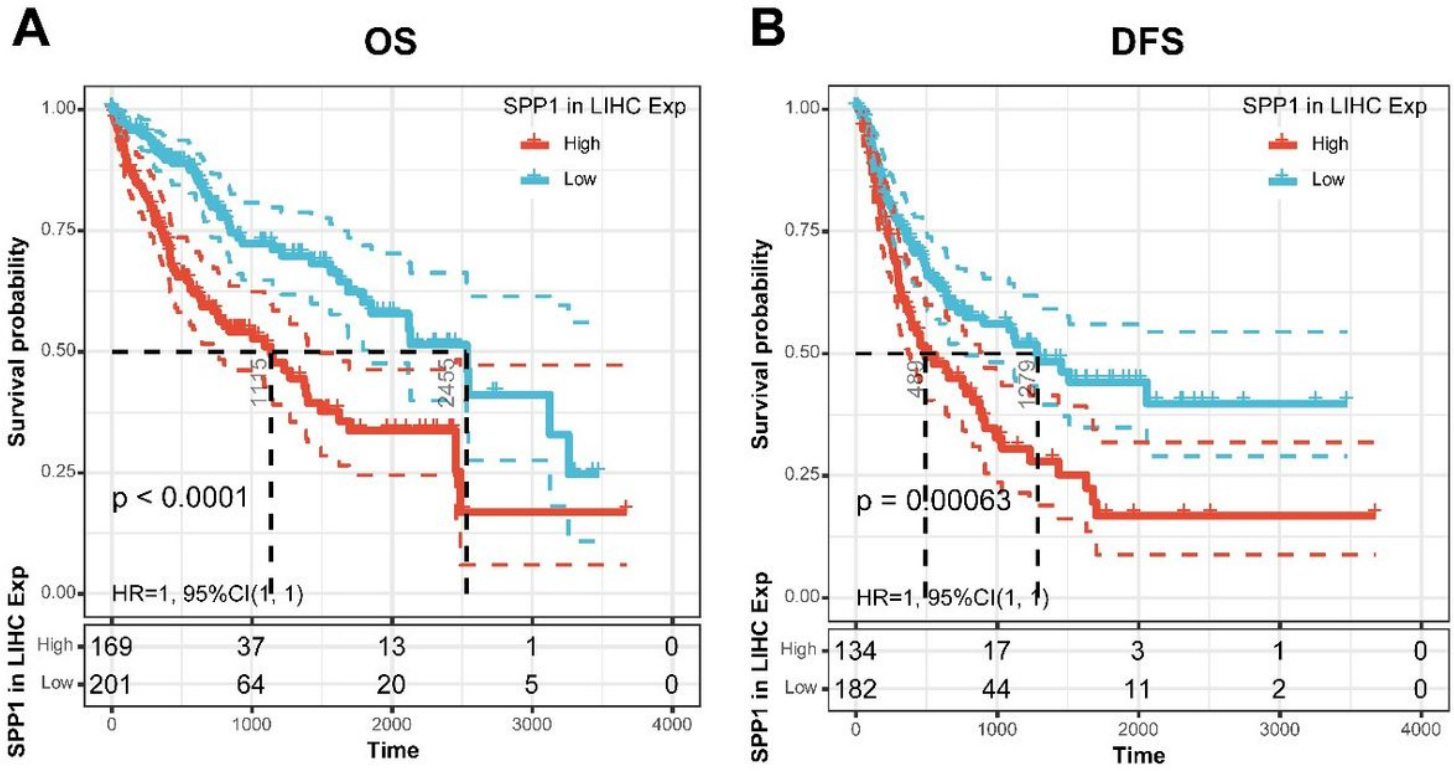

Figure 5

Kaplan-Meier survival curves for LIHC patients based on TCGA datasets. (A) OS of LIHC patients; (B) DFS of LIHC patients. OS: Overall survival; DFS: Disease-free survival; HR: Hazard ratio; CI: Confidence inter; LIHC: Liver hepatocellular carcinoma.

