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ORIGINAL ARTICLE

Meta-analysis and systematic review of prognostic significance of Glypican-3 in patients with hepatitis B-related hepatocellular carcinoma

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Abstract Hepatocellular carcinoma (HCC) is a common malignant cancer and the second cause of cancer-related death worldwide. Glypican-3 (*GPC3*) is established as an important prognostic factor for HCC but the results are still controversial. Moreover, its utility as an immunohistochemical marker for HCC is not conclusive. Herein we aimed to find the prognostic significance of *GPC3* in HCC patients. The PubMed, Web of Science, EMBASE, SCOPUS and Cochrane library databases were searched and eligible studies based on the *GPC3* expression and survival outcome of HCC (odds ratios or hazard ratios) included in the current meta-analysis. The STATA 12.0 and RevMan 5.3 software were used for statistical evaluations. 17 articles contained 2618 patients, were included in the recent meta-analysis. Our findings revealed a significant association between tumor stage, higher tumor grade, presence of vascular invasion, shorter overall survival, shorter disease-free survival and high expression of *GPC3*. The subgroup analyses based on sample size, cutoffs and follow-up period were also conducted to examine the association between *GPC3* and OS and also to increase the homogeneity of study. Current study found a significant association between *GPC3* expression and poor prognosis of HCC and specially related to the HCC invasion and progression. It was recommended to design more prospective studies based on the relationship between *GPC3* and HCC to confirm our results.

Keywords *GPC3* · HCC · Meta-analysis

Abbreviations

GPC3	Glypican-3
HCC	Hepatocellular carcinoma
HR	Hazard ratio
DFS	Disease-free survival
OS	Overall survival
OR	Odds ratio

Introduction

Hepatocellular carcinoma (HCC) as a common malignant cancer, is the second cause of cancer-related death, ranking fifth in the global incidence of malignant tumors. HCC is related to the 70–90% of primary liver cancer [30, 31]. Hepatitis B (HBV) and C (HCV) viruses are the main causes of HCC and is a prevalent malignancy in Asia [1]. The best treatments for HCC are surgical resection, transplantation, radiotherapy, chemotherapy, and radiofrequency but many patients lose the chance of treatment because their cancer is detected at advanced stages due to the lack of proper diagnostic methods and recurrence is frequent. On the other hand, the clinical manifestation of

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HCC is not clear and the probability of metastasis is very high. Therefore, the survival rate of patients reaches 5 years at 7% [37]. Early diagnosis of HCC is likely to improve the patient's survival and prevent cancer. Nowadays, measurement of serum biomarkers and imaging techniques are the most common cancer screening techniques [32, 37]. However, the sensitivity and specificity of serological biomarkers are very low. For example, alpha fetoprotein (AFP), as one of the most common HCC markers, remains in normal range in 40% of patients with early stage and 15–30% with advanced HCC [51]. Also, in patients with chronic hepatitis B and/or C, AFP levels increase [33]. Ultrasonography is another cost-effective way to detect HCC in early stage, but, this method cannot detect nodules smaller than 3 cm [38]. CT and MRI are also improved sensitivity and specificity in the early stages to an acceptable level (91% and 96% respectively). However, these methods are expensive and because of exposure to radiation, routine use, especially in large-scale screening is not common [16]. Therefore, identification of a non-invasive and cost-effective diagnostic method is essential and introduction of high-sensitivity diagnostic markers also improves detection and screening techniques for HCC.

