

Review Article

Prognostic significance of Golgi protein 73 in hepatocellular carcinoma after hepatectomy in the Chinese population: a meta-analysis

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Abstract: The Golgi protein 73 (GP73) has been shown to play an important role in tumor initiation and progression in multiple cancers. However, its prognostic value, specifically in hepatocellular carcinoma (HCC) patients treated with hepatectomy, remains controversial. Thus, in this meta-analysis study, we have tried to determine if GP73 overexpression has any prognostic value for Chinese HCC patients. In order to investigate this relationship, we have evaluated GP73 association with overall survival (OS), disease-free survival (DFS), tumor invasion and other invasion-related clinicopathological features, based on the information derived from eight different studies that included data of 1187 HCC patients. These studies were identified by searching Embase, PubMed, Web of Science and Cochrane Library databases, until Oct 1, 2015. The hazard ratio (HR) or odds ratio (OR) with corresponding 95% confidence intervals (95% CIs) were calculated from these studies using random-effects model. Our results from the meta-analysis revealed that higher GP73 expression correlated with poor OS [HR 1.958, 95% CI 1.548 to 2.368, $I^2=0.0\%$] and DFS [HR 1.775, 95% CI 1.416 to 2.133, $I^2=0.0\%$] of HCC patients. The aberrant GP73 expression also correlated with tumor invasion [OR 1.286, 95% CI 0.618 to 2.679, $I^2=82.7\%$], advanced TNM stage [OR 10.084, 95% CI 1.884 to 53.976, $I^2=93.0\%$] and advanced tumor grade [OR 7.912, 95% CI 5.492 to 11.399, $I^2=26.0\%$]. Overall, these findings led us to conclude that GP73 overexpression indicate a poor clinical outcome in HCC patients after hepatectomy and may have a predictive value for HCC invasion and metastasis.

Keywords: Hepatocellular carcinoma, Golgi protein 73, prognosis, meta-analysis, Chinese patient population

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the second largest contributor toward cancer-related deaths [1]. In China alone, HCC accounted for more than 50% of the total cancer cases [1]. According to the estimates of the National Central Cancer Registry (NCCR) of China, there were 355,595 new liver cancer cases and 322,416 deaths in 2011. The liver cancer incidence and mortality were 26.39/100,000 and 23.93/100,000, respectively, which make HCC the third most common cancer and the second leading cause of death in China [2]. Due to improved diagnostic and surgical techniques, more HCC patients undergo curative surgery at

an early stage; however, the overall prognosis of HCC patients remains disappointing due to its aggressive behaviors and higher recurrence rate [3]. Thus, an early prediction of the probability of recurrence following curative resection is critical for the management of HCC; and thus there is an urgent need to identify new predictive tumor markers.

GP73, a Golgi apparatus-associated protein, is an oncoprotein that promotes the malignant transformation of primary cell lines, tumor growth, migration and metastasis [4, 5]. Previous studies reported that GP73 overexpression not only enhanced tumorigenesis of various cancers but also correlated with poor survival rates [6-9]. In 2005, Marrero *et al.*

identified Golgi protein 73 (GP73) as a novel HCC serum marker. The GP73 mRNA was first identified during an analysis of upregulated hepatic genes in a patient with syncytial giant cell hepatitis [10]. Subsequent studies suggested GP73 to be an accurate serum marker for the detection of not only HCC directly but also the chances of recurrence after surgery, with higher sensitivity and specificity. The levels of GP73 usually decrease following surgical resection of HCC lesions but increase with tumor recurrence [11]. This study specifically identified a positive correlation between the level of GP73 expression and the prognosis of HCC patients, suggesting its role as a prognostic marker for HCC.

Prognostic biomarkers provide essential information to facilitate and help clinicians in deciding on further treatment strategies. These biomarkers also hold great promise for enhancing the effectiveness of cancer therapies. During HCC, these prognostic markers can help balance the threshold of determining if patients need further treatment with curative hepatectomy alone or insufficient curative treatment.

There have been many recent additional studies analyzing the relationship between GP73 overexpression and HCC progression, metastasis, and prognosis [12-22]. Despite numerous studies pointing towards the prognostic relevance of GP73 in patients with HCC who undergo curative hepatectomy [12-17], confusion remains regarding its role due to the presence of heterogeneous and conflicting results [23-25]. Additionally, a study by Sun *et al.* suggested that an increased expression of GP73 in HCC tissue correlates with tumor aggression and patient survival [26]. Moreover, few of the published studies that evaluated the role of GP73 in HCC had small study populations and thus their conclusion may not be very predictive. Also, there has been no exclusive effort thus far to study the correlation between GP73 expression and HCC in a Chinese population, which has one of the largest incidence rates. We undertook this meta-analysis to address these limitations.

In this paper, by pooling results from published studies that included Chinese HCC patients who underwent hepatectomy with curative intent, we attempted to make an objective assessment of the prognostic significance of

elevated tissue levels of GP73 for vascular invasion, tumor grade, clinical stage, overall survival and disease-free survival. Additionally, we hope that our study could shed some light by providing useful information on the unclear association between GP73 and HCC in a Chinese population.

Material and methods

Identification of relevant articles

All relevant studies were identified by searching the Embase, PubMed, Web of Science and Cochrane Library databases up to October 1, 2015. The studies were searched for using the following Medical Subject Headings (MESH) and/or text words: 'GP73', 'Golgi protein 73', 'GOLM1', 'golp2', 'HCC', 'carcinoma, hepatocellular'. The reference lists of the retrieved articles were also searched to identify additional studies. Studies in all languages were reviewed. In studies in which key information relevant to the meta-analysis was missing, the investigators were contacted to supply additional data. If an eligible article provided the data from different populations, we treated each population as a separate study.

Inclusion and exclusion criteria

We included studies in our meta-analysis based on the following criteria: (1) cohort or case-control design, (2) studies on human beings, (3) studies analyzing GP73 expression in the HCC using immunohistochemistry (IHC), (4) patients underwent hepatectomy with curative intent, (5) information on survival was provided, (6) a maximum follow-up time exceeding 2 years, and (7) studies conducted on a Chinese population. If serial studies of the same population from the same group were reported, the latest study only was included. When a study reported results on different subpopulations based on geographical region or ethnicity, we treated each subpopulation as a separate comparison. Reports or articles from non-peer reviewed sources and studies investigating only response rates were excluded.

