TIMP3 promoter hypermethylation contributes to the risk of renal cell carcinoma: A meta-analysis

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Abstract

Background/aim: The purpose of our study is to assess the association between the methylation level of tissue inhibitor of metalloproteinase-3 (*TIMP3*) and risk of renal cell carcinoma (RCC).

Methods: A systematic review of relevant literature from PubMed, EMBASE, Web of Science, and the Cochrane Library databases was conducted. Odds ratio (OR) and its corresponding 95% confidence interval (CI) were calculated to analyze the data and acquire comprehensive results.

Results: Ten eligible kinds of literature including 495 RCC specimens and 409 normal controls were analyzed in this meta-analysis. The results indicated that *TIMP3* promoter hypermethylation was correlated with RCC-increased risk (OR=12.16, 95% CI: 3.64-40.66, P<0.001). The results of subgroup analysis revealed that the OR of the urine subgroup (OR=22.16) was greater than that of the tissue (OR=13.15) and blood subgroups (OR=3.20). Furthermore, the results of five studies containing RCC clinicopathological features indicated that the methylation level of *TIMP3* gene was related to low tumor stage (OR=2.77, 95% CI: 1.48-5.18, P=0.001).

Conclusion: Our results suggested that the methylation level of *TIMP3* promoter region was significant in RCC. Moreover, the finding is related to early tumor stage. The detection of *TIMP3* promoter methylation may be a good strategy for the diagnosis of RCC at its early stages. Nevertheless, additional well-designed studies are needed to further prove our conclusion.

Introduction

RCC is a potentially lethal tumor, accounting for approximately 90% of all kidney cancers, and the third most frequent malignancy in the urinary system [1,2]. The incidence and mortality rates of RCC are gradually increasing at approximately 2-3% per decade worldwide [3]. In the USA, approximately 62,700 newly diagnosed subjects of kidney cancer and 14,240 deaths due to kidney cancer were reported in 2016 [4]. To date, surgical resection is the mainstream therapy for RCC and shows a good curative effect on patients with localized cancer. However, RCC in the early stage is a serious problem during diagnosis because RCC is usually asymptomatic. Approximately 20-30% of patients with RCC have already developed into distant metastasis when newly diagnosed [5]. However, no reliable biomarkers, which can support clinicians to detect RCC at an early stage, are available.

DNA methylation is one of the important epigenetic mechanisms that regulate gene expression without DNA sequence alteration and is involved in the occurrence and development of tumors [6]. Gene promoter hypermethylation accounts for most of DNA abnormal methylation and is correlated with some functional gene inactivations, particularly those involving tumor suppressor genes in human cancers [7,8]. The tissue inhibitor of metalloproteinase-3 (*TIMP3*) gene mapped to the chromosome 22q13.2 is a tissue inhibitor of the metalloproteinase gene family [9]. *TIMP3* protein is involved in cancer progression; it disrupts the activity of vascular endothelial growth factor signaling pathway [10]. Methylation-associated silencing of *TIMP3* gene plays a tumor inhibitor role in different cancers including RCC [11]. Notably, *TIMP3* promoter methylation detected in urine specimens is significantly more enhanced in patients with RCC than in healthy people [12-14], indicating that it may be a noninvasive

biomarker for RCC. However, the results of related studies about *TIMP3* gene methylation in RCC remained controversial. In this study, we searched meticulously for relevant literature in some widely used electronic databases and conducted a meta-analysis to obtain comprehensive data.

Materials and methods

Literature review

Related literature was identified by a systematical search in well-known electronic databases, such as PubMed, EMBASE, the Cochrane Library, and Web of Science databases, before April 2017. The following keywords were used: (methylation OR hypermethylation) AND (renal OR kidney) AND (cancer OR carcinoma OR neoplasm) AND (*TIMP3* OR metalloproteinase inhibitor 3). The inclusion criteria were summarized as follows: (1) The included articles can provide sufficient information regarding *TIMP3* methylation levels detected in the tissue, blood, or urine samples of patients with RCC and normal controls or regarding the estimation of the correlation between *TIMP3* promoter methylation and clinicopathological characteristic of patients with RCC. (2) Studies must be published in English. (3) The RCC cases had a definite diagnosis by pathology. Recent studies or studies with large

data were included only when they had repetitive data. Articles without case-control data were excluded.