Glypican family comprises six members, GPC1 to GPC6, are proteins with heparan sulfate proteoglycan subunit and glycosyl-phosphatidylinositol anchor which can bind to the outer surface of the cell membrane [19, 33]. The *GPC3* gene is located on the human *X* chromosome (*Xq26*) encoding a 70-kDa core protein. The mutation in the *GPC3* gene is related to the human Simpson-Golabi-Behmel syndrome [36]. *GPC3* is produced in the placenta and fetal liver, but it is not expressed in other adult tissues. The role of *GPC3* in cancer is widespread and depends on cellular content as well as cellular signaling pathways. *GPC3* is involved in signaling pathways such as tumor growth factor, Hedgehog, bone morphogenetic protein, Wnt/β-catenin and fibroblast growth factor through a lipase called Notum. *GPC3* has different roles in various cancers. It can inhibit cell proliferation and induce pro-apoptotic functions in mesothelioma, breast and ovarian cancers [8]. In HCC, the expression of *GPC3* is increased as an oncogene [25, 33]. *GPC3* is a precise diagnostic marker for HCC and can differentiate between the early stage of HCC and precancerous state [9]. It can distinguish HCC from a number of pathological conditions such as cholangiocellular carcinoma, hepatocellular adenoma, focal nodular hyperplasia and cirrhosis [10, 24, 48]. In patients with hepatectomy, *GPC3* is a strong diagnostic marker for HCC [11]. The *cDNA* microarray analysis has been revealed an overexpression of *GPC3* in HCC, whereas its expression has been reduced in preneoplastic and non-neoplastic lesions [28, 42]. Some studies have reported that high *GPC3* expression was related to the poor prognosis of HCC [11, 14, 43]. However, some other studies have revealed different results, distinctly [3, 18, 35, 44, 50]. The prognostic significance of *GPC3* in patients with hepatocellular carcinoma is still unclear and it remains to be elucidated. Six retrospective studies and tow meta-analyses have reported that high *GPC3* expression was related to the poor prognosis in patients with HCC [11, 14, 26, 34, 39, 43, 46, 49]. Moreover, there were also some studies reported different conclusions distinctly [3, 18, 27, 35, 44, 50]. In the current meta-analysis, we explored the correlation between *GPC3* and prognostic significance in HCC by adding the latest data from current studies.

Methods and materials

Search strategy and inclusion criteria

The studies were included from PubMed, Web of Science, EMBASE, Cochrane Library and SCOPUS databases. These databases were systematically searched until November 1th, 2018 without time restriction. Resources search was done using the following keywords: (*GPC3* [MESH] or *GPC3* [TEXT WORD] or *GPC3* protein or glypican-3 [All Fields]) AND (carcinoma, hepatocellular, liver cancer or hepatoma [MESH] or HCC [TEXT WORD]). If the study meet the following inclusion criteria, it would enter the meta-analysis: (1) the studies had to be published as cohort study in English with the full text available, (2) the technique for measuring *GPC3* should be immunohistochemistry (IHC), (3) the sample size should preferably be greater than 20, (4) the studies included HCC patients with surgical resection (SR) or liver transplantation (LT), (5) the relationship between *GPC3* and disease-free survival (DFS) and/or overall survival (OS) of patients with HCC and 95% confidence interval (CI) was evaluated or studies must provide sufficient information to estimate HR and 95% CI, (6) if several studies reused the same patient, the study that has the most data was included in the meta-analysis. Non-english-language papers, reviews, conference abstracts, or articles with insufficient information for calculating the HR and 95% CI of OS or DFS were considered as ineligible. The method for selecting eligible studies is shown in Supplementary Fig. 1.

Data extraction and quality assessment

Two reviewers, independently, extracted all the data based on the inclusion criteria. In cases where the two reviewers disagreed, the third reviewer announced the final decision. The information was extracted from the study included: first author, year of publication, country, the number of patients, the numbers of different clinicopathological

