Prognostic value of \( p53 \) mutation for poor outcome of Asian primary liver cancer patients: evidence from a cohort study and meta-analysis of 988 patients

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Abstract: Several previous studies have investigated the association between gene \( p53 \) (\( p53 \)) mutation and the poor outcome of primary liver cancer (PLC) patients; however, the results remain inconsistent. In the present study, \( p53 \) mutation in 60 paired tumor and corresponding nontumor tissues derived from a cohort of 60 PLC patients was systematically analyzed. The results showed that \( p53 \) mutation was only an independent risk factor for overall survival (OS), not for recurrence-free survival (RFS), and a meta-analysis was performed to verify this. Online databases were searched up to July 1, 2016. Studies about the association between \( p53 \) mutation and the postsurgery survival of PLC patients were collected. A total of 988 patients from eight studies were analyzed; among them, 341 (34.51%) patients had \( p53 \) mutation. Pooled hazard ratios (HRs) and 95% confidence intervals (CIs) were 2.03 (1.64, 2.41) and 2.36 (1.31, 3.42) for OS and RFS, respectively. In conclusion, both the cohort study and meta-analysis suggested that the \( p53 \) mutation was associated with postsurgery OS in Asian PLC patients. However, the relationship between \( p53 \) mutation and recurrence should be confirmed by further studies.

Keywords: \( p53 \), primary liver cancer, survival, meta-analysis

Introduction
Primary liver cancer (PLC), ranked as the second and sixth leading cause of cancer deaths in males and females, respectively, is one of the most lethal human malignancy worldwide, and it is especially common in Asia.\(^1,2\) Although improved diagnostic techniques and treatment approaches have contributed more patients to receive treatment at early stages, such as liver resection, the mortality rate still remains high due to the frequent recurrence of tumor.\(^3-5\) Therefore, the identification of effective prognostic factors is extremely needed for the proper management of PLC patients.

Currently, the available main prognostic factors include the size of tumor, the number of tumor nodules, portal vein tumor thrombus (PVTT), serum level of \( \alpha \)-fetoprotein (AFP) and serum ferritin, and whether the patient has metastasis or not. However, the prognostic significance of each single variable has been found to be unsatisfactory so far; some findings even remain controversial.\(^4-8\)

The gene of \( p53 \) residing on chromosome 17p13.1 is a well-known tumor suppressor gene that controls response to several different cellular stresses, including DNA damage, hypoxia, and oncogene activation.\(^9,10\) The mutations of \( p53 \) had been found in...
many kinds of cancer and repeatedly reported as a prognostic factor for the clinical outcome of PLC patients.\textsuperscript{11,12} To explore the role of \textit{p53} mutation in predicting the outcome of PLC patients, \textit{p53} mutation in paired tissues of 60 Chinese PLC cases was screened for, and then the association between \textit{p53} mutation and patients’ long-term clinical outcome was analyzed. Moreover, a meta-analysis including 988 PLC patients to verify the consequence was carried out.

**Materials and methods**

**Study population and their clinical data collection**

Tumor tissue specimens were obtained from 60 Chinese patients who had underwent surgical treatment in Henan Cancer Hospital, Zhengzhou, China, between December 2008 and December 2009. All the patients were diagnosed with PLC by pathological diagnosis, and none of them received any chemotherapy or radiotherapy before surgery. By reading the electronic medical record, clinical features and biochemical indicators were collected.

The age of the patients ranged from 34 to 70 years (median 50.82 years). The male to female ratio was 43:17. Serological tests showed that out of the 60 patients, three patients (5.00\%) were HBeAg positive and 59 patients (98.33\%) were serum hepatitis B surface antigen positive, whereas serum anti-hepatitis C virus (HCV) antibody was present in only one (1.67\%) patient. What is more, 58 (96.67\%) patients had cirrhosis diagnosed by pathology.

This study was approved by the ethics committee of Peking University Health Science Center, Beijing, China. Written informed consents were obtained from all the participants.