Data extraction

Each article was reviewed by two independent reviewers (Yang L and Jiang XH), who analyzed the articles based on the inclusion criteria, discussed any discrepancies, and reached a con-

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Table 1. Newcastle-Ottawa quality assessment scale

Selection

- Representativeness of the exposed cohort
 - Truly representative of the average HCC patients who underwent hepatectomy in the community*
 - Somewhat representative of the average HCC patients who underwent hepatectomy in the community*
 - Selected group of users, e.g., nurses, volunteers
 - No description of the derivation of the cohort
- Selection of the non-exposed cohort
 - Drawn from the same community as the exposed cohort*
 - Drawn from a different source
 - No description of the derivation of the non-exposed cohort
- Ascertainment of exposure
 - Secure record (e.g., surgical records)*
 - Structured interview*
 - Written self report
 - No description
- Demonstration that outcome of interest was not present at start of study
 - Yes*
 - No

Comparability

- Comparability of cohorts on the basis of the design or analysis
 - Study controls for metastasis or recurrence*
 - Study controls for any additional factor (tumor grade, clinical stage, vascular invasion, etc.)*

Outcome

- Assessment of outcome
 - Independent blind assessment*
 - Record linkage*
 - Self report
 - No description
- Was follow-up long enough for outcomes to occur?
 - Yes (2 years)*
 - No
- Adequacy of follow up of cohorts
 - Complete follow up - all subjects accounted for*
 - Subjects lost to follow up unlikely to introduce bias- small number lost- (25%) follow up, or description provided of those lost*
 - Follow up rate (<75%) and no description of those lost
 - No statement

A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability. *Identify 'high' quality choices with a star.

sensus to extract the following information from each included study: (1) first author, publication year and geographical areas of the study origin, (2) study design and population characteristics, (3) overall survival, (4) disease-free survival, and (5) tumor vascular invasion, Edmondson-Steiner grade and tumor-node-metastasis (TNM) stage.

Quality assessment

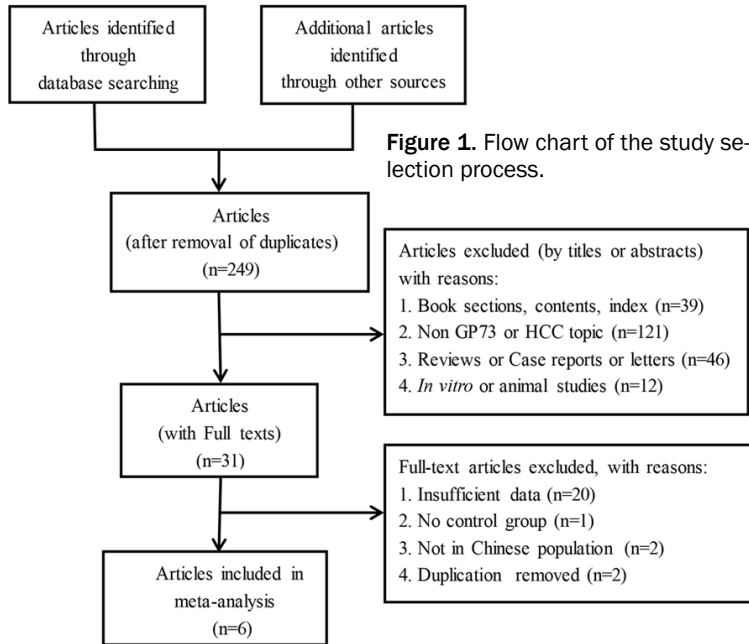
The quality of each study was assessed by the same two reviewers (Yang L and Jiang XH) according to the Newcastle-Ottawa quality

assessment scale (NOS), which enabled the assessment of patient population and selection criteria, comparison between studies, follow up, and outcome of interest from cohort studies. A score of 0 and 9 correlated with the lowest and highest quality, respectively, and studies with scores of 6 or more were graded as the high quality studies based on the scale (**Table 1**).

Statistical analysis

Hazard ratios (HR) and Odds ratio (OR) were used to analyze the quantitative aggregation of

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the survival results and clinicopathological parameters, respectively. If the HR and its variance were not provided directly in the included study, we calculated these values from the available data using the method designed by Tierney [27]. According to the assumptions of inter-study heterogeneity, the random-effects model was applied because it is more conservative due to a wider CI around the pooled HR or OR than the fixed effect analysis model [28]. Summary HR or OR estimates with corresponding 95% confidence intervals (CIs) were calculated with the method devised by DerSimonian and Laird [28]. The results were considered statistically significant if the *P* value was <0.05. The heterogeneity between studies was measured using an I-squared (I^2) statistic ($I^2 < 25\%$, no heterogeneity; $25\% < I^2 \leq 50\%$, moderate heterogeneity; and $I^2 > 50\%$, strong heterogeneity) [29].

Additionally, subgroup analyses were performed to investigate the potential sources of heterogeneity. Subgroup analyses were stratified based on the 1) geographical areas of the study origin (North versus South) and 2) sample size (≥ 170 versus < 170). When the compared subgroups included three studies, a test of interaction was carried out to assess the risk of bias for the individual studies [30]. Furthermore, a sensitivity analysis was performed by excluding studies that had the highest weight, the highest

or lowest estimates, or the largest sample size. Meta-regression analyses could not be performed due to the small number of included studies.

Publication bias was assessed by both Egger's test and Begg's funnel plot [31, 32]. A meta-analysis was performed using STATA, version 11.0 (STATA, College Station, Texas, USA) software.

Results

Eligible studies

A total of 374 potentially relevant articles were initially identified from the database search based on the search criteria. Among these articles, 125 were

duplicates and therefore excluded. After reviewing the titles and abstracts of the remaining 249 articles by two independent authors, 218 were excluded due to their obvious irrelevance with the aims of our study. Based on the inclusion criteria, an additional 25 articles were further excluded after reviewing and evaluating the full texts. Among these articles, 4 did not have relevant survival data, whereas another 4 had overlapping data sets. One article had a very small sample size and other 7 articles did not have significant information. Additionally, three other articles detected GP73 expression in the serum and thus did not match our inclusion criteria, whereas one other article lacked a control group. In another article, the HCC patients did not undergo hepatectomy, whereas the remaining two articles reported on duplicate cohorts. The last two articles that were excluded were not on a Chinese population. Thus, finally, 6 eligible articles [12-17] consisting of 8 different studies were included in our meta-analysis. The complete process of study selection is summarized in **Figure 1**.

