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ORIGINAL RESEARCH

TSC2 Mutations Were Associated with the Early Recurrence of Patients with HCC Underwent Hepatectomy

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Purpose: To explore the value of Tuberous sclerosis complex 2 (*TSC2*) mutations in evaluating the early recurrence of hepatocellular carcinoma (HCC) patients underwent hepatectomy.

Patients and Methods: A total of 183 HCC patients were enrolled. Next-generation sequencing was performed on tumor tissues to analyze genomic alterations, tumor mutational burden and variant allele fraction (VAF). The associations between *TSC2* mutations and recurrence rate within 1 year, RFS and OS after hepatectomy were analyzed.

Results: Our results showed that *TSC2* mutation frequency in HCC was 12.6%. Compared to patients without *TSC2* mutation, the proportion of microvascular invasion (MVI) and Edmondson grade III–IV was significantly higher in patients with a *TSC2* mutation ($p<0.05$). The VAF of mutated *TSC2* was higher in patients with maximum diameter of tumor >5cm or MVI than that of other patients ($p<0.05$). The frequency of *TP53* mutation was significantly higher in patients with a *TSC2* mutation than those without *TSC2* mutation ($p=0.003$). Follow-up analysis showed that patients with a *TSC2* mutation had significantly higher recurrence rate within 1 year ($p=0.015$) and poorer median recurrence-free survival (RFS) ($p=0.010$) than patients without *TSC2* mutation. TSC2 mutations did not significantly affect overall survival of patients ($p=0.480$). The multivariate analysis results showed that the Barcelona Clinic Liver Cancer (BCLC) B-C stage, *TSC2* mutations and preoperative serum alpha-fetoprotein level ≥400μg/L were independently associated with recurrence within 1 year after hepatectomy (HR=8.628, 95% CI: 3.836–19.405, $p=0.000$; HR=3.885, 95% CI: 1.295–11.653, $p=0.015$; HR=2.327, 95% CI: 1.018–5.323, $p=0.045$; respectively), and poorer RFS after hepatectomy (HR=3.070, 95% CI: 1.971–4.783, $p=0.000$; HR=1.861, 95% CI: 1.061–3.267, $p=0.030$; HR=1.715, 95% CI: 1.093–2.693, $p=0.019$; respectively).

Conclusion: *TSC2* mutations were significantly associated with MVI in liver para-carcinoma tissue and Edmondson grade III–IV in patients with HCC and were independently associated with recurrence within 1 year and poorer RFS after hepatectomy. The TSC2 mutation may be a potential predictor for early recurrence in HCC patients underwent hepatectomy.

Keywords: hepatocellular carcinoma, tuberous sclerosis complex 2, next-generation sequencing, gene mutation, early recurrence

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the fourth most common cause of cancer-related death worldwide.[1] Surgery is the main

treatment for HCC patients, including liver transplantation, liver resection and ablation. However, the risk of recurrence after surgical treatment is high.[2,3]

Tuberous sclerosis complex 2 (*TSC2)* is an important tumor-suppressor gene, which was firstly found in tuberous sclerosis complex.[4] Many studies have demonstrated that *TSC2* closely related with several cancers. For example, Mehta et al[5] reported that the expression of *TSC2* was downregulated in aggressive breast cancer. Chakraborty et al[6] found that the methyltransferase inhibitor 5-azacytidine could significantly increase the expression of *TSC2* in oral squamous cell carcinoma cell lines. In a prognostic model for lung adenocarcinoma established by Geng et al,[7] *TSC2* was a biomarker to predict a poor prognosis. Lee et al[8] also reported that *TSC2* rs30259G > A mutation could predict shorter OS and DFS of non-small cell lung cancer patients after curative surgery.

Currently, some studies have reported that *TSC2* could be a therapeutic target in HCC.[9–11] However, the value of *TSC2* in predicting the prognosis after hepatectomy was rarely reported. In this study, we aimed to detect the genomic variations (GAs) of HCC and evaluated the potential value of *TSC2* in predicting the prognosis of HCC patients after hepatectomy.

Table 1 Clinicopathological Characteristics of HCC Patients

Clinicopathological Characteristics	Number of Patients
Age (<65/≥65)	144/39
Gender (male/female)	156/27
Hypertension (no/yes)	137/46
Diabetes (no/yes)	162/21
Family history of cancer (no/yes)	128/55
History of alcoholism (no/yes)	116/67
HBsAg (negative/positive)	22/161
HBV-DNA (<1E+003/≥1E+003IU/mL)	126/57
Anti-hepatitis virus treatment (no/yes)	103/80
AFP (<400/≥400μg/L)	130/53
Tumor number (single/multiple)	130/53
Tumor size (≤5cm/>5cm)	118/65
BCLC (0-A/B-C)	127/56
Macrovascular invasion (no/yes)	163/20
Edmondson grade (I–II/III–IV)	93/90
MVI (no/yes)	93/90

Patients and Methods

Patients

A total of 183 HCC patients who were treated by hepatectomy at the Affiliated Hospital of Qingdao University from March 2017 to February 2020 were enrolled in this study and no extrahepatic metastasis was found in all patients before surgery. Among the enrolled patients, 161 patients were infected by hepatitis B virus (HBV), while those infected by hepatitis C virus and underwent antitumor therapy before liver resection were excluded. The surgical margins of all patients were achieved R0. The preoperative serological results and clinicopathological characteristics were shown in Table 1.

