ORIGINAL RESEARCH

Overexpression of Taspase 1 Predicts Poor Prognosis in Patients with Hepatocellular Carcinoma

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Background: Taspase 1 (TASP1) is a highly conserved protease involved in site-specific proteolysis. Existing researches have revealed a link between TASP1 expression and carcinogenesis. However, limited data are available regarding the prognosis and functions of TASP1 in hepatocellular carcinoma (HCC).

Methods: Western Blotting and qRT-PCR were employed to evaluate the level of TASP1 in HCC cell lines and clinical specimens. TASP1 expression was further calculated in clinical specimens by immunohistochemistry and the mRNA level of TASP1 in HCC was analyzed using Oncomine and UALCAN databases. The TASP1 promoter methylation modification was shown via MEXPRESS and UALCAN. The association between TASP1 expression and postoperative prognosis was evaluated using Kaplan–Meier and Cox regression analysis in clinical patients. The effect of TASP1 on HCC prognosis was analyzed via Kaplan-Meier plotter, GEPIA and UALCAN. Additionally, the regulators, kinases, miRNA and transcription factor targets of TASP1 were identified using LinkedOmics. Moreover, cBioPortal was used to detect the genetic alteration of TASP1. Finally, TIMER was utilized to assess the relation between TASP1 and the immune cell infiltration, whereas the correlation of TASP1 with three immune factors was detected through TISIDB.

Results: TASP1 expression was increased in HCC cell lines and HCC tissues. CNV and DNA methylation of TASP1 were changed. Survival analysis revealed that high TASP1 expression was correlated with overall survival (OS). Functional network analysis about TASP1 in HCC showed that the double-strand break repair, peptidyl-threonine modification, spindle organization, peptidyl-lysine modification and microtubule-based movement were modulated. Furthermore, TASP1 expression revealed puissant relation to the infiltration of immune cells and three immune factors in HCC.

Conclusion: These data indicate that TASP1 may act as a potential prognostic marker in HCC and regulate HCC via multiple mechanisms.

Keywords: hepatocellular carcinoma, TASP1, prognosis, biological functional networks

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Introduction

Liver cancer is one of the most common malignant tumors worldwide, and its incidence is rising especially in developing countries.[1,2] Hepatocellular carcinoma (HCC), accounting for almost 90% of the primary liver cancer, is difficult to be diagnosed at an early stage and many patients have a poor prognosis due to the high likelihood of metastasis and recurrence.[3,4] Though many types of treatments, including surgery, liver transplantation and radiation, are used in clinical patients, the underlying mechanisms of HCC remain unknown, leading to the difficulty for effective treatments.[5] Therefore, it is greatly important to identify novel markers to diagnose HCC, which may help find new targets for prognosis and treatment.

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http://doi.org/10.2147/CMAR.S296069

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Cancer Management and Research 2021:13 2517–2537

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Site-specific proteolysis regulates critical aspects of biology by carrying out selective cleavage of specific substrates.[6] Site-specific proteolysis is also essential for many pathological processes, including cancers.[7] However, there is limited knowledge about the correlation between proteolysis and tumor biology. Therefore, it is remarkably important to understand the underlying mechanisms of proteolysis in cancers, so as to explore better therapeutic methods for cancers.

Taspase 1 (threonine aspartase 1; TASP1), encoding a 50 kDa proenzyme, is one of the highly conserved proteases, which cleaves the substrates to modulate diverse biological processes.[8] TASP1 was originally recognized as the protease to regulate homeobox (HOX) expression through cleaving mixed lineage leukemia 1 (MLL1). Except MLL1, some other TASP1 substrates, such as MLL2, transcription factor IIA (TFIIA), TFIIA-Like Factor (ALF), and host cell factor 1 (HCF-1), are identified.[9–11] These substrates are broad-acting nuclear factors which regulate gene transcription levels, indicating that TASP1 plays an important role in orchestrating numerous biological processes.[12] TASP1 has been shown to affect the development of glioblastoma and melanoma through modulating apoptosis and proliferation.[13] It has also been reported that the expression of TASP1 is abnormal in various cancers, such as leukaemia, breast cancer, gallbladder cancer and breast cancer.[13–15] In addition, though one study shows that TASP1 has been involved in the networks of Hepatocyte Nuclear Factor 4 Alpha (HNF-4α) in HCC,[16] the detailed functions and mechanisms of TASP1 in HCC are still unknown.

In this study, we identified that the TASP1 expression levels were increased in HCC tissues. Additionally, Kaplan–Meier and Cox regression multivariate analyses indicated that TASP1 expression is an independent prognostic factor of overall survival (OS) in HCC patients. Furthermore, the bioinformatics approaches were also utilized to explore the expression and mutation of TASP1 in HCC via various online databases. In addition, we evaluated genomic alterations, function networks, and prognostic value of TASP1 and explored the effect of TASP1 on tumor immunity in HCC by multi-dimensional methods. Therefore, we may provide new understandings about TASP1 in HCC, and reveal a potentially diagnostic, prognostic and therapeutic target for HCC.

Materials and Methods
Tissue Samples
A total of 100 patients, who were surgically treated for HCC between October 2005 and May 2011 at the Fifth Affiliated Hospital of Zhengzhou University, were enrolled in this study. Patients, who received anticancer therapy, such as chemotherapy, radiotherapy, and other adjuvant treatments, were excluded. Patient clinical data were obtained and this study was approved by the Ethics Committee of the Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou, China. All the participants signed an informed consent form before this study. And this study was conducted in accordance with the Declaration of Helsinki.

Reverse-Transcription Quantitative PCR (qPCR)
Total RNA was extracted from the HCC cell lines, HCC patients and their paired adjacent normal tissues, using Trizol (Takara). Total RNA was reversely transcribed into cDNA with the PrimeScriptTM RT Reagent Kit (Takara). qPCR was carried out using SYBR Green (Takara), and the results were normalized to GAPDH expression. The primer sequences of TASP1 are: FORWARD (5′-3′) GTTGCCAACAGACTCTTATGTG, REVERSE (5′-3′) GTATTCCATGATCTACTGCCCA. The primer sequences of GAPDH are FORWARD (5′-3′) AATGGGCAGCCG TTAGGAAA, REVERSE (5′-3′) GCGCCCAATACGA CCAAATC.

Western Blot Analysis
Proteins of the liver tissue of HCC patients, paired adjacent normal tissues and cultured cells were extracted with RIPA lysis buffer (Beyotime) supplemented with a protease inhibitor (Beyotime) and were quantified by means of the BCA Protein Assay Kit (Thermo Scientific). Western blotting was performed using antibodies against TASP1 (1: 1000; #16739-1-AP; Proteintech), GAPDH (1: 25000; #60004-1-lg; Proteintech) and then with horseradish peroxidase-conjugated secondary antibodies (Thermo Fisher, USA) for 1 hr at room temperature. ChemiDoc XRS systems (Bio-Rad Laboratories, CA, United States) and the AlphaView software were used to image and analyze the Western blot.

Immunohistochemistry

Briefly, the specimens were dewaxed routinely and boiled in citrate buffer. After the endogenous peroxidase activity in the specimen was inactivated with 3% H_2O_2, the specimen was incubated with polyclonal antibody against human TASP1 (1: 200; #16739-1-AP; Proteintech) for 1 h at 37°C. After washed with PBS, the specimen was incubated with secondary antibody for 0.5 h at 37°C. The specimens were stained with diaminobenzidine (DAB) and counterstained with hematoxylin. And the film was sealed. Two pathologists were assigned to evaluate the stained slides in a double-blinded manner. The IHC staining intensity scoring criteria were as follows: 0–3 (none = 0, weak = 1, medium = 2, and strong = 3). The scoring criteria for the proportion of positive tumor cells were as follows: 0, <5%; 1, 5–25%; 2, 26–50%; 3, 51–75%; and 4, >75%.

Oncomine Analysis

The Oncomine database (www.oncomine.org) was utilized for detecting the mRNA level and copy number variation of TASP1 in different cancers, including HCC.[17] We paid attention to several datasets, such as Chen liver, Guichard Liver, Roessler liver. Differences associated with $p < 0.05$ were regarded statistically significant.

Kaplan–Meier Survival Curve Analysis

An online survival analysis tool that Kaplan–Meier Plotter (http://kmplot.com/analysis) was utilized to assess the prognostic significance of genes in 21 different cancers containing over 10,000 samples, including 371 LIHC.[18] HCC patients were categorized into two groups according to the median expression level of TASP1 (high vs low) to analyze survival rate via Kaplan–Meier Plotter. A hazard ratio (HR) with a 95% confidence interval and log-rank P-value were calculated. P-value <0.05 was considered significant.

GEPIA Analysis

The Gene Expression Profiling Interactive Analysis (GEPIA) dataset (http://gepia.cancer-pku.cn/) is an interactive web server to visualize and analyze RNA sequencing expression data of 9736 tumors and 8587 normal samples from the TCGA and GTEx projects.[19] GEPIA was utilized to examine the expression of TASP1 and generate the survival rate based on the TASP1 mRNA level in HCC.

UALCAN Analysis

UALCAN (http://ualcan.path.uab.edu) draws on TCGA level 3 RNA-seq and clinical data from 31 cancer types to analyze the gene expression data in depth.[20] UALCAN was utilized to detect the relative expression and methylation of TASP1 across HCC tissues and normal tissues, as well as in various sub-groups defined according to cancer age, race, gender, weight, disease stage, tumor grade, nodal metastasis and TP53 mutation. $P < 0.05$ was regarded statistically significant.

MEXPRESS Analysis

MEXPRESS (https://mexpress.be/) is a web tool for integrating and visualizing the expression, DNA methylation and clinical data, as well as the relationships between them.[21] In this study, MEXPRESS stool was utilized for detecting the relationship between the TASP1 expression and methylation status in HCC.

