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Original Article

The diagnostic value of suppressor of cytokine signaling-1 (SOCS-1) methylation for human hepatocellular carcinoma (HCC): a meta-analysis based on data from 15 studies

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Abstract: AIM: To assess the potential diagnostic value of suppressor of cytokine signaling-1 (*SOCS-1*) methylation in human hepatocellular carcinoma (HCC). Methods: Search results were filtered with inclusion criteria. Relative study data was collected. Quality of included studies was assessed by QUADAS2; sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) were calculated with 95% CI. A summary receiver operating characteristic (SROC) curve was used to assess the diagnostic power. Meta-regression was applied to analyze observed heterogeneity, and publication bias was evaluated with Deeks' test. Results: 807 HCC and 664 non-tumor samples were included. The sensitivity, specificity, PLR, NLR, and DOR of the *SOCS-1* methylation test were 0.619 (0.522-0.708), 0.803 (0.558-0.930), 3.148 (1.278-7.750), 0.474 (0.361-0.622), and 6.639 (2.213-19.917), respectively. The area under the curve (AUC) was 0.71 (0.67-0.75). Four factors of study were included in meta-regression and none of them were significant ($P > 0.05$). No significant publication bias was detected using Deeks' test ($P = 0.194$). Conclusion: Our results indicate that SOCS-1 methylation has a potential diagnostic value in HCC. *SOCS-1* methylation together with α-fetoprotein (AFP) may increase diagnostic sensitivity and specificity; however, further investigation is needed.

Keywords: Hepatocellular carcinoma, suppressor of cytokine signaling-1, methylation, tumor suppressor genes, meta-analysis

Introduction

Hepatocellular carcinoma (HCC) is a very common cancer worldwide whose incidence is increasing dramatically in many parts of the world. HCC is the sixth leading cause of cancer-related death [1]. Given the prevalence of viral hepatitis, including chronic hepatitis C (HCV) and hepatitis B (HBV) in China and India, the morbidity and mortality of HCC in South East Asia is among the highest in the world. Thus, HCC has become a very serious public health challenge. Since detection at early stage is limited, and the lack of awareness towards health check is frequent, HCC is more likely to be found in advanced stages, when prognosis is poor.

Methylation is one of the most important epigenetic alterations, essential for the control of gene expression. Changes in the pattern of DNA methylation are critical in cancer development [2]. Indeed, the aberrant methylation of tumor suppressor genes (TSG) is characteristic of different types of cancers [3-6]. Therefore, a variety of TSG have been the focus of research in order to understand the underlying molecular mechanism of carcinogenesis. Moreover, TSG are of particular clinical importance as they can be used as predictive biomarkers for early diagnosis or ongoing prognosis of human cancers [7-9].

SOCS-1, an intracellular protein that negatively regulates the JAK/STAT signaling pathway,

which is a principal cytokine signaling transduction pathway, has been widely studied. *SOCS-1* has been found to have essential roles in cell function, including apoptosis and tumor suppressing [10]. For instance, *SOCS-1* regulates cytokine expression and signaling pathways involving IL-6 and IFN-γ [11, 12].

Recent studies have proven the relevance of epigenetic changes in the development of HCC. However, the potential diagnostic or therapeutic value of TSG in HCC remains unclear [13]. Up to now, there is a lack of a meta-analysis on the potential diagnostic value of SOCS-1 in HCC. Herein, we performed a meta-analysis study to investigate the diagnostic sensitivity and specificity of SOCS-1 methylation in liver tissues and tumor-adjacent tissues from a cohort of HCC patients and case control studies.

Methods

Study selection

PRISMA statement guidelines were followed for conducting and reporting meta-analysis data [14]. We searched for relevant articles in PubMed, EMBASE, Web of Science, and the Cochrane Library until March 20th, 2015. Keywords used in the title and abstract were: "hepatocellular carcinoma/hepatocellular carcinomas/hepatoma/hepatomas/HCC/liver cancer/liver cancers/liver cell carcinomas" and "SOCS1/SOCS-1/suppressor of cytokine signaling 1/STAT-induced STAT inhibitor 1/SSI1/JAK binding protein/JBP". We tried to use "genome-wide", "genome-wide association study (GWAS)" or such keywords instead of SOCS-1 beforehand so that we would not miss any important study, but all relevant results were also included in the keywords mentioned above. Furthermore, we tried to use these keywords in the three major Chinese databases: Wanfang, CNKI, Weipu, obtaining only one search result which was directly ruled out after reading the abstract. Therefore, this result is not included into the figure. All search results were also brought for further manual identification (see below). All studies were assessed by two independent reviewers.

