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Review Article

SALL4 expression is associated with poor outcome in hepatocellular carcinoma

Yu-Liang Jiang[1*], Wei Li[2*], Hu-Gang Shen[3*], Rui Ren[1], Zhi-Hua Xu[1], Dai-Wei Wan[1], Ye Han[1], Yu-Ting Kuang[1], Qiao-Ming Zhi[1]

Departments of [1]General Surgery, [2]Oncology, The First Affiliated Hospital of Soochow University, Suzhou 215006, China; [3]Department of General Surgery, Kunshan Hospital of Traditional Chinese Medicine, Kunshan 215300, China. [*]Equal contributors.

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Abstract: Spalt-like transcriptional factor 4 (SALL4), which is located on chromosome 20q13.12-13.31 and encodes a C2H2 zinc-finger transcription factor, has been identified as a putative stem cell marker. In published studies, SALL4 has been proved to contribute to the development of hepatocellular carcinoma (HCC). To systematically evaluate the clinical and prognostic role of SALL4 in HCC, we performed this meta-analysis. A total of 8 studies containing 290 positive and 1063 negative cases were included in our meta-analysis. The quality assessment of included studies was performed by the Newcastle-Ottawa scale (NOS), with an average NOS score of 7.25. Our data indicated that SALL4 expressing level was not associated with the gender (pooled OR = 0.764, 95% CI = 0.506-1.153, P = 0.200, fixed effect), histological differentiation (pooled OR = 0.420, 95% CI = 0.132-1.337, P = 0.142, random effect) and Child-Pugh score (pooled OR = 2.014, 95% CI = 0.848-4.784, P = 0.113, fixed effect) in HCC patients. However, high SALL4 statistically related to hepatitis B virus infection (pooled OR = 2.083, 95% CI = 1.300-3.338, P = 0.002, fixed effect) and vascular invasion (pooled OR = 1.685, 95% CI = 1.219-2.328, P = 0.002, fixed effect), which led to a lower 1-year disease free survival (1-year DFS, RR = 1.715, 95% CI = 1.078-2.728, P = 0.023) and 1-year overall survival (1-year OS, RR = 2.495, 95% CI = 1.636-3.805, P = 0.000). These findings suggested that SALL4-immunopositive expression might lead to a poorer patient survival in HCC. SALL4 could be served as an efficient marker for prognostic indicator in HCC, and might be a promising target for future HCC therapies.

Keywords: SALL4, clinicopathological characteristics, prognosis, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second leading cause of cancer mortality worldwide [1, 2]. Multiple risk factors for HCC are the most frequent chronic viral hepatitis (B and C), alcohol abuse, serum alpha-fetoprotein (AFP) concentration, obesity, diabetes and exposure to aflatoxins [3, 4]. To the best of our knowledge, HCC staging affects treatment decisions and patient prognosis largely. Due to lack of distinctive symptoms in early stages, less than 30-40% of HCC patients are eligible for potentially curative therapies including surgical resections and transplantations [5, 6]. As to the patients who lost the surgery opportunity, palliative treatments such as transarterial chemoembolization, sorafenib, small molecular target agents, monocolonal antibodies and Chinese herbal medicine, offer a relative survival benefit [7-10]. Because the molecular mechanisms underlying hepatocarcinogenesis are not well characterized, all the combined treatments do not reach an ideal objective. Hence, it is urgent to elucidate pathogenic mechanisms and find high specificity and sensitivity biomarkers for early detection of HCC, which may benefit the therapy and prognosis of HCC patients.

Stem cells are generally defined as clonogenic cells which are capable of both self-renewal and multilineage differentiation [11]. Given their properties, stem cells are unit of biological organization, product of new and replacement cells for tissues during development and homeostasis [12]. Cancer stem cells (CSCs), a subpopulation of cancer cells which have the

