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ORIGINAL ARTICLE

Aberrant promoter methylation of SOCS-1 gene may contribute to the pathogenesis of hepatocellular carcinoma: a meta-analysis

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Summary

Purpose: *We conducted this meta-analysis of published case-control studies aiming to evaluate the relationship between abnormal suppression of cytokine signaling-1 (SOCS-1) promoter methylation and the risk of hepatocellular carcinoma (HCC).*

Methods: *Relevant studies were retrieved from PubMed, Embase, Web of Science, Cochrane Library, Chinese National Knowledge Infrastructure (CNKI) and China Biological Medicine (CBM) databases without language restrictions. Meta-analysis was conducted using the STATA 12.0 software. We calculated odds ratio (OR) and its 95 % confidence interval (95% CI) to estimate the correlations.*

Results: *Sixteen case-control studies with a total of 941 HCC patients and 114 individuals with benign liver diseases met our inclusion criteria. Our results demonstrated that the frequency of SOCS-1 promoter methylation in cancer tissues was significantly higher than in adjacent non-tumorous tissues and benign tissues (cancer tissue vs adjacent tissue: OR=3.05, 95%CI 1.62-5.77, p=0.001; cancer tissue vs benign tissue: OR=11.55, 95%CI 5.93-22.49, p=0.000). Subgroup analyses by ethnicity, detecting method and sample size also suggested that abnormal SOCS-1 promoter methylation was correlated to the risk of HCC in the majority of these subgroups.*

Conclusion: *Our findings provide empirical evidence that abnormal SOCS-1 promoter methylation may contribute to the pathogenesis of HCC. Thus, detection of SOCS-1 promoter methylation may be a valuable diagnostic biomarker for HCC.*

Key words: *hepatocellular carcinoma, meta-analysis, promoter methylation, SOCS-1*

Introduction

HCC is one of the most common malignancies worldwide and the second leading cause of cancer-related mortality in China. About 600,000 people are diagnosed with HCC every year [1,2]. Despite significant improvements in the diagnostic methods and treatments for HCC, the prognosis remains unsatisfactory and the 5-year survival rate is only 3-5% according to cancer registries in developing countries. The main risk factors associated with HCC are chronic hepatitis and cirrhosis by infection with either hepatitis B virus (HBV) or hepatitis C virus (HCV) [3].

As it is well known, tumorigenesis is caused by activation of proto-oncogenes and inactivation of tumor suppressor genes (TSGs). Recent advances in genetics have proved that CpG island

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methylation in the promoter regions of TSGs is a common epigenetic mechanism of transcriptional regulation and is involved in the deregulation of many cellular processes that lead to the initiation and progression of human cancers [4-6]. Moreover, evidence from epidemiological studies has indicated the silencing of multiple TSGs by gene promoter methylation may contribute to the pathogenesis of HCC [7-9]. Such epigenetic findings also have been observed in non-tumorous liver tissues of HCC patients, which support the concept that methylation-induced epigenetic silencing may be involved in the early stages of HCC [10].

The Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway mediates signaling by cytokines, which play a key role in survival, proliferation and differentiation of various cell types. To date, the potential role of the JAK/STAT pathway in carcinogenesis has been proposed in many kinds of tumors [11]. There has been a large amount of evidence demonstrating that the JAK/STAT pathway may be involved in carcinogenesis. The constitutive activation of the JAK/STAT pathway has been observed in numerous transformed cells [12]. The cytokine signalling (SOCS) family has been identified as a negative feedback protein of this cytokine-induced signalling pathway. SOCS protein can be activated by STATs and negatively regulate the JAK/STAT pathway by directly inhibiting the JAKs or blocking the access of the STATs [13]. The SOCS family consists of eight members, including SOCS1-7 and CIS (cytokine inducible SH2-containing protein). SOCS-1 is a negative regulator of IL-6 signals. The silencing of SOCS-1 leads to constitutive activation of the JAK/STAT pathway. Without negative feedback by SOCS-1, the target genes and downstream pathways are strongly activated [14]. Although the precise mechanism by which SOCS-1 protein regulates cytokine signaling has been studied to an extent, its biological role continues to be investigated. There are several lines of evidence supporting the idea that SOCS-1 expression is suppressed through aberrant promoter methylation of the CpG islands and thus leading to carcinogenesis due to persistent activation of JAK/STAT pathway [15,16].

Up to now, a large number of human studies have suggested that SOCS-1 promoter methylation may play a crucial role in the susceptibility to HCC , but contradictory results have also been reported due to limited sample size [9,16-19]. Though Liu et al. [20] published a meta-analysis evaluating the association of SOCS-1 promoter methylation and HCC risk, we added 5 more studies and evaluated the quality of each included study by a insured score, making our results of meta-analysis more reliable and robust. In consideration of the conflicting evidence on this issue, we performed a meta-analysis of all available case-control studies to determine the relationships between SOCS-1 promoter methylation and hepatocarcinogenesis.