**Follow-up**

After patients were discharged from hospital, by telephoning or checking patients’ medical records or visiting patients or their relatives, their medical and survival information were collected.

The first and second follow-ups were carried out 1 month and 3 months after hospital discharge, respectively. The patients were then followed up every 3 months for the first year and every 6 months for the following 5 years. The last follow-up was carried out in July 2015. Five of the patients were lost to follow-up and 44 died during the past 5 years.

**Method**

DNAs were extracted from 60 paired frozen PLC tissues, and for the array-based comparative genomic hybridization (a-CGH) study, the genomic DNAs were extracted using the Genomic DNA PURIFICATION Kit (Qiagen NV, Venlo, the Netherlands). Four independent polymerase chain reactions (PCRs) were used to amplify exons 2–11 of \textit{p53}. The PCR products were directly sequenced to identify mutation. Chromosome aberration was comprehensively analyzed via a-CGH. In the assay, each corresponding paired adjacent nontumor tissue DNA was used as reference DNA.

In the following analysis, compared to the adjacent nontumor tissue DNA sequences, loss of heterozygosity (LOH) or point mutation of \textit{p53} would be defined as \textit{p53} mutation of the patients.

**Statistical analysis**

Chi-square test was used to assess the significance of the associations between \textit{p53} mutation and clinical features. Cumulative survival was tested by the Kaplan–Meier method, and the Mantel–Cox model was used to assess the effects of \textit{p53} mutation on survival and recurrence. A two-sided \(P\)-value < 0.05 was defined as statistical significance. These tests were done by SPSS 21.0 (IBM Corporation, Armonk, NY, USA). Stata version 12 (StataCorp LP, College Station, TX, USA) was used, and a random-effect model was conducted if heterogeneity was substantial with the value of \(I^2\) > 50\% and \(\chi^2\) \(P\)-value < 0.10; if not, fixed-effects model was used for secondary analysis.\textsuperscript{13} Publication bias was considered if \(P\)-value was < 0.05 in Egger’s test.

**Search strategy and study selection**

Comprehensive literature search was conducted in the databases of PubMed, Cochrane Library, and Web of Science with an upper date limit to July 1, 2016. By using the random combination of the search terms “primary liver cancer or hepatocellular carcinoma”, “PLC or HCC”, “\textit{p53} or \textit{p53} mutation”, “\textit{TP53} or \textit{TP53} mutation”, and “prognosis, survival, recurrence or outcome”, a total of 653 literatures were found.

Eligible studies were required to match the following criteria: 1) proven pathology diagnosis of PLC patients; 2) reported explicit methods for the detection of \textit{p53} mutation; and 3) provided hazard ratio (HR), 95\% confidence interval (CI), or crude data.

**Data extraction and quality assessment**

According to the guidelines of Meta-Analysis of Observational Studies in Epidemiology,\textsuperscript{14} two reviewers...
independently extracted the data from each eligible study and a consensus was achieved through discussion in case of disagreement between these two reviewers. Data extracted from the literatures included author, publication year, clinical features, assay method, HR, and 95% CI. If the studies did not report the raw data, HR and CI were extracted according to survival curves by using the previous published methods.15,16 The quality of each study was identified according to the Newcastle–Ottawa Quality Assessment Scale (NOS) for cohort studies.14 However, the literature’s use was limited in the following analysis if the data were not completely reported in the literatures.

Results

Status of p53 mutation and the clinical features of the patients

A total of 29 out of 60 patients (48.33%) were detected carrying p53 mutation in the present cohort. As the results shown in Table 1 and similar to several previous studies reported, poor mean overall survival (OS) time and short mean recurrence-free survival (RFS) time were observed among patients carrying p53 mutation (P<0.05). No other clinical features were found to be statistically different between the two groups.

