Overexpression of chaperonin containing TCP1 subunit 7 has diagnostic and prognostic value for hepatocellular carcinoma.

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Abstract

Background

Chaperonin containing TCP1 subunit 7 (CCT7), a member of the chaperonin containing TCP1 complex (CCT), has been reported regulating the expression of many tumor-related proteins. In this study, we investigated the diagnostic and prognostic value of CCT7 expression for hepatocellular carcinoma (HCC).

Methods

We investigated the CCT7 expression in HCC in The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO) and our cohort. The diagnostic and prognostic value were verified by receiver operating characteristic curve (ROC) analysis and Kaplan-Meier analysis, respectively. The association between CCT7 expression with DNA methylation status was investigated in the TCGA database. Gene ontology (GO), The Kyoto Encyclopedia of Genes and Genomes (KEGG), and gene set enrichment analysis (GSEA) analysis were employed to identify the potential pathway in which CCT7 is involved in tumorigenesis and progression.

Results

CCT7 expression in HCC was significantly higher than adjacent normal tissues, and elevated CCT7 expression correlated with tumor stages and tumor grade. Furthermore, the ROC curve showed CCT mRNA expression has a better diagnostic value for HCC with early-stage and low alpha-fetoprotein expression. Positive predictive value (PPV) of CCT7 was higher than alpha-fetoprotein both in the GEO and TCGA database. The Multivariate Cox Regression analysis of clinicopathologic characteristics revealed that both high mRNA and protein expression of CCT7 were independent risk factors for overall survival (OS) and recurrence-free survival (RFS). High DNA methylation of CpG site(cg19515186) was associated with low CCT7 expression and better OS in HCC. The GO, KEGG and GSEA analysis demonstrated that CCT7 mRNA expression was associated with Spliceosome signaling pathway.

Conclusions

The findings of this study demonstrated that CCT7 has diagnostic and prognostic value for HCC.

Background

Hepatocellular carcinoma is one of the most common malignant tumors and has caused a huge economic and healthy burden for a long time around the world. According to data released by the American Cancer Society in 2021, the 5-year survival rate of all stages of HCC patients is still less than 20%, and cancer-related mortality ranks fifth[1]. The main reason for the poor prognosis of HCC should

attribute to the low diagnosis rate in the early stage of the disease[2]. Most HCC patients miss potential curative therapeutic interventions at the time of diagnosis was made. Currently, liver ultrasound examination and serum alpha-fetoprotein (AFP) detecting were recommended to screen patients with early stages of HCC[3]. However, the sensitivity of both is not enough to screen the early lesions. Therefore, the search for more sensitive diagnostic molecular biomarkers for the early stage of HCC is of great significance to improve the prognosis.

Chaperonin-containing TCP-1 (CCT), composed of 8 subunits (α, β, γ, δ, ε, ζ, η, and θ, which were coded by CCT1, CCT2, CCT3, CCT4, CCT5, CCT6, CCT7, CCT8, respectively), was a kind of an intracellular chaperonin[4, 5]. Chaperonin-containing CCT was responsible for promoting the folding of intracellular proteins in the cytoplasm, mainly cytoskeletal proteins such as tubulin and actin[6]. Since cell division, directed cell migration, and invasion were the main driving factors for tumorigenesis and progression, and all these processes depend on the microtubules and actin filaments of the cytoskeleton, CCT activities are fundamentally involved in cancers[5-7]. Existing studies have shown that CCT3 promotes the progression of HCC by co-acting upstream with Yes-associated protein (YAP) and transcription factor CP2 (TFCP2) and serves as a potential therapeutic target and biomarker for HCC[8]. In addition, Guang Xu and their colleagues have shown that CCT3 was closely related to the proliferation and metastasis of breast cancer, and may be a novel therapeutic target[9]. Previous studies demonstrated that CCT2, CCT5, CCT6A, CCT7 were found overexpressed in various tumors, and its high expression was related to the prognosis[10-14]. Studies through bioinformatics analysis found that CCT7 is overexpressed in HCC and associated with worse survival, but its clinical prognosis and diagnostic value, and gene function were not illustrated[15, 16].

In the present study, we analyzed the Gene Expression Omnibus (GEO), the Cancer Genome Atlas (TCGA), and other public databases, combined with the immunohistochemical staining of a HCC cohort, finding that the mRNA and protein level of CCT7 significantly overexpressed in HCC tissues compared with adjacent normal tissues, and its high expression was closely related to multiple clinical characteristics and predicted a worse clinical outcomes of HCC patients. At the same time, we identified a CpG site(cg19515186) and found a negative correlation between the methylation status of CCT7 with the OS of HCC. Next, we investigated the gene function of CCT7 by performing the gene ontology (GO), The Kyoto Encyclopedia of Genes and Genomes (KEGG), and gene set enrichment analysis (GSEA) analysis to identify the potential pathway in which CCT7 is involved in tumorigenesis and progression.

Methods

Gene expression analysis.

We analyzed the mRNA expression level of CTT7 in the UALCAN database(http://ualcan.path.uab.edu/analysis)[17], which visualized data from the TCGA database[18]. The mRNA expression level of CCT7 in metastatic tissues and non-metastatic tumors was compared in the TNMplot database (https://tnmplot.com/analysis/)[19]. The associations between CCT7 mRNA level

with OS and RFS were analyzed in the GEPIA database (http://gepia.cancer-pku.cn/)[20]. Then, we queried the protein expression level of CTT7 in the Human Protein Atlas database (https://www.proteinatlas.org/)[21]. We also analyzed the mRNA level and compared the diagnostic efficiency of CCT7 and AFP in the GEO (GSE76247, GSE54236, GSE136247, GSE25097, and GSE63898 datasets)[22] and the TCGA database.

Prognostic value analysis using CCT7 mRNA expression and clinicopathological data in the TCGA database.

We downloaded the gene expression RNAseq and clinical characteristics dataset from the TCGA database to study the relationship between the expression of CCT7 and the clinical outcome of HCC patients. According to the mRNA expression level, 372 HCC patients were divided into high and low CCT7 mRNA expression groups to evaluate the prognostic value of CTT7. We defined overall survival (OS) as the time interval between surgery and death or between surgery and the last observation point. We defined recurrence-free survival (RFS) as the time interval between the date of surgery and the date of diagnosis of any type of recurrence[23].

Prognostic value analysis using CCT7 protein expression and clinicopathological data in HCC cohort with 118 patients.

To further investigated the association between CCT7 protein expression with the clinical outcomes, we performed immunohistochemistry (IHC) staining using 118 HCC tissues and paired adjacent normal liver tissues which were collected from patients who underwent hepatectomy from February 2013 to November 2014 in 900 Hospital of the Joint Logistics Team. The shortest follow-up time is 5-years. We obtain follow-up data through re-examinations, telephone calls, and the Social Security Death Index. Assessment of liver function and tumor stages using Child-Pugh classification and the 2010 International Union Against Cancer Tumor-Node-Metastasis (TNM) classification system, respectively[24, 25]. The inclusion criteria were: only one lesion or multiple lesions confined to one liver lobe; patients without any distant metastasis; did not receive chemotherapy or TACE or immunotherapy before surgery; postoperative pathology confirmed hepatocellular carcinoma. This study has been performed in accordance with the principles of the Declaration of Helsinki and approved by the Human Research Ethics Committee of 900 Hospital of the Joint Logistics Team (Fuzhou, China). All participants were given written informed consent before surgery and the collection of the specimens.

Immunohistochemistry (IHC) analyses.

118 HCC specimens were cut into 4 μm sections and fixed in the special microscope glass slide. Then, the slides were deparaffinized and dehydrated using gradient concentrations of malondialdehyde and ethanol. Next, the slices were immersed in a boiled solution of Tris/EDTA (pH 9.0) for 20 minutes for antigen retrieval. The slides were immersed in 3% H2O2 for 10 minutes to inhibit endogenous peroxidase. Then, the slides were incubated with the primary antibody (1:250; 15994-1-AP, Proteintech, Wuhan, China) and the secondary antibody (1:50,000; KIT-5010; anti-rabbit/mouse IgG; Maixin Biotechnology Development Co., Ltd., Fuzhou, China) in turn, and washed three times with PBS. Finally, the sections were stained with 3,3'-diaminobenzidine and substrate chromogen (Dako) for 2 minutes at room temperature and then counterstained with hematoxylin for 40 seconds. The slides incubated with only a second antibody without primary antibody were as negative control. The IHC staining was assessed by two separate pathologists who are not informed of any patient information. A 5-point scale system was used to assess the protein expression of CCT7, as 0, 1, 2, 3, and 4 represented no positive cells, <25% positive cells, 26–50% positive cells,51-75% positive cells, and >75% positive cells, respectively.

DNA methylation and genetic alteration of CCT7 in HCC.

We downloaded gene expression and DNA methylation data from gene expression RNAseq and Illumina Human Methylation 450 datasets in the TCGA database (https://xenabrowser.net/datapages/)[26], respectively. Then, we analyzed the correlation between CCT7 mRNA and DNA methylation and identified CpG sites that affect mRNA expression in the MethSurv database, which was A web tool to perform multivariable survival analysis using DNA methylation data[27]. Next, we investigated genetic alteration and mutation hotspots of CCT7 in the Liver Hepatocellular Carcinoma (TCGA, Firehose Legacy) dataset from the cBioPortal database (http://www.cbioportal.org/)[28]. Then, we analyzed the correlation between the genetic alteration of CCT7 and the prognosis of HCC patients.

GO and KEGG enrichment analysis and PPI network construction.

We analyzed the correlated gene of CCT7 using the liver hepatocellular carcinoma (LIHC) dataset from the cBioportal database and the LinkedOmics database, respectively[29]. Then, the overlapping correlated gene with Spearman's correlation greater than 0.6 in the cBioportal and the LinkedOmics database was screened as the co-expressed genes of CTT7. Next, the Functional Annotation Tool in the DAVID database was utilized to perform GO and KEGG enrichment analysis on the overlapping co-expressed genes of CTT7 to explore the gene role which may regulate the tumorigenesis and progression in HCC[30]. We constructed the protein-protein interaction (PPI) network on overlapping co-expressed genes of CCT7 in the STRING database and visualized it in the Cytoscape software (Version 3.7.2)[31]. In the process of GO and KEGG enrichment analysis, $P<0.05$ and false discovery rate (FDR)<0.25 were considered statistically significant.