LinkedOmics Analysis

The LinkedOmics database (http://www.linkedomics.org/login.php) is a web-based platform for analyzing 32 TCGA cancer-associated multi-dimensional datasets.[22] The LinkFinder module of LinkedOmics was used to study genes that were expressed differentially in correlation with LIHC via Pearson's correlation coefficient. The LinkFinder was also utilized to create plots for individual genes. And the results were represented in the form of volcano plots, heatmaps, or scatter plots. The LinkInterpreter, another module of LinkedOmics, was drawn on to conduct pathway and network analyses. The comprehensive functional category database in the Web-based Gene Set AnaLysis Toolkit (WebGestalt) was applied. Gene set enrichment analysis (GSEA) was used to perform analysis of GO (CC, BP and MF), KEGG pathways, kinase-target enrichment, miRNA-target enrichment and transcription factor-target enrichment based on the data from the LinkFinder results. The rank criterion was an FDR <0.05, and 500 stimulations were conducted.

cBioPortal Analysis

The cBio Cancer Genomics Portal (cBioPortal) (http://cbioportal.org) is an open-access resource characterized by the multidimensional cancer genomics data sets, containing 225 cancer studies.[23] The TASP1 alterations in TCGA LIHC, were analyzed using cBioPortal, and the search parameters consisted of mutation, copy number

variation (CNV) and mRNA expression. In addition, the tab OncoPrint represents an overview of genetic alterations per sample in TASP1.

String Analysis

The Search Tool for the Retrieval of Interacting Genes (STRING) (https://string-db.org) was applied to perform the protein–protein interaction (PPI) network.[24] As for the combined score >0.4 for PPI pairs in our study, we further used built Cytoscape to build a PPI network model and to identify hub genes.

TIMER Analysis

The TIMER dataset (https://cistrome.shinyapps.io/timer/), a comprehensive web server, is used to analyzing the tumor-infiltrating immune cells (TILCs) by gene expression profiling in 32 cancer types from over 10,000 samples.[25] The TIMER database was employed to investigate the TASP1 expression and its relationship with the abundance of TILCs, including B cells, CD8$^+$ T cells, CD4$^+$ T cells, macrophages, neutrophils and dendritic cells, through the "gene" module.

TISIDB Analysis

The mRNA expression of TASP1 and its correlation with immunomodulators and chemokines were analyzed using TISIDB database (http://cis.hku.hk/TISIDB/index.php), which is a web portal for integrating genes of tumor and immune system interactions from seven public databases.[26]

Statistical Analysis

The Chi-square test was used to evaluate the correlations between TASP1 expression and clinicopathological features. The Kaplan–Meier method and Log rank test were used to analyze survival curves. The Cox repression analyses were utilized to assess the risk of death associated with clinically related factors. $P < 0.05$ was considered statistically significant. All Statistical analyses were performed using SPSS 20.0software package.

Results
Clinical Characteristics of HCC Patients

A total of 100 patients with HCC were enrolled in this study, including 54 males and 46 females; the median age of HCC patients was 63 (55–69) years. In respect of their disease features, 49 patients were AJCC stage I + II and 51 patients were AJCC stage III + IV; 72 patients were T stage I + II and 28 patients were T stage III + IV; 75 patients were pathologic differentiation moderate + well and 25 patients were pathologic differentiation poor; 68 patients had single tumor and 32 patients had multiple tumor; there were 64 patients with lymph node metastasis, and 36 patients without lymph node metastasis; there were 43 patients with vascular invasion, and 57 patients without vascular invasion; the largest tumor size of 56 patients was less than 5.0 cm, while that of 44 patients was equal to or greater than 5.0 cm. And the median levels of TBIL, Creatinine, AFP, INR and MELD were 21.38 (0.76–1.89) mg/dL, 0.97 (0.8–1.20) mg/dL, 52.5 (23.75–126.25) ng/mL, 1.21 (1.03–1.91) and 10 (8–14), respectively. The detailed clinicopathological characteristic data of HCC patients were shown in Table 1.

TASP1 Was Overexpressed in HCC Tissues and Correlated with Poor Prognosis of HCC Patients

Firstly, we used qRT-PCR and Western blotting to detect the mRNA and protein expression of TASP1 in HCC cell lines and normal liver cell line. The results showed that the mRNA and protein expression of TASP1 in HCC cell lines including SMMC7721, Huh7, HepG2, LM3 and Hep3B, was higher than that in the normal liver cell line LO2 ($p < 0.05$) (Figure 1A–C). Next, we examined TASP1 mRNA and protein expression in tumor and adjacent tissues, finding that TASP1 expression level was significantly higher in tumor tissues compared with adjacent tissues ($p < 0.05$) (Figure 1D–F).

Since the expression of TASP1 was different between HCC and normal tissues, we further performed immunohistochemistry to detect TASP1 protein expression in tumor and adjacent tissues. Results revealed that the expression of TASP1 in the tumor tissue was higher than that in the adjacent tissue (Figure 2A–D). In our clinical samples, we also found that upregulation of TASP1 expression was associated with pathologic differentiation ($p= 0.028$) and levels of AFP ($p < 0.001$) (Table 2). However, TASP1 expression was not correlated with sex, age, AJCC stage, T stage, lymph node metastasis, metastasis, vascular invasion, HBV, HCV, alcohol consumption, cirrhosis, tumor size, tumor number (Table 2). Then, we evaluated the prognostic value of TASP1 expression in HCC patients. Kaplan–Meier survival analysis showed that HCC patients with high TASP1 expression in our

Table 1 Clinicopathological Characteristics of HCC Patients

Clinicopathological Parameter	HCC Patients (n=100)
Sex, N (%)	
Male	54 (54)
Female	46 (46)
Age (years), median (IQR)	63 (55–69)
AJCC stage, N (%)	
I + II	49 (49)
III + IV	51 (51)
T stage, N (%)	
I + II	72 (72)
III + IV	28 (28)
Pathologic differentiation, N (%)	
Poor	25 (25)
Moderate + Well	75 (75)
Lymph node metastasis, N (%)	
No	36 (36)
Yes	64 (64)
Metastasis, N (%)	
No	57 (57)
Yes	43 (43)
Vascular invasion, N (%)	
No	57 (57)
Yes	43 (43)
HBV, N (%)	
Negative	37 (37)
Positive	63 (63)
HCV, N (%)	
Negative	78 (78)
Positive	22 (22)
Alcohol consumption, N (%)	
No	47 (47)
Yes	53 (53)
Cirrhosis, N (%)	
No	58 (58)
Yes	42 (42)
Tumor size, N (%)	
<5cm	56 (56)
≧5cm	44 (44)
Tumor number, N (%)	
Single	68 (68)
Multiple	32 (32)
Total Bilirubin (mg/dL), median (IQR)	1.38 (0.76–1.89)
Creatinine (mg/dL), median (IQR)	0.97 (0.8–1.20)

(*Continued*)

Table 1 (Continued).

Clinicopathological Parameter	HCC Patients (n=100)
INR, median (IQR)	1.21 (1.03–1.91)
AFP (ng/mL), median (IQR)	52.5 (23.75–126.25)
TASP1 expression, N (%)	
Low	51 (51)
High	49 (49)
MELD, median (IQR)	10 (8–14)

experiment had a poor prognosis (Figure 2E). These results collectively show that TASP1 was upregulated in HCC tumor tissues and was clinically significant.

TASP1 Was an Independent Risk Factor for Prognosis in Clinical HCC Patients

To test whether TASP1 performed a clinically independent prognostic value in HCC patients, we used univariate and multivariate analysis to analyzed independent prognostic factors, including TASP1, for survival in patients with HCC. The univariate analysis results (Table 3) showed that age (HR =1.936, 95% CI [1.146–3.271]; $p = 0.014$), pathologic differentiation (HR = 0.332, 95% CI [0.182–0.605]; $p < 0.001$), and TASP1 expression (HR = 2.025, 95% CI [1.169–3.508]; $p = 0.012$) were significantly associated with the OS of HCC patients. Multivariate survival analysis (Table 3) showed that TASP1 expression was statistically significant predictor of OS (HR = 1.832, 95% CI [1.028–3.267]; $p = 0.040$) and that age (HR = 2.032, 95% CI [1.187–3.477]; $p = 0.010$), and pathologic differentiation (HR = 0.387, 95% CI [0.209–0.717]; $p = 0.003$) were independent predictive factors for OS. In summary, TASP1 expression may be an independent prognostic factor in HCC patients.