Methods

Literature search

To identify all potentially eligible studies, we searched PubMed, Embase, Web of Science, Cochrane Library, Chinese National Knowledge Infrastructure (CNKI) and China Biological Medicine (CBM) databases (last updated on April 9th, 2015) without language restrictions. We used the following search terms: ("SOCS1" or "SOCS-1" or "suppressor of cytokine signaling 1") and ("methylation" or "DNA methylation" or "hypermethylation" or "promoter methylation") and ("HCC" or "hepatocellular carcinoma" or "liver cancer" or "liver neoplasm" or "hepatoma"). A manual search for references identified in the included articles was also performed to find other potential articles. In such cases, if the retrieved articles did not include sufficient data, an email was sent to the corresponding authors to get additional information. If our request was refused or no reply was received, the item was excluded.

Selection criteria

The following criteria were used to determine eligibility for including studies: (1) studies must address the correlation between SOCS-1 promoter methylation and the pathogenesis of HCC; (2) diagnosis of HCC patients should be confirmed by histopathologic examination; (3) studies should provide sufficient information to estimate OR and 95% CI. The exclusion criteria included the following: (1) letters, reviews, case reports, conference abstracts, editorials, and expert opinions; (2) publications regarding in vitro studies, cell lines, and human xenografts were also excluded. Articles that met the exclusion criteria and did not meet the inclusion criteria were excluded. When authors published several studies using the same subjects, either the most recent or largest sample size publication was included.

Data extraction and methodological assessment

Two authors (Zhao and Zhou) used a standardized form to extract the following data from included studies: language of publication, the first author's surname, publication year of the article, geographical location, ethnicity of subjects, detection method of methylation,

Table 1. Main characteristics and methodological quality of all eligible studies

First author	*Year*	*Country*	*Ethnicity*	*Method*	*Total*	*Cancer*		*Adjacent*		*Benign*		*Material*	NOS score
						M	*U*	*M*	*U*	*M*	*U*		
Okochi [16]	2003	Japan	Asian	MSP	100	30	20	0	50	-	-	Frozen Tissue	6
Yang [9]	2003	USA	Caucasian	MSP	65	33	18	1	6	1	6	PPFE Tissue	8
Miyoshi [17]	2004	Japan	Asian	MSP	16	9	1	-	-	1	5	Frozen and PPFE	7
Lehmann [18]	2005	Germany	Caucasian	qMSP	90	38	3	19	2	23	5	Frozen and PPFE	7
Liu [19]	2006	China	Asian	MSP	98	39	11	24	24	-	-	Frozen Tissue	7
Nomoto [26]	2007	Japan	Asian	MSP	142	38	36	2	49	0	17	Frozen Tissue	6
Lou [27]	2008	China	Asian	MSP	130	35	25	32	28	3	7	Frozen Tissue	8
Vivekanandan [28]	2008	USA	Caucasian	MSP	40	13	23	2	2	-	-	Frozen Tissue	7
Kiran [29]	2009	India	Asian	MSP	43	23	0	-	-	18	2	Frozen Tissue	6
Chu [30]	2010	China	Asian	MSP	92	18	28	19	27	-	-	Frozen Tissue	7
Formeister [31]	2010	USA	Asian	MSP	98	39	4	41	4	0	10	Frozen Tissue	7
Li [32]	2010	China	Asian	MSP	174	95	20	24	24	1	10	Frozen Tissue	8
Sakamoto [33]	2010	Brazil	Caucasian	qMSP	25	8	12	-	-	0	5	PPFE Tissue	5
Nishida [34]	2012	Japan	Asian	COBRA	354	66	111	24	153	-	-	Frozen and PPFE	9
Saelee [35]	2012	Thailand	Asian	MSP	58	17	12	0	29	-	-	Frozen Tissue	6
Zhang [36]	2014	China	Asian	qMSP	232	65	51	63	53	-	-	Frozen Tissue	7

MSP: methylation-specific polymerase chain reaction, *qMSP*: quantitative methylation-specific polymerase chain reaction, *COBRA*: combined bisulfite restriction analysis, *PPFE*: formalin-fixed, paraffin-embedded, *NOS*: Newcastle-Ottawa Scale

total number of cases, methylation frequencies and type of samples.

The same authors evaluated the methodological quality of the included studies using the Newcastle-Ottawa Scale (NOS) criteria, independently [21]. The NOS criteria are scored based on three aspects: (1) subject selection: 0-4, (2) comparability of subject: 0-2, and (3) clinical outcome: 0-3. Total NOS scores ranged from 0 to 9, with scores ≥7 indicating good quality.

Statistics

Statistical analyses were conducted using the STATA statistical software (version 12.0, Stata Corporation, College Station, TX, USA). We calculated the ORs and their 95 %CI to estimate the correlation between SOCS-1 promoter methylation and the pathogenesis of HCC. Z test was used to estimate the statistical significance of the pooled ORs. P values tailed less than 0.05 were considered statistically significant. The frequency of SOCS-1 methylation in HCC tissues was compared with the

