

# Promoter hypermethylation of Wnt inhibitory factor-1 in patients with lung cancer

## A systematic meta-analysis

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### Abstract

**Background:** Promoter hypermethylation of Wnt inhibitory factor-1 (*WIF-1*)—a tumor suppressor gene—has been detected in several types of human tumors. However, the association between *WIF-1* promoter hypermethylation and lung cancer remains to be elucidated. Therefore, we conducted this study to evaluate the clinical significance of *WIF-1* promoter hypermethylation in lung cancer.

**Methods:** A comprehensive literature search was conducted to obtain eligible studies. The combined odds ratios (ORs) or hazard ratios and 95% confidence intervals were used to estimate the strength of associations.

**Results:** A total of 8 eligible publications with 626 cases and 512 controls were included in our study. The combined ORs revealed that *WIF-1* promoter hypermethylation was significantly higher in lung cancer than in controls (OR 10.53,  $P < 0.001$ ). Moreover, *WIF-1* promoter hypermethylation was significantly associated with smoking behavior (OR 1.88,  $P = 0.002$ ). No significant correlation was found between *WIF-1* promoter hypermethylation and sex status, age status, tumor stage, and pathological types in cancer. Multivariate analysis results indicated the absence of correlation between *WIF-1* promoter hypermethylation and with relapse-free survival and overall survival. Subgroup analysis by sample type demonstrated that promoter hypermethylation of *WIF-1* was significantly associated with an increased risk of lung cancer in the tissue (OR 7.89,  $P < 0.001$ ), blood (OR 21.83,  $P = 0.034$ ), and pleural effusion subgroups (OR 157.43,  $P = 0.001$ ).

**Conclusions:** Promoter hypermethylation of *WIF-1* may play a crucial role in lung cancer carcinogenesis. It may be a noninvasive biomarker using blood or pleural effusion detection. *WIF-1* promoter hypermethylation is correlated with smoking behavior, but not with sex status, age status, tumor stage, pathological types, and the prognosis of lung cancer patients in terms of relapse-free survival and overall survival. More investigations, including a larger number of subjects, are required to further confirm the findings of our analysis.

**Abbreviations:** AC = adenocarcinoma, CIs = confidence intervals, HRs = hazard ratios, LCC = large cell carcinoma, nMSP = nested methylation-specific polymerase, NSCLC = nonsmall cell lung cancer, ORs = odds ratios, OS = overall survival, RFS = relapse-free survival, SCC = squamous cell carcinoma, TSGs = tumor suppressor genes, *WIF-1* = Wnt inhibitory factor-1, Wnt = Wingless-type.

**Keywords:** biomarker, clinical significance, hypermethylation, lung cancer, *WIF-1*

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**Author contributions:** YZ and XY contributed to the conception and design of the study. DC, XL, YJ, YX, BS, QZ, and XL contributed to the retrieval of articles, the extraction of data, the calculation of data, and the design of the figures and tables. All the authors approved the final version of the manuscript for submission.

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## 1. Introduction

Lung cancer is the most frequent malignant disease worldwide, accounting for approximately 13% of all cancer diagnoses in 2012, and the top leading cause of cancer-related deaths.<sup>[1]</sup> Lung cancer includes 2 main histological types: nonsmall cell lung cancer (NSCLC) and small cell lung cancer. The former accounts for about 85% of all lung cancer cases and is subclassified into adenocarcinoma (AC), squamous cell carcinoma (SCC), large cell carcinoma (LCC), and others.<sup>[2]</sup> However, since most patients are diagnosed with an advanced stage or metastatic lung cancer,<sup>[3]</sup> their 5-year relative survival rate is only approximately 18%.<sup>[4]</sup>

Accumulating evidence reveals that epigenetic alterations may play a vital role in cancer initiation, progression, and prognosis.<sup>[5–8]</sup> DNA methylation—a major molecular mechanism of epigenetic changes—has been recognized as an important event in many cancer types, including lung cancer.<sup>[9,10]</sup> Studies have shown that gene methylation in the promoter regions regulates the silencing of tumor suppressor genes (TSGs), leading to the down-regulation of gene expression.<sup>[11,12]</sup> Dysregulation of the Wntless-type (Wnt) signaling pathway has been reported to be associated with many types of human cancers, such as lung, prostate, and breast cancer.<sup>[13,14]</sup> Located at 12q14, Wnt inhibitory factor-1 (*WIF-1*) gene, encoding a secreted protein that is a key Wnt antagonist, inhibits Wnt/ $\beta$ -catenin signaling pathway via binding to Wnt proteins.<sup>[15,16]</sup> Thus, the over-expression of *WIF-1* may be involved in the inhibition of cell growth in various cancer cell lines.<sup>[17,18]</sup> In previous studies, the expression of *WIF-1* was down-regulated through promoter methylation in several types of human cancers, including lung cancer.<sup>[15,19,20]</sup>

Interestingly, promoter hypermethylation of *WIF-1* is frequent in Western patients with lung cancer,<sup>[15,21]</sup> whereas it is rare in Japanese patients.<sup>[22,23]</sup> Thus, we first performed this study to evaluate the relationship between *WIF-1* promoter hypermethylation and lung cancer risk. In addition, we determined whether *WIF-1* promoter hypermethylation was associated with sex status, age status, tumor stage, smoking behavior, pathological types, and relapse-free survival (RFS) and overall survival (OS) in cancer.