Inclusion and exclusion criteria

We manually filtered the search results with the following inclusion criteria: 1) liver tissue samples for the methylation test were obtained from HCC patient; 2) there was a control group which was obtained from the non-tumor liver tissue in HCC patients; 3) since every study would be an epigenetic research, the study could be either a case-control study or a cohort study fulfilling the requirements above; 4) all liver tissues were obtained from adult patients; and 5) the study was published in English.

We excluded studies lacking HCC patients and only using HCC cell lines. Also, studies which met the search criteria, but data on SOCS-1 was incomplete or even not qualified were excluded. We ignored the difference between health control and the control group with chronic liver disease, since it was not the focus of our research. Finally, two independent reviewers made a final decision on inclusion or exclusion in the study, and identified any applicable conflicts.

Data extraction and quality assessment

The following data were extracted: author's name, year of publication, sample type, test method, sequence, geographic area, methylation number, and total number in HCC, non-tumor samples, and in recurrence and survival samples. All data were extracted independently by two reviewers. Any conflict was resolved after discussion.

The quality of each study was assessed with the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) [15]. The risk of bias in several features of the study was judged and rated. The QUADAS-2 was completed by revman5.3.

Statistical analysis

All raw methylation data were converted into the following: true-positive (TP), false-positive (FP), false-negative (FN), and true negative (TN). TP refers to the HCC samples whose methylation test was positive. FP refers to the non-tumor samples whose methylation test was positive. FN was defined as the HCC samples whose methylation test was negative. Finally, TN refers to the non-tumor samples whose methylation test was negative. Sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) were worked out with 95% CI. Forrest Plots were illustrated for sensitivity,