Mutation of p53 predicts poor OS in PLC patients

To further evaluate the association of p53 mutation with patients’ poor prognosis after surgical treatment, Kaplan–Meier test was conducted. The results showed that patients with p53 mutation had significantly shorter OS than those without p53 mutation (P=0.002), but RFS did not differ (P=0.738); survival curves are shown in Figure 1.

Next, Cox regression multivariate analysis was performed with six other potential clinical variables, including age (≤60 years/>60 years), tumor size (≤5 cm/>5 cm), tumor number (1/≥2), serum level of AFP (≤400 ng/mL/>400 ng/mL), PVTT (+/-), and cirrhosis (+/-). The results are shown in Table 2. Both p53 mutation and large tumor size were the independent predictors for short OS. Other factors, including age, PVTT, cirrhosis, tumor number, and AFP, tended to have influence on survival to some extent, but without significant P-values.

Meta-analysis

Nine studies including the results reported earlier were eligible for meta-analysis; one was performed in the UK and other eight studies including 988 patients were performed in Asia. The basic data are listed in Table 3.11,12,17–23 The mean rate of p53 mutation was 34.51%. One of these studies combined the patients from Asia and Europe including 336 Chinese patients and 73 White patients, and the data about Asian patients were used in the following study.21 The follow-up time postsurgery ranged from 2 to 8 years, and the median time was 4.8 years. Remarkable differences identified among the studies were positive rate of virus infection and cirrhosis. The positive rate of Hepatitis B virus (HBV) ranged from 15.5% to 98.3%, and that of HCV ranged from 1.49% to 77.1%. Excluding one study that did not report cirrhosis rate, the cirrhosis rates ranged from 44.4% to 95.1%. The cohort study research was pooled in the follow-up analyses. Figure 2 shows the forest plots for the association

Table I Preoperative clinical features stratified by p53 status

| Clinical features | Total patients (N=60) | p53 mutation (-) patients, (n=31) | p53 mutation (+) patients, (n=29) | P-value |
|-------------------|----------------------|----------------------------------|----------------------------------|---------|
| Age (years), mean ± SD | 50.82±8.40 | 50.90±8.90 | 50.72±8.00 | 0.705 |
| Gender (M/F) | 43/17 | 21/14 | 22/7 | 0.485 |
| PVTT (+/-) | 45/14 | 24/6 | 21/8 | 0.491 |
| Cirrhosis (+/-) | 2/58 | 2/29 | 0/29 | 0.164 |
| Size (<5 cm/>5 cm) | 17/42 | 8/23 | 9/20 | 0.653 |
| Tumor number (<2/>2) | 31/29 | 18/13 | 17/12 | 0.965 |
| AFP (≤400/>400), ng/mL | 25/34 | 11/20 | 14/14 | 0.260 |
| AST (≤40/>40), U/L | 17/43 | 9/22 | 8/21 | 0.901 |
| ALT (≤40/>40), U/L | 26/34 | 17/14 | 9/20 | 0.063 |
| ALP (≤150/>150), U/L | 44/16 | 23/8 | 21/8 | 0.876 |
| OS time, mean ± SD | 32.79±24.85 | 38.71±28.07 | 24.05±16.15 | <0.001 |
| RFS time, mean ± SD | 13.02±18.86 | 13.98±23.85 | 8.28±9.77 | 0.048 |

Abbreviations: M, male; F, female; PVTT, portal vein tumor thrombus; AFP, α-fetoprotein; AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; OS, overall survival; RFS, recurrence-free survival; SD, standard deviation.
between \( p53 \) mutation and OS or RFS. Egger’s test was carried out to assess the publication bias of the studies, and it showed that the publication bias was absent in both the analyses. The \( P \)-values of the bias for OS and RFS were 0.405 and 0.830, respectively; funnel plot was also done to value the publication bias (Figure 3).