Identification of Genetic Alterations, TMB, and VAF

Formalin-fixed, paraffin-embedded (FFPE) tissues were collected from patients for next-generation sequencing (NGS). The genes were captured and sequenced by genomic profile produced using the NGS-based YuanSu 450 gene panel. Genetic alterations (GAs) were identified as follows: single nucleotide variants (SNVs) were identified by MuTect (v1.7); Insertion-deletions (InDels) were identified by using PINDEL (V0.2.5). The functional impact of GAs was annotated by SnpEff3.0. Copy number variations (CNV) regions were identified by Control-FREEC (v9.7). Gene rearrangement/fusion was detected through an in-house developed pipeline. Tumor mutational burden (TMB) was estimated by counting the coding somatic mutations, including SNVs and Indels, per megabase of the sequence examined in each patient. Variant allele fraction (VAF) was calculated by dividing the number of mutated bases by the total base number of the site. The concept of VAF was only for SNVs and short InDels due to biological information algorithms. Thus, there was no VAF on the CNV or gene rearrangement/fusion.

Follow-Up

All patients enrolled in this study were followed up regularly after surgery. During the first 3 months after liver resection, the patients were followed up once a month; during 3–24 months after liver resection, they were followed up every 3 months; and after 2 years, they were followed up every 6 months. The follow-up examination

included serum alpha-fetoprotein (AFP), liver function, ultrasonic examination of liver and computed tomography of lung. Patients received the contrast-enhanced computed tomography (CT) scan of upper abdomen annually. When suspected signs of recurrence were found, contrast-enhanced CT or magnetic resonance imaging (MRI) was performed to clarify the diagnosis. Recurrence-free survival (RFS) was confirmed by imaging examination. The patients were followed up until August 31 2020 or died.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 (IBM). Kaplan–Meier curves were drawn using GraphPad Prism 7.0. Chi-square test or Fisher's exact test was used for qualitative data in univariate analysis and logistic regression was used for multivariate analysis. Mann–Whitney *U*-test was used to analyze the correlation between VAF of *TSC2* and clinicopathological characteristics. Kaplan–Meier curve analysis and Log rank test were used to compare RFS and OS in different groups. Variables associated with RFS were assessed by Cox regression model and variables with p values <0.05 in univariate analysis were subjected to multivariate analysis. $P < 0.05$ was considered to be statistically significant.

Results

Baseline Data of HCC Patients

In this cohort, a total of 183 HCC patients were enrolled. The main characteristics of patients were shown in Table 1. Among them, there were 161 patients with serum hepatitis B surface antigen (HBsAg) positive, 53 patients with preoperative serum AFP level above 400μg/L and 20 patients with macrovascular invasion. The BCLC stage of 22, 105, 36, 20 patients was 0, A, B and C, respectively. In pathological results, the Edmondson grades of tumors were I–II (n=93) and III–IV (n=90), and the MVI were found in liver para-carcinoma tissues of 90 patients.

The Correlation Between *TSC2* Mutations and Clinicopathological Characteristics

Out of 183 specimens, 23 (12.6%) were harboring *TSC2* mutations, including 15 SNVs, 7 InDels, and 1 CNV (Table 2). Compared to patients without *TSC2* mutation, the proportion of MVI and Edmondson grade III–IV was significantly higher in patients with a *TSC2* mutation (p=0.011 and p=0.036, respectively) (Table 3). We did not find a significant association between *TSC2* mutations and other clinicopathological characteristics (Table 3).