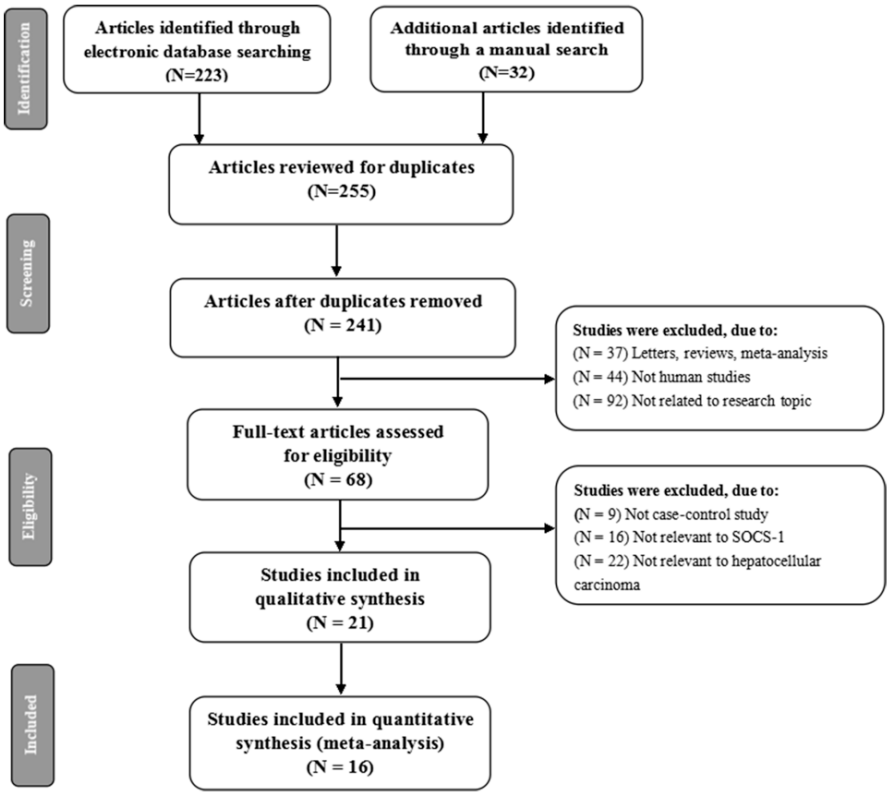

Figure 1. Flow chart showing the study selection procedure. Sixteen case-control studies were included in this meta-analysis.

corresponding adjacent non-tumorous tissues and resected tissues from benign liver diseases, respectively. Heterogeneity among studies was estimated with the Cochran's Q statistic and the I2 metric [22,23]. When $p<0.1$ for Q statistic or $I^2>50\%$, indicating presence of significant heterogeneity, the random-effect model was applied. Otherwise, the fixed-effect model was used [24]. We explored potential sources of heterogeneity using subgroup analyses. In order to evaluate the influence of a single study on the overall estimate, a sensitivity analysis was performed. One single study was excluded each time to reflect the influence of the individual dataset to the pooled ORs. Funnel plots, Begg's test and Egger's linear regression test were applied to investigate publication bias [25]. Asymmetric funnel-shaped plots or $p<0.05$ were considered as showing existence of publication bias.

Results

Baseline characteristics of the included studies

The flow chart of study selection is illustrated in Figure 1. Based on the predetermined search strategies, a total of 255 articles were identified. We reviewed the titles and abstracts of all papers and excluded 187 articles. After systematically examining the remaining full texts, we excluded another 47 articles. Five studies were also excluded due to lack of data integrity. Finally, 16 case-control studies [9,16-19,26-36], which enrolled a total of 941 HCC patients and 114 individuals with benign liver diseases met our inclusion criteria and were included for quantitative data analysis. The publication years of eligible studies ranged from 2003 to 2014. Of the 16 studies, 12 were conducted among Asian populations and the other 4 among Caucasians. Frozen tissues and formalin-fixed, paraffin-embedded (PPFE) tissues were variously used to detect SOCS-1 promoter methylation in these studies. 7 studies used adjacent non-tumor tissue as control, 3 studies used benign hepatic diseases such as chronic hepatitis and liver cirrhosis as control, and 6 studies used both. The status of SOCS-1 promoter methylation was determined by methylation-specific polymerase chain reaction (MSP) for most studies. The NOS scores of all included studies were ≥5. Detailed characteristics and methodological qualities of the included studies are summarized in Table 1.

