SPP1 is a Prognostic Related Biomarker and Correlated with Tumor-Infiltrating Immune Cells in Hepatocellular Carcinoma

Xiaofeng Wang
the First Affiliated Hospital of Zhengzhou University
Kun Zhang
Hainan General Hospital
Li Geng
the First Affiliated Hospital of Zhengzhou University
DongLi Liu (dongli.liu@shgh.cn)
Shanghai General Hospital

Research Article

Keywords: SPP1, hepatocellular carcinoma, prognostic, tumor immune infiltration, bioinformatics database.

Posted Date: December 13th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1156469/v1

Abstract

Background: Secreted phosphoprotein 1 (SPP1) functions as a tumor promoter in varies tumors, but little is known whether it is an actual player on driving immune infiltration in hepatocellular carcinoma.

Methods: In this study, we identified the expression of SPP1 by Oncomine, GEPIA and TIMER databases, and assessed SPP1 immumohistochemical staining analysis by The HPA database. We evaluated the clinical outcomes between SPP1 expression and hepatocellular carcinoma patients via Kaplan-Meier Plotter. We also tested the relationship between SPP1 and critical oncogenes by TIMER and GEPIA databases. Then we explored immune infiltration analyses using TIMER and TISIDB datasets. In addition, we performed functional enrichment analyses with Metascape and GeneMANIA databases.

Results: We found that SPP1 overexpressed in hepatocellular carcinoma tissues and high SPP1 expression was correlated with shorter OS and PFS survivals in hepatocellular carcinoma patients. SPP1 expression is positive correlation with critical oncogenes related stemness associated genes, cell cycle and proliferation, therapeutic resistance, metastasis, and tumor angiogenesis in hepatocellular carcinoma. Importantly, SPP1 expression was positively correlated with infiltrating levels of CD4+ T cells, CD8+ T cells, macrophages, neutrophils, and dendritic cells. Furthermore, SPP1 expression showed strong correlations with diverse immune hallmark sets in hepatocellular carcinoma. Notably, functional enrichment analysis suggested that SPP1 strong related with immune response.

Conclusions: These findings imply that SPP1 is correlated with prognosis and immune cell infiltrating, offering a new potential immunotherapeutic target in hepatocellular carcinoma.

Background

Primary liver cancer is the sixth most commonly diagnosed cancer and the third leading cause of cancer death worldwide in 2020, and the incidence is the highest in East Asia including China. Amount 75-85% of primary liver cancer cases are liver hepatocellular carcinoma (LIHC, also well-known as HCC) [1]. There were 392,868 new-diagnosed cases and 368,960 HCC-related death cases in China in 2018[2].The main risk factors for HCC are chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), aflatoxin-contaminated foods, heavy alcohol intake, excess body weight, type 2 diabetes, and smoking[1]. Patients with HCC have poor survival outcomes with surgical interventions and medical treatment regimens. There is an unmet clinical need to find better treatment options for HCC. Immunotherapy is an emerging treatment against cancer. Different cancer types of patients, treated with immune checkpoint inhibitors have a better survival prognosis. These types of cancer include lung cancer, melanoma, hepatocellular carcinoma, urothelial and renal carcinomas, Hodgkin's lymphoma and gastrointestinal cancers, etc. Nivolumab, pembrolizumab and camrelizumab have been approved for the treatment of HCC due to their exact efficacy, however, only a minority of patients with HCC can benefit from immunotherapy [3-5]. The underlying mechanisms remains unknown and needs be clarified.

Secreted phosphoprotein 1 (SPP1), also known as early T-lymphocyte activation 1 or protein Osteopontin (OPN), is encoded by a single-copy gene, *spp1*, on human chromosome 4 near the centromere [6]. It is a multifunctional secretory acidic glycoprotein, widely expressed in osteoclasts; osteoblasts; epithelial cells of the breast, kidney, and skin, nerve cells, vascular smooth muscle cells, endothelial cells, and fibroblasts. A broad range of immune cells such as macrophages, lymphocytes, natural killer cells, eosinophils, dendritic cells, and microglia express SPP1[6-9]. It is reported that SPP1 is abnormally overexpressed in lung cancer, gastric cancer, colon cancer, breast cancer and

liver cancer [10-12]. SPP1 has been widely implicated in cancer invasion and metastasis, and its expression is associated with poor prognosis in many types of cancer. SPP1 promote cancer invasion and metastasis through angiogenesis, degradation of (ECM), the formation of lamellipodia, and switching phenotypes towards either cancer-associated fibroblasts or stem cells. In head and neck, lung, colorectal, and breast cancer, SPP1 has been implicated in treatment resistance[13-16]. SPP1 is secreted by HCC cells and serum SPP1 increases in HCC patients. The plasmatic SPP1 concentration was referred not as a potential biomarker for hepatocellular carcinoma diagnosis, but as an independent prognostic parameter for survival [17-19]. Yet, the underlying mechanisms are still unclear and it is urgent to study the depth profile.

SPP1 can regulate the host immune system via upregulating IL-12 and IFNγ in mouse macrophages and NK cells which indicates that SPP1 may act as a potential role in host immunity [20]. SPP1 is upregulated in human glioma-associated macrophages, and mediate macrophage polarization and facilitate immune escape by upregulating PD-L1 in lung adenocarcinoma [21, 22]. It is reported that SPP1 knockdown could regulate M2 macrophage polarization via upregulating insulin-like growth factor 1 and leukemia inhibitory factor [23]. However, the molecular mechanisms of SPP1 by modulating immune infiltration cell and prognosis of hepatocellular carcinoma were still not fully elucidated.

In our present study, we comprehensively analyzed SPP1 expression and correlation with prognostic value of HCC patients in databases including Oncomine, GEPIA, TIMER, HPA and Kaplan-Meier plotter. We found SPP1 expression is positive correlation with critical oncogenes in hepatocellular carcinoma. We demonstrated the association of SPP1 with tumor infiltration immune cells in the HCC microenvironments via TIMER and TISIDB. Moreover, functional enrichment analysis suggested that SPP1 strong related with immune response. Our findings in this report highlight the vital role of SPP1 in HCC and further offer a probable relationship and underlying mechanisms between SPP1 and tumor-immune interactions.

Results

Assessment of SPP1 expression levels in different cancers

In order to explore the SPP1 expression levels in different cancers and the corresponding normal tissues, three different online databases were used in our study. The Oncomine database showed that the expression of SPP1 significantly higher in cancer samples than normal tissues in most datasets (Fig. 1a). The similar results were showed in GEPIA database (Fig. 1b). The expression of SPP1 was distinctly up-regulated in bladder cancer (BLCA), brain and CNS cancer, breast cancer (BRCA), cervical cancer (CESC), colorectal cancer (COAD and READ), esophageal cancer (ESCA), glioblastoma multiforme (GBM), stomach cancer (STAD), head and neck cancer (HNSC), kidney renal papillary cell carcinoma(KIRP), brain Lower Grade Glioma(LGG), liver hepatocellular cancer (LIHC), lung cancer (LUAD and LUSC), lymphoma, melanoma, ovarian cancer (OV), and pancreatic adenocarcinoma（PAAD）, skin cutaneous melanoma (SKCM), testicular germ cell tumors(TGCT), thyroid carcinoma(THCA), uterine corpus endometrial carcinoma(UCEC) and uterine carsinosarcoma(UCS) than in corresponding normal tissues. We next evaluate the expression of SPP1 between different tumors and matched normal samples in TIMER dataset with RNA-seq data from TCGA (Fig. 1c). The results are mostly consistent with the above two online databases. Interestingly, SPP1 expression in the metastasis was higher than that in the primary tumor tissue in SKCM. However, the expression levels of SPP1 in some cancers were controversial. SPP1 was significantly lower expressed in kidney renal clear cell carcinoma (KIRC), kidney chromophobe (KICH) than in control normal samples in GEPIA and TIMER

database. Taken together, these results demonstrated that SPP1 was up-regulated in multiple cancers suggested that SPP1 may play a crucial biological role in tumor progression.