GSEA enrichment analysis.

We downloaded the normalized gene expression dataset in the TCGA database and divided 373 HCC samples into high and low expressed groups based on the median of CTT7 expression as the critical point. Then, we performed KEGG enrichment analysis utilizing the GSEA software (Version 4.1.2). In this process, "c2.cp.kegg.v7.0.symbols.gmt" was selected as a functional gene set, and the number of permutations was set as 1000. We set all other parameters as the default settings. The pathway and gene enrichment with a normal p-value<0.05 and FDR q-value<0.25 were considered significantly enriched.

Statistical analysis.

statistical analysis and generated the statistical figures using GraphPad Prism 6.0(GraphPad Software, Inc., San Diego, CA, USA). The association between the mRNA expression of CCT7 with the clinicopathological characters was analyzed using the two-tail Student t-test, Fisher's Exact test, or Wilcox test. Pearson's chi-square test was utilized to compare the categorical variables. Kaplan-Meier method with the log-rank test was utilized to estimate the survival curve of OS, RFS. Univariate and multivariate analysis with cox's proportional regression model was utilized to predict the risk factors or independent risk factors. Receiver operating characteristic (ROC) curve with area under the curve (AUC) was utilized to estimate the diagnostic value of mRNA expression of CCT7 and DNA methylation of cg19515186 for HCC. P<0.05 was defined as a statistically significant difference unless otherwise stated.

Results

CCT7 mRNA is significantly upregulated in HCC and associated with poor survival in the TCGA database.

We analyzed the mRNA expression of CCT7 in the TCGA database and visualized it in the UALCAN database. The result showed a significant upregulation of CCT7 in the HCC compared with normal liver samples (Figure 1A). In addition, the mRNA expression of CCT7 in HCC samples incrementally upregulated with increasing cancer stages (Figure 1B) and tumor grade (Figure 1C). Besides, the TNMplot database showed that CCT7 mRNA expression in metastatic samples was higher than tumor samples as well as normal samples (Figure 1D).

High mRNA expression of CCT7 was correlated with poor survival and clinical outcomes in HCC.

We analyzed the association between mRNA expression of CCT7 with the clinical outcomes in the TCGA database. The survival curve showed that high mRNA expression of CCT7 correlated to worse OS

(Figure 1E) and RFS (Figure 1F). We noticed that high mRNA expression was associated with vascular invasion (P=0.015), TNM staging (P=0.049), tumor grade (P=0.007), serum AFP level (P<0.001), family cancer history (P=0.011), adjacent hepatic inflammation (P=0.027), fibrosis (P=0.002), recurrence (P=0.037), and survival (P=0.038). Age, gender, radiation, pharmaceutical was not associated with the mRNA level of CCT7 (Table 1). Univariate Cox Regression analysis found that vascular invasion (P=0.003), TNM staging (P<0.001), tumor grade (P<0.001), and high mRNA expression of CTT7 (P<0.001) were risk factors for OS of HCC patients. The Multivariate Cox Regression analysis revealed that TNM staging (HR (95%CI): 2.047(1.342-3.124); P=0.001), tumor grade (HR (95%CI): 1.808(1.191-2.744); P=0.005), and high mRNA expression of CTT7 (95%CI): 2.031(1.327-3.110); P=0.001) were independent risk factors for OS. For the RFS, the Univariate Cox Regression analysis demonstrated that vascular invasion (P<0.001), TNM staging (P<0.001), tumor grade (P=0.001), pharmaceutical (P=0.002), TACE of postoperative (P=0.002), fibrosis (P=0.027), and high mRNA expression of CTT7 (P<0.001) were risk factors. The Multivariate Cox Regression analysis confirmed that vascular invasion (HR (95%CI): 1.528(0.998-2.338); P=0.049), TNM staging (HR (95%CI): 1.790(1.076-2.714); P=0.023), tumor grade (HR (95%CI): 1.571(1.045-2.363); P=0.030), TACE of postoperative (HR (95%CI): 2.314(1.296-4.133); P=0.005), and high mRNA expression of CTT7 (HR (95%CI): 1.460(1.039-2.052); P=0.029) were independent risk factors for RFS of HCC patients (Table 2).

High protein expression of CCT7 correlated with poor survival and clinical outcomes in a cohort with 118 HCC patients.

We found the protein expression of CTT7 in HCC tissues (Figure 1H-I) significantly higher than normal liver tissues (Figure 1G) in the Human Protein Atlas database, and this finding was supported by our IHC staining (Figure 2A-B). We divided 118 HCC patients into high CCT7 protein group (n=57) and low CCT7 protein group (n=61) based on the IHC score. High protein expression of CCT7 correlated to TNM staging (P=0.043), serum AFP level (P<0.001), tumor differentiation (P=0.010), vascular invasion (P=0.029), and recurrence (P=0.005) of HCC patients (Table 3). In addition, the Univariate Cox Regression analysis revealed that the tumor size (P=0.038), TNM staging (P=0.006), tumor differentiation (P=0.012), vascular invasion (P=0.040), and high protein expression of CTT7 (P=0.048) were risk factors for OS of HCC patients. The Multivariate Cox Regression analysis showed that tumor differentiation (HR (95%CI): 3.232(1.273-8.208); P=0.014), vascular invasion (HR (95%CI): 2.224(1.253-3.949); P=0.006), and high protein expression of CTT7 (HR (95%CI): 1.754(1.047-2.937); P=0.033) were independent risk factors for OS. For RFS of HCC patients, Tumor differentiation (HR (95%CI): 2.840(1.110-7.264); P=0.029), Vascular invasion (HR (95%CI): 2.106(1.186-3.426); P=0.010), Tumor encapsulation (HR (95%CI): 0.303(0.179-0.511); P<0.001), and high CCT7 expression (HR (95%CI): 1.695(1.012-2.839); P=0.045) were both risk factors and independent risk factors (Table 4). In addition, the group of high CCT7 protein expression exhibited a worse OS time (Figure 2C) and RFS time (Figure 2D).

CCT7 is a diagnostic biomarker for HCC.

We analyzed the mRNA expression of CCT7 in the GSE76427 (Figure 3A), GSE54236 (Figure 3C), and GSE136247 (Figure 3E) datasets from the GEO database and the result showed that its mRNA level significantly upregulated in HCC tissues compared with the non-HCC tissues (all P<0.001). The ROC curve exhibited a well diagnostic significance with the AUC value of 0.847 (Figure 3B), 0.673 (Figure 3D), and 0.793 (Figure 3F), respectively. Furthermore, the heat map showed the mRNA expression of CCT7 in HCC tissues was 90% higher than paired adjacent normal liver tissues in the GSE76427 dataset (Figure 3G).

CCT7 has higher positive predictive value than AFP for HCC diagnosis.

We compared the diagnostic efficiency of CCT7 and AFP mRNA expression for HCC in GEO and TCGA databases. The mRNA expression of CCT7 in HCC was significantly higher than normal liver tissues in the GSE25097 (Figure 4A), GSE63898 (Figure 4D), and the TCGA LIHC datasets (Figure 4G). Furthermore, the ROC curve showed that the AUC value of CCT7 was also significantly higher than that of AFP in the GSE25097 (0.719 vs 0.677, Figure 4B), GSE63898 (0.803 vs 0.567, Figure 4E), and TCGA LIHC datasets (0.743 vs 0.616, Figure 4H). The best cut-off values for the diagnosis of CTT7 and AFP were identified based on the sensitivity and specificity of the ROC curve. We found that the CCT7 has a higher positive predictive value (PPV) than that of the AFP both in the GSE25097 (54.9% vs 44.1%, Figure 4C), GSE63898 (64.5% vs 28.1%, Figure 4F), and TCGA LIHC datasets (55.8% vs 41.3%, Figure 4I), even though they have the statistically similar negative predictive value (NPV). All these results revealed that CCT7 has a higher sensitivity for the diagnosis of HCC than AFP.

CCT7 has a better diagnostic value for HCC patients with low AFP expression.

As we know that AFP is increased in no more than 70% of patients with HCC. we evaluated the diagnostic value of CCT7 in HCC patients with low AFP expression using GSE25097 and GSE63898 datasets in GEO database. The AFP expression in cirrhosis and HCC patients was similar in the two datasets (Figure 5A, D). CCT7 mRNA expression in HCC was significantly higher than the cirrhosis patients (Figure 5B, E). The ROC analysis revealed that the AFP expression has no diagnostic value in both the GSE25097 and GSE63898 datasets with the AUC value of 0.588 and 0.535 (P>0.05). The CCT7 mRNA expression in two datasets has a significant diagnostic value with the AUC value of 0.724 (P<0.001, Figure 5C) and 0.803 (P<0.001, Figure 5F). These results demonstrated that CCT can be used as an accurate diagnostic biomarker for low AFP expression HCC patients.

CCT7 is a better diagnostic value than AFP for early-stage HCC patients.

We evaluated the diagnostic efficiency of CCT mRNA for early-stage HCC patients using the TCGA database. The ROC curve analysis exhibited that the AUC value of CCT for stage 1 HCC patients was significantly higher than that of AFP (Figure 5G). In addition, we found that CCT7 mRNA expression in stage 1 HCC patients has a higher PPV than that of AFP in the TCGA database (50.3% vs 42.1%), and NPV of CCT7 and AFP were similar (92.0% vs 94.0%, Figure 5H). Moreover, we investigated the correlations of CCT7 with other diagnostic biomarkers (AFP, r=0.240; ACE, r=0.066; GPC3, r=-0.150; GPT, r=-0.126, Figure 5I-L) which were identified by previous research[32-34]. The results showed that CCT7 expression may be an independent diagnostic biomarker for patients with HCC.

Dysregulation of CCT7 expression was associated with DNA methylation status in patients with HCC.