## 2. Materials and methods

### 2.1. Ethics approval

Ethics approval was not required because the present study does not involve human subjects.

### 2.2. Search strategy

We extensively searched PubMed, EBSCO, Wanfang, and Embase electronic databases to identify relevant studies published before July 9, 2016, without language limitation. The following keywords and free-text word searching terms were used: (*WIF1* OR *WNT* inhibitory factor 1 OR *Wif-1*) AND (methylation OR hypermethylation) AND (lung cancer OR lung carcinoma OR lung tumor). Manual searches of the references in the selected studies were also conducted to identify other potentially eligible investigations.

### 2.3. Eligible criteria

The following inclusion criteria were used to identify the available studies eligible for inclusion in our analysis: patients met the diagnostic criteria for primary lung cancer without

restriction of sample type; original papers with a case-control or cohort study design; the studies provide sufficient data regarding the methylation levels of *WIF-1* promoter to evaluate the correlation between *WIF-1* promoter hypermethylation and lung cancer risk; if authors reported the use of the same sample data in more than 1 article, only the most recently published article or the article with the largest sample size was selected for inclusion in the current study.

### 2.4. Data extraction

To determine the eligibility of the included studies, we extracted the following information on *WIF-1* promoter hypermethylation for the following: first author's surname, publication year, country, patients' ethnicity, tumor histology, type of sample, sample size, method for detection of methylation, sex status, smoking behavior, age status, tumor stage, RFS, OS, and frequency of methylation. Two authors (DC and XL) independently extracted and assessed the relevant data from the eligible studies. Disagreements were resolved by discussion among 3 authors (YJ, YX, and BS).

### 2.5. Statistical analysis

All data were statistically analyzed using STATA 12.0 software (Stata Corporation, College Station, TX). The combined odds ratios (ORs) with their corresponding 95% confidence intervals (95% CIs) were calculated to determine the relationship between *WIF-1* promoter hypermethylation and lung cancer risk, and the association in relation to sex status, age status, tumor stage, tumor histology, and smoking behavior in cancer. Furthermore, the pooled hazard ratios (HRs) with 95% CIs were calculated to establish whether *WIF-1* promoter hypermethylation was associated with RFS or OS of lung cancer patients. Between-study heterogeneity was evaluated according to the Cochran  $Q$ -statistic and  $I^2$  tests.<sup>[24]</sup> The random-effects model was applied when there was significant heterogeneity ( $I^2 \geq 50\%$  or  $P < 0.1$ ); otherwise, the fixed-effects model was used when there was no substantial heterogeneity.<sup>[25,26]</sup> Subgroup meta-analyses were conducted based on ethnicity, sample type, and detection method, and a meta-regression analysis was carried out to explore the possible sources of heterogeneity. The potential publication bias was measured using Egger test.<sup>[27]</sup> A  $P$  value  $< 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Baseline characteristics of eligible studies

In all, 91 potential studies were retrieved by the original search of the databases and the manual search. After selection according to our inclusion criteria, a total of 8 case-control studies comprising 626 cases and 512 controls were included in the present analysis<sup>[15,21,22,28–32]</sup> (Fig. 1). Two of these 8 studies were performed in a Caucasian population and 6 in an Asian population. Regarding the methylation detection methods, nested methylation-specific polymerase chain reaction (nMSP) was used in 2 of the studies included, whereas methylation-specific polymerase chain reaction (MSP) was employed in the remaining 6 studies. Eight studies analyzed the relationship between *WIF-1* promoter hypermethylation and lung cancer risk and 6 the association of *WIF-1* promoter hypermethylation with sex status. In addition, 6 studies evaluated the correlation of *WIF-1* promoter hypermethylation with smoking behavior in cancer

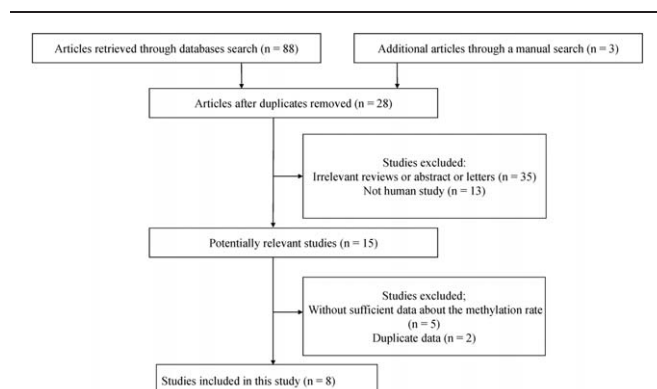


Figure 1. Flow diagram of the included studies.

patients and 5 studies examined the correlation of *WIF-1* promoter hypermethylation with tumor histology in cancer. Two investigations assessed the relationship of *WIF-1* promoter hypermethylation with age status and tumor stage. The basic characteristics of the 8 eligible studies included in our analysis are summarized in Table 1.