Data collection

Two reviewers (S.M. and Z.S.) extracted the data independently. Useful information from each included study was extracted as follows: surname of the first author, year of publication, region, sample source, tumor histology type, sample size, tumor stage, nuclear grade, and the method of methylation detection. The specimens of normal controls were derived from healthy people or from normal tissues adjacent to RCC. Tumor stage (T1-2) was regarded as a low stage, otherwise high stage. Histological grade (G1-2) was classified as low grade, otherwise high grade.

Statistical analysis

The statistical results revealed the correlation between the *TIMP3* gene methylation level and the risk of RCC. Pooled odds ratio (OR) and 95% confidence interval (CI) were used to represent the results. The Cochrane's Q test and Higgins I^2 statistic were performed to examine statistical heterogeneity [15], which was analyzed by using subgroup analysis and meta-regression. Different effect models were selected according to the results of p-value and I^2 statistic. If P>0.05 or $I^2 \leqq 50\%$, a fixed-effects model (Mantel-Haenszel method) suitable for low heterogeneity was selected for use. Otherwise, a random-effects model (DelSimonian-Laird method) was selected. Furthermore, the stability of our results was evaluated by sensitivity analysis. Publication bias was inferred roughly with a funnel plot and examined quantitatively with Egger's test and Begg's test. All statistical data were analyzed using STATA 12.0 software (Stata Corporation, TX, USA). P<0.05 represented a significant statistical level.

Results

Study filtration

The process of searching and selecting articles was shown in the flow diagram **(Figure 1)**. A total of 305 kinds of literatures were identified initially by searching the databases. Then, the articles, which were duplicate or irrelevant or had insufficient data, were excluded. Eventually, 10 articles [11-14,16-21] published between 1999 and 2013 met our criteria. Among them, two articles [12,13] detected *TIMP3* gene methylation not only in the tissue group but also in the urine group or blood group or both. We analyzed each group independently. The useful data of all eligible studies are listed in Table 1.

Study heterogeneity

Significant heterogeneity existed in our meta-analysis (I^2=80.4%). Subsequently, we proceeded with subgroup analysis and meta-regression to find the heterogeneity sources. As reported in Table 2, the data were stratified by testing method, material, and region. The detection method of quantitative methylation-specific polymerase chain reaction (qMSP) and the sample of tissue accounted for parts of heterogeneity (I^2 for qMSP was 76.3% and I^2 for tissue was 85.5%). In the meta-regression analysis (Table 3), heterogeneity was attributed to methods (P=0.026) but not to materials heterogeneity (P=0.607). Thus, a portion of heterogeneity was derived from the method of methylation detection.

Table 1. Characteristics of the included studies. Abbreviation: RCCs: unasserted renal cell carcinoma; PC: papillary cell cancer; M: methylation; U: unmethylation; MSP: methylation-specific polymerase chain reaction; QMSP: quantitative methylation-specific polymerase chain reaction; aStudy that evaluated TIMP3 promoter methylation in urine; bStudy that evaluated TIMP3 promoter methylation in blood.

First author	Year	Region	Method	Sample type	Histology type	Sample size	Case		Control	
							M	U	M	U
Bachman	1999	USA	MSP	tissue	RCCs	36	28	8	0	27
Esteller	2001	USA	MSP	tissue	RCCs	36	28	8	0	36
Battagli	2003	USA	MSP	tissue	RCCs	45	26	19	0	10
Battagli-u[a]	2003	USA	MSP	urine	RCCs	45	23	22	0	24
Dulaimi	2004	USA	MSP	tissue	RCCs	86	50	36	0	15
Hoque-u	2004	USA	QMSP	urine	RCCs	17	9	8	8	83
Hoque-b[b]	2004	USA	QMSP	blood	RCCs	17	3	14	0	30
Cairns	2004	USA	MSP	urine	RCCs	50	30	20	0	24
Costa	2007	Portugal	QMSP	tissue	RCCs	75	13	62	15	47
Onay	2009	Turkey	MSP	tissue	RCCs	21	4	17	2	19
Ellinger	2010	Germany	QMSP	tissue	PC	32	2	30	0	15
Hauser	2013	Germany	QMSP	blood	RCCs	35	20	15	21	33

Table 2. Subgroup analysis of the association between TIMP3 promoter methylation and RCC risk.