Table 2 Alterations of TSC2 in 23 Patients

Patients	Alteration Type	Coding DNA Change	VAF
1	SNV	139-2A>G	0.31
2	SNV	2299del	0.29
3	SNV	1906G>T	0.08
4	SNV	319G>A	0.12
5	SNV	849-2A>C	0.12
6	SNV	3496del	0.19
7	SNV	1643del	0.15
8	InDel	1716+1904_3035del	0.49
9	SNV	337-2A>C	0.20
10	SNV	4037C>A	0.44
11	InDel	110_139-344del	0.22
12	CNV	Gene deletion	–
13	InDel	3560_3561del	0.07
14	SNV	5138G>A	0.63
15	InDel	1717-121_1840-167del	0.97
16	InDel	1362-133_1716+507del	0.11
17	SNV	648+1G>T	0.29
18	SNV	3651_3652insA	0.25
19	InDel	exon2_exon3del	–
20	SNV	65G>C	0.01
21	SNV	1257+2T>A	0.09
22	InDel	1444-235_1665del	0.13
23	SNV	2242G>T	0.26

The Correlation Between VAF of *TSC2* Mutations and Clinicopathological Characteristics

In the subgroup with *TSC2* mutations, VAF of mutated *TSC2* could be calculated in 21 cases. The median VAF was 0.20 (range, 0.01–0.97). By Mann–Whitney *U*-test, we found VAF of mutated *TSC2* was associated with MVI and tumor size. The VAF of mutated *TSC2* was significantly higher in patients with MVI and maximum diameter of tumor > 5cm (p<0.05) (Table 4).

Table 3 The Correlation Between TSC2 Mutations and Clinicopathological Characteristics

Clinicopathological Characteristics	TSC2			
	Wild Type	Mutant	χ^2	*P*
Age (<65/≥65)	125/35	19/4	–	0.788
Gender (male/female)	138/22	18/5	–	0.345
Hypertension (no/yes)	116/44	21/2	3.779	0.052
Diabetes (no/yes)	141/19	21/2	–	1.000
Family history of cancer (no/yes)	111/49	17/6	0.197	0.657
History of alcoholism (no/yes)	99/61	17/6	1.256	0.262
HBsAg (negative/positive)	22/138	0/23	–	0.081
AFP (<400/≥400μg/L)	115/45	15/8	0.433	0.510
Tumor number (single/multiple)	115/45	15/8	0.433	0.510
Tumor size (≤5cm/>5cm)	102/58	16/7	0.297	0.586
BCLC (0-A/B-C)	112/48	15/8	0.217	0.642
Macrovascular invasion (no/yes)	143/17	20/3	–	0.722
Edmondson grade (I–II/III–IV)	86/74	7/16	4.374	0.036
MVI (no/yes)	87/73	6/17	6.438	0.011

Table 4 The Correlation Between VAF of TSC2 Mutations and Clinicopathological Characteristics

Clinicopathological Characteristics	VAF of TSC2		
	Median	*U*	*P*
Age (<65/≥65)	0.220/0.195	48.500	0.654
Gender (male/female)	0.210/0.130	25.500	0.240
Family history of cancer (no/yes)	0.190/0.255	29.000	0.698
History of alcoholism (no/yes)	0.200/0.200	42.500	0.850
AFP (<400/≥400μg/L)	0.225/0.130	38.000	0.443
Tumor number (single/multiple)	0.170/0.220	35.500	0.322
Tumor size (≤5cm/>5cm)	0.150/0.400	19.000	0.045
BCLC (0-A/B-C)	0.170/0.220	35.500	0.322
Edmondson grade (I–II/III–IV)	0.235/0.190	44.500	0.970
MVI (no/yes)	0.080/0.235	13.000	0.025

The Correlation Between *TSC2* Mutations and Other Genes

The most commonly mutated genes of enrolled patients were *TP53* (54.1%), *TERT* (41.0%), *CTNNB1* (23.0%), *AXIN1* (14.8%) and *TSC2* (12.6%). The mutated mTOR pathway-related genes were *TSC2* (n=23), *PTEN* (n=7), *TSC1* (n=5), *mTOR* (n=5), *PIK3CA* (n=3), *NF1* (n=3), *STK11* (n=3), *AKT2* (n=2). This result demonstrated that *TSC2* gene was the most frequently mutant gene among mTOR pathway-related genes in our study.

In this study, we found that co-mutations between *TSC2* and *TP53* were detected in 19 patients, 9 patients had co-mutations between *TSC2* and *TERT*, 3 patients had co-mutations between *TSC2* and *CTNNB1*, 5 patients had co-mutations between *TSC2* and *AXIN1*. We also found *TSC1* mutations in 5 patients and no patient had co-mutation of *TSC2* and *TSC1*. Univariate analysis identified the correlation between *TSC2* mutations and *TP53* mutations. Compared to patients without a *TSC2* mutation, the proportion of patients with a *TP53* mutation was significantly higher in patients with a *TSC2* mutation (p=0.003) (Table 5).

TMB values were calculated in all 183 HCC specimens, and the 75% TMB threshold value was 8.5 mutations/Mb. TMB value higher than 8.5 mutations/Mb was defined as TMB-H, and those lower than 8.5 mutations/Mb was defined as TMB-L. The patients with a TSC2 mutation was account for 13.5% in the TMB-L group, while was account for 10.0% in the TMB-H group. There was no correlation between TMB and TSC2 mutations (p=0.520) (Table 5).

