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SPP2 plays a role in the tumorigenesis of hepatocellular carcinoma: A bioinformatic based analysis

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ABSTRACT

Objective: Hepatocellular carcinoma (HCC) patients at the same stage exhibit different prognosis, and the underlying molecular mechanism remains unclear. This study aims to identify the key genes impacting the prognosis of HCC patients.

Methods: Differentially expressed gene analyses were performed between HCC samples and normal ones, and between patients with long overall survival (OS) and those with short OS, in TCGA-LIHC and GSE14520 datasets. The Kaplan-Meier method with log-rank test was used to evaluate the role of secreted phosphoprotein 2 (SPP2) in the prognosis of HCC patients. Gene set enrichment analysis (GSEA) was used to understand the difference of enriched signaling pathways between SPP2-stratified HCC subgroups. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to predict the potential functional pathways in which SPP2 might participate.

Results: SPP2 was significantly down-regulated in tumors when compared with normal tissues, or in tumor samples with short OS when compared with those with long OS [fold change (FC) >2 and false discovery rate (FDR) <0.05]. Low expression of SPP2 was associated with worse clinicopathological features like vascular invasion (P=1.6e-05), poor cancer status (with tumor, P=0.021), advanced T stage (T3 or T4, P=4.5e-04), advanced TNM stage (stage III or IV, P=3.1e-04), and with unfavorable prognosis (shorter OS, P=0.002). Gene enrichment analyses revealed that SPP2 might involve in the metabolic homeostasis of HCC and in the development of liver fibrosis and cirrhosis.

Conclusion: SPP2 might inhibit the development of liver fibrosis and cirrhosis and the tumorigenesis of HCC, and analogs of SPP2 might be potential drugs in the prevention of these diseases.

KEY WORDS secreted phosphoprotein 2; hepatocellular carcinoma; gene set enrichment analysis; tumorigenesis

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基于生物信息学分析SPP2在肝癌发生过程中的作用

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[摘 要] 目的：分期一致的肝细胞癌(hepatocellular carcinoma，HCC)患者其预后可能不同，其分子生物学机制仍不明确。本研究旨在鉴定影响肝癌预后的关键核心基因。**方法：**在TCGA-LIHC和GSE14520数据集中，对HCC样本与正常样本、总生存期(overall survival，OS)长与OS短的患者进行差异表达基因分析。采用Kaplan-Meier法结合log-rank检验评价分泌型磷蛋白2(secreted phosphoprotein 2，SPP2)在HCC患者预后中的作用；基因集富集分析(gene set enrichment analysis，GSEA)了解SPP2分层的HCC亚组间富集信号通路的差异；基因本体论(Gene Ontology，GO)和京都基因与基因组数据库(Kyoto Encyclopedia of Genes and Genomes，KEGG)分析预测SPP2可能参与的信号分子通路。**结果：**与正常组织相比，肿瘤组织中分泌的SPP2明显下调，且与长OS相比，短OS肿瘤样本中分泌的SPP2明显下调[表达倍数>2和伪发现率(false discovery rate，FDR)<0.05]。SPP2低表达与较差的临床病理特征相关，如血管侵犯(*P*=1.6e-05)、较差的肿瘤状态(合并肿瘤)(*P*=0.021)、较晚期的T期(T3或T4期)(*P*=4.5e-04)、较晚期的TNM期(III或IV期)(*P*=3.1e-04)、较差的预后(较短的生存期)(*P*=0.002)。基因富集分析表明SPP2可能参与了HCC的代谢稳态以及肝纤维化和肝硬化的发生、发展。**结论：**SPP2可能抑制肝纤维化和肝硬化的发展以及HCC的肿瘤发生，且SPP2类似物可能是预防肝硬化和肝细胞癌发生的潜在药物。

[关键词] 分泌型磷蛋白2；肝细胞癌；基因集富集分析；肿瘤发生

Hepatocellular carcinoma (HCC) accounts for more than 80% of primary liver cancer, a leading cause of cancer-related death worldwide[1-2]. Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, nonalcoholic fatty liver disease (NAFLD), alcohol, aflatoxin, and aristolochic acid are the most important causes of HCC[2]. These varied risk factors, together with genetic susceptibilities, morphological diversity, inconsistent molecular and signal transduction network disorders, and microenvironmental discrepancies, contribute to the extraordinarily heterogeneity of the disease and partially result in different outcomes of HCC patients at the same stage[3-4]. In recent years, the rapid progress of molecular biotechnologies, particularly the next generation sequence, has been helpful to identify different molecular mechanisms and various biomarkers related to the initiation and progression of HCC. For example, whole-genome sequencing indicates that 30% to 40% of liver cancer exhibits tumor protein p53 (TP53) mutation, which plays essential role in the initiation of HCC, favors proliferation and invasion of the disease, and down-regulates immune response in HCC[5-7]. In addition, Bcl-2 associated athanogene 2 (BAG2), over-expressing in many types of cancer, promotes accumulation of mutant *TP53* by inhibiting murine double minute 2 (MDM2) -mediated ubiquitination and degradation of the mutant protein[8]. Although above studies have intended to understand the carcinogenesis and progression of HCC, the underlying mechanism has not yet been fully elucidated.

Due to the accumulative data deposited in public databases, particularly the Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) database, reanalyzing and integrating these shared data might provide novel insights into the mechanism of cancer development and provide biomarkers in predicting the prognosis of patients[9-10]. For instance, Hao, et al. [11] have identified that DNA methylation could be useful for the diagnosis of 4 common cancers (breast, colon, liver, and lung) via using whole-genome methylation data from TCGA data sets. Cursons, et al.[12] have evaluated natural killer (NK) cell infiltration in tumors by using a gene-set scoring method to investigate RNA-seq data from TCGA, and metastatic cutaneous melanoma patients have better prognosis when their tumor shows evidence of NK cell infiltration. Liu, et al.[13] have developed a six-gene signature, which could stratify HCC patients into a high- and low-risk group and exhibit prognostic significance via TCGA and GEO.

Cancer patients, including HCC patients, have different outcomes even if they are at the same clinical or pathological stage and receive similar treatment. For instance, 54% HCC patients reoccur at a median time of 22 months from primary resection and have significantly shorter overall survival (OS) while the rest have a much longer OS[14].

This study aims to identify the key genes impacting the prognosis of HCC patients, and to understand the molecular mechanism between HCC patients with longer OS and those with shorter OS by reanalyzing the gene expression profiles and clinical information from TCGA and GEO database.