| Variables	Studies	Tumor		Control		Pooled Effect			Heterogeneity	
		M	U	M	U	OR (95%)	Z	P	I² (%)	P
Total	12	236	259	46	363	12.16(3.64-40.66)	4.06	0.000	80.4%	0.000
Method										
MSP	7	189	130	2	155	38.30(9.02-162.64)	4.94	0.000	51.5%	0.056
QMSP	5	47	129	44	208	2.93(0.83-10.34)	1.67	0.094	76.3%	0.002
Material										
Tissue	7	151	180	17	169	13.15(1.53-112.72)	2.35	0.019	85.5%	0.000
Blood	2	23	29	21	63	3.20(0.65- 15.67)	1.43	0.020	33.3%	0.221
Urine	3	62	50	8	131	22.16(5.57-88.10)	4.40	0.000	24.8%	0.264
Region										
America	8	197	135	8	249	32.33(13.32-78.45)	7.69	0.000	8.9%	0.361
Europe	4	39	124	38	114	1.33(0.63-2.79)	0.75	0.456	29.4%	0.236
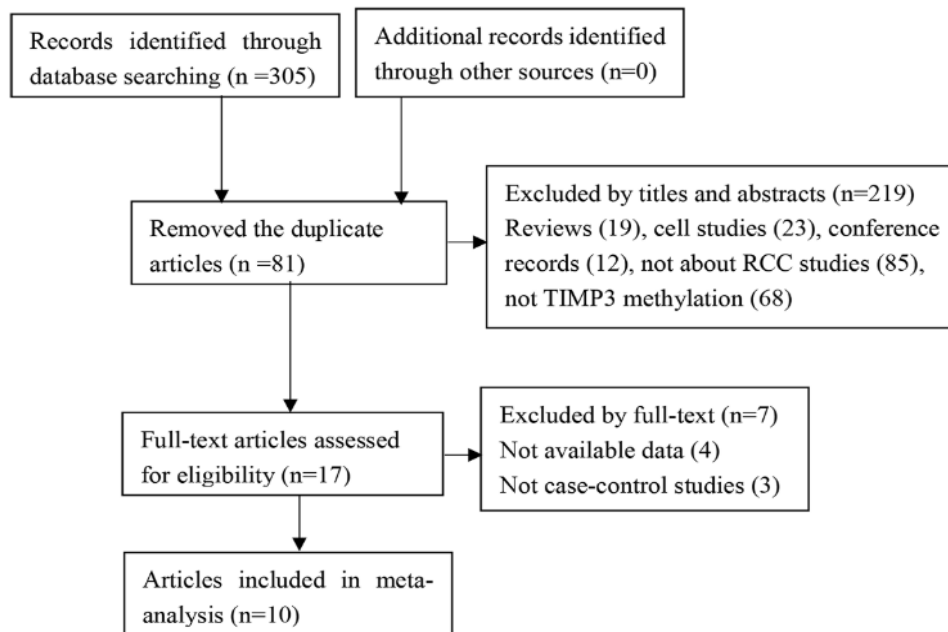
Figure 1. Flow chart of literature search and selection.

Relation between *TIMP3* promoter methylation and RCC risk

Frequency of *TIMP3* promoter hypermethylation in RCC cases and normal controls. As shown in forest plot (Figure 2), *TIMP3* promoter hypermethylation was significantly correlated with the incidence rate of RCC (OR=12.16, 95% CI: 3.64-40.66, P<0.001, random-effects model). In subgroup analysis, the OR of urine sample subgroup (OR=22.16) was greater than that of tissue (OR=13.15) and blood sample subgroups (OR=3.20).

Association between *TIMP3* promoter hypermethylation with RCC clinicopathological characteristic. The data on RCC clinicopathological features (tumor stage and histological grade) are shown in Table 4. Five studies containing definite clinicopathological information were included in the analysis, and the results indicated that *TIMP3* promoter methylation was associated with low tumor stage (OR=2.77, 95% CI: 1.48-5.18, P=0.001, fixed-effects model) (Figure 3) but not associated with histological grade (OR=1.70, 95% CI: 0.98-2.95, P=0.06, fixed-effects model) (Figure 4).

Sensitivity analyses

Sensitivity analysis was performed to reflect the stability and credibility of the statistical results by assessing the effect of the removal of a single study on the total pooled ORs. As presented in Figure 5, the pooled ORs were not changed obviously, indicating that the results of our meta-analysis were stable.