Characteristics of included studies

The individual characteristics of each study are listed in **Table 1**. The included studies were published between the years 2013 and 2015 and contained a total of 1,187 HCC patients (median sample size: 173 [62-217]; mean:

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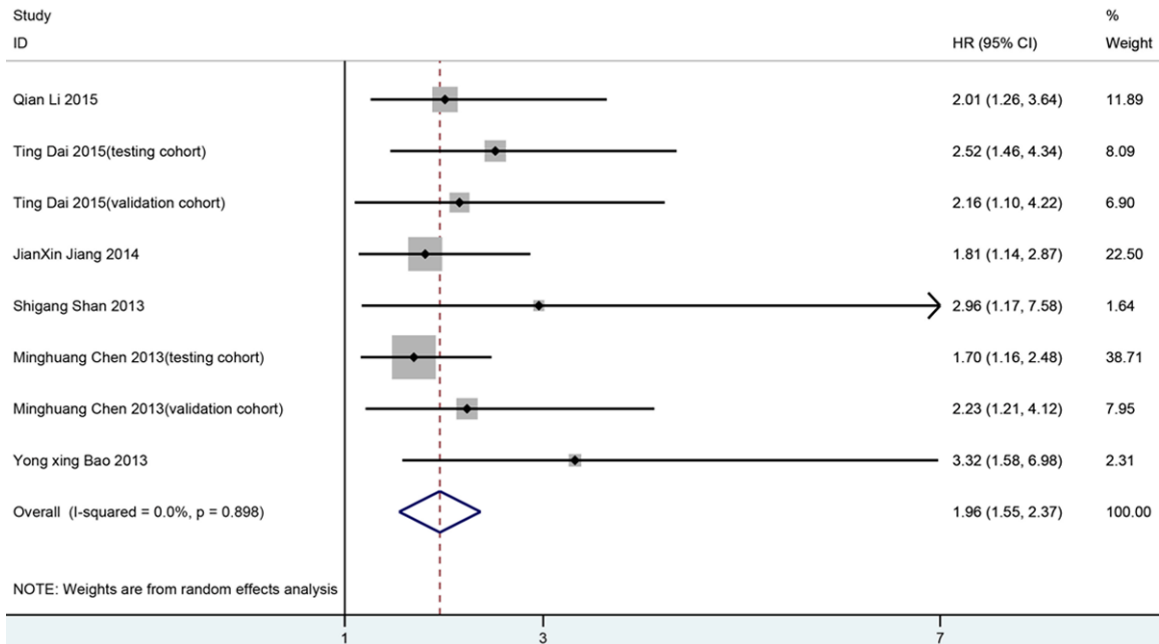


Figure 2. Meta-analysis of the association between GP73 overexpression and HCC patients overall survival.

148). These studies were carried out in the provinces of Guangdong, Taiwan (2), Shandong (1), Guizhou (1), Tianjin (1) and Sichuan (1), and all of the studies investigated the expression of GP73 using immunohistochemistry (IHC) analysis. However, each study used different antibody types. Seven studies used the polyclonal antibody, whereas one trial used the monoclonal. Each individual study reported a correlation between a high GP73 expression and patient survival; however, the method used for setting a cut-off value to determine high GP73 differed for each study. All eight studies reported data on OS, whereas only 4 studies had data on DFS.

Correlation analysis between GP73 expression and OS in HCC patients

All eight included studies reported data on GP73 expression and OS in HCC patients. An analysis of the combined data from all eight studies showed that increased GP73 levels were significantly correlated with poor OS, and the pooled HR estimate was 1.958 [95% confidence interval (CI): 1.548-2.368, $P=0.000$; **Figure 2**]. No heterogeneity was observed between these studies ($\chi^2=2.58$, $I^2=0.0\%$, $P=0.898$). Additionally, we also performed a subgroup analysis based on the geographical location of the study and sample size. The

effect of the geographical location on the correlation between GP73 expression and OS appeared to be larger in studies from the North (pooled HR 2.299, 95% CI 1.268 to 3.330, $P=0.626$, 3 studies, $I^2=0.0\%$) than those from the South (HR 1.894, 95% CI 1.447 to 2.341, $P=0.842$, 5 studies, $I^2=0.0\%$, [Supplementary Figure 1A](#)). However, the difference between these two geographical locations was not statistically significant, as a risk of bias was not observed (P for interaction =0.001). Moreover, due to a lack of any heterogeneity between these studies, no sensitivity analyses were undertaken. Interestingly, a similar trend was observed between GP73 expression and OS when a subgroup analysis was performed based on the sample size (as observed in [Supplementary Figure 1B](#)).

Correlation analysis between GP73 expression and DFS in HCC patients

Only four studies reported data on GP73 expression and DFS in HCC patients [14-16]. As a result of our meta-analysis, we observed that high GP73 expression was significantly correlated with poor DFS; the pooled HR estimate was 1.775 (95% CI: 1.416-2.133, $P=0.000$; **Figure 3**). There was no significant heterogeneity between these four studies ($\chi^2=0.40$, $I^2=0.0\%$, $P=0.939$). Based on the subgroup

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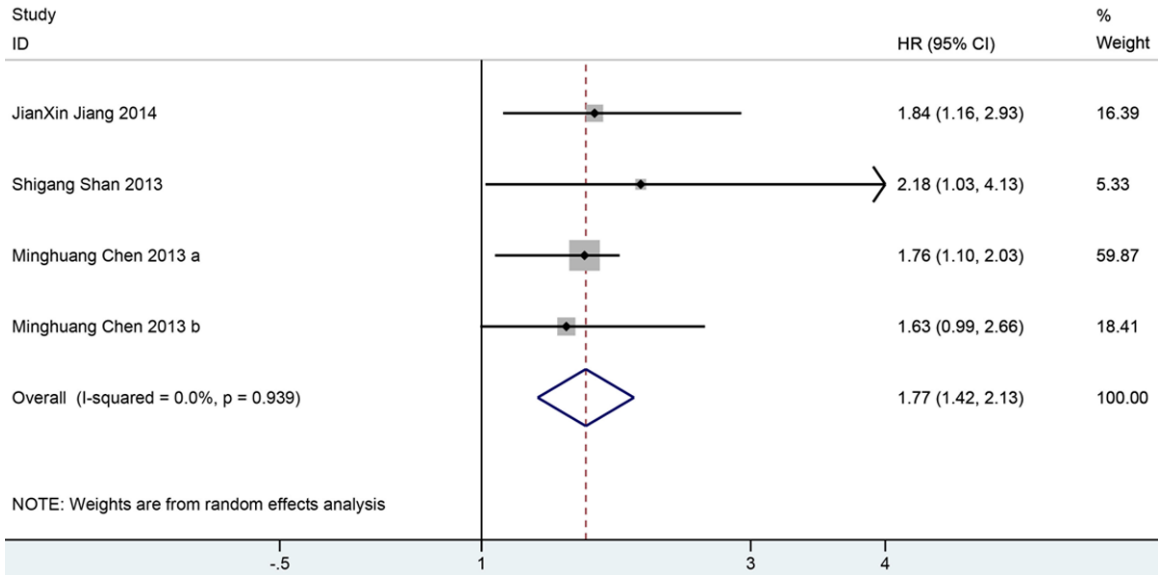


Figure 3. Meta-analysis of the association between GP73 overexpression and disease-free survival (DFS) of HCC patients.