### 3.2. *WIF-1* promoter hypermethylation and lung cancer risk

The random-effects model was used in all 8 studies with 626 cases and 512 controls, because significant heterogeneity was present in the comparison with lung cancer versus controls ( $I^2 = 73.0\%$ ,  $P = 0.001$ ). Significantly higher OR was observed for *WIF-1* promoter hypermethylation observed in lung cancer than in nonmalignant samples (OR 10.53, 95% CI 4.24–26.14,  $P < 0.001$ ) (Fig. 2), indicating that *WIF-1* promoter hypermethylation was significantly correlated with an increased risk of lung cancer.

### 3.3. Subgroup analyses of *WIF-1* promoter hypermethylation in cancer versus controls

Subgroup analysis by ethnicity (Asians and Caucasians), methylation detection methods (MSP and nMSP), and sample types (tissue, blood, and pleural effusion) was carried out to find the strength of correlation between *WIF-1* promoter hypermethylation in lung cancer versus controls (Table 2). The subgroup analysis on the basis of ethnicity revealed the presence of a significant association between *WIF-1* promoter hypermethylation and lung cancer in Asians and Caucasians (OR 9.14,

Table 1

The general characteristics of 8 eligible studies.

First author, y	Country	Ethnicity	Method	Histology	Control	Cases N (M %)	Controls N (M %)	≤63 y		≥63 y		Male M/N	Female M/N	Smoking M/N	Nonsmoking M/N	AC M/N	SCC M/N	Stage I M/N	Stage II to IV M/N
								M/N	M/N	M/N	M/N								
Mazieres, 2004 <sup>[15]</sup>	USA	Caucasians	MSP	NSCLC	AT	26 (84.6)	26 (7.7)	—	—	—	—	—	—	—	—	—	—	—	—
Suzuki, 2007 <sup>[30]</sup>	Japan	Asians	MSP	NSCLC	AT	238 (27.7)	175 (1.1)	—	—	50/168	16/70	52/171	14/67	30/135	28/87	25/85	41/153	—	
Licchesi, 2008 <sup>[21]</sup>	USA	Caucasians	MSP	NSCLC	AT+NT	19 (68.4)	50 (28)	—	—	—	—	—	—	—	—	—	—	—	—
Xu, 2008 <sup>[32]</sup>	China	Asians	nMSP	LC	AT+NT	66 (50)	35 (17.1)	—	—	29/54	4/12	25/41	8/25	4/8	27/47	—	—	—	—
Yoshino, 2009 <sup>[22]</sup>	Japan	Asians	MSP	NSCLC	AT	44 (15.9)	32 (6.2)	3/21	4/23	4/22	3/22	5/23	2/21	4/30	3/11	—	—	—	—
Yang, 2009 <sup>[29]</sup>	China	Asians	MSP	NSCLC	BPE	36 (69.4)	35 (0)	—	—	14/20	11/16	9/13	16/23	19/30	2/2	—	—	—	—
Liu, 2009 <sup>[31]</sup>	China	Asians	nMSP	LC	NB	58 (34.5)	20 (0)	—	—	15/40	5/18	15/31	5/27	—	—	—	—	—	—
Lee, 2013 <sup>[26]</sup>	Korea	Asians	MSP	NSCLC	AT	139 (47.5)	139 (20.9)	30/67	36/72	48/101	18/38	51/104	15/35	36/79	30/60	45/85	21/54	—	

AC=adenocarcinoma, AT=adjacent tissue, BPE=benign pleural effusion, LC=lung cancer, M=methylation, MSP=methylation-specific polymerase chain reaction, N=number of sample, NB=normal blood, nMSP=nested methylation-specific polymerase chain reaction, NSCLC=non-small cell lung cancer, NT=normal tissue, SCC=squamous cell carcinoma.

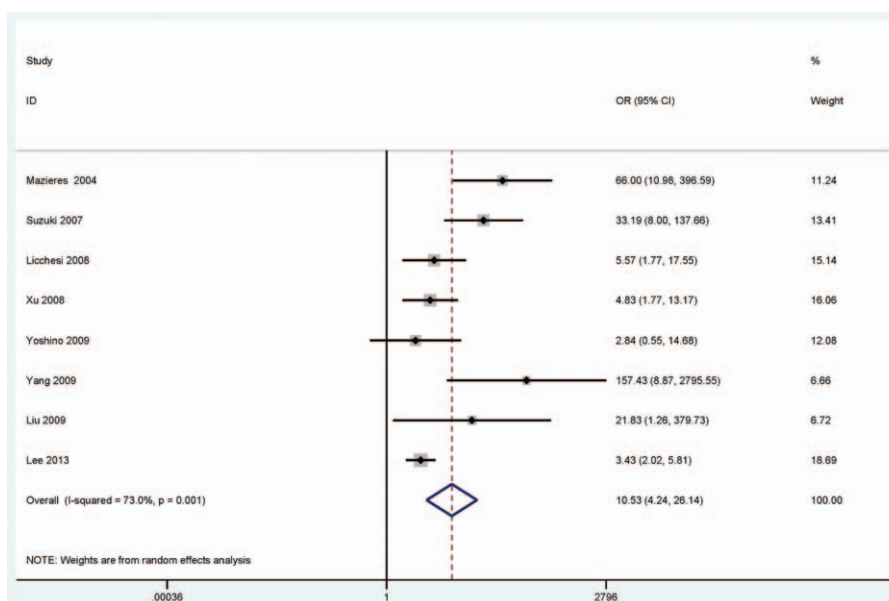


Figure 2. Forest plot of the combined OR from 8 studies including 626 lung cancer patients and 512 controls in cancer versus controls ( $I^2 = 73.0\%$ , OR 10.53, 95% CI 4.24–26.14,  $P < 0.001$ ). CI=confidence interval, OR=odds ratio.

