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ORIGINAL RESEARCH

Clinicopathological significance and potential drug targeting of CDH1 in lung cancer: a meta-analysis and literature review

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Background: CDH1 is a protein encoded by the *CDH1* gene in humans. Mutations in this gene are linked with several types of cancer. Loss of CDH1 function contributes to the progression of cancer by increasing proliferation, invasion, and/or metastasis. However, the association between and clinicopathological significance of *CDH1* promoter methylation and lung cancer remains unclear. In this study, we systematically reviewed the studies of *CDH1* promoter methylation and lung cancer, and evaluated the association between *CDH1* promoter methylation and lung cancer using meta-analysis methods.

Methods: A comprehensive search of the PubMed and Embase databases was performed up to July 2014. The methodological quality of the studies was also evaluated. The data were extracted and assessed by two reviewers independently. Analyses of pooled data were performed. Odds ratios (ORs) were calculated and summarized.

Results: Finally, an analysis of 866 patients with non-small cell lung cancer from 13 eligible studies was performed. The *CDH1* methylation level in the cancer group was significantly higher than in the controls (OR 3.89, 95% confidence interval [CI] 2.87–5.27, $P<0.00001$). However, there were no correlations between *CDH1* promoter methylation and clinicopathological characteristics (sex status, OR 0.78, 95% CI 0.41–1.50, $P=0.46$; smoking history, OR 0.97, 95% CI 0.53–1.79, $P=0.93$; pathological type, OR 0.97, 95% CI 0.59–1.60, $P=0.91$; clinical staging, OR 1.48, 95% CI 0.81–2.68, $P=0.2$; lymph node metastasis, OR 0.68, 95% CI 0.13–3.63, $P=0.65$; or differentiation degree, OR 1.01, 95% CI 0.34–3.02, $P=0.99$).

Conclusion: The results of this meta-analysis suggest that *CDH1* methylation is associated with an increased risk of lung cancer. *CDH1* hypermethylation, which induces inactivation of the *CDH1* gene, plays an important role in carcinogenesis and may serve as a potential drug target in lung cancer. However, *CDH1* methylation does not correlate with other factors, such as smoking history, clinical stage, pathological type, sex status, lymph node metastasis, or degree of differentiation.

Keywords: CDH1, methylation, lung cancer, meta-analysis, tumor suppressor gene, odds ratio

Introduction

Lung cancer is a leading cause of cancer mortality in the USA and other developed countries.[1] The two major forms of the disease are non-small cell lung cancer and small cell lung cancer, which account for 85% and 15% of all lung cancers, respectively.[2] Despite advances in preliminary detection and standard treatment with combination chemotherapy and radiotherapy, the prognosis for patients with lung cancer has not improved significantly in the last 20 years,[3,4] and survival rates have changed little over the past 2 decades. Currently, an enormous amount of research is aimed at understanding the molecular and cellular biology of lung cancer; however, much more

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work is needed to better understand the correlation between gene regulation and lung cancer.

DNA methylation is an important epigenetic mechanism for gene silencing. Growing evidence shows that aberrant hypermethylation in 5′-CpG islands in the promoter regions is a major mechanism for silencing tumor suppressor or other cancer-associated genes in many kinds of human cancer.[5–8] Loss of function in cancer suppressor genes may hinder inhibition of growth of cancer cells, which leads to malignant transcription and translation during replication of DNA. A number of genes, including the cyclin-dependent kinase inhibitor (p16), the tumor suppressor gene Ras association domain family protein 1A, Kelch-like ECH-associating protein 1, the DNA repair gene *MGMT*, and the cystic fibrosis transmembrane conductance regulator are demonstratively methylated in lung cancer.[9–12] CDH1, a member of the transmembrane glycoprotein family, also known as cadherin-1, CAM 120/80, epithelial cadherin (E-cadherin), or uvomorulin, is encoded by the *CDH1* gene (16q22.1).[13] CDH1 is a calcium-dependent cell-cell adhesion glycoprotein containing three domains, ie, five extracellular cadherin repeats, a transmembrane region, and a highly conserved cytoplasmic tail.[14] *CDH1* as a tumor suppressor gene plays an essential role in maintaining cell adhesion and adherent junctions in normal tissues. *CDH1* expression is frequently absent in a variety of epithelial tumors, and loss of normal intercellular junctions results in promotion of cancer invasion and metastasis and is correlated with several types of cancers.[15–17] However, the association between and clinicopathological significance of CDH1 promoter hypermethylation and lung cancer remains unclear. In this study, we systematically investigate studies of *CDH1* promoter hypermethylation and lung cancer, and validate the association between *CDH1* promoter hypermethylation and lung cancer using meta-analysis methods. In addition, we summarize these findings and discuss tumor suppressor function, as well as the clinicopathological significance of *CDH1* in lung cancer.