The Expression of TASP1 in HCC and Other Cancers in Public Databases

To clarify the role of TASP1 in HCC, we performed bioinformatics analysis to detect the transcription level of TASP1 both in HCC tissues and normal tissues using Oncomine database. We found that the mRNA level of TASP1 was significantly higher in tumor tissues of HCC than that in normal tissues (Figure 3A–C). Aligned with the result, we also confirmed that the high expression of TASP1 in tumor tissues via UALCAN database (Figure 4A). Additionally,
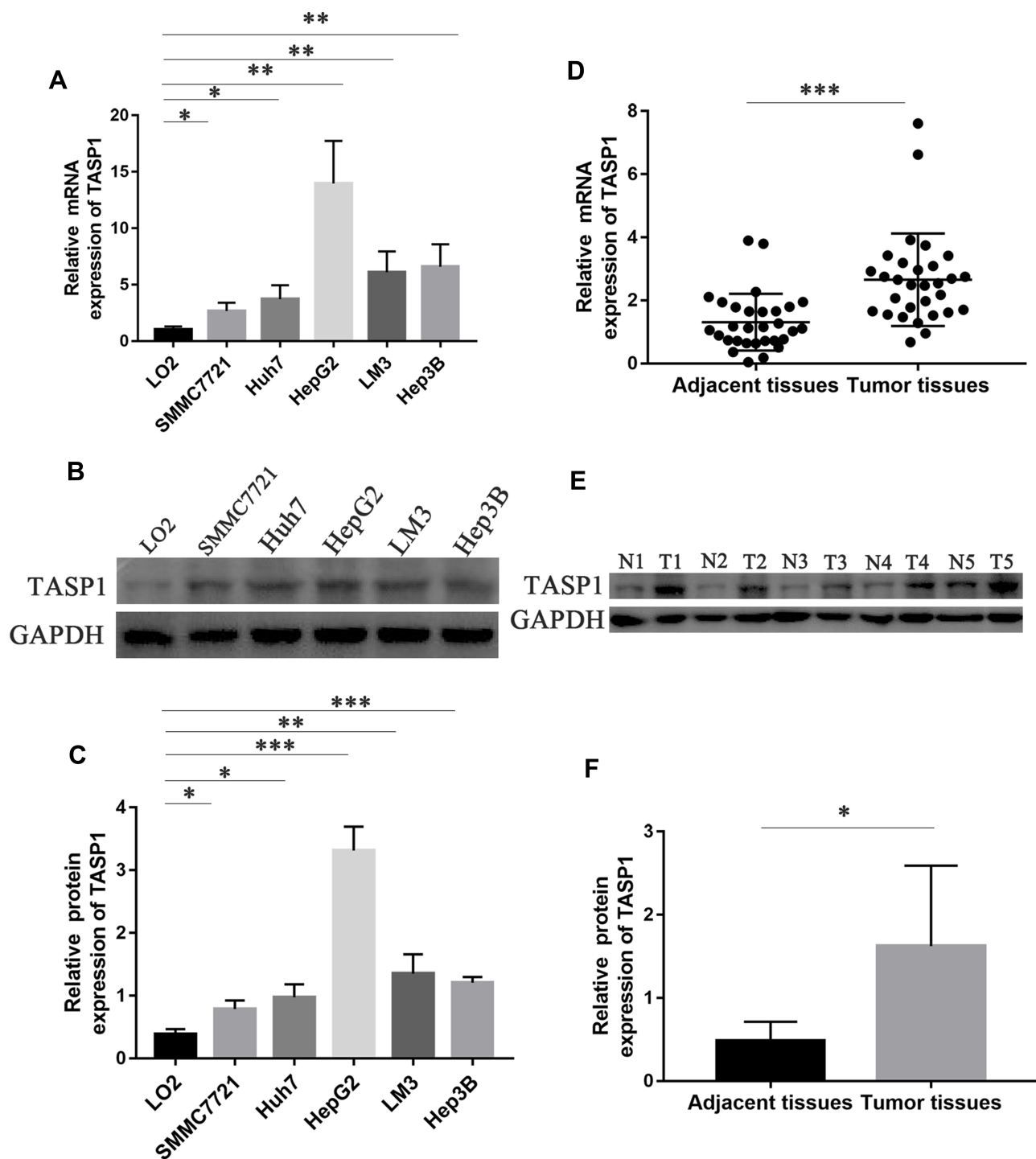
Figure 1 The mRNA and protein expression of TASP1 in HCC cell lines, HCC tissues and the adjacent tissues.

Notes: (**A**) Relative mRNA expression of TASP1 in five HCC cell lines and the normal human liver cell line LO2. (**B** and **C**) TASP1 protein expression in five HCC cell lines and the normal human liver cell line LO2. (**D**) Relative mRNA expression of TASP1 in HCC tissues and the adjacent tissues. (**E** and **F**) TASP1 protein expression in HCC tissues and the adjacent tissues. *$P<0.05$, **$P<0.01$, ***$P<0.01$.

Abbreviations: T, tumor tissue; N, adjacent tissue.

we partially verified the relationship between TASP1 and clinicopathological parameters by conducting a comprehensive analysis of the expression of TASP1 in HCC via UALCAN and the results showed that TASP1 mRNA expression in HCC samples was higher than that in normal samples based on age, race, gender, weight, disease stage, tumor grade, nodal metastasis and TP53 mutation (Figure 4B–I). In addition, we focused on the
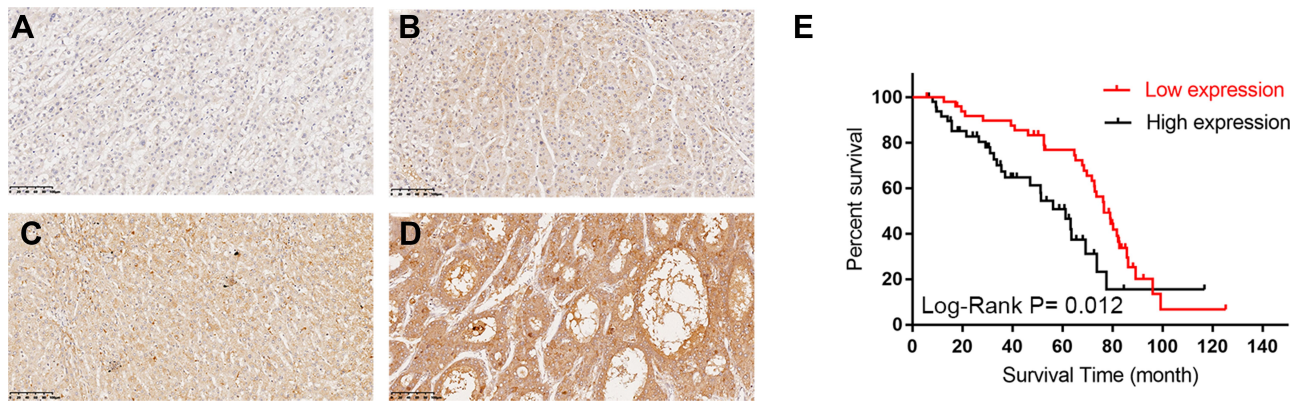
Figure 2 The Immunohistochemical staining of TASP1 expression in HCC tissues and survival curve.
Notes: (**A–D**) TASP1 expression by immunohistochemical staining; (**A**) negative expression of TASP1 in tumor cells of HCC; (**B**) weak TASP1 positive staining in tumor cells of HCC; (**C**) moderate TASP1 positive staining in tumor cells of HCC; (**D**) strong TASP1 positive staining in tumor cells of HCC; (**E**) Kaplan–Meier analysis of overall survival (OS) with variable TASP1 expression in HCC patients. The red curve represents TASP1 low expression group, and the back curve represents TASP1 high expression group.

difference of DNA levels of TASP1, including copy number variation (CNV) and methylation. The results from Guichard livers in Oncomine database represented that TASP1 CNV in HCC tissues was higher compared with that in normal tissues (Figure 3D–F). Furthermore, we identified that TASP1 promoter methylation in tumor tissues was lower compared to that in normal tissues using UALCAN (Figure 4J). Similar results were found in MEXPRESS (Figure 5A). Interestingly, the expression of TASP1 was positively related with DNA methyltransferase (DNMT) expression (Figure 5B–E). Taken together, TASP1 may act as a potential diagnostic biomarker for HCC.

Prognostic Significance of TASP1 Expression in HCC Patients in Public Databases

In cancers, the survival rate of patients is significantly correlated with the abnormal expression of genes. Then we used Kaplan–Meier plotter to examine the prognostic significance of TASP1 expression in HCC, finding that HCC patients with high TASP1 expression had a shorter overall survival, but not a disease-free survival, than those with low TASP1 expression (Figure 6A and B). Furthermore, we verified the results using GEPIA database (Figure 6C and D). Similarly, the result that patients with high TAPS1 expression had poor survival was also validated (Figure S1). These results indicated that TASP1 was tightly correlated with the prognosis of HCC.

Genomic Alterations of TASP1 in HCC

The alternation of TASP1 was analyzed using cBioPortal. TASP1 was altered in 22 of 367 (6%) HCC patients and the

mutation, copy-number variations, and mRNA expression were the main parameters of TASP1 alternation (Figure 7A). Additionally, the expression of TASP1 in gain group was higher than that in the diploid group (Figure 7B). Moreover, we detected the frequency distribution of TASP1 CNV patients in different grade and stage groups (Figure 7C), and the results indicated that TASP1 CNV alteration had a strong correlation with the high occurrence and an early-event.

The Biological Function of Co-Expression Genes Related with TASP1 in HCC

In order to explore the biological meaning of TASP1 in HCC and the correlation between TASP1 and TASP1-related genes, we used the function module of LinkedOmics to investigate the mRNA sequencing data of 371 LIHC patients in TCGA project. The volcano plot showed the correlated genes of TASP1 including 7549 positive genes and 2941 negative genes (Figure 8A). In addition, the results represented the top 50 significant genes positively and negatively correlated with TASP1 in the heatmap (Figure 8B and C). Furthermore, the all details of co-expressed genes were in Table S1. Thus, the result indicated that TASP1 played an important role in the transcriptome. TASP1 was positively correlated with top three genes, including *ESF1* (r = 0.5959, *p* = 4.415E-36), *CSNK2A1* (r = 0.5911, *p* = 2.2E-35), and *XRN2* (r = 0. 5888, *p* = 4.676E-35) (Figure 8D–F), while TASP1 expression showed a negative association with expression of *BLOC1S1* (r =-0.5188, *p* = 2.908E-26), *RILP* (r = -0.4794, *p* = 3.863E-22), and *UQCRQ* (r = -0.4773, *p* = 6.173E-22) (Figure 8G–I). These genes were correlated with TASP1 reflect changes in cell adhesion, proliferation, transcription

Table 2 Association Between TASP1 Expression and Clinicopathological Characteristics of Patients

Clinicopathological Parameter	Total	Expression of TASP1		P value
		Low	High	
Sex				0.558
Male	54	29	25	
Female	46	22	24	
Age				0.715
<60	61	32	29	
≧60	39	19	20	
AJCC stage				0.421
I + II	49	27	22	
III + IV	51	24	27	
T stage				0.057
I + II	72	41	31	
III + IV	28	10	18	
Pathologic differentiation				0.028*
Poor	25	8	17	
Moderate + Well	75	43	32	
Lymph node metastasis				
No	36	14	22	
Yes	64	37	27	
Metastasis				0.977
No	57	29	28	
Yes	43	22	21	
Vascular invasion				0.977
No	57	29	28	
Yes	43	22	21	
HBV				0.195
Negative	37	22	15	
Positive	63	29	34	
HCV				0.915
Negative	78	40	38	
Positive	22	11	11	
Alcohol consumption				0.680
No	47	25	22	
Yes	53	26	27	
Cirrhosis				0.865
No	58	30	28	
Yes	42	21	21	
Tumor size				0.859
<5cm	56	29	27	
≧5cm	44	22	22	

(*Continued*)

Table 2 (Continued).