Publication bias

As shown in Figure 6, the shape of funnel plot exhibited obscured symmetry, then, Egger's and Begg's tests were performed to evaluate publication bias quantitatively. The p-value of Egger's test was 0.002, whereas the that of Begg's test was 0.858. Trim and fill analysis was conducted to determine the conditions wherein the conclusions of the two tests are inconsistent. The results of the relationship between *TIMP3* methylation and RCC risk also had statistical significance after increasing 5 imputed studies in trim and fill analysis (OR=1.29, 95%CI: 0.26-2.35, P=0.02). Thus, the results of Begg's test and trim and fill

analysis demonstrated that there were no significant publication bias in our meta-analysis.

Discussion

DNA aberrant methylation regulates gene expression without changing DNA sequences and is extensively involved in oncogenesis. The hypermethylation of gene promoter region is associated with transcriptional silencing and inactivation of some functional genes, such as tumor suppressor genes and DNA damage repair genes in human cancers [22,23]. *TIMP3* gene, found in chromosome 22q13.2, is a tumor suppressor gene. It is involved in the inhibition of the growth, angiogenesis, invasion, and metastasis of human cancers [24-26]. Decrease in *TIMP3* expression is associated with abnormal promoter methylation in various malignant tumors, including kidney cancer [11,27], bladder cancer [28], choriocarcinoma [29,30], esophageal squamous cell carcinoma [31], meningioma [32], and gastric cancer [33].

However, studies on the relationship between *TIMP3* promoter methylation and RCC risk have inconsistent results. During meta-analysis, *TIMP3* promoter methylation was detected in tissue, blood, and urine samples through the testing method of methylation-specific polymerase chain reaction (MSP) or qMSP. The results were correlated with the increased risk of RCC (OR=12.16, P=0.000). However, distinct heterogeneity was presented in this meta-analysis. Meta-regression and subgroup analysis showed that parts of heterogeneity were derived from the method of methylation detection. Usually, the detection method of qMSP has higher sensitivity than that of MSP [34]. As previous studies reported, *TIMP3* promoter methylation occurred in some normal renal tissue samples when the qMSP method was used; this result was difficult to obtained when MSP was performed [16,19]. Meanwhile, morphologically normal human tissues might undergo processes that can lead to tumorigenesis, particularly aberrant methylation because of the "field-effect" (also known as field cancerization) and gradual cytogenetic alteration [35]. The results of subgroup analysis stratified by the testing method revealed that *TIMP3* promoter methylation detected only by MSP is significantly associated with RCC risk (OR=38.30). This finding suggests that our results were not influenced

Table 3. Meta-regression analysis.

Variables	Coefficient	95% Confidence interval	I^2-residual	Adjusted R^2	P value
Method	-2.53	(-4.67,-0.38)	65.24%	45.93%	0.026
Material	0.38	(-1.21,1.96)	75.70%	-7.38%	0.607
Region	-3.24	(-4.69,-1.79)	8.57%	88.78%	0.001

Table 4. Clinicopathological characteristics of five studies. Abbreviation: M: methylation; U: unmethylation.