Quantitative data synthesis

The random-effect model was applied for the

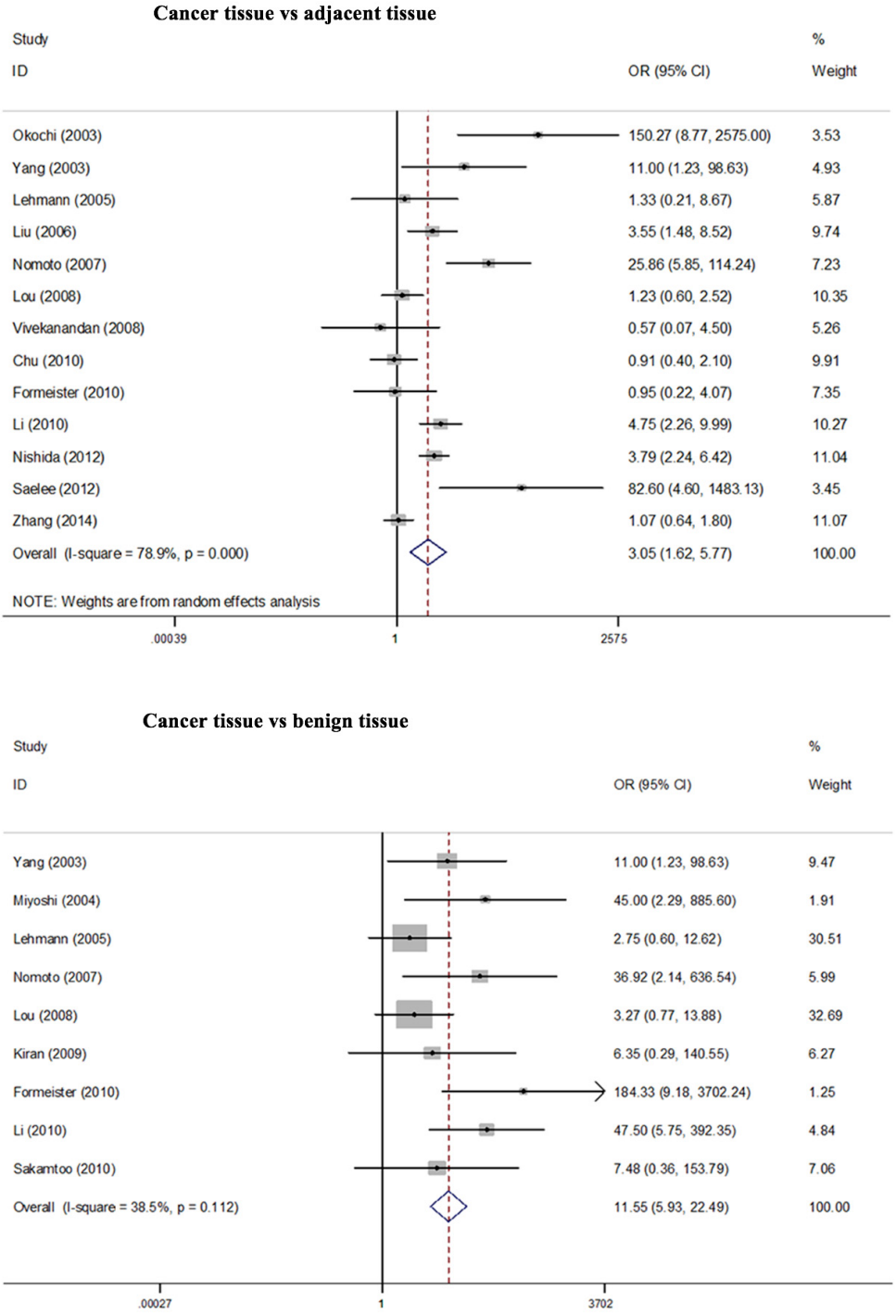

Figure 2. Forest plots for the relationships between SOCS-1 promoter methylation and the pathogenesis of hepatocellular carcinoma. The center of each square represents the OR, the area of the square is the number of samples, and thus the weight used in this analysis and the horizontal line indicate the 95% CI.

assessment of the association between SOCS-1 promoter methylation and the risk of HCC in comparison to cancer tissues and adjacent tissues, because significant heterogeneity was observed (I^2=78.9%, P_H=0.000). On the contrary, the fixed-effect model was used for comparison of cancer tissues and benign tissues when absence of heterogeneity was noticed (I^2=38.5%, P_H=0.112) . Our results demonstrated that the frequency of SOCS-1 promoter methylation in cancer tissues was significantly higher than in adjacent non-tumor tissues and benign tissues (cancer tissue vs adjacent tissue: OR=3.05, 95%CI 1.62-5.77, p=0.001; cancer tissue vs benign tissue: OR=11.55, 95%CI 5.93-22.49, p=0.000) (Figure 2).

To further comprehensively evaluate the as-

Table 2. Pooled ORs and 95% CIs of stratified meta-analysis

Stratification	*N*	*Cancer tissue vs adjacent tissue*				*Cancer tissue vs benign tissue*			
		OR (95%CI)	I^2 *(%)*	P_H	*P*	*OR (95%CI)*	I^2 *(%)*	P_H	*P*
Total	16	**3.05 (1.62-5.77)$^{\Delta}$**	78.9	0.000	0.001	**11.55 (5.93-22.49)**	38.5	0.112	0.000
Ethnicity									
Asian	10	**3.36 (1.66-6.80)$^{\Delta}$**	83.0	0.000	0.001	**20.87 (5.28-82.51)$^{\Delta}$**	47.0	0.093	0.000
Caucasian	3	2.22 (0.78-6.36)	50.0	0.135	0.136	**5.12 (1.68-15.67)**	0.0	0.558	0.004
Method									
MSP	10	**4.03 (1.66-9.76)$^{\Delta}$**	80.0	0.000	0.002	**16.31 (7.29-36.49)**	36.7	0.148	0.000
Others	3	1.87 (0.67-5.22)$^{\Delta}$	82.5	0.003	0.233	3.64 (0.96-13.81)	0.0	0.556	0.057
Sample size									
≥100	6	**4.17 (1.66-10.46)$^{\Delta}$**	87.1	0.000	0.002	**14.67 (2.03-105.95)$^{\Delta}$**	63.2	0.066	0.008
<100	7	2.23 (0.85-5.82)$^{\Delta}$	65.2	0.008	0.102	**10.57 (4.40-25.35)**	33.8	0.183	0.000

N: number of involved studies, OR: odds ratio, 95%CI: 95% confidence interval, P_H: p value of heterogeneity test, P: p value of significance test, Δ: estimates for random effects model, MSP: methylation-specific polymerase chain reaction. The results marked in boldface indicate statistical significance.

sociation of SOCS-1 promoter methylation and the pathogenesis of HCC, we also carried out subgroup analysis based on ethnicity, detecting method and sample size. The results of stratified analysis based on ethnicity and sample size indicated that aberrant promoter methylation of SOCS-1 was closely linked to the pathogenesis of HCC in the majority of subgroups. Furthermore, method-stratified subgroup analysis revealed that the frequency of aberrant SOCS-1 promoter methylation was correlated with the pathogenesis of HCC among studies which detected methylation with MSP method in both comparisons. Nevertheless, no association was observed among subgroups using other methods in none of the comparisons (Table 2).