1 Materials and methods

1.1 TCGA and GEO data sets

Normalized RNA-seq data (424 cases, HTSeq-FPKM), phenotype information (469 cases), and survival data (463 cases) of the liver hepatocellular carcinoma (LIHC) project of TCGA (TCGA-LIHC) were downloaded. Normalized gene expression data (log_2-transformed RMA-calculated signal intensity) of the GSE14520/GPL571 and GSE25097 (series matrix file) data sets were downloaded from the GEO database through the GEO query package in the R software (version 3.6.2). All the data used in this study were obtained from public databases, and further approval by an ethics committee was not required.

1.2 Data processing

The first sample according to the label was selected if the same patient had 2 or more samples in TCGA-LIHC data set. Normalized gene expression values in TCGA-LIHC were converted to transcripts per million (TPM) and log-transformed [log_2(TPM+1)]. The normalized data between arrays package in R (version 3.6.2) were used to normalize expression intensities (method= "quantile") if they were not similarly distributed across samples[15]. For genes with multiple probes in TCGA-LIHC, GSE14520/GPL571 and GSE25097 data sets, the probe detected in the largest number of tumors was retained[16]. For prognosis analysis, samples without survival information were not included, and 385 tumor samples from TCGA-LIHC and 220 tumor samples for GSE14520/GPL571 were used for further analyses. Unavailable or unknown clinicopathological information in these 2 data sets was regarded as missing values.

1.3 Identification of key genes impacting prognosis of HCC patients

To screen the potential genes that involve in the tumorigenesis and development of HCC, differentially expressed genes (DEGs) in TCGA-LIHC and GSE14520 data sets with available survival information were analyzed. The DEGs were analyzed between normal tissues and tumor ones, or between samples with long OS (OS>3 years) and those with short OS (<1 year), with the following criteria: Fold change (FC)>2 and false discovery rate (FDR)<0.05.

1.4 Oncomine and Gene Expression Profiling Interactive Analysis (version 2, GEPIA 2) database analysis

The Oncomine database was used to validate transcription level of the interested genes in HCC by retrieving expression data (log_2-transformed) in 5 cohorts of HCC vs normal tissues for statistical comparison, with default thresholds: P<1e-4, FC>2 and the gene ranks in the top 10%. The online database GEPIA2 was used to validate the transcriptional change of interested genes between HCC tumors and normal samples, and to evaluate their prognostic significance.

1.5 Gene set enrichment analysis, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis

Gene set enrichment analysis (GSEA) was used to determine whether an a priori defined set of genes shows statistically significant, concordant differences between the high- and low- group, based on the median expression level of interested genes[17]. Gene expression data was loaded into the GSEA software (version 4.0.3) and gene sets database, permutations were performed 1 000 times for each analysis. In the whole process, the expression level of interested genes was regarded as a phenotype. The pathways were regarded significant enriched with the following criteria: Normalized enrichment score (NES)>1, nominal P<0.05 and FDR Q<0.25. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

were conducted by the clusterProfiler package in R (version 3.6.2)[18].

Since secreted phosphoprotein 2 (SPP2) was considerably down-regulated in HCC and in tumor samples with short OS, we then explored whether the expression of SPP2 correlated with the prognosis of HCC patients. After data filtering, 365 cases of gene expression data, survival information and phenotype data from the TCGA-LIHC, and 220 cases from the GSE14520, were included in the following analysis. We set the medium expression as the cut-off value and divided the samples into high and low expression groups. For prognosis analysis, the HCC patients in each dataset were divided into 2 subgroups based on the median value of SPP2 in the dataset, and the Kaplan-Meier method with log-rank test was used to evaluate the role of SPP2 in the prognosis of HCC patients.

1.6 Construction of target gene of protein-protein interaction network

Based on NES, we constructed a protein-protein interaction (PPI) network between SPP2 and 38 HNF3B signaling-related genes in the STRING database (http://string-db.org).

1.7 Statistical analysis

Online data analyses were performed by default as described by web resources. The rest data analyses were performed by R software (version 3.6.2). Correlation analysis between the gene expression of interested genes and that of the rest genes was conducted with Spearman method. Wilcoxon test was conducted to compare gene expression between groups. Packages in R used for data analysis and graph plotting included ggstatsplot, ggplot2, ggpubr, limma, survminer, survival, tidyverse, dplyr, and plyr. $P<0.05$ was considered statistically significant.

2 Results

2.1 Down-regulated SPP2 in HCC

The result showed that only SPP2 was dramatically down-regulated in tumors when compared with normal tissues, or in tumor samples with short OS when compared with those with long OS (Figure 1A). The expression pattern of SPP2 in HCC was confirmed by mining the GEPIA2 database, with the default setting ($|\log_2FC|$cut off=1, P=0.01, Figure 1B). We further analyzed data from the Oncomine database, which validated that SPP2 was significantly down-regulated in HCC tissues when compared with the corresponding normal ones across 5 independent cohorts (Figure 1C). Since cirrhosis was regarded as a pivotal process in the tumorigenesis of HCC, we further investigated the expression of SPP2 in non-tumor samples, cirrhosis tissues, and HCC samples. As shown in Figure 1D, SPP2 was obviously down-regulated in cirrhosis tissues when compared with non-tumor samples, indicating SPP2 might involve in the tumorigenesis of HCC.

2.2 Low expression of SPP2 predicted dismal prognosis and correlated with worse clinicopathological features

As shown in Figure 2A and 2B, high expression of SPP2 significantly correlated with a longer OS (P=0.002) and disease-free survival (DFS) (P=0.013). Besides, high expression of SPP2 was found to be correlated with a lower risk of death (Figure 2C, P=0.029). The prognostic value of SPP2 was further validated in the GSE14520 data set (Figure 2D, 2E), and consistently, patients with elevated expression of SPP2 had a significantly lower risk of death (P=0.003, Figure 2F).