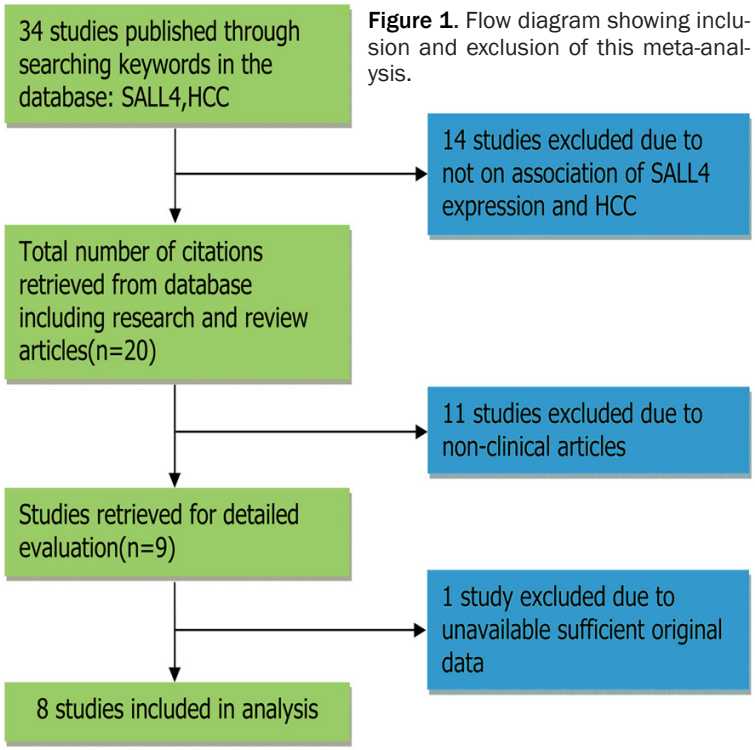


Figure 1. Flow diagram showing inclusion and exclusion of this meta-analysis.

Table 1. Characteristics of included studies in this meta-analysis

Study	Year	Methods	Tumor stage	Total	SALL4 positive (n)	SALL4 negative (n)
Nilda	2012	IHC	I-IV	69	32	37
Zeng	2013	IHC	I-IV	144	43	101
Han	2014	IHC	I-IV	38	18	20
Liu	2014	IHC	-	236	3	233
Shibahara	2014	IHC	-	337	47	290
Park	2015	IHC	I-IV	190	39	151
Yin	2016	IHC	I-IV	126	58	68
Jung	2016	IHC	I-IV	213	50	163

same properties, share with normal stem cells [13]. The stem/progenitor cells in cancer tissues have dual effects on developing embryogenic stem cell and activating or suppressing tumor [14]. CSCs play a crucial role in controlling tumor proliferation, invasion, metastasis and oncogenicity [15]. Current cancer treatments are limited to eliminate CSCs populations completely, which increase the risk of tumor re-growth and disease relapse [16, 17]. CSCs, as important indicators of poor prognosis, have been found in several tumor types, including colorectal cancer, gastric cancer, hepatocellular carcinoma and breast cancer [18-21].

As a marker of stem cells, Spalt-like transcriptional factor 4 (SALL4) is located on chromosome 20q13.12-13.31 and encodes a C2H2 zinc-finger transcription factor [22]. SALL4 protein is expressed in fetal, but it is silenced in normal adult. However, in 2006, Ma et al. firstly reported that SALL4 re-expressed in leukemia and was associated with the poor prognosis of AML patients [23]. Afterwards, SALL4 were gradually unveiled in some solid tumors, including breast cancer, lung cancer, colorectal cancer and hepatocellular carcinoma [24-27]. In 2013, Nilda et al. pointed that these were no correlations between SALL4 expression and clinicopathologic characteristics in HCC, such as histologic grade, vascular invasion and pathologic T stage [28]. However, Yin et al. found that the expression of SALL4 was relevant to the prognosis of HCC patients, and patients with higher expression levels of SALL4 and AFP have worse prognosis [29]. Other similar studies also referred to the expressions of SALL4 in HCC [28-35], but its clinical significance and potential related tumor characteristics were not fully elucidated. Therefore, we investigated this meta-analysis to assess the clinical roles of SALL4 in HCC.

Materials and methods

Literature search

For obtaining potentially eligible studies, several databases were retrieved including PubMed, Embase, Cochrane Library and the European Society for Medical Oncology We used a combination of relevant keywords to construct the search strategy including “SALL4” and “Hepatocellular carcinoma”. A study was considered eligible for inclusion if: (1) articles