Sensitivity analysis and publication bias

In the sensitivity analysis, no single study could influence the overall pooled estimates (Figure 3). For data on cancer tissue vs adjacent tissue comparison, funnel plots showed no evidence of obvious asymmetry (Figure 4). Begg's test and Egger's test also showed no evidence of publication bias (Begg's test: p=0.127; Egger's test: p=0.180). However, with regard to cancer tissue vs benign tissue comparison, funnel plots demonstrated apparent asymmetry and Egger's test also showed evidence of publication bias (Begg's test: p=0.602; Egger's test: p=0.030).

Discussion

Human SOCS-1 gene is located on chromosome 16p12-p13.1. This gene transcribes a 1215-nt mRNA which encodes a protein of 211 amino acid residues [37]. The SOCS-1 protein is a negative feedback inhibitor of the JAK/STAT signaling pathway. The SH2 domain of SOCS-1 binds to a JH1 domain of JAK2 and inhibits the phosphorylation of JAK2, thus negatively regulating the JAK/STAT signaling pathway [38-40]. There is a variety of gene products in the downstream of the JAK/STAT pathway, including c-myc or c-fos [41]. Activation of this pathway may result in activation of a series of oncogenes and growth-associated genes and eventually lead to carcinogenesis. Several studies have indicated that dysregulation of the JAK/STAT pathway is involved in the malignant transformation for several commonly-encountered human cancers, such as HCC [42], non-small-cell lung cancer (NSCLC) [43,44], and head and neck squamous cell carcinoma (HNSCC) [45]. It has been reported that SOCS-1 expression was suppressed through aberrant methylation of the CpG island in several HCC cell lines [42,46]. Moreover, Yoshikawa et al. [42] found that the SOCS-1 gene is frequently silenced by methylation of the CpG island in human HCC. These findings all suggested that SOCS-1 expression is suppressed through aberrant promoter methylation of the CpG islands and thus leading to carcinogenesis due to persistent activation of JAK/STAT pathway.

In the present meta-analysis, we attempted to explore the role of aberrant methylation of SOCS-1 promoter in the pathogenesis of HCC. Our meta-analysis results demonstrated that the