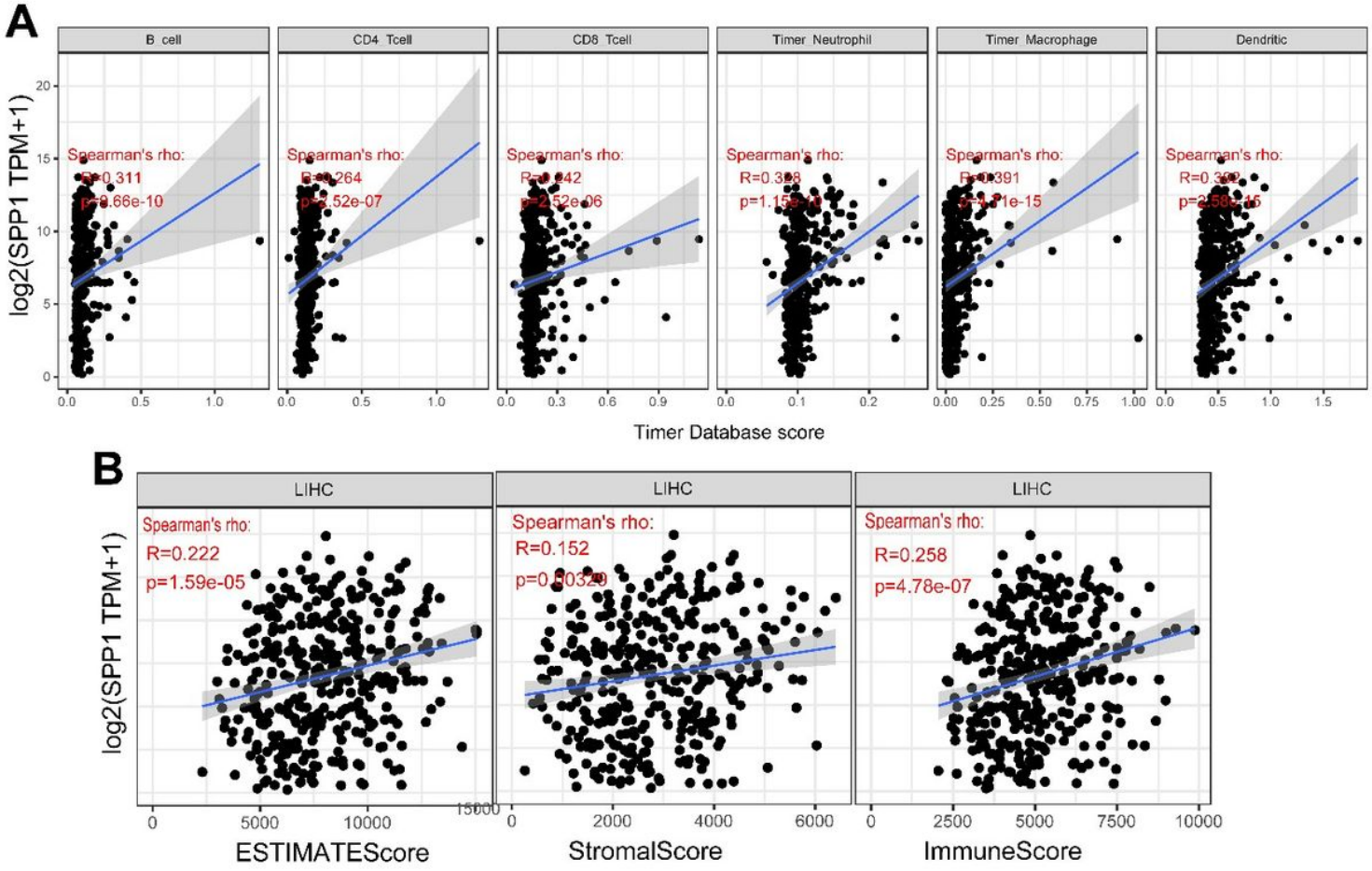

Figure 6

Relationship between SPP1 and LIHC immuno-microenvironment based on TIMER database. (A) Associations between SPP1 expression level and 6 immune cells infiltration. (B) Relationship between SPP1 expression and ESTIMATEScore, StromalScore and ImmuneScore. LIHC: Liver hepatocellular carcinoma; TPM: Transcripts Per Million; TIMER: Tumor Immune Estimation Resource.