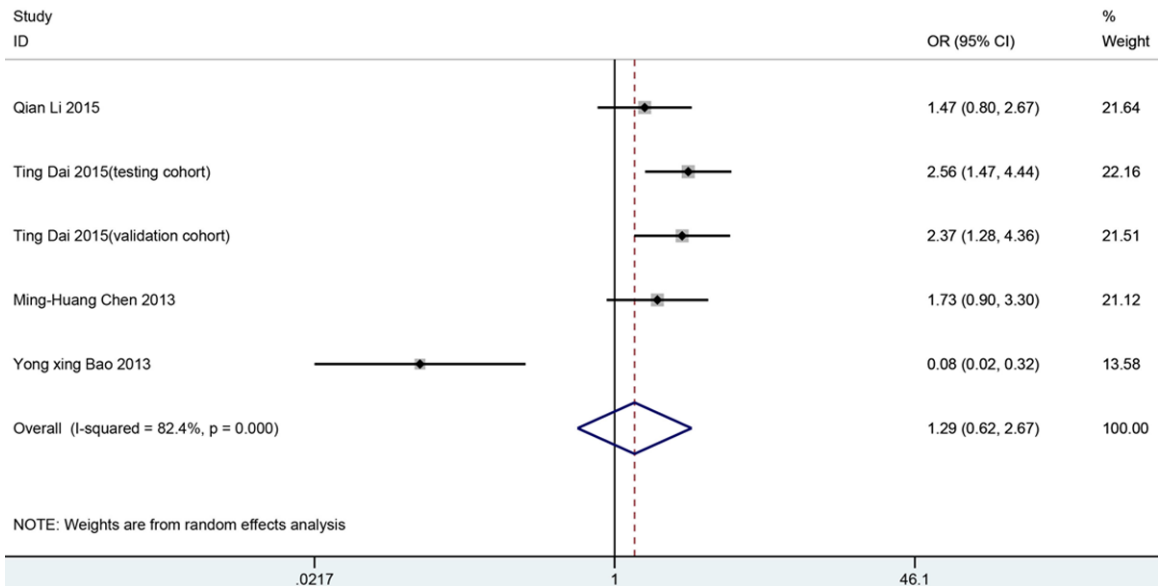


Figure 4. Meta-analysis of the association between GP73 overexpression and tumor vascular invasion in HCC patients.

analysis, the three studies from the south still showed an association between GP73 and DFS with a similar pooled HR value (1.75 (95% CI: 1.38-2.12)) as observed above (Supplementary Figure 1C). However, a subgroup analysis of the studies from the North was not performed because there was only one study in this subgroup. Hence, a test of interaction was also not carried out to assess the risk of bias for individual studies. Similar findings were observed

for GP73 expression and DFS in a subgroup analysis of HCC patients based on sample size, as shown in Supplementary Figure 1D.

Association between GP73 expression and tumor vascular invasion in HCC patients

Next, we assessed the association between GP73 expression and tumor vascular invasion in HCC patients based on five studies with rel-

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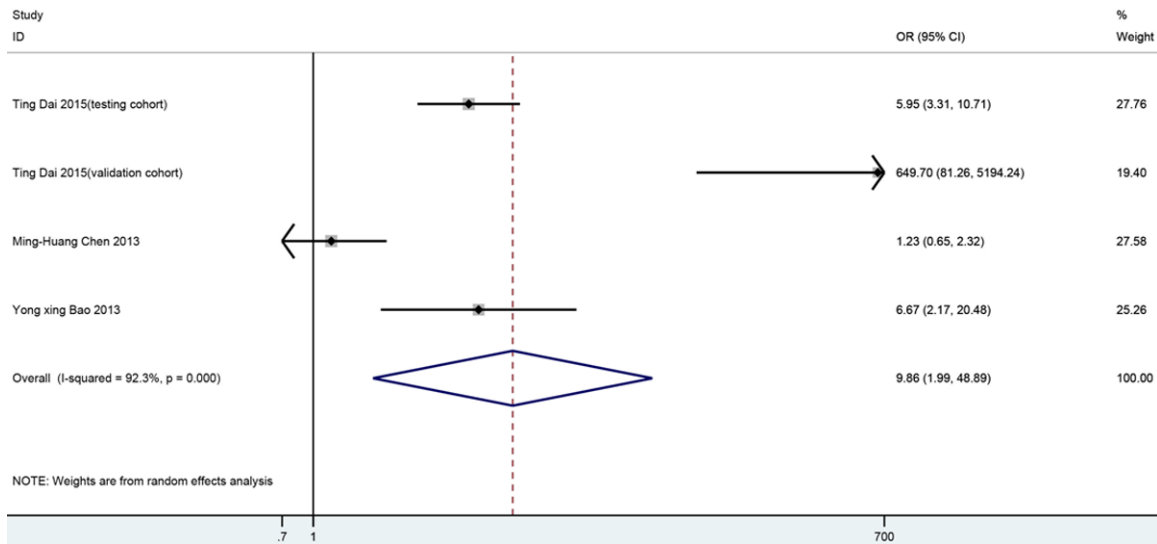


Figure 5. Meta-analysis of the association between GP73 overexpression and TNM stage of HCC patients.

evant data [12, 13, 16, 17]. The meta-analysis showed no statistically significant association between GP73 overexpression and the presence of vascular invasion (OR: 1.286, 95% CI: 0.618-2.679, $P=0.501$; **Figure 4**). Heterogeneity was evident ($\chi^2=23.7$, $I^2=82.7\%$, $P=0.000$). Notably, we observed a trend of association between high GP73 expression and vascular invasion (although only four studies were included in the analysis). However, the analysis based on two studies only showed a significant association. Thus, we performed sensitivity analyses by restricting the eligible criteria and observed that the overall analysis was driven by the individual study by Bao *et al.* [17]. This study had an OR value of <1 and was of low quality. The removal of this particular study resulted in an OR value of 2.006 (95% CI 1.487 to 2.706, $P=0.001$; **Supplementary Figure 2A**) with no significant heterogeneity ($\chi^2=2.28$, $I^2=0.0\%$, $P=0.516$).