Table 2. NOS score of included studies in this meta-analysis

Column	Entries	First author							
		①	②	③	④	⑤	⑥	⑦	⑧
	Is the definition adequate	☆	☆	☆	☆	☆	☆	☆	☆
	Representativeness of the cases	☆	☆	☆	☆	☆	☆	☆	☆
Section	Selection of controls	-	-	-	-	-	-	-	-
	Definition of controls	☆	☆	☆	☆	☆	☆	☆	☆
Comparability	Comparability of cases and controls on the basis of the design and analysis	☆	☆	☆☆	☆	☆	☆	☆☆	☆
	Ascertainment of exposure	☆	☆	☆	☆	☆	☆	☆	☆
Exposure	Same method of ascertainment for cases and controls	☆	☆	☆	☆	☆	☆	☆	☆
	Non-Response rate	☆	☆	☆	☆	☆	☆	☆	☆
Total scores		7	7	8	7	7	7	8	7

Notes: ①. Nilda 2012; ②. Zeng 2013; ③. Han 2014; ④. Liu 2014; ⑤. Shibahara 2014; ⑥. Park 2015; ⑦. Yin 2016; ⑧. Jung 2016.

were published before July 2016; (2) articles were published as original research; (3) studies were written in English; (4) the study provided sufficient information which reported the relationships between SALL4 expression and potential prognostic factors (or DFS/OS); (5) articles must be the full-text manuscripts. The following studies were excluded: studies (1) were researched by RT-PCR or other non-immunohistochemistry methods; (2) could not be acquired the relevant original data or could not be used for statistical analyses; (3) were reviews, letters, case reports and expert opinions.

Data extractionand quality assessment

With the standard protocol, the eligible articles were assessed independently by two experienced investigators (Yuliang Jiang and Wei Li). General information extracted from the eligible studies include: authors, the year of publications, the methods of measuring SALL4 expression in HCC tissues, tumor stage, the total number of HCC patients in each study, the number of SALL4 positive and negative in HCC patients, the correlations between SALL4 and clinicopathological parameters, the 1-year DFS rate and OS rate. The quality of eligible studies was assessed by Newcastle-Ottawa quality assessment scale (NOS). Assessed items included selection, outcome and comparability with a score range of 0-9. Studies with a NOS score of five or greater were regarded as moderate to high quality studies, whereas those with a NOS score of less than five score were considered as low quality studies.