As shown in Figure 3A－3I, patients with vascular invasion (P=1.6e-05), poor cancer status (with tumor, P=0.021), advanced T stage (T3 or T4, P=4.5e-04), and advanced TNM stage (stage III or IV, P=3.1e-04) were associated with significantly lower expression of SPP2. No correlation was found between the level of SPP2 and level of alpha fetoprotein (AFP), adjacent hepatic tissue inflammation, N stage or M stage. In the GSE14520 data set, patients with advanced TNM stage (stage III or IV, P=3e-06), high risk of metastasis (P=4.6e-14), large tumor (main tumor size >5 cm, P=3.1e-05), cirrhosis (P=0.042), advanced Barcelona clinic liver cancer (BCLC) stage (stage B or C, P=9e-05), and elevated level of alanine aminotransferase (ALT) (P=0.014) were also significantly associated with lower expression of SPP2 (Figure 4A－4F). Consistently, no correlation was observed

between the expression of SPP2 and level of AFP (P=0.270, Figure 4G). In addition, patients with multi-nodules tended to show a lower expression of SPP2, although the significance was not reached (P=0.053, Figure 4H).

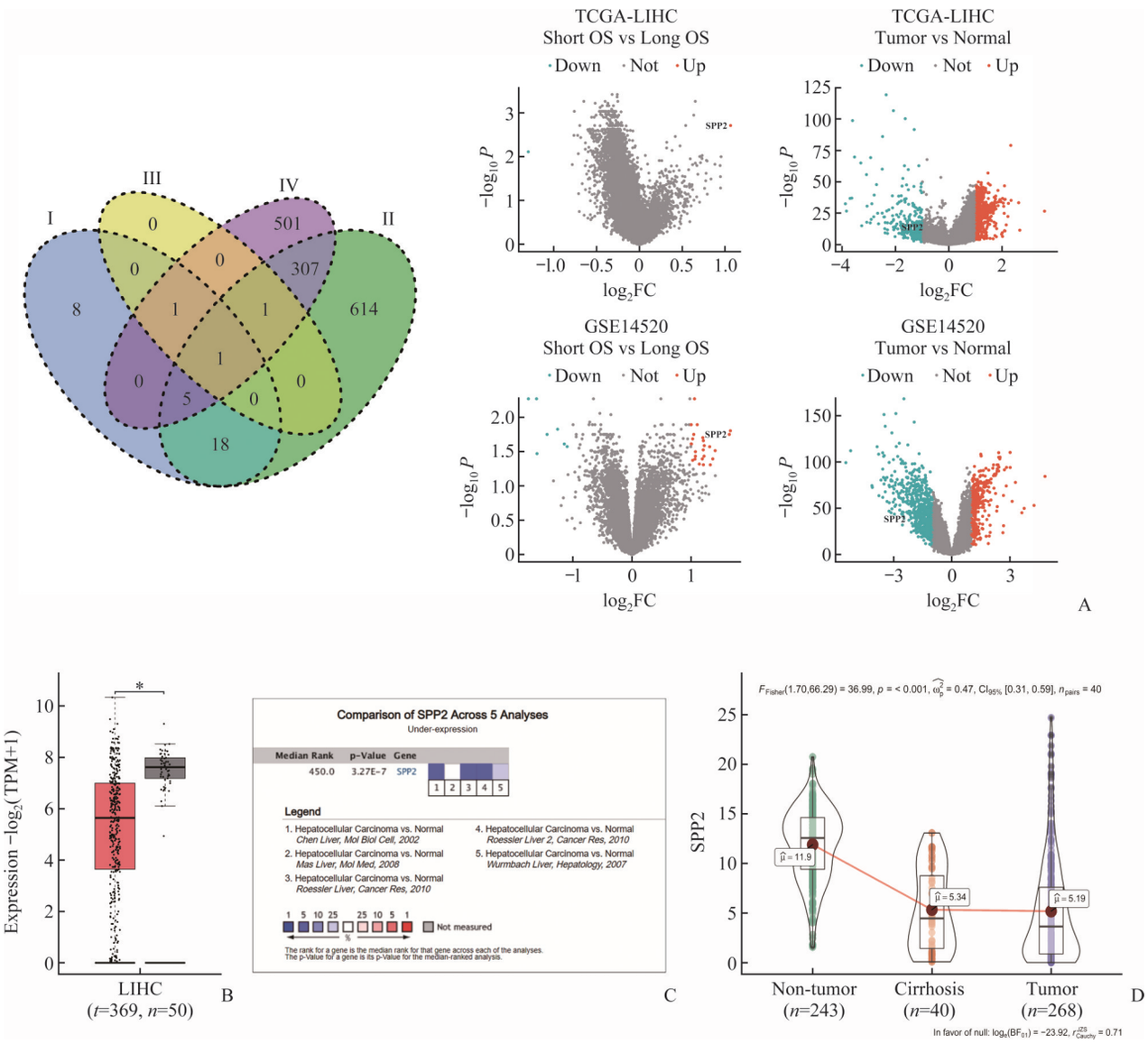

Figure 1 SPP2 was down-regulated in HCC

A: Venn graph and volcano graph of differentially expressed genes between normal tissues and tumor ones, or between samples with long overall survival (OS) (>3 years) and those with short OS (<1 year), in TCGA-LIHC and GSE14520 data sets, with fold change (FC)>2 and false discovery rate (FDR)<0.05; B: Data from the database validating that SPP2 was significantly down-regulated in HCC tissues; C: Data from the Oncomine database showing that SPP2 was significantly down-regulated in HCC tissues; D: Data from the GSE25097 data set showing that SPP2 was significantly down-regulated in cirrhosis livers. SPP2: Secreted phosphoprotein 2; HCC: Hepatocellular carcinoma; TCGA: The Cancer Genome Atlas; LIHC: Liver hepatocellular carcinoma.