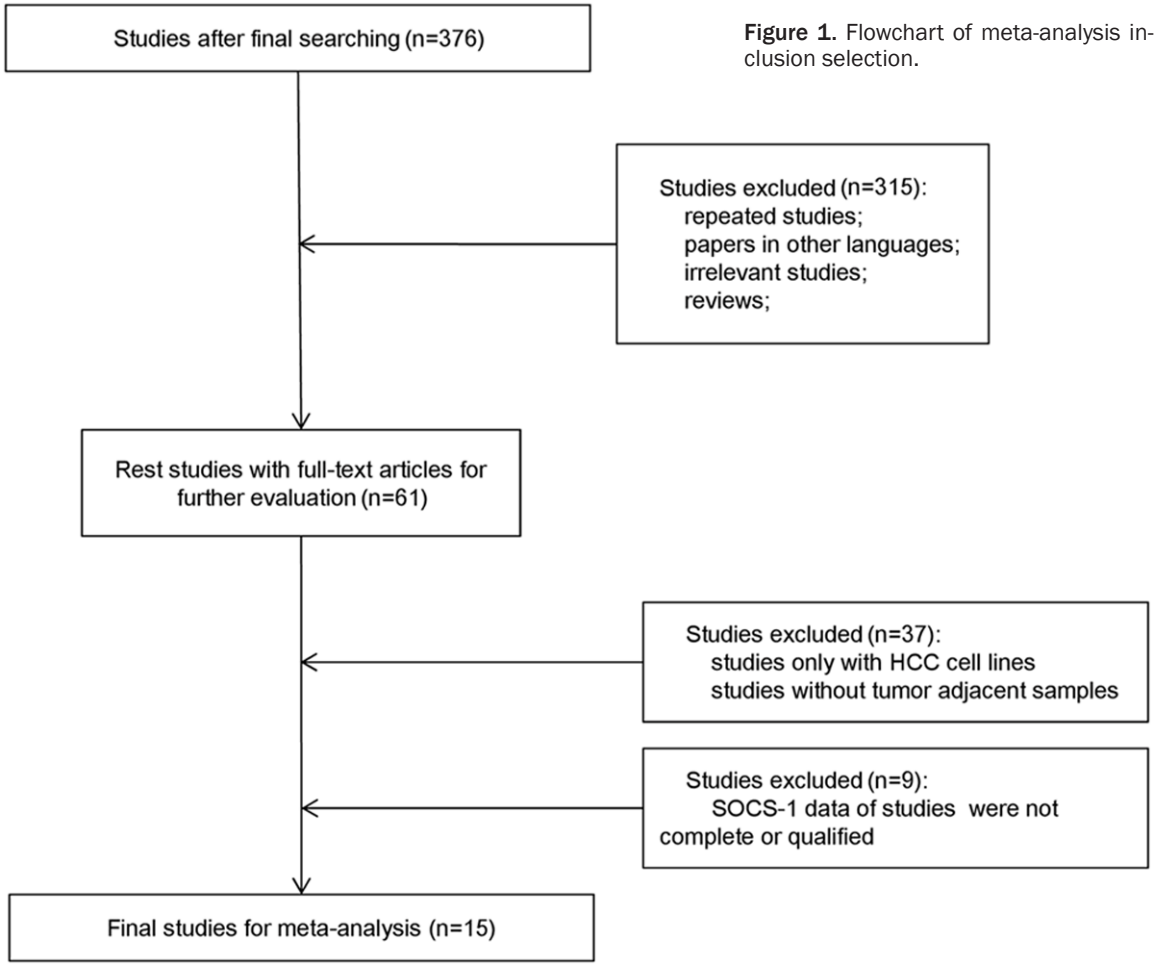

Figure 1. Flowchart of meta-analysis inclusion selection.

Table 1. Characteristics of the selected studies

Author	Year	Region	Method	Sample origin	Sample storage	Diagnosis	Samples (n)	Adjacent Controls (n)
Calvisi DF	2006	Asian	MSP	hepatectomy	not mentioned	HCC	80	80
Chu PY	2010	Asian	MSP	hepatectomy	-80°C	HCC	46	46
Formeister EJ	2010	Asian	MSP	hepatectomy	-80°C	HCC	43	45
Lehmann U	2005	Europe	real-time-PCR	hepatectomy	-80°C + formalin	HCC	41	21
Lehmann U	2007	Europe	real-time-PCR	biopsy	not mentioned	HCC	10	17
Li B	2010	Asian	MSP	hepatectomy	-80°C	HCC	115	48
Lou C	2009	Asian	MSP	hepatectomy	-80°C	HCC	60	60
Miyoshi H	2004	Asian	MSP	hepatectomy	-80°C	HCC	10	10
Nishida N	2008	US	COBRA	hepatectomy	-80°C	HCC	75	77
Nomoto S	2007	Asian	MSP	hepatectomy	-80°C	HCC	74	51
Okochi O	2003	Asian	MSP	hepatectomy	-80°C	HCC	50	50
Saelee P	2012	Asian	MSP	not mentioned	-80°C	HCC	29	22
Vivekanandan P	2008	US	MSP	hepatectomy	-80°C	HCC	32	4
Yang B	2003	US	MSP	hepatectomy + biopsy	formalin & ethanol-based fixative	HCC	26	15
Zhang X	2014	Asian	MethylScreen	hepatectomy	not mentioned	HCC	116	116

specificity, PLR, NLR, and DOR. SROC curve was illustrated and area under the curve (AUC) was calculated to assess the diagnostic value.

Publication bias was assessed by Deek's test and was displayed in the funnel plot. To assess the heterogeneity, Q test and I^2 was performed

Table 2. SOCS-1 primer sequences used in methylation test of the selected studies

Author	Year	Method	Forward	Reverse
Calvisi DF	2006	MSP	not available	not available
Chu PY	2010	MSP	5'-TTGTTCGGAGGTCGGATTT-3'	5'-ACTAAAACGCTACGAAACCG-3'
Formeister EJ	2010	MSP	5'-TTCGCGTGTATTTTTAGGTCGGTC-3'	5'-CGACACAACTCCTACAACGACG-3'
Lehmann U	2005	RT-PCR	not available	not available
Lehmann U	2007	RT-PCR	not available	not available
Li B	2010	MSP	5'-TTCGCGTGTATTTTTAGGTCGGTC-3'	5'-CGACACAACTCCTACAACGACCG-3'
Lou C	2009	MSP	5'-TTCGCGTGTATTTTTAGGTCGGTC-3'	5'-CGACACAACTCCTACAACGACCG-3'
Miyoshi H	2004	MSP	5'-TTCGCGTGTATTTTTAGGTCGGTC-3'	5'-CGACACAACTCCTACAACGACCG-3'
Nishida N	2008	COBRA	not available	not available
Nomoto S	2007	MSP	5'-CGCGCGGGGTTTTCGTAGTA-3'	5'-CTAACTCCAACCGTCCGACC-3'
Okochi O	2003	MSP	5'-TTCGCGTGTATTTTTAGGTCGGTC-3'	5'-CGACACAACTCCTACAACGACCG-3'
Saelee P	2012	MSP	5'-GGATGGTAGTCGCGAGAGTTTC-3'	5'-ACGCGACGCTAACGCAACG-3'
Vivekanandan P	2008	MSP	5'-TCGTTCGTACGTCGATTATC-3'	5'-AAAAAAATACCCACGAACTCG-3'
Yang B	2003	MSP	5'-TTCGCGTGTATTTTTAGGTCGGTC-3'	5'-CGACACAACTCCTACAACGACCG-3'
Zhang X	2014	MethylScreen	5'-GCGTGAAGATGGCCTCGGGACC-3'	5'-GATGCGCTGGCGGCACAGCTCC-3'

