Prognostic significance of TP53 expression for patients with hepatocellular carcinoma: a meta-analysis

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

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

Results: We performed a meta-analysis of 24 studies that evaluated the correlation between p53 expression and survival in patients with HCC. Combined hazard ratios (HRs) suggested that p53 expression had an unfavorable impact on overall survival (OS) [HR =1.64, 95% confidence interval (CI): 1.40-1.85], and disease free survival (DFS) (HR =1.57, 95% CI: 1.26-1.87) in patients with HCC.

Conclusions: p53 expression indicates a poor prognosis for patients with HCC.

Keywords: TP53; expression; hepatocellular carcinoma (HCC); prognosis; 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. Studies have focused on the potential role of new biological factors involved in the carcinogenic process as prognostic markers in patients with HCC.

Tumor 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 tumor differentiation, vascular invasion, tumor stage, Child-Pugh class and serum AFP in HCC (4-7).

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

Materials and methods

Search strategy and study selection

The electronic databases PubMed was searched for studies to include in the present meta-analysis. An upper date limit of Dec 30, 2013 was applied; we used no lower date limit. Searches included the terms "hepatocellular or liver", "cancer or carcinoma or tumor or neoplasm", "p53", "expression" 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 expression in the primary HCC; (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 expression 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 (32). The data extraction and quality assessment could refer to our previous published meta-analysis (33-36).

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 expression on survival by hazard ratio (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*. (37).

Heterogeneity of the individual HRs was calculated with χ^2 tests according to Yusuf *et al*.'s method (38). 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 (39) 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% 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*. (40) and of Begg *et al*. (41). 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, USA).

Results

Study selection and characteristics

Twenty-four studies (8-31) published between 1996 and 2012 were eligible for this meta-analysis. All reported the prognostic value of p53 expression status for survival in HCC patients. The total number of patients included was 2,585, ranging from 20 to 400 patients per study (median 107). The major characteristics of the 24 eligible publications are reported in *Table 1*.

All of the studies reported the prognostic value of p53 expression status for survival in patients with HCC tissue. Of the 24 studies, 18 directly reported HRs (multivariate analysis), while the other six studies provided survival curves. Among them, the proportion of patients exhibiting p53 overexpression in individual studies ranged from 16% to 43%. Estimation using survival curves were segregated

Table 1 Main characteristics and results of the eligible studies

First author [year] (references)	Patients source	N pts	Stage	Method	p53 mutation, %	HR estimation	HR (95% CI)
Srivastava [2012] (8)	Singapore	121	I-IV	IHC	30.0	PFS and OS	OS, 1.93 (1.03-3.63); PFS, 1.93 (1.03-3.63)
Zhang [2009] (9)	China	181	NA	IHC	71.0	OS	OS, 1.20 (0.86-1.68)
Yeh [2009] (10)	China	154	NA	IHC	16.0	PFS	PFS, 1.83 (1.29-2.59)
Anzola [2004] (11)	Spain	78	I-IV	IHC	23.0	PFS and OS	OS, 1.41 (0.69-2.90); PFS, 1.35 (0.62-2.97)
Chiu [2003] (12)	China	22	NA	IHC	68.0	OS	OS, 3.16 (1.29-7.76)
Endo [2000] (13)	Japan	107	NA	IHC	32.0	OS	OS, 2.25 (1.24-4.08)
Gianni [2005] (14)	Italy	91	I-IV	IHC	31.9	OS	OS, 2.31 (1.40-3.81)
Guo [2006] (15)	China	90	NA	IHC	33.0	PFS	PFS, 1.12 (0.64-1.97)
Guzman [2005] (16)	USA	20	NA	IHC	30.0	PFS	PFS, 11.7 (2.83-23.9)
Hu [2007] (17)	China	124	I-IV	IHC	42.0	PFS and OS	OS, 1.90 (1.17-3.09); PFS, 1.63 (1.07-2.49)
Kobayashi [2002] (18)	Japan	63	I-IV	IHC	42.9	PFS	PFS, 1.40 (0.78-2.53)
Liu [2009] (19)	China	400	NA	IHC	43.0	PFS	PFS, 2.67 (1.14-6.25)
Mise [1998] (20)	Japan	80	NA	IHC	23.0	PFS and OS	OS, 2.49 (1.26-4.94); PFS, 2.17 (0.97-4.86)
Naka [1998] (21)	Japan	126	I-IV	IHC	37.0	OS	OS, 1.97 (1.18-3.28)
Osada [2004] (22)	Japan	153	NA	IHC	41.7	OS	OS, 1.36 (0.93-1.99)
Qin [2002] (23)	China	244	NA	IHC	50.0	OS	OS, 1.73 (1.34-2.24)
Qin [2005] (24)	China	47	II-IV	IHC	38.3	OS	OS, 1.34 (0.68-2.65)
Schöniger-Hekele [2005] (25)	Austria	81	I-IV	IHC	43.0	OS	OS,1.76 (1.10-2.86)
Soini [1996] (26)	Finland	33	I-IV	IHC	23.0	OS	OS, 1.62 (0.61-4.30)
Stroescu [2008] (27)	Romania	47	NA	IHC	32.0	PFS and OS	OS, 5.49 (2.33-12.96); PFS, 4.41 (1.87-10.4)
Sung [2005] (28)	Korea	105	NA	IHC	19.0	PFS and OS	OS, 1.62 (0.71-3.68); PFS, 1.35 (0.63-2.89)
Terris [1997] (29)	France	113	NA	IHC	22.0	OS	OS, 1.34 (0.43-4.13)
Umemura [2008] (30)	Japan	43	I-IV	IHC	30.0	OS	OS, 1.18 (0.55-2.54)
Wu [1999] (31)	China	62	NA	IHC	53.0	OS	OS, 1.34 (0.83-2.17)