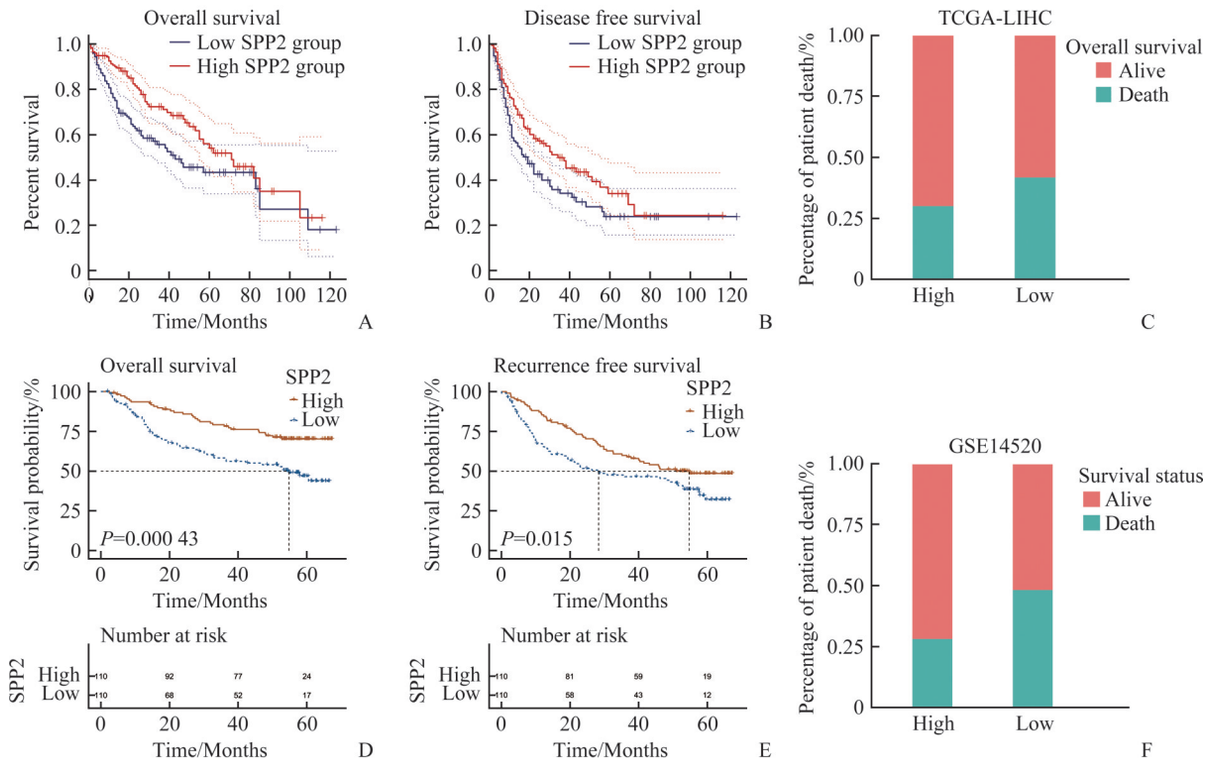

Figure 2 Low expression of SPP2 was associated with unfavorable prognosis in HCC

A−C: Low expression of SPP2 was significantly related to shorter overall survival (A), shorter disease free survival (B), and higher risk of death (C) in TCGA-LIHC. D−F: Low expression of SPP2 was significantly related to shorter overall survival (D), shorter disease free survival (E), and higher risk of death (F) in GSE14520. SPP2: Secreted phosphoprotein 2; HCC: Hepatocellular carcinoma; TCGA: The Cancer Genome Atlas; LIHC: Liver hepatocellular carcinoma.

2.3 SPP2-related signaling pathways by GSEA analysis

The significantly enriched signaling pathways were included, and the top 9 most significantly enriched signaling pathways based on their NES were shown in Figure 5A−5I. In particular, SPP2 was found to be related to the hepatocyte nuclear factor 3-β (HNF3B, also known as forkhead box A2, FOXA2) pathway, fatty acid metabolism, cytochrome P450 pathway, clycine serine and threonine metabolism, peroxisomal protein import, bile acid and bile salt metabolism, and peroxisome.

The PPI network between SPP2 and 38 HNF3B signaling-related genes in the STRING database as showed in Figure 6A. The network shows that SPP2 might interact with tyrosine aminotransferase (TAT), apolipoprotein A1 (APOA1), solute carrier family 2 member 2 (SLC2A2), albumin (ALB), insulin like growth factor binding protein 1 (IGFBP1), CCAAT enhancer binding protein alpha (CEBPA), AFP, and transthyretin (TTR). Particularly, a strong positive correlation was observed between SPP2 and APOA1, SLC2A2 or TTR at the transcriptional level (correlation coefficient >0.5, Figure 6B−6G).

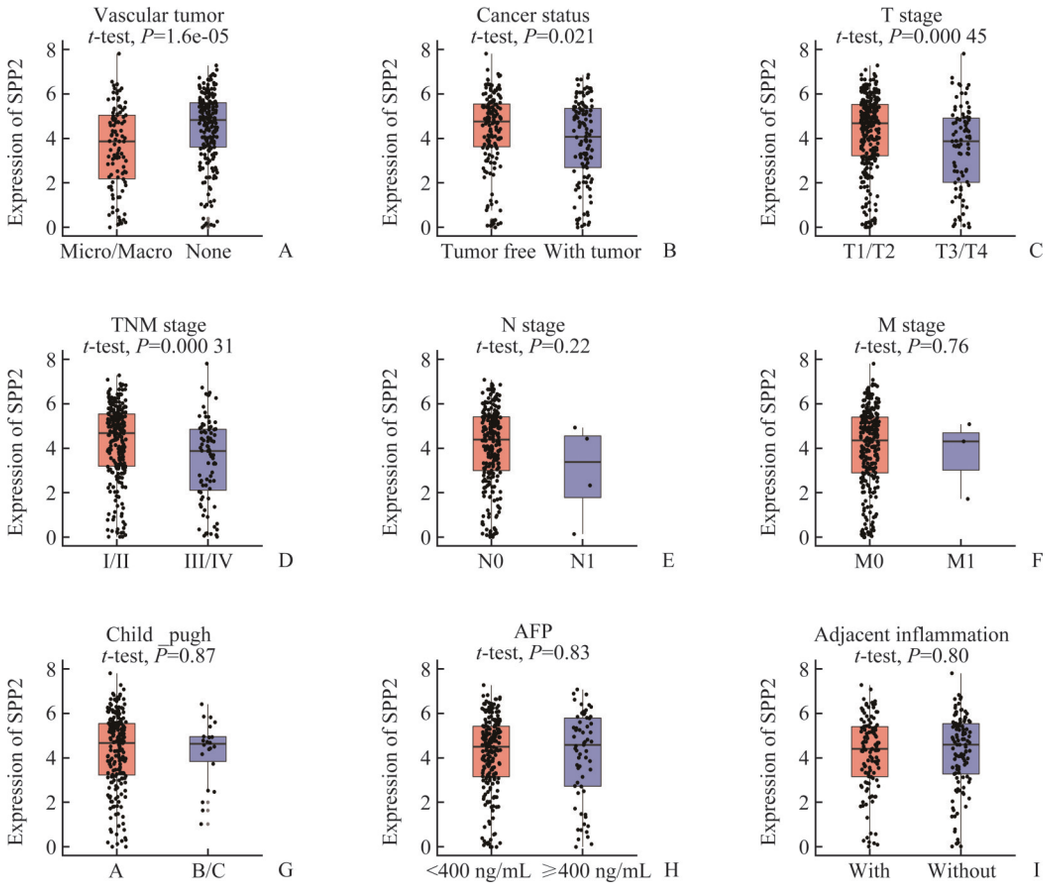

Figure 3 HCC patients with worse clinicopathologic features had significantly lower expression of SPP2 in TCGA-LIHC

A－D: Patients with vascular invasion (A), poor cancer status (B), advanced T stage (C), and advanced TNM stage (D) were associated with significantly lower expression of SPP2. E−I: No correlation was found between the level of SPP2 and N stage (E), M stage (F), Child_pugh grade (G), level of AFP (H), or adjacent hepatic tissue inflammation (I). SPP2: Secreted phosphoprotein 2; TCGA: The Cancer Genome Atlas; LIHC: Liver hepatocellular carcinoma; AFP: Alpha fetoprotein.