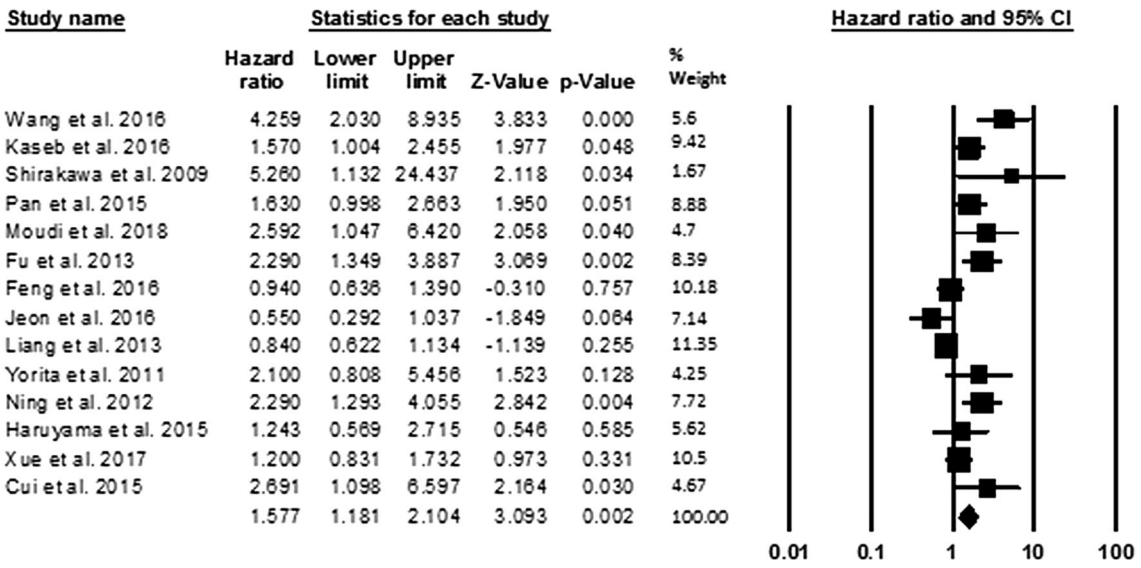

Fig. 1 The association between high expression of GPC3 and OS in patients with HCC. OS was reported in 14 studies with a total of 2432 HCC patients. Because of the heterogeneity in the study, pooled HR was calculated by the random effect model. The summary HR and 95% CIs were shown. *CI* confidence interval, *GPC3* glypican-3, *HCC* hepatocellular carcinoma, *HR* hazard ratio

parameters, cutoff, follow-up period, HR and OR with 95% CI. In articles where the survival data had not been directly examined, data were extracted using Kaplan–Meier curves and GetData Graph Digitizer 2.24 software (http://getdata-graphdigitizer.com). All analyzes were based on previously published studies and there was no need for informed consent and ethical approval. Macro- or microscopic vascular invasion was referred to the tumor vascular invasion. The interval between the medical treatment and last observation of patients/death was considered as OS. The interval between the treatment and detection of tumor recurrence was measured as DFS. Tumors were grouped according to the Edmondson Steiner grading system as follows: well/moderately (I/II) and poorly (III/IV) differentiated [6]. To find out whether *GPC3* expression was low or high, we referred to the articles. Quality assessment was performed by the standard Newcastle–Ottawa quality assessment scale. Numbers from 0 to 9 were used to evaluate the quality of articles. Patient selection and ascertainment of outcome were awarded 1 point and comparability awarded 2 points. When it was rated 0 to 4 and 5 to 9, quality was considered low and high, respectively.

Statistical analysis

Meta-analysis was done using STATA 12.0 (StataCorp LP, College Station, TX, USA) and RevMan 5.3 software (Cochrane Collaboration, Oxford, UK). The heterogeneity between studies was shown using I^2 statistic. We used random effect model if $I^2 \geq 50\%$ and fixed effect model in other situations. HR with 95% CI evaluated the association between GPC3 and HCC survival outcome. OR with 95% CI revealed the possible relationship between GPC3 and clinicopathological parameters for HCC. The stability of the results was assessed by analyzing the sensitivity. In this regard, we deleted 1 study each time and therefor examined the influence of each data on the pooled HR [23]. The bias was tested by the Egger linear regression using Begg funnel plots with significant publication bias defined as $P < 0.05$ [2].