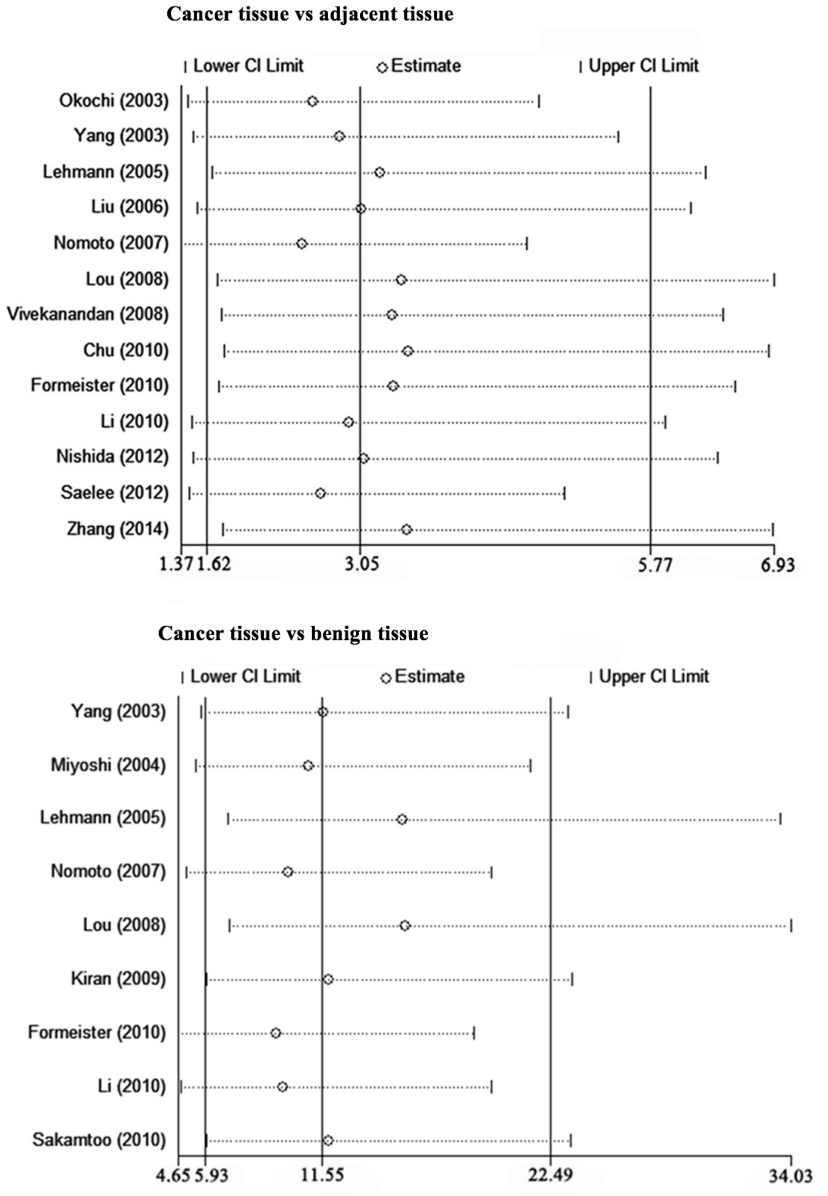

Figure 3. Sensitivity analysis of the summary odds ratio coefficients on the relationships between SOCS-1 promoter methylation and the risk of hepatocellular carcinoma. Results were computed by omitting each study in turn. Bars indicate 95% CI.

frequency of SOCS-1 promoter methylation in cancer tissues was significantly higher than in adjacent non-tumorous tissues and benign tissues, indicating that aberrant methylation of the SOCS-1 gene can be considered as a potential candidate for predicting the risk of HCC. Considering the possible impact of heterogeneity on the outcomes, we carefully performed stratified analyses based on ethnicity, detecting method and sample size. When stratified by ethnicity, the results suggested that there was a significant association between aberrant promoter methylation of the SOCS-1 gene and the pathogenesis of HCC among Asians. When it comes to the subgroup of Caucasians, the results turned out to be conflicting for two comparisons. Only for cancer tissue vs benign tissue comparison the status of methylation showed significant difference between HCC tissues and benign tissues, while for the other comparison, the frequency of SOCS-1 gene methylation of cancer tissues was not significantly higher than that of adjacent tissues. The null result may be due to the limited number of studies with only 3 studies from Caucasians available in our me-

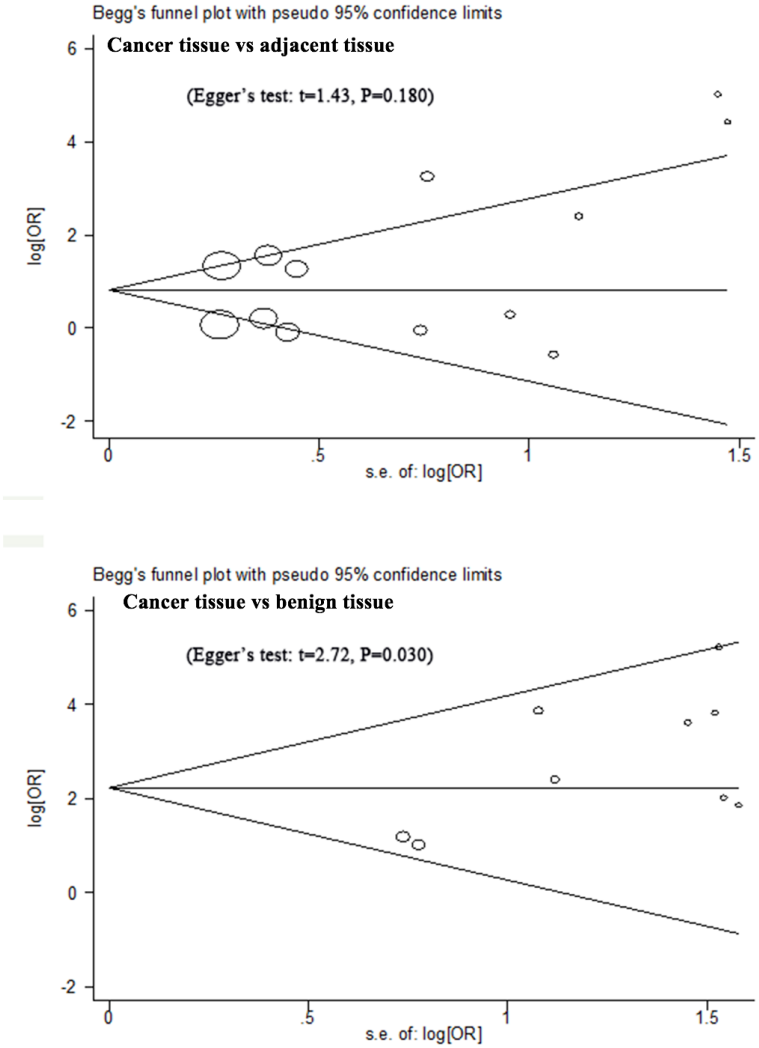

Figure 4. Funnel plot of publication bias on the relationships between SOCS-1 promoter methylation and the pathogenesis of hepatocellular carcinoma. Log OR: natural logarithm of OR. Horizontal line: mean effect size.