Table 3. QUADAS2 assessment of the selected studies

Study	Year	Risk of bias				Applicability concerns		
		Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Calvisi DF	2006	-[a]	?[b]	+[c]	+	?	+	+
Chu PY	2010	-	?	+	+	?	+	+
Formeister EJ	2010	-	?	+	?	?	+	+
Lehmann U	2005	-	-	+	?	?	-	+
Lehmann U	2007	-	?	+	-	?	?	+
Li B	2010	-	?	+	+	+	?	+
Lou C	2009	-	?	+	+	?	?	+
Miyoshi H	2004	-	?	+	-	?	?	+
Nishida N	2008	-	-	+	-	-	?	+
Nomoto S	2007	-	?	+	?	?	+	+
Okochi O	2003	-	?	+	+	?	?	+
Saelee P	2012	-	?	+	-	?	?	+
Vivekanandan P	2008	-	?	+	+	-	?	+
Yang B	2003	-	?	+	-	?	?	+
Zhang X	2014	-	-	+	?	?	-	+

[a]: - high risk; [b]: + low risk; [c]: ? unknown risk.

and meta-regression was also included for further discussion. The data was analyzed by STATA 12.0 (STATA Corp., USA). All *p* values were two-tailed and considered significant if $P < 0.05$.

Results

Study statistics

We identified 376 studies during the initial search. We then excluded 315 repeated studies, papers in other languages, irrelevant studies, and reviews after reading the abstract. Out of the remaining 61 papers, 37 were excluded after analyzing the full text because they contained no patient tissues but only HCC cell lines, or were studies without non-tumor sample in HCC patients. Additionally, we excluded nine studies in which SOCS-1 data were not complete or qualified (**Figure 1**). Finally, we selected 15 studies according to the inclusion criteria [16-30]. 807 HCC and 664 non-tumor samples from HCC patients were included for meta-analysis. The summarized data was collected from 3 areas all over the world, using 3

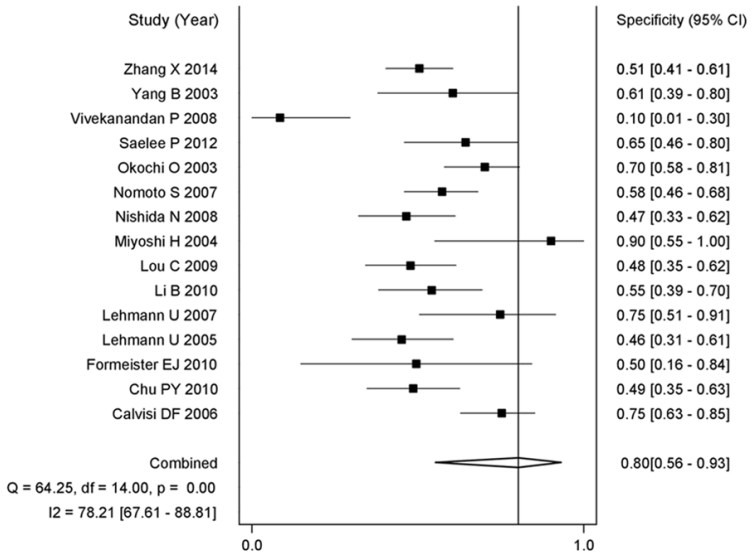

Figure 2. Forest plot showing the specificity of SOCS-1 methylation in the diagnosis of HCC.