**Table 2****Subgroup analyses of *WIF-1* promoter hypermethylation in lung cancer versus controls.**

Subgroup	Studies	Pooled OR (95% CI)	Heterogeneity ( $I^2$ ; $P$ )	$P$	Cases	Controls
Race						
Caucasians	2	17.36 (1.54–195.32)	80.8%; 0.023	0.021	45	76
Asians	6	9.14 (3.12–26.78)	73.3%; 0.002	<0.001	581	436
Method						
MSP	6	12.47 (3.74–41.53)	79.9%; <0.001	<0.001	502	457
nMSP	2	5.90 (2.05–17.03)	5.9%; 0.303	0.001	124	55
Sample						
Tissue	6	7.89 (3.24–19.18)	73.0%; 0.002	<0.001	532	457
Pleural effusion	1	157.43 (8.87–2795.55)	NA; NA	0.001	36	35
Blood	1	21.83 (1.26–379.73)	NA; NA	0.034	58	20

CI=confidence interval, MSP=methylation-specific polymerase chain reaction, NA=not applicable, nMSP=nested methylation-specific polymerase chain reaction, OR=odds ratio, *WIF-1*=Wnt inhibitory factor-1.

95% CI 3.12–26.78,  $P < 0.001$ ; OR 17.36, 95% CI 1.54–195.32,  $P = 0.021$ , respectively). Further, the subgroup analysis based on the methylation detection methods demonstrated that *WIF-1* promoter hypermethylation was significantly correlated with lung cancer in both MSP (OR 12.47, 95% CI 3.74–41.53,  $P < 0.001$ ) and nMSP (OR 5.90, 95% CI 2.05–17.03,  $P = 0.001$ ) subgroups. On the contrary, the subgroup analysis by sample types showed that *WIF-1* promoter hypermethylation was significantly correlated with an increased risk of lung cancer in tissue (OR 7.89, 95% CI 3.24–19.18,  $P < 0.001$ ), blood (OR 21.83, 95% CI 1.26–379.73,  $P = 0.034$ ), and pleural effusion subgroups (OR 157.43, 95% CI 8.87–2795.55,  $P = 0.001$ ). Nevertheless, the results obtained for the subgroups of the Caucasian population, nMSP, and blood and pleural effusion should be interpreted with caution as only 1 or 2 studies with smaller sample size were analyzed in this research.

### 3.4. Meta-regression analysis of *WIF-1* promoter hypermethylation in cancer versus controls

Meta-regression analysis of *WIF-1* promoter hypermethylation based on ethnicity (Asians and Caucasians), methylation detection methods (MSP and nMSP), and sample types (tissue, blood, and pleural effusion) was conducted to elucidate the potential sources of heterogeneity (Table 3). The results showed that methylation detection method, ethnicity, and sample type could not explain the sources of heterogeneity (all  $P > 0.1$ ).

### 3.5. Correlation between *WIF-1* promoter hypermethylation and clinicopathological characteristics of lung cancer

No significant heterogeneity was detected in relation to the clinicopathological features of lung cancer (all  $I^2 = 0.0\%$ ); thus,

the fixed-effects model was applied. Based on the findings of 6 studies with 405 male and 176 female patients with lung cancer, we established that *WIF-1* promoter hypermethylation had a significantly similar OR in male and female lung cancer patients (OR 1.34, 95% CI 0.90–1.99,  $P = 0.15$ ) (Fig. 3). The combined OR from 6 studies involving 383 smoking and 198 nonsmoking patients with lung cancer showed significantly higher *WIF-1* promoter hypermethylation in smoking patients with lung cancer than in nonsmoking patients with lung cancer (OR 1.88, 95% CI 1.26–2.79,  $P = 0.002$ ) (Fig. 4). Furthermore, the overall OR from 5 studies with 282 AC and 207 SCC cases indicated that *WIF-1* promoter hypermethylation had a slightly similar OR in AC and SCC (OR 0.67, 95% CI 0.44–1.01,  $P = 0.058$ ) (Fig. 5). The overall OR from 2 studies with 183 lung cancer patients exhibited no correlation between *WIF-1* promoter hypermethylation and age status ( $\leq 63$  vs  $\geq 63$  years; OR 0.81, 95% CI 0.44–1.50,  $P = 0.499$ ) (Fig. 6). The pooled OR from 2 studies with 170 stage I lung cancer patients and 207 stage II to IV lung cancer patients showed that *WIF-1* promoter hypermethylation was not correlated with tumor stage (OR 1.37, 95% CI 0.88–2.14,  $P = 0.165$ ) (Fig. 6).

Therefore, altogether the results obtained evidenced that *WIF-1* promoter hypermethylation was significantly correlated with smoking status, but not with sex status, tumor stage, pathological types, and age status of lung cancer patients.