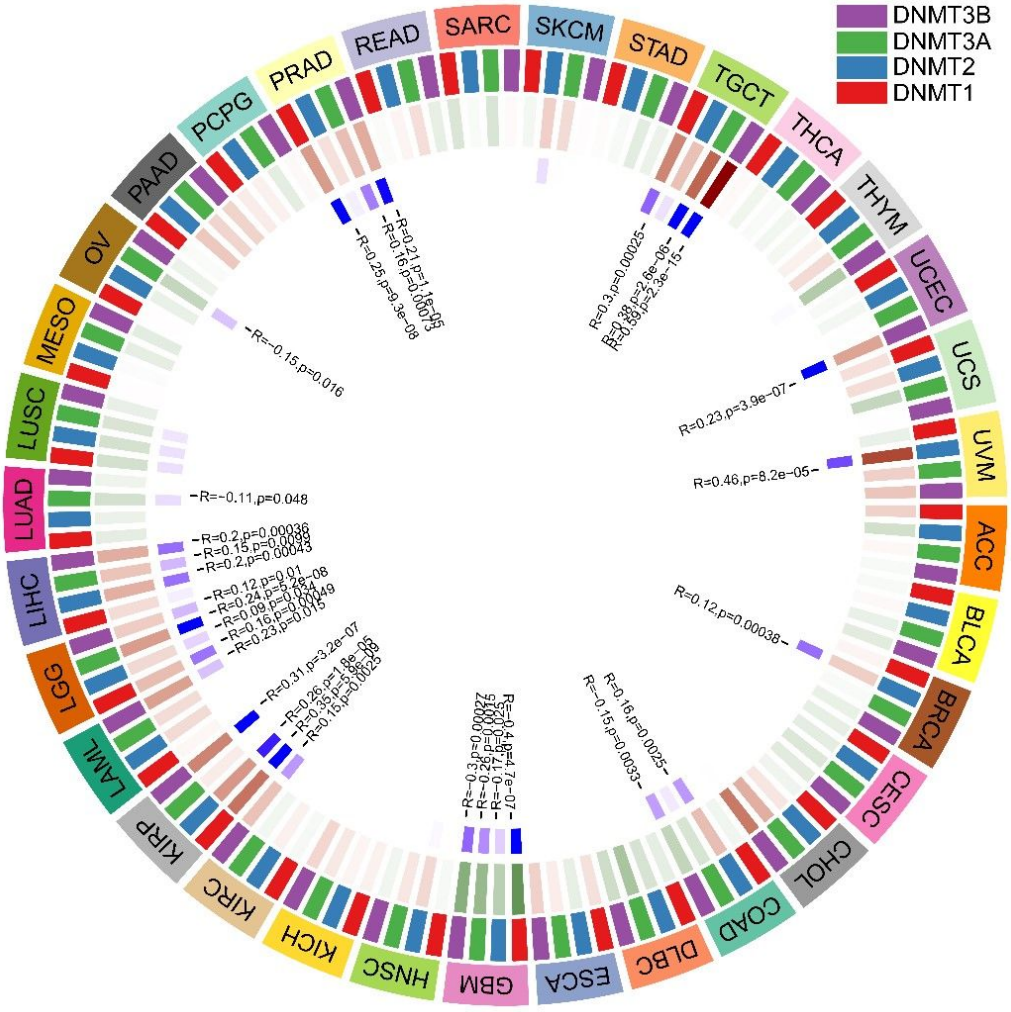

Figure 7

Correlation between SPP1 expression and DNA methylation regulatory genes in various cancers.