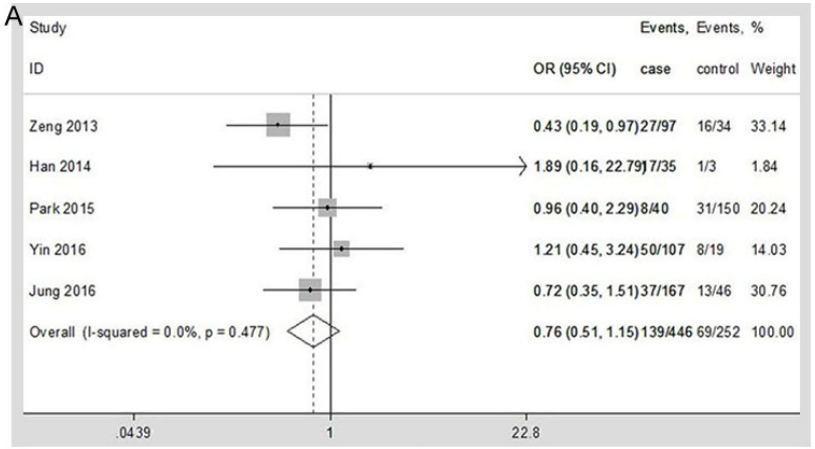
Statistical analysis

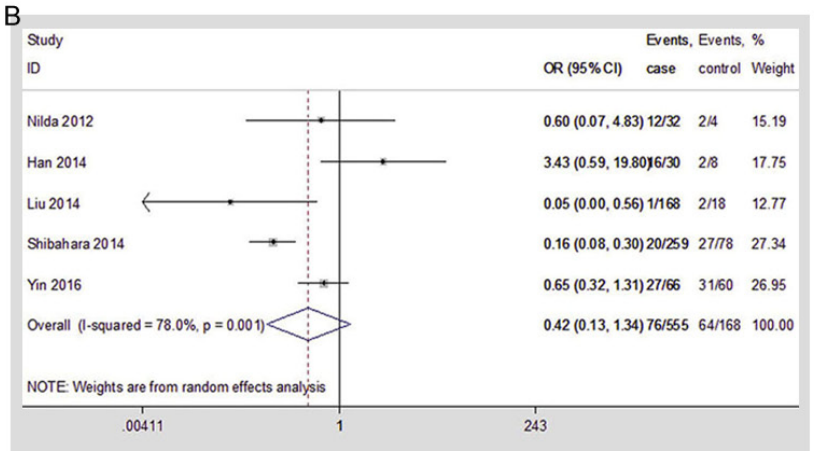
The relative risk (RR) and its 95% confidence interval (CI) were calculated for the associations between SALL4 expression and clinicopathological parameters in HCC, including gender, histological differentiation, Child-Pugh score, Hepatitis B and vascular invasion. Moreover, RRs with 95% CI were used to estimate the relationships between SALL4 expression and DSF/OS. Among them, histological differentiation was divided into two groups: well/moderate as differentiated and poor as undifferentiated. Similarly, Child-Pugh score was also simplified as A and non-A. Heterogeneity was assessed by the Chi-squared test in our meta-analysis. We defined that I^2 = 0-50% or *P*-values greater than 0.05 meant no statistically significant heterogeneity. Otherwise, the random-effects model was applied. To prove the reliability and evaluate the influence of individual study to overall results, sensitivity analysis was performed. Begg' test and Funnel plots were used to measure the publication bias for each study. We defined that the shape of graphics had apparent asymmetry or *P*-values less than 0.05 meant significant publication bias. All the statistical tests in this meta-analysis were performed with STATA software version 11.0 (STATA Corporation, College Station, TX, USA).

Results

Study characteristics

The process of study selection was shown in **Figure 1**. According to the criteria for selection, 26 studies were discarded (14 articles lacked correlations between SALL4 expressions and HCC, 11 articles were classified as non-clinical articles, 1 study showed unavailable sufficient data). Finally, 8 studies [28-35] were included in this meta-analysis, and the major characteristics were listed in **Table 1**. Our 8 included studies contained 1353 patients, ranging from





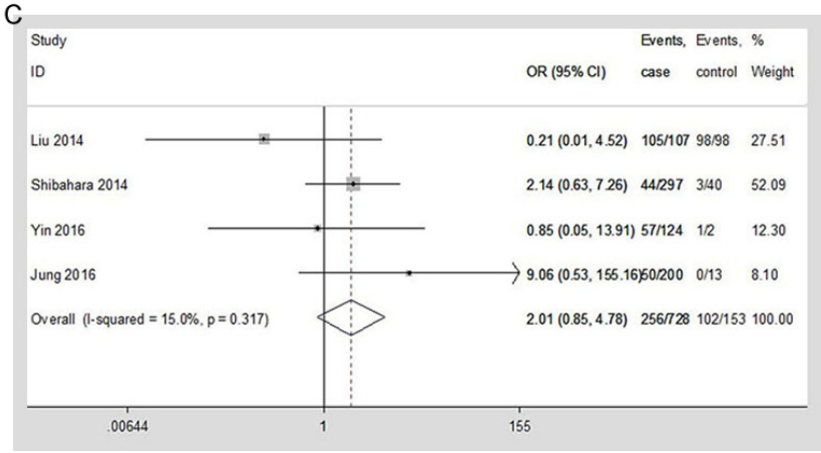


Figure 2. Forrest plot of ORs for the association of SALL4 expression with the (A) gender; (B) Histological differentiation; (C) Child-Pughscore in HCC patients.