Elevated expression of SPP1 correlated with poor clinical outcomes of hepatocellular carcinoma

To clarify the relationship between SPP1 expression and prognosis of patients with hepatocellular carcinoma, we first evaluated the expression of SPP1 in hepatocellular carcinoma and normal liver tissue using GEPIA database. The results showed that SPP1 was significantly overexpressed in hepatocellular carcinoma (Fig. 2 a). However, we found that there was no relationship between SPP1 expression and tumor stage (Fig. 2b). In addition, we examined the expression of SPP1 using IHC via HPA database. We found that SPP1 existed in both cell membrane and cytoplasm of hepatocellular cancer cell, and about 45.8% (167/365) hepatocellular cancer patients with SPP1 high expression, while the expression of SPP1 was not detected in normal liver tissue (Fig. 2c and 2d).

We next investigated the correlation between SPP1 expression and clinical outcomes of hepatocellular carcinoma. We observed that higher SPP1 expression was correlated with overall survival (OS), progression free survival (PFS) and with stage, grade, T stage, vascular invasion, gender, race, alcohol consumption and Hepatitis virus (Table 1). Kaplan-Meier analysis showed that the PFS and OS of SPP1 high patients were shorter than those of SPP1 low patients in hepatocellular cancer (HR=1.59, p=0.0017; HR=2.27, p=3.5e-06; Fig. 3a and 3b). In different hepatitis B virus infection status and different race population, further analysis confirmed thdat patients with high level of SPP1 had worse OS. Compared to SPP1 low patients, SPP1 high patients were correlated with poor OS in HBV+ patients (HR=3.16, p=0.00024), HBV- patients (HR=2.51, p=0.00014), Asia population (HR=3.3, p=5.7e-05) and white population (HR=1.9, p=0.016) (Fig. 3c-3f). Taken together, all results implied that SPP1 is a potential prognostic factor of hepatocellular cancer.

SPP1 expression is positive correlation with critical oncogenes in hepatocellular carcinoma.

It was reported that SPP1 participated in maintaining cancer stem cell phenotype [24], cell cycle progression [25], chemoresistance [26], cancer metastasis [27], and tumor angiogenesis [28, 29]. So we analyzed the association between SPP1 and cancer stemness associated genes (PROM1, CD44, NANOG, Olig2, L1CAM), cell cycle and proliferation oncogenes (MKI67, HELLS, NEK2, MELK, FOXM1), therapeutic resistance genes (EZH2, EGFR, VIM, AURKA, CHEK1), metastasis (MMP2, MMP7, MMP9, TWIST1, SNAI1), and tumor angiogenesis genes (HIF1A, VEGFA, VEGFB, VEGFC) in TIMER database. We found the elevated SPP1 expression was related with CD44, PROM1, Olig2, L1CAM, MKI67, HELLS, NEK2, MELK, FOXM1, EZH2, VIM, AURKA, CHEK1, MMP2, MMP7, MMP9, TWIST1, SNAI1, HIF1A, VEGFA, VEGFB, VEGFC(p□0.05) (Table 2). The similar correlation analysis results in LIHC were found in GEPIA database (Supplementary Fig. 1a-1f). These results implied that SPP1 played an important role in regulating malignant phenotypes in hepatocellular carcinoma.

SPP1 expression is correlated with infiltrating immune cells in hepatocellular carcinoma

Previous studies showed that tumor survival could be predicted by tumor-infiltrating lymphocytes (TILs) [30-33]. Emerging reports indicated that SPP1 functions in the tumor microenvironment through regulating macrophages and T cells [34, 35]. Based on this, we further investigated the relationship between SPP1 expression and TIL abundance via TIMER database and TISIDB database. We analyzed the relation between SPP1 expression with the levels of infiltrating B cells, CD8+ cells, CD4+ cells, macrophages, neutrophils and dendritic cells in hepatocellular carcinoma. We observed that SPP1 was significantly associated with tumor purity (r=0.177, p=9.73e-04), and the levels of infiltrating B cell (r=0.474, p=1.24e-20), CD8+ cells (r=0.284, p=9.30e-08), CD4+ cells (r=0.378, p=3.84e-13),

macrophages (r=0.436, p=3.22e-17), neutrophils (r=0.374, p=7.02e-13) and dendritic cells (r=0.453, p=1.38e-18). (Fig. 4a). We then detected the correlation between infiltrating cell and SPP1 expression by Kaplan-Meier plots using TIMER database. However, we didn't observe that infiltrating immune cells were significantly related to the prognosis of hepatocellular cancer (Fig. 4b). In addition, we compared the immune cell infiltration levels with different somatic copy number alterations of SPP1 in hepatocellular carcinoma. We found that the infiltration levels of CD4+ T cells was correlated with SPP1 arm-level deletion (Fig. 4c).

Next, we evaluated the associations between SPP1 expression and immune subtypes in hepatocellular carcinoma by TISIDB database. The cells were divided into six immuno-phenotypes C1 (wound healing), C2 (IFN-gamma dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet), C6 (TGF-β dominant). But we didn't find that SPP1 expression was correlated with C1, C2, C3 and C4 in hepatocellular carcinoma (Fig. 4d). We further investigated the associations between SPP1 expression and molecular subtypes in hepatocellular carcinoma. The results showed that SPP1 expression wasn't correlated with iCluster:1, iCluster:2 and iCluster:3 (Fig. 4e). These results demonstrated that SPP1 could recruit tumor infiltration immune cells in hepatocellular carcinoma.

Correlation analysis between SPP1 and immune marker expression

To further explore the effects of SPP1 expression on tumor infiltration immune cells, we used TIMER, TISIDB and GEPIA online database. The heatmap of relationship between SPP1 expression and TILs in various cancers was showed in Fig. 5a). We observed that there was a strong correlation between SPP1 expression and abundance of 20 TILs types in hepatocellular carcinoma (Fig. 5b-5g), Supplementary Table 1). Next, we assessed the correlations between SPP1 expression and immunoinhibitors of hepatocellular carcinoma in TISIDB（Fig. 5h）. The results showed that a significant positive correlation between SPP1 expression and immunoinhibitors, such as PDCD1 (PD-1), CTLA4, and TIGIT (Fig. 5i-5n), Supplementary Table 2), suggesting that SPP1 expression were significantly associated with immune-checkpoint and SPP1 may play an important role in immune escape of hepatocellular carcinoma.

We further investigated the relationship between SPP1 expression and particular cell subsets including CD8+ T cells, general T cells, monocytes, TAM, macrophages, neutrophils, natural killer cells, dendritic cells, Th1 cells, Th2 cells and exhaustion T cells. The results were adjusted based on tumor purity. We observed a significant correlation between SPP1 expression and markers of CD8+ T cell (CD8A, CD8B), T cell (CD3D, CD3E, CD2), monocyte (CD86, CD115), TAM (CCL2, CD68, IL10), M1 macrophage (IRF5, COX2), M2 macrophage (CD163, VSIG4, MS4A4A), neutrophils (CD11b), DC (HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1), Th1 (STAT4, STAT1, IFN-γ, TNF-α), Th2 (GATA3, STAT6, TGFβ), T cell exhaustion (PD-1, CTLA4, TIM-3) in hepatocellular carcinoma (Supplementary table 3). These results suggested that SPP1 may participate the regulation of macrophage polarization and DC infiltration. Taken together, these findings indicated that SPP1 expression significantly correlated with immune microenvironment and may promote tumor immune escape process in hepatocellular carcinoma.

Functional enrichment analysis of SPP1 in patients with hepatocellular carcinoma

To better understand the interplay functions of SPP1 and neighboring genes, we accessed SPP1 networks using GeneMANIA online dataset. The results demonstrated that the extracellular matrix gene FN1, integrin family gene ITGA5, ITGA8, ITGAV, ITGA9, ITGB8, apoptosis genes CASP3, CASP8, extracellular matrix disassembly gene MMP7, integrin-mediated signaling pathway genes MAP3K1, MAP3K14, leukocyte migration genes PDLIM7, SYK and oncogenes BRCA1, RIMS4, ETV4 and DSEL were closely associated with SPP1 (Fig. 6a). Among them, ITGA5 and F2 were found as the top two significant hallmarks in the PPI network relating to SPP1.