Materials and methods

Publication selection

A systematic literature searching was performed using PubMed, Embase, and the Web of Science up to August 13, 2014 without any language restrictions. The following keywords and terms were used: [methylation or DNA methylation or hypermethylation or de-methylation] and [CDH1 or cadherin-1 or CAM 120/80 or epithelial cadherin (E-cadherin) or uvomorulin] and [lung cancer or lung carcinoma or lung tumor]. References from these publications were manually searched to identify additional studies. The published scientific articles were restricted to English language, and conference abstracts were excluded due to lack of sufficient data. Titles, abstracts, and key words in the articles were initially evaluated for inclusion criteria. Details and additional information were identified and collected from the full text of these articles.

Inclusion and exclusion criteria

A study included for meta-analysis needed to have: evaluated the correlation between *CDH1* methylation and lung cancer; included a clinical cohort and controls; included at least three patients and controls; used methylation-specific polymerase chain reaction or quantitative methylation-specific polymerase chain reaction to examine *CDH1* methylation and expression; and used tissue data rather than blood data. Studies that did not meet our inclusion criteria were excluded. When the same groups of patients were reported in multiple papers, only the most recent and complete paper was selected to avoid overlap.

Data extraction and quality assessment

Two researchers independently collected the information and extracted the data regarding authorship, year, source of publication, inclusion criteria, *CDH1* methylation frequency, sex status, smoking history, pathological type, clinical staging, degree of differentiation, lymph node metastasis, epidermal growth factor receptor status, and prognosis in patients and controls. Any discrepancy was resolved by discussion until agreement was reached. The data are summarized in Table 1 according to the criteria mentioned above. Methodological evaluation was assessed by the researchers according to the REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies) guidelines and ELCWP (European Lung Cancer Working Party) score.[18,19]

Data analysis

The meta-analysis was performed using Reviewer Manager 5 (Cochrane Collaboration, Oxford, UK). Pooled odds ratios (ORs) and confidence intervals (CIs) were calculated to assess the correlation between *CDH1* methylation and lung cancer. Cochran's Q test and I^2 were used to assess heterogeneity among the studies.[20] A Q test showing $P<0.05$ or an I^2 test $>50\%$ indicated significant heterogeneity and a fixed-effects model was used to calculate the parameters. Otherwise, a random-effects model was used to pool data and

Table 1 Characteristics of the included studies

Authors	Years	Samples		Sex status		Smoking history		Pathological types		Clinical staging		
		Cases	Controls	Male	Female	Yes	No	Squamous cell carcinomas	Adenocarcinomas	I	II	III and IV
Zochbauer-Muller et al[24]	2001	107	104	76	31	98	9	43	45	61	21	25
Yanagawa et al[25]	2003	75	75	54	21	20	55	29	43	24	33	42
Russo et al[26]	2005	49	49	–	–	22	5	–	–	–	–	–
Tsou et al[27]	2005	7	11	–	–	–	–	–	–	–	–	–
Kim et al[28]	2007	88	88	70	18	72	16	53	35	51	37	
Tan et al[29]	2007	20	10	–	–	–	–	–	–	–	–	–
Feng et al[30]	2007	49	49	26	23	–	–	14	20	21	17	–
Wang et al[31]	2007	28	12	17	11	–	–	7	15	–	–	–
Wang et al[32]	2008	95	95	19	76	–	–	35	45	31	26	34
Vaissiere et al[33]	2009	209	164	171	38	173	36	121	58	–	–	–
Begum et al[34]	2011	76	30	40	36	–	–	26	36	41	17	11
Guzman et al[35]	2012	26	33	26	33	–	–	25	7	–	–	–
Zheng et al[36]	2012	37	37	26	11	–	–	15	17	–	–	–