The summary HR and 95% CI for OS and RFS were 2.03 (1.64, 2.41) and 2.36 (1.31, 3.42), respectively. The result supported \( p53 \) mutation as a kind of risk factor for the poor outcome of PLC patients who underwent surgery, which is consistent with a previous report.9

### Sensitivity and subgroup analysis

To ensure the reliability of this study, sensitivity analysis was performed, by excluding every individual study, the studies with a score of NOS \( \leq 5 \), the largest effect study, and studies with follow-up \( \leq 5 \) years. No significant change had happened, and it suggested that no individual study significantly affected the pooled HR, which indicated that the results were statistically robust.

To the authors’ knowledge, the conditions of virus infection and cirrhosis are strong factors for the OS of PLC patients and may contact with \( p53 \) conditions.24–26 Therefore, subgroup analyses were performed to eliminate the variances among clinical features in this study.

By dividing the research according to the rate of cirrhosis, HBV infection, HCV infection, number of the patients, and the test method, subgroup analyses showed that the heterogeneity comes from the difference in HBV and HCV infection rate experiment methods and patient number in each research; all the \( P \)-values were \( >0.10 \) in these subgroups (Table 4).

### Discussion

A comprehensive understanding of the influential factors for the OS and RFS is crucial for the improved management

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**Table 2** Multivariate analysis for predictors of prognosis of PLC patients

| Covariate     | Coefficient | SE  | HR      | 95% CI     | \( P \)-value |
|---------------|-------------|-----|---------|------------|---------------|
| **OS**        |             |     |         |            |               |
| \( p53 \) mutation \((-/+)\) | 0.95        | 0.37 | 2.58    | (1.26, 5.27) | 0.010         |
| Age \((<60/\geq60)\) | 0.69        | 0.44 | 1.98    | (0.84, 4.70) | 0.119         |
| Size \((<5 \text{ cm/} \geq 5 \text{ cm})\) | 1.04        | 0.50 | 2.83    | (1.06, 7.58) | 0.038         |
| Tumor number \((<2/\geq2)\) | 0.33        | 0.37 | 1.39    | (0.68, 2.84) | 0.374         |
|AFP \((\leq400/>)400\) | 0.34        | 0.40 | 1.41    | (0.64, 3.10) | 0.394         |
| PVTT \((-/+)\) | –0.55       | 0.40 | 0.61    | (0.26, 1.29) | 0.178         |
| Cirrhosis \((-/+)\) | 0.32        | 0.34 | 1.38    | (0.71, 2.65) | 0.341         |
| **RFS**       |             |     |         |            |               |
| \( p53 \) mutation \((-/+)\) | 0.49        | 0.50 | 1.62    | (0.61, 4.30) | 0.501         |
| Age \((<60/\geq60)\) | –0.14       | 0.60 | 0.87    | (0.27, 2.84) | 0.815         |
| Size \((<5 \text{ cm/} \geq 5 \text{ cm})\) | 1.78        | 0.68 | 5.95    | (1.56, 22.68) | 0.009         |
| Tumor number \((<2/\geq2)\) | 1.25        | 0.50 | 3.48    | (1.30, 9.29) | 0.013         |
|AFP \((\leq400/>)400\) | 0.39        | 0.58 | 1.48    | (0.47, 4.61) | 0.501         |
| PVTT \((-/+)\) | –0.97       | 0.75 | 0.38    | (0.09, 1.63) | 0.192         |
| Cirrhosis \((-/+)\) | 0.35        | 0.40 | 1.42    | (0.64, 3.13) | 0.388         |

**Abbreviations:** PLC, primary liver cancer; SE, standard error; HR, hazard ratio; CI, confidence interval; OS, overall survival; AFP, \( \alpha \)-fetoprotein; PVTT, portal vein tumor thrombus; RFS, recurrence-free survival.

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**Figure 1:** Kaplan–Meier survival curves for primary liver cancer patients with and without \( p53 \) mutation.