Abbreviations: NA, not applicable; HR, hazard ratio; CI, confidence interval; OS, overall survival; PFS, progress-free survival.

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	19	1.64 (1.40-1.85)	0.920
Overall for DFS	11	1.57 (1.26-1.87)	0.490

Abbreviations: HR, hazard ratio; Nb, number of studies; OS, overall survival; DFS, disease-free survival.

according to either OS or DFS. A HR on DFS and OS could be extracted for 11 publications and 19 publications of studies, respectively.

Meta-analysis

The results of the meta-analysis were shown in *Table 2* and *Figures 1,2*. Overall, the combined HR for all 19 eligible
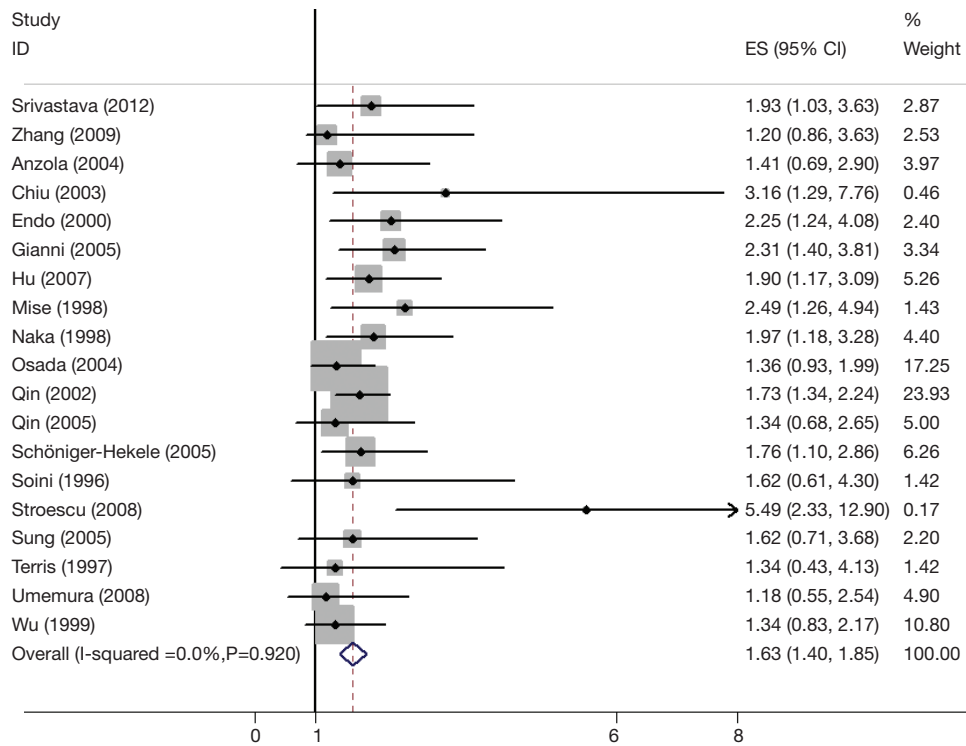
Figure 1 Meta-analysis (Forest plot) of the 19 evaluable studies assessing p53 expression in hepatocellular carcinoma for overall survival.
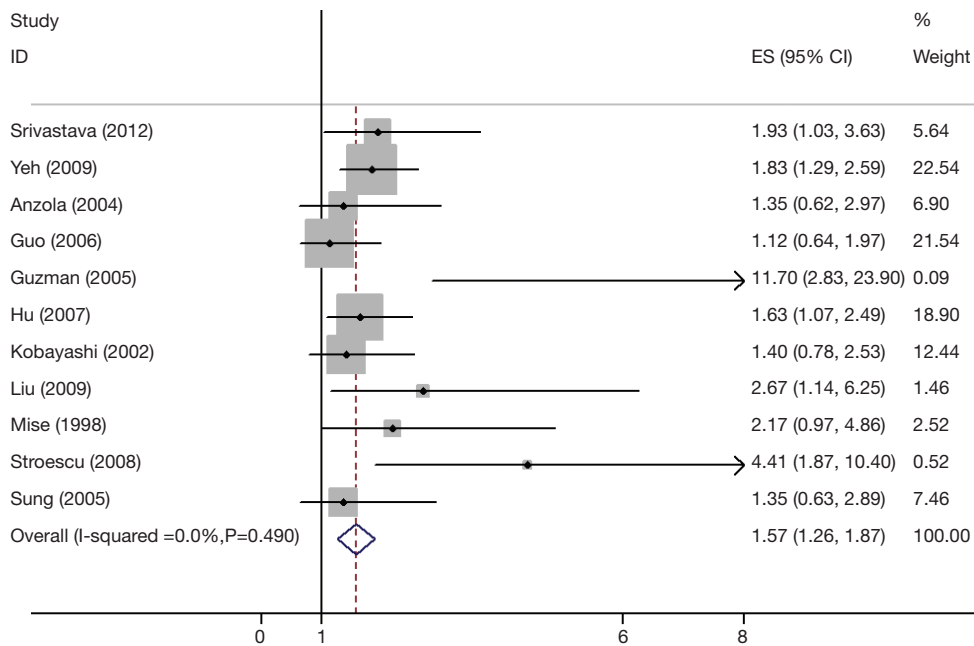