Note: "–" means data not available.

attempt to identify potential sources of heterogeneity based on subgroup analyses.[21,22] Publication bias was assessed by Begg's test and use of funnel plots.[23] The analysis of meta-regression and publication bias was performed using Stata version 10.0 (Statacorp, College Station, TX, USA).

Results

The process followed to select the papers used in this report is shown in Figure 1. Ninety papers were identified by electronic database searching and 20 further papers by manual searching. Eighty-five papers were excluded for being duplicate publications, being an irrelevant title or abstract, or being a relevant title and abstract but not published in the English language. After retrieval of the full-text articles, eleven papers were excluded for not having a large enough study population or for being reviews. Thirteen further studies were screened out (involving 866 cases and 757 controls) based on the inclusion and exclusion criteria in the pooled analysis.

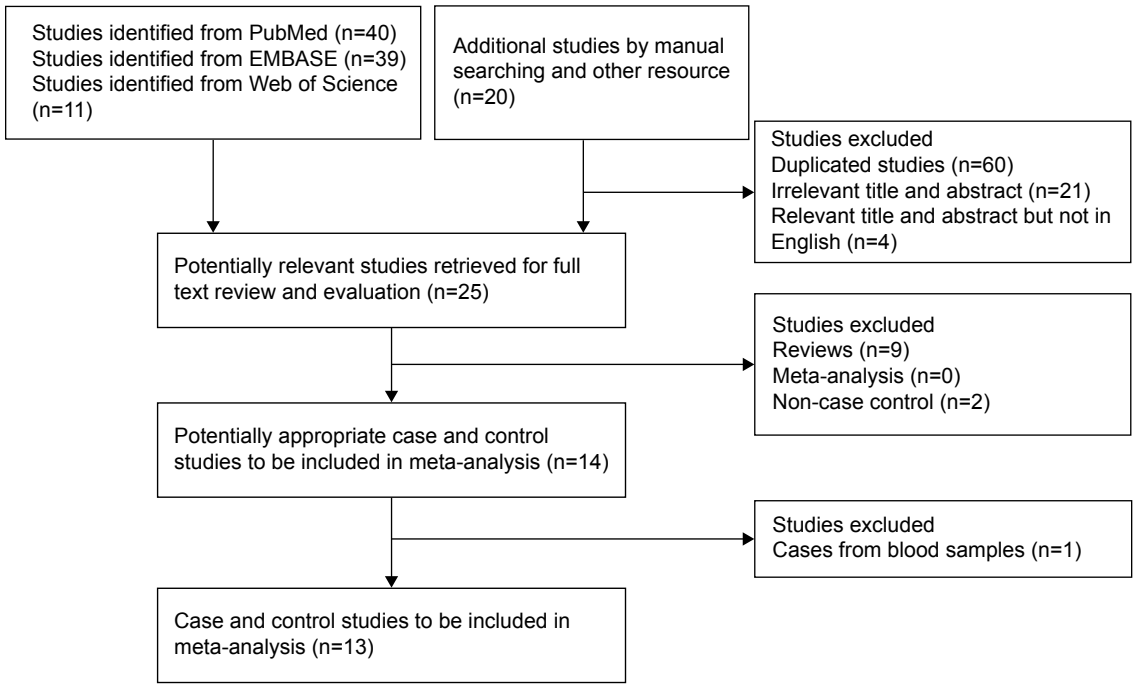


Figure 1 Flow diagram of the literature search strategy and assessment of studies identified for meta-analysis.
Abbreviation: EMBASE, Excerpta Medica dataBASE.