Association of GP73 expression with tumor TNM stage in HCC patients

The association between GP73 expression and tumor TNM stage was analyzed based on four studies [13, 16, 17]. Our meta-analysis suggested that a high level of GP73 expression was significantly associated with advanced tumor stage (OR: 10.084, 95% CI: 1.884-53.976, $P=0.007$; **Figure 5**). However, there was significant heterogeneity between these studies ($\chi^2=42.8$, $I^2=93.0\%$, $P=0.000$). A sensi-

tivity analysis excluding the studies with the highest [13] and lowest [16] OR estimates resulted in an overall OR value of 6.099 (95% CI: 3.625-10.262, $P=0.001$; **Supplementary Figure 2B**) with very little heterogeneity ($\chi^2=0.03$, $I^2=0.0\%$, $P=0.861$). Moreover, due to the presence of a single study each from the North region and of a <170 sample size, a test of interaction between different subgroups was not performed. However, the effect size was similar (P for interaction = 0.001, respectively; **Supplementary Figure 3A, 3B**).

Association between GP73 expression and Edmondson-Steiner grade in HCC patients

The association between GP73 expression and Edmondson-Steiner grade in HCC patients was based on the data from four studies [12, 13, 17]. The combined data from these studies revealed that high GP73 expression was significantly associated with a negative effect on high tumor grade (III+IV) (OR: 7.912, 95% CI: 5.492-11.399, $P=0.000$; **Figure 6**), and low to moderate or no heterogeneity was observed across the studies ($\chi^2=4.06$, $I^2=26.0\%$, $P=0.255$). Additionally, the effect of geographical location on advanced tumor grade appeared to be reduced among studies from the North (OR 2.131, 95% CI 1.633 to 2.783, $P=0.092$, $I^2=64.8\%$) than those from the South (OR 2.966, 95% CI 2.272 to 3.873, $P=0.621$, $I^2=0.0\%$, **Supplementary Figure 3C**). However, this difference was not statistically significant

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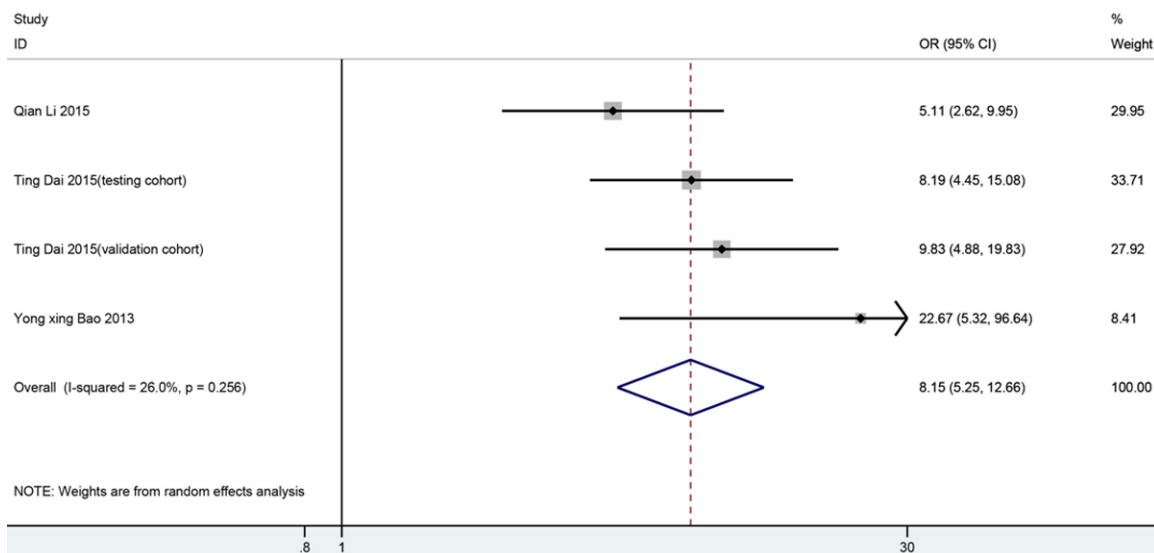


Figure 6. Meta-analysis of the association between GP73 overexpression and Edmondson-Steiner grade of HCC patients.

(P for interaction = 0.001). Similarly, the same trend was observed in the subset of studies analyzed based on the sample size ([Supplementary Figure 3D](#)).

Publication bias

Finally, we assessed the publication bias and observed no bias in the studies analyzing the correlation between GP73 expression and DFS using Begg's and Egger's plot analysis ([Supplementary Figure 4C, 4D](#)) ($P=0.461$), whereas an OS analysis showed some publication bias ($P=0.001$) ([Supplementary Figure 4A, 4B](#)).

Discussion

HCC, a disease of multi-factorial etiology, presents many surveillance and management challenges. The survival rates for HCC are generally poor, as only 10-20% of patients show complete recovery after the surgical resection of primary tumors. In other cases, the disease usually leads to mortality within 3 to 6 months [33], partially due to metastasis and recurrence. Thus, due to the lack of definitive biomarkers to diagnose the recurrence of HCC after surgical resection [34], we specifically analyzed the utility of GP73 as a tentative marker for HCC tumor progression, invasion and recurrence in the Chinese population.

To our knowledge, this is the first comprehensive meta-analysis to evaluate the correlation

between a high GP73 expression with OS and DFS in Chinese HCC patients who received surgical treatments. We identified that HCC patients in a Chinese population have high GP73 expression and a shorter OS and DFS than those with low GP73 expression. This resulted in poor patient outcomes. Further subgroup analyses stratified based on the geographical location and sample size also showed a similar pattern for the correlation between high GP73 expression and OS and DFS in HCC patients. These results were consistent with previous published studies [14-16] and also with the data of the National Central Cancer Registry (NCCR) of China, who reported that the survival rate of patients with liver cancer was generally low in both developed and developing regions [2]. However, the reasons for the differences in OS and DFS among HCC patients with high and low GP73 expression remain unclear. Many factors usually correlate with poor HCC prognosis, such as tumor invasion and metastasis [4, 5], and interestingly our study also showed that overall high GP73 expression correlated with increased vascular invasion after the exclusion of one study (Bao, Cao *et al.* 2013). Consistent with this observation, Jin *et al.* [35] demonstrated that GP73 promoted cell invasion by enhancing CREB-MMP-13 expression, which eventually potentiates HCC cell metastasis. Another study also indicated that GP73 may play a critical role in the regulation of HCC aggressiveness and angiogenesis by activating the NF- κ B signaling

pathway, an activity that could be responsible, at least partially, for the development and/or progression of human HCC [13].

Further, we also analyzed the association between high GP73 expression and other invasion-related parameters (such as TNM stage and Edmondson-Steiner grade). As expected, the results suggested a trend toward higher GP73 expression in late TNM stage HCC patients. However, there was a considerable heterogeneity across studies and the sensitivity analysis after excluding studies with the highest [13] or lowest [16] estimates, which strengthened this association. Moreover, we also observed substantial heterogeneity when the subgroup analysis was performed based on the geographical locations and sample size. None of the studies had a definitive explanation for the heterogeneity, but the differences in the baseline characteristics of the included patient population can contribute towards such a large heterogeneity. We also noticed an obvious association between high GP73 expression and advanced Edmondson-Steiner grade.