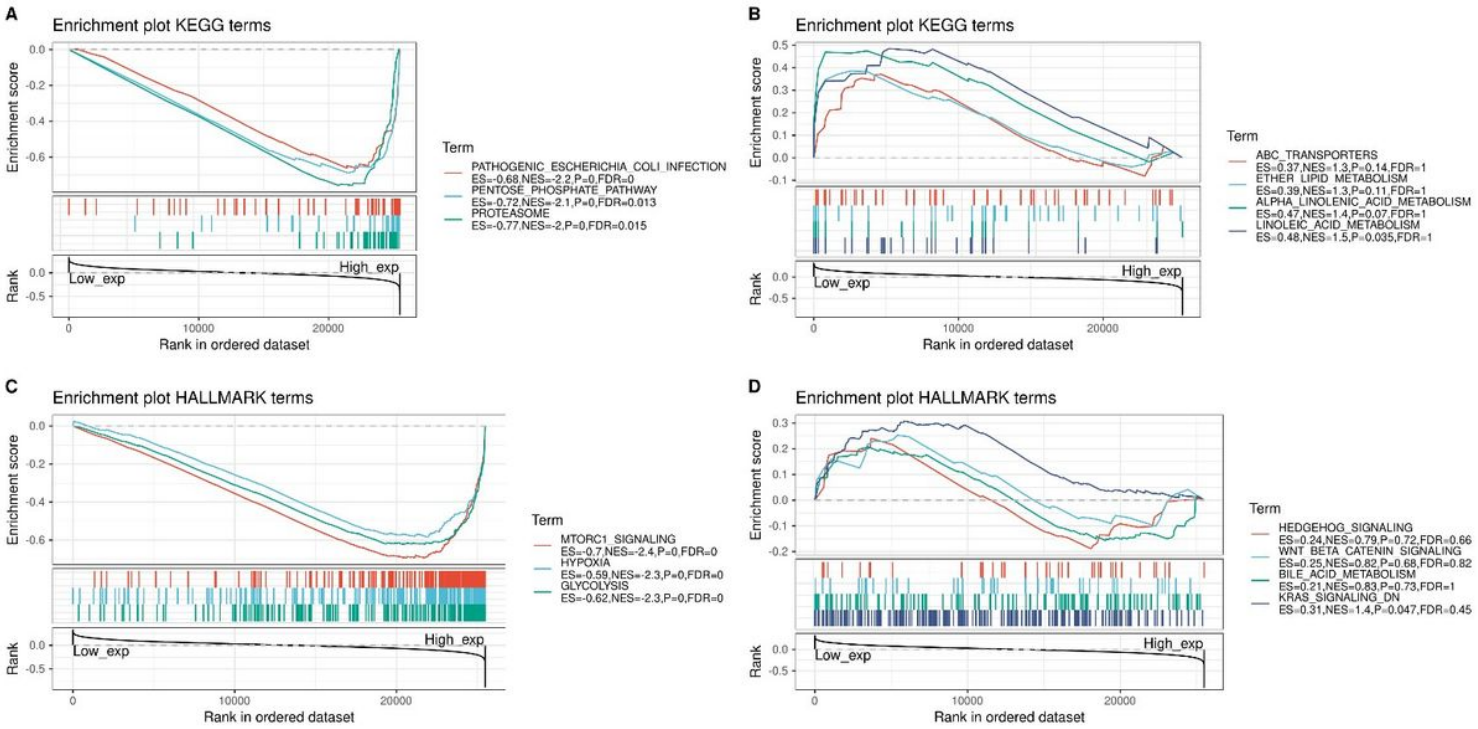

Figure 8

GSEA for samples with high SPP1 expression and low expression. (A) The enriched gene sets in KEGG of high-expressed SPP1; (B) The enriched gene sets in KEGG of low-expressed SPP1; (C) The enriched gene sets in HALLMARK collection of high-expressed SPP1; (D) The enriched gene sets in HALLMARK collection of low-expressed SPP1. GSEA: Gene Set Enrichment Analysis.

Supplementary Files

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