Next, functional enrichment analysis were predicted by analyzing GO and KEGG in Metascape. The top 20 GO enrichment items were classified into three functional groups: 10 items of biological process group, 5 items of molecular function group and 5 items of cellular component group. Consistent with our preceding analysis，the results showed strong relationship with immune response. Top enriched ontology clusters of SPP1 and its neighboring genes included immune response-activating signal transduction, immune system process, immune-regulatory interactions between a lymphoid and a non-lymphoid cell, regulation of cell activation. Moreover, all the pathways achieved from the KEGG analysis were related with immune response (Fig. 6b-6e).

Discussion

In view of high prevalence of HBV, liver cancer is the leading incidence cancer in China. And survival outcome of HCC patients is still poor receiving with existing therapies [2]. Therefore, the determination of molecular markers has attracted much attention in the treatment and prognosis of hepatocellular cancer. SPP1 is a secreted arginine glycine aspartic acid containing phosphorylated glycoprotein overexpressed in various malignant neoplasms, and it is involved in various functions, such as in cell adhesion and migration, apoptosis, metastasis, tumor angiogenesis, and initiating cell self-renewal. SPP1 is often overexpressed in multiple cancers including pancreatic cancer, lung cancer, gastric cancer, breast cancer and colon cancer [10, 36, 37]. SPP1 mRNA and protein increase in HCC. SPP1 is secreted by HCC cells and serum SPP1 increases in HCC patients [17, 18]. As the combination of AFP and SPP1 significantly improved diagnosis performance when compared with AFP alone, SPP1 was considered as a promising serological biomarker for hepatocellular carcinoma diagnosis.[38] And SPP1 was also referred as an independent prognostic parameter for survival in HCC [19]. Previous study implied SPP1 promotes progression and cancer stem cell-like phenotype in hepatocellular carcinoma cells via the αvβ3–NF-κB–HIF-1α pathway [39]. However，the underlying mechanisms are still unclear and need be fully elucidated.

Recently, tumor immunotherapy such as anti-PD-1/PD-L1/CTLA-4 monoclonal antibody has extensively focused as a monotherapy, or an important part of combined therapy. Immunotherapy is fundamentally different from targeted therapy or chemotherapy [40]. Instead of directly targeting cancer cells, it recruits and activates core immune guardian T cells to recognize and eliminate cancer cells through antigen antibody response [41]. Unfortunately, only about 20% patients respond to immunotherapy, especially in hepatocellular carcinoma [42]. Therefore, it is urgent to identify new potential targets for immune-related therapy. It is reported that there is a positive correlation between the SPP1 and PD-L1 expression, and SPP1 expression and TAM infiltration in tumor tissues from patients with HCC [17]. To gain more detailed insights into the potential immune functions of SPP1 in hepatocellular carcinoma and its regulatory network, we performed the bioinformatics analysis of public data to guide future research in hepatocellular carcinoma.

In this study, we analyzed the expression and prognosis of SPP1 in 33 different types of cancers by three different online databases: Oncomine database, GEPIA database and TIMER database. Oncomine database showed elevate expression of SPP1 levels in many cancers except for sarcoma (Fig. 1a). GEPIA database showed that SPP1 expression was up-regulated in BLCA, brain and CNS cancer, BRCA, CESC, COAD, READ, ESCA, GBM, STAD, HNSC, KIRP, LGG, LIHC, LUAD, LUSC, OV, PAAD, SKCM, TGCT, THCA, UCEC and UCS compared to normal tissues (Fig. 1b). TIMER database revealed that SPP1 was high expressed in BLCA, BRCA, CHOL, COAD, ESCA, HNSC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA and UCEC relative to normal tissues, whereas expression was decreased in KIRP and KIRC than in control samples (Fig. 1c). HPA database indicated that SPP1 existed in both cell membrane and cytoplasm, and about 45.8% hepatocellular carcinoma patients with SPP1 high expression, while SPP1

expression was not detected in normal liver tissue (Fig. 2c-2d). Kaplan-Meier plotter database found elevated SPP1 was associated with poor outcomes (Fig. 3a-3f). The above results together imply that SPP1 may have an important value as a prognostic biomarker of hepatocellular carcinoma.

Previous study showed that SPP1 is associated with tumor development, progression and metastasis [43]. SPP1 affect the receptors of different integrin and CD44 to cause tumor progression, and SPP1 is required in maintaining stem-like properties in HCC cells [39, 44]. In breast cancer, SPP1 control cancer progression and angiogenesis through ILK and NF-κB-mediated HIF1α-dependent VEGF expression [29]. To verified the role of SPP1 in tumor development, progression, metastasis and stemness, we analyzed the relationship between SPP1 and critical oncogenes related stemness associated genes, cell cycle and proliferation, therapeutic resistance, metastasis, and tumor angiogenesis in hepatocellular carcinoma. We found SPP1 overexpression was related with CD44, PROM1, L1CAM, MKI67, HELLS, NEK2, MELK, FOXM1, EZH2, VIM, CHEK1, MMP2, MMP7, MMP9, TWIST1, SNAI1, HIF1A, VEGFA, VEGFC (Table 2). These results referred that SPP1 played an important role in regulating malignant phenotypes in hepatocellular carcinoma.

Immune infiltrating cells in the tumor microenvironment (TME) have been implied to play a vital role in tumor progression and influence clinical outcomes in cancer patients [45]. In our report, we found that SPP1 expression was correlated with TILs abundance. We demonstrated that SPP1 could recruit CD8+ cells, CD4+ cells, macrophages, neutrophils and dendritic cells in hepatocellular carcinoma (Fig. 4a). However, we didn't observe that infiltrating immune cells were significantly related to the prognosis of hepatocellular cancer (Fig. 4b). Then, we found that the infiltration levels of CD4+ T cells was correlated with SPP1 arm-level deletion (Fig. 4c). In addition, we observed that there was a strong correlation between SPP1 expression and abundance of 20 TILs types in hepatocellular carcinoma (Fig. 5a-5g), Supplementary Table 1), and a significant positive correlation between SPP1 expression and immunoinhibitors, such as PDCD1 (PD-1), CTLA4, and TIGIT (Fig. 5h-5n, Supplementary Table 2), suggesting that SPP1 may play an important role in immune escape of hepatocellular carcinoma. Taken together, these findings indicated that SPP1 expression significantly correlated with tumor immune microenvironment and may promote tumor immune escape process.

Enrichment analysis of target gene sets can help reveal important networks of transcription factors, target genes and pathway hallmarks. Our study suggested that neighboring gene network of SPP1 was associated with extracellular matrix, integrin, apoptosis, integrin-mediated signaling pathway and leukocyte migration (Fig. 6a). Functional enrichment analysis suggested strong relationship with immune response including immune response-activating signal transduction, immune system process, immune-regulatory interactions. (Fig. 6b-6e). The findings further highlighted that SPP1 was closely related with immune response.

Conclusions

SPP1 may be a promising prognostic biomarker and an important regulator of tumor immune cell infiltration for hepatocellular carcinoma patients, offering a new potential immunotherapeutic target in hepatocellular carcinoma.

Methods

Oncomine database analysis

The expression levels of SPP1 in HCC were analyzed using the ONCOMINE database (www.oncomine.org)[46, 47]. The cutoff p-valueand fold-change values were as follows: *P*-value: 1E-4; fold change:2.0; gene rank: 10%.

The Human Protein Atlas database

The SPP1 immumohistochemical (IHC) staining analysis was assessed by The Human Protein Atlas (HPA) database (https://www.proteinatlas.org/) [48]. We evaluated the protein expression in HCC and normal liver tissue, separately. The SPP1 antibody was HAP027541.

GEPIA database analysis

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia2.cancer-pku.cn/#index) is an online database to analyze the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the The Cancer Genome Atlas (TCGA) and the Genotype- Tissue Expression (GTEx) database [49]. We use this dataset to evaluate SPP1 expression levels in hepatocellular carcinoma and we also evaluate SPP1 expression in different tumor stages of HCC.

