TP53 mutation is associated with a poor outcome for patients with hepatocellular carcinoma: evidence from a meta-analysis

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Background: Various studies examined the relationship between p53 mutation with the clinical outcome in patients with hepatocellular carcinoma (HCC), but yielded conflicting results.

Methods: Electronic databases updated to July 2013 were searched to find relevant studies. A meta-analysis was conducted with eligible studies which quantitatively evaluated the relationship between p53 mutation and survival of patients with HCC. Survival data were aggregated and quantitatively analyzed.

Results: We performed a meta-analysis of 9 studies that evaluated the correlation between p53 mutation and survival in patients with HCC. Combined hazard ratios suggested that p53 mutation had an unfavorable impact on overall survival (OS) [hazard ratio (HR) =1.46, 95% confidence interval (CI): 1.15-1.76], and disease free survival (DFS) (HR =2.57, 95% CI: 1.46-3.68) in patients with HCC. The significant heterogeneity (P=0.035) was observed among 8 studies for OS, however no significant heterogeneity (P=0.597) was observed among 5 studies for DFS.

Conclusions: p53 mutation indicates a poor prognosis for patients with hepatocellular carcinoma.

Keywords: Hepatocellular carcinoma (HCC); p53; mutation; survival; meta-analysis

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third most common cause of death from cancer and the third largest cause of cancer-related deaths worldwide (1). Although liver resection is an effective treatment for patients with advanced HCC, the long-term postoperative prognosis remains poor because of the high recurrence rate and lack of effective systemic therapy for HCC patients with metastases. The main prognostic factors are clinicopathological characteristics of the disease, including tumor size, stage, and grade. However, the prognostic factors do not fully predict individual clinical outcome. There is the need for better markers to identify patients with poor prognosis at the time of diagnosis. Researches have focused on the potential

role of new biological factors involved in the carcinogenic process as prognostic markers in patients with HCC.

Tumour suppressor gene p53, its wild-type protein is responsible for cell-cycle regulation and apoptosis after DNA damage. If p53 is mutated, however, the cell with DNA damage can escape from apoptosis and turn into cancer cells (2). Furthermore, the mutant p53 protein, which lost the function of wild-type protein, can accumulate in cell nuclei and is regarded as a highly specific indicator of malignancy (3). To date, some studies have documented that p53 alterations are correlated with tumour differentiation, vascular invasion, tumour stage, Child-Pugh class and serum AFP in HCC (4-7).

Many studies have evaluated whether p53 mutation may be a prognostic factor for survival in patients with HCC. However, the results of the studies are inconclusive and

Table 1 Main characteristics and results of the eligible studies

First author-year	Patients source	N pts	Stage	Method	p53 mutation, %	HR estimation	HR (95% CI)
Hayashi-1995	Japan	90	I-IV	PCR-SSCP	27.8	RFS and OS	OS 2.78 (1.08-7.13); PFS 4.29 (1.87-9.86)
Honda-1998	UK	42	NA	PCR-SSCP, DNA sequencing	23.8	OS	OS 2.89 (1.42-5.88)
Sugo-1999	Japan	98	NA	PCR-SSCP	26	RFS and OS	OS 2.68 (1.42-5.05); PFS 1.98 (1.02-3.85)
Park-2001	Korea	20	NA	PCR-DNA sequencing	45	RFS and OS	OS 4.21 (0.84-21.01); PFS 3.50 (1.22-10.04)
Yuan-2006	China	156	I-IV	DNA sequencing	46	OS	OS 2.04 (1.37-3.02)
Yano-2007	Japan	83	NA	PCR-SSCP DNA sequencing	19.3	RFS and OS	OS 13.88 (3.69-52.25); PFS 5.74 (2.38-13.84)
Su-2008	China	54	NA	PCR-SSCP, DNA sequencing	26	RFS	PFS 2.83 (1.28-6.25)
Woo-2011	China	409	NA	DNA sequencing	37.2	OS	OS 1.86 (1.37-2.52)
Yuan-2013	China	187	NA	DNA sequencing	44.9	OS	OS 0.93 (0.60-1.45)

NA, not applicable; HR, hazard ratio; CI, confidence interval; OS, Overall survival; DFS, disease-free survival

no consensus has been reached. It is unknown whether differences in these investigations have been mostly due to their limited sample size or genuine heterogeneity. Thus, we conducted a meta-analysis of all available studies relating VEGF with the clinical outcome in patients with HCC.