Importantly, this study found considerable heterogeneity when some parameters were analyzed; however, this may not conflict with the overall observation of GP73 being a potential indicator to predict patient prognosis. The potential explanation for higher heterogeneity is the variability in the use of IHC methods to analyze GP73 expression across the included studies. Each study used different antibodies, different antibody dilutions and variable scoring systems for immunopositivity. All of these variables made the interpretation difficult because the patients with the some values of immunopositivity may be classified to have high GP73 expression in some studies but low in others. Therefore, the adoption of a universal and consensus cut-off value for analyzing GP73 expression could facilitate the replication of the results from different studies. This indicated the need for a clear protocol for analyzing GP73 expression to develop and validate its use as a clinical biomarker.

Furthermore, despite our comprehensive evaluation of the correlation between high GP73 expression and outcomes in HCC patients receiving surgical treatments, there were some limitations to our systematic review and meta-analysis. First is the presence of a tentative

potential risk bias in this systematic review. As is well known, positive results were more acceptable by journals than negative ones, which were often rejected or were not even submitted for review. Herein, attempted to identify potential sources of bias and heterogeneity by carrying out several sensitivity and subgroup analyses; however, we were still unable to conduct sufficient analyses due to the lack of relevant information. Additionally, a meta-regression could not be performed due to the small number of included studies, which may impact the validity of our analysis. Our publication bias estimate using both Begger's plot and Egger's test further showed the presence of publication bias in studies analyzing high GP73 expression and its association with OS. Second, as a retrospective study, our meta-analysis only focused on the summary of published data from previous cohort studies, which were difficult to control for confounders. Several studies did not provide HRs, which we estimated using their reported data; this may have influenced the authentic prognostic value of GP73 overexpression in HCC. Third, all of the studies included only patients from surgical series and had information regarding tissue-based GP73 levels only, which can potentially limit the wide implications of our results. The more advanced stages in HCC patients do not permit curative treatment; thus, we were unable to assess this population of patients. Additionally, the measurement of serum GP73 as an indirect marker of the tissue levels, which can be easily measured before and after surgical treatment, poses a question in regard to whether serum-based GP73 levels were superior to tissue-based GP73 expression in predicting HCC invasion and prognosis. This remains to be investigated in future studies. Moreover, our analysis was based on data from Chinese patients, a majority of which had hepatitis B virus-related HCC. In contrast, hepatitis C virus-related or alcohol-related HCC is the predominant cancer type in Western countries. Thus, our results may have limitations in terms of universal application and cannot be extended to patients from Western countries.

In summary, our study concluded that high GP73 expression is a clinically important prognostic marker that has negative prognostic impact on Chinese HCC patients. Moreover, our study indicates a need for the adoption of a

standardized detection technique to assess GP73 expression in large patient cohorts to reduce high heterogeneity. Further clinical trials are required to prospectively assess the value of a serum-based GP73 level as a simple method to monitor prognosis as well as responses to systemic therapy and tumor progression.

Acknowledgements

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Disclosure of conflict of interest

None.

