Over-Expression of TTC21A Correlates Poor Prognosis of Liver Cancer

Mingyi Li

The First Affiliated Hospital of Jiamusi University

Wen Yan (✉ yw1696330918@126.com)

Peking University International Hospital

Research Article

Keywords: Hepatocellular carcinoma, TTC21A, Immune cell infiltration, Nomogram, Bioinformatics analysis

Posted Date: February 22nd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1331743/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Liver hepatocellular carcinoma (LIHC) is one of the most severe malignant tumors worldwide.

Method: RNA sequencing data is obtained from TCGA database to analyze the expression of TTC21A and differentially expressed genes (DEG). Protein-protein interaction (PPI) network is used to screen out the related genes of TTC21A. A single-sample GSEA is used to analyze the relationship between TTC21A and immune cell infiltration. Receiver operating characteristic (ROC) curve and area under the curve (AUC) values are applied to evaluate the value of TTC21A as a binary classifier. Finally, Cox regression, Kaplan-Meier analysis, and nomogram plot are used to determine the prognosis value of TTC21A for LIHC.

Results: Compared to normal tissues, the expression of TTC21A in liver cell carcinoma is significantly higher ($P < 0.05$). The expression of TTC21A mRNA is different from T, M clinical stage, pathological grade, and histological grade ($P < 0.001$). High expression of TTC21A in liver cancer is correlated to T staging. PPI detected its potential co-expressed gene network. IHC staining suggested that TTC21A is expressed higher in tumor tissues than in normal tissues, significantly correlating with the immune cell infiltration, including macrophages and T cells. ROC curve displayed that TTC21A has a robust diagnostic ability for hepatocellular carcinoma ($AUC = 0.778$). Kaplan-Meier analysis displayed that the prognosis of patients with high TTC21A expression is poor ($HR = 1.81$, $P = 0.001$).

Conclusion: Our results suggest that over-expression of TTC21A is closely related to the poor prognosis of patients with liver cancer, indicating that TTC21A could possess an excellent diagnostic value in patients with LIHC.

1. Introduction

Due to the uncontrollable vascular invasion and acquired drug resistance, liver cancer has become one of the most common causes of cancerous deaths (around 600,000 deaths every year) globally, ranking third in mortality and seventh in incidence rate according to the data of the World Health Organization (WHO) [1]. Notably, the incidence rate of liver disease is higher in men than women in developing countries [2–4]. In this regard, several risk factors include fatty liver, hepatitis B virus, alcohol-related smoking, hepatitis C virus, obesity, diabetes, and liver cirrhosis [5–6]. Among these risk factors, 75–85% of liver cancer patients are related to the incidence of chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection [7, 8]. The expressions of various genes would be correlated to the risk factors of different cancers at the molecular level. One of the protein-coding genes is TTC21A, with many tetrapeptide repeat (TPR) domains, often found in IFT protein. The intraflagellar transport (IFT) is a kind of rapid movement of multi-subunit protein particles along the flagellar microtubules, an assembly of eukaryotic flagella requiring maintenance [9]. In addition, one of the homologous genes is TTC21B, which is expressed in a variety of tissues and organs. Previous studies have indicated that this pathogenic gene, TTC21A causes human asthenozoospermia [10]. Notably, seven additional genes, including TTC21A, were found near

brain-derived neurotrophic factor (BDNF), which plays a vital role in brain development and function [11]. Moreover, several reports also demonstrated that this pathogenic gene, TTC21A, is related to cilia biogenesis [12]. Correlating to these aspects, numerous human tumor tissue specimens presented an up-regulation of primary cilia functions and structural defects, indicating an association between the primary cilia function and cancer development [13]. To the best of our knowledge, no studies have reported that TTC21A is stringently related to the occurrence of liver cancer.

In this study, we are intended to demonstrate the correlation between TTC21A and LIHC. To explore this aspect, we compared the expression of TTC21A between liver cell carcinoma tissues and normal tissues. Using high-throughput RNA-seq data obtained from TCGA for bioinformatics analysis, we assessed the correlation between the clinical characteristics of LIHC patients and the expression of TTC21A. Further, we determined the predictive value of TTC21A for LIHC patients' overall survival, progression-free interval, and disease-specific survival. Then, we employed enrichment analysis, molecular interaction network analysis, and immune penetration correlation analysis to determine its biological significance. After searching on PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), we observed that no researchers revealed the role of TTC21A in LIHC. Our results indicated that TTC21A is a promising diagnostic and prognostic biomarker.