Materials and methods

Search strategy and study selection

The electronic databases PubMed and China National Knowledge Infrastructure (CNKI) were searched for studies to include in the present meta-analysis. An upper date limit of July 15, 2013 was applied; we used no lower date limit. Searches included the terms "hepatocellular or liver", "cancer or carcinoma or tumour or neoplasm", "p53", "mutation" and "prognosis". We also reviewed the Cochrane Library for relevant articles. The references reported in the identified studies were also used to complete the search.

Studies eligible for inclusion in this meta-analysis met the following criteria: (I) measure p53 mutation in the primary hepatocellular carcinoma; (II) provide information on survival [i.e., disease free survival (DFS) and/or overall survival (OS), studies investigating response rates only were excluded] and (III) When the same author reported results obtained from the same patient population in more than

one publication, only the most recent report, or the most complete one, was included in the analysis. Two reviewers (P.Z. and Y.J.) independently determined study eligibility. Disagreements were resolved by consensus.

Data extraction and quality assessment

Data retrieved from the reports included author, publication year, patient source, study design, test method, p53 mutation positive ratio and survival data (*Table 1*). If data from any of the above categories were not reported in the primary study, items were treated as "not applicable". We did no contact the author of the primary study to request the information. We did not use prespecified quality-related inclusion or exclusion criteria and did not weigh each study by a quality score, because the quality score has not received general agreement for use in a meta-analysis, especially observational studies (8). The data extraction and quality assessment could refer to our previous published meta-analysis (9-12).

Statistical methods

Included studies were divided into two groups for analysis: those with data regarding OS and those regarding DFS. For the quantitative aggregation of the survival results, we measured the impact of p53 mutation on survival by

Table 2 Meta-analysis: HR value of OS and DFS in hepatocellular carcinoma

	Nb	Random effects HR (95% CI)	χ^2 heterogeneity test (P)
Overall for OS	8	1.46 (1.15-1.76)	0.035
Overall for DFS	5	2.57 (1.46-3.68)	0.597
HR, hazard ratio; Nb, number of studies; OS, overall survival; DFS, disease-free survival			

HR between the two survival distributions. HRs and 95% confidence intervals (CIs) were used to combine as the effective value. If the HRs and their 95% CIs were given explicitly in the articles, we used crude ones. When these variables were not given explicitly, they were calculated from the available numerical data using methods reported by Parmar *et al.* (13).

Heterogeneity of the individual HRs was calculated with χ^2 tests according to Peto's method (14). Heterogeneity test with inconsistency index (I^2) statistic and Q statistic was performed. If HRs were found to have fine homogeneity, a fixed effect model was used for secondary analysis; If not, a random-effect model was used. DerSimonian-Laird random effects analysis (15) was used to estimate the effect of p53 mutation on survival. By convention, an observed HR >1 implies worse survival for the group with p53 mutation. The impact of p53 mutation on survival was considered to be statistically significant if the 95% confidence interval (CI) did not overlap with 1. Horizontal lines represent 95% CIs. Each box represents the HR point estimate, and its area is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with CI represented by its width. The unbroken vertical line is set at the null value (HR=1.0).

Evidence of publication bias was sought using the methods of Egger *et al.* (16) and of Begg *et al.* (17). Intercept significance was determined by the t-test suggested by Egger (P<0.05 was considered representative of statistically significant publication bias). All of the calculations were performed by STATA version 11.0 (Stata Corporation, College Station, TX)

Results

Study selection and characteristics

Nine studies (18-26) published between 1995 and 2013 were eligible for this meta-analysis. All reported the prognostic value of p53 mutation status for survival in hepatocellular carcinoma patients. The total number of patients included was 1,139, ranging from 20 to 409 patients per study

(median 126). The major characteristics of the 9 eligible publications are reported in *Table 1*. The studies were conducted in four countries (China, Japan, South Korea and UK). Among the 9 studies, eight studies were performed in Asian populations.