Follow-Up Results Analysis

HCC Recurrence

The median follow-up time of 183 patients was 15.5 months (range, 4.6–40.7 months). The recurrence was found in 87

Table 5 The Correlation Between TSC2 Mutations and Other Genes

Other Mutant Genes	TSC2			
	Wild Type	Mutant	χ^2	*P*
TP53 (Wild type/Mutant)	80/80	4/19	8.611	0.003
TERT (Wild type/Mutant)	94/66	14/9	0.037	0.847
CTNNB1 (Wild type/Mutant)	121/39	20/3	1.460	0.227
AXIN1 (Wild type/Mutant)	138/22	18/5	–	0.345
TMB (<8.5/≥8.5 mutations/Mb)	115/45	18/5	0.413	0.520

Table 6 The Correlation Between Different Factors and Recurrence Within 1 Year After Hepatectomy

Variables	Univariate Analysis			Multivariate Analysis		
	n/n	χ^2	P	HR	95% CI	P
Age (<65/≥65)	46/11	0.202	0.653			
Gender (male/female)	50/7	0.316	0.574			
Hypertension (no/yes)	49/8	3.638	0.056			
Diabetes (no/yes)	53/4	1.213	0.271			
Family history of cancer (no/yes)	42/15	0.774	0.379			
History of alcoholism (no/yes)	36/21	0.000	0.995			
HBsAg (negative/positive)	3/54	3.179	0.075			
HBV-DNA (<1E+003/≥1E+003IU/mL)	35/22	1.197	0.274			
Anti-hepatitis virus treatment (no/yes)	34/23	0.167	0.682			
AFP (<400/≥400μg/L)	32/25	10.844	0.001	2.327	1.018–5.323	0.045
BCLC (0-A/B-C)	23/34	37.065	0.000	8.628	3.836–19.405	0.000
Liver fibrosis (S1S2/S3S4)	14/41	0.277	0.598			
Edmondson grade (I–II/III–IV)	24/33	4.318	0.038	–	–	0.933
MVI (no/yes)	19/38	11.376	0.001	–	–	0.161
TP53 (Wild type/Mutant)	23/34	0.993	0.319			
TERT (Wild type/Mutant)	31/26	0.914	0.339			
CTNNB1 (Wild type/Mutant)	45/12	0.106	0.744			
AXIN1 (Wild type/Mutant)	50/7	0.161	0.688			
TSC2 (Wild type/Mutant)	45/12	5.922	0.015	3.885	1.295–11.653	0.015
TMB (<8.5/≥8.5 mutations/Mb)	45/12	0.035	0.852			

patients. Among our cohort, 160 patients were followed up for more than 1 year and 57 patients of them were found with HCC recurrence within 1 year after surgery. By Chi-square test, the results showed that the recurrence rate within 1 year in patients with a *TSC2* mutation was significantly higher than patients without a *TSC2* mutation (60% vs 32%, p=0.015) (Table 6). Besides, some other clinicopathological factors were significantly associated with recurrence within 1 year, including serum AFP, BCLC stage, Edmondson grade and MVI (p<0.05) (Table 6). By logistic regression analysis, we found BCLC B-C stage (HR=8.628, 95% CI: 3.836–-19.405, p=0.000), *TSC2* mutations (HR=3.885, 95% CI: 1.-295–11.653, p=0.015) and serum AFP ≥400μg/L (HR=2.327, 95% CI: 1.018–5.323, p=0.045) were independently associated with recurrence within 1 year after surgery (Table 6).

RFS and OS

By Kaplan–Meier analysis, the median RFS of patients with a *TSC2* mutation was 7.4 months, while the median RFS of patients without a *TSC2* mutation was 30.8 months. Patients with a *TSC2* mutation had significantly poorer RFS than patients without a *TSC2* mutation (p=0.010) (Figure 1). However, TSC2 mutations did not significantly affect overall survival of patients (p=0.480) (Figure 2).

In univariate analysis, we found some factors which were significantly correlated with RFS, including *TSC2* mutations, BCLC stage, MVI, Edmondson grade, serum AFP and hypertension (p<0.05) (Table 7). By multivariate Cox regression analysis, the results showed that BCLC B-C stage (HR=3.070, 95% CI: 1.971–4.783, p=0.000), *TSC2* mutation (HR=1.861, 95% CI: 1.061–3.267,