2. Experimental Section

2.1. Data collection

Liver hepatocellular carcinoma (LIHC) patient data sets, including gene expression profiles and clinical information, were collected. Since the relevant data provided by TCGA [14] is publicly available, the approval of the local ethics committee is not obligatory. In this context, 424 specimens from the publicly available "Cancer Genome Atlas" (TCGA) were downloaded, including 50 normal tissues and 374 tumor tissues. Notably, cases with insufficient or missing age and overall survival data were excluded in the follow-up treatment.

2.2. Identification of differentially expressed genes (DEG)

According to the median of TTC21A, 424 specimens of liver cancer patients were divided into high-expression and low-expression groups. The "DESeq2" package was employed to identify the DEG between the two groups, where the logarithmic change greater than 1.5 and the adjusted P value less than 0.05 were considered the threshold. The R packages "pheatmap" (Kolde, 2019) and "Enhanced Volcano" (Blighe, 2019) were used to display the results in the form of heat maps and volcano maps.

2.3. Functional annotation of related DEGs

The functional annotation processing on the recognized DEG was performed on Metascape database 2 and online tools [15]. Notably, the analysis threshold was set as the minimum count greater than 3, the enrichment factor greater than 1.5, and the AP value of less than 0.01.

2.4. Relationship between the TTC21A expression and clinical characteristics

The expression of TTC21A in different clinical subgroups was analyzed by the Wilcoxon rank-sum test to analyze the difference of TTC21A expression in liver cancer tumor tissue and normal tissue. Further, TTC21A expression and pathological grade, clinical T staging, clinical M staging, clinical M staging, and histological grade were analyzed, and the relationship between the TTC21A expression and clinical characteristics was established.

2.5. Immunohistochemical (IHC) Analysis

Immunohistochemical staining of TTC21A in liver cancer and the paracancerous tissues was obtained from HPA database (<http://www.proteinatlas.org/>)(Figure 4).

2.6. The Human Protein Atlas analysis

HPA contains normal tissues and tumor tissues information regarding the expression profiles of human genes on protein level. In this study, we conducted HPA to compare the protein expression of TTC21A between liver cancer and the paracancerous tissues.

2.7. Protein-protein interaction (PPI) network construction

The PPI network of TTC21A was constructed using the Interaction Gene/Protein Search (STRING) database search tool for research [16]. In this study, we conducted STRING to search co-expression genes and construct PPI networks. An interaction with a combined score > 0.4 was considered statistically significant.

2.7. Immune cell infiltration analysis

Initially, a single-sample gene set enrichment analysis (SsgSEA) was applied to infiltrate and enrich 24 typical immune cells, including dendritic cells, neutrophils, macrophages, type 2 T helper cells (Th2) cells, Th cells, and Tcm memory cells, among others. Further, the spearman correlation analysis was used to study the correlation between TTC21A and immune cell infiltration. The Wilcoxon rank-sum test was used to compare the degree of immune cell infiltration in the TTC21A high-expression group and the low-expression group. Finally, the logistic analysis was employed to evaluate the relationship between TTC21A expression and clinicopathological characteristics (Table 2).

2.8. Clinical significance of TTC21A expression

Several analyses were performed to explore the clinical significance of TTC21A expression. Initially, a receiver operating characteristic (ROC) analysis was used to compare the expression of TTC21A in LIHC tumor and paracancerous tissues and test the predictive value. Including overall survival, progression-free interval, and disease-specific survival, the prognosis analysis was demonstrated using Kaplan-Meier (K-M) analysis and univariate and multivariate Cox regression analysis. In this vein, the Random forest

regression uses the R software package "random forest" (Svetnik et al., 2003). The R package "RMS" (Harrell, 2020) was used to construct the nomogram and calibration maps. The R program package "ForestPlot" (Max Gordon, 2020) was employed as its often preferred in clinical pathological subtype research. Notably, the above statistical analysis was performed with R (v4.0.0), in which $P < 0.05$ was considered significant.