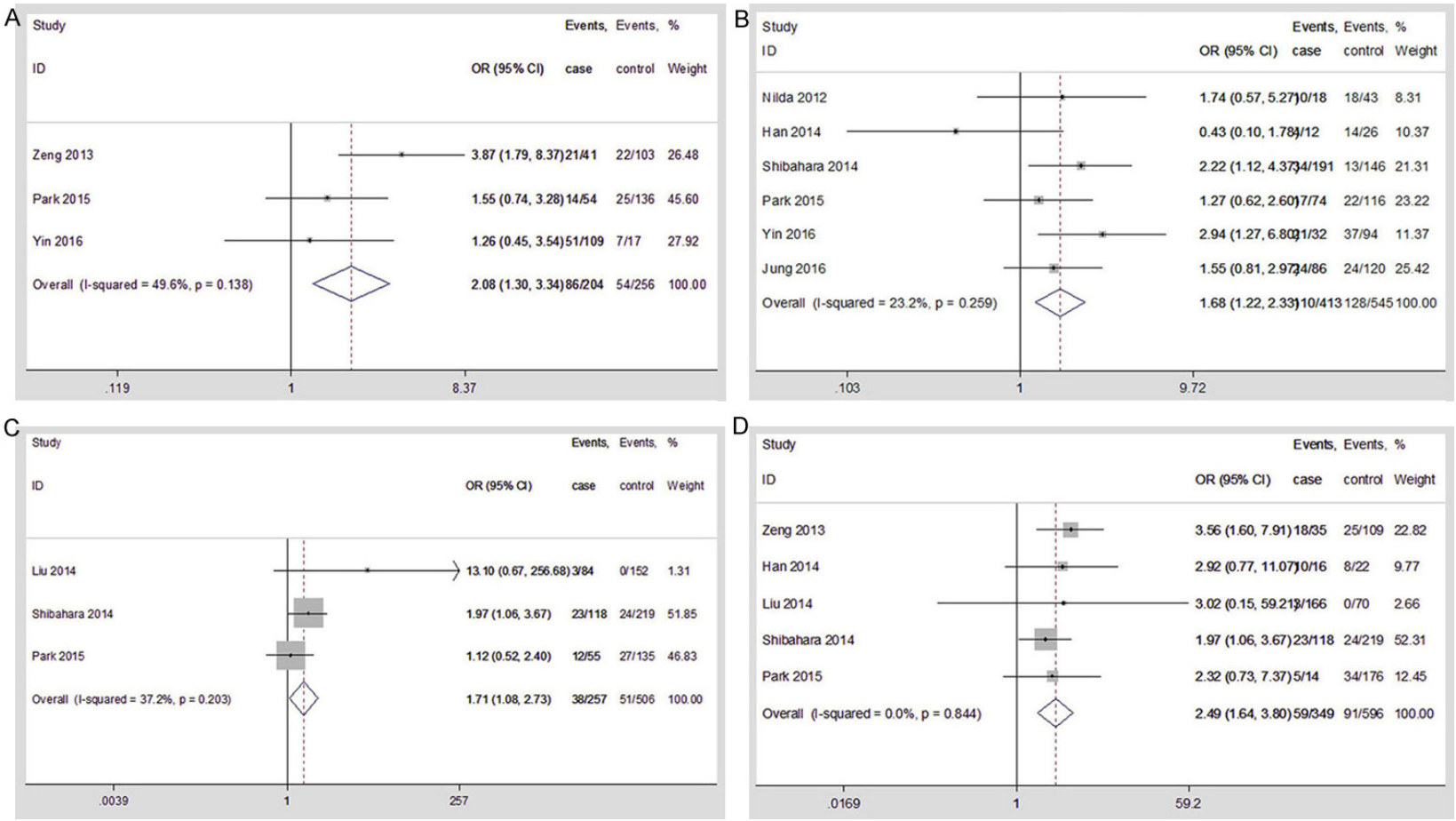


Figure 3. Forrest plot of ORs for the association of SALL4 expression with the (A) Hepatitis B virus infection; (B) Vascular invasion; (C) 1-year DFS; (D) 1-year OS in HCC patients.

38 to 337 patients per study, with 290 SALL4 positive and 1063 SALL4 negative HCC patients. The details of the quality evaluation for eligible studies are shown in **Table 2**. The average NOS score was 7.25. Immunohistochemistry (IHC) was the only applied method in this meta-analysis.

Correlations of SALL4 expression with clinicopathological parameters in HCC

As shown in **Figure 2A-C**, our results indicated that SALL4 expression was not associated with the gender (pooled OR = 0.764, 95% CI = 0.506-1.153, P = 0.200, fixed effect), histological differentiation (pooled OR = 0.420, 95% CI = 0.132-1.337, P = 0.142, random effect) and Child-Pughscore (pooled OR = 2.014, 95% CI = 0.848-4.784, P = 0.113, fixed effect) in HCC patients. However, the SALL4-immunopositive expression showed a connection towards Hepatitis B virus infection (pooled OR = 2.083, 95% CI = 1.300-3.338, P = 0.002, fixed effect). Similar positive results were also found in vascular invasion (pooled OR = 1.685, 95% CI = 1.219-2.328, P = 0.002, fixed effect) (**Figure 3A**, **3B**). In the process of evaluation of the related studies, no significant heterogeneity was observed among the studies on gender (I^2 = 0.0%, P = 0.477), histological differentiation (I^2 = 23.2%, P = 0.259), Child-Pugh score (I^2 = 15.0%, P = 0.317), Hepatitis B virus infection (I^2 = 49.6%, P = 0.138) and vascular invasion (I^2 = 23.2%, P = 0.259).