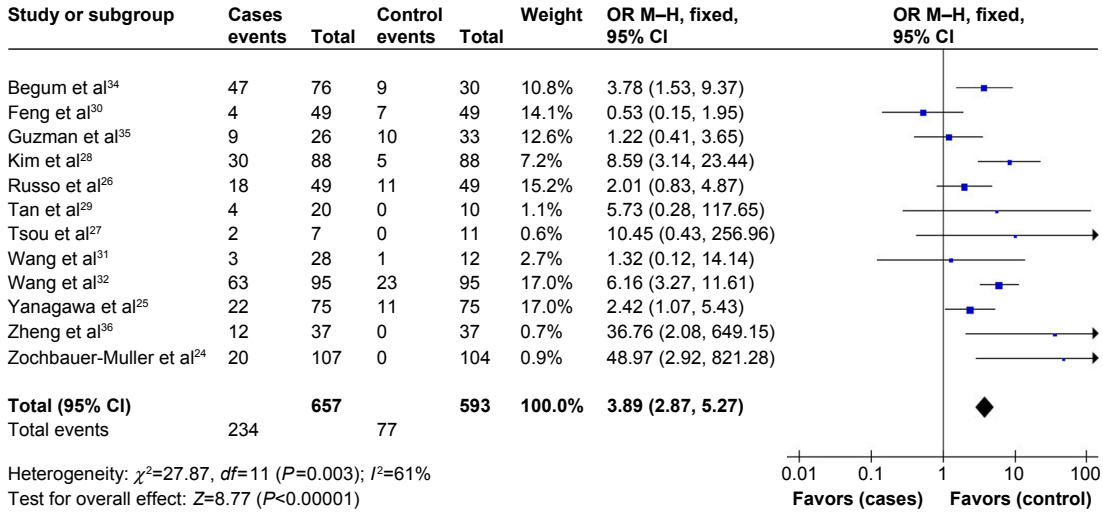


Figure 2 Estimates for *CDH1* methylation frequency associated with lung cancer in the meta-analysis.
Abbreviations: CI, confidence interval; M–H, Mantel–Haenszel; OR, odds ratio.

The characteristics of the studies are shown in Table 1.[24–36] Six papers were used to study the correlation between *CDH1* methylation and sex status. Five papers provided smoking history data, allowing an investigation of the influence of smoking on *CDH1* methylation. Six studies included pathological typing (squamous small carcinoma or adenocarcinoma). Three studies investigated the effect of clinical stage, ie, stage I, II, III, or IV. One paper discussed lymph node metastasis and another discussed degree of differentiation.

Analyzing tissue samples from 657 patients and 593 controls, the mean frequency of *CDH1* methylation was 32% (range 8.33% to 66.32%) in tumor tissue and 9% (range 0.00%–27.78%) in tissue from controls. This result indicates that the occurrence of *CDH1* methylation is higher in tumor tissue than in normal tissue. Using the fixed model, meta-analysis showed that 657 cases and 593 controls from 12 studies were pooled OR as shown in Figure 2 (OR 3.89, 95% CI 2.87–5.27, $P<0.00001$). These findings indicate that *CDH1* methylation is a key molecular event in tumor tissue but not in normal tissue.

The results also show heterogeneity across the included studies (I^2 is 61%, ie, more than 50%). Given this significant heterogeneity, a subgroup analysis was performed to investigate sex status, smoking history, pathological type, clinical stage, differentiation degree, and lymph node metastasis to observe the relationship between *CDH1* methylation and clinical characteristics (see Figure 3). However, there was no correlation between *CDH1* promoter methylation and any of these factors (sex status, OR 0.78, 95% CI 0.41–1.50, P=0.46; smoking history, OR 0.97, 95% CI 0.53–1.79, P=0.93; pathological type, OR 0.97, 95% CI 0.59–1.60, P=0.91; clinical stage, OR 1.48, 95% CI 0.81–2.68, P=0.2; lymph node metastasis, OR 0.68, 95% CI 0.13–3.63, P=0.65; and differentiation degree, OR 1.01, 95% CI 0.34–3.02, P=0.99).