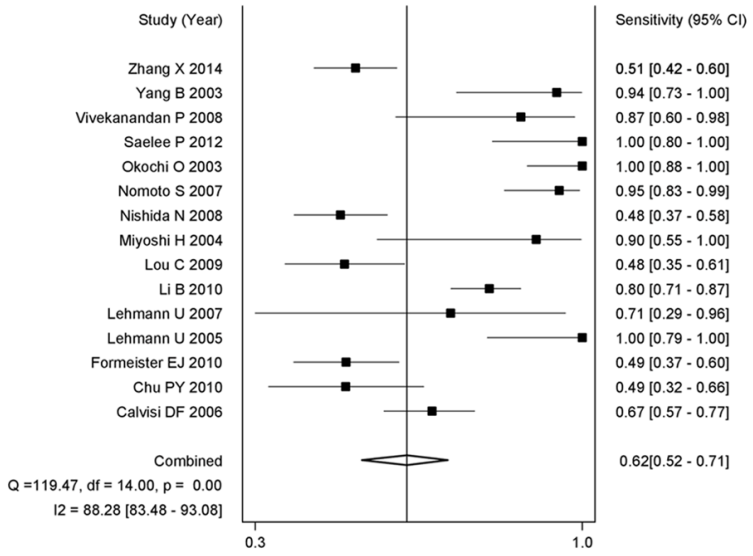

Figure 3. Forest plot showing the sensitivity of SOCS-1 methylation in the diagnosis of HCC.

different methods, and were published from 2003 to 2013. Importantly, all studies used liver tissues after hepatectomy and/or liver biopsy; 11 studies used methylation-specific PCR (MSP), two studies used real-time (RT)-PCR, one study used MethylScreen which is also based on RT-PCR, and one study used combined bisulfite restriction analysis (COBRA). Main characteristics of the included studies are shown in **Table 1**. The methylation test sequences are listed in **Table 2**.

Quality assessment

All studies included in our research were case control, which may have increased the risk in patient selection bias. Each study consisted of diagnosed patients using the pathology evaluation of the HCC tissue, so that the risk of bias of the reference standard for all studies was considered be 'low'. The criteria are summarized in **Table 3**.

Diagnostic accuracy analysis

The sensitivity and specificity of the SOCS-1 methylation test in these 15 studies was 0.619 (95% CI: 0.522-0.708) and 0.803 (95% CI: 0.558-0.930). Furthermore, PLR, NLR, and DOR were 3.148 (95% CI: 1.278-7.750), 0.474 (95% CI: 0.361-0.622), and 6.639 (95% CI: 2.213-19.917), respectively. The χ^2 value of sensitivity, specificity, PLR, NLR, and DOR were 119.47 ($P < 0.01$), 64.25 ($P < 0.01$), 103.87 ($P < 0.01$), 86.54 ($P < 0.01$), and 9.2e+06 ($P < 0.01$), respectively, which indicated significant heterogeneity between studies. The forest plots of the data above are shown in **Figures 2**-**6**. The SROC curve is shown in **Figure 7**. Additionally, the AUC was 0.71 (0.67-0.75). Altogether, these results indicated that SOCS-1 may have a relatively good diagnostic accuracy.

Heterogeneity, meta-regression, and publication bias

The general chi-square value obtained from our study was 185.503 ($P = 0.000$) and the I-square was 98.92 (98.39-99.47). The above mentioned results indicated that there was significant heterogeneity between studies. Furthermore, we made a meta-regression with the goal to find out the heterogeneity source. As a representative index in the study, DOR was selected

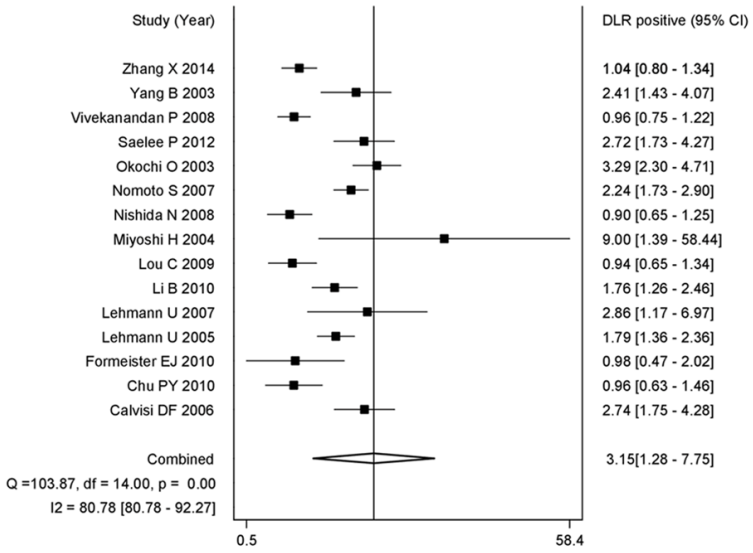

Figure 4. Forest plot showing the positive likelihood ratio (PLR) of SOCS-1 methylation in the diagnosis of HCC.