Correlations of SALL4 expression with DFS and OS in HCC

Because RRs were not described directly in include studies, we extracted the related date from the Kaplan-Meier cures or calculated from original papers [36, 37]. Based on our calculations, the high tissue SALL4 level in HCC patients was statistically related to the 1-year disease free survival (1-year DFS, RR = 1.715, 95% CI = 1.078-2.728, P = 0.023) and 1-year overall survival (1-year OS, RR = 2.495, 95% CI = 1.636-3.805, P = 0.000) with no significant heterogeneity (1-year DFS: I^2 = 37.2%, P = 0.203; 1-year OS: I^2 = 0.0%, P = 0.844) (**Figure 3C**, **3D**). This finding indicated that SALL4-immunopositive expression might lead to a poorer patient survival in HCC.

Sensitivity analysis and publication bias

A sensitivity analysis was performed to validate the stability of the pooled results. The corresponding pooled ORs and HRs were not significantly altered, which indicated the stability of our analyses (**Figure 4A-G**). There was no evidence of publication bias as suggested by Begg's tests for gender (P = 0.142), histological differentiation (P = 1.000), Child-Pugh score (P = 0.497), Hepatitis B virus infection (P = 0.602) and vascular invasion (P = 0.573). Similar results were also found in OS analysis 1-year DFS analysis (P = 0.602) and 1-year OS analysis (P = 0.624) (**Figure 5A-G**), which suggested that no evidence of publication bias were existed in our meta-analysis (**Figure 5A-G**).

Discussion

It is widely accepted that HCC is a multi-factorial disease, and millions of people die every year. SALL4, a member of the SALL gene family, is essential for the proliferation and stabilization of embryonic stem cells [38, 39]. SALL4 regulates the lineage commitment of hepatoblasts differentiating into hepatocytes or cholangiocytes through interacting with OCT3/4, SOX2 and NANOG [40-42]. SALL4 is expressed not only in fetal liver, but also re-expressed in HCC as a valuable marker. SALL4 is expressed in both the nucleus and cytoplasm [43]. By using loss-of-function studies, Yong et al. confirmed that SALL4 might serve as a transcription repressor through interacting with the NuRD (nucleosome remodeling and histone deacetylase, HDAC) complex to affect phosphatase, tensin homologue (PTEN) and phosphatidylinositol 3-kinase (PI3K)-AKT signaling [44]. These mechanistic findings suggested that SALL4 might be a potential marker of stem cell-driven hepatocarcinogenesis and contributed to the poor prognosis. Similarly, Oikawa et al. elucidated that SALL4 expression correlated with the tumor growth and referred to the resistance to 5-FU, and its suppression resulted in differentiation and slowing tumor growth [43]. Further discoveries of SALL4 are essential and may help us better understand the clinical significance of SALL4 in HCC.

In our study, we found that the SALL4 expressing level was not associated with the gender, histological differentiation and Child-Pugh score in HCC patients. In 2012 Hoshida et al. pointed that histological grades of HCC turned out to be an objective method for characterizing their biological behavior or prognosis [45]. But our results concluded that SALL4 was not involved or sufficient in HCC histological differ-