The stability of the results was tested by sensitivity analysis. The OR ranged from 0.78 to 3.89, which was not a significant change, suggesting that the results of our meta-analysis were not significantly unstable. The funnel plot shown in Figure 4 is partially symmetric, indicating low publication bias regarding *CDH1* methylation in lung cancer.

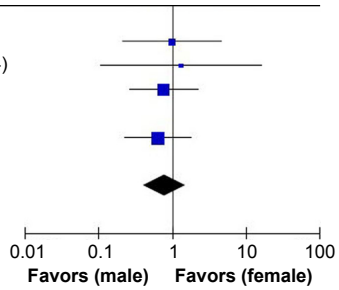
Discussion

DNA methylation is an important epigenetic mechanism for regulation of gene expression. An imbalance of gene methylation can cause a variety of human diseases. Hypermethylation of tumor suppressor genes and hypomethylation of oncogenes are two essential components of the molecular mechanism involved in the epigenomic regulation of initiation and progression of cancer. As a tumor suppressor gene, *CDH1* maintains cell-cell adhesion and keeps epithelial cells arranged in normal arrangement and layer. In vitro studies demonstrate that loss of expression or function of *CDH1* can activate transcription factors associated with epithelial-mesenchymal transition, leading to metastasis of cancer cells.[37] *CDH1* methylation has been detected in several types of carcinoma, including breast cancer, gastric cancer, and lung cancer.[38–40] A comprehensive evaluation of markers of methylation in lung cancer is needed to better understand the relationship between *CDH1* methylation and lung cancer. Although a large number of studies have demonstrated a possible relationship between

A

Study or subgroup	Male events	Total	Female events	Total	Weight	OR M–H, fixed, 95% CI
Kim et al[28]	20	70	10	0		Not estimable
Vaissiere et al[33]	9	171	2	38	15.6%	1.00 (0.21, 4.83)
Wang et al[32]	2	17	1	11	5.4%	1.33 (0.11, 16.74)
Yanagawa et al[25]	15	54	7	21	36.7%	0.77 (0.26, 2.28)
Zheng et al[36]	0	0	0	0		Not estimable
Zochbauer-Muller et al[24]	12	76	7	31	42.2%	0.64 (0.23, 1.83)
Total (95% CI)		**388**		**101**	**100.0%**	**0.78 (0.41, 1.50)**
Total events	58		27			

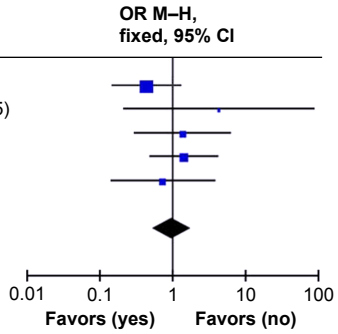
Heterogeneity: χ^2=0.40, *df*=3 (*P*=0.94); I^2=0%
Test for overall effect: *Z*=0.74 (*P*=0.46)



B

Study or subgroup	Yes events	Total	No events	Total	Weight	OR M–H, fixed, 95% CI
Kim et al[28]	22	72	8	16	43.4%	0.44 (0.15, 1.32)
Russo et al[26]	6	22	0	5	2.7%	4.33 (0.21, 90.05)
Vaissiere et al[33]	13	173	2	36	14.6%	1.38 (0.30, 6.40)
Yanagawa et al[25]	7	20	15	55	24.8%	1.44 (0.48, 4.29)
Zochbauer-Muller et al[24]	17	98	2	9	14.5%	0.73 (0.14, 3.85)
Total (95% CI)		**385**		**121**	**100.0%**	**0.97 (0.53, 1.79)**
Total events	65		27			