All of the studies reported the prognostic value of p53 mutation status for survival in patients with HCC tissue. Of the 9 studies, 8 directly reported HRs (multivariate analysis), while the other 1 study provided survival curves. Among them, the proportion of patients exhibiting p53 mutation in individual studies ranged from 19.3% to 44.9%. Estimation using survival curves were segregated according to either OS or DFS. A HR on DFS and OS could be extracted for 5 publications and 8 publications of studies, respectively. Seven of the 9 studies identified p53 mutation as an indicator of poor prognosis, and the other 2 studies showed no statistically significant impact of p53 mutation on survival.
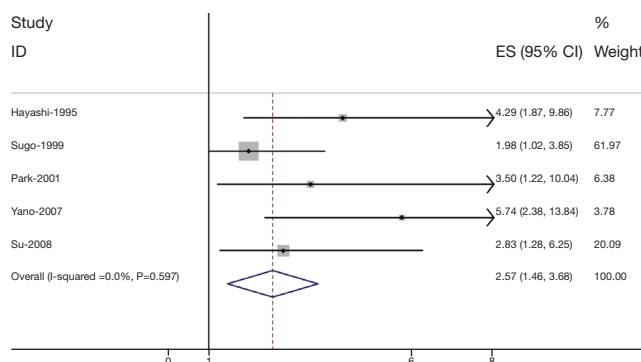
Meta-analysis

The results of the meta-analysis were shown in *Table 2* and *Figures 1,2*. Overall, the combined HR for all 8 eligible studies evaluating p53 mutation on OS was 1.46 (95% CI: 1.15-1.76), suggesting that p53 mutation was an indicator of poor prognosis for hepatocellular carcinoma. However, significant heterogeneity was observed among the studies (Q=5.49, I^2=53.5%, P=0.035). Meanwhile, for DFS analysis, statistically significant effect of p53 mutation (HR=2.57, 95% CI: 1.46-3.68) in patients with HCC was also observed. (Q=3.53, I^2=0.0%, P=0.597).

Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias in the literature. All 8 eligible studies investigating p53 mutation on OS yielded a Begg's test score of P=0.083 and an Egger's test score of P=0.100, meanwhile according to the funnel plot (*Figure 3*), the absence of publication bias was found. For DFS analysis, no publication biases were found for investigating p53
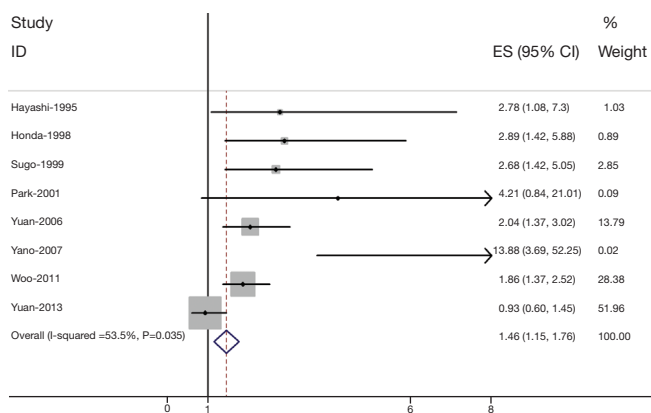
Figure 1 Meta-analysis (Forest plot) of the 8 evaluable studies assessing p53 mutation in hepatocellular carcinoma for overall survival.