Clinicopathological Parameter	Total	Expression of TASP1		P value
		Low	High	
Tumor number				0.115
Single	68	31	37	
Multiple	32	20	12	
AFP (ng/mL)				< 0.001*
< 20	22	20	2	
≧20	78	31	47	

Note: *Statistical significance (P value <0.05).

and cell cycle. Additionally, the expression of top genes also affected the overall survival on Kaplan–Meier plotter (Figure S2). Furthermore, GO term analysis of GSEA showed that genes differentially expressed correlated with TASP1 were located mainly in double-strand break repair, peptidyl-threonine modification, spindle organization, peptidyl-lysine modification and microtubule-based movement (Figure 9A–C and Tables S2–4). And the KEGG pathway was enriched in Fanconi anemia pathway, TGF-β pathway, Thyroid hormone signaling pathway, Hippo signaling pathway, et al (Figure 9D and Table S5).

TASP1 Networks of Kinases, miRNAs, Transcription Factor Targets in HCC

To further explore the regulators of TASP1 in HCC, we analyzed the kinases, miRNAs, and transcription factors using the GSEA module in LinkedOmics. The top 5 significant kinase targets of *TASP1* were ATM serine/threonine kinase (ATM), ATR serine/threonine kinase (ATR), cyclin dependent kinase 1 (CDK1), polo like kinase 1 (PLK1) and checkpoint kinase 1 (CHEK1) (Table 4 and Table S6). Interestingly, the expression levels of these kinases, were higher in HCC tissues than those in normal tissues via UALCAN (Figure S3), and except ATM, they were also related with the OS of HCC via Kaplan-Meier plotter (Figure S4). The top 5 correlated miRNA-target networks were (ATAGGAA) MIR-202, (ATGTACA) MIR-493, (ATAAGCT) MIR-21, (ATAACCT) MIR-154, and (TTGGAGA) MIR-515-5P, MIR-519E (Table 4 and Table S7). And the top 5 enrichments of transcript factors were TAANNYSGCG_UNKNOWN, V$E2F1_Q6, V$E2F1DP1_01, V$E2F1DP2_01 and V$E2F4DP2_01 (Table 4 and Table S8). In addition, Gustavo et al study

Table 3 Univariate and Multivariate Analysis of Various Prognostic Parameters in Patients with HCC

Character	n	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	P	HR (95% CI)	P
Sex					
Male	54	I			
Female	46	0.956 [0.566–1.613]	0.865		
Age					
< 60	61	I		I	
≧60	39	1.936 [1.146–3.271]	0.014*	2.032 [1.187–3.477]	0.010*
AJCC stage					
I + II	49	I			
III + IV	51	1.062 [0.632–1.783]	0.821		
T stage					
I + II	72	I			
III + IV	28	0.850 [0.482–1.496]	0.572		
Pathologic differentiation					
Poor	25	I		I	
Moderate+ Well	75	0.332 [0.182–0.605]	<0.001*	0.387 [0.209–0.717]	0.003*
Lymph node metastasis					
No	36	I			
Yes	64	0.907 [0.530–1.551]	0.907		
Metastasis					
No	57	I			
Yes	43	1.114 [0.660–1.881]	0.686		
Vascular invasion					
No	57	I			
Yes	43	1.114 [0.660–1.881]	0.686		
HBV					
Negative	37	I			
Positive	63	1.037 [0.616–1.745]	0.893		
HCV					
Negative	78	I			
Positive	22	1.060 [0.559–2.011]	0.858		
Alcohol consumption					
No	47	I			
Yes	53	1.422 [0.843–2.397]	0.187		
Cirrhosis					
No	58	I			
Yes	42	0.764 [0.450–1.296]	0.319		
Tumor size					
<5cm	56	I			
≧5cm	44	1.219 [0.730–2.035]	0.450		
Tumor number					
Single	68	I			
Multiple	32	1.070 [0.629–1.821]	0.803		

(*Continued*)

Table 3 (Continued).

Character	n	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	*P*	HR (95% CI)	*P*
AFP (ng/mL)					
< 20	22	1			
≧20	78	1.056 [0.572–1.947]	0.862		
TASP1					
Low		1		1	
High		2.025 [1.169–3.508]	0.012*	1.832 [1.028–3.267]	0.040*

Note: *Statistical significance (P value <0.05).

found that E2F-driven transcriptional program played a critical role in the development and progression of HCC. Therefore, TASP1 may affect the development and progression of HCC by modulating these kinases, microRNAs and transcript factors.

Networks Analysis of TASP1 with Interacted Genes

We performed PPI network to identify the interactions between TASP1 and co-expressed genes in HCC using STRING database. The top 20 genes evaluated via connectivity degrees from the PPI network through Cytoscape were selected as hub genes, including KMT2A, WDR5, MEN1, PSIP1, KMT2B,

MLLT3, RBBP5, MLLT1 and AFF1. The results indicated that these top genes with TASP1 were mainly enriched in transcriptional misregulation in cancers (Figure 10).

Correlations Between TASP1 Expression and Immune Infiltrations in HCC

Growing studies showed that lymphocyte infiltration plays a key role in cancers, acting as an independent predictive factor for overall survival. Therefore, we conducted a comprehensive analysis to reveal the correlation between TASP1 expression and immune infiltration levels in HCC based on TIMER. The most six frequently immune infiltrating cells, including CD4 $^+$ T cell, CD8 $^+$ T cell,
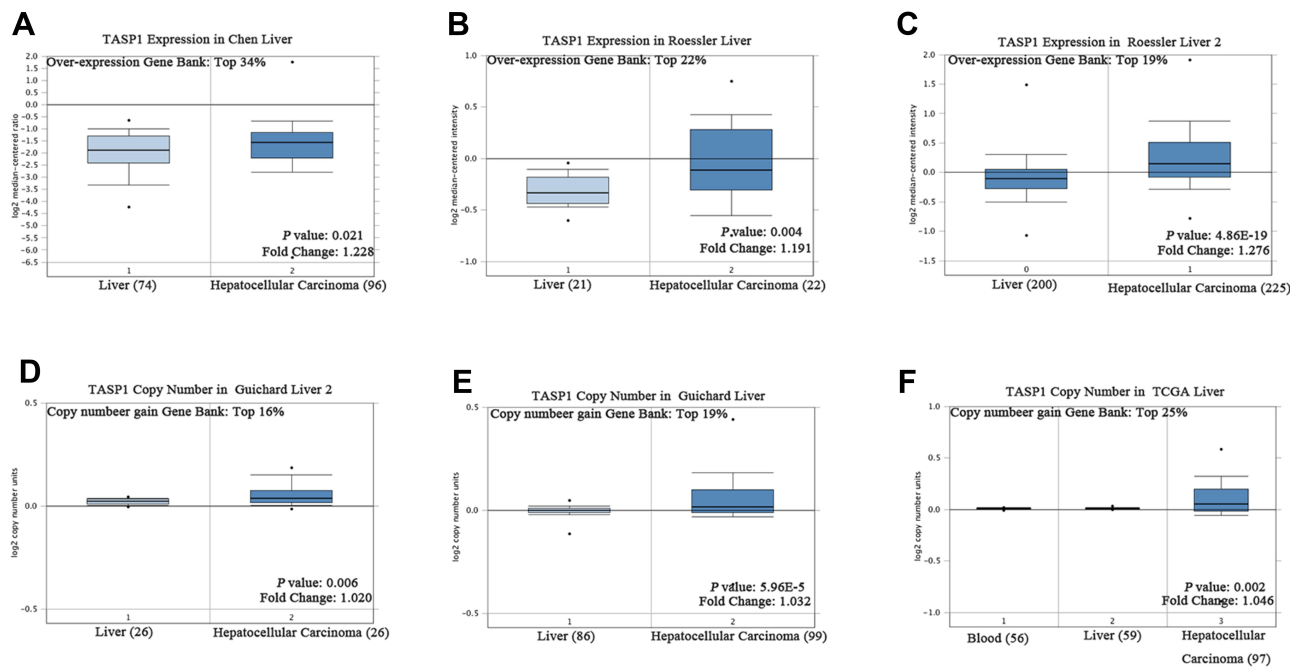
Figure 3 TASP1 transcription in hepatocellular carcinoma (Oncomine).
Notes: (**A**–**C**) Box plot showing TASP1 mRNA levels in, respectively, the Chen liver, Guichard Liver, Roessler liver datasets. (**D**–**F**) Box plot showing TASP1 copy number in The Cancer Genome Atlas (TCGA) Liver and Guichard Liver and Guichard Liver 2 datasets, respectively.
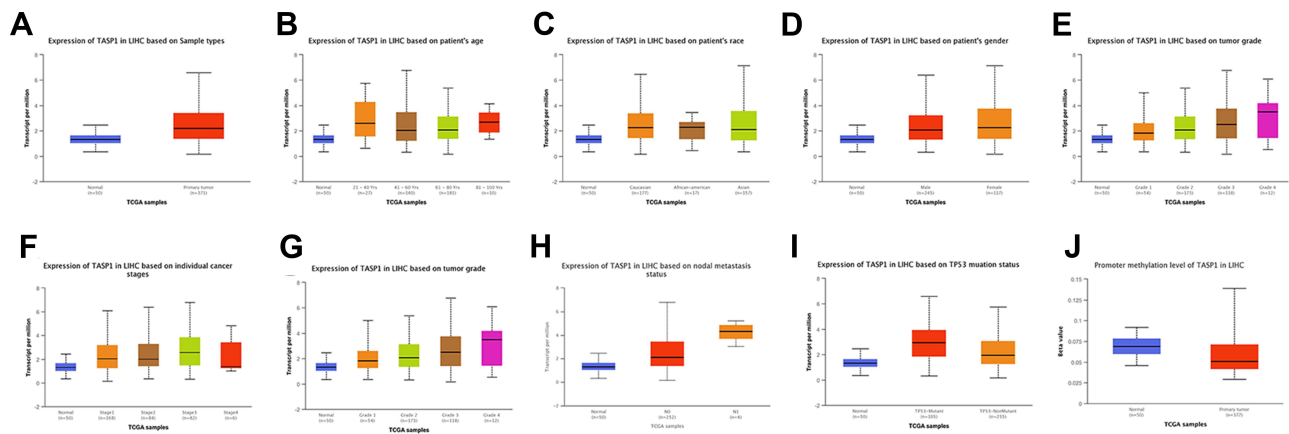
Figure 4 TASP1 expression in hepatocellular carcinoma (HCC) (UALCAN).