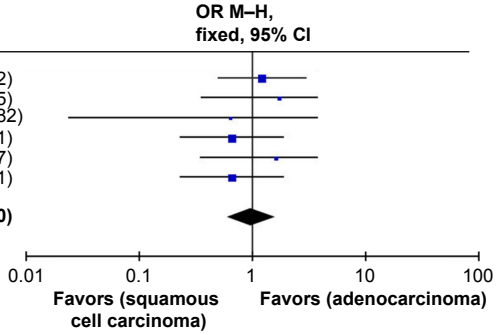
Heterogeneity: χ^2=3.73, *df*=4 (*P*=0.44); I^2=0%
Test for overall effect: *Z*=0.09 (*P*=0.93)



C

Study or subgroup	Squamous cell carcinoma events	Total	Adenocarcinoma events	Total	Weight	OR M–H, fixed, 95% CI
Kim et al[28]	19	53	11	35	26.9%	1.22 (0.49, 3.02)
Vaissiere et al[33]	7	121	2	58	8.1%	1.72 (0.35, 8.55)
Wang et al[32]	0	7	1	15	3.0%	0.64 (0.02, 17.82)
Yanagawa et al[25]	7	29	14	43	27.1%	0.66 (0.23, 1.91)
Zheng et al[36]	5	15	4	17	7.9%	1.63 (0.34, 7.67)
Zochbauer-Muller et al[24]	7	29	14	43	27.1%	0.66 (0.23, 1.91)
Total (95% CI)		**254**		**211**	**100.0%**	**0.97 (0.59, 1.60)**
Total events	45		46			

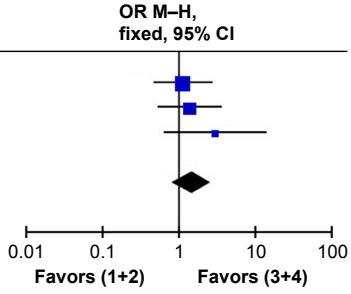
Heterogeneity: χ^2=2.23, *df*=5 (*P*=0.82); I^2=0%
Test for overall effect: *Z*=0.12 (*P*=0.91)



D

Study or subgroup	I+II events	Total	III+IV events	Total	Weight	OR M–H, fixed, 95% CI
Kim et al[28]	18	51	12	37	49.0%	1.14 (0.46, 2.79)
Yanagawa et al[25]	14	57	8	42	37.8%	1.38 (0.52, 3.68)
Zochbauer-Muller et al[24]	17	82	2	25	13.2%	3.01 (0.64, 14.04)
Total (95% CI)		**190**		**104**	**100.0%**	**1.48 (0.81, 2.68)**
Total events	49		22			

Heterogeneity: χ^2=1.16, *df*=2 (*P*=0.56); I^2=0%
Test for overall effect: *Z*=1.28 (*P*=0.20)



E

Study or subgroup	Yes events	Total	No events	Total	Weight	OR M–H, fixed, 95% CI
Wang et al[32]	1	22	2	6	91.1%	0.10 (0.01, 1.32)
Zheng et al[36]	1	1	11	36	8.9%	6.65 (0.25, 175.98)
Total (95% CI)		**23**		**42**	**100.0%**	**0.68 (0.13, 3.63)**
Total events	2		13			

Heterogeneity: χ^2=4.01, *df*=1 (*P*=0.05); I^2=75%
Test for overall effect: *Z*=0.45 (*P*=0.65)

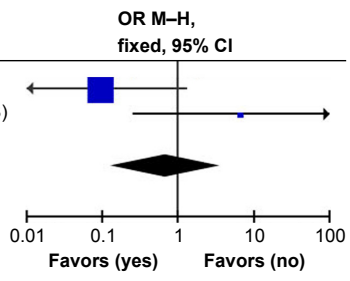


Figure 3 (*Continued*)