### 3.6. Prognosis of *WIF-1* promoter hypermethylation in lung cancer patients

We also determined whether *WIF-1* promoter hypermethylation was correlated with the prognosis of RFS and OS of lung cancer patients (Table 4). The authors of a study with 44 lung cancer patients found no significant association between *WIF-1* promoter hypermethylation and RFS (HR 1.480, 95% CI 0.307–7.133,  $P = 0.625$ ) after they conducted a multivariate analysis. The pooled HR estimate for OS was 2.02 (95% CI 0.48–8.58,  $P = 0.340$ ) by multivariate analysis, including 183 lung cancer patients.

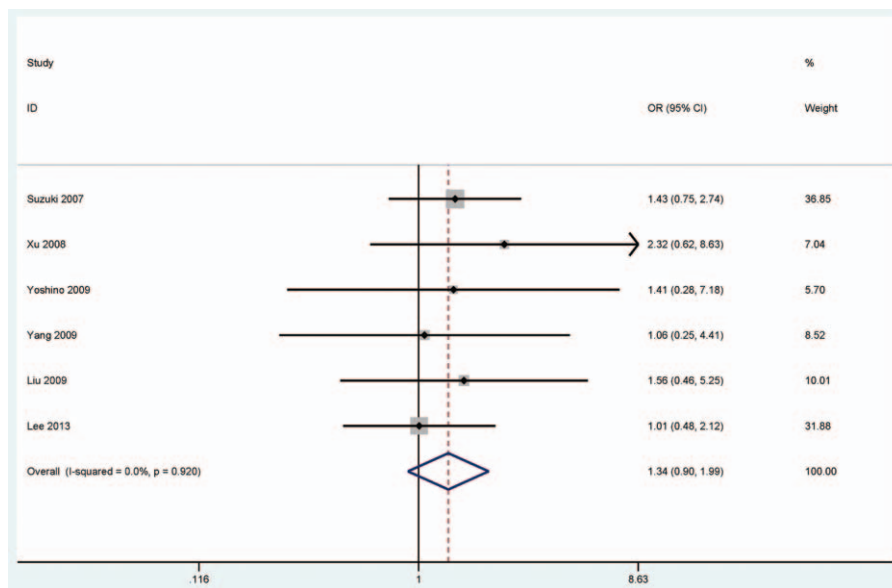
### 3.7. Publication bias

The results from the Egger test indicated that a slight publication bias was present in lung cancer versus controls ( $P = 0.027$ ) (Fig. 7). However, there was no evidence of publication in relation to smoking behavior, sex status, and the pathological types of cancer ( $P > 0.05$ ) (Figs. 7 and 8).

**Table 3****Meta-regression analysis of *WIF-1* promoter hypermethylation in lung cancer versus controls.**

	Coefficient (95% CI)	$t$	$P$
Testing methods	−0.990 (−5.108, 3.127)	−0.67	0.541
Ethnic population	−0.648 (−4.328, 3.031)	−0.49	0.65
Sample materials	1.336 (−1.579, 4.251)	1.27	0.272

CI=confidence interval, *WIF-1*=Wnt inhibitory factor-1.



**Figure 3.** Forest plot of the combined OR from 6 studies including 405 male and 176 female patients with lung cancer in relation to sex status in cancer ( $I^2=0.0\%$ , OR 1.34, 95% CI 0.90–1.99,  $P=0.15$ ). CI=confidence interval, OR=odds ratio.

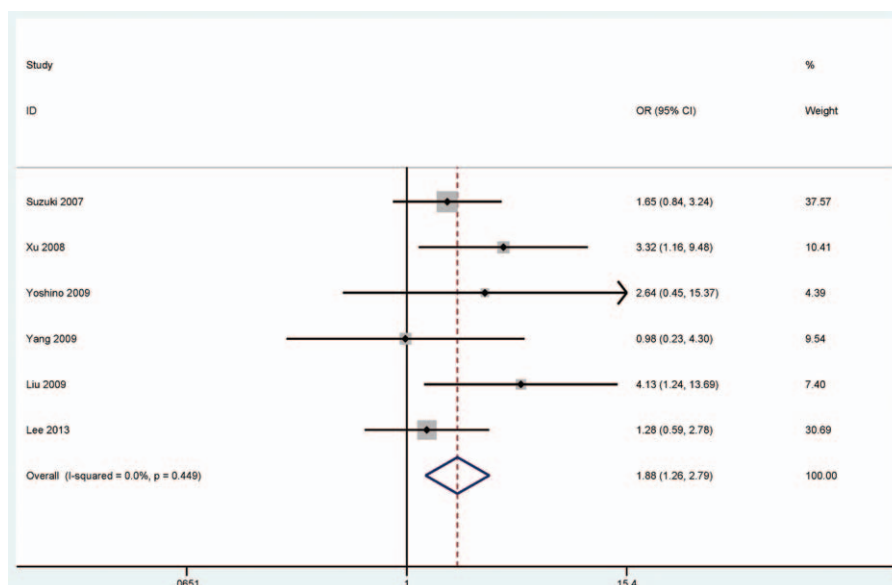
#### 4. Discussion

The silencing of TSGs via promoter hypermethylation has been shown to facilitate the initiation and progression of cancer.<sup>[33]</sup> Promoter hypermethylation of TSG *WIF-1* is a common early event in a range of human tumors, such as laryngeal SCC,<sup>[34]</sup> adrenocortical tumor,<sup>[35]</sup> and gastric cardia AC.<sup>[36]</sup> Inconsistent and even controversial results existed concerning *WIF-1* gene methylation frequencies in the promoter region in lung cancer cases, which ranged from 15.9%<sup>[22]</sup> to 84.6%.<sup>[15]</sup> Therefore, to the best of our knowledge, the current study was the first to determine whether *WIF-1* promoter hypermethylation was significantly correlated with an increased risk of lung cancer.