Results

Selection and characteristics of literature

During the first process of retrieving the articles using the considered keywords, 367 articles on the relationship between *GPC3* and HCC were obtained. In the next step, by evaluating the title and abstract of articles, 315 articles were deleted because they were not reported original articles and English language. The full texts were reviewed and evaluated and 5 papers were deleted due to a lack of information on patient survival data. In two studies, the same patients were used [43, 44]. To avoid duplicate counting, only the study was selected that contained more information. Finally, 17 articles contained 2618 patients, who had the inclusion criteria, were selected for the recent meta-analysis [3, 5, 7, 11, 14, 18, 21, 27, 33–35, 40, 44, 45, 47, 49, 50] (Fig. 1). Geographical distribution of articles was as follows: 9 literature from China [5, 7, 11, 27, 34, 35, 44, 45, 47], 4 from Japan [3, 14, 40, 49], 1 from USA [21], 1

from Iran [33], 1 from Taiwan [50] and 1 from Korea [18]. In all articles, IHC was used to evaluate *GPC3* expression in the liver tissue. To evaluate low or high GPC3 expression, information from each article was used and the amount of cutoff was calculated. In this study, a unified amount of high *GPC3* cutoff was not selected. In the selected articles, *GPC3* was often expressed in the cytoplasm and in some articles, it was also expressed in the cell membrane. OS, DFS and their 95% CIs with HRs were extracted by the methods as mentioned above. The main treatment for HCC patients was SR that was performed in 13 articles and LT in 2 articles. The sample size varied from 31 to 362 in all studies. The number of patients with the highest levels of GPC3 expression were 20 to 270. The mean age was 43 to 69 years and the number of males was 29 to 324. The average follow-up time was 3 years. Quality assessment was performed by the standard Newcastle–Ottawa quality assessment scale and was 5 to 8. Primary parameters and clinicopathological properties of the 17 articles are summarized in Supplementary Table 1.

Relevance between high expression of GPC3 and clinicopathological features

In order to determine the effect of *GPC3* on diagnosis of HCC, we assessed clinicopathological parameters. In many of the studies that were selected for the recent meta-analysis, the relationship between clinicopathological properties (HBV/HCV infection, tumor number, tumor size, histological grade, vascular invasion, Child–Pugh grade) and high expression of *GPC3* was evaluated. Our findings revealed significant association between tumor stage and high expression of *GPC3* (OR = 1.02, 95% CI 1.09–2.74, $P = 0.04$). Also, there was a significant relationship between high expression of *GPC3* and higher tumor grade (OR = 1.86, 95% CI 1.42–3.45, $P < 0.001$). On the other hand, the presence of vascular invasion was significantly associated with the high expression of GPC3 (OR = 1.36, 95% CI 1.09–3.02, $P = 0.04$) (Table 1).

Relevance between high expression of GPC3 and OS in patients with HCC

In this meta-analysis, 14 studies, including 2432 HCC patients, reported the relationship between GPC3 expression and OS. In evaluations the results, it was found that there is heterogeneity in the current study ($I^2 = 72\%$, $P < 0.001$), therefore, the random effect model was used for pooled HR calculation. Our findings revealed significant association between overexpression of *GPC3* and decreased OS (pooled HR: 1.57, 95% CI 1.18–2.10, $P = 0.002$) (Fig. 1).

Relevance between high expression of GPC3 and DFS in patients with HCC

In this meta-analysis, 7 studies, including 829 HCC patients, reported the relationship between *GPC3* expression and DFS. In evaluations the results, it was found that there is significant heterogeneity in the current study ($I^2 = 81.0\%$, $P < 0.001$), therefore, the random effect model was used for pooled DFS calculation. Our findings revealed a significant association between overexpression of *GPC3* and poor DFS (pooled HR: 1.93, 95% CI 1.09–3.43, $P = 0.02$) (Fig. 2).