Kaplan-Meier plotter analysis

The clinical outcomes between SPP1 mRNA level and HCC patients were evaluated with Kaplan-Meier Plotter (www.kmplot.com)[50]. RNA-Seq ID of SPP1 is 6696 in Start KM Plotter for 364 HCC patients with OS data and 370 HCC patients with PFS data. The overall survival (OS) and Progression Free Survival (PFS) of patients with HCC were determined by dividing the patient samples into two groups based on best cutoff (high vs. low expression). P-value 0.05 was considered a statistical significance.

Spearman relationship analysis

Using Tumor Immune Estimation Resource (TIMER) database (https://cistrome.shinyapps.io/timer/) and GEPIA database,

we analyzed Spearman relationship between SPP1 and critical oncogenes related stemness associated genes, cell cycle and proliferation, therapeutic resistance, metastasis, and tumor angiogenesis in hepatocellular carcinoma. P-value0.05 was considered a statistical significance.

Immune infiltration analysis

The expression of SPP1 in HCC and the abundances of B cell, CD8+ T cell, CD4+ T cell, macrophage, neutrophil and dendritic cell were evaluated by TIMER database (https://cistrome.shinyapps.io/timer/) [51]. TISIDB dataset

(http://cis.hku.hk/TISIDB/) , a web portal for tumor and immune system interaction, was used to assess the correlations between SPP1 expression and tumor infiltration lymphocytes (TILs) of HCC. Spearman correlations between expression of SPP1 and immunoinhibitors across HCC were also performed by TISIDB dataset[52].

Functional enrichment analysis

The GeneMANIA project is a biological network integration for gene prioritization and predicting gene function (http://genemania.org/). In this study, protein–protein interaction (PPI) network of SPP1 was analyzed with the GeneMANIA[53].We used the Metascape database (https://metascape.org) to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analyses of SPP1[54]. Terms with a p-value < 0.01, a minimum count of 3, and an enrichment factor > 1.5 are collected and grouped into clusters based on their membership similarities.

Abbreviations

SPP1: Secreted phosphoprotein 1

OPN: Osteopontin

GEPIA: Gene Expression Profiling Interactive Analysis

TIMER: Tumor Immune Estimation Resource

HPA: The Human Protein Atlas

HCC: liver hepatocellular carcinoma

LIHC: liver hepatocellular carcinoma

ECM: extracellular matrix

IHC: immumohistochemical

OS: overall survival

DFS: progression free survival

TILs: tumor-infiltrating lymphocytes

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

AFP: alpha fetoprotein

TAM: tumor-associated macrophage

Declarations

Author information

Affiliations

XiaoFeng Wang, Li Geng

Department of Medical Oncology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China.

Kun Zhang

Department of Geriatrics, Hainan General Hospital, Haikou, China.

DongLi Liu

Department of Radiation Oncology, Shanghai General Hospital, China.

Contributions

DL Liu and L Geng conceived and designed the study. XF Wang and K Zhang analyzed the data and prepared the manuscript. All authors read and approved the final manuscript.

XiaoFeng Wang and Kun Zhang contributed equally to this work and should be considered co-first authors.

Corresponding author

Correspondence to DongLi Liu or Li Geng.

Ethics declarations

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

The project is supported by the National Natural Science Foundation of China (82003117).

Availability of data and materials

All data are included in the manuscript. However, the raw data used and/or analyzed in the present study are available from the corresponding author on reasonable request.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians* 2021.
2. Feng RM, Zong YN, Cao SM, Xu RH. Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? *Cancer communications (London, England)* 2019; 39(1):22.
3. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet (London, England)* 2017; 389(10088):2492-2502.
4. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *The Lancet Oncology* 2018; 19(7):940-952.
5. Xu J, Shen J, Gu S, Zhang Y, Wu L, Wu J, et al. Camrelizumab in Combination with Apatinib in Patients with Advanced Hepatocellular Carcinoma (RESCUE): A Nonrandomized, Open-label, Phase II Trial. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2021; 27(4):1003-1011.
6. Young MF, Kerr JM, Termine JD, Wewer UM, Wang MG, McBride OW, et al. cDNA cloning, mRNA distribution and heterogeneity, chromosomal location, and RFLP analysis of human osteopontin (OPN). *Genomics* 1990;

7(4):491-502.

7. Moorman HR, Poschel D, Klement JD, Lu C, Redd PS, Liu K. Osteopontin: A Key Regulator of Tumor Progression and Immunomodulation. *Cancers* 2020; 12(11).
8. Almozyan S, Colak D, Mansour F, Alaiya A, Al-Harazi O, Qattan A, et al. PD-L1 promotes OCT4 and Nanog expression in breast cancer stem cells by sustaining PI3K/AKT pathway activation. *International journal of cancer* 2017; 141(7):1402-1412.
9. Oldberg A, Franzén A, Heinegård D. Cloning and sequence analysis of rat bone sialoprotein (osteopontin) cDNA reveals an Arg-Gly-Asp cell-binding sequence. *Proceedings of the National Academy of Sciences of the United States of America* 1986; 83(23):8819-8823.
10. Fedarko NS, Jain A, Karadag A, Van Eman MR, Fisher LW. Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer. *Clin Cancer Res* 2001; 7(12):4060-4066.
11. Lin F, Li Y, Cao J, Fan S, Wen J, Zhu G, et al. Overexpression of osteopontin in hepatocellular carcinoma and its relationships with metastasis, invasion of tumor cells. *Mol Biol Rep* 2011; 38(8):5205-5210.
12. Hu Z, Lin D, Yuan J, Xiao T, Zhang H, Sun W, et al. Overexpression of osteopontin is associated with more aggressive phenotypes in human non-small cell lung cancer. *Clin Cancer Res* 2005; 11(13):4646-4652.
13. Le QT, Sutphin PD, Raychaudhuri S, Yu SC, Terris DJ, Lin HS, et al. Identification of osteopontin as a prognostic plasma marker for head and neck squamous cell carcinomas. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2003; 9(1):59-67.
14. Mack PC, Redman MW, Chansky K, Williamson SK, Farneth NC, Lara PN, Jr., et al. Lower osteopontin plasma levels are associated with superior outcomes in advanced non-small-cell lung cancer patients receiving platinum-based chemotherapy: SWOG Study S0003. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2008; 26(29):4771-4776.
15. Ng L, Wan T, Chow A, Iyer D, Man J, Chen G, et al. Osteopontin Overexpression Induced Tumor Progression and Chemoresistance to Oxaliplatin through Induction of Stem-Like Properties in Human Colorectal Cancer. *Stem cells international* 2015; 2015:247892.
16. Insua-Rodríguez J, Pein M, Hongu T, Meier J, Descot A, Lowy CM, et al. Stress signaling in breast cancer cells induces matrix components that promote chemoresistant metastasis. *EMBO molecular medicine* 2018; 10(10).
17. Zhu Y, Yang J, Xu D, Gao XM, Zhang Z, Hsu JL, et al. Disruption of tumour-associated macrophage trafficking by the osteopontin-induced colony-stimulating factor-1 signalling sensitises hepatocellular carcinoma to anti-PD-L1 blockade. *Gut* 2019; 68(9):1653-1666.
18. Song Z, Chen W, Athavale D, Ge X, Desert R, Das S, et al. Osteopontin takes center stage in chronic liver disease. *Hepatology (Baltimore, Md)* 2020.
19. Bruha R, Vitek L, Smid V. Osteopontin - A potential biomarker of advanced liver disease. *Annals of hepatology* 2020; 19(4):344-352.
20. Rabinowich H, Lin WC, Amoscato A, Herberman RB, Whiteside TL. Expression of vitronectin receptor on human NK cells and its role in protein phosphorylation, cytokine production, and cell proliferation. *J Immunol* 1995; 154(3):1124-1135.
21. Zhang Y, Du W, Chen Z, Xiang C. Upregulation of PD-L1 by SPP1 mediates macrophage polarization and facilitates immune escape in lung adenocarcinoma. *Exp Cell Res* 2017; 359(2):449-457.
22. Szulzewsky F, Pelz A, Feng X, Synowitz M, Markovic D, Langmann T, et al. Glioma-associated microglia/macrophages display an expression profile different from M1 and M2 polarization and highly express Gpnmb and Spp1. *PloS one* 2015; 10(2):e0116644.