Figure 2 Meta-analysis (Forest plot) of the 5 evaluable studies assessing p53 mutation in hepatocellular carcinoma for disease-free survival.
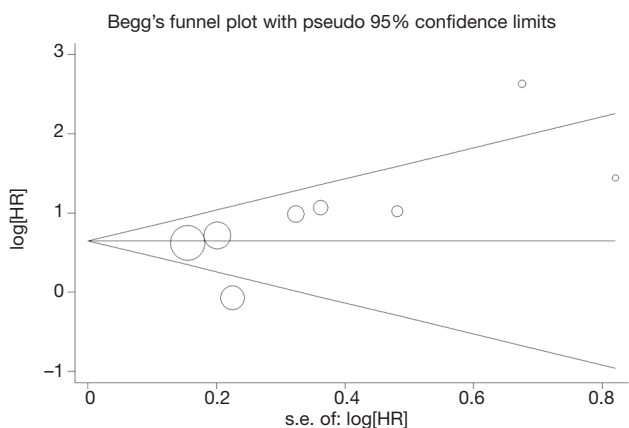
Figure 3 Funnel plot of the 8 evaluable studies assessing p53 mutation in hepatocellular carcinoma for overall survival.
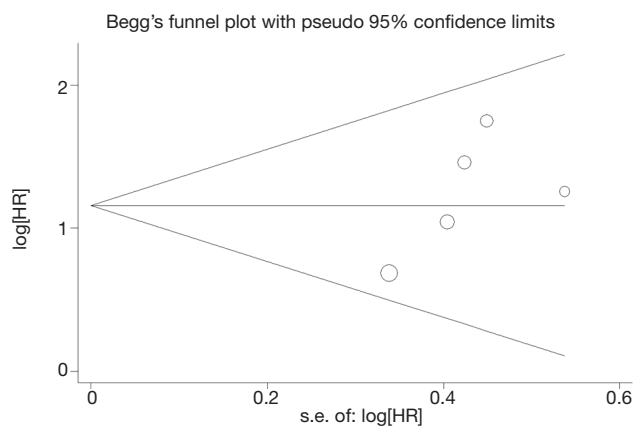
Figure 4 Funnel plot of the 5 evaluable studies assessing p53 mutation in hepatocellular carcinoma for disease-free survival.

mutation (a Begg's test score of $P=0.142$ and an Egger's test score of $P=0.191$) (*Figure 4*).

Discussion

Hepatocellular carcinoma has poor prognosis and high recurrence rate, regardless of the treatment. Therefore, it is imperative for clinicians and scientists to find new ways to stratify patients for appropriate treatment. Previous reports have attempted to build a model based on the prognostic value of putative hepatic stem cell biomarkers in HCC (27). Traditionally, however, tumour staging system (TNM and BCLC staging), tumour size and serum AFP levels are used to predict the outcome of HCC patients, which sometimes cannot accurately predict the outcome of all HCC patients (28). Till date, there is neither any

molecular marker routinely incorporated to staging systems, nor there is a molecular prognostic model. The present meta-analysis has combined 9 publications including 1,139 patients to yield statistics, indicating a statistically significant role of p53 mutation on overall survival and disease-free survival in HCC.

Our data were consistent with the results of a previous meta-analysis (29) published in 2011 that showed an association between p53 aberration and poor survival of patients with HCC. This analysis included only 7 studies. We have improved upon that previous meta-analysis by including more recent related studies and by generally using a more comprehensive search strategy. Screening, study selection and quality assessment were performed independently and reproducibly by two reviewers. We also explored heterogeneity and potential publication bias in

accordance with published guidelines.

The heterogeneity issue was complicated in the systematic review and meta-analysis was. We found no significant heterogeneity among all studies included and subgroup analysis. Another potential source of bias is related to the method of HR and 95% CI extrapolation. If these statistics were not reported by the authors, we calculated them from the data available in the article. If this was not possible, we extrapolated them from the survival curves, necessarily making assumptions about the censoring process. Data for multivariate survival analysis reported in the article were included in the present systematic review and meta-analysis; if these data were not available, data calculated from survival curves by univariate analysis were included. These results should be confirmed by an adequately designed prospective study. Furthermore, the exact value of p53 mutation status needs to be determined by appropriate multivariate analysis.

Publication bias (30) is a major concern for all forms of meta-analysis; positive results tend to be accepted by journals, while negative results are often rejected or not even submitted. The present analysis does not support publication bias; the obtained summary statistics likely approximate the actual average. However, it should be noted that our meta-analysis could not completely exclude biases. For example, the study was restricted to papers published in English and Chinese, which probably introduced bias.

In conclusion, our meta-analysis estimated the association between prognostic significance of p53 mutation and patients with HCC. As determined in our meta-analysis, we concluded that p53 mutation was associated with poor overall survival and disease-free survival. To strengthen our findings, well-designed prospective studies with better standardized assessment of prognostic markers should help to explore the relation between p53 mutation and survival of hepatocellular carcinoma.

Acknowledgements

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