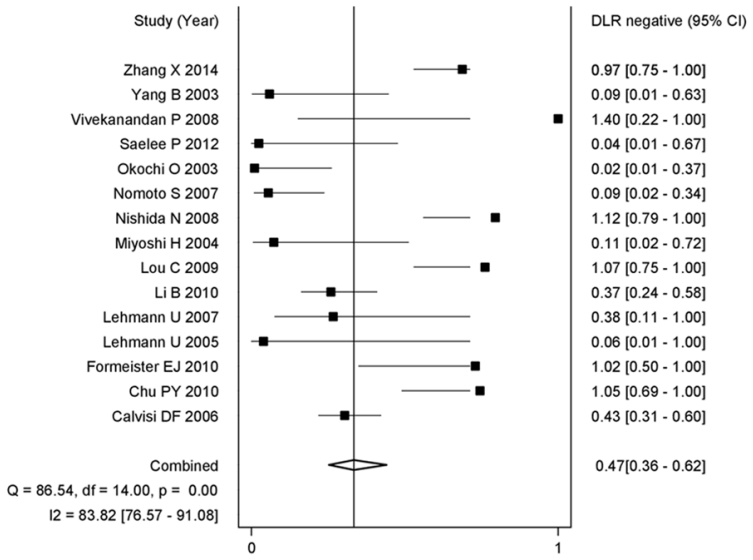

Figure 5. Forest plot showing the negative likelihood ratio (NLR) of SOCS-1 methylation in the diagnosis of HCC.

for meta-regression. In these studies, MSP was the main method for the detection of methylation. In addition, the living area, sampling methods, and storage method may also affect the accuracy for the diagnosis of HCC. Therefore, we chose these factors for the meta-regression. As the results showed, the total estimate of variance Tau-squared dropped from 1.3270 to 0.5990, which might explain 54.86% of the (inner) heterogeneity. However, none of these factors were considered to be statistically significant (**Table 4**). For the detection of publication bias, we used the Deek's test. The result showed no statistical significance ($P = 0.194$) with the tunnel plot shown (**Figure 8**).

Subgroup analysis

Since we hypothesized that SOCS-1 methylation may have diagnostic value in the survival and recurrence of HCC, we next divided these studies into two subgroups. However, we were able to include only two studies in each of these two subgroups, and the data were not sufficient to make meta-analysis. Thus, we manually summarized the statistical data and performed a Chi-square test. Our data shows that Chi-square of survival and recurrence was 0.354 and 0.399, and *p*-value was 0.614 and 0.67, respectively. At the same time, the DOR of survival and recurrence was 1.387 (0.391-3.398) and 1.308 (0.488-2.584). All of these data indicate that SOCS-1 has no value in the prognosis of survival and recurrence of HCC after hepatectomy.

Discussion

Many studies have targeted the function of SOCS-1 in a variety of cancers, including HCC. It is now accepted that aberrant methylation of SOCS-1 is a common feature of HCC. However, the diagnostic value of SOCS-1 has not been determined and a meta-analysis has not been performed to date. AFP has been the most commonly used marker for HCC. Nonetheless, pathological tests are the most direct and reliable way of diagnosing HCC, thus they are still the reference standard for other tests. Due to the limitations of pathological tests, health professionals rely on the combination of AFP and other tests, including imaging tests, to diagnose HCC in clinical practice. However, the sensitivity of AFP is suboptimal [31]. Many HCC patients are diagnosed in the late stage in part

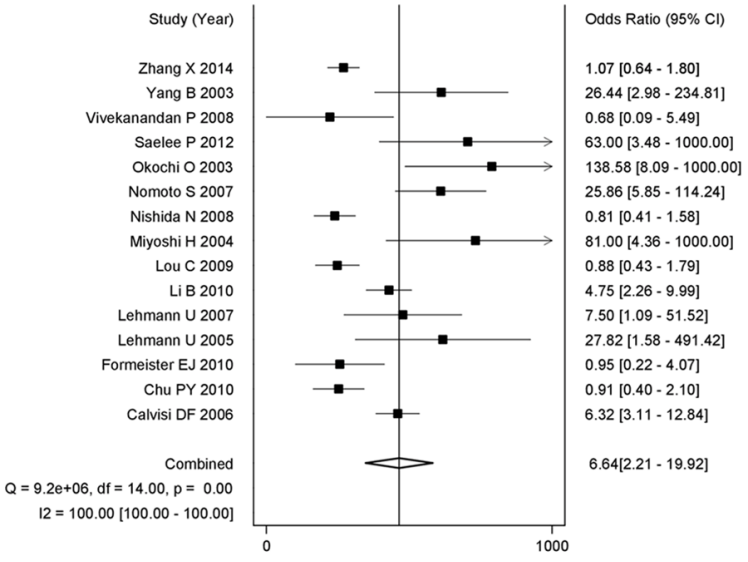

Figure 6. Forest plot showing the diagnostic odds ratio (DOR) of SOCS-1 methylation in the diagnosis of HCC.