Notes: (**A**) Boxplot showing the relative expression of TASP1 in normal and LIHC samples. (**B**) Boxplot showing relative expression of TASP1 in normal individuals of any ethnicity or in LIHC patients of Caucasian, African-American or Asian ethnicity. (**C**) Boxplot showing relative expression of TASP1 in normal individuals of either gender or male or female LIHC patients. (**D**) Boxplot showing relative expression of TASP1 in normal individuals of any age or in LIHC patients aged 21–40, 41–60, 61–80, or 81–100 yr. (**E**) Boxplot showing relative expression of TASP1 in normal individuals or in LIHC patients in stages 1, 2, 3 or 4. (**F**) Boxplot showing relative expression of TASP1 in normal individuals or LIHC patients with grade 1, 2, 3 or 4 tumors. (**G**) Boxplot showing relative expression of TASP1 in normal individuals or in LIHC patients in normal weight, extreme weight, obese, or extremely obese. (**H**) Boxplot showing relative expression of TASP1 in normal individuals or in LIHC patients with or without nodal metastasis. (**I**) Boxplot showing relative expression of TASP1 in normal individuals or in LIHC patients with or without TP53 mutation. (**J**) Boxplot showing the promoter methylation level of TASP1 in normal individuals or in LIHC patients.
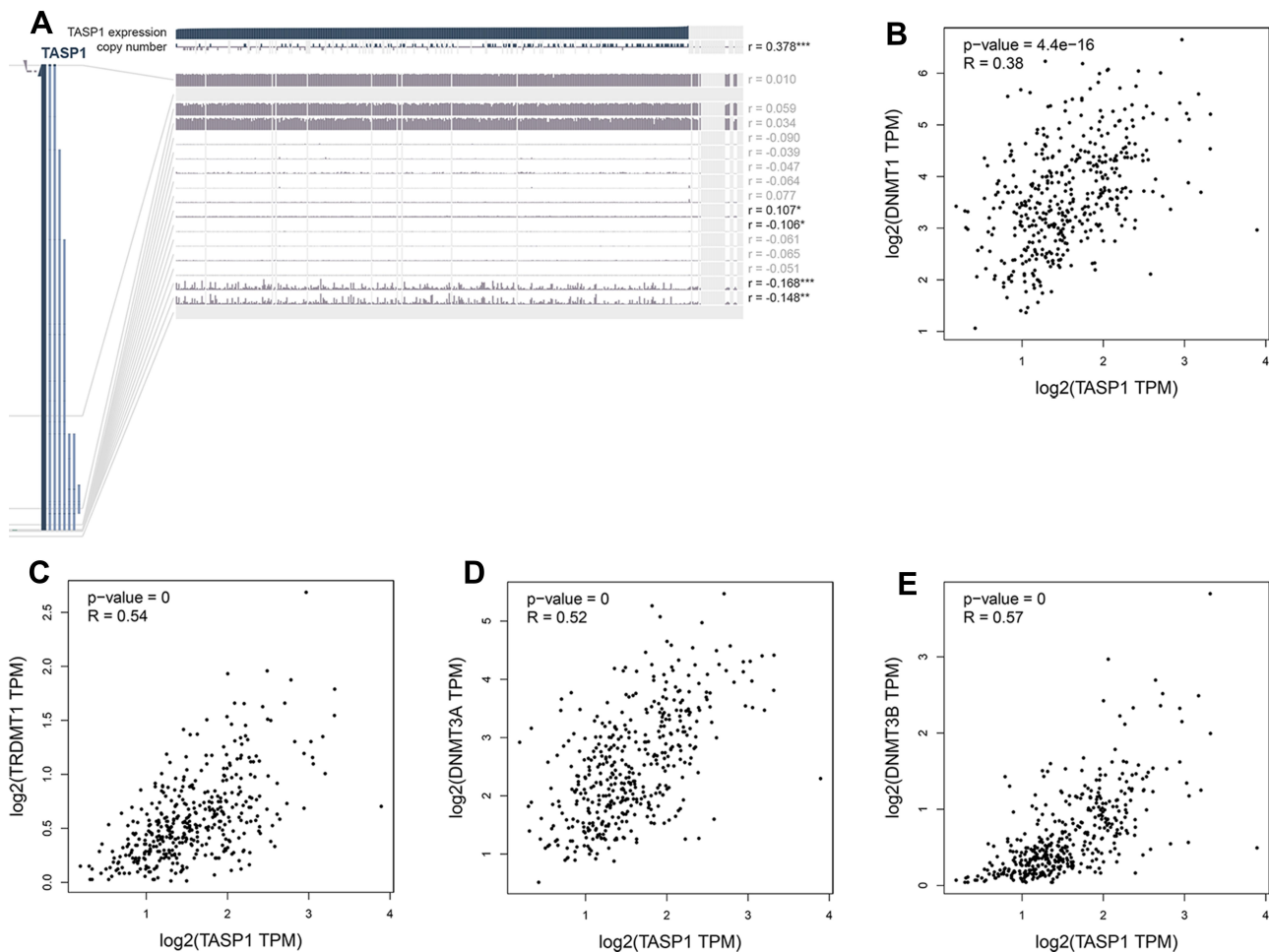
Figure 5 The methylation modification of TASP1 promoter in HCC.

Notes: (**A**) TASP1 DNA methylation level in HCC via MEXPRESS. (**B-E**) The relationship between TASP1 transcription level and DNA methyltransferase (DNMT) expression via GEPIA..
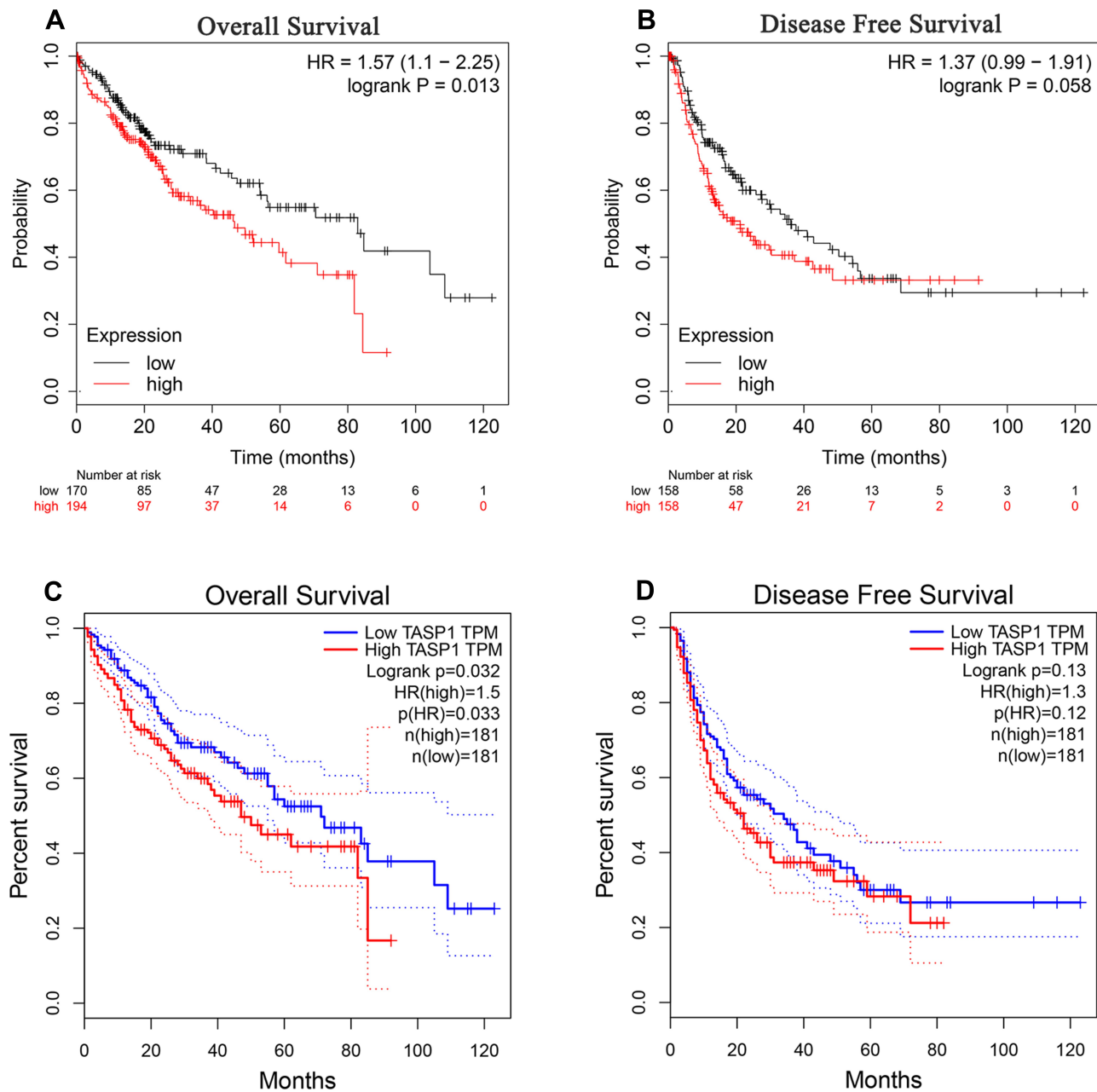
Figure 6 The survival outcome of TASP1 in HCC.
Notes: (**A**) Overall survival (OS) (**B**) disease-free survival (DFS) of TASP1 in Kaplan–Meier Plotter database. (**C**) OS (**D**) DFS of TASP1 in GEPIA.

neutrophil, macrophage, B cell and Dendritic cell, were selected and we found that the expression of TASP1 had a correlation with tumor purity (Cor = 0.099, p = 6.6E-02) in HCC. Additionally, the mRNA level of TASP1 had a remarkably positively correlation with the infiltration of B cell (Cor = 0.287, p = 6.31E-08), CD8^{+} T cell (Cor = 0.251, p =2.65E-06), CD4^{+} T cell (Cor = 0.406, p = 4.76E-15), macrophage (Cor = 0.409, p = 3.38E-15), neutrophil (Cor = 0.446, p = 2.69E-18) and dendritic cell (Cor = 0.407, p = 5.27E-15) (Figure 11). These findings

suggested that TASP1 may play an important role in the tumor microenvironment by affecting the infiltration of immune cells. In addition, the top 3 positive or negative genes of TASP1 showed the significant correlations with varying degrees with immune cells (Figure S5).