23. Capote J, Kramerova I, Martinez L, Vetrone S, Barton ER, Sweeney HL, et al. Osteopontin ablation ameliorates muscular dystrophy by shifting macrophages to a pro-regenerative phenotype. *J Cell Biol* 2016; 213(2):275-288.
24. Lamour V, Henry A, Kroonen J, Nokin MJ, von Marschall Z, Fisher LW, et al. Targeting osteopontin suppresses glioblastoma stem-like cell character and tumorigenicity in vivo. *International journal of cancer* 2015; 137(5):1047-1057.
25. Xu C, Sun L, Jiang C, Zhou H, Gu L, Liu Y, et al. SPP1, analyzed by bioinformatics methods, promotes the metastasis in colorectal cancer by activating EMT pathway. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2017; 91:1167-1177.
26. Yang MC, Wang HC, Hou YC, Tung HL, Chiu TJ, Shan YS. Blockade of autophagy reduces pancreatic cancer stem cell activity and potentiates the tumoricidal effect of gemcitabine. *Molecular cancer* 2015; 14:179.
27. Kothari AN, Arffa ML, Chang V, Blackwell RH, Syn WK, Zhang J, et al. Osteopontin-A Master Regulator of Epithelial-Mesenchymal Transition. *Journal of clinical medicine* 2016; 5(4).
28. Zhao H, Chen Q, Alam A, Cui J, Suen KC, Soo AP, et al. The role of osteopontin in the progression of solid organ tumour. *Cell death & disease* 2018; 9(3):356.
29. Raja R, Kale S, Thorat D, Soundararajan G, Lohite K, Mane A, et al. Hypoxia-driven osteopontin contributes to breast tumor growth through modulation of HIF1α-mediated VEGF-dependent angiogenesis. *Oncogene* 2014; 33(16):2053-2064.
30. Azimi F, Scolyer RA, Rumcheva P, Moncrieff M, Murali R, McCarthy SW, et al. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. *J Clin Oncol* 2012; 30(21):2678-2683.
31. Oble DA, Loewe R, Yu P, Mihm MC, Jr. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human melanoma. *Cancer Immun* 2009; 9:3.
32. Ohtani H. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human colorectal cancer. *Cancer Immun* 2007; 7:4.
33. Uppaluri R, Dunn GP, Lewis JS, Jr. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in head and neck cancers. *Cancer Immun* 2008; 8:16.
34. Wei J, Marisetty A, Schrand B, Gabrusiewicz K, Hashimoto Y, Ott M, et al. Osteopontin mediates glioblastoma-associated macrophage infiltration and is a potential therapeutic target. *The Journal of clinical investigation* 2019; 129(1):137-149.
35. Klement JD, Paschall AV, Redd PS, Ibrahim ML, Lu C, Yang D, et al. An osteopontin/CD44 immune checkpoint controls CD8+ T cell activation and tumor immune evasion. *The Journal of clinical investigation* 2018; 128(12):5549-5560.
36. Kolb A, Kleeff J, Guweidhi A, Esposito I, Giese NA, Adwan H, et al. Osteopontin influences the invasiveness of pancreatic cancer cells and is increased in neoplastic and inflammatory conditions. *Cancer biology & therapy* 2005; 4(7):740-746.
37. Wang X, Zhang F, Yang X, Xue M, Li X, Gao Y, et al. Secreted Phosphoprotein 1 (SPP1) Contributes to Second-Generation EGFR Tyrosine Kinase Inhibitor Resistance in Non-Small Cell Lung Cancer. *Oncology research* 2019; 27(8):871-877.
38. Zhu M, Zheng J, Wu F, Kang B, Liang J, Heskia F, et al. OPN is a promising serological biomarker for hepatocellular carcinoma diagnosis. *Journal of medical virology* 2020.
39. Cao L, Fan X, Jing W, Liang Y, Chen R, Liu Y, et al. Osteopontin promotes a cancer stem cell-like phenotype in hepatocellular carcinoma cells via an integrin-NF-κB-HIF-1α pathway. *Oncotarget* 2015; 6(9):6627-6640.

40. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; 366(26):2455-2465.
41. Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol* 2016; 17(6):717-726.
42. Wing-Sum Cheu J, Chak-Lui Wong C. Mechanistic Rationales guiding Combination Hepatocellular Carcinoma Therapies involving Immune Checkpoint Inhibitors. *Hepatology (Baltimore, Md)* 2021.
43. Icer MA, Gezmen-Karadag M. The multiple functions and mechanisms of osteopontin. *Clinical biochemistry* 2018; 59:17-24.
44. Rangaswami H, Bulbule A, Kundu GC. Osteopontin: role in cell signaling and cancer progression. *Trends in cell biology* 2006; 16(2):79-87.
45. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nature medicine* 2013; 19(11):1423-1437.
46. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia* 2004; 6(1):1-6.
47. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, et al. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia* 2007; 9(2):166-180.
48. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. *Science* 2015; 347(6220):1260419.
49. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic acids research* 2017; 45(W1):W98-w102.
50. Nagy A, Lanczky A, Menyhart O, Gyorffy B. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. *Sci Rep* 2018; 8(1):9227.
51. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer Res* 2017; 77(21):e108-e110.
52. Ru B, Wong CN, Tong Y, Zhong JY, Zhong SSW, Wu WC, et al. TISIDB: an integrated repository portal for tumor-immune system interactions. *Bioinformatics* 2019; 35(20):4200-4202.
53. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic acids research* 2010; 38(Web Server issue):W214-220.
54. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun* 2019; 10(1):1523.

Tables

Table 1. Correlation between SPP1 expression and clinicopathological factors in hepatocellular cancer by Kaplan-Meier plotter database.

Clinicopathological characteristics	Progression free survival (N=370)					Overall survival (N=364)				
	N	SPP1 expression		Hazard ratio	P value	N	SPP1 expression		Hazard ratio	P value
		low	*high*				*low*	*high*		
Stage										
1	170	108	62	1.95(1.11-3.21)	0.079	170	108	62	2.9(1.55-5.4)	***0.00046***
2	85	59	26	1.84(1-3.4)	***0.047***	83	59	24	1.75(0.78-3.9)	0.17
3	85	23	62	0.71(0.4-1.27)	0.24	83	42	41	2.11(1.14-3.91)	***0.015***
4	5	-	-	-	-	4	-	-	-	-
Grade										
1	55	40	15	1.67(0.73-3.86)	0.22	55	40	15	2.1(0.81-5.45)	0.12
2	177	93	84	1.79(1.16-2.78)	***0.0083***	174	91	83	2.07(1.21-3.53)	***0.0066***
3	121	29	92	1.56(0.86-2.83)	0.14	118	86	32	3.44(1.88-6.29)	***2e-05***
4	12	-	-	-	-	12	-	-	-	-
AJCC T										
1	180	108	73	2.04(1.26-3.31)	***0.0033***	180	113	67	2.86(1.58-5.18)	***0.00028***
2	93	63	30	1.7(0.97-3)	0.062	92	62	28	1.86(0.89-3.87)	0.092
3	80	19	61	0.7(0.37-1.3)	0.25	78	25	53	2.31(1.16-4.61)	***0.015***
4	13	-	-	-	-	13	-	-	-	-
Vascular invasion										
none	205	129	76	2.17(1.39-3.4)	***5e-04***	203	129	74	2.33(1.38-3.92)	***0.0011***
micro	92	37	55	0.54(0.3-0.96)	***0.033***	90	23	67	5.29(1.25-22.42)	***0.011***
macro	16	-	-	-	-	16	-	-	-	-
Gender										
male	249	186	63	1.79(1.21-2.64)	***0.0031***	246	146	100	2.66(1.69-4.17)	***1.1e-05***
female	121	69	52	1.51(0.91-2.53)	0.11	118	68	50	1.99(1.12-3.52)	***0.016***

Race										
asia	157	85	72	2.06(1.28-3.29)	***0.0022***	155	91	64	3.3(1.78-6.11)	***5.7e-05***
white	184	53	131	1.66(1.04-2.65)	***0.032***	181	56	125	1.9(1.12-3.24)	***0.016***
Alcohol consumption										
yes	117	31	86	1.96(1.04-3.71)	***0.035***	125	37	78	3.03(1.38-6.63)	***0.0037***
none	203	122	83	1.75(1.17-2.62)	***0.0061***	202	114	88	2.36(1.47-3.79)	***0.00024***
Hepatitis virus										
yes	153	90	63	2.05(1.29-3.26)	***0.0018***	150	111	39	3.16(1.65-6.05)	***0.00024***
none	169	73	96	1.51(0.97-2.37)	0.068	167	82	85	2.51(1.54-4.08)	***0.00014***

- : Sample number of these subtypes was too low for meaningful analysis.