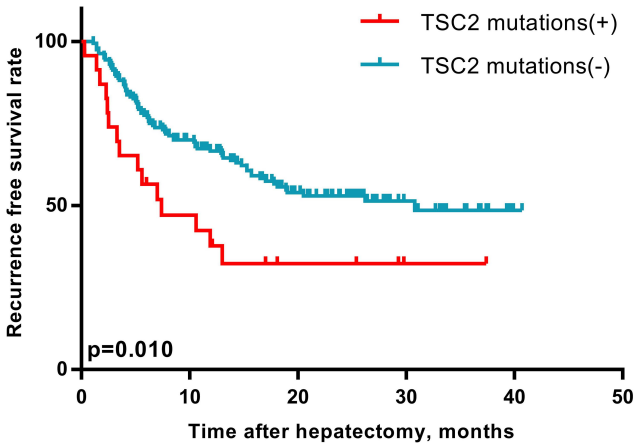

Figure 1 Survival curves of RFS according to *TSC2* mutational status evaluated by Kaplan–Meier method (n=183). There were 23 patients with a *TSC2* mutation and 160 patients without *TSC2* mutation. The median RFS of patients with a *TSC2* mutation was 7.4 months, while the median RFS of patients without *TSC2* mutation was 30.8 months. Patients with a *TSC2* mutation had significantly poorer RFS than others (p=0.010).

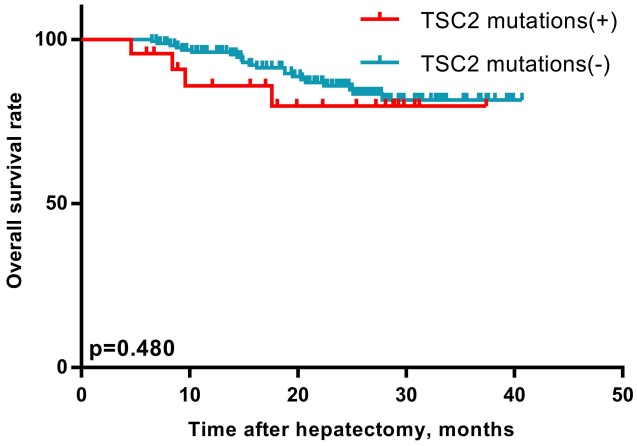

Figure 2 Survival curves of OS according to *TSC2* mutational status evaluated by Kaplan–Meier method (n=183). There were 23 patients with a *TSC2* mutation and 160 patients without *TSC2* mutation. *TSC2* mutations did not significantly affect overall survival of patients (p=0.480).

p=0.030) and preoperative serum AFP level ≥400μg/L (HR=1.715, 95% CI: 1.093–2.693, *p*=0.019) were independent risk factors for poor RFS in HCC patients after hepatectomy (Table 7).

There were 8 patients with a *TSC2* mutation and 45 patients without *TSC2* mutation on the patients with serum AFP ≥400μg/L group (n=53). The corresponding median RFS time was 3.3 months and 10.7 months, respectively, in patients with or without a *TSC2* mutation. The difference approached near significance (*p*=0.061) (Figure 3). In the subgroup with BCLC stage B-C (n=55), there were 8 patients with a *TSC2* mutation and corresponding median RFS was 2.5 months. The median RFS of patients without a *TSC2* mutation was 6.8 months. But the difference was not significant (*p*=0.118) (Figure 4).

Discussion

TSC2 was firstly identified in tuberous sclerosis complex in 1993.[12] Nowadays, it is known that *TSC2* is a key regulator in the upstream signaling of PI3K/AKT/mTOR pathway, which plays an important role on HCC carcinogenesis and metastasis.[13,14] Activation of the PI3K/AKT/mTOR signaling pathway could induce many biological processes, which induced oncogenic transformation, such as accelerating cell proliferation, protecting cells against apoptosis, metabolic reprogramming, suppressing autophagy and senescence.[15] As a downstream molecular of *TSC2*, mTORC1 was a key component in regulating a series of cancer-promoting biological processes by phosphorylation of proteins such as S6K1, 4E-BP1.[16] The complex of *TSC2* and *TSC1* can inhibit mTORC1 and downstream signaling of PI3K/AKT/mTOR pathway.[17,18] Therefore, *TSC2* was an important negative regulator of PI3K/AKT/mTOR signaling pathway.

In our study, mutations of *TSC2* were found in 12.6% of HCC patients. The mutation frequency of *TSC2* was higher than previous reports, including 5% from Schulze et al[19] and 5% from Totoki et al[20] and 3.0% to 4.5% from the cBioPortal (2019) database. This difference may be due to the background of different viral hepatitis. There were 88% of patients with HBsAg positive in this study, while the HBsAg positive only accounted for 14% and 23% in the study of Schulze et al[19] and Totoki et al.[20] Our results showed that *TSC2* was the most commonly mutated gene of PI3K/AKT/mTOR signaling pathway. This is consistent with previous study of Ho and colleagues.[21]