The CCT7 mRNA expression in HCC was frequently upregulated, this dysregulation associated with copy number alteration (Figure 6A). The analysis of Illumina Human Methylation 450 datasets in the TCGA database demonstrated that CCT7 mRNA level was negatively associated with DNA methylation status (Figure 6B). We identified three CCT7-related methylated CpG sites (cg15777261, cg07135469, and cg19515186) in HCC using the MethSurv database (Figure 6C). A CpG site (cg19515186) was found associated with survival time of HCC (P<0.001, HR (95%CI):0.49 (0.34-0.72); Figure 6D). In addition, the correlation analysis revealed that the methylation status of cg19515186 was negatively associated with the mRNA expression of CCT7 (Figure 6E). Furthermore, the ROC curve analysis exhibited a significant diagnostic value of the methylation status of cg19515186 for HCC in the TCGA database (AUC=0.821, P<0.001, Figure 6F). Finally, the survival analysis revealed that the high methylation status of cg19515186 was associated with better OS (Figure 6G). These results demonstrated that dysregulation of CCT7 expression was associated with DNA methylation status in patients with HCC.

Genetic alteration of CCT7 associated with poor survival in patients with HCC.

We queried the CCT alteration in a cohort of 348 HCC patients using the cBioPortal database and found that CCT7 altered in 143 (41%) of queried patients, including 1 case of missense, 14 cases of low expression, and 128 cases of high expression (Figure 7A). Meanwhile, a mutational hotspot of 1479F/Missense was found in 104 samples (Figure 7B). We also found the somatic mutation of CCT7 is 0.3%. In addition, the Kaplan-Meier curve showed that compared with patients with no CCT7 alterations, HCC patients with CCT7 alterations have poor overall survival (P=6.568e-03, Figure 7C), disease-free survival (P=5.715e-03, Figure 7D), progression-free survival (P=2.150e-02, Figure 7E) and disease-specific survival (P=4.0e-02, Figure 7F). Finally, Spearman's correlation analysis revealed that CCT7 mRNA expression positively correlated with other prognostic biomarkers (Ki67: r=0.230, P<0.001; PCNA: r=0.307, P<0.001; Figure 7G-H).

Analysis of co-expressed genes of CTT7 in patients with HCC.

We screen out the correlated gene of CCT7 using the liver hepatocellular carcinoma (LIHC) dataset in the LinkedOmics and the cBioPortal database, respectively. 11319 negatively and 8670 positively correlated genes with CCT in the LinkedOmics database were exhibited in the volcano plot and heat map (Figure 7I-K). Then, 45 overlapping genes from two database with Spearman's value greater than 0.55 were identified as co-expressed gene of CCT7 (Figure 7L). Next, we investigated the significant interactions of CCT 7 with 45 co-expressed genes with the highest confidence score (greater than 0.9) in the STRING database. A PPI network with 42 nodes and 288 edges was constructed and visualized in Cytoscape software. We noticed that 8 proteins (CCT2, CCT3, CCT4, CCT5, NOP56, RPL8, RPL27, and RUVBL1) directly interacted with CTT7 in the PPI network (Figure 7M). In addition, the mRNA expression of these eight genes incrementally upregulated with normal, tumor, and metastatic tissues in HCC analyzed in the TNMplot database (Figure 8A). Furthermore, we performed survival analysis in the GEPIA database and found that high expression of these eight genes was associated with worse OS in patients with HCC (Figure 8B). Finally, the significantly positive correlations between CCT7 with these eight genes were validated in the TCGA database (Figure 8C).

High CCT7 expression correlated with Spliceosome signaling pathway.

Previous research has suggested that CCT7 promoted the progression of endometrial cancer through Spliceosome signaling pathway[35]. In addition, Spliceosome pathway plays a significant role in the tumorigenesis and progression of several tumors[36-39]. We performed GO and KEGG analysis on 45 co-expressed genes of CCT7 in the DAVID database. AS shown in Figure 9A, co-expressed genes were most enriched in mRNA splicing via spliceosome, rRNA processing, protein folding, translation etc by GO-BP analysis. We also performed enrichment analysis for cellular component (CC) and molecular functions (MF) (Additional Figure 1). The Ribosome, Spliceosome, Purine metabolism, and Ribosome biogenesis in eukaryotes were greatly enriched in KEGG analysis (Figure 9B). We next analyzed the KEGG pathway of genes enriched that are most relevant to the survival of HCC in the GEPIA database. KEGG analysis revealed that the Spliceosome pathway significantly correlated with the prognosis of patients with HCC (Figure 9C). The genes that are most relevant to the survival of HCC were listed in Additional Table. 1. We further investigated the potential pathway that CCT7 regulate the tumorigenesis and development of HCC by performing GSEA. The co-expressed genes with CCT7 in HCC tissues are shown in Figure 9D. As shown in Figure 9E, we exhibited the top eight differentially regulated pathways. We noticed that the normalized enrichment score (NES) for the Spliceosome signaling pathway was 2.09, which demonstrated that Spliceosome pathway positively correlates with HCC tumorigenesis and progression. These results revealed that mRNA expression of CCT7 positively correlates with the Spliceosome signaling pathway in HCC.

Discussion

A large number of studies have demonstrated that several members of the CCT subunit play a significant role in tumor proliferation and migration in various cancers[10, 40, 41]. Huang et al reported that CCT8 was upregulated in HCC and promoted proliferation[42]. In addition, Zhang et al. revealed that CCT3 promotes HCC cell proliferation by facilitating mitotic and suppressing apoptosis eventually[43]. Moreover, CCT4, CCT6A and CCT6B have diagnostic and prognostic values for HCC[16]. CCT7 was highly expressed in HCC through the method of bioinformatics analysis[15], but its clinical diagnostic, prognostic value and gene function were rarely analyzed. This study is the first systematic investigation of diagnostic value, clinical significance, and the gene function of CCT7 in HCC.

We analyzed the mRNA expression of CCT7 in the UALCAN database and revealed that its expression in HCC samples was significantly higher than in normal tissues. Besides, its expression exhibited incrementally upregulated with increasing cancer stages and tumor grade. Moreover, its expression in metastatic samples was higher than tumor samples. High mRNA expression correlated with poor OS. We further analyzed the association between CCT7 mRNA expression with clinical outcomes in the TCGA database. The results suggested CCT7 expression correlated with worse clinicopathological features and was an independent risk factor for worse OS and RFS. Next, we analyzed the protein expression of CCT7 in the Human Protein Atlas database and an HCC cohort with 118 patients. The results showed CCT7 protein was significantly upregulated in HCC tissues compared with the adjacent normal tissues. In addition, high protein of CCT7 was also associated with poor clinicopathological features and was an independent risk factor for worse OS and RFS of HCC. These results demonstrated that CCT7 was a diagnostic and prognostic biomarker for HCC.

We evaluated the diagnostic efficiency of CCT7 using independent datasets in the GEO and TCGA database. The ROC curve analysis showed that CCT7 has a higher AUC value than AFP, the current golden biomarker of diagnosis for HCC[44, 45]. In addition, the positive predictive value of CCT7 was better than AFP. We also found that CCT7 was highly expressed in HCC patients with low APF expression, suggesting that CCT7 has a better diagnostic significance for these patients. For HCC patients with early-stage, CCT7 also exhibited a better AUC value (0.715 vs 0.599) and positive predictive value (50.3% vs 42.1%) than HCC in the TCGA database. These results demonstrated that CCT7 was an effective biomarker for HCC diagnosis, especially for low AFP expressed patients and early-stage patients. Moreover, Spearman's correlations analysis suggested that CCT7 expression may be an independent diagnostic biomarker for patients with HCC.

It is commonly known that DNA methylation was abnormal in all forms of cancer and numerous studies have demonstrated a broad range of genes silenced by DNA methylation in different cancer types[46–48]. Therefore, it is reasonable to speculate that dysregulation of CCT7 expression was associated with DNA methylation status in patients with HCC. We found that upregulation of CCT7 mRNA expression was correlated with hypomethylation status of the CpG site of cg19515186. In addition, the hypomethylation status of cg19515186 was associated with better OS in HCC patients. These results demonstrated that dysregulation of CCT7 expression was associated with DNA methylation status in patients with HCC.

We then explored the genetic alteration of CCT7 in HCC patients to gain more insight into the gene function. Our results showed that 41% of queried patients exhibited the CCT7 alteration in the cBioPortal database. Furthermore, its alteration predicted a worse prognosis that has poor overall survival, disease-free survival, progression-free survival and disease-specific survival. Therefore, it is reasonable to assume that dysregulation of CCT7 expression was associated with genetic alteration in HCC.

Next, we investigated the role of CCT7 in tumorigenesis and progression of HCC by performing GO, KEGG, and GSEA analysis. The results demonstrated that CCT7 was involved in the signaling pathway of Spliceosome both exhibited by GO, KEGG and GSEA. Interestingly, Spliceosome was one of the pathways of genes enriched that were most relevant to the survival of HCC in the GEPIA database. These results revealed that CCT7 play a role of oncogene which involved in tumorigenesis and progression through the Spliceosome signaling pathway.

Conclusion

In summary, this present study demonstrated that CCT7 mRNA and protein expression were significantly upregulated in HCC compared with adjacent normal liver tissues. High CCT7 expression was associated with poor clinical outcomes and prognosis. CCT7 was an effective biomarker for HCC diagnosis and prognosis, especially for low AFP expressed patients and early-stage patients. Upregulation of CCT7 was associated with the hypomethylation status of the CpG sites of cg19515186, and that demethylation increased the expression of CCT7. CCT7 play a role of oncogene which involved in tumorigenesis and progression through the Spliceosome signaling pathway.

Abbreviations

CCT7: Chaperonin containing TCP1, subunit 7; HCC: hepatocellular carcinoma; IHC: immunohistochemistry; GSEA: Gene set enrichment analysis; AFP: Serum alpha-fetoprotein; GEO: Gene Expression Omnibus; GO: gene ontology; KEGG: The Kyoto Encyclopedia of Genes and Genomes; GEPIA: Gene Expression Profiling Interactive Analysis; TCGA: The Cancer Genome Atlas; OS: overall survival; RFS: recurrence-free survival; DAVID: Database for Annotation, Visualization and Integrated Discovery; PPI: protein-protein interaction; ROC: receiver operating characteristic; AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; YAP: yes-associated protein; TFCP2: transcription factor CP2; TNM: Tumor-Node-Metastasis; FDR: false discovery rate.