Table 2. SPP1 expression is positive correlation with critical oncogenes.

Stemness					
	CD44	PROM1	Olig2	NANOG	L1CAM
r	0.494	0.285	0.138	-0.038	0.257
p value	***3.08e-24***	***2.39e-08***	***0.0076***	0.465	***5.48e-07***
Cell cycle					
	MKI67	HELLS	NEK2	MELK	FOXM1
r	0.237	0.195	0.258	0.279	0.212
p value	***3.89e-06***	***1.53e-04***	***4.80e-07***	***4.55e-08***	***3.92e-05***
Therapeutic resistance					
	EZH2	EGFR	VIM	AURKA	CHEK1
r	0.198	-0.091	0.274	0.133	0.239
p value	***1.27e-04***	0.0792	***8.49e-08***	***0.0101***	***3.15e-06***
Metastasis					
	MMP2	MMP7	MMP9	TWIST1	SNAI1
r	0.276	0.568	0.484	0.236	0.192
p value	***6.46e-08***	***4.22e-33***	***3.64e-23***	***4.38e-06***	***2.02e-04***
Tumor angiogenesis					
	HIF1A	VEGFA	VEGFB	VEGFC	
r	0.359	0.152	0.128	0.188	
p value	***9.96e-13***	***0.00334***	***0.0137***	***2.61e-04***	

Figures

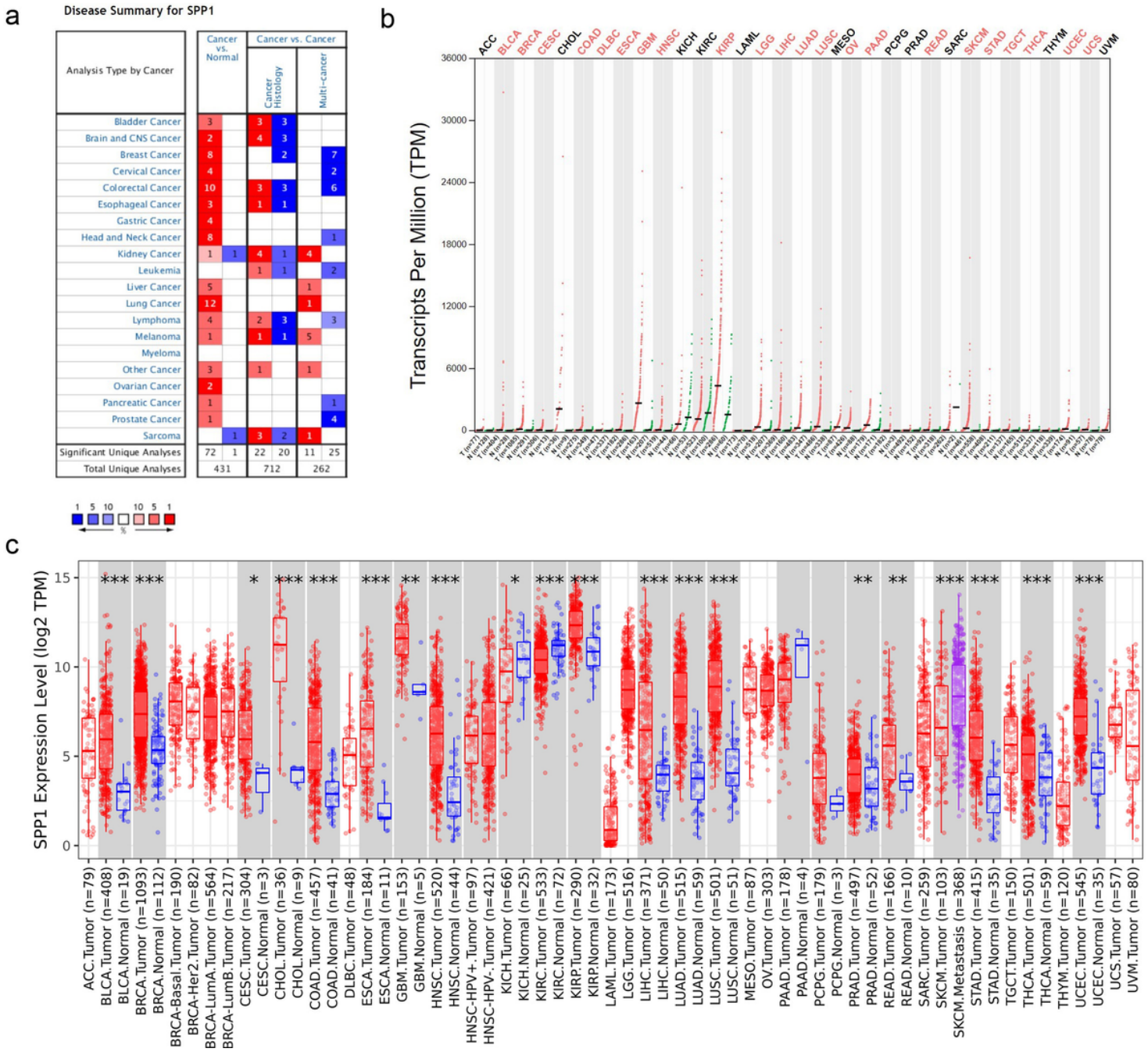

Figure 1

The expression level of SPP1 in different types of tumor tissues and normal tissues. a The expression of SPP1 in different types of tumor tissues and normal tissues in the Oncomine database. (P-value: 1E-4; fold change:2.0; gene rank: 10%.) b The expression of SPP1 in different types of tumor tissues and normal tissues in GEPIA database. c The expression of SPP1 in different types of tumor tissues and normal tissues in TIMER database (*P < .05, **P < .01, ***P < .001).

Figure 2

The expression level of SPP1 in hepatocellular carcinoma. a. The expression level of SPP1 in hepatocellular carcinoma and normal tissues in GEPIA database. b. The expression level of SPP1 in different stages of

hepatocellular carcinoma. (c-d) The distribution of SPP1 in liver cancer. Representative IHC images of SPP1 expression in normal liver tissue and hepatocellular cancer tissue.