The Correlations of TASP1 Expression with Immune Factors

Some studies have indicated that chemokines and their receptor families are important mediators for recruiting immune

A

B

C
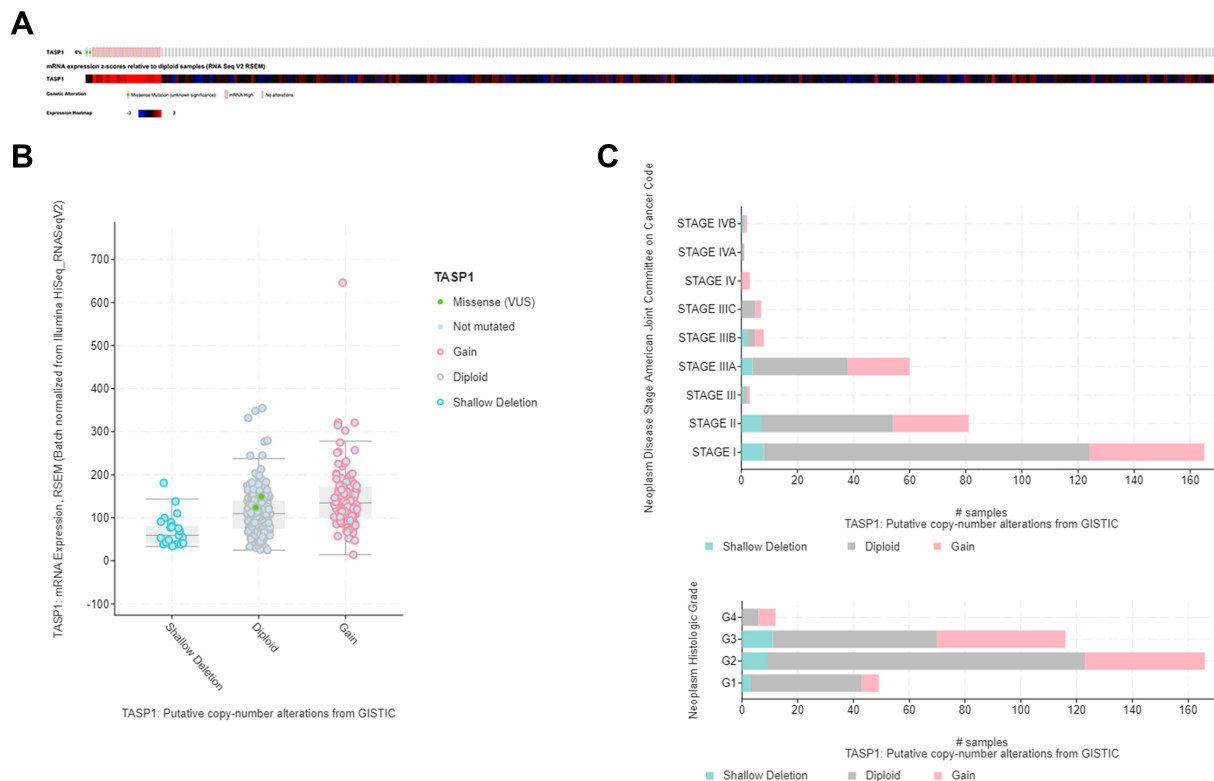
Figure 7 Visual summary of TASP1 alterations in HCC (cBioPortal).
Notes: (**A**) OncoPrint of TASP1 alterations in LIHC cohort. (**B**) Boxplot showing the copy-number variation (CNV) of TASP1 in HCC by GISTIC. (**C**) The frequency of TASP1 CNV in subgroups such as stage and grade.

cells, thus providing an ideal microenvironment for tumor biology. Moreover, different chemokines have different roles in cancers: some chemokines stimulate the development and progression of cancers, whereas some chemokines show opposite functions. Thus, TISIDB database was utilized for analyzing the correlation between TASP1 expression and chemokines. The heatmap of chemokines in various cancers suggested that TASP1 acted a key role in chemokines in cancers and we analyzed the correlation among them after filtering for $|\pm rho| \geq 0.1$ and $p < 0.05$. In this study, the results indicated that TASP1 had a strong negatively correlation with CCL2, CCL5, CCL8, CCL11, CCL13, CCL14, CCL15, CCL16, CCL19, CCL21, CXCL2, CXCL16 and XCL2, and the relationship between TASP1 and CCL20 was positive (Figure 12A). The results showed that TASP1 may be involved in regulating the tumor immunology via chemokines.

Except for chemokines, immune checkpoints, also known as coinhibitory molecules, modulate the tumor microenvironment and help tumor cells evade immune surveillance in many studies. Furthermore, antibodies targeting coinhibitory molecules have become popular and

promising. To determine whether TASP1 may play a role in cancers via immune checkpoints, we investigated the relationship between TASP1 expression and immune checkpoints through TISIDB database. Surprisingly, as shown in the heatmaps, TASP1 was significantly correlated with immune checkpoint genes including immunoinhibitory genes and immunostimulatory genes. We found that TASP1 had a negative correlation with C10orf5, CD48, KLRK1, TNFRSF4, TNFRSF14, TNFRSF17, TNFRSF18 and TNFSF9, and a positive correlation with CD80, IL6R, TNFRSF9 and TNFSF15 of immunostimulants (Figure 12B); In addition, TASP1 was negatively related with CD160, CD244, CSF1R, IL10RB, LGALS9 and PVRL2, and positively related with TGFBR1 of immunoinhibitors (Figure 12C). These findings revealed that TASP1 may affect tumor biology via the regulation of immune checkpoints.

Discussion

Due to a difficulty in detecting HCC at an early stage, HCC, one of the most common form of cancers, is a big
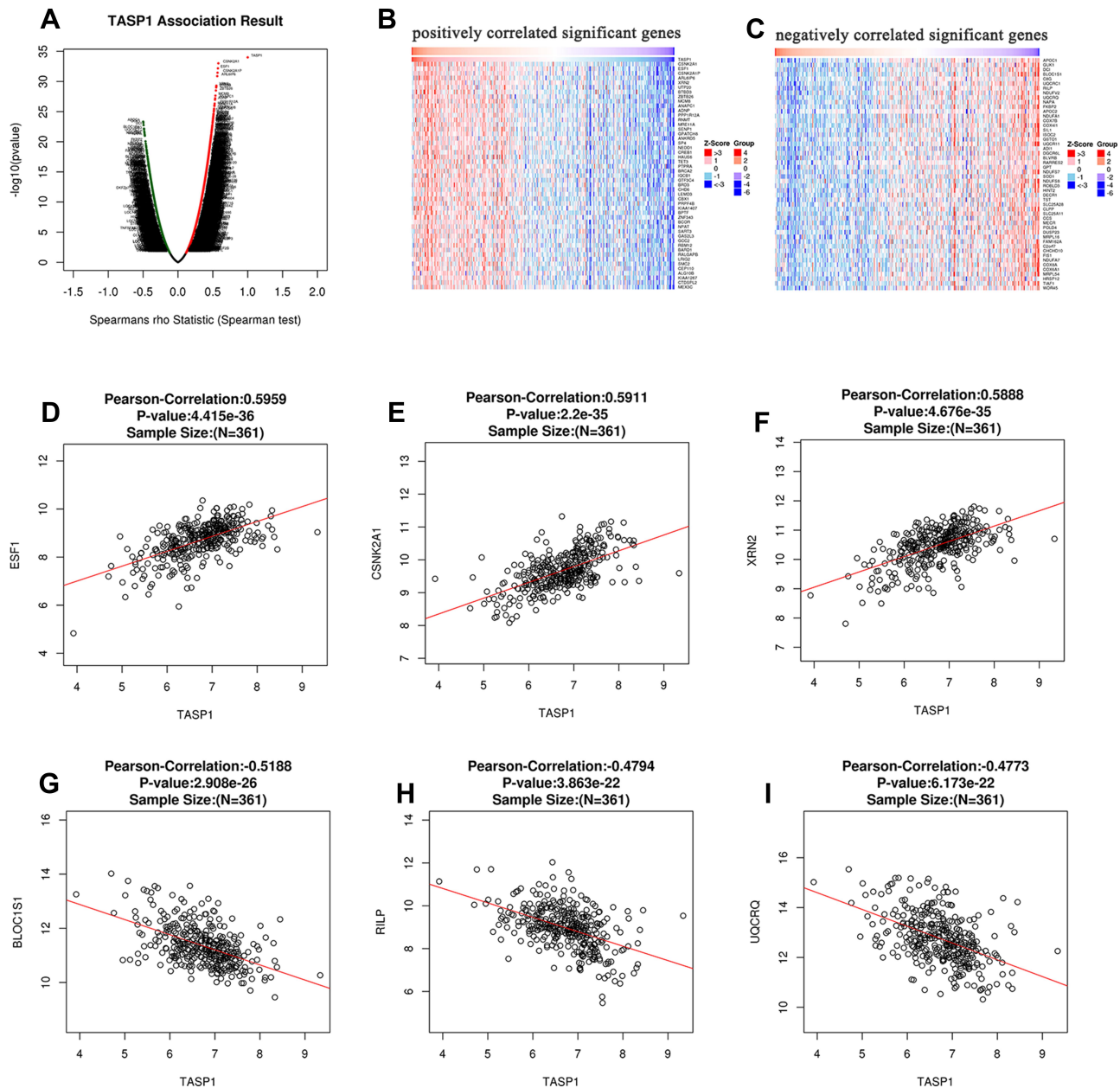
Figure 8 The co-expression genes of TASP1 in HCC.
Notes: (**A**) A person test was used to explore correlations between TASP1 and genes differentially expressed in HCC. (**B** and **C**) The top 50 genes were positively or negatively correlated with TASP1 in HCC were shown in heat maps. (**D**–**F**) The positive correlation of TASP1 with the top 3 genes (SF1, CSNK2A1, XRN2). (**G**–**I**) The negative correlation of TASP1 with the top 3 genes (BLOC1S1, RILP, UQCRQ).

threat to human health. Because the molecular and cellular heterogeneity in HCC is complex, the underlying mechanisms of HCC are still unknown. Site-specific proteolysis has been reported to play an important role in regulating numerous physiological and pathological conditions caspases activation and cancers.[27–29] TASP1, a highly conserved protease, mediates proteolytic cleavage occurs, then leading to the development and progression of various cancers.[8,13] However, the role of TASP1 in HCC has not

been understood well. To better understand the potential functions and the regulatory network of TASP1 in HCC, we collect HCC cells lines, clinical specimens and conduct bioinformatics analysis to provide novel insights for further research.