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

Conception and design: Yi Jiang, Xinghua Huang; administrative support: Yi Jiang; Xinghua Huang; provision of study materials or patients: Huaxiang Wang, Fengfeng Xu, Lizhi Lv; collection and assembly of data: Huaxiang Wang, Fengfeng Xu, Lizhi Lv, Tingting Liu; data analysis and interpretation: Huaxiang Wang, Fengfeng Xu, Tingting Liu, Huanzhang Hu, Ruling Wang, Bin Jiang; supplementary mechanism experiment: Huaxiang Wang, Fengfeng Xu, Lizhi Lv, Ruling Wang, Bin Jiang, Huanzhang Hu; manuscript writing: Huaxiang Wang, Fengfeng Xu, Lizhi Lv; writing - review & editing: Yi Jiang, Xinghua Huang; final approval of manuscript: All authors.

Ethics approval and consent to participate

This study was performed according to the relevant medical ethics regulations and approved by the Human Research Ethics Committee of 900 Hospital of the Joint Logistics Team (Fuzhou, China). All participants gave written informed consent prior to surgery and collection of the specimens.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Correlation between CCT7 expression and clinical outcomes in HCC in the TCGA database

Characteristics			CCT7 level		χ^2	*P-Value
		N	high(n)	low(n)		
Gender	Male	251	132	119	2.07	0.15
	Female	121	54	67		
Age(years)	>50	301	151	150	0.017	0.895
	<=50	71	35	36		
Vascular invasion	Yes	105	61	44	5.897	0.015
	No	211	92	119		
TNM staging	I/II	269	126	143	3.88	0.049
	III/□	103	60	43		
Tumor grade	G1/G2	135	80	55	7.267	0.007
	G3/G4	237	106	131		
Serum AFP level(ng/ml)	>400ng/ml	82	54	28	13.62	<0.001
	<=400ng/ml	228	96	132		
Family cancer history	Yes	111	68	43	6.409	0.011
	No	207	96	111		
Adjacent hepatic inflammation	Yes	134	75	59	4.859	0.027
	No	121	51	70		
Radiation	Yes	10	7	3	1.644	0.200
	No	362	179	183		
Pharmaceutical	Yes	24	14	10	0.713	0.399
	No	348	172	176		
Fibrosis	Yes	186	106	80	9.558	0.002
	No	123	48	75		
BMI (kg/m^2)	>=24	111	46	65	4.359	0.037
	<24	226	121	105		
Recurrence	Yes	180	100	80	4.306	0.038
	No	192	86	106		
Survival	Alive	245	105	140	14.646	<0.001

	Dead	147	81	46

Abbreviations: CCT7: Chaperonin containing TCP1, subunit 7. AFP - alpha fetoprotein, TNM - tumor, node, metastasis. *P-Value<0.05 were considered statistically significant. BMI, body mass index.

Table 2 Univariate and Multivariate Cox Regression analysis of overall survival and Recurrence-free survival in TCGA database.

variables		Overall survival	*P-Value	Recurrence-free survival	*P-Value
		HR (95%CI)		HR (95%CI)	
Univariate analysis					
Age(years)	>55 vs. <=55	1.248(0.786-1.979)	0.348	0.993(0.689-1.431)	0.969
Gender	Male vs. female	1.224(0.855-1.754)	0.270	1.019(0.746-1.392)	0.904
Vascular invasion	Yes vs. no	1.863(1.242-2.793)	0.003	2.134(1.523-2.989)	<0.001
TNM staging	I/II vs. III/	2.515(1.771-3.573)	<0.001	1.954(1.439-2.653)	<0.001
Serum AFP level(ng/ml)	>400 vs <=400	1.441(0.940-2.210)	0.093	1.337(0.938-1.905)	0.108
Tumor grade	G1/G2 vs. G3/G4	2.032(1.431-2.885)	<0.001	1.675(1.249-2.247)	0.001
Family cancer history	Yes vs. no	1.130(0.779-1.639)	0.519	0.893(0.642-1.241)	0.500
Adjacent hepatic inflammation	Yes vs. no	1.415(0.892-2.244)	0.141	1.213(0.854-1.725)	0.281
Radiation	Yes vs. no	1.063(0.392-2.883)	0.904	1.491(0.699-3.180)	0.302
Pharmaceutical	Yes vs. no	1.100(0.558-2.168)	0.784	2.098(1.302-3.379)	0.002
TACE of postoperation	Yes vs. no	0.763(0.352-1.657)	0.495	1.015(1.006-1.024)	0.002
Fibrosis	Yes vs. no	0.946(0.634-1.412)	0.786	1.463(1.044-2.050)	0.027
BMI (kg/m^2)	>=24 vs.	0.853(0.570-	0.438	0.982(0.710-	0.912

	<24	1.275)		1.358)	
CCT7	High vs. low	2.143(1.490-3.081)	<0.001	1.719(1.279-2.310)	<0.001
Multivariate analysis					
Vascular invasion	Yes vs. no	1.386(0.912-2.107)	0.126	1.528(0.998-2.338)	0.049
TNM staging	I/II vs. III/	2.047(1.342-3.124)	0.001	1.790(1.076-2.714)	0.023
Tumor grade	G1/G2 vs. G3/G4	1.808(1.191-2.744)	0.005	1.571(1.045-2.363)	0.030
Pharmaceutical	Yes vs. no			1.486(0.640-3.449)	0.357
TACE of postoperation	Yes vs. no			2.314(1.296-4.133)	0.005
Fibrosis	Yes vs. no			1.102(0.722-1.679)	0.653
CCT7	High vs. low	2.031(1.327-3.110)	0.001	1.460(1.039-2.052)	0.029

Abbreviations: CCT7: Chaperonin containing TCP1, subunit 7. AFP - alpha fetoprotein, TNM - tumor, node, metastasis. BMI, body mass index. HR, hazard ratio; CI, confidential interval. **P*-Value<0.05 were considered statistically significant.

Table 3 Correlation between CCT7 protein expression and clinical outcomes in HCC patients(n=118)

Characteristics			CCT7 level		
		N	high(n)	low(n)	*P-Value
Age (year)	>55	80	40	40	0.593
	<=55	38	17	21	
Gender	Male	103	48	55	0.332
	Female	15	9	6	
Tumor size (cm)	>5cm	67	37	30	0.085
	<=5cm	51	20	31	
TNM staging	I/II	79	33	46	0.043
	III	39	24	15	
Serum AFP level	>400ng/ml	53	36	17	<0.001
	<=400ng/ml	65	21	44	
Tumor location	Left	39	23	16	0.103
	Right	79	34	45	
Tumor differentiation	Low	12	8	4	0.01
	Median	81	43	38	
	High	15	6	19	
Vascular invasion	Yes	52	31	21	0.029
	No	66	26	40	
Tumor encapsulation	Yes	76	32	44	0.070
	No	42	25	17	
Recurrence	Yes	63	38	25	0.005
	No	55	19	36	
Survival	Alive	46	27	19	0.071
	Dead	72	30	42	

Abbreviations: CCT7: Chaperonin containing TCP1, subunit 7. AFP - alpha fetoprotein, TNM - tumor, node, metastasis. *P-Value<0.05 were considered statistically significant.

Table 4 Univariate and Multivariate Cox Regression analysis of overall survival and Recurrence-free survival in HCC patients.

variables		Overall survival	*P-Value	Recurrence-free survival	*P-Value
		HR (95%CI)		HR (95%CI)	
Univariate analysis					
Age (year)	>55 <=55	0.799(0.439-1.456)	0.464	0.755(0.451-1.264)	0.285
Gender	Male vs. female	0.821(0.324-2.080)	0.678	1.533(0.779-3.017)	0.216
Tumor size (cm)	>5 vs. <=5	1.913(1.038-3.527)	0.038	1.527(0.921-2.530)	0.101
TNM staging	I/II vs. III	2.262(1.267-4.039)	0.006	1.573(0.943-2.624)	0.083
Serum AFP level	>400 vs <=400	1.709(0.956-3.054)	0.071	1.205(0.734-1.980)	0.461
Tumor location	Left vs. right	0.825(0.449-1.514)	0.535	1.182(0.690-2.024)	0.543
Tumor differentiation	High vs. median/low	4.492(1.393-14.491)	0.012	4.211(1.686-10.519)	0.002
Vascular invasion	Yes vs. no	1.877(1.030-3.419)	0.040	2.359(1.400-3.974)	0.001
Tumor encapsulation	Yes vs. no	0.837(0.462-1.515)	0.556	0.258(0.155-0.431)	<0.001
CCT7	High vs. low	1.810(1.005-3.259)	0.048	2.062(1.242-3.422)	0.005
Multivariate analysis					

Tumor size (cm)	>5 vs. <=5	0.832(0.420-1.651)	0.420		
TNM staging	I/II vs. III	1.462(0.755-2.829)	0.260		
Tumor differentiation	High vs. median/low	3.232(1.273-8.208)	0.014	2.840(1.110-7.264)	0.029
Vascular invasion	Yes vs. no	2.224(1.253-3.949)	0.006	2.106(1.186-3.426)	0.010
Tumor encapsulation	Yes vs. no			0.303(0.179-0.511)	<0.001
CCT7	High vs. low	1.754(1.047-2.937)	0.033	1.695(1.012-2.839)	0.045

Abbreviations: CCT7: Chaperonin containing TCP1, subunit 7. AFP - alpha fetoprotein, TNM - tumor, node, metastasis. HR, hazard ratio; CI, confidential interval. *P-Value<0.05 were considered statistically significant.