Subgroup analysis

The results of the subgroup analyses based on sample size, cutoffs and follow-up period are shown in Table 2 to examine the association between *GPC3* and OS and also to increase the homogeneity of the study. In regard to the sample size, the combined HR of the studies with ≤ 200 cases was 1.49 (95% CI 1.32–2.21, $P = 0.019$) and the combined HR based on studies with more than 200 cases was 1.33 (95% CI 0.76–1.68, $P = 0.814$). In subgroup analyses based on the follow-up period, only studies with a shorter follow-up period (≤ 60 months) showed a significant association between *GPC3* and poor OS with HR = 1.73 (95% CI 1.42–2.67, $P = 0.001$). Moreover, subgroup analysis related to the *GPC3* cutoff values revealed that the pooled OS was varied in the included studies.

Publication bias and sensitivity analyses

In our meta-analysis, the bias was tested by the Egger linear regression using Begg funnel plots with significant publication bias defined as $P < 0.05$. In all studies, an obvious symmetry was seen in the funnel plot ($P = 0.05$ for the Egger test) (Fig. 3) which means that the current meta-analysis did not have a significant publication bias.

Discussion

A large number of literatures reported that the expression of *GPC3* was lower or even absent in the normal tissue compared with malignant specimen and it distinctly expressed in HCC [15, 17, 20, 22]. In some tissues, *GPC3* acts as a tumor suppressor gene, whereas in others, it acts as an oncofetal protein. *GPC3* immunohistochemistry can aid in the differentiation of testicular germ cell tumors, being expressed in all yolk sac tumors but not in seminomas. *GPC3* expression has also been identified in some squamous cell carcinomas of the lung and clear cell carcinomas of the ovary. The role of *GPC3* in melanomas is

Table 1 Association between high expression of GPC3 and clinicopathological features

	Effect model	OR (95% CI)	*P* value	Heterogeneity test	
				I^2 (%)	*P* value
HBV (±)	Fixed	1.41 (0.98–1.84)	0.09	38	0.12
HCV (±)	Random	1.05 (0.65–2.72)	0.81	72	0.03
Child–Pugh grade (B or C/A)	Fixed	1.317 (0.87–2.01)	0.19	0	0.57
Tumor size (≥ 5 cm/< 5 cm)	Fixed	1.08 (0.86–1.54)	0.79	7	0.46
Tumor number (multiple/single)	Random	1.08 (0.76–2.52)	0.23	59	0.01
Hepatic cirrhosis (positive/negative)	Fixed	1.42 (0.73–1.84)	0.16	43	0.17
Stage (III–IV/I–II)	Random	1.02 (1.09–2.74)	0.04	58	0.02
Histological grade (G2–3/G1)	Fixed	1.86 (1.42–3.45)	< 0.001	48	0.24
Vascular invasion (positive/negative)	Random	1.36 (1.09–3.02)	0.04	55	0.006

This table evaluated the associations between GPC3 high expression and infection of HBV or HCV, Child–Pugh grade, tumor size, tumor number, stage, histological grade, and vascular invasion

CI confidence interval, *GPC3* glypican-3, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *N* number, *OR* odds ratio