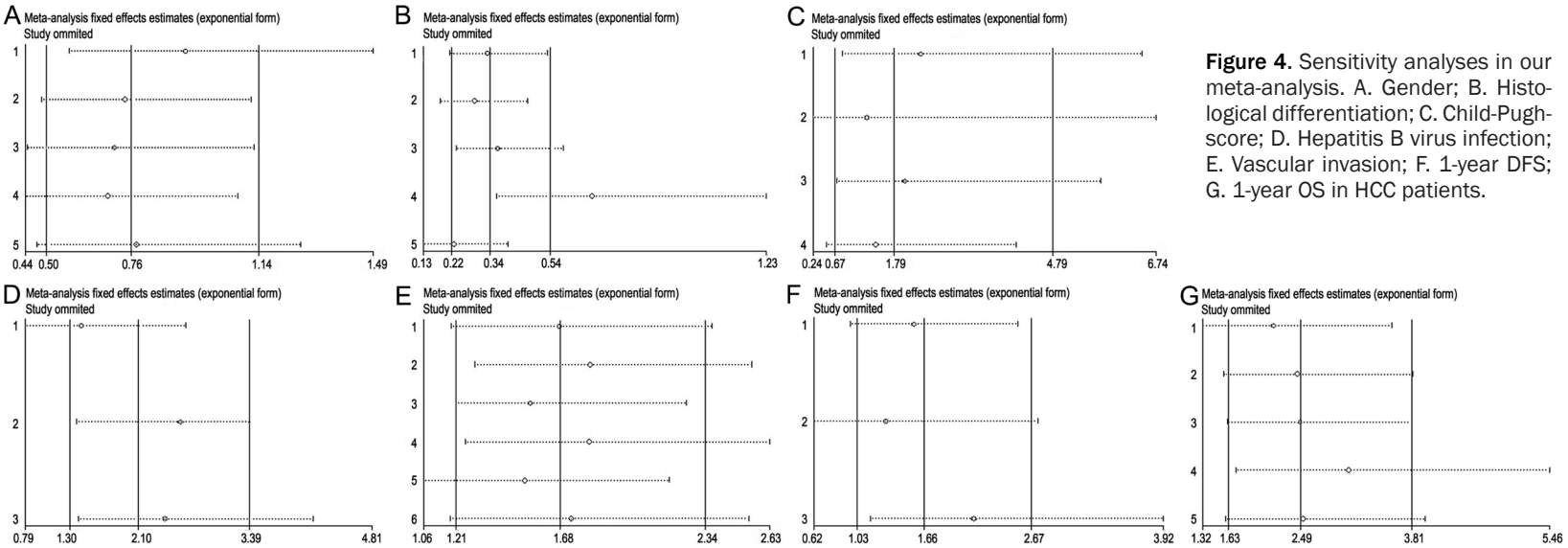


Figure 4. Sensitivity analyses in our meta-analysis. A. Gender; B. Histological differentiation; C. Child-Pughscore; D. Hepatitis B virus infection; E. Vascular invasion; F. 1-year DFS; G. 1-year OS in HCC patients.