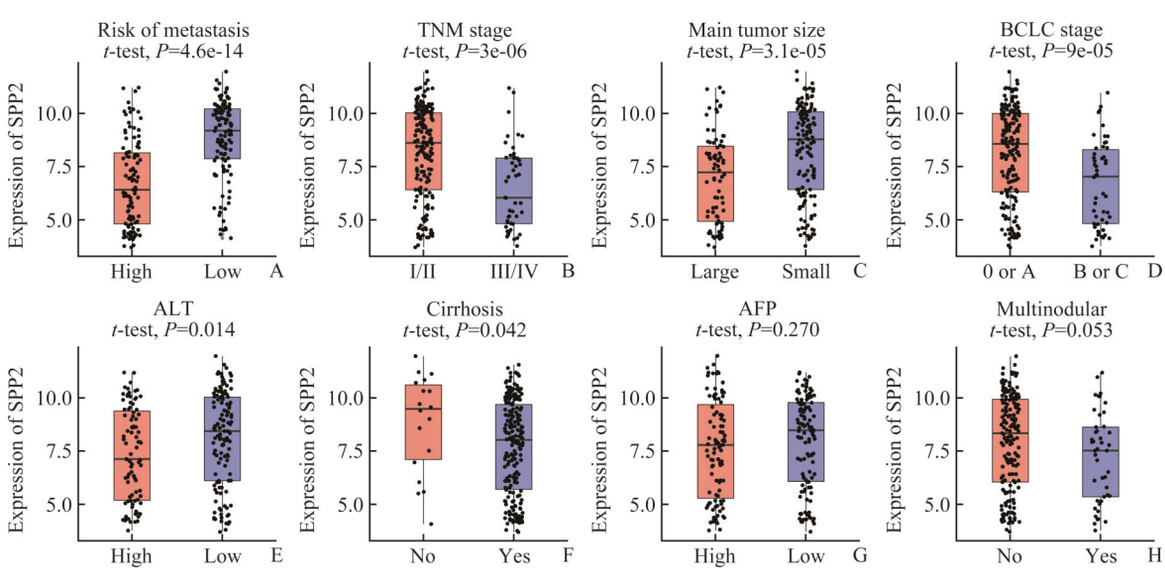

Figure 4 HCC patients with worse clinicopathologic features had significantly lower expression of SPP2 in GSE14520

A−F: Patients with high risk of metastasis (A), advanced TNM stage (B), large tumor size (C), advanced BCLC stage (D), high ALT level (E), and cirrhosis (F) were associated with significantly lower expression of SPP2. G−H: No correlation was found between the level of SPP2 and level of AFP (G), or number of nodules (H). SPP2: Secreted phosphoprotein 2; BCLC: Barcelona clinic liver cancer; ALT: Aminotransferase; AFP: Alpha fetoprotein.