2.9. Statistical analysis

All the data were subjected to R software (v.3.6.3) at a defined level of statistical significance of $P < 0.05$. Chi-square and Fisher's exact test were used for clinical bioinformatics analysis. In addition, the Wilcoxon rank-sum test was also performed.

3. Results

3.1. Expression profiles of TTC21AmRNA and related DEGs

Based on the TCGA database, the expression of TTC21A mRNA was measured in different tumors. As depicted in Figure 1A, among 33 specimens of cancerous, TTC21A mRNA was significantly overexpressed in most of the cancers. Notably, it was observed that the expression of TTC21A in liver cell carcinoma tissues was significantly higher ($P < 0.001$) than that of the adjacent tissues (Figure 1B). Further, 424 LIHC patients were randomly divided into two groups, a high-expression group and a low-expression group, and the expressions of mRNA and lncRNA between the two groups were compared. Finally, 561 mRNAs (537 up-regulated, 24 down-regulated, Figure 1C), 388 lncRNAs (281 up-regulated, 107 down-regulated, Figure S1A) were identified as DEGs (absolute value of fold change > 1.5 , $P < 0.05$), illustrated by a heat map (Figure 1D and Figure S1B).

3.2. Functional annotation of TTC21A-related DEGs

Further, Metascape software was used to evaluate the functions of TTC21A-related DEGs in LIHC patients. As presented in Figure 2A-C, it was observed that several pathways related to cancers were enriched, including epidermal development (GO: 00085444, $P < 0.001$), negative regulation of endopeptidase activity (GO: 0010951, $P < 0.001$), and endocrine hormones (GO: 0046879, $P < 0.001$).

3.3. Expression of TTC21A in different clinical subgroups

Notably, the expression of TTC21A in patients with LIHC is often related to clinicopathological characteristics. To demonstrate this aspect, the Wilcoxon rank-sum test was employed to analyze the differences in the expression of TTC21A in liver cancer tumor tissues and normal tissues. The results showcased that there exist significant differences in TTC21A expression and pathological grade ($P < 0.05$), clinical T stage ($P < 0.001$), clinical M stage ($P < 0.001$), histological grade (Figure 3A-F). The baseline characteristics of hepatocellular carcinoma patients from TCGA were listed (Table 1). Further, the Univariate analysis showed that the increased expression of TTC21A (based on the median value) was associated with clinicopathological features of poor prognosis (Table 2).

3.4. Immunohistochemistry and PPI network construction

From the immunohistochemical staining of TTC21A in the HPA database, it was observed that the expression of TTC21A in tumor tissues was significantly higher than that of normal tissues (Figure 4). Further, we employed string tools to construct a protein-protein interaction network of TTC21A and its related potential co-expressed gene network (Figure 5). Among them, TTC21A was considered the central gene related to other 10 genes, including ARMC2, CFAP43, IFT122, IFT140, IFT120, ITF52, IFT74, IFT81, WDR19, and WDR35, with their scores of above 0.7 (Table 4).

3.5. Relationship between TTC21A and immune cell penetration

GSEA package was used to determine the correlation between HSPB1 and other immune cells, displaying that the expression levels of TTC21A in the LIHC microenvironment were positively correlated with the quantitative immune cell infiltration levels of ssGSEA. Specifically, TTC21A expression was positively correlated with macrophages, TFH, and NK CD56 bright cells (Figure 6A). As depicted in Figure 6B-G, macrophages ($R = 0.290$, $P < 0.001$), Tem ($R = 0.210$, $P < 0.001$), Th1 ($R = 0.220$, $P < 0.001$) and Th2 ($R = 0.200$, $P < 0.001$) were all significantly correlated to the expression of TTC21A. Contrarily, B cells ($R = +0.061$, $P > 0.001$), NK cells ($R = 0.074$, $P > 0.001$), showed a negative correlation with TTC21A.