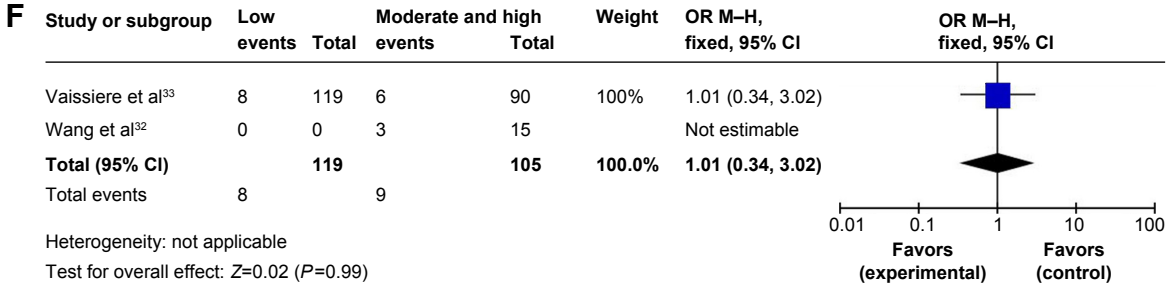


Figure 3 Clinicopathological significance of *CDH1* hypermethylation rate in patients with lung cancer. Forest plots for the relationship between *CDH1* methylation frequency and lung cancer. (**A**) Sex status, (**B**) smoking history, (**C**) pathological type, (**D**) clinical staging, (**E**) degree of differentiation, and (**F**) lymph node metastasis.
Abbreviations: CI, confidence interval; M–H, Mantel–Haenszel; OR, odds ratio.

CDH1 methylation and lung cancer, a meta-analysis can summarize the relevant studies and compare different subgroup characteristics.

In this meta-analysis, we mainly focus on the correlation between *CDH1* methylation and lung cancer. We analyzed data from 13 studies that included 657 tumor tissue samples and 593 control samples. The results show that the *CDH1* methylation level in the cancer group was significantly higher than in the control group. The pooled OR using the fixed-effect model was 3.89 (95% CI 2.87–5.27 versus the control group). *CDH1* methylation plays a key role in the induction of lung cancer due to silencing of the tumor suppressor gene *CDH1*. This conclusion is consistent with that of a previous study.[36] Since changes in *CDH1* promoter hypermethylation are reversible, drug treatment promoting demethylation may be useful for delaying carcinogenesis and progression. Treatment with 5-aza-2′-deoxycytidine showed that migration of A549 cells decreased markedly upon restoration of CDH1.[41] These preclinical studies show the therapeutic potential of restoration of tumor suppressor expression via