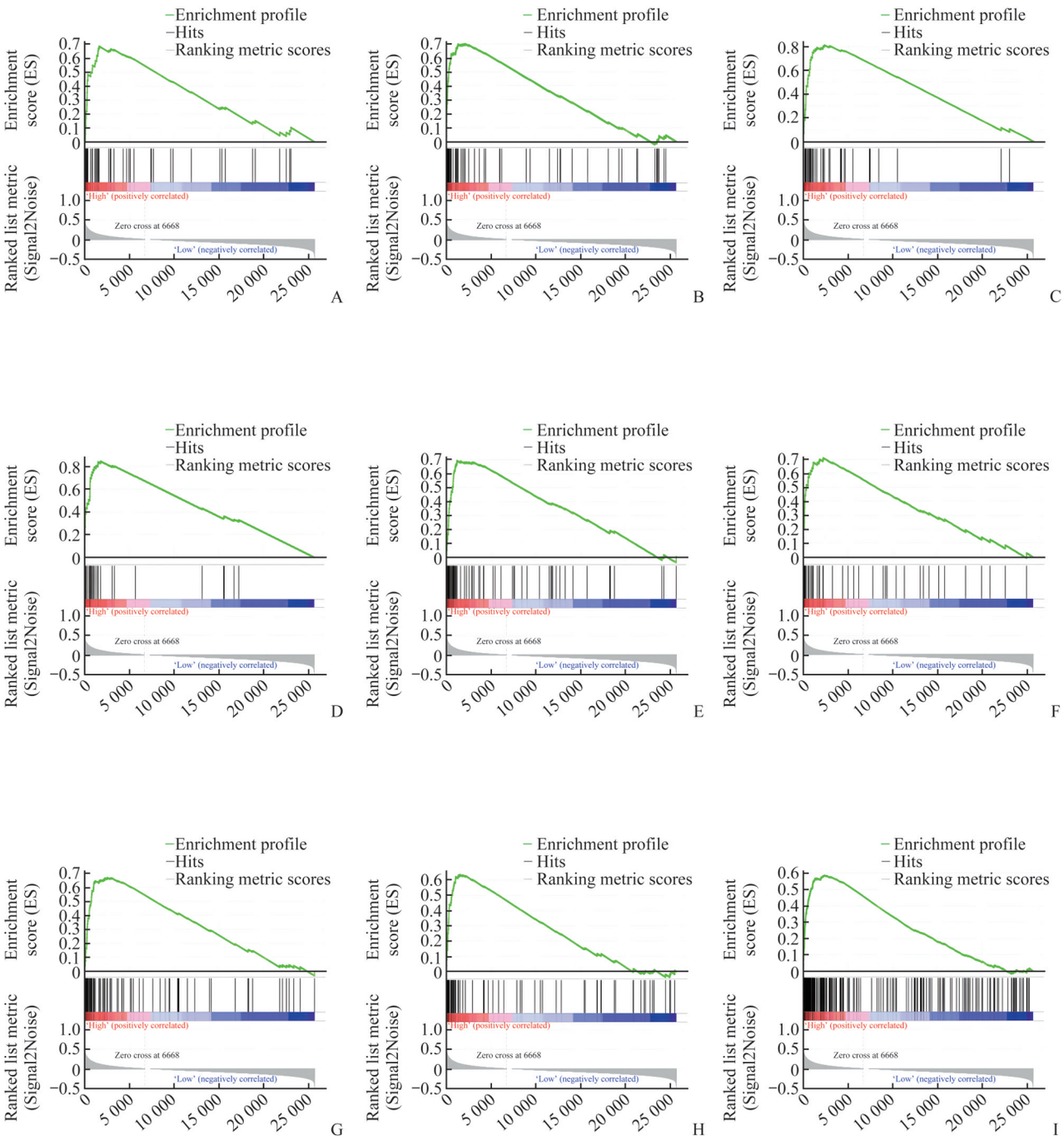

Figure 5 GSEA of gene expression data from TCGA-LIHC and GSE14520

A−I: HNF3B pathway (A), cytochrome p450 arranged by substrate type (B), fatty acid metabolism (C), glycine serine and threonine metabolism (D), peroxisomal protein import (E), bile acid and bile salt metabolism (F), peroxisome (G), PPAR signaling pathway (H), and biological oxidations (I) were enriched in the high expression group of SPP2. SPP2: Secreted phosphoprotein 2; TCGA: The Cancer Genome Atlas; LIHC: Liver hepatocellular carcinoma.

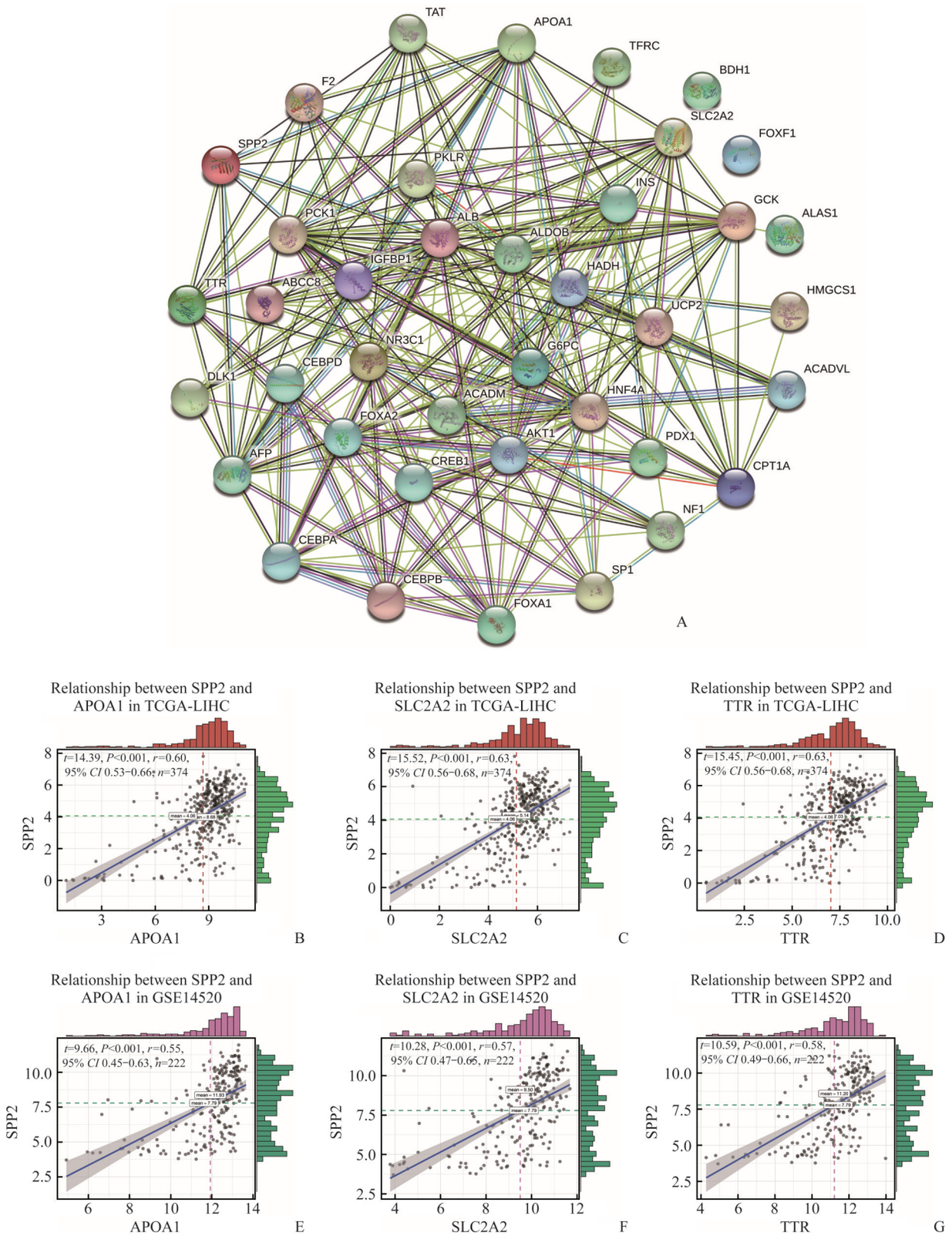

Figure 6 PPI network construction of SPP2 and HNF3B signaling-related genes

A: PPI network construction of SPP2 and 38 HNF3B signaling-related genes in the STRING database. B−D: SPP2 was strongly positive correlated with APOA1 (B), SLC2A2 (C), and TTR (D) at transcription level in TCGA-LIHC. E−G: SPP2 was strongly positive correlated with APOA1 (E), SLC2A2 (F), and TTR (G) at transcription level in GSE14520. SPP2: Secreted phosphoprotein 2; APOA1: Apolipoprotein A1; SLC2A2: Solute carrier family 2 member 2; TTR: Transthyretin; TCGA: The Cancer Genome Atlas; LIHC: Liver hepatocellular carcinoma.