Figures

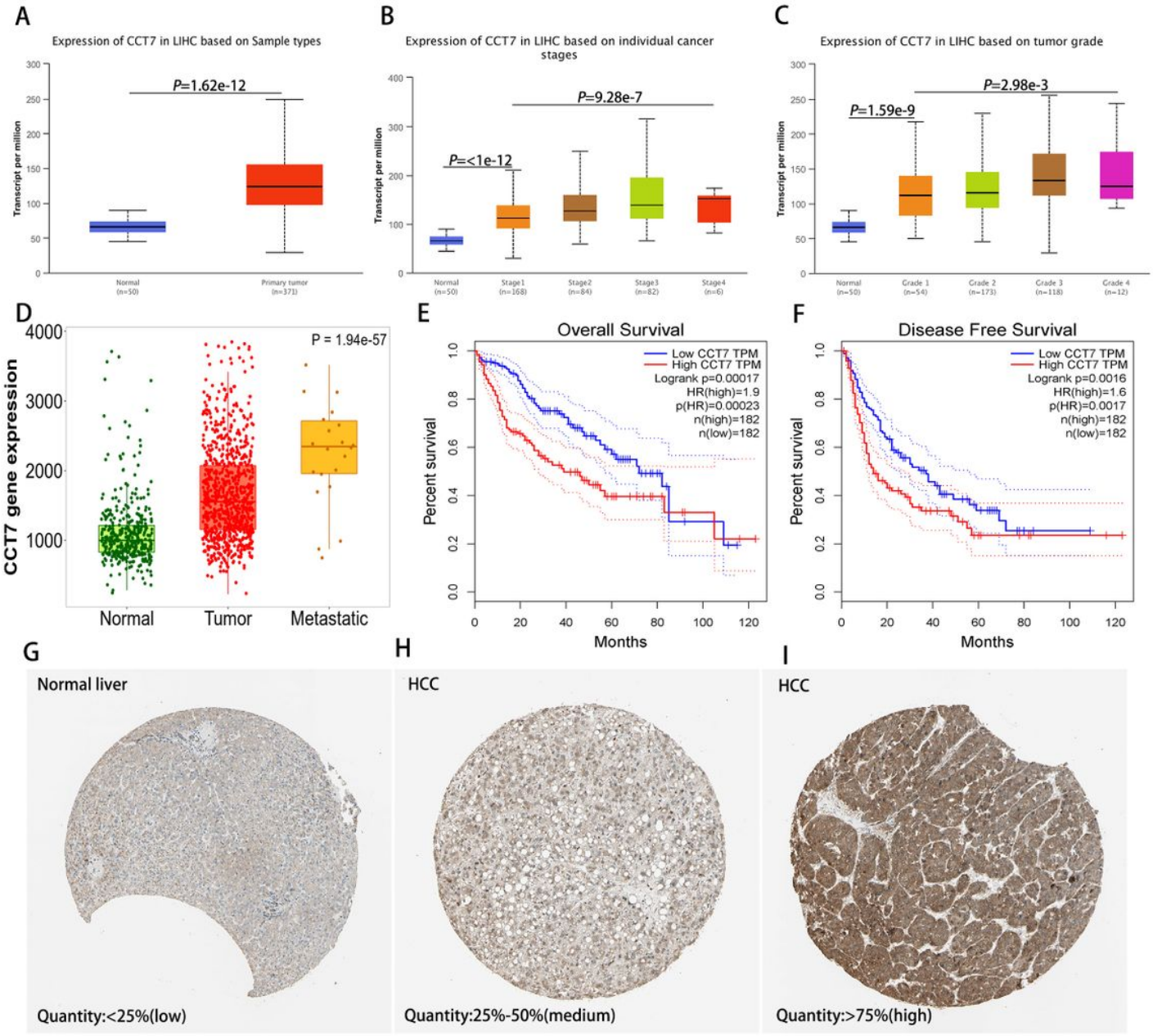

Figure 1

CCT7 expression level in HCC and adjacent normal liver tissues. (A) mRNA expression of CCT7 in HCC was significantly higher than normal liver tissues. (B-C) mRNA expression of CCT7 in HCC tissues incrementally upregulated with increasing cancer stages (B) and tumor grade (C). (D) CCT7 mRNA expression in metastatic samples was higher than tumor samples. (E-F) High mRNA expression of CCT7 was correlated to worse overall survival (E) and recurrence-free survival (F). (G-I) Representative images of immunohistochemical staining of CCT7 protein expression in normal liver tissues (G, expression quantity <25%), low expression HCC tissues (H, expression quantity 25-50%), and high expression HCC tissues in the Human Protein Atlas database (I, expression quantity >75%).

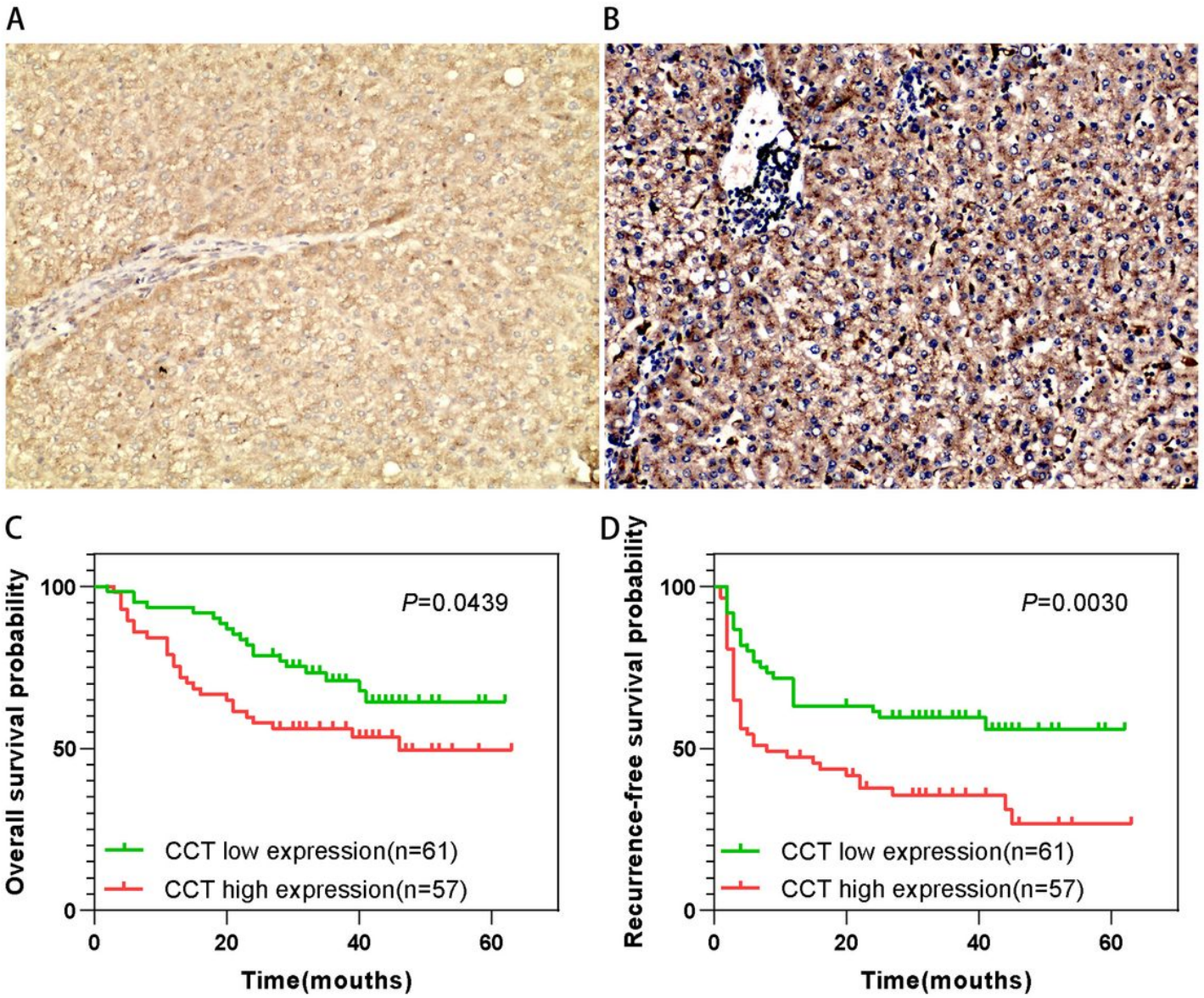

Figure 2

Prognostic value of CCT7 protein expression in a cohort with 118 HCC patients. (A-B) Representative images of immunohistochemical staining of CCT7 protein expression in low (A)/high (B) expression HCC tissues (x200 magnification). (C-D) High CCT7 protein expression associated with worse overall survival (C) and reference-free survival (D).