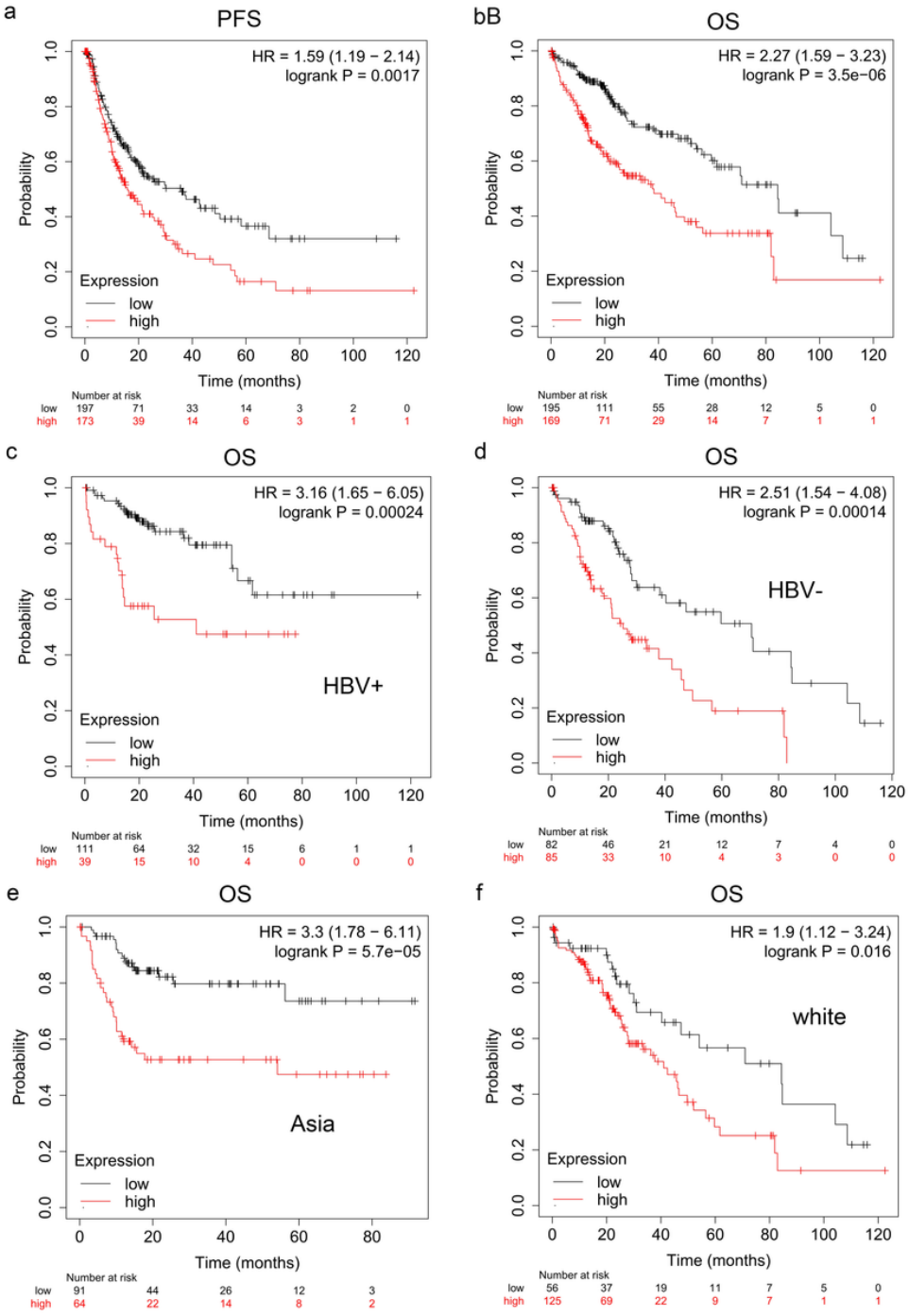

Figure 3

The prognostic value of SPP1 in hepatocellular carcinoma patients in Kaplan-Meier Plotter database. (a-b) Survival curves of OS and PFS of hepatocellular carcinoma in TCGA. (c-d) Survival curves of OS of hepatocellular carcinoma of different HBV infection status in TCGA. (e-f) Survival curves of OS of different race patients with hepatocellular carcinoma in TCGA.

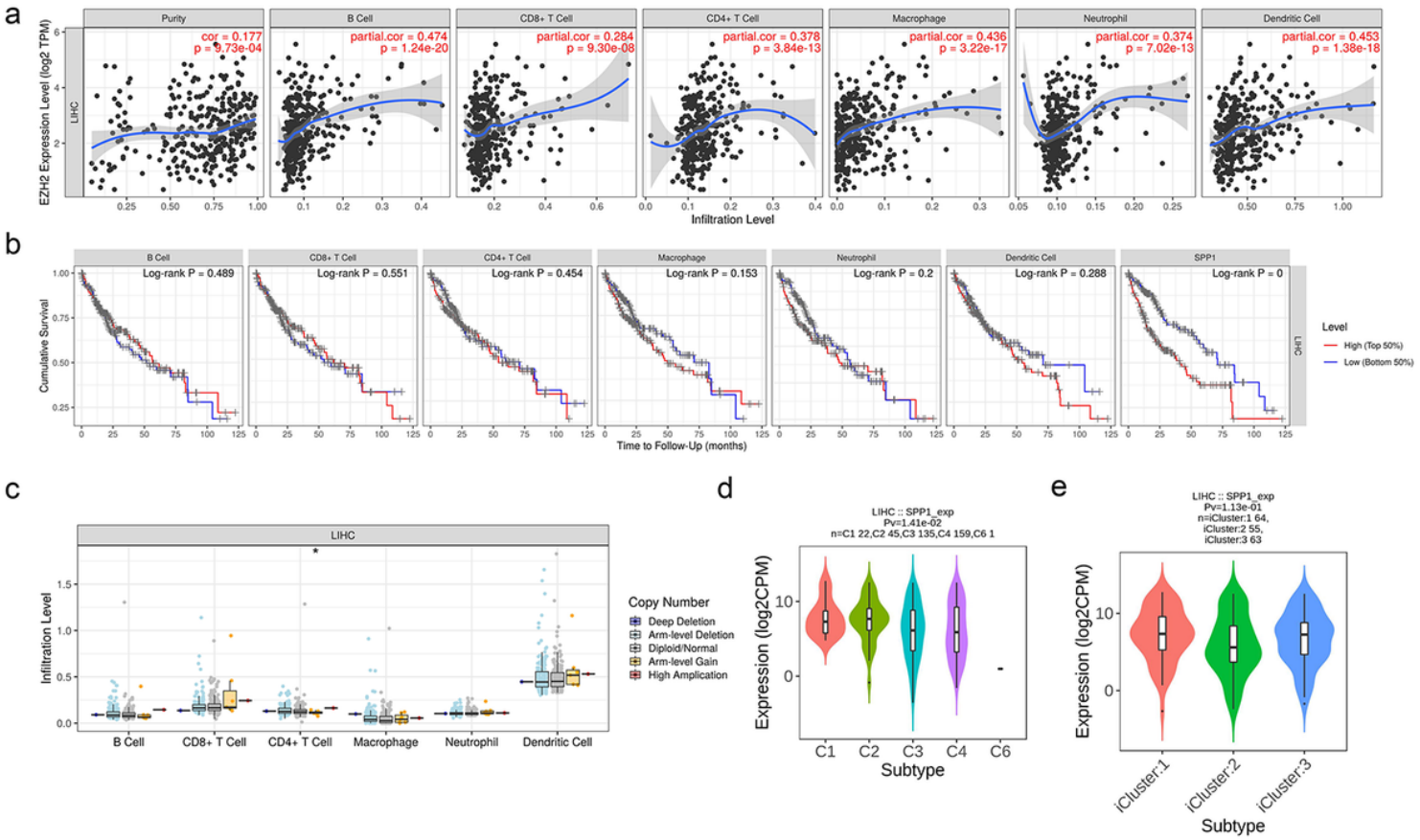

Figure 4

SPP1 expression is correlated with the level of immune infiltration in hepatocellular carcinoma. a SPP1 expression is correlated with the level of immune infiltration in hepatocellular carcinoma. b Kaplan-Meier plots of immune infiltration and SPP1 expression levels in hepatocellular carcinoma. c The comparison of tumor infiltration levels among tumors with different somatic copy number alterations for SPP1. The infiltration level for each SCNA category is compared with the normal using a two-sided Wilcoxon rank-sum test. (*P < 0.05, **P <0.01, ***P < .0001). d Correlation of SPP1 expression and immune subtypes (C1: wound healing, C2: IFN-gamma dominant, C3: inflammatory, C4: lymphocyte depleted) in hepatocellular carcinoma. e Correlation of SPP1 expression and immune subtypes (differentiated, immunoreactive, mesenchymal, proliferative) in hepatocellular carcinoma.