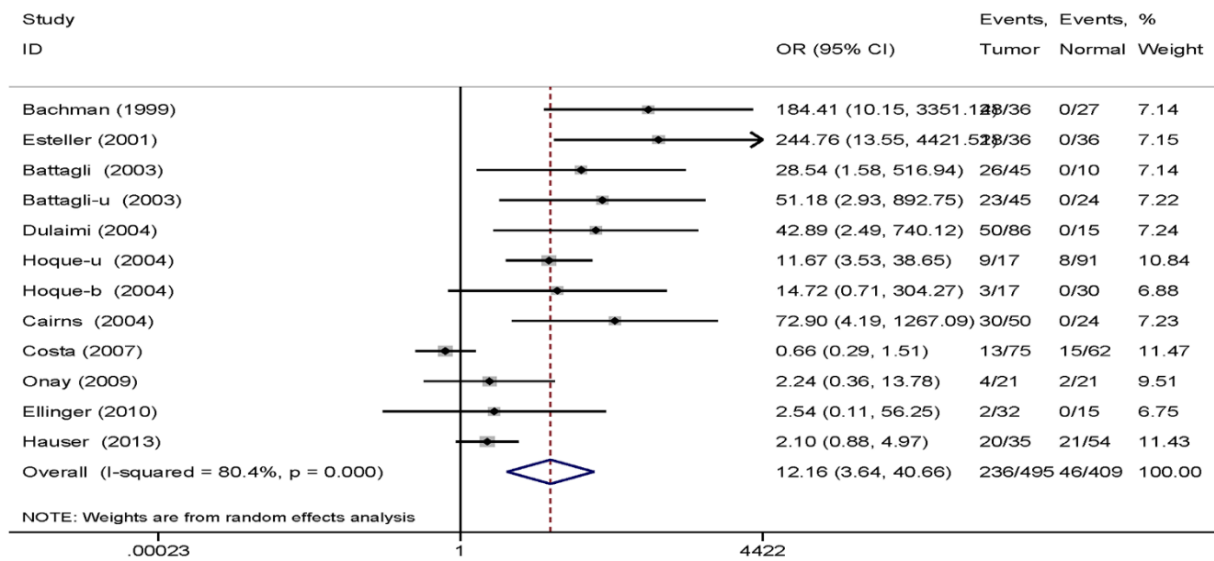| First author	Year	Method	Sample type	Low Grade		High Grade		Low stage		High stage	
				M	U	M	U	M	U	M	U
Bachman	1999	MSP	tissue	14	5	14	3	20	5	8	3
Battagli	2003	MSP	tissue	18	8	8	11	23	11	3	8
Battagli-u	2003	MSP	urine	16	10	7	12	20	14	3	8
Dulaimi	2004	MSP	tissue	29	18	21	18	39	22	11	14
Onay	2009	MSP	tissue	4	15	0	2	4	14	0	3

Figure 2. Pooled OR from 10 studies including 495 RCC cases and 409 normal controls. OR=12.16, 95% CI: 3.64–40.66, P<0.001.
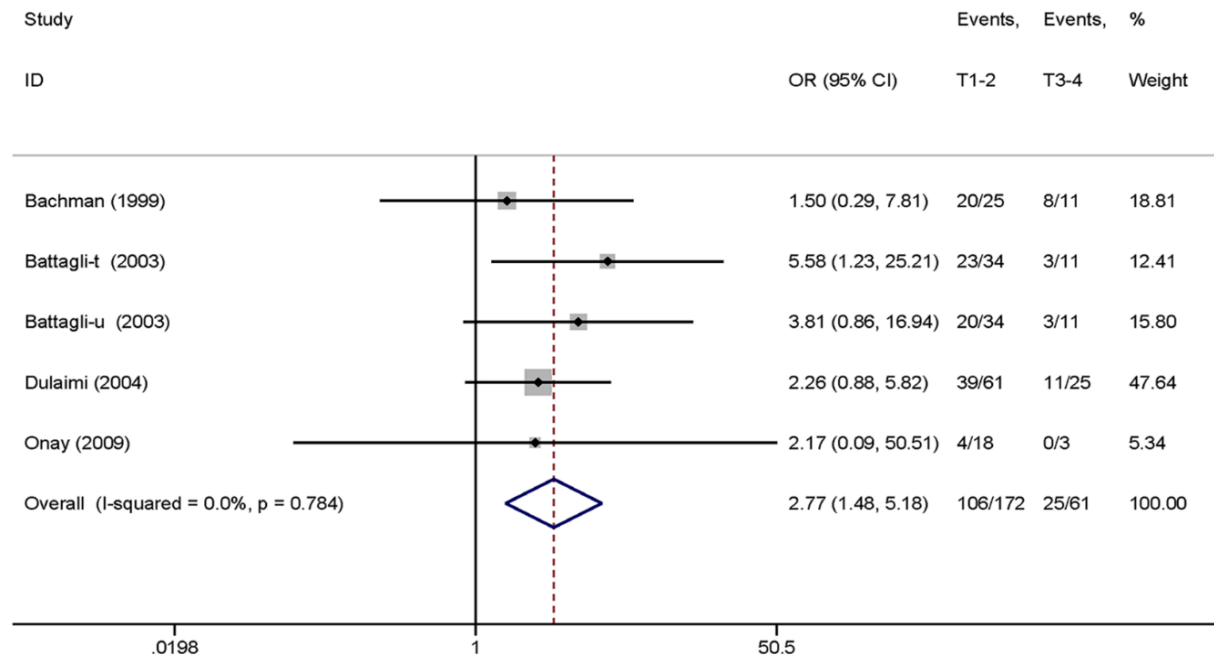