In addition, the expression of TTC21A showed a good discrimination ability. The ROC curve of TTC21A expression in normal tissues and tumor tissues had shown an AUC value of 0.778; while the AUC values of stage I, II, and III subgroup analysis were 0.752, 0.810, and 0.814, respectively, which could distinguish tumors and normal tissues (Figure 7). Moreover, the Kaplan-Meier survival analysis showed that the high TTC21A expression group was more correlated with poor prognosis than the low TTC21A expression group (Figure 8). In the univariate COX model, high expression TTC21A levels were associated with high T staging ($P < 0.001$) (Table 3).

3.6. Independent risk and diagnostic value of TTC21A expression

The univariate survival analysis showed that TTC21A expression age (HR=1.205, $P=0.295$), gender (HR=0.793, $P=0.2$), T stage (HR=2.598, $P < 0.001$), M stage (HR=2.029, $P = 0.324$), and N stage (HR=4.077, $P=0.017$) were important factors to be considered in predicting the survival chances in correlation to the TTC21A expression (Figure 9).

3.7. Nomogram chart of survival rate of liver cancer patients

Based on the TCGA training cohort, age, tumor stage, tumor status, and risk score model were also added to construct a prognostic prediction nomogram and predict the probability of OS in 1, 3, and 5 years (Figure 10A). As depicted in Figure 10B, the calibration curve showed that, in all cohorts, the OS predicted by the nomogram was in good agreement with the actual observations in 1, 3, and 5 years.

4. Discussion

Liver cancer, *i.e.*, hepatocellular carcinoma (HCC), is a major health problem globally, accounting for 75–85% of the total deaths. Among them, the main risk factors for HCC include cirrhosis, obesity, and diabetes, in developing countries. Due to the lack of early specific symptoms, The median survival time of patients with advanced HCC is less than 6 months, as most patients are diagnosed late. Several conventional treatment options have been under practice to eradicate HCC, such as surgical resection, ablation, chemical embolization, systemic chemotherapy, administration of molecularly targeted drugs, and liver transplantation. The treatment results indicated that in the cases of radical resection of the liver, 60–70% of patients still face recurrence within 5 years. To this end, chemotherapy based on drugs solely or in combination has been employed, which, however, remained far from satisfactory in terms of HCC eradication. In this context, early detection and appropriate treatment options at the right time are inevitable. According to the guidelines issued by the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL), liver cancer can be early diagnosed by imaging [17]. Therefore, developing highly innovative sensitive biomarkers is of great significance for the early diagnosis of liver cancer.

This study aims to clarify the expression of TTC21A in liver cell carcinoma and its potential therapeutic and prognostic values and establish the theranostic products. In the current research, high-throughput RNA-seq data obtained from TCGA for bioinformatics analysis was employed, focusing on the expression profile, clinicopathological association, and clinical significance of factor TTC21A. It was observed that the expression of TTC21A in tumor tissues was higher than that in normal tissues. In addition, in TTC21A patients, the expression of TTC21A was correlated with clinical T staging. Finally, the predictive value of TTC21A for LIHC patients' overall survival, progression-free interval, and disease-specific survival were systematically determined. Given the limited data on the function of TTC21A, we have carried out functional annotations, functional annotations of TTC21A-related DEGs, epidermal development, negative regulation of endopeptidase activity, and endocrine hormones that are closely related to the expression of TTC21A.

Notably, the tumor microenvironment is an important interface that mediates the physiological response of cancer cells [18, 19]. In this regard, immune cells play a key role in the microenvironment of liver cancer and exhibit complex crosstalk with cancer cells. Given the key role of the tumor microenvironment in mediating tumor progression, tumor-infiltrating immune cells are an indispensable part of the tumor microenvironment. The potential relationship between TTC21A expression and immune cell infiltration was revealed. There was a positive correlation with T cells and macrophages and a negative correlation with DCs and CD8 T cells. As the most effective antigen-presenting cells, DCs activated CD8 + T cells through cross primers to initiate anti-tumor immunity [20]. In contrast, T cells [21] and macrophages [22] grafted together to act against tumors.