2.4 Mechanism of SPP2 in the tumorigenesis of hepatocellular carcinoma

To explore the potential regulatory mechanism of SPP2 in the disease, we analyzed the DEGs between non-tumor samples (n=243) and cirrhosis samples (n=40) in the GSE25097 data set, and 1 005 genes were found to be up-regulated in the cirrhosis tissues (Figure 7A). We also conducted correlation analysis between the expression of SPP2 and that of the rest genes in the cirrhosis samples of the GSE25097 data set, and 9 297 genes were found to be significantly correlated with SPP2 in the gene transcription (P<0.05). Then the genes that had a strong negative correlation with SPP2 in the transcription level (correlation coefficient< − 0.5, P< 0.05) were intersected with the above up-regulated DEGs to obtain the shared 626 genes (Figure 7B). These 626 genes were then underwent GO and KEGG analysis.

The GO analysis revealed that these genes were significantly enriched in the biological process of binding a set of materials including growth factor, integrin, glycosaminoglycan, collagen, actin, cadherin and cell adhesion molecule, and in the molecular function of activating T cell and neutrophil, integrin-mediated signaling pathway, and extracellular matrix organization (Figure 8A). KEGG enrichment analysis indicated that these genes participate in a range of signaling pathways including focal adhesion, phagosome, ECM-receptor interaction, proteoglycans in cancer, leukocyte transendothelial migration, tight junction, antigen processing and presentation, PI3K-Akt signaling pathway, chemokine signaling pathway, NK cell mediated cytotoxicity (Figure 8B), suggesting down-regulation of SPP2 was accompanied with modification in the immune-environment, and cell proliferation and invasion.

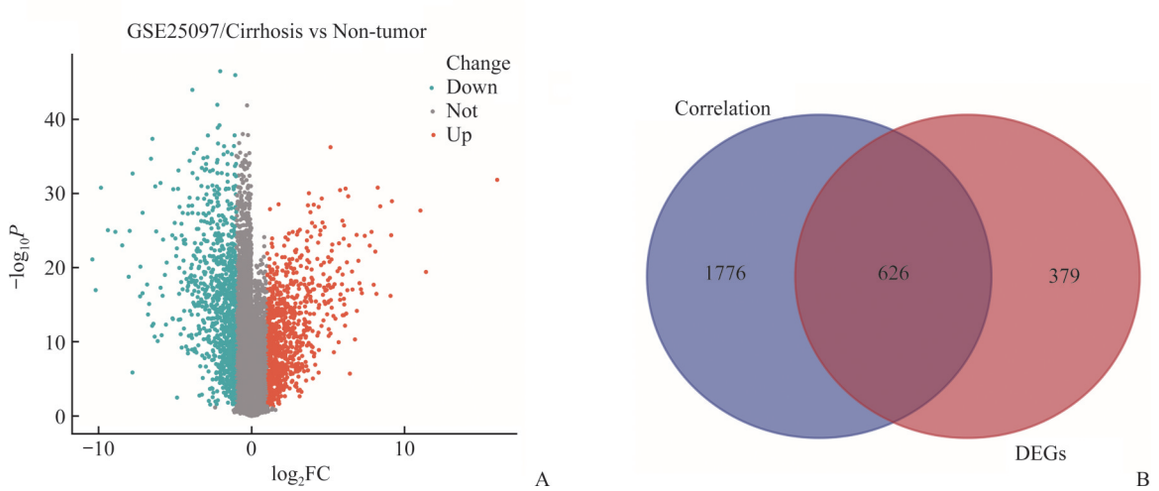

Figure 7 Co-expression analysis of SPP2-related genes in cirrhosis liver

A: Volcano graph of DEGs between cirrhosis liver and non-tumor samples; B: Venn graph of DEGs and strongly negative correlated genes of SPP2 in cirrhosis livers. SPP2: Secreted phosphoprotein 2; DEGs: Differentially expressed genes.