Figure 3. Pooled OR from five studies assessing the association between *TIMP3* promoter methylation and tumor stage. OR=2.77, 95% CI: 1.48–5.18, P=0.001.
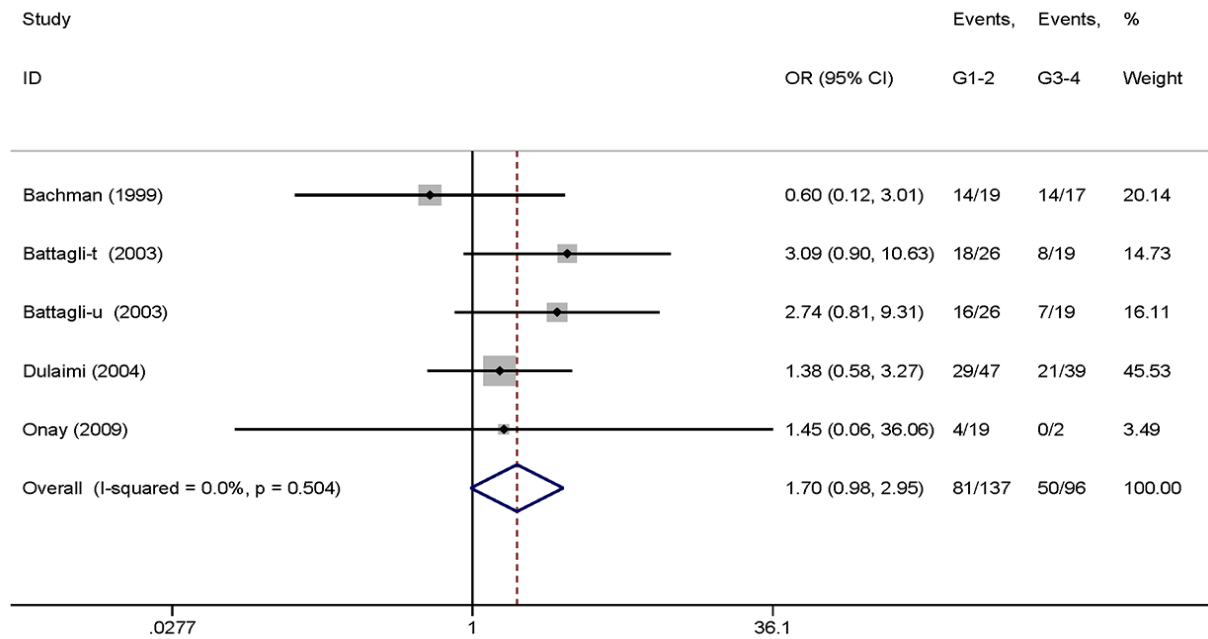
Figure 4. Pooled OR from five studies assessing the association between *TIMP3* promoter methylation and histological grade. OR=1.70, 95% CI: 0.98-2.95, P=0.06.
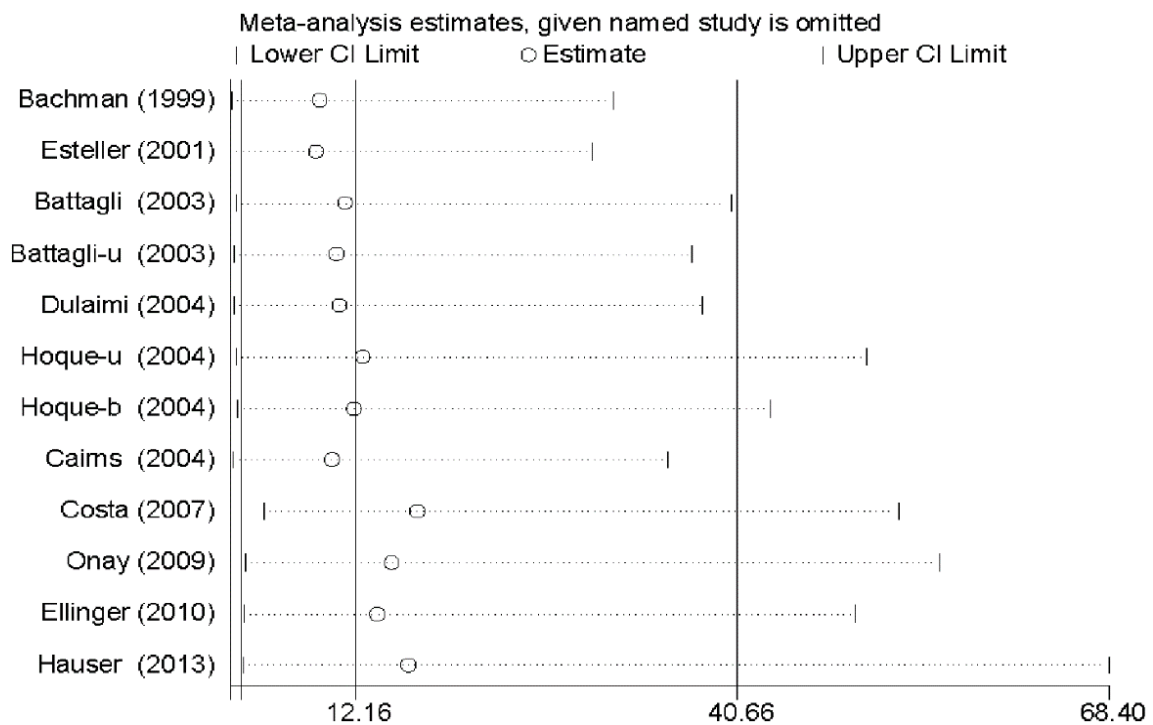