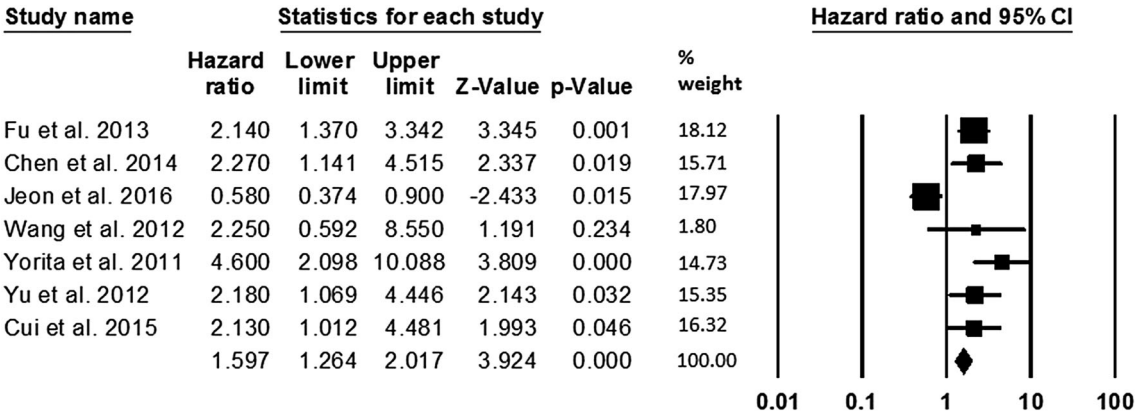

Fig. 2 The association between high expression of GPC3 and DFS in patients with HCC. DFS was reported in 7 studies with a total of 829 HCC patients. Because of the heterogeneity in the study, pooled HR was calculated by the random effect model. The summary HR and 95% CIs were shown. *CI* confidence interval, *GPC3* glypican-3, *HCC* hepatocellular carcinoma, *HR* hazard ratio

Table 2 Subgroup analyses for GPC3 on HCC overall survival

	No. of studies	Effect model	HR (95% CI)	*P* value	Heterogeneity test	
					I^2 (%)	*P* value
Overall Sample size	14	Random	1.62 (1.26–2.30)	0.03	69.1	0.001
≤ 200	10	Random	1.49 (1.32–2.21)	0.019	63.4	0.021
> 200	4	Random	1.33 (0.76–1.68)	0.814	53.7	0.125
Duration of follow-up						
≤ 60 months	6	Fixed	1.73 (1.42–2.67)	0.001	41.5	0.12
> 60 months	6	Random	1.54 (0.57–1.69)	0.196	70.2	0.001
Cut-off value						
5%	1	–	0.60 (0.33–1.29)	0.071	–	–
10%	8	Random	1.29 (0.86–2.10)	0.81	71.4	0.008
20%	4	Fixed	1.48 (0.71–2.67)	0.176	0	0.601
25%	1	–	2.14 (1.21–3.43)	0.002	–	–

Subgroup analyses for the association between GPC3 and OS, based on sample size, follow-up period, and cut-offs, were conducted in this table

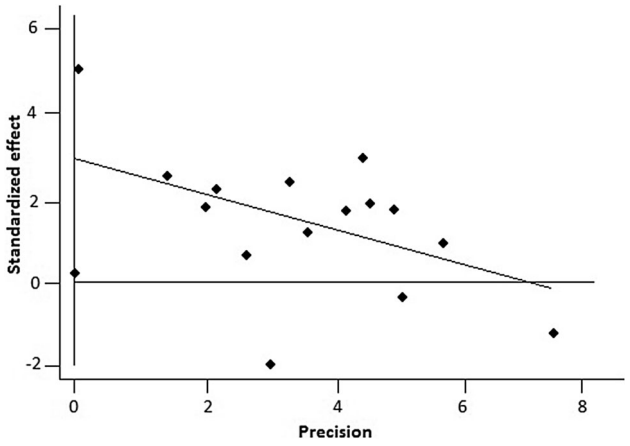

Fig. 3 Funnel plots of Egger to detect publication bias on overall estimate. Test of publication bias in our meta-analysis was performed using Begg funnel plot and Egger regression method. There was no asymmetry observed in funnel plot, indicating no evidence of significant publication bias

still controversial [20]. Studies have shown that *GPC3* expression contribute to the pathogenesis of HCC through proliferation, invasion, and progression cancer. However, there are contradictions in the results of various studies and the prognostic significance of GPC3 in patients with hepatocellular carcinoma is still unclear. Thus, in the current meta-analysis, we explored the correlation between *GPC3* and prognostic significance in HCC using available researches. Current study, comprehensively revealed the relationship between *GPC3* expression in liver tissue and HCC.