ta-analysis. Moreover, as reported by Kondo et al. [10], the silencing of multiple TSGs by gene promoter methylation was observed not only in HCC tissues but also in non-tumorous liver tissues of HCC patients. This finding supported the concept that methylation-induced epigenetic silencing may be involved in the early stages of HCC, which could explain why the difference of frequency of methylation between HCC tissues and adjacent tissues in our meta-analysis was not so evident. It is critical that larger and well-designed multicentric studies based on Caucasian patients should be performed to re-evaluate the association. Similar results were observed in the subgroup analysis of sample size. When we compared methylation frequency between cancer tissues and adjacent tissues, we failed to observe differences in the subgroup of sample size less than 100. This may be partially due to that studies with smaller sample size are more likely failing to detect the statistical differences. Interestingly, we found that researches applying the MSP method observed that aberrant methylation of SOCS-1 in cancer tissues was significantly related to the pathogenesis of HCC in both comparisons, but studies using other methods didn't. To the best of our knowledge, though the MSP method is most widely used, other methods such as qMSP and COBRA (combined bisulfite restriction analysis) have higher efficiency of detecting the frequency of methylation. The paradoxical results may be explained by the limited number of included studies with only 3 studies using these other methods.

Some limitations of our study still should be addressed. First, our results lacked sufficient statistical power to assess the relationship between SOCS-1 promoter methylation and the pathogenesis of HCC due to the relatively small

sample size of available data. Second, we failed to obtain the original data from retrieved studies, thus restraining further investigation of the role of SOCS-1 methylation in the pathogenesis of HCC. Last, as a retrospective study, results of our meta-analysis may have been inevitably affected by subject selection bias. Despite possessing the above limitations, this is the first meta-analysis focusing on the association of SOCS-1 promoter methylation and the susceptibility of HCC. Most importantly, our meta-analysis employed a comprehensive literature search strategy, strict selection criteria and rigorous statistical analyses, which ensured the robustness and reliability of our results.

In summary, our findings provided empirical evidence that aberrant promoter methylation of SOCS-1 gene may contribute to the pathogenesis of HCC. Thus, detection of SOCS-1 promoter methylation may be a valuable method for the early diagnosis of HCC. Nevertheless, given the limitations listed above, more studies with larger sample size and more integral data are needed to provide a more comprehensive and reliable statistical analysis.

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References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer 2001;94:153-156.
2. He J, Gu D, Wu X et al. Major causes of death among men and women in China. NEJM 2005;353:1124-1134.
3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
4. Issa JP. CpG island methylator phenotype in cancer. Nat Rev Cancer 2004;4:988-993.
5. Ushijima T. Detection and interpretation of altered methylation patterns in cancer cells. Nat Rev Cancer 2005;5:223-231.
6. Baylin SB, Ohm JE. Epigenetic gene silencing in cancer - a mechanism for early oncogenic pathway addiction? Nat Rev Cancer 2006;6:107-116.
7. Yu J, Ni M, Xu J et al. Methylation profiling of twenty promoter-CpG islands of genes which may contribute to hepatocellular carcinogenesis. BMC Cancer 2002;2:29.
8. Lee S, Lee HJ, Kim JH, Lee HS, Jang JJ, Kang GH. Aberrant CpG island hypermethylation along multistep hepatocarcinogenesis. Am J Pathol 2003;163:1371-1378.
9. Yang B, Guo M, Herman JG, Clark DP. Aberrant promoter methylation profiles of tumor suppressor genes in hepatocellular carcinoma. Am J Pathol 2003;163:1101-1107.
10. Kondo Y, Kanai Y, Sakamoto M, Mizokami M, Ueda R, Hirohashi S. Genetic instability and aberrant DNA methylation in chronic hepatitis and cirrhosis--A comprehensive study of loss of heterozygosity and microsatellite instability at 39 loci and DNA hypermethylation on 8 CpG islands in microdissected specimens from patients with hepatocellular carcinoma. Hepatology 2000;32:970-979.
11. Constantinescu SN, Girardot M, Pecquet C. Mining for JAK-STAT mutations in cancer. Trends Biochem Sci 2008;33:122-131.
12. Kishimoto T, Kikutani H. Knocking the SOCS off a tumor suppressor. Nat Gen 2001;28:4-5.
13. Dimitriou ID, Clemenza L, Scotter AJ et al. Putting out the fire: coordinated suppression of the innate and adaptive immune systems by SOCS1 and SOCS3 proteins. Immunol Rev 2008;224:265-283.
14. Greenhalgh CJ, Miller ME, Hilton DJ, Lund PK. Suppressors of cytokine signaling: Relevance to gastrointestinal function and disease. Gastroenterology 2002;123:2064-2081.
15. Ko E, Kim SJ, Joh JW, Park CK, Park J, Kim DH. CpG island hypermethylation of SOCS-1 gene is inversely associated with HBV infection in hepatocellular carcinoma. Cancer Lett 2008;271:240-250.
16. Okochi O, Hibi K, Sakai M et al. Methylation-mediated silencing of SOCS-1 gene in hepatocellular carcinoma derived from cirrhosis. Clin Cancer Res 2003;9:5295-5298.
17. Miyoshi H, Fujie H, Moriya K et al. Methylation status of suppressor of cytokine signaling-1 gene in hepatocellular carcinoma. J Gastroenterol 2004;39:563-569.
18. Lehmann U, Wingen LU, Brakensiek K et al. Epigenetic defects of hepatocellular carcinoma are already found in non-neoplastic liver cells from patients with hereditary haemochromatosis. Hum Molec Genetics 2007;16:1335-1342.