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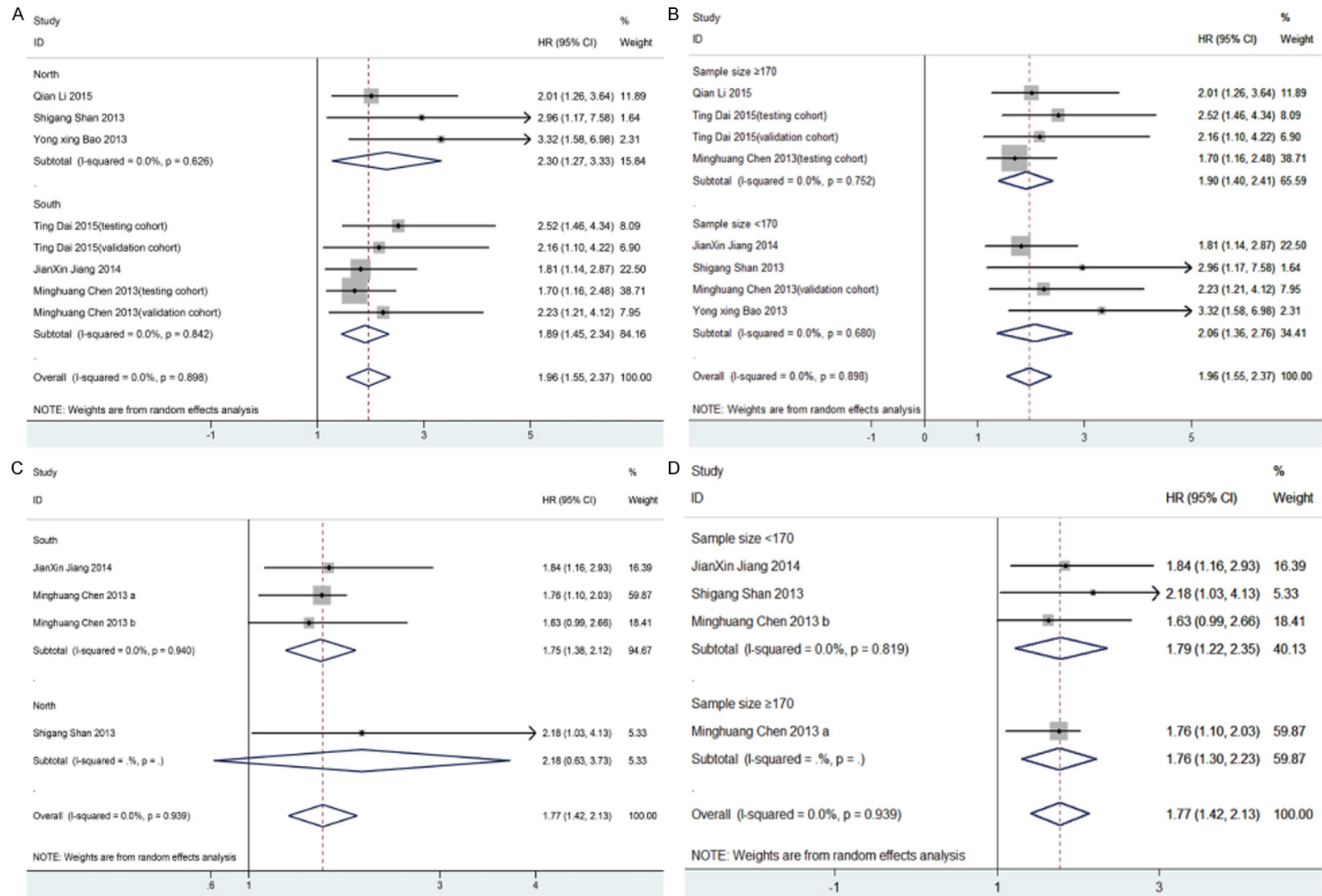
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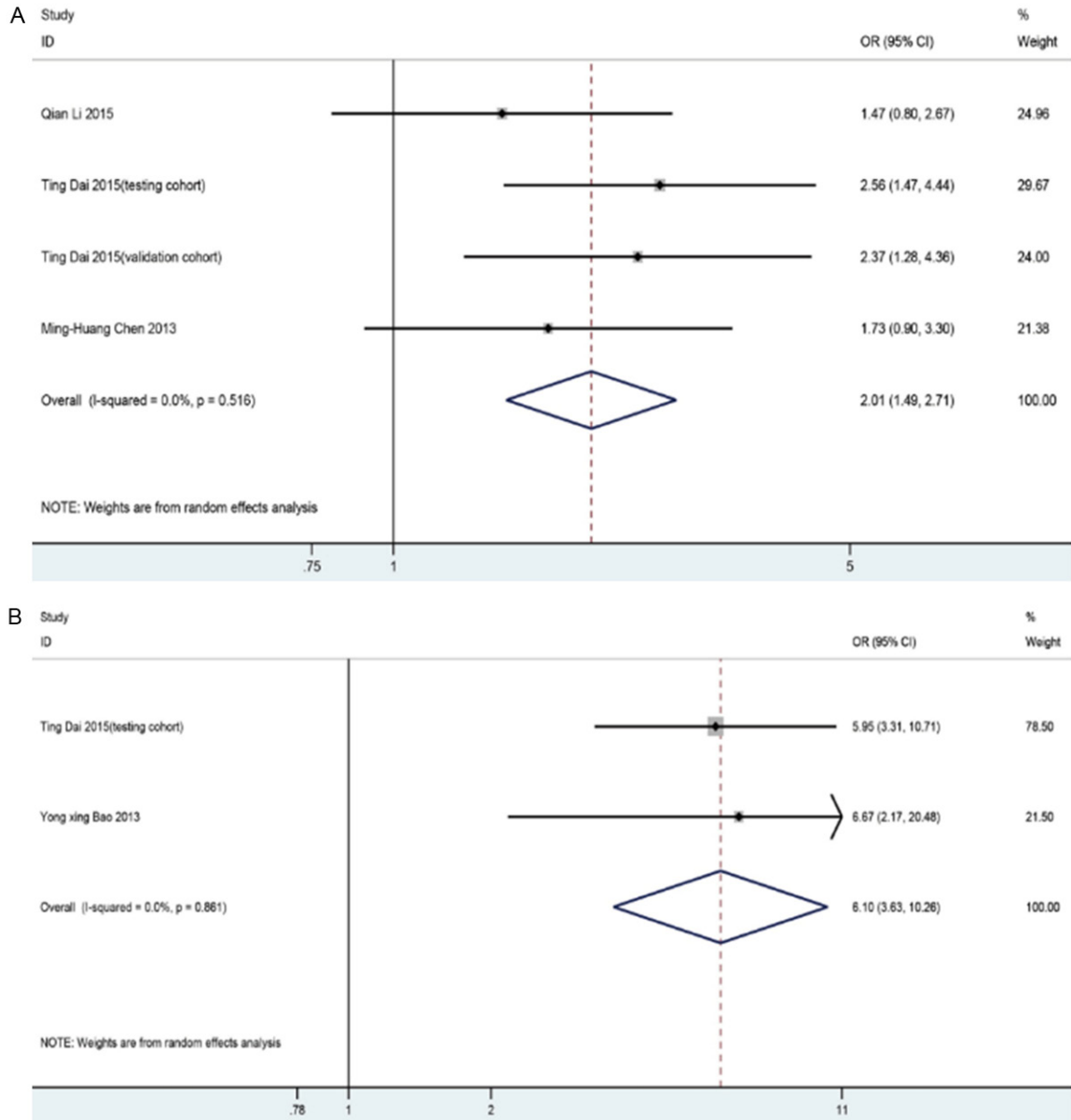
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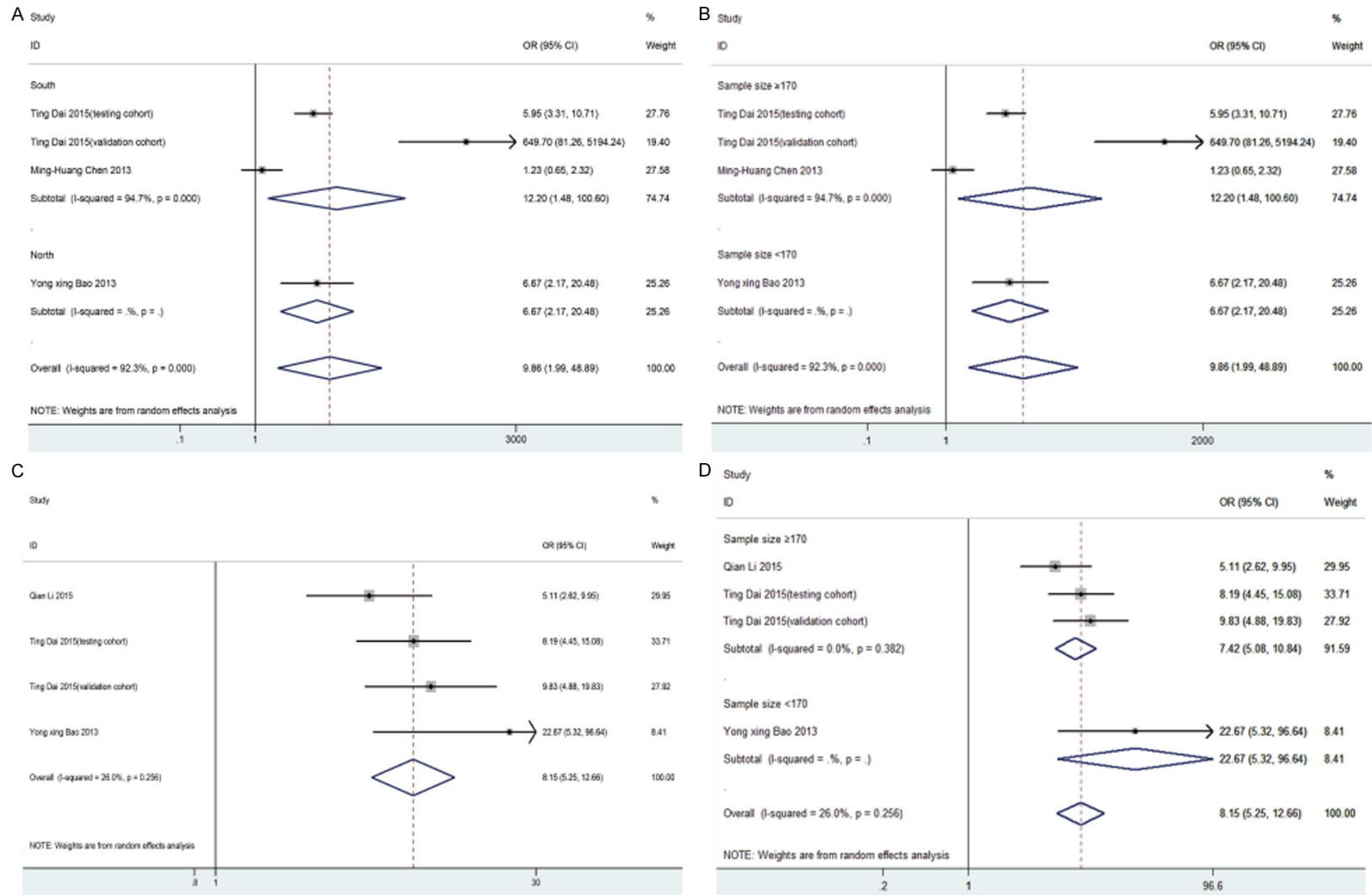
Supplementary Figure 1. Subgroup analysis of association between GP73 overexpression and HCC patients. (A) OS based on geographical location, (B) OS based on the sample size, (C) DFS based on the geographical location, and (D) DFS based on the sample size.