In this study, 17 papers containing 2618 patients were analyzed, with more cases than the previous studies [29, 46, 52]. Analysis the relevance between expression of *GPC3* and clinicopathological properties indicated a significant association between higher expression of GPC3 and the presence of vascular invasion, later tumor stage and higher tumor grade. On the other hand, there was no significant relationship between *GPC3* and ORs of some pathological properties (HBV and or HCV infection, tumor size, tumor number, Child–Pugh grade). It means that higher *GPC3* levels can be a reliable marker for assessing the invasiveness of HCC. Our results are inconsistent with the results of Cheng et al. [4], Gauglhofer et al. [13], Galli et al. [12] and Sun et al. [41] studies. They showed that higher expression of *GPC3* could promote the growth of cancer cells through FGF activity, insulin growth factor signaling and Wnt signaling pathway, in vivo and in vitro. Meanwhile, down regulation of *GPC3* expression, decreases cell proliferation and cell cycle progression at the G1 phase through phosphorylation of SMAD2/3 in HCC cell lines [41]. Another valuable result found in the recent study was the promising relationship between *GPC3* expression and the OS/DFS of HCC. Our results showed that higher *GPC3* expression could increase the risk of poor OS and poor DFS 1.57 and 1.93 times, respectively compared to the patients with lower *GPC3* expression. Our pooled HR for OS was different from previous meta-analysis. This discrepancy may be due to some reasons as follows: in our recent study, we analyzed more articles than previous meta-analysis, which can increase the statistical strength of the research and provide reliable conclusions. Also, in this study, more and varied geographical populations have been assessed than previous meta-analysis that can provide more valuable results. Although, a significant relationship was seen between higher *GPC3* expression and poorer HCC survival in the current study, however, the diagnostic value of *GPC3* should be discussed more than ever clinical situations.

In our meta-analysis, also subgroup analyses were carried out to examine the association between *GPC3* and OS and also to increase the homogeneity of the study because our results had a significant heterogeneity. In subgroup analyses based on the follow-up period, only studies with follow-up period $\leq$ 60 months showed significant association between *GPC3* and poor OS and studies with follow-up period $>$ 60 months were not significant. It means that *GPC3* expression may be able to predict short-termoutcome of HCC. Nevertheless, in regard to the sample size, the combined HR of the studies with $\leq$ 200 cases was significant and indicated a significant relationship between higher *GPC3* and poor prognosis, which means that significant prognostic value of GPC3 was related to the studies with $\leq$ 200 patients. Therefore, in future studies, we needed to use a larger sample size to calculate the exact value of *GPC3* in predicting the OS for HCC patients.

There are limitations in this study that should be taken into consideration. However, the publication bias was not significant in this study, but the most obvious limitation of the recent meta-analysis was publication bias because only the articles were used whit complete text and in English language. On the other hand, the method of HR extraction can be another factor of bias. In this regard, survival results related to the survival curves can reveal such imprecision. Also, in the eligible studies that were analyzed, the standard amount was not raised with cutoff values. Therefore, *GPC3* cutoff values of each article were used to perform subgroup analysis. Moreover, given the fact that subgroup information was incomplete in some articles, we could not accurately report the amount of cutoff for predicting OS. Since the number of analyzed articles was limited, we were unable to include factors such as the primary antibody, treatments, laboratory infrastructure in subgroup analyses. All of the above factors can cause bias. To remove the bias sources, we need to increase the sample size and meta-analysis done in a multi-center manner.

In conclusion, despite all the limitations mentioned, the current study found a significant association between *GPC3* expression and poor prognosis of HCC and specially related to the HCC invasion and progression. It was recommended to design more prospective studies based on the relationship between *GPC3* and HCC to confirm our results.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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