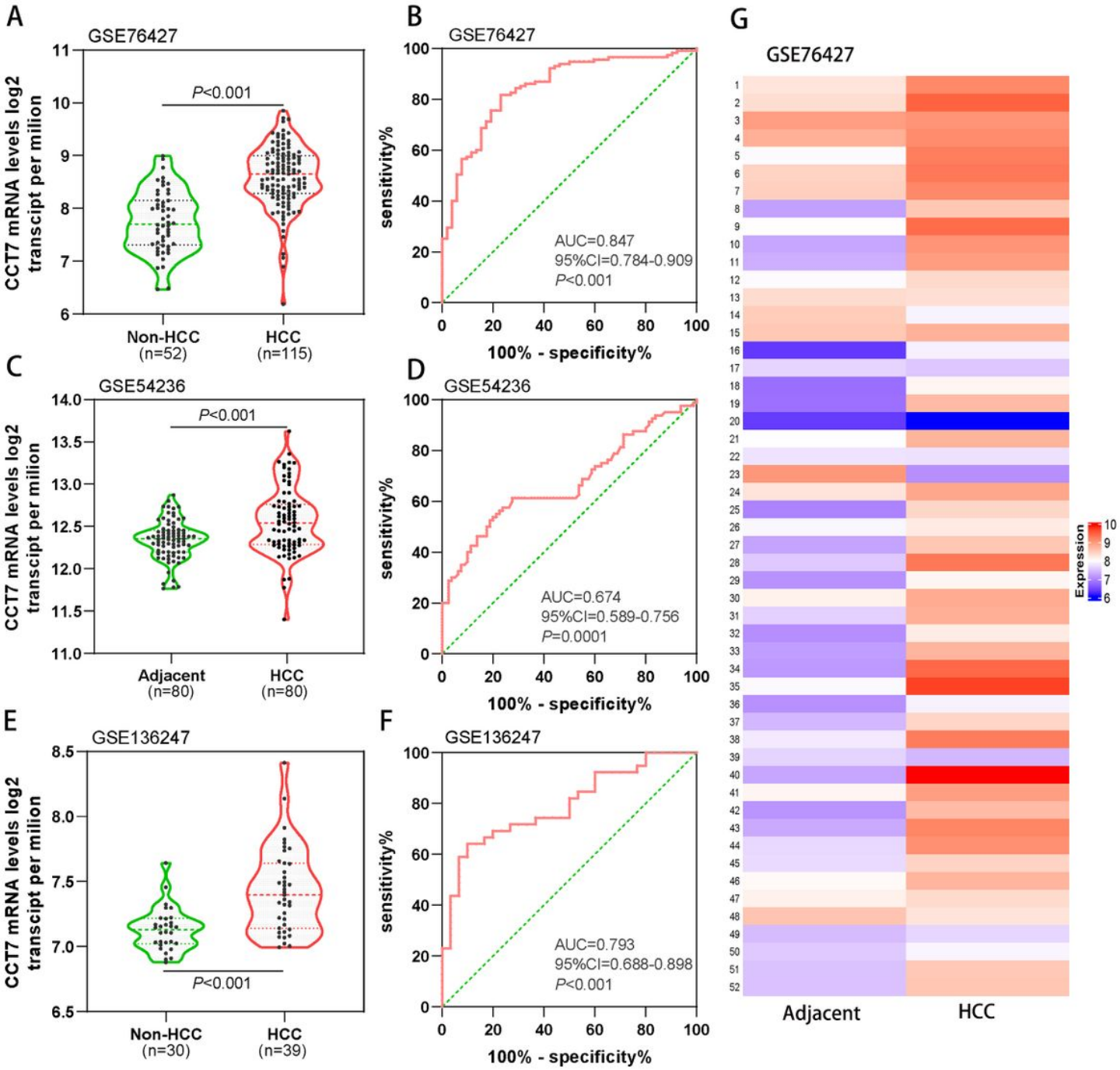

Figure 3

Diagnostic value of CCT7 mRNA expression for HCC in the GEO database. CCT7 mRNA expression significantly upregulated in HCC compared with the non-HCC tissues in GSE76427 (A), GSE54236 (C) and GSE136247 (E). The ROC curve exhibited a well diagnostic significance for HCC in GSE76427 (B), GSE54236 (D) and GSE136247 (F). (G) The heat map shows CCT7 mRNA expression in 52 paired HCC and corresponding adjacent normal tissues.

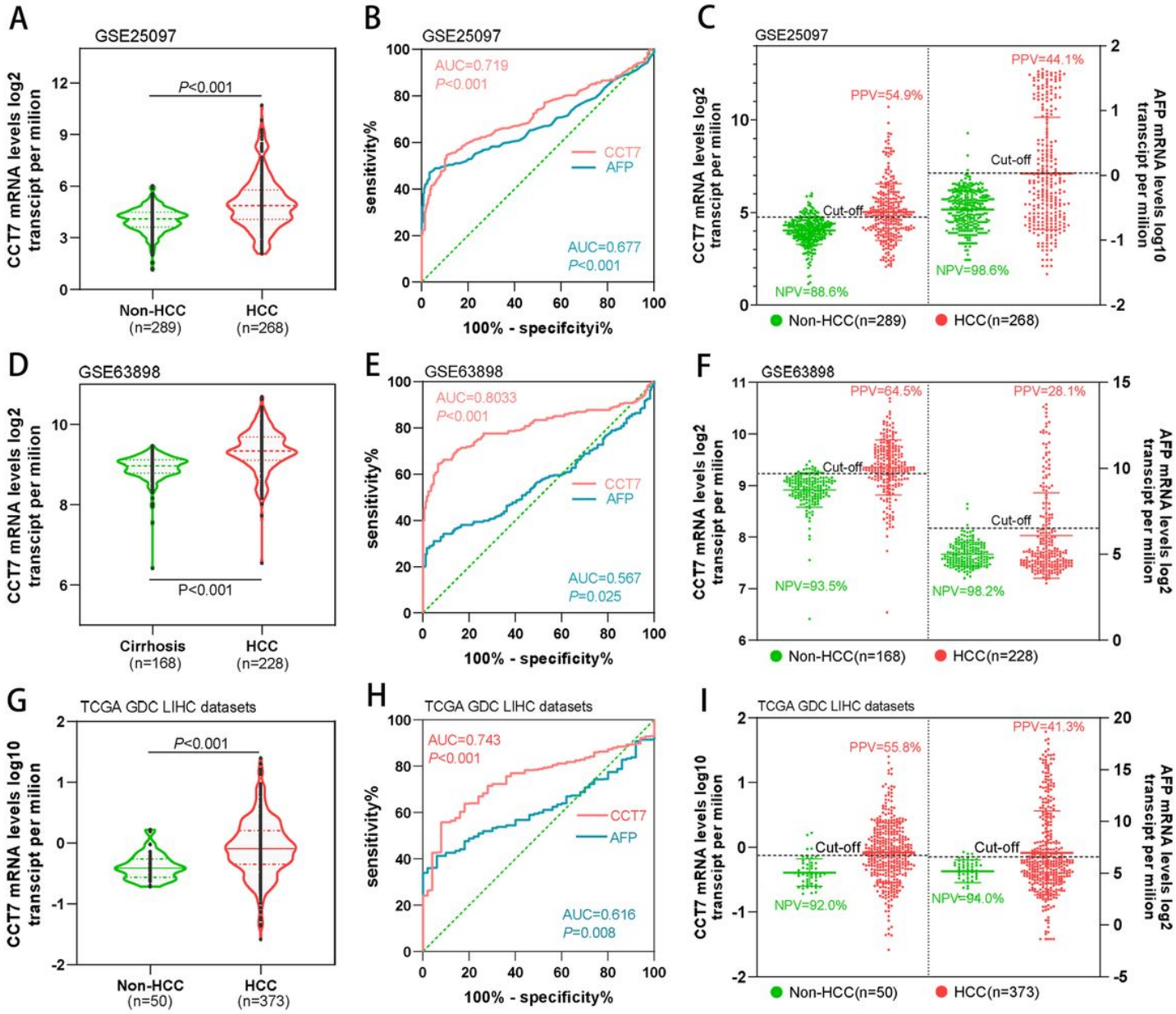

Figure 4

CCT7 has higher positive predictive value than AFP for HCC diagnosis. CCT7 mRNA expression significantly upregulated in HCC compared with the non-HCC tissues in GSE25097 (A), GSE63898 (D) and TCGA LIHC datasets (G). AUC value of CCT7 significantly higher than that of AFP in the GSE25097 (0.719 vs 0.677, B), GSE63898 (0.803 vs 0.567, E), and TCGA LIHC datasets (0.743 vs 0.616, H). CCT7 has a higher positive predictive value (PPV) than that of the AFP in the GSE25097 (54.9% vs 44.1%, C), GSE63898 (64.5% vs 28.1%, F), and TCGA LIHC datasets (55.8% vs 41.3%, I).

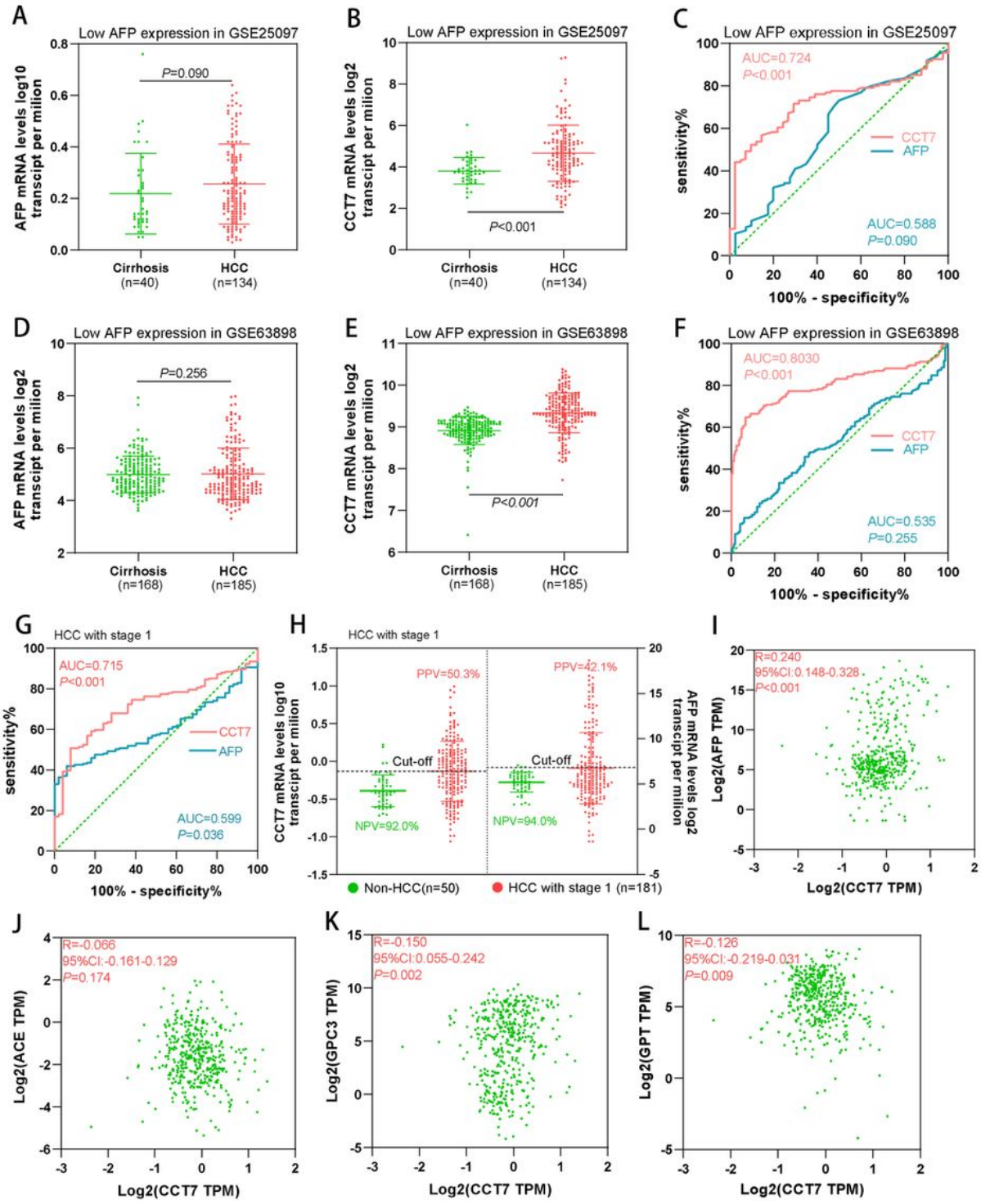

Figure 5

CCT7 has a better diagnostic value for HCC patients with low AFP expression and early stage. (A-B) AFP (A) and CCT7 (B) mRNA expression in the cirrhosis tissues (n=40) and HCC patients with low AFP expression (n=134) in the GSE25097 dataset. (C) ROC curve analysis shows the diagnostic values of AFP and CCT7 in HCC patients with low AFP expression in the GSE25097 dataset. (D-E) AFP (D) and CCT7 (E) mRNA expression in the cirrhosis tissues (n=168) and HCC patients with low AFP expression (n=185) in

the GSE63898 dataset. (C) ROC curve analysis shows the diagnostic values of AFP and CCT7 in HCC patients with low AFP expression in the GSE63898 dataset. (G) ROC curve analysis shows the diagnostic values of AFP and CCT7 in stage 1 HCC patients from the TCGA database. (H) The positive/negative predictive value of AFP and CCT7 in stage 1 HCC patients from the TCGA database. (I-L) The correlations of CCT7 with AFP (I), ACE (J), GPC3 (K) and GPT (L).