Consequently, the over-expression of TTC21A seemed to inhibit tumor immunity, help cancer cells escape elimination, and ultimately accelerate the occurrence of tumors. Therefore, we speculate that TTC21A may affect the prognosis of patients by regulating the immune infiltration in LIHC. Further, to study the biological functions of TTC21A in LIHC, the protein-protein interaction network (PPI) construction showed

the participation of TTC21A and its related potential co-expressed gene network. It is noteworthy to discuss another issue, *i.e.*, the clinical significance of TTC21A in LIHC. In this regard, the area under the ROC curve (AUC) was calculated as 0.778, suggesting that the TTC21A could be established as a convincing biomarker for the diagnosis of LIHC. In addition, it was observed that the high expression of TTC21A was related to the clinicopathological characteristics of pathological grade, clinical T staging, clinical M staging, and histological grades. Multivariate Cox regression analysis and construction of a prognostic nomogram chart further illustrated the diagnostic value of TTA21A in patients with LIHC. Together, we firmly believe that the TTC21A is an independent prognostic factor, substantially affecting the survival of liver cancer patients. Despite the advantages, this research still has some room in exploring the biological functions of TTC21A *in vitro* and *in vivo*, needing further experimental verifications.

In summary, this study has demonstrated that the overexpression of TTC21A is correlated to the poor prognosis of LIHC, which is also closely related to immune cell infiltration. Therefore, our results suggested that the expression of TTC21A may have obvious prognostic value for liver cancer patients and may become a potential target for liver cancer immunotherapy. Further validations in terms of the biological functions of TTC21A *in vitro* and *in vivo* should be carried out to explore the influence of TTC21A overexpression in LIHC.

Declarations

Availability of data and materials

Data were analyzed from ,TCGA (<https://portal.gdc.cancer.gov>), Metascape (<http://metascape.org/gp/index.html>) and Kaplan Meier plots (<https://string-db.org/>) and Kaplan Meier plots (<https://kmplot.com/analysis/>). We declare that the data and materials in this study will be provided free of charge to scientists for noncommercial purposes.

Statement of data

The data is available upon reasonable request

Acknowledgments

Not applicable.

Funding

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author contributions

Mingyi Li and Wen Yan designed the study and wrote the first draft of the manuscript and conducted the statistical analysis. Mingyi Li performed the data collection and took part in statistical analysis. Mingyi Li provided critical input into the data analysis and interpretation of the results. Mingyi Li and Wen Yan participated in conception, designed of the study and revised it critically for important intellectual content. All authors have read the draft critically to make contributions and also approved the final manuscript.

References

1. R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2019, *CA Cancer J Clin*, 69 (2019) 7–34.
2. B.Q. Starley, C.J. Calcagno, S.A. Harrison, Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection, *Hepatology* (Baltimore, Md.), 51 (2010) 1820–1832.
3. Omar Massoud, Michael Charlton. Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis and Hepatocellular Carcinoma[J]. *Clinics in Liver Disease*, (2018),22(1):
4. Altekruse, S.F.; McGlynn, K.A.; Reichman, M.E. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J. Clin. Oncol.* 2009, 27, 1485–1491.
5. Massoud O, Charlton M.*Clin Liver Dis.* 2018 Feb;22(1):201–211.
6. M.M. Center, A. Jemal, International trends in liver cancer incidence rates, *Cancer Epidemiol Biomarkers Prev*, 20 (2011) 2362–2368.
7. Michielsen, P.P.; Francque, S.M.; van Dongen, J.L. Viral hepatitis and hepatocellular carcinoma. *World J. Surg. Oncol.* 2005, 3, 27.
8. Kumar, R.; Saraswat, M.K.; Sharma, B.C.; Sakhuja, P.; Sarin, S.K. Characteristics of hepatocellular carcinoma in India: A retrospective analysis of 191 cases. *Mon. J. Assoc. Phys. (QJM)* 2008, 101, 479–485.
9. Pazour G J, Dickert B L, Vucica Y, et al. Chlamydomonas IFT 88 and its mouse homologue, polycystic kidney disease gene Tg 737, are required for assembly of cilia and flagella[J]. *The Journal of cell biology*, 2000, 151(3): 709–718.
10. Liu W, He X, Yang S, et al. **Bi-allelic mutations in TTC21A induce asthenoteratospermia in humans and mice**[J]. *The American Journal of Human Genetics*, 2019, **104**(4): 738–748.
11. The genetics of circulating BDNF: towards understanding the role of BDNF in brain structure and function in middle and old ages and flagella, *J. Cell Biol.* 151 (3) (2000) 709–718.
12. G.J. Pazour, B.L. Dickert, Y. Vucica, et al., Chlamydomonas IFT88, and its mouse homologue, polycystic kidney disease gene Tg737, are required for assembly of cilia and flagella, *J. Cell Biol.* 151 (3) (2000) 709–718
13. B. Yasar, K. Linton, C. Slater, R. Byers, Primary cilia are increased in number and demonstrate structural abnormalities in human cancer, *J. Clin. Pathol.* (2017), 70(7), 571–574