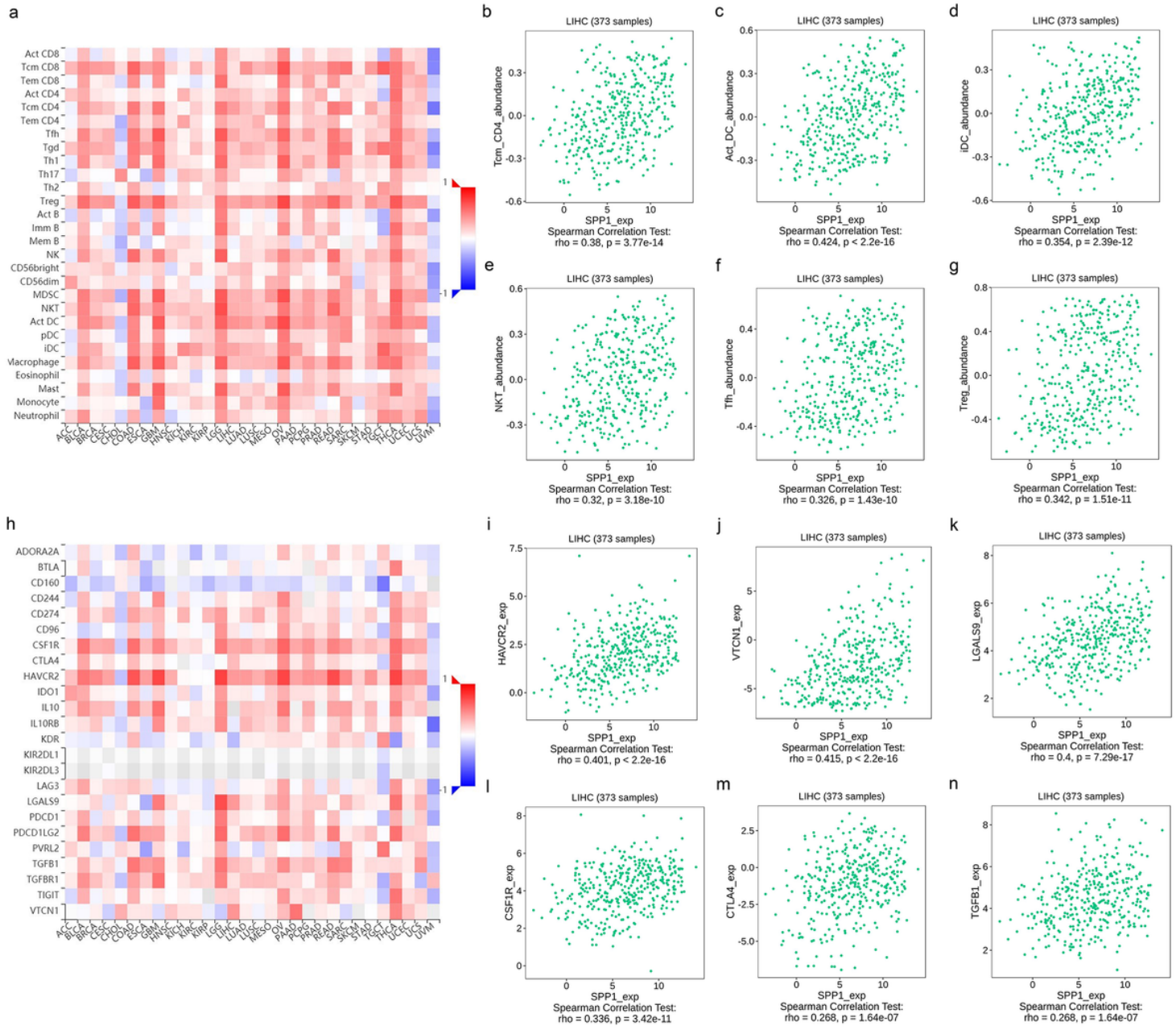

Figure 5

Correlation of SPP1 expression with immune cells in hepatocellular carcinoma. a The landscape of relationship between SPP1 expression and TILs in hepatocellular carcinoma r (red is positive correlated and blue is negative correlated). (b–g) SPP1 expression was positively closely related with infiltrating levels of Tcm_CD4, act_DC, iDC, NKT, Tfh, Treg. h The landscape of relationship between SPP1 expression and immunoinhibitors in hepatocellular carcinoma (red is positive correlated and blue is negative correlated). (i–n) SPP1 expression was positively closely related with infiltrating levels of HAVCR2, VTCN1, LGALS, CSF1R, CTLA4, and TGFB1.

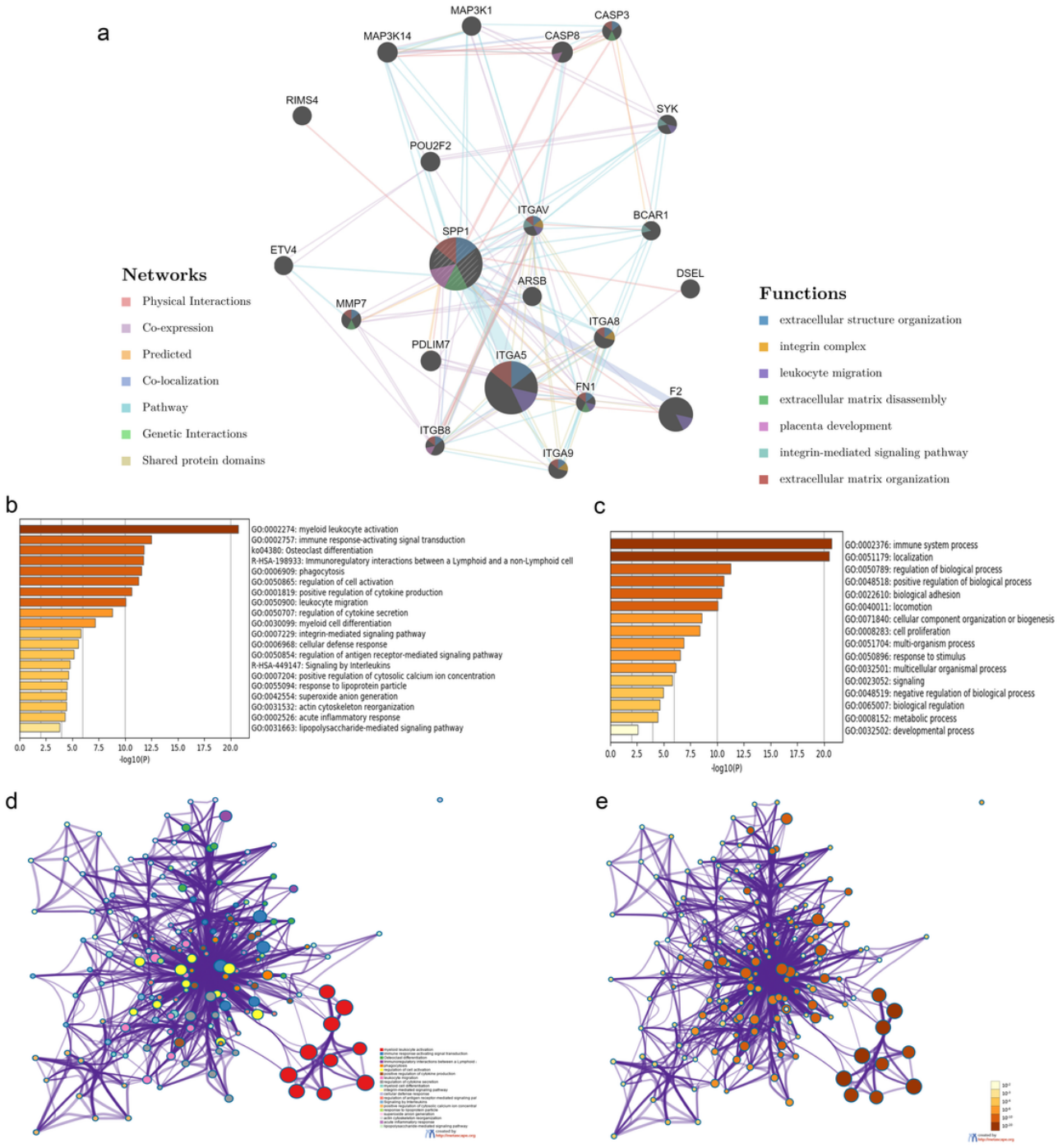

Figure 6

Functional enrichment analysis of SPP1 in cancer patients. a Protein-protein interaction network of SPP1 networks. The different colors for the network nodes indicate the biological functions of the set of enrichment genes. b Bar graph of Gene Ontology (GO) enriched terms colored by p-values. c Bar graph of the top level Gene Ontology (GO) biological processes. d Network of GO enriched terms colored by cluster ID. e Network of GO enriched terms colored by p-value.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementaryfiugresandtables.docx