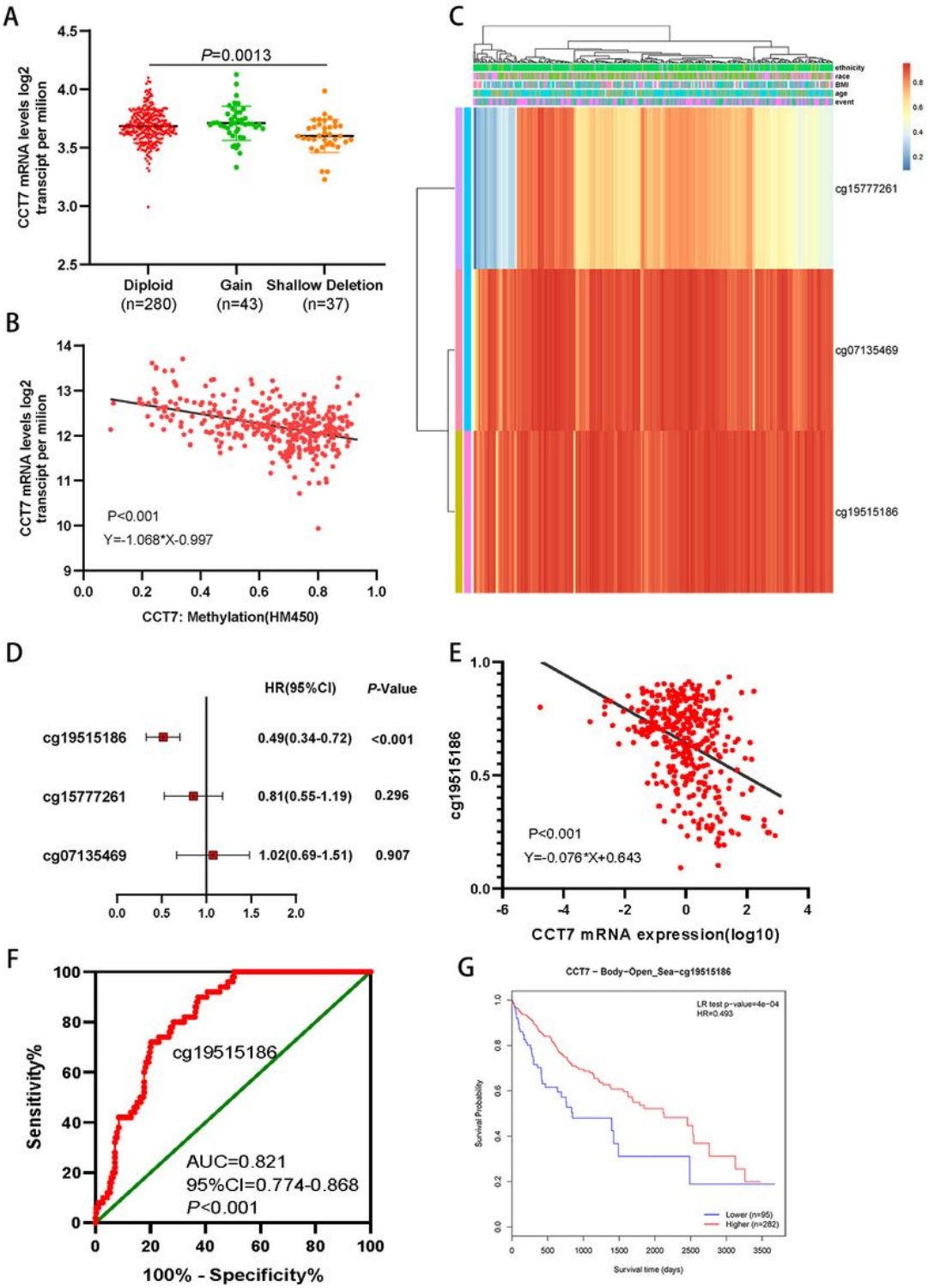

Figure 6

Dysregulation of CCT7 expression was associated with DNA methylation status in patients with HCC. (A) CCT7 mRNA expression in different copy number group. (B) The correlation between CCT7 mRNA expression with DNA methylation status. (C) The heat map shows CCT7-related methylated CpG sites in HCC. (D) The forest map shows the correlation between methylation of CpG site with survival time of HCC. (E) The correlation between CCT7 mRNA expression with methylation status of cg19515186. (F) ROC curve analysis shows a significant diagnostic value of the methylation status of cg19515186 for HCC in the TCGA database. (G) The survival analysis shows that high methylation status of cg19515186 correlated with better OS.

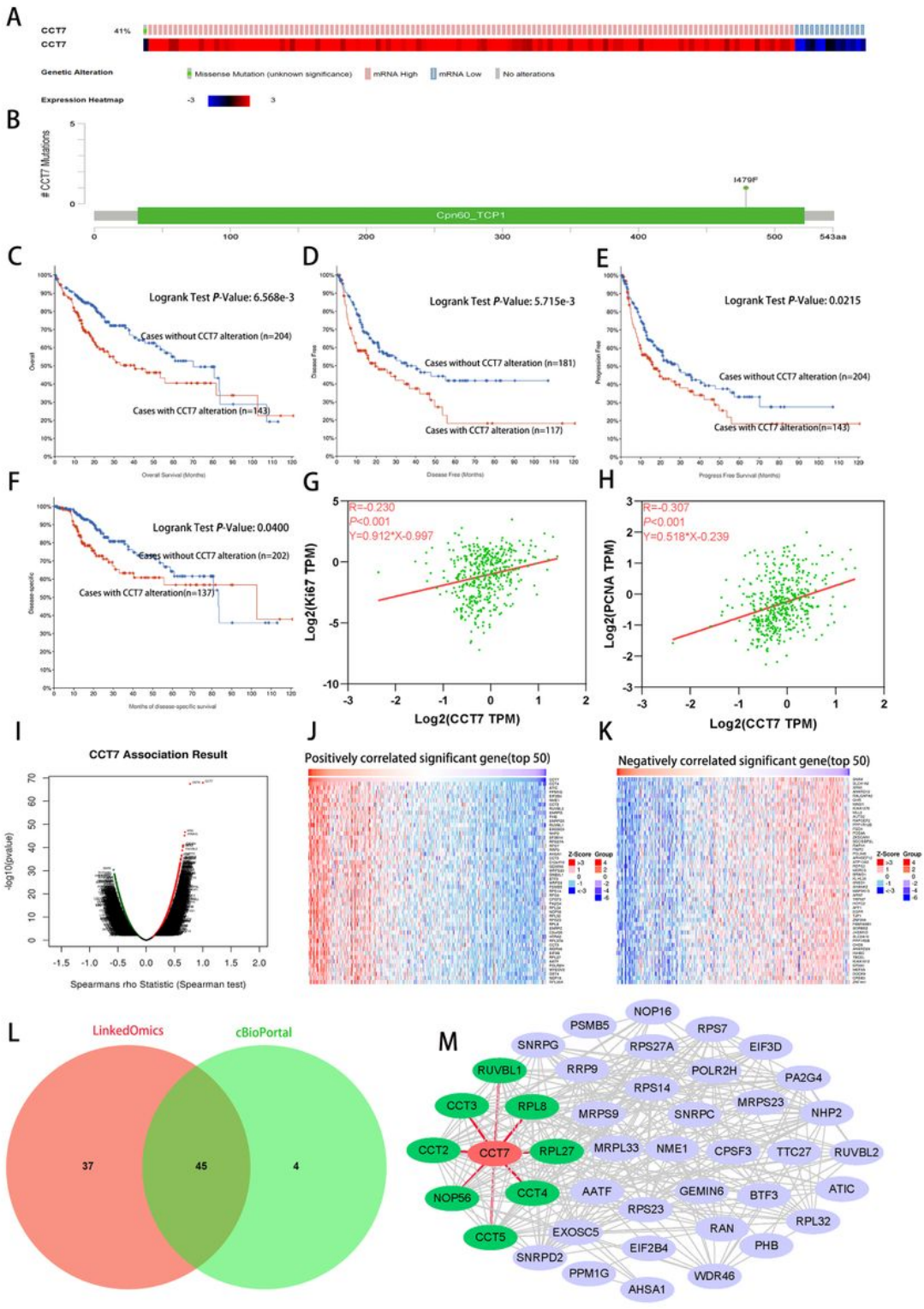

Figure 7

Genetic alteration of CCT7 associated with poor survival in patients with HCC. (A) CCT7 altered in 143 (41%) of queried patients in a cohort of 348 HCC patients. (B) A mutational hotspot of 1479F/Missense was found in 104 patients. (C-F) Survival analysis shows HCC patients with CCT7 alterations have poor overall survival (C), disease-free survival (D), progression-free survival (E) and disease-specific survival (F). (G-H) Spearman's correlation analysis reveals that CCT7 mRNA expression positively correlated with

Ki67 (G)and PCNA (H). (I-K) CCT7 expression associated target genes analysis in the LinkedOmics database. (I) Volcano chart exhibited CCT7 expression positively/negatively correlated significant genes. (J) Top 50 genes that are positively associated with CCT7 expression. (K) Top 50 genes that are negatively associated with CCT7 expression. (L) Venn plot shows overlapping genes from the LinkedOmics and the cBioPortal database with Spearman's value greater than 0.55. (M) PPI network for 45 co-expressed genes of CCT7 was constructed and visualized. CCT2, CCT3, CCT4, CCT5, NOP56, RPL8, RPL27, and RUVBL1 protein can interact with CCT7.