Moreover, we analyzed the association between *WIF-1* promoter hypermethylation and the clinicopathological features of lung cancer patients.

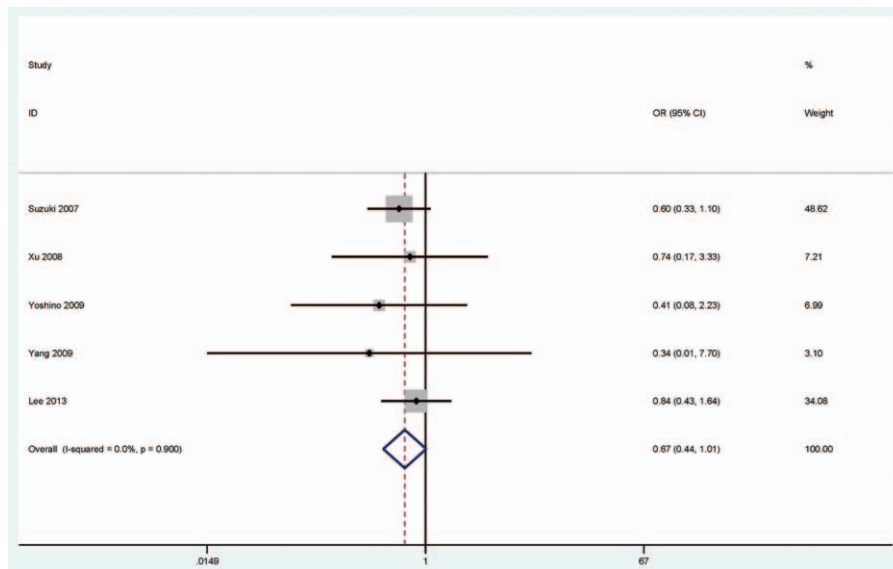
Our findings revealed that *WIF-1* promoter hypermethylation was significantly higher in lung cancer than in nonmalignant samples, suggesting that *WIF-1* promoter hypermethylation may play a pivotal role in lung cancer carcinogenesis.

Next, subgroup analyses by ethnic population, testing methods, and sample types were conducted to find the difference in lung cancer versus controls. Subgroup analysis by ethnic population showed that significant correlation was available between *WIF-1* promoter hypermethylation and ethnic subgroups. However, the subgroup of the Caucasian population had



**Figure 4.** Forest plot of the combined OR from 6 studies including 383 smoking and 198 nonsmoking patients with lung cancer in relation to smoking behavior in cancer ( $I^2=0.0\%$ , OR 1.88, 95% CI 1.26–2.79,  $P=0.002$ ). CI=confidence interval, OR=odds ratio.

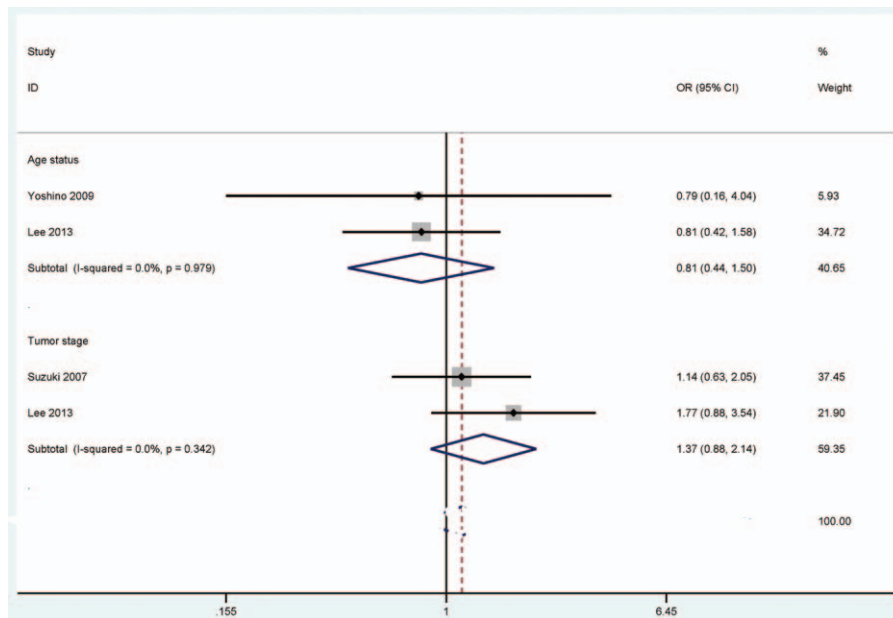




**Figure 5.** Forest plot of the combined OR from 5 studies with 282 adenocarcinoma (AC) and 207 squamous cell carcinoma (SCC) in relation to pathological types in cancer ( $I^2=0.0\%$ , OR 0.67, 95% CI 0.44–1.01,  $P=0.058$ ). CI=confidence interval, OR=odds ratio.