As a negative regulator of the PI3K/AKT/mTOR pathway, low expression or loss of *TSC2* implied overactivation of this pathway. It would inevitably lead to a series of biological processes conducive to the development of cancer. Some studies found that loss and mutations of *TSC2* led to the loss function of *TSC2* in HCC.[11,21] In this study, we found that *TSC2* mutations were significantly correlated with MVI and poorer Edmondson grade ($p<0.05$). Similarly, a study reported *TSC2* alterations were associated with HCC belonging to transcriptomic G3 subclasses characterized by poorly differentiation.[22] This result indicated that *TSC2* mutations were associated with poor biological characteristics of tumor in HCC patients. In patients with a *TSC2* mutation, we found patients with MVI or maximum diameter of tumor

Table 7 Univariate and Multivariate Cox Regression Analysis of Clinicopathological Characteristics and Gene Mutations with RFS of HCC Patients

Variables	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (<65/≥65)	0.741	0.430–1.274	0.278			
Gender (male/female)	0.674	0.349–1.303	0.241			
Hypertension (no/yes)	0.474	0.263–0.855	0.013	–	–	0.109
Diabetes (no/yes)	0.642	0.296–1.390	0.261			
Family history of cancer (no/yes)	0.892	0.560–1.419	0.629			
History of alcoholism (no/yes)	0.881	0.564–1.376	0.578			
HBsAg (negative/positive)	1.939	0.895–4.200	0.093			
HBV-DNA (<1E+003/≥1E+003IU/mL)	1.090	0.644–1.846	0.749			
Anti-hepatitis virus treatment (no/yes)	0.937	0.611–1.436	0.765			
AFP (<400/≥400μg/L)	2.274	1.478–3.498	0.000	1.715	1.093–2.693	0.019
BCLC (0-A/B-C)	3.513	2.290–5.388	0.000	3.070	1.971–4.783	0.000
Liver fibrosis (S1S2/S3S4)	1.162	0.709–1.903	0.551			
Edmondson grade (I–II/III–IV)	1.542	1.009–2.358	0.046	–	–	0.807
MVI (no/yes)	1.947	1.264–2.998	0.002	–	–	0.483
TP53 (Wild type/Mutant)	0.948	0.622–1.444	0.804			
TERT (Wild type/Mutant)	1.093	0.713–1.675	0.685			
CTNNB1 (Wild type/Mutant)	1.209	0.755–1.936	0.430			
AXIN1 (Wild type/Mutant)	0.914	0.497–1.681	0.772			
TSC2 (Wild type/Mutant)	2.043	1.169–3.570	0.012	1.861	1.061–3.267	0.030
TSC1 (Wild type/Mutant)	1.405	0.444–4.447	0.563			
TMB (<8.5/≥8.5 mutations/Mb)	0.685	0.412–1.139	0.145			

>5 cm had higher *TSC2* VAF than others ($p<0.05$). This result indicated that high mutation load of *TSC2* might correlate with poor biological characteristics of HCC.

We observed co-mutation between *TSC2* mutations and *TP53* mutations in the current study. *TP53* gene is a key regulator in *TP53*/cell-cycle pathway and its mutations are major drivers of HCC.[23,24] *TP53*/cell-cycle pathway also plays a role in the occurrence and development of liver cancer. Previous studies have suggested possible associations between different genes. For example, Huang et al[25] found the association between *TSC2* and *GSK3* beta expression. Peng et al[26] found different combinations between *TP53* polymorphisms and *MDM2* polymorphisms were significantly correlated with the risk of HCC development. Our study found a higher proportion of *TSC2* mutations in *TP53* mutated HCC patients, indicating the potential correlation between them, which has not been reported before. Although Ho et al[21] did not identify the correlation of *TSC2* mutations and *TP53* mutations in 95 patients with HBV-related HCC, the co-mutation of *TSC2* and *TP53* was still worthy of further investigation.

In this study, our results showed that the BCLC B-C stage, *TSC2* mutations and preoperative serum AFP ≥400μg/L were independent risk factors for poor RFS of HCC patients after hepatectomy. We did not find the correlation between these factors. It has been a consensus that BCLC staging and serum AFP level are extremely

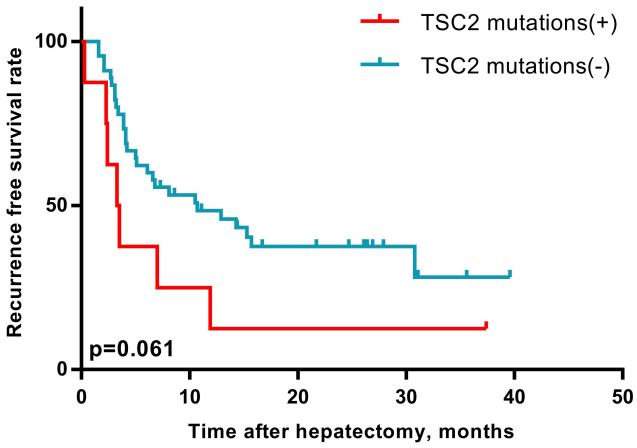

Figure 3 Survival curves of RFS according to *TSC2* mutational status evaluated by Kaplan–Meier method in patients with preoperative serum AFP level above 400μg/L (n=53). Median RFS between patients with a *TSC2* mutation and patients without *TSC2* mutation was 3.3 vs 10.7 months (p=0.061).