19. Liu WJ, Wang L, Wang JP et al. [Correlations of CpG island methylator phenotype and OPCML gene methylation to carcinogenesis of hepatocellular carcinoma]. Ai zheng (Chinese J Cancer) 2006;25:696-700.
20. Liu M, Cui LH, Li CC, Zhang L. Association of APC, GSTP1 and SOCS1 promoter methylation with the risk of hepatocellular carcinoma: a meta-analysis. Eur J Cancer Prev 2015;24:470-483.
21. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25:603-605.
22. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Statist Medicine 2002;21:1539-1558.
23. 23. Jackson D, White IR, Riley RD. Quantifying the impact of between-study heterogeneity in multivariate meta-analyses. Statist Medicine 2012;31:3805-3820.
24. 24. Zintzaras E, Ioannidis JP. Heterogeneity testing in meta-analysis of genome searches. Genet Epidemiol 2005;28:123-137.
25. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Comparison of two methods to detect publication bias in meta-analysis. JAMA 2006;295:676-680.
26. Nomoto S, Kinoshita T, Kato K et al. Hypermethylation of multiple genes as clonal markers in multicentric hepatocellular carcinoma. Br J Cancer 2007;97:1260-1265.
27. Lou C, Yang B, Gao YT et al. [Aberrant methylation of multiple genes and its clinical implication in hepatocellular carcinoma]. Zhonghua zhong liu za zhi [Chinese J Oncol]. 2008;30:831-836.
28. Vivekanandan P, Torbenson M. Epigenetic instability is rare in fibrolamellar carcinomas but common in viral-associated hepatocellular carcinomas. Mod Pathol 2008;21:670-675.
29. Kiran M, Chawla YK, Kaur J. Methylation profiling of tumor suppressor genes and oncogenes in hepatitis virus-related hepatocellular carcinoma in northern India. Cancer Genetics Cytogenetics 2009;195:112-119.
30. Chu PY, Yeh CM, Hsu NC, Chang YS, Chang JG, Yeh KT. Epigenetic alteration of the SOCS1 gene in hepatocellular carcinoma. Swiss Med Weekly 2010;140:w13065.
31. Formeister EJ, Tsuchiya M, Fujii H, Shpyleva S, Pogribny IP, Rusyn I. Comparative analysis of promoter methylation and gene expression endpoints between tumorous and non-tumorous tissues from HCV-positive patients with hepatocellular carcinoma. Mutation Res 2010;692:26-33.
32. Li B, Liu W, Wang L et al. CpG island methylator phenotype associated with tumor recurrence in tumor-node-metastasis stage I hepatocellular carcinoma. Ann Surg Oncol 2010;17:1917-1926.
33. Sakamoto LH, Dec B, Cajaiba M, Soares FA, Vettore AL. MT1G hypermethylation: a potential prognostic marker for hepatoblastoma. Pediatric Res 2010;67:387-393.
34. Nishida N, Kudo M, Nagasaka T, Ikai I, Goel A. Characteristic patterns of altered DNA methylation predict emergence of human hepatocellular carcinoma. Hepatology 2012;56:994-1003.
35. Saelee P, Chuensumran U, Wongkham S, Chariyalertsak S, Tiwawech D, Petmitr S. Hypermethylation of suppressor of cytokine signaling 1 in hepatocellular carcinoma patients. Asian Pacific J Cancer Prev 2012;13:3489-3493.
36. Zhang X, Wang J, Cheng J et al. An integrated analysis of SOCS1 down-regulation in HBV infection-related hepatocellular carcinoma. J Viral Hepatitis 2014;21:264-271.
37. Yandava CN, Pillari A, Drazen JM. Radiation hybrid and cytogenetic mapping of SOCS1 and SOCS2 to chromosomes 16p13 and 12q, respectively. Genomics 1999;61:108-111.
38. Starr R, Willson TA, Viney EM et al. A family of cytokine-inducible inhibitors of signalling. Nature 1997;387:917-921.
39. Endo TA, Masuhara M, Yokouchi M et al. A new protein containing an SH2 domain that inhibits JAK kinases. Nature 1997;387:921-924.
40. Naka T, Narazaki M, Hirata M et al. Structure and function of a new STAT-induced STAT inhibitor. Nature 1997;387:924-929.
41. Darnell JE, Jr., Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994;264:1415-1421.
42. Yoshikawa H, Matsubara K, Qian GS et al. SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. Nat Genetics 2001;28:29-35.
43. He B, You L, Uematsu K et al. SOCS-3 is frequently silenced by hypermethylation and suppresses cell growth in human lung cancer. Proc Natl Academy of Sciences USA 2003;100:14133-14138.
44. He B, You L, Xu Z, Mazieres J, Lee AY, Jablons DM. Activity of the suppressor of cytokine signaling-3 promoter in human non-small-cell lung cancer. Clin Lung Cancer 2004;5:366-370.
45. Weber A, Hengge UR, Bardenheuer W et al. SOCS-3 is frequently methylated in head and neck squamous cell carcinoma and its precursor lesions and causes growth inhibition. Oncogene 2005;24:6699-6708.
46. Nagai H, Kim YS, Lee KT et al. Inactivation of SSI-1, a JAK/STAT inhibitor, in human hepatocellular carcinomas, as revealed by two-dimensional electrophoresis. J Hepatol 2001;34:416-421.