In this study, we examined the mRNA and protein expression of TASP1 in HCC cell lines and normal liver cell line, finding that the mRNA and protein expression of TASP1 in HCC cell lines was higher than that in the
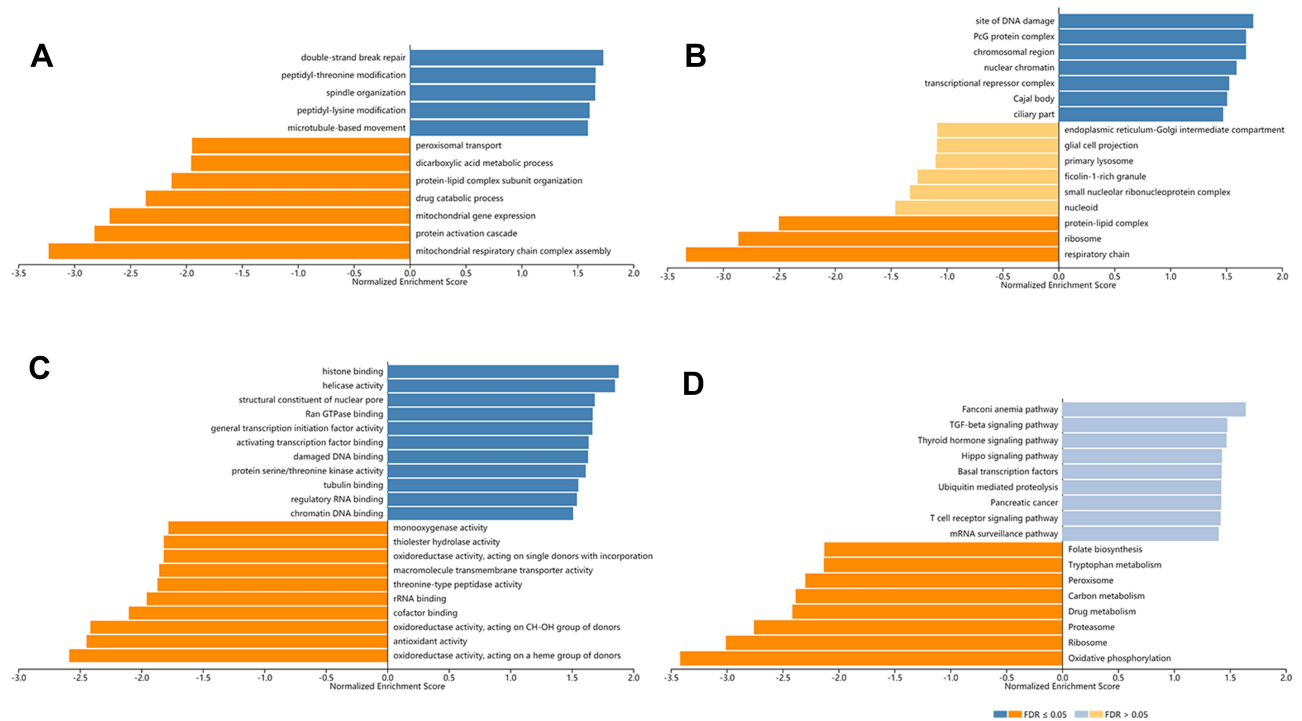
Figure 9 Biological function of TASP1 correlated genes in HCC (LinkedOmics).
Notes: GO annotations and KEGG pathways by GSEA were performed to clarify the biological function of TASP1 correlated genes in HCC. (**A**) Cellular components. (**B**) Biological processes. (**C**) Molecular functions. (**D**) KEGG pathway analysis.

normal liver cell line. And based on clinical samples and public databases, the expression levels of TASP1 in HCC tissues were significantly higher than those in normal liver tissues. And high TASP1 expression was strongly correlated with pathologic differentiation in our clinical patients. Additionally, the mRNA levels of TASP1 in HCC were strongly correlated with clinic pathological features under subgroups, including age, race, gender, weight, disease stage, tumor grade, nodal metastasis and TP53 mutation from public databases. Furthermore,

Table 4 The Kinase, miRNA and Transcription Factor-Target Networks of TASP1 in Hepatocellular Carcinoma (LinkedOmics)

| Enriched Category	Geneset	LeadingEdgeNum	FDR
Kinase Target	Kinase_ ATM	59	0.0023
	Kinase_ ATR	29	0.0127
	Kinase_CDK1	96	0.0154
	Kinase_PLK1	41	0.0161
	Kinase_CHEK1	62	0.0175
MiRNA Target	ATAGGAA, MIR-202	47	0
	ATGTACA, MIR-493	163	0
	ATAAGCT, MIR-21	45	0
	ATAACCT, MIR-154	29	0
	TTGGAGA, MIR-515-5P, MIR-519E	52	0
Transcription Factor Target	TAANNYSGCG_UNKNOWN	34	0
	V$E2F1_Q6	78	0.0017
	V$E2F1DP1_01	103	0.0018
	V$E2F1DP2_01	103	0.0018
	V$E2F4DP2_01	103	0.0018
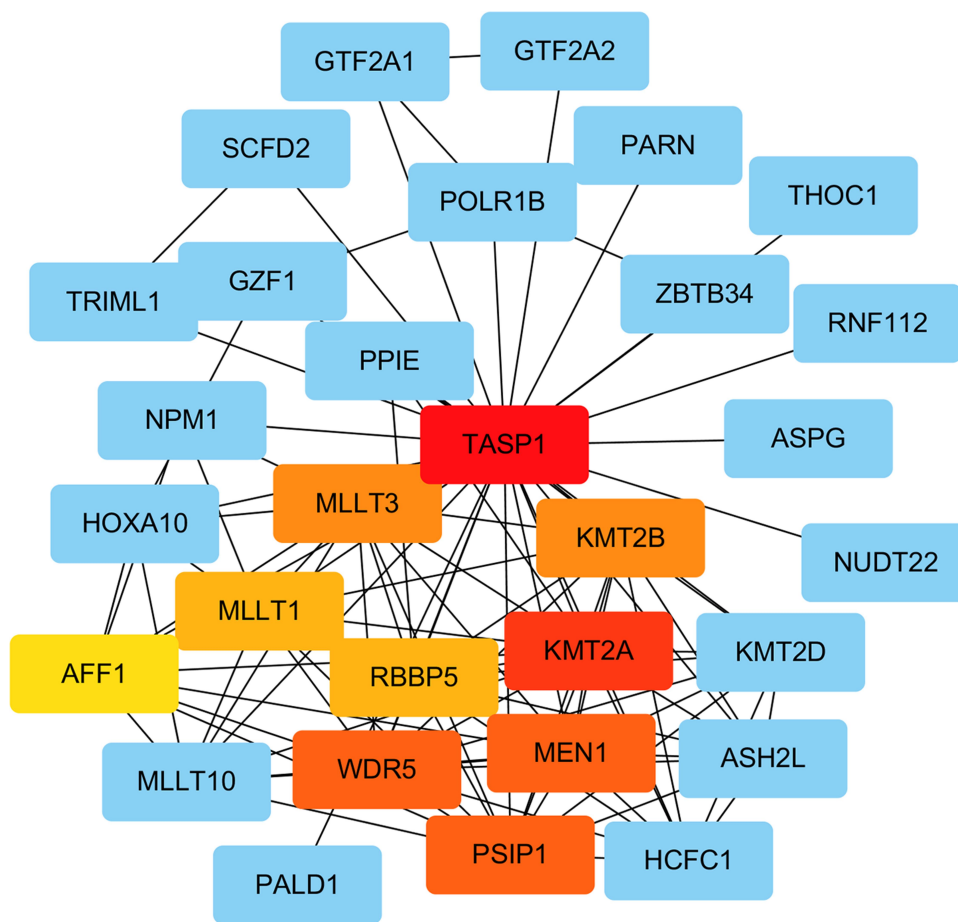
Figure 10 Analysis of TASP1 related proteins based on PPI network (STRING).
Note: On line tool of STRING database analysis of the PPI network for the differentially expressed genes.
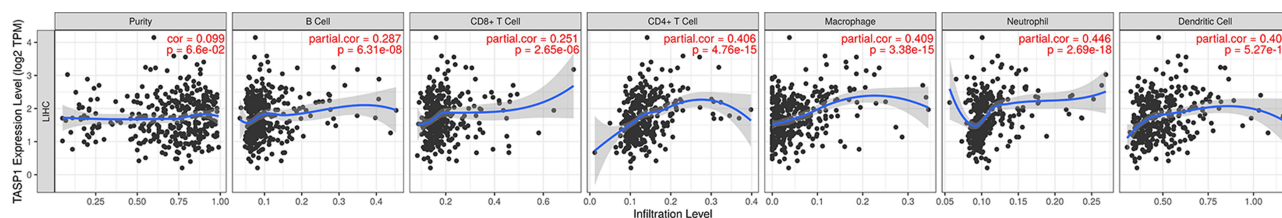

Figure 11 Correlation of TASP1 expression with immune cell infiltration in HCC (TIMER).
Notes: TASP1 is related with tumor purity and TASP1 expression has positive correlations with the infiltrating levels of B cells, CD8 $^+$ T cells, CD4 $^+$ T cells, macrophages, neutrophils and dendritic cells.