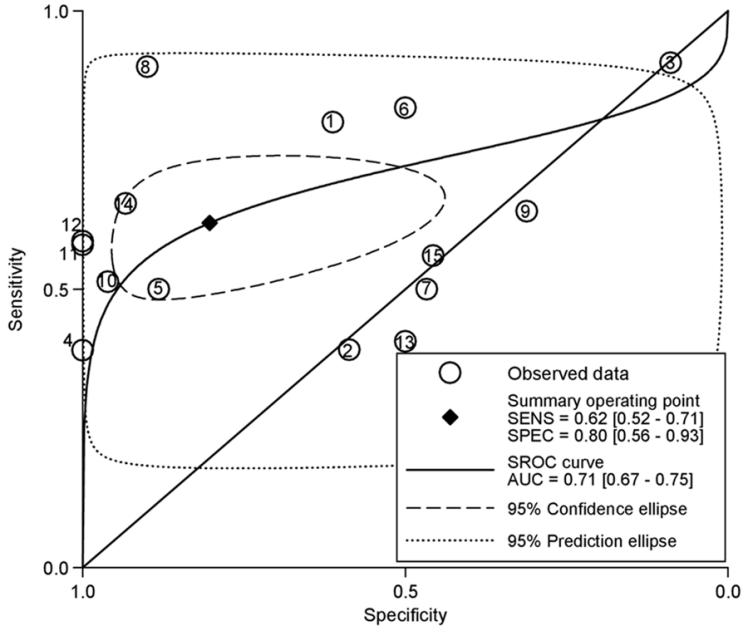

Figure 7. Summary receiver operation characteristic curve with Confidence and Predictive Ellipses for SOCS-1 methylation test of the selected studies which numbered by their order in **Table 1**.

because conventional methods fail to detect HCC at earlier stages in development. Thus, novel methods for the detection and early diagnosis of HCC are needed.

In our analysis, SOCS-1 methylation showed good sensitivity and specificity. Since AFP has a very good specificity, SOCS-1 methylation could be combined with AFP, increasing the sensitivity and specificity of HCC detection at early stages of development. One drawback of our analysis is the fact that we only investigated SOCS-1 methylation status in the liver tissue, since relevant studies in serum are not sufficient. The diagnostic value of SOCS-1 methylation status in serum for HCC still needs further investigation.

SOCS are a family of genes, which are cytokine-inducible negative regulators of cytokine signaling. As the first member of this family, SOCS-1, also known as STAT-induced STAT inhibitor (SSI1) or JAK binding protein (JBP), plays an important role in negative regulation in the JAK/STAT pathway [32-34]. Increasing evidence has demonstrated the molecular mechanism of SOCS-1 in gastric cancer, prostate cancer, melanoma, colorectal cancer, and other kinds of cancers [35, 36]. In HCC, most researchers have demonstrated aberrant methylation of SOCS-1 compared with the tumor adjacent tissues [17, 23, 29]. However, few reports have addressed the potential diagnostic value of SOCS-1 methylation.

With recent developments in detection technology, faster and easier methods are blooming for either qualitative or quantitative detection of SOCS-1 methylation. COBRA, Methylight, Methylscreen, quantitative MSP, and other methylation real-time PCR methods provide more accurate ways to detect methylation and are able to quantify the methylation levels of SOCS-1 in each sample for further study [19, 24, 26, 37, 38]. However, it should be noted that the procedures, primers, and the cut-off values from different studies might vary. Challenges arise as we try to draw conclusions from studies with

Table 4. Meta-regression results of meta-analysis

Source	Co-efficient (95% CI)	SE	T	P
Method	-0.7893147 (-2.137002, -0.5583722)	0.604849	-1.3	0.221
Area	0.7170803 (-1.531292, 2.965453)	1.009081	0.71	0.494
Origin	-1.280584 (-5.320215, 2.759047)	1.813007	-0.71	0.496
Storage	-.1093016 (-2.658334, 2.43973)	1.144018	-0.1	0.926