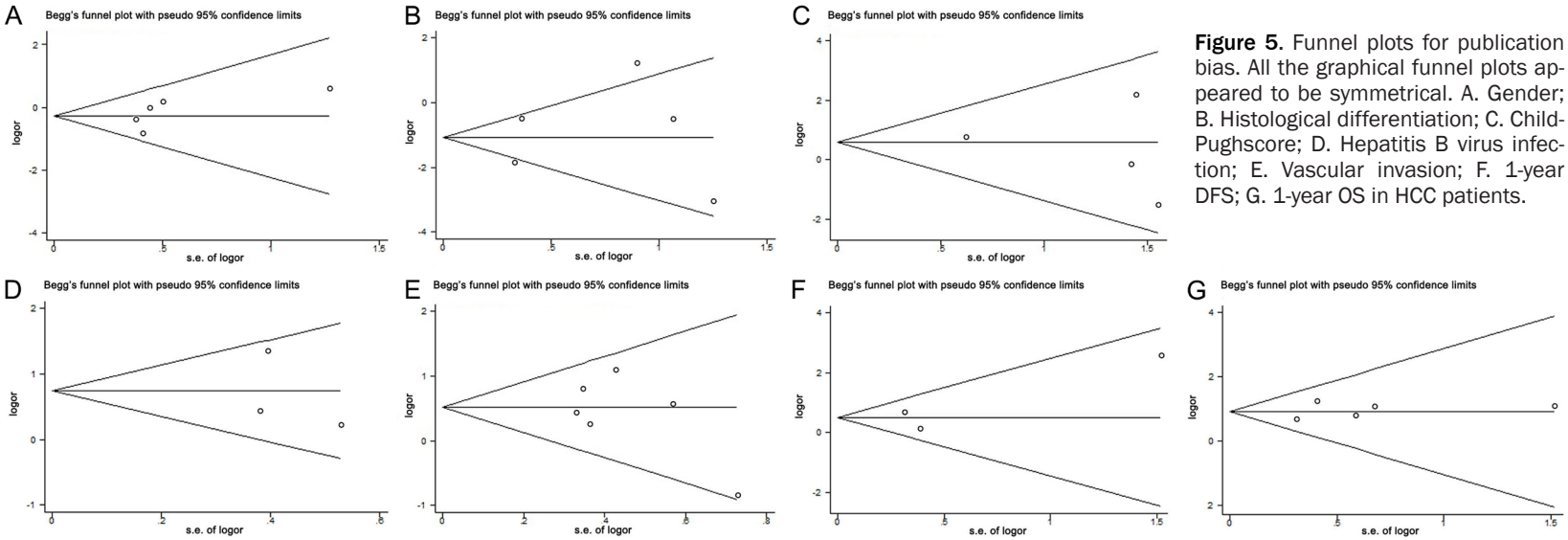


Figure 5. Funnel plots for publication bias. All the graphical funnel plots appeared to be symmetrical. A. Gender; B. Histological differentiation; C. Child-Pughscore; D. Hepatitis B virus infection; E. Vascular invasion; F. 1-year DFS; G. 1-year OS in HCC patients.

entiation, which was consistent with the results of some similar studies [28, 29, 31]. Emerging evidence showed that some CSCs markers, such as K19 and EpCAM, had been proved to be significantly higher in cirrhotic patients of Child-Pugh Class B or C than those of Child-Pugh Class A, which might promote the cancer recrudescence in HCC [46]. However, no reports specifically discussed the potential relationships or mechanisms between SALL4 and Child-Pugh score until now. Our results also confirmed no relevance between them.

It is known that the antecedent HBV-related chronic hepatitis is a common precursor condition for HCC. Recently, Minuk et al. addressed a possible association between HBV and CSCs in HCC [47]. As a proved biomarker of CSCs in HCC, we speculated that SALL4-positivity might be more frequently associated with hepatitis B virus infection. Though the potential role or exact mechanisms of SALL4 in HBV infection need further explored, our data firstly confirmed their correlations in HCC. As we know, a tumor cell or tumor cell colonies that initiates a metastatic colony at the distant organ must firstly (a) detach from the primary mass, (b) invade the local host tissue stroma, (c) penetrate local lymphatic and blood vessels. Afterwards, these survived tumor cells within the circulation become transported until they arrest in the capillaries of a distant organ [48, 49]. Experimental studies have shown that these CSCs or stem-like cells are more capable of escaping from the primary tumor (intravasation) and disseminating to the blood or lymphatic system [49-51]. Fortunately, our data also confirmed that SALL4 up-regulation worsen the vascular invasion in our study. Similarly, because of its related characteristics of CSCs, we hypothesized that SALL4 immuno-positive cells might lead to more tumor invasion and metastasis. Similar results were also confirmed by Shibahara or Yin, in which high SALL4 expressions related with more intrahepatic metastasis or advanced clinical stages respectively [29, 33]. Furthermore, our data also demonstrated that SALL4-positive expression was statistically associated with the disease free survival and overall survival in HCC. This finding strongly supported that high SALL4 might be served as an independent prognostic predictor for HCC patients.

In interpreting our results from our meta-analysis, there were still some limitations should be addressed. Firstly, IHC was the only applied method in our study and the cutoff values were defined differently. Secondly, the small amount of studies and case samples might relatively lead to higher heterogeneity. Thirdly, lacking the available data from our included studies, the potential relationships between SALL4 and some other clinicopathological features shall be further researched, such astumor size, liver cirrhosis, EpCAM and serum AFP. These key factors may help us better understand the molecular mechanisms of SALL4 in HCC. Finally, lack of directly obtained disease free survival data or survival data, we calculated DFS/OS from the available data or Kaplan-Meier curves, which might be less reliable. What's more, a longer period of DFS/OS rather than only 1 year might be more statistically significant. All these factors could potentially affect the results of our meta-analysis. Therefore, more well-designed studies or researches, which refer to the expression or mechanisms of SALL4, are needed for our deep understanding of CSCs in HCC.

Our meta-analysis is the preliminary one to explore the relationships between SALL4 expression and clinicopathological characteristics (or DFS/OS) in HCC. Our data indicated that SALL4 expressing level was not associated with the gender, histological differentiation and Child-Pugh score in HCC patients. However, high SALL4 statistically related to hepatitis B virus infection and vascular invasion, which led to a poorer patient survival in HCC. These findings suggested that SALL4 could be served as an efficient marker for prognostic indicator in HCC, and might be a promising target for future HCC therapies.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yu-Ting Kuang and Qiao-Ming Zhi, Department of General Surgery, The

First Affiliated Hospital of Soochow University, 188 Shizi Road, Suzhou 215006, China. Tel: +86 512 67780166; E-mail: sdyt_kuang@163.com (YTK); strexboy@163.com (QMZ)

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