Figure 2 Meta-analysis (Forest plot) of the 11 evaluable studies assessing p53 expression in hepatocellular carcinoma for disease-free survival.
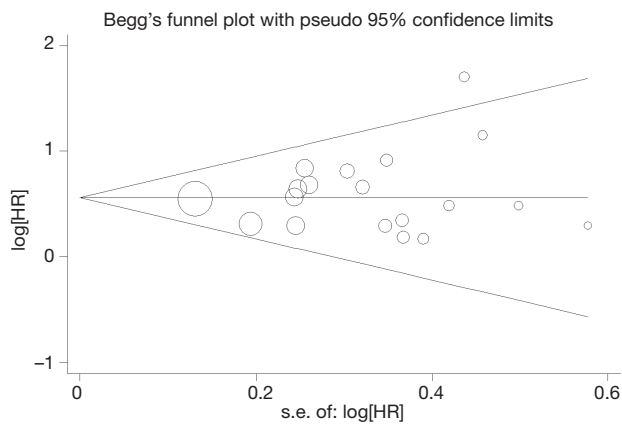

Figure 3 Funnel plot of the 19 evaluable studies assessing p53 expression in hepatocellular carcinoma for overall survival.

Figure 4 Funnel plot of the 11 evaluable studies assessing p53 expression in hepatocellular carcinoma for disease-free survival.

studies evaluating p53 overexpression on OS was 1.64 (95% CI: 1.40-1.85), suggesting that p53 overexpression was an indicator of poor prognosis for HCC. No significant heterogeneity was observed among the studies (Q=2.24, I^2=0.0%, P=0.920). Meanwhile, for DFS analysis including 11 studies, statistically significant effect of p53 overexpression (HR =1.57, 95% CI: 1.26-1.87) in patients with HCC was also observed (Q=5.27, I^2=0.0%, P=0.490).

Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias in the literature. All 19 eligible studies investigating p53 overexpression on OS yielded a Begg's test score of P=0.105 and an Egger's test score of P=0.262, 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 overexpression of 11 studies [a Begg's test score of P=0.208 and an Egger's test score of P=0.148 (*Figure 4*)].

Discussion

HCC 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 (42). Traditionally, however, tumor staging system (TNM and BCLC staging), tumor size and serum AFP levels are used to predict the outcome of HCC patients, which sometimes cannot
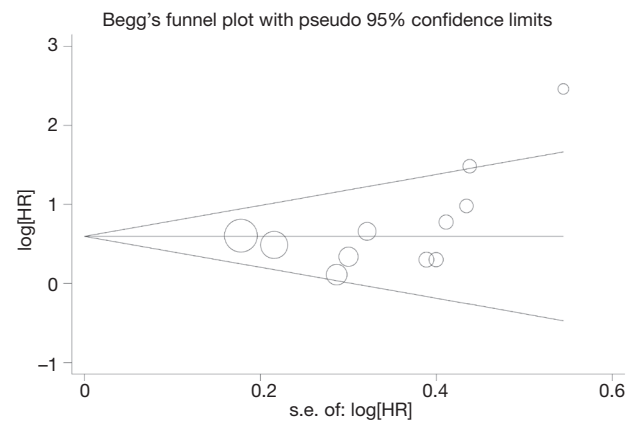
accurately predict the outcome of all HCC patients (43). Up to date, there is neither any molecular marker routinely incorporated to staging systems, nor there is a molecular prognostic model. The present meta-analysis has combined 24 publications including 2,585 patients to yield statistics, indicating a statistically significant role of p53 overexpression on OS and DFS in HCC.

Our data were consistent with the results of a previous meta-analysis (44) published in 2011 that showed an association between p53 aberration and poor survival of patients with HCC. 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 expression needs to be determined by appropriate multivariate analysis.

Publication bias (45) 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 expression and patients with HCC. As determined in our meta-analysis, we concluded that p53 expression was associated with poor OS and DFS.

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