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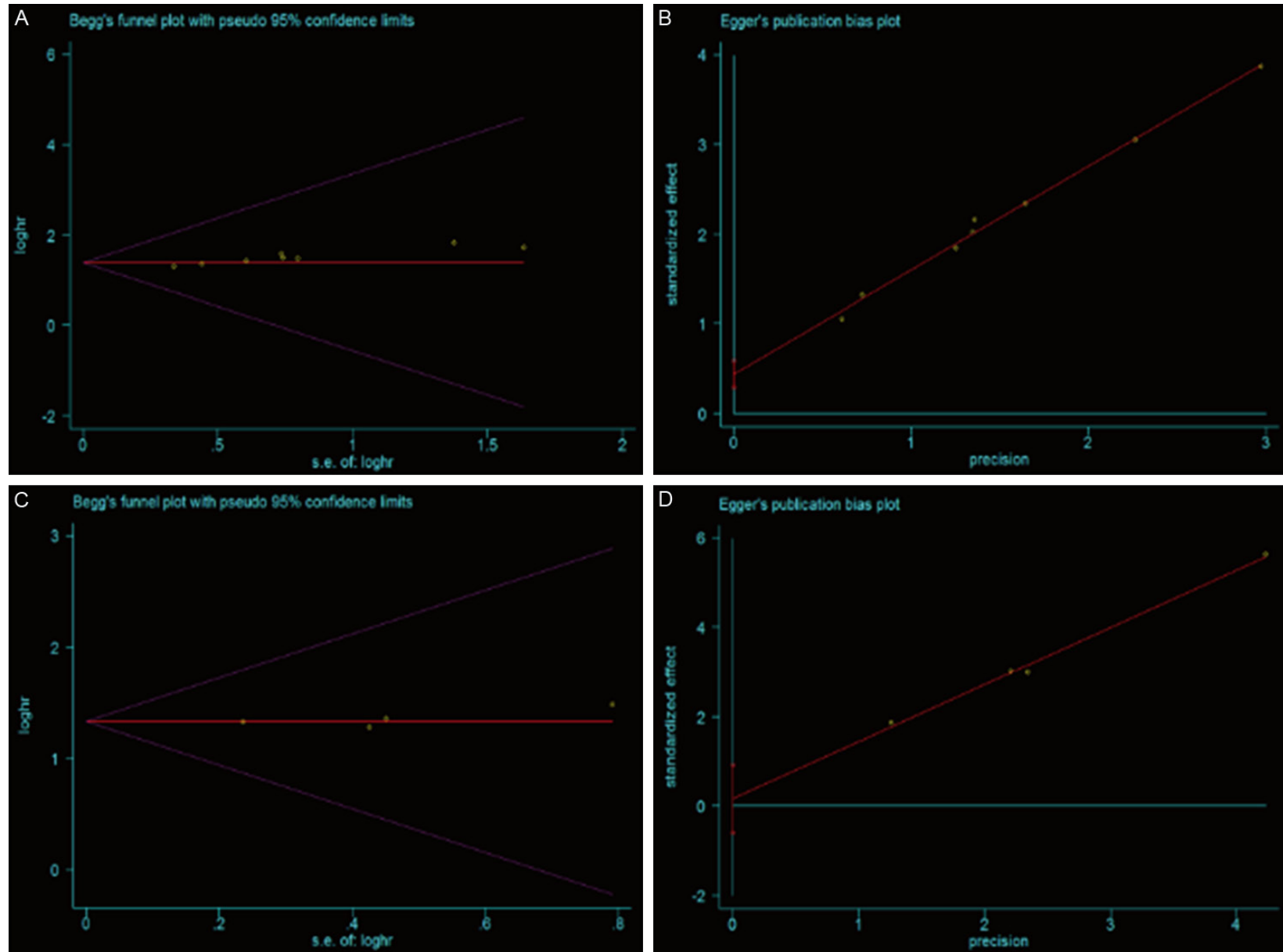
Supplementary Figure 2. Sensitivity analysis. A. Evaluating the association between GP73 overexpression and DFS in HCC after excluding a study with an OR value of <1 and of low quality; B. Assessing the association of GP73 overexpression with TNM stage in HCC after excluding the studies with the highest or lowest estimates.

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Supplementary Figure 3. Subgroup analysis of the association between GP73 overexpression with TNM stage and Edmondson-Steiner grade in HCC patients. (A) TNM stage of HCC patients based on geographical location, (B) TNM stage of HCC patients based on sample size, (C) Edmondson-Steiner grade in HCC patients based on the geographical areas, and (D) Edmondson-Steiner grade in HCC patients based on the sample size.

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Supplementary Figure 4. Assessment of publication bias. Begg's funnel plot assessing the publication bias between (A) the overall survival studies of HCC patients and (C) the DFS studies of HCC patients. Egger's plot analysis of the publication bias between (B) the overall survival studies of HCC patients and (D) the DFS studies of HCC patients.