14. Tomczak K, Czerwińska P, Wiznerowicz M. *Contemp Oncol (Pozn)*. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. 2015, 19(1A):A68-77. doi: 10.5114/wo.2014.47136.
15. Yingyao Zhou, Bin Zhou, Lars Pache, Max Chang, Alireza Hadj Khodabakhshi, Olga Tanaseichuk, Christopher Benner, Sumit K. Chanda. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets[J]. *Nature Communications*, 2019, 10(1):
16. He WQ, Gu JW, Li CY, Kuang YQ, Kong B, Cheng L, Zhang JH, Cheng JM, Ma Y. The PPI network and clusters analysis in glioblastoma. *Eur Rev Med Pharmacol Sci*. 2015 Dec;19(24):4784–90.
17. José, Vera Ruth, Sangro Bruno, Bruix Jordi. Diagnosis and treatment of hepatocellular carcinoma. Update of the consensus document of the AEEH, AEC, SEOM, SERAM, SERVEI, and SETH.[J]. *Medicina Clinica*, 2021, 156(9):
18. Hinshaw Dominique C, Shevde Lalita A. The Tumor Microenvironment Innately Modulates Cancer Progression.[J]. *Cancer Research*, 2019, 79(18)
19. Liu Jibing, Kuang Shuwen, Zheng Yiling, Liu Mei, Wang Liming. Prognostic and predictive significance of the tumor microenvironment in hepatocellular carcinoma[J]. *Cancer Biomarkers*, 2021, 32(1):
20. Guo Shuli, Xiao Pengli, Li Bo, Wang Wanli, Wang Songyun, Lv Tao, Xu Xiaoyan, Chen Cong, Huang Lei, Li Zhi, Tang Li, Peng Liang, Wang Huirui. Co-immunizing with PD-L1 induces CD8 + DCs-mediated anti-tumor immunity in multiple myeloma[J]. *International Immunopharmacology*, 2020, 84:
21. Kebriaei Partow, Kelly Susan S, Manuri Pallavi, Jena Bipulendu, Jackson Rineka, Shpall Elizabeth, Champlin Richard, Cooper Laurence J N. Chimeric antibody receptors (CARs): driving T-cell specificity to enhance anti-tumor immunity.[J]. *Frontiers in bioscience (Scholar edition)*, 2012, 4:
22. Ying Yao Zhou, Bin Zhou, Lars Pache, Max Chang, Alireza Hadj Khodabakhshi, Olga Tanaseichuk, Christopher Benner, Sumit K. Chanda. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets[J]. *Nature Communications*, 2019, 10(1):

Tables

Table 1
Baseline characteristics data of TCGA-LIHC patients.