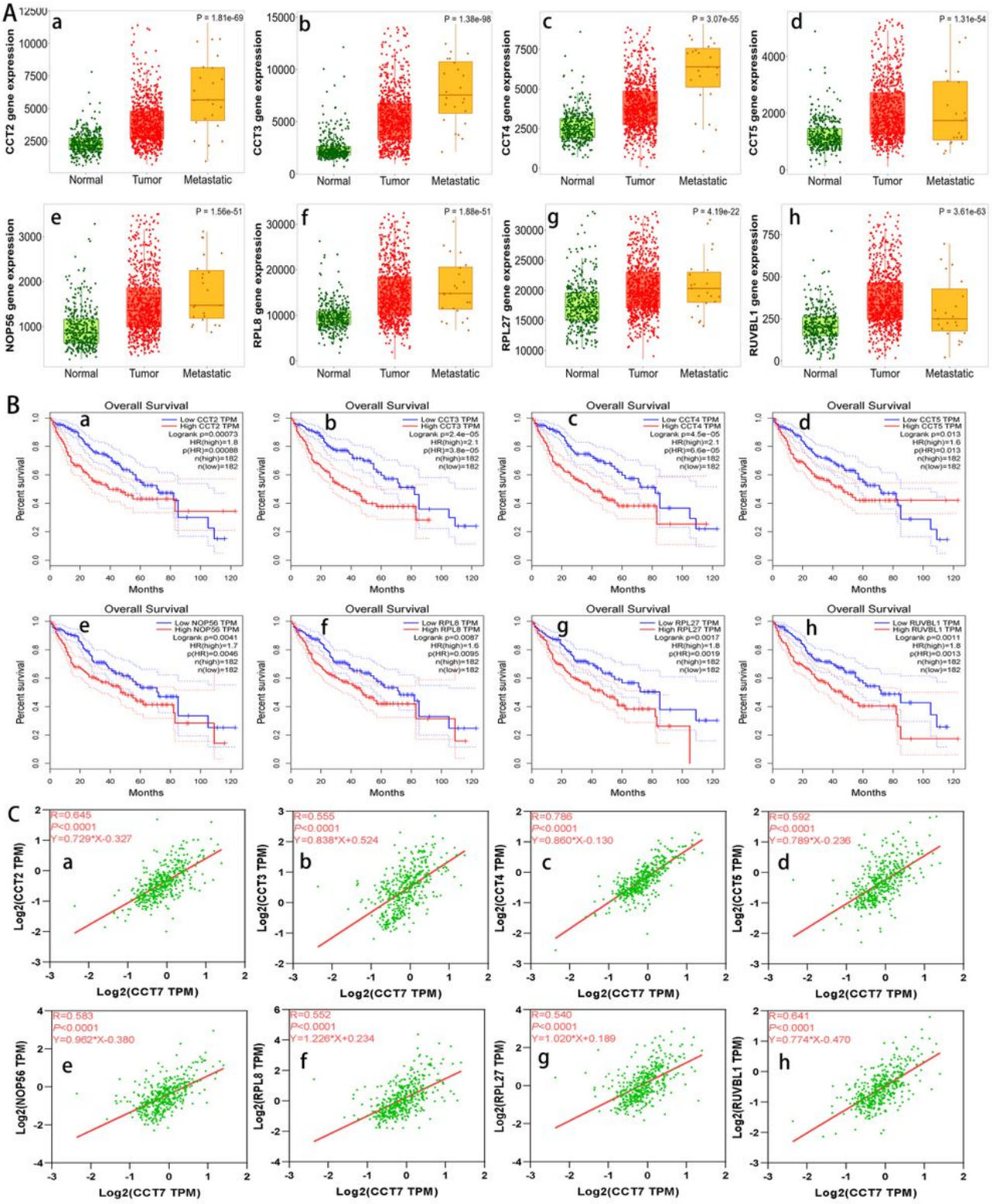

Figure 8

Analysis of co-expressed genes of CTT7 in patients with HCC. (A) mRNA expression of CCT2 (a), CCT3 (b), CCT4 (c), CCT5 (d), NOP56 (e), RPL8 (f), RPL27 (g), and RUVBL1 (h) in normal, tumor and metastatic tissues. (B) Survival analysis shows the mRNA expression of CCT2 (a), CCT3 (b), CCT4 (c), CCT5 (d), NOP56 (e), RPL8 (f), RPL27 (g), and RUVBL1 (h) associated with the overall survival in HCC patients (all P<0.05). (C) Correlation between CCT7 with CCT2 (a), CCT3 (b), CCT4 (c), CCT5 (d), NOP56 (e), RPL8 (f), RPL27 (g), and RUVBL1 (

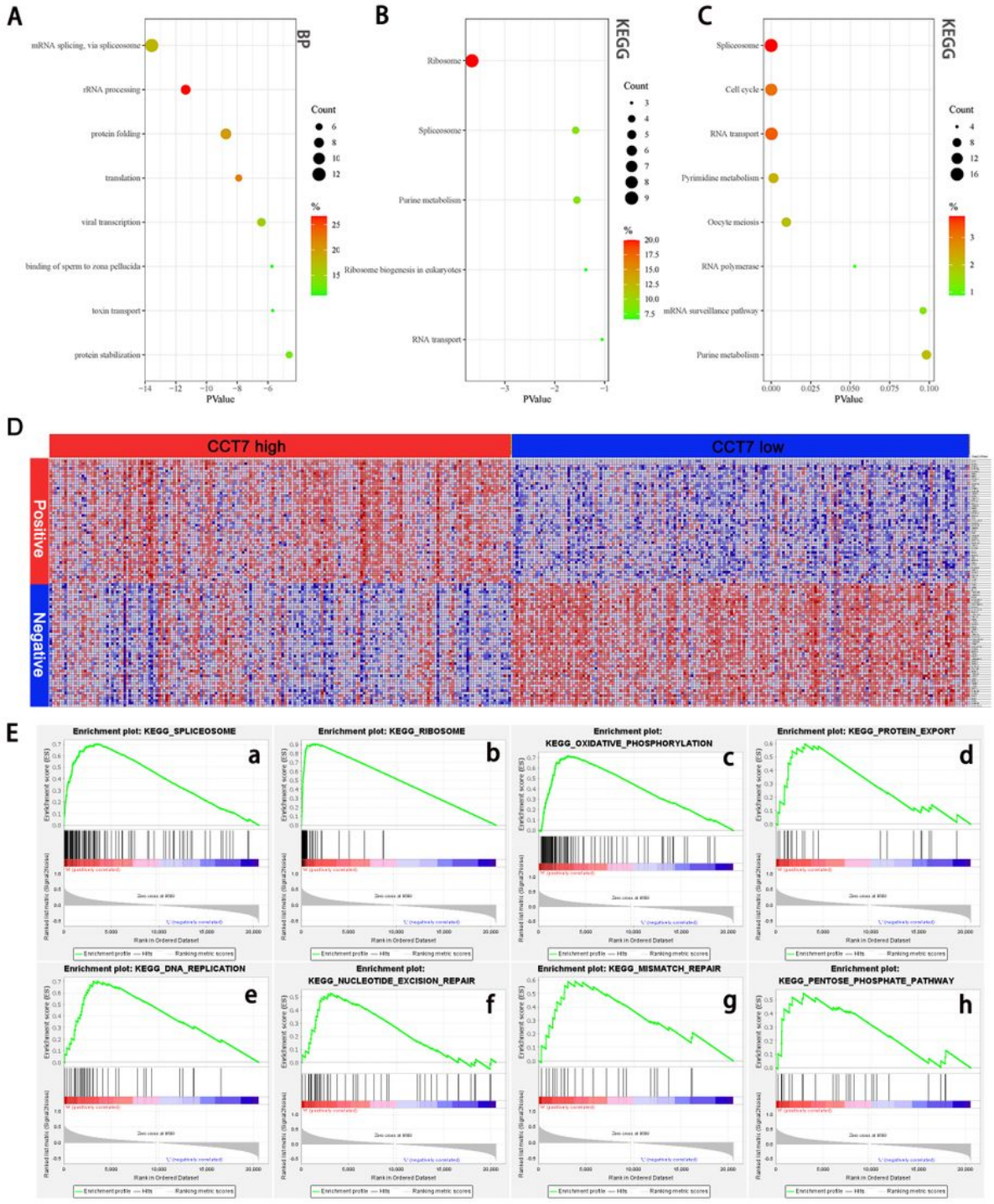

Figure 9

High CCT7 expression positively correlates with Spliceosome in HCC patients. (A-B) The 45 co-expressed genes of CCT7 in the HCC tissues based on the BP-GO analysis (A) and KEGG pathway (B)are shown. (C) The most significant survival-associated genes in the HCC tissues according to analysis using the GEPIA database based on the KEGG pathway are shown. (D) Heat map shows the median mRNA expression of genes that co-express with CCT7 in HCC tissues in the GSEA. (E) The main enriched KEGG pathways of CCT7 using GSEA. SPLICEOSOME (a), RIBOSOME (b), OXIDATIVE PHOSPHORYLATION (c), PROTEIN EXPORT (d), DNA REPLICATION (e), NUCLEOTIDE EXCISION PEPAIR (f), MISMATCH REPAIR (g), PENTOSE PHOSPHATE PATHWAY (h).

Supplementary Files

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

- AdditionalFigure.1.jpg
- AdditionalTable1.xlsx