previous studies have shown that somatic mutation is a key role in the development and progression of cancers.[30,31] Copy-number variation, one of the major alterations in cancers, also significantly affects the somatic mutations, then causing the phenotypic differences via changing the original state of the genome.[32,33] DNA methylation, one of the most popular epigenetic changes, is another aspect affecting tumor biological behaviors.[34,35] One previous study indicated that the methylation of LY6K promoter significantly affected the glioblastoma

tumorigenicity via ERK1/2 signaling pathway.[36] The recurrence in HCC was strongly involved in the CpG methylation.[37] Our results showed that the promoter methylation levels of TASP1 in liver tumor tissues are decreased compared with that in normal tissues. Moreover, the expression of TASP1 was strongly correlated with the expression of DNMTs, indicating that the gene TASP1 was modulated by DNA methylation. Thus, we speculate that TASP1 may be a potential marker for diagnosis of HCC.
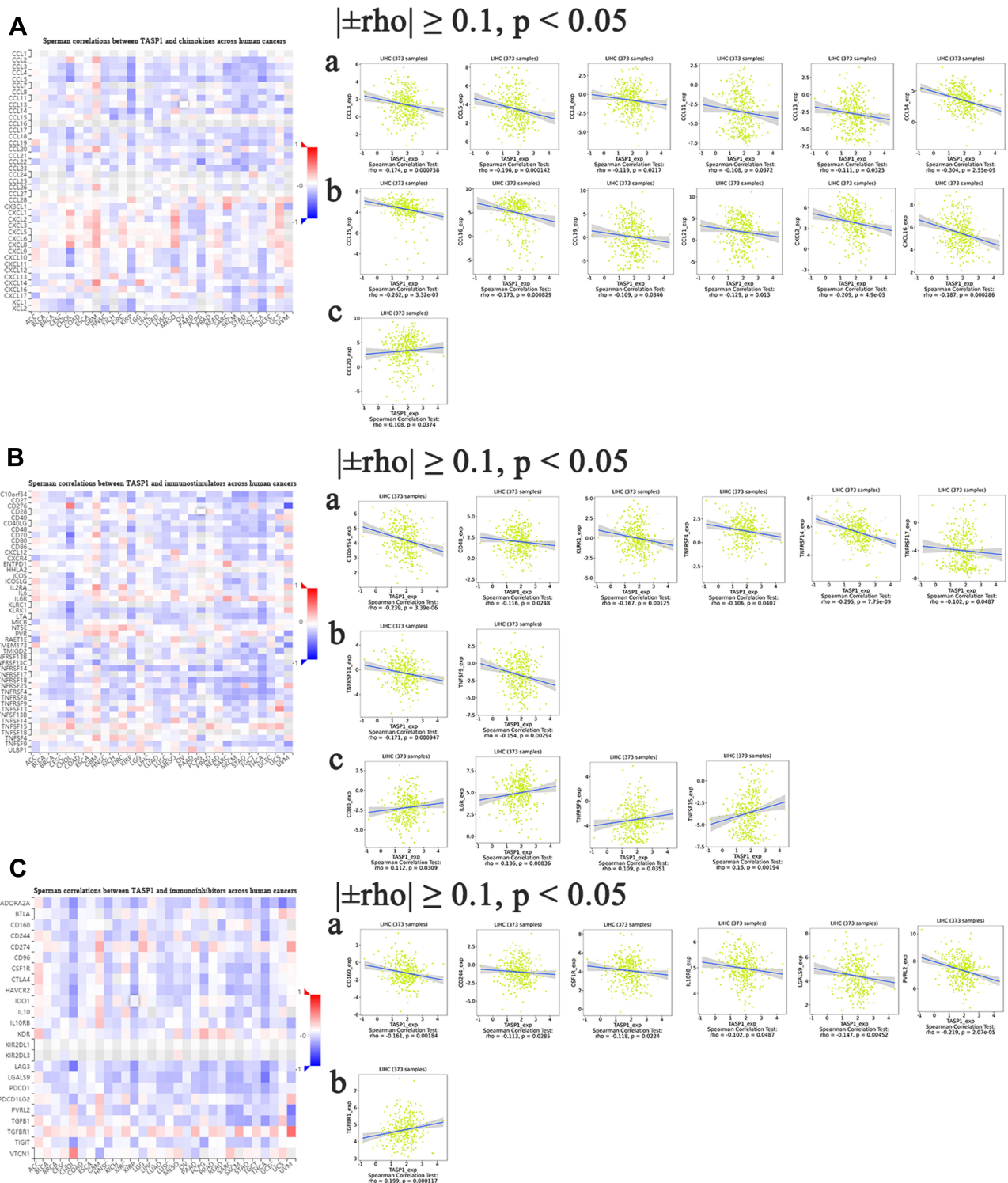
Figure 12 Correlation analyses between dysregulated TASP1 and cancer-related immune factor in HCC (TISIDB).
Notes: (**A**) The association between TASP1 and chemokines was assessed in HCC. (**B**) The association between TASP1 and immunostimulators was assessed in HCC. (**C**) The association between TASP1 and immunoinhibitors was assessed in HCC.

In our study, we analyzed TASP1 expression and its relationship with prognosis using our clinical patients and public databases. Survival analysis revealed that patients with high expression of TASP1 usually had poorer survivals than those with low TASP1 expression. Thus, we speculate that TASP1 expression may act as a prognostic biomarker for HCC. To better understand the mechanism of TASP1 in HCC, we performed

bioinformatic analysis, including GO and KEGG pathway enrichment analysis, and PPI analysis, to detect the function of TASP1 in HCC and TASP1-related genes and potential molecular pathways. We found that the functional network of TASP1 participated primarily in the TGF-β pathway, Thyroid hormone signaling pathway, and Hippo signaling pathway. Accumulated evidence indicated that TGF-β signaling pathway frequently occurs in various cancers, including HCC, and it was highly associated with overall survival.[38,39] Also, hippo signaling pathway plays a crucial role in biological process related with tumorigenesis and metastasis, including self-renewal, proliferation, migration, and invasion.[40] These findings suggest that TASP1 is a potential drug target for therapy.

Because the tumor cells have some fundamental characteristics, such as genomic instability and mutagenesis,[41,42] we conducted GSEA to identify some key kinases and kinase-related signaling pathways, which may stabilize and repair genomic DNA. The results represented that TASP1 had a strong correlation with a network of kinases, including ATM, ATR, CDK1, PLK1, CHEK1. Interestingly, several studies have confirmed that these kinases modulate genomic stability, DNA replication and cell cycle.[43–46] All these kinases, related with TASP1, have been focused on in various studies. For example, CDK1 regulates the mitosis, differentiation and somatic reprogramming. Interestingly, some CDK1 inhibitors have been used to perform clinical trials for the treatment of various cancers.[47] Additionally, CDK1-PLK1, together with CDK1, have been found to participate in the regulation of cell cycle in HCC.[48]

Furthermore, we performed GSEA to detect the networks of miRNAs related with TASP1 in HCC and find miR-202, miR-493, miR-21, miR-154, miR-515-5p and miR-519e. miRNAs, post-transcriptionally regulating gene expression, are involved in the carcinogenesis. These miRNAs detected in this study display a role in the development and progression of cancers via tumor proliferation, apoptosis, cell cycle, invasion, metastasis and angiogenesis.[49–52] In fact, miR-202, miR-493 and miR-21 had been reported that they could be used to diagnose and predict the prognosis of HCC.[53–55] Also, miR-21 promotes the growth of HCC via TETs-PTENp1-PTEN signaling pathway.[56] This indicates that TASP1 affect HCC through the miRNAs dysregulation and changes of these miRNAs may be promising targets for HCC.

Except kinases and miRNAs, we analyzed transcription factors of TASP1 in HCC. The results showed that among significant transcription factors, E2F1 acts an important role due to the dysregulation of TASP1, and modulates the cell cycle in HCC. E2F, a common oncogenic signaling pathway, drives the development and progression of HCC.[57] Furthermore, a previous study represented that the copy number of E2F1 was changed, leading the progression of HCC.[58]

Immune components play a critical role in the regulating tumor responses and more and more studies have focused on the interactions between tumors and immune cells. Thus, we investigated whether TASP1 can regulate the immune microenvironment in HCC. Then, we analyzed the correlation between TASP1 and the infiltration of immune cells, and found that TASP1 expression was strongly associated with the immune cell infiltration, including B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells. Our result was aligned with other studies, indicating that the immunotherapy and clinical outcomes of cancers are dependent on the immune cell infiltration.[59,60] Previous studies showed that chemokines can modulate the tumor immunity and tumor biological phenotypes by mediating the infiltration of immune cells.[61–63] Further study showed that TASP1 expression was correlated with some chemokines in HCC, such as CCL2, CCL5, CCL8, CCL14, et al. And a study has been found that CCL14 served as an independent prognostic biomarker of HCC.[64] In addition, CCL14 regulates angiogenesis and metastasis of breast cancer via the JARID1B/LSD1/NuRD complex.[65] Above the mentioned results, we speculate that TASP1 may affect the HCC biological behaviors through immune response, such as the infiltration of immune cell and chemokine expression.

Many factors take part in the tumor microenvironment and immunological checkpoints are included. The expression levels of inhibitory immune checkpoint proteins in tumor tissues are changed compared to that in normal tissues, reflecting that immune checkpoints are involved in tumor microenvironment.[66] And immune checkpoints lead to the development and progression and promote cancers from evading immune response.[67] To confirm that TASP1 acts as a multimodality regulator of tumor immune response, we detect the relationship between TASP1 and immunological checkpoints. And we find that different kinds of immunological checkpoints, such as C10orf5, CD48, IL-6R, CD160,

CD244 and et al, are involved in TASP1, indicating that immunological checkpoints act as an important role in HCC.

Our study has some limitations. In vitro and in vivo experiments are needed to confirm the functions and mechanisms of TASP1 in HCC.

Conclusion

In conclusion, the results showed that the expression of TASP1 in HCC was increased and TASP1 may be an independent biomarker for prognosis in HCC patients. Moreover, TASP1 could play a critical role in the development and progression of HCC via numerous regulators including kinases, miRNAs and transcript factors. In addition, TASP1 affected the tumor microenvironment by the immune cell infiltration, chemokines, and immune checkpoints. To our knowledge, we found that TASP1 was an important modulator in HCC and TASP1 was a target with greatly therapeutic promise for HCC.

Acknowledgments

Funding

Disclosure

The authors report no conflicts of interest in this work.

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