Notes: (A) Overall survival curves \((P=0.002)\). (B) Recurrence-free survival curves \((P=0.738)\).
| Country | Cases | HBV infection (%) | HCV infection (%) | Cirrhosis (%) | p53 mutation rate (%) | Assay method | Follow-up years (up to) | OS HR (95% CI) | RFS HR (95% CI) |
|---------|-------|-------------------|-------------------|--------------|-----------------------|--------------|-----------------------|----------------|----------------|
| Japan   | 12    | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |
| South Korea | 20  | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |
| China   | 83    | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP, direct sequencing | 2.58 (1.26, 5.27)     |                |                |
| Japan   | 54    | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |
| China   | 367   | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |
| China   | 187   | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |
| China   | 60    | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |

Notes: The results of this current study were included in a meta-analysis and are shown in the table as “Wen et al (2015).” En-dashes indicate that the research had not reported this information or the result could not be calculated.

Abbreviations: PLC, primary liver cancer; HBV, hepatitis B virus; HCV, hepatitis C virus; RFS, recurrence-free survival; HR, hazard ratio; CI, confidence interval; OS, overall survival; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism; a-CGH, array-based comparative genomic hybridisation.

### Table 3 - Characteristics of studies on p53 mutation and Plc patients

| References | Country | Cases | HBV infection (%) | HCV infection (%) | Cirrhosis (%) | p53 mutation rate (%) | Assay method | Follow-up years (up to) | OS HR (95% CI) | RFS HR (95% CI) |
|------------|---------|-------|-------------------|-------------------|--------------|-----------------------|--------------|-----------------------|----------------|----------------|
| Hayashi et al (1995) | Japan | 90    | 76.67             | 18.89             | 62.22        | 2.77 (1.11-23.38)      | PCR-SSCP     | 5.69 (1.10-28.37)     | 1.96 (1.02-9.19) | 1.96 (1.02-9.19) |
| Sugio et al (1999) | Japan | 98    | 77.55             | 19.39             | 89.88        | 2.53 (1.11-23.38)      | PCR-SSCP     | 5.69 (1.10-28.37)     | 1.96 (1.02-9.19) | 1.96 (1.02-9.19) |
| Park et al (2001) | South Korea | 20  | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |
| Yano et al (2007) | Japan | 83    | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |
| Su et al (2008) | China | 54    | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |
| Wen et al (2015) | China | 367   | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |
| Wen et al (2013) | China | 187   | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |
| Wen et al (2015) | China | 60    | 75.00             | 5.00              | 13.8         | 1.49                  | PCR-SSCP     | 2.58 (1.26, 5.27)     |                |                |

Notes: The possible mechanisms attributed to the prognosis-predicting value of p53 might associate with the biological function of p53. As known, p53 is an important tumor suppressor gene responding to genotoxic and oncogenic stress by inducing cell cycle arrest or apoptosis. p53 has the capacity to regulate the expression of several hundred genes, many of which are involved in mediating or regulating cell growth, division, survival, and programmed cell death.30–32 What is more, p53-null mice were susceptible to spontaneous tumor formation, and the removal of p53 would lead to rapid tumor development and death for various specific cancers.33–35 However, it is worthwhile to notice that cancer relapse is a complicated process and is affected by multiple factors, such as surgery conditions, chemotherapy, radiotherapy, and underlying diseases.36–39 Hence, basic conditions would be considered when using p53 mutation or not as a predict factor for PLC patients.

For the meta-analysis, heterogeneity was also a common problem. According to a previous study, the value of $I^2$, which is adjusted by freedom, would be a better indicator of heterogeneity.40 In this study, the $I^2$ values of OS and RFS were 26.3% and 0, respectively. The background of patients, including gender, age, race, cirrhosis status, and virus infection status, were possible reasons of heterogeneity.41–42 Interestingly, the heterogeneity was
absent in OS after adjusting the studies by HBV and HCV infection rates, patient number, and experiment method, indicating that these factors might partially account for the appreciable heterogeneity. Cirrhosis might be a potential factor of heterogeneity, however most of the Asian PLC patients had hepatitis virus infection and cirrhosis, and all of the patients in the eight studies analyzed had a high cirrhosis rate, so subgroup analyses did not consider the cirrhosis rate.