Figure 5. Sensitivity analysis of overall odds ratio coefficients for **TIMP3** methylation.

by the heterogeneity caused by the method of methylation detection. Basing on the results of the subgroup analysis stratified by material, we also found that *TIMP3* promoter methylation detected in the urine subgroup was highly correlated with RCC-increased risk (OR=22.16). Thus, *TIMP3* promoter methylation might be developed into a noninvasive and convenient biomarker to diagnose RCC.

Previous studies reported that the hypermethylation level of *TIMP3* gene is associated with tumor stage [11,13,18] and histological grade [18], whereas others reported no correlation [12,16]. Five studies were conducted to produce RCC clinicopathological data to assess the relationship between the frequency of *TIMP3* promoter methylation and the clinicopathological features of RCC. The results suggested
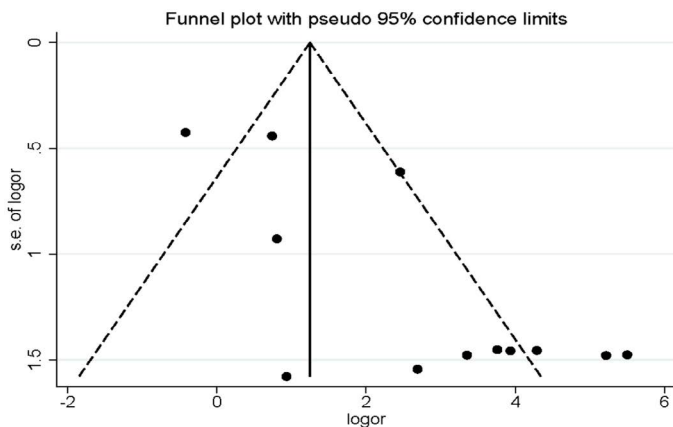
Figure 6. Funnel plot of publication biases on the correlation between *TIMP3* promoter methylation and RCC risk.

that the methylation level of *TIMP3* promoter region is related to low tumor stage (P=0.001). We speculated that aberrant methylation of *TIMP3* gene is involved in the early stage of renal tumorigenesis. Given that some tumors are accompanied with DNA methylation alteration in clinical specimens, such as blood, urine, and ascites [36,37], research on *TIMP3* promoter methylation might offer a promising and noninvasive approach for the early detection and surveillance of RCC.

A few potential limitations in our study were as follows: selection bias was inevitable as we searched only in English literature and might miss some important studies published in other languages; included articles were published several years ago (1999 to 2013), and newly published studies were suggested to be included in the analysis; other clinical data, such as cancer subtypes, age, and gender, were not presented in our meta-analysis and were not correlated with *TIMP3* gene methylation.

Overall, our study indicated that *TIMP3* promoter hypermethylation is closely related with the risk of RCC and correlated with low tumor stage. Additional well-designed studies with sufficient sample size may clearly confirm the role of *TIMP3* promoter methylation in RCC.

Acknowledgment

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