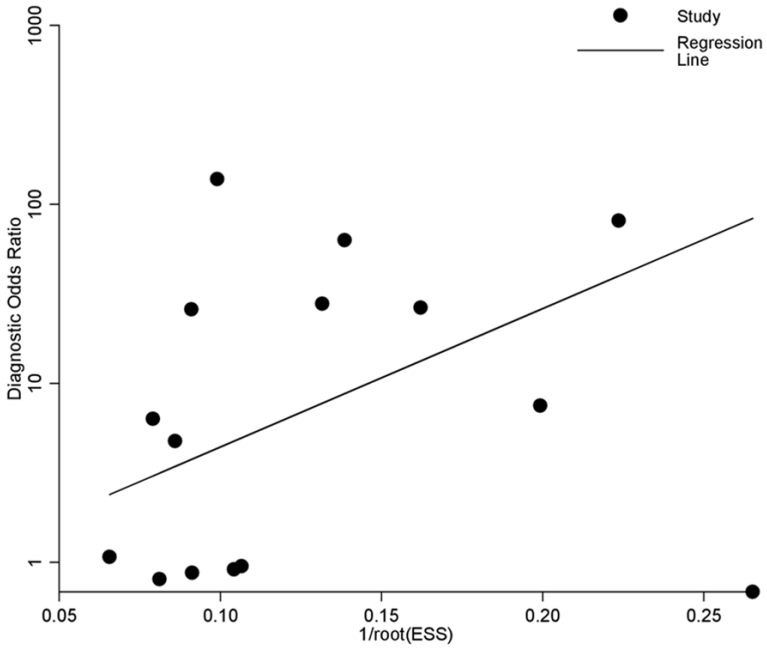

Figure 8. Assessment of publication bias in SOCS-1 methylation test of the selected studies with Log odds ratio versus 1/sqrt (Effective sample size) (Deeks test).

different methods or same method with different procedures, primers, and cut-off values. Although traditional MSP may have an increased risk for false positives and low quantitative accuracy, MSP remains a basic and popular qualitative method, which has a relative intuitive and clear criteria for simply 'positive' and 'negative', and can reduce the heterogeneity between studies [39]. Nevertheless, concerns exist since the MSP methodology is not strictly identical between studies. A standard protocol, which could be universally accepted, to prevent bias in studies would be needed for a biomarker to be further used in clinical practice. If more studies with quantitative and unified test methods could be included, the heterogeneity and bias caused can be greatly reduced, and a more convincing conclusion can be drawn.

As the summarized data showed, there was significant heterogeneity between studies according to the results including I-square and Chi-square tests. Next, we performed the meta-regression trying to identify the source of this heterogeneity. Unfortunately, none of the factors we included were statistically significant, and thus, we were able to interpret only about half of the heterogeneity. The other half still needs further exploration. On the other hand, we did not find a significant publication bias after the Deek's test either. It should be noted that all selected studies were case-control studies, since the study was designed to have all HCC samples and tumor adjacent normal liver tissues, it is very likely to have bias in patient selection. At the same time, the percentage of primary diseases varies between studies, with variations also in the test type, procedures, primers, and the cut-off values mentioned before, all of which together may account for the heterogeneity. In addition to these findings, it has been reported that the time of sampling may affect the methylation level, but we were not able to get enough relevant data to analyze this variable [40]. Besides these factors, the limited number of selected studies and samples led to the impossibility of performing further analysis. This limitation may affect the selection bias and thus play an important role in the heterogeneity.

Another limitation of the present studies is that all of the chosen studies were based on the samples obtained after hepatectomy. Such invasive test methods are not suitable for conventional regular HCC screening. Serum tests together with imaging tests are vital for the diagnosis of HCC at early stages of the disease. Novel peripheral blood test methods are necessary to increase diagnostic sensitivity together with AFP. Although we found no relevant study based on serum SOCS-1 methylation levels, some studies have already used peripheral blood to test SOCS-1 methylation in other liver

diseases [41, 42]. Further relevant studies with easier peripheral blood tests are essential for clinical practice.

In the subgroup study, we performed Chi-square test, which indicated that SOCS-1 may not be a suitable marker of prognosis of survival and recurrence of HCC after hepatectomy, in accordance with a previous study [37].

In conclusion, SOCS-1 methylation is a potential diagnostic biomarker, but needs further targeted high-quality investigations to discover its value in clinical practice. Overall, the combination of SOCS-1 methylation test, AFP, and other clinical parameters may be of interest for the early detection of HCC.

Disclosure of conflict of interest

None.

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