a higher OR (OR 17.36,  $P=0.021$ ) than the subgroup of the Asian population (OR 9.14,  $P<0.001$ ), indicating that Caucasians may be more susceptible to the expression of *WIF-1* gene. The subgroup analysis of testing methods demonstrated that *WIF-1* promoter hypermethylation had a significant association in both the MSP and the nMSP subgroups. Furthermore, the OR of the MSP subgroup (OR 12.47,  $P<0.001$ ) was higher than that of the nMSP subgroup (OR 5.90,  $P=0.001$ ), suggesting that MSP may be a more sensitive methylation testing method for hypermethylated *WIF-1*. The subgroup analysis of sample types

indicated that *WIF-1* promoter hypermethylation was significantly associated with an increased risk of lung cancer in the tissue, blood, and pleural effusion subgroups. Interestingly, the ORs of the blood and pleural effusion subgroups (OR 21.83,  $P=0.034$ ; OR 157.43,  $P=0.001$ , respectively) were significantly higher than that of the tissue subgroup (OR 7.89,  $P<0.001$ ), suggesting that *WIF-1* promoter hypermethylation may be a promising noninvasive biomarker for lung cancer for detection in blood or pleural effusion samples. However, the results should be interpreted cautiously because of 1 or 2 studies with small



**Figure 6.** Forest plot demonstrating the combined OR from 2 studies with 183 lung cancer patients in relation to age status in cancer ( $I^2=0.0\%$ , OR 0.81, 95% CI 0.44–1.50,  $P=0.499$ ); the pooled OR from 2 studies with 170 stage I lung cancer patients and 207 stage II to IV lung cancer patients in relation to tumor stage in cancer ( $I^2=0.0\%$ , OR 1.37, 95% CI 0.88–2.14,  $P=0.165$ ). CI=confidence interval, OR=odds ratio.

**Table 4**

**Prognosis of *WIF-1* promoter hypermethylation using multivariate analysis.**

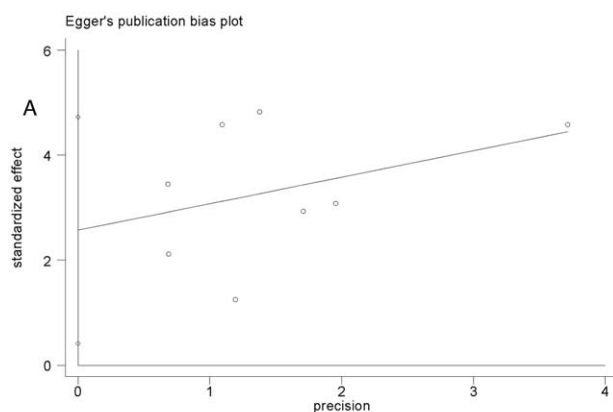
First author, y	Patients	Multivariate analysis for RFS	Multivariate analysis for OS
Yoshino, 2009 <sup>[22]</sup>	44	1.480 (0.307–7.133)	5.155 (1.125–23.810)
Lee, 2013 <sup>[28]</sup>	139	NA	1.13 (0.68–1.89)

The pooled HR in RFS: HR 1.480, 95% CI 0.307–7.133,  $P=0.625$ ; the pooled HR in OS: HR 2.02, 95% CI 0.48–8.58,  $I^2=70.7\%$ ,  $P=0.340$ . OS=overall survival, *WIF-1*=Wnt inhibitory factor-1.

subjects in the analyses of Caucasian population, nMSP, blood, and pleural effusion subgroups. The future conducting of additional studies with larger samples of subjects is indispensable.

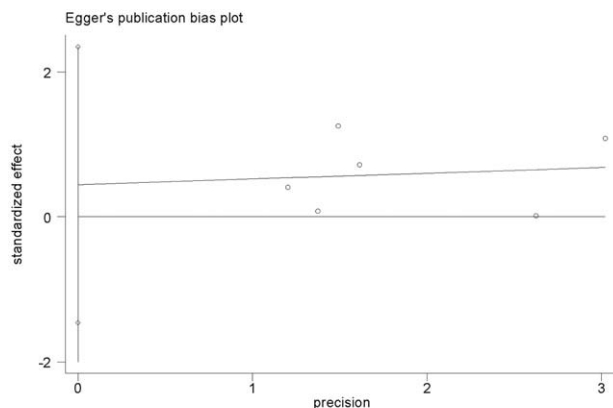
In addition, to explore the possible sources of heterogeneity, meta-regression analysis was implemented in our study, but the results obtained for the ethnic population, testing method, and sample type failed to explain the heterogeneity. However, we identified different methylation rates detected in samples from adjacent tissues. For example, Suzuki et al<sup>[30]</sup> reported that the methylation rate of *WIF-1* promoter was 1.1% in adjacent tissues, whereas Lee et al<sup>[28]</sup> discovered a methylation rate of 20.9%. This discrepancy could possibly be due to the collection of major control tissue samples from adjacent tissues which may have been differently contaminated by lung cancer cells. Therefore, the adjacent tissues selected for laboratory analysis could be one potential source of heterogeneity.