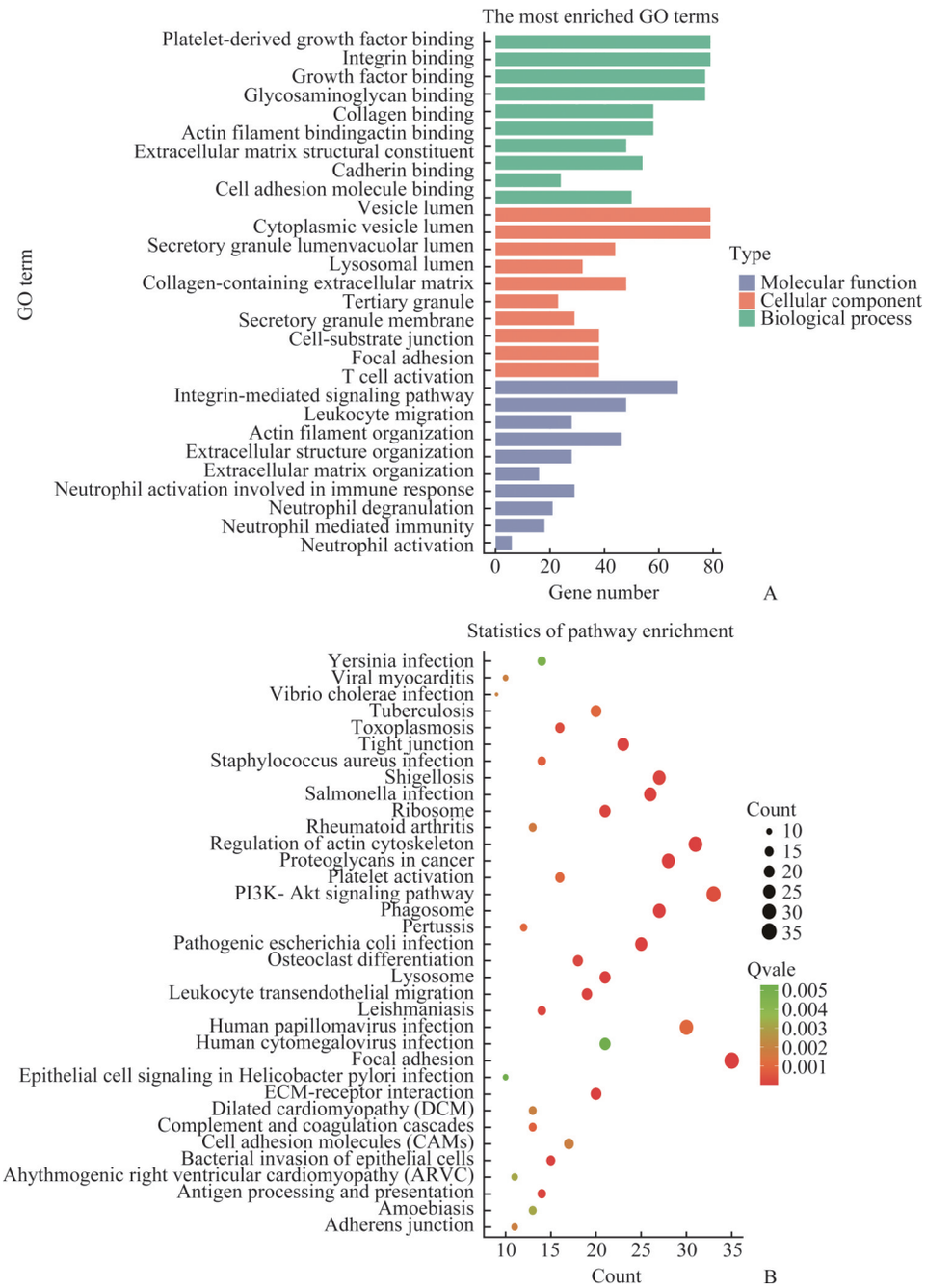

Figure 8 GO and KEGG enrichment analysis of SPP2-related genes in cirrhosis liver

A: GO enrichment analysis of SPP2 related genes in cirrhosis livers; B: KEGG enrichment analysis of SPP2 related genes in cirrhosis livers. SPP2: Secreted phosphoprotein 2; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

3 Discussion

Cancer patients exhibit different prognosis, even when they are at same stage and receive similar treatment. By reanalyzing public data sets with gene expression profiling and clinical information (TCGA-LIHC and GSE14520), we identify that only the expression of SPP2 is consistently and obviously decreased in tumors when compared with normal tissues, and in tumor patients with short OS when compared to those with long OS. In addition, decreased expression of SPP2 in HCC is associated with advanced clinicopathological features, short survival time, and short recurrence free survival (RFS), indicating SPP2 might act as a tumor-suppressor in HCC. In early studies[19-20], SPP2 is reported to be generated primarily

in the liver and delivered to other tissues, and is identified as a bone matrix protein that plays an essential role in the regulation of bone metabolism. Recently, several studies[21-24] show that SPP2 could inhibit tumor growth and induce tumor apoptosis by abolishing the pro-tumor function of bone morphogenetic protein 2 (BMP-2) signaling. Indeed, SPP2 contains a BMP-2 binding transforming growth factor-β (TGF-β) receptor II homology 1 (TRH1) domain (the cystatin domain which is preserved in all of the N-terminal products) similar to those described in fetuin and the TGF-β receptor II[19]. In addition, SPP2 and its C-terminal truncation products could all bind to transforming growth factor-β1 TGF-β1, TGF-β2 and BMP-2, and the C-terminal fragment is distinct from the cystatin domain and could independently bind to BMP-2 and TGF-β[25-26]. Wu, et al.[27] report that BMP2 could result in the expansion of myeloid-derived suppressor cells (MDSCs) in peripheral blood, which infiltrates HCC and facilitates tumor growth by secreting interleukin-6 (IL-6). Zuo, et al. [28] have demonstrated that BMP-2 can enhance angiogenesis in HCC through p38, ERK and Akt/m-TOR pathway. In addition, numerous studies[29-34] have found that activation of TGF-β receptor (TGFβR) signaling in cancers, including HCC, facilitates the proliferation and invasion of tumors. Since SPP2 could act as a pseudoreceptor for BMP-2 and TGF-β, it might suppress the development of HCC by inhibiting the BMP/TGF-β signaling pathway[19-21].

However, SPP2 might have other functions. For instance, early studies[19, 35] show that the acidic residues of the cystatin domain of SPP2 may form a negatively charged planar β-sheet that inhibits basic calcium phosphate precipitation in a manner similar to that observed for the D1 region of the structurally similar cystatin domain of fetuin. Besides, SPP2 is extremely labile to proteolysis, which results in N-terminally intact degradation products ranging in size from 14 to 23 kD, and some of these products could modulate signaling pathways, like Gi-protein coupled receptor-Erk1/2 signaling pathway, that are independent of full length of SPP2[36]. In this case, we use TCGA data and GEO data for GSEA and explore the functions of SPP2 other than regulation on BMP/TGF-β signaling pathway. GSEA analysis of both gene expression data from TCGA-LIHC and GSE14520 data sets indicate that SPP2 might regulate HNF3B pathway, oxidation, peroxisome, and metabolism of lipid and bile acid. When a PPI network is constructed between SPP2 and HNF3B pathway related genes, we found that SPP2 is strongly correlated with TTR, SLC2A2, and APOA1 in transcription level, and might interact with these proteins. APOA1 participates in the lipid metabolism[37]. TTR has been well-known for its role in transporting thyroid hormones (THs) and retinol (vitamin A), and recent studies indicate that it has other biological functions such as regulating glucose metabolism[38-39]. SLC2A2 regulates glucose metabolism and high expression of SLC2A2 correlates with good prognosis in HCC patients[40-41]. Although the exact regulatory mechanism of SPP2 in metabolism is not clear at the present time, but its strong correlation with TTR, SLC2A2, and APOA1 raises the hypothesis that SPP2 might involve in the metabolism of glucose and lipid, and future studies are required to provide evidence.

Since SPP2 is significantly down-regulated in cirrhotic tissues and HCC samples with cirrhosis, the down-regulation of SPP2 might occur prior to HCC, thus we conduct gene enrichment analysis for the genes that are significantly over-expressed in cirrhosis tissues and strongly negatively correlated with SPP2. GO and KEGG enrichment analysis show that these up-regulated genes contribute to the fibrosis and cirrhosis of liver by binding to extracellular matrix like collagen and integrin[42-44], stimulate cell proliferation by binding with growth factors and activating the PI3K-Akt signaling pathway[45], and modulate immune microenvironment like activating T cells and neutrophils that are important for liver inflammation and fibrosis[46].

In summary, this study has shown that SPP2 is obviously down-regulated in cirrhosis samples and in liver cancers when compared with normal tissues, and low expression of SPP2 is related to worse clinicopathological features and unfavorable prognosis. SPP2 might inhibit the development of liver fibrosis and cirrhosis and the tumorigenesis of HCC, highlighting analogs of SPP2 might be potential drugs in the prevention of liver cirrhosis and tumorigenesis.

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