Characteristics	Low expression of TTC21A	High expression of TTC21A	p-value
n	185	186	
T stage, n (%)			0.249
T1	97 (26.4%)	84 (22.8%)	
T2	40 (10.9%)	54 (14.7%)	
T3	37 (10.1%)	43 (11.7%)	
T4	8 (2.2%)	5 (1.4%)	
N stage, n (%)			1.000
N0	123 (48%)	129 (50.4%)	
N1	2 (0.8%)	2 (0.8%)	
M stage, n (%)			0.059
M0	130 (48.1%)	136 (50.4%)	
M1	4 (1.5%)	0 (0%)	
Gender, n (%)			0.530
Female	57 (15.4%)	64 (17.3%)	
Male	128 (34.5%)	122 (32.9%)	
Age, n (%)			1.000
<=60	89 (24.1%)	88 (23.8%)	
> 60	96 (25.9%)	97 (26.2%)	
Pathologic stage, n (%)			0.223
Stage I	91 (26.2%)	80 (23.1%)	
Stage II	38 (11%)	48 (13.8%)	
Stage III	38 (11%)	47 (13.5%)	
Stage IV	4 (1.2%)	1 (0.3%)	
Tumor status, n (%)			0.518
Tumor free	104 (29.5%)	97 (27.6%)	
With tumor	72 (20.5%)	79 (22.4%)	
Histologic grade, n (%)			0.032

Characteristics	Low expression of TTC21A	High expression of TTC21A	p-value
G1	36 (9.8%)	19 (5.2%)	
G2	85 (23.2%)	92 (25.1%)	
G3	58 (15.8%)	64 (17.5%)	
G4	3 (0.8%)	9 (2.5%)	
Vascular invasion, n (%)			0.350
No	113 (35.9%)	93 (29.5%)	
Yes	53 (16.8%)	56 (17.8%)	
Age, median (IQR)	61 (52, 69)	61 (51, 68)	0.839

Table 2

Logistic regression analysis of the correlation between clinicopathological characteristics of LIHC patients and TTC21A expression.

Characteristics	Total (N)	Odds Ratio (OR)	P value
Age (> 60 vs. ≤60)	373	0.969 (0.645–1.455)	0.878
Gender (Male vs. Female)	374	0.929 (0.602–1.434)	0.74
Pathologic stage (Stage III & Stage IV vs. Stage I & Stage II)	350	1.434 (0.887–2.334)	0.143
T stage (T3 & T4 vs. T1 & T2)	371	1.268 (0.792–2.037)	0.324
N stage (N1 vs. N0)	258	2.862 (0.361–58.271)	0.365
Residual tumor (R1 & R2 vs. R0)	345	1.620 (0.622–4.500)	0.331
Histologic grade (G2 & G3 vs. G1)	357	2.783 (1.517–5.325)	0.001
Adjacent hepatic tissue inflammation (Mild & Severe vs. None)	237	0.803 (0.481–1.338)	0.4
Vascular invasion (Yes vs. No)	318	1.573 (0.990–2.511)	0.056
Prothrombin time (> 4 vs. ≤4)	297	0.916 (0.555–1.507)	0.731

Table 3
COX regression analysis (single factor/multiple factors).

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	373				
<=60	177	Reference			
> 60	196	1.205 (0.850–1.708)	0.295		
Gender	373				
Female	121	Reference			
Male	252	0.793 (0.557–1.130)	0.200		
T stage	370				
T1&T2	277	Reference			
T3&T4	93	2.598 (1.826–3.697)	< 0.001	2.780 (1.798–4.296)	< 0.001
N stage	258				
N0	254	Reference			
N1	4	2.029 (0.497–8.281)	0.324		
M stage	272				
M0	268	Reference			
M1	4	4.077 (1.281–12.973)	0.017	2.056 (0.631–6.704)	0.232
Histologic grade	368				
G1&G2	233	Reference			
G3&G4	135	1.091 (0.761–1.564)	0.636		
TTC21A	373				
Low	187	Reference			
High	186	1.324 (0.938–1.869)	0.111		

Table 4
Details of *TTC21A* related genes

Gene symbol	Annotation	Score
ARMC2	Required for sperm flagellumaxoneme organization and function	0.753
CFAP43	<i>Cilia and flagella associated protein 43</i>	0.738
IFT122	<i>Intraflagellar transport protein 122 homolog</i>	0.776
IFT140	<i>Intraflagellar transport protein 140 homolog</i>	0.88
IFT20	<i>Intraflagellar transport protein 20 homolog</i>	0.781
IFT52	<i>Intraflagellar transport protein 52 homolog</i>	0.743
IFT74	<i>Intraflagellar transport protein 74 homolog</i>	0.826
IFT81	<i>Intraflagellar transport protein 81 homolog</i>	0.849
WDR19	<i>WD repeat-containing protein 19</i>	0.808
WDR35	<i>WD repeat-containing protein 35</i>	0.831