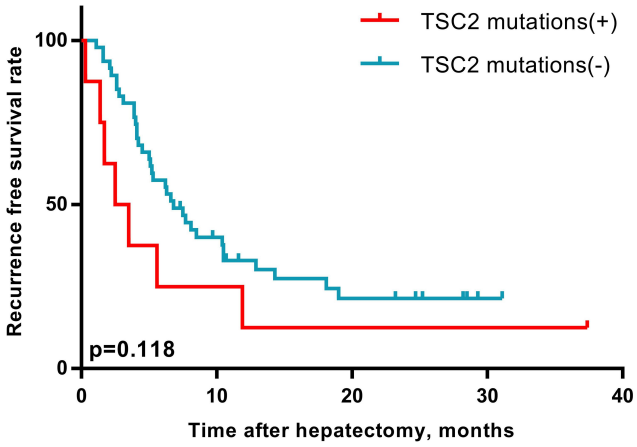

Figure 4 Survival curves of RFS according to *TSC2* mutational status evaluated by Kaplan–Meier method in patients with BCLC stage B-C (n=55). Median RFS between patients with a *TSC2* mutation and patients without *TSC2* mutation was 2.5 vs 6.8 months (p=0.118).

valuable for the prognostic evaluation of HCC patients. We found that patients with a *TSC2* mutation exhibited shorter RFS time after hepatectomy than those without a *TSC2* mutation. The mutation of *TSC2* was one of independent risk factors for both HCC recurrence within 1 year and poor RFS time after hepatectomy. This result showed that *TSC2* mutations in HCC tissue might be one of the early recurrence factors for HCC patients underwent liver resection. Huang et al[25] also reported that low expression of *TSC2* was associated with poor prognosis of HCC patients. The mutation of *TSC2* means the dysfunction of *TSC2* and may promote the invasion and aggression of HCC, supporting that *TSC2* could predict early recurrence of HCC patients. In subgroup with AFP level above 400μg/L, the median RFS was shorter in patients with a *TSC2* mutation than those without *TSC2* mutation, and the difference was trends clinically significant. The *TSC2* mutations may predict poorer RFS for HCC patients with AFP level above 400μg/L. We inferred that the HCC patients with *TSC2* mutation might be a group at high risk of early recurrence after hepatectomy. For these patients, surveillance was more important for detecting recurrence and early intervention.

This study has some limitations. Firstly, this is a monocenter study and lacks representativeness. Secondly, the follow-up time of this study was relatively shorter. Thus, multicenter study is necessary to enrich the results. We expect that we can acquire more convincing results with the extension of follow-up time and the increase of sample size.

Conclusion

In conclusion, *TSC2* mutations were significantly associated with MVI in liver para-carcinoma tissue and Edmondson grade III–IV in patients with HCC and were independently associated with recurrence within 1 year and poorer RFS after hepatectomy. The TSC2 mutation may be a potential predictor for early recurrence in HCC patients underwent hepatectomy.

Ethics Approval and Consent

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (ethics approval number: QDFYKYLLL-20161212). Informed consent was obtained from all patients included in our study. All participants and contributors of this study have signed informed consent for publication.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the

