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

Introduction
Hepatocellular carcinoma (HCC) is the sixth most common cancer and the fourth most common cause of cancer-related death worldwide. 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.  

Tuberous sclerosis complex 2 (TSC2) is an important tumor-suppressor gene, which was firstly found in tuberous sclerosis complex.  

Many studies have demonstrated that TSC2 closely related with several cancers. For example, Mehta et al. reported that the expression of TSC2 was downregulated in aggressive breast cancer. Chakraborty et al.  

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.,  

TSC2 was a biomarker to predict a poor prognosis. Lee et al. 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. However, the value of TSC2 in predicting the prognosis after heptectomy 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 heptectomy.

**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 anti-tumor 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 SnvEff3.0. 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

| 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 |
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).

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 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 |
Table 3 The Correlation Between TSC2 Mutations and Clinicopathological Characteristics

| Clinicopathological Characteristics | TSC2 | \( \chi^2 \) | P  |
|-------------------------------------|------|--------------|----|
|                                    | Wild Type | Mutant |        |
| 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/≥400 μg/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 |
| Macrophase 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 |

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).

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

| Clinicopathological Characteristics | VAF of TSC2 | \( U \) | P  |
|-------------------------------------|-------------|--------|----|
|                                    | Median      |        |    |
| 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)  | 0.190/0.253 | 29.000 | 0.698 |
| History of alcoholism (no/yes)     | 0.200/0.200 | 42.500 | 0.850 |
| AFP (<400/≥400 μg/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 |

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

| Variables                        | Univariate Analysis |                               |                               |
|----------------------------------|---------------------|--------------------------------|--------------------------------|
|                                  | n/n                | $\chi^2$ | 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 |

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,
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 al19 and 5% from Totoki et al20 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 al19 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 poor 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 (S1/S2/S3/S4) | 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 al25 found the association between TSC2 and GSK3 beta expression. Peng et al26 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 al21 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
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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