Figures

Figure 1

(A) The expression level of *TTC21A* in different cancer tissues and its corresponding normal tissues; (B) The expression levels of *TTC21A* in different types of human tumors in cancer tissues and its corresponding normal tissues. Note: * $P < 0.001$, ** $P < 0.01$, and *** $P < 0.05$. The expression difference of *TTC21A* in 374 liver cancer tumor tissues and 50 normal tissues, Note: * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$. (C)

Volcano map showing the median expression of *TTC21A* in LIHC tumors, 424 LIHC patients from the Cancer Genome Atlas-Liver Hepatocellular Carcinoma Project (TCGA-LIHC) stratified into high and low *TTC21A* expression groups. (D) The heat map of the top 10 significantly differentially expressed genes in the high expression and low expression groups. Heat maps (Figure 1D and Supplementary Figure B) The blue and red dots represent down-regulated and up-regulated genes, respectively.

Figure 2

Functional annotation of differentially expressed genes (DEGs) in liver hepatocellular carcinoma (LIHC) patients with different *TTC21A* levels. According to the Metascape database, 561 mRNAs differentially expressed between the high and low *TTC21A* expression groups were used for functional annotation. Then, based on the threshold of 0.3 kappa score, they were clustered hierarchically into trees. Pick out those with a higher degree of enrichment, then group them into several categories based on functional relevance and build a network based on relevance and similarity. In the figure, different colors were used to represent different classes. The above figure is colored by the degree of enrichment. The darker the color, the more genes enriched in this type of pathway or biological process.

Figure 3

*The expression of TTC21A in different clinical subgroups related to clinicopathological characteristics. (A) The Wilcoxon rank-sum test was used to analyze the differences in the expression of TTC21A between liver tumor tissues and normal tissues. The expression of TTC21A was related to pathologic grade (B), clinical T staging (C), clinical M staging (D), clinical N staging (E), and the relationship between histologic grades (F) * $P < 0.001$, ** $P < 0.01$, and *** $P < 0.05$.*

Figure 4

Immunohistochemical staining of TTC21A from HPA database, normal tissue (A, B) tumor tissue (C, D).

Figure 5

Comprehensive analysis of protein-protein interaction (PPI) gene network of TTC21A using string tools to analyze TTC21A and its potential co-expressed gene network.

Figure 6

(A) ssGSEA method to determine the correlation between *TTC21A* and immune cell penetration of liver cell carcinoma tumors among 24 immune cell types in China. Comparison of the penetration levels of most related immune cells, including dendritic cells (B), Neutrophils (C), macrophages (D), type 2 T helper cells (Th2) cells (E), Th cells (F), and Tcm memory cells (G) between the high and low *TTC21A* expression groups. (Note: DC, dendritic cells; aDC, activated DC; iDC, immature DCs; pDC, plasma cell DCs; Th, T

helper cells; Th1, type 1 Th cells; Th2, type 2 Th cells; Th17, Type 17 Th cells; Treg, regulatory T cells; Tgd, T central memory T cells; Tem, effector memory T cells; Tfh, T follicular helper T cells; NK, natural killer)

Figure 7

ROC curve of TTC21A expression in the liver cancer cohort. (A-D) ROC curves of TTC21A expression in normal and tumor tissues; subgroup analysis of stage I, II, and III.

Figure 8

The independent risk and diagnostic value of *TTC21A* expression in LIHC. (A) Overall survival analysis (OS) of TCGA-LIHC database; (B) Kaplan-Meier survival analysis of OS; (C) Disease-specific survival rate (DSS); (D) Progression-free survival rate (PFS); and (E) Recurrence-free survival rate (RFS).

Figure 9

Univariate survival analysis of the overall survival probability of TTC21A expression in patients with different subgroups of tumor stages.

Figure 10

(A) Nomogram of the overall survival of patients with liver cancer in 1 year, 3 years, and 5 years. (B) The nomogram was used to predict the likelihood of the OS diagram of the calibration diagram

Supplementary Files

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

- [SupplementaryInfo.docx](#)