Notably, there are some limitations in this study. First, only five articles and the cohort study data with 405 patients can be used for the meta-analysis of RFS. Second, it is difficult to eliminate the effects of publication bias during the meta-analysis; even Egger’s test showed that the publication bias was absent.43
Table 4 Subgroup analysis of the eligible studies on p53 mutation with OS and RFS

| Analysis                        | Studies (n) | Pooled HR | CI     | I² statistic (%) | P-value for heterogeneity |
|---------------------------------|-------------|-----------|--------|-----------------|---------------------------|
| OS                              |             |           |        |                 |                           |
| HBV infection                   |             |           |        |                 |                           |
| <50%                            | 4           | 3.41      | (1.78, 5.05) | 0.37         | 0.561                     |
| ≥50%                            | 3           | 1.90      | (1.55, 2.34) | 0             | 0.205                     |
| HCV infection                   |             |           |        |                 |                           |
| <50%                            | 5           | 3.46      | (1.56, 2.35) | 14.1         | 0.322                     |
| ≥50%                            | 3           | 2.47      | (1.71, 5.04) | 0             | 0.369                     |
| Method                          |             |           |        |                 |                           |
| SSCP                            | 2           | 2.58      | (0.56, 4.60) | 0             | 0.732                     |
| Direct sequencing               | 3           | 1.78      | (1.49, 2.36) | 0             | 0.743                     |
| Mix                             | 2           | 2.81      | (1.98, 3.64) | 59.2         | 0.117                     |
| Follow-up time (months)         |             |           |        |                 |                           |
| <60                             | 2           | 2.40      | (1.33, 6.13) | 0             | 0.601                     |
| ≥60                             | 5           | 2.02      | (1.64, 2.41) | 49.0         | 0.098                     |
| Number of patients              |             |           |        |                 |                           |
| <100                            | 5           | 2.79      | (2.03, 3.56) | 0             | 0.598                     |
| ≥100                            | 2           | 1.77      | (1.32, 2.21) | 0             | 0.632                     |
| HBV infection                   |             |           |        |                 |                           |
| <50%                            | 3           | 2.82      | (1.14, 4.49) | 31.7         | 0.231                     |
| HCV infection                   |             |           |        |                 |                           |
| ≥50%                            | 3           | 2.06      | (0.70, 3.43) | 0             | 0.730                     |
| <50%                            | 3           | 2.06      | (0.70, 3.43) | 0             | 0.730                     |
| ≥50%                            | 3           | 2.82      | (1.14, 4.49) | 31.7         | 0.231                     |
| Method                          |             |           |        |                 |                           |
| SSCP                            | 2           | 2.44      | (0.70, 4.18) | 0             | 0.450                     |
| Direct sequencing               | 1           | 3.65      | (0.61, 2.31) | –            | –                         |
| Mix                             | 3           | 2.25      | (0.88, 3.61) | 38.2         | 0.198                     |
| Follow-up time (months)         |             |           |        |                 |                           |
| <60                             | 3           | 2.73      | (0.74, 4.71) | 0             | 0.725                     |
| ≥60                             | 3           | 2.22      | (0.97, 3.47) | 37.6         | 0.201                     |
| Number of patients              |             |           |        |                 |                           |
| <80                             | 3           | 2.06      | (0.70, 3.43) | 0             | 0.730                     |
| ≥80                             | 3           | 2.82      | (1.14, 4.49) | 31.7         | 0.231                     |

Abbreviations: OS, overall survival; RFS, recurrence-free survival; HR, hazard ratio; CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; SSCP, single-strand conformation polymorphism.
Conclusion
This study suggested that p53 mutation was associated with postsurgery OS in Asia PLC patients, and it might be a new potential prognosis factor for PLC patients.

Disclosure
The authors report no conflicts of interest in this work.

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