We also determined whether *WIF-1* promoter hypermethylation was associated with clinicopathological features, such as sex status, smoking behavior, age status, tumor stage, and pathological types of lung cancer. Our findings indicated that *WIF-1* promoter hypermethylation was not correlated with sex status, age status, tumor stage, and pathological types, but was correlated with smoking status, and was higher in smoking than in nonsmoking patients (OR 1.88, 95% CI 1.26–2.79,  $P=0.002$ ). In a previous meta-analysis on the association between gene methylation and smoking behavior in patients with NSCLC, Huang et al<sup>[37]</sup> revealed that the hypermethylation of 7 genes, including *WIF-1*, was correlated with the smoking behavior of NSCLC patients (OR 1.62, 95% CI 1.04–2.53,  $P=0.03$ ). The variations between the results of the pooled ORs may be due to the different numbers of studies included. We analyzed 6 studies, with 581 lung cancer patients, whereas Huang et al selected 5 studies including 507 patients.



cancer vs. control group ( $P=0.027$ )

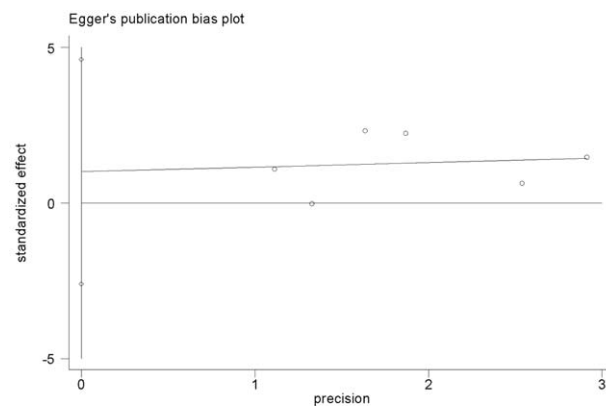
**A**



gender status in cancer ( $P=0.552$ )

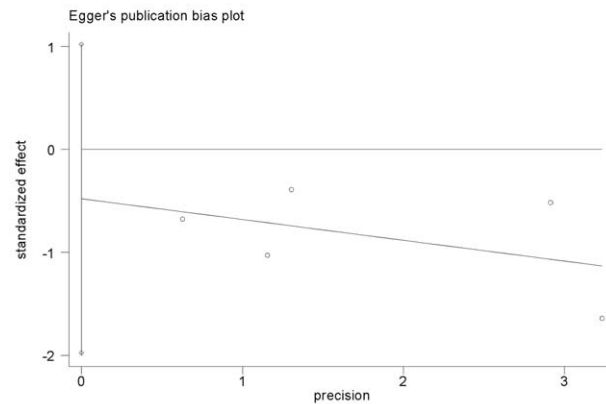
**B**

**Figure 7.** Funnel plot of the publications included in this study using Egger test: (A) cancer versus control group ( $P=0.027$ ); (B) sex status in cancer ( $P=0.552$ ).



smoking behavior in cancer ( $P=0.485$ )

**A**



pathological types in cancer ( $P=0.385$ )

**B**

**Figure 8.** Funnel plot of publication using Egger test in this study: (A) smoking behavior in cancer ( $P=0.485$ ); (B) pathological types in cancer ( $P=0.385$ ).

Finally, we investigated whether *WIF-1* promoter hypermethylation was correlated with the prognosis of lung cancer patients based on the determination of RFS and OS as predictors of outcome by multivariate analysis. The results revealed that *WIF-1* promoter hypermethylation was not associated with RFS and OS of lung cancer patients.

Nevertheless, several limitations of the present meta-analysis should be acknowledged. First, a slight publication bias was detected in lung cancer versus controls ( $P=0.027$ ). Since eligible studies published only in English or Chinese were included in our study, we could have missed relevant papers published in other languages. In addition, articles with positive results are more easily published than articles with negative results, which also could have caused a bias. Second, due to the limited number of studies, the relationship between *WIF-1* promoter hypermethylation and other clinicopathological characteristics, such as tumor grade, was not evaluated in this research. Third, only 1 or 2 studies with small sample sizes were included in the subgroup analyses of the Caucasian population, nMSP, blood, and pleural effusion. Finally, due to the limited subject sample size ( $n < 1000$ ) in our meta-analysis, additional studies with larger numbers of subjects are essential to further validate the results we obtained.

In conclusion, our findings suggest that *WIF-1* promoter hypermethylation is significantly associated with an increased risk of lung cancer. Moreover, promoter hypermethylation of *WIF-1* may become a noninvasive biomarker for lung cancer used for detection in blood or pleural effusion samples. Promoter hypermethylation of *WIF-1* is associated with smoking status, but not with sex status, age status, tumor state, pathological types, RFS, and OS. Further large-scale, multicenter cohort studies are absolutely essential to further confirm the role of *WIF-1* promoter hypermethylation in lung cancer.

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