version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare no conflicts of interest in this work.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424. doi:10.3322/caac.21492
2. Mazzola A, Costantino A, Petta S, et al. Recurrence of hepatocellular carcinoma after liver transplantation: an update. *Future Oncol*. 2015;11(21):2923–2936. doi:10.2217/fon.15.239
3. Kumar AM, Fredman ET, Coppa C, El-Gazzaz G, Aucejo FN, Abdel-Wahab M. Patterns of cancer recurrence in localized resected hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int*. 2015;14(3):269–275. doi:10.1016/s1499-3872(15)60382-4
4. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J*. 2008;412(2):179–190. doi:10.1042/bj20080281
5. Mehta MS, Vazquez A, Kulkarni DA, et al. Polymorphic variants in TSC1 and TSC2 and their association with breast cancer phenotypes. *Breast Cancer Res Treat*. 2011;125(3):861–868. doi:10.1007/s10549-010-1062-1
6. Chakraborty S, Mohiyuddin SM, Gopinath KS, Kumar A. Involvement of TSC genes and differential expression of other members of the mTOR signaling pathway in oral squamous cell carcinoma. *BMC Cancer*. 2008;8:163. doi:10.1186/1471-2407-8-163
7. Geng H, Li S, Guo Y, et al. Survival prediction for patients with lung adenocarcinoma: A prognostic risk model based on gene mutations. *Cancer Biomark*. 2020;27(4):525–532. doi:10.3233/cbm-191204
8. Lee YH, Do SK, Lee SY, et al. TSC2 genetic variant and prognosis in non-small cell lung cancer after curative surgery. *Thorac Cancer*. 2019;10(2):335–340. doi:10.1111/1759-7714.12951
9. Zhang Y, Jia QA, Kadel D, Zhang XF, Zhang QB. Targeting mTORC1/2 complexes inhibit tumorigenesis and enhance sensitivity to 5-fluorouracil (5-FU) in hepatocellular carcinoma: a preclinical study of mTORC1/2-targeted therapy in hepatocellular carcinoma (HCC). *Med Sci Monit*. 2018;24:2735–2743. doi:10.12659/msm.907514
10. Cho J, Lee J, Kim J, et al. Loss of tuberous sclerosis complex 2 (TSC2) as a predictive biomarker of response to mTOR inhibitor treatment in patients with hepatocellular carcinoma. *Transl Oncol*. 2016;9(5):466–471. doi:10.1016/j.tranon.2016.08.009
11. Huynh H, Hao HX, Chan SL, et al. Loss of tuberous sclerosis complex 2 (TSC2) is frequent in hepatocellular carcinoma and predicts response to mTORC1 inhibitor everolimus. *Mol Cancer Ther*. 2015;14(5):1224–1235. doi:10.1158/1535-7163.mct-14-0768
12. European Chromosome 16 Tuberous Sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell*. 1993;75(7):1305–1315. doi:10.1016/0092-8674(93)90618-z.
13. Dimri M, Satyanarayana A. Molecular signaling pathways and therapeutic targets in hepatocellular carcinoma. *Cancers (Basel)*. 2020;12(2):491. doi:10.3390/cancers12020491
14. Couri T, Pillai A. Goals and targets for personalized therapy for HCC. *Hepatol Int*. 2019;13(2):125–137. doi:10.1007/s12072-018-9919-1
15. Aoki M, Fujishita T. Oncogenic roles of the PI3K/AKT/mTOR axis. *Curr Top Microbiol Immunol*. 2017;407:153–189. doi:10.1007/82_2017_6
16. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell*. 2012;149(2):274–293. doi:10.1016/j.cell.2012.03.017
17. Ashworth RE, Wu J. Mammalian target of rapamycin inhibition in hepatocellular carcinoma. *World J Hepatol*. 2014;6(11):776–782. doi:10.4254/wjh.v6.i11.776
18. Tee AR, Fingar DC, Manning BD, Kwiatkowski DJ, Cantley LC, Blenis J. Tuberous sclerosis complex-1 and −2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. *Proc Natl Acad Sci U S A*. 2002;99(21):13571–13576. doi:10.1073/pnas.202476899
19. Schulze K, Imbeaud S, Letouzé E, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet*. 2015;47(5):505–511. doi:10.1038/ng.3252
20. Totoki Y, Tatsuno K, Covington KR, et al. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. *Nat Genet*. 2014;46(12):1267–1273. doi:10.1038/ng.3126
21. Ho DWH, Chan LK, Chiu YT, et al. TSC1/2 mutations define a molecular subset of HCC with aggressive behaviour and treatment implication. *Gut*. 2017;66(8):1496–1506. doi:10.1136/gutjnl-2016-312734
22. Calderaro J, Couchy G, Imbeaud S, et al. Histological subtypes of hepatocellular carcinoma are related to gene mutations and molecular tumour classification. *J Hepatol*. 2017;67(4):727–738. doi:10.1016/j.jhep.2017.05.014
23. Zheng Y, Lv P, Wang S, Cai Q, Zhang B, Huo F. LncRNA PLAC2 upregulates p53 to induce hepatocellular carcinoma cell apoptosis. *Gene*. 2019;712:143944. doi:10.1016/j.gene.2019.143944
24. Bykov VJN, Eriksson SE, Bianchi J, Wiman KG. Targeting mutant p53 for efficient cancer therapy. *Nat Rev Cancer*. 2018;18(2):89–102. doi:10.1038/nrc.2017.109
25. Huang KT, Huang YH, Li P, et al. Correlation between tuberous sclerosis complex 2 and glycogen synthase kinase 3 beta levels, and outcomes of patients with hepatocellular carcinoma treated by hepatectomy. *Hepatol Res*. 2014;44(11):1142–1150. doi:10.1111/hepr.12256
26. Peng Q, Lao X, Chen Z, et al. TP53 and MDM2 gene polymorphisms, gene-gene interaction, and hepatocellular carcinoma risk: evidence from an updated meta-analysis. *PLoS One*. 2013;8(12):e82773. doi:10.1371/journal.pone.0082773