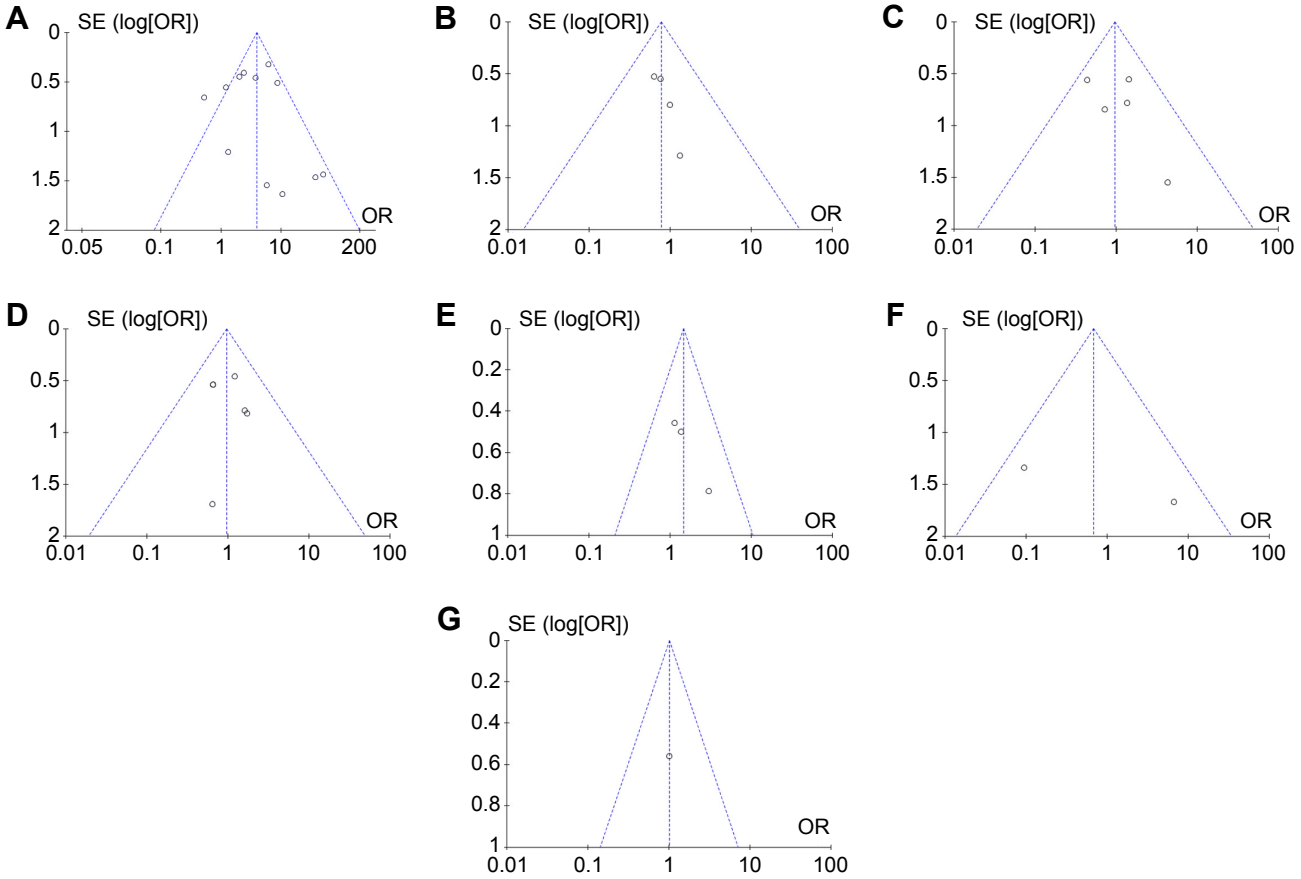


Figure 4 Funnel plot analysis of *CDH1* hypermethylation rate in patients with lung cancer. Funnel plot for assessment of publication bias in the meta-analysis. (**A**) Cases versus control, (**B**) sex status, (**C**) smoking history, (**D**) pathological type, (**E**) clinical staging, (**F**) degree of differentiation, and (**G**) lymph node metastasis, respectively.
Abbreviations: OR, odds ratio; SE, standard error of the mean.

epigenetic modulation. This approach may bring hope for patients with cancer through gene-targeted therapy.

We further determined the clinicopathological significance of *CDH1* promoter hypermethylation in patients with lung cancer. For smoking history, the summary OR was 0.97 (95% CI 0.53–1.79) in the 65 cases and 27 controls. The results show that methylation level of *CDH1* is not associated with smoking history. Lung cancer is a complicated disease with different genetic and epigenetic profiling. Smoking is not the only risk factor for lung cancer. Smoking may target other specific genes for methylation or mutation, for example, methylenetetrahydrofolate reductase.[33] Other subgroup meta-analysis was performed, including for sex status (OR 0.78, 95% CI 0.41–1.50), pathological type (OR 0.97, 95% CI 0.59–1.60), clinical stage (OR 1.48, 95% CI 0.81–2.68), lymph node metastasis (OR 0.68, 95% CI 0.13–3.63), and degree of differentiation (OR 1.01, 95% CI 0.34–3.02). *CDH1* methylation determined by clinical staging shows a slightly more significant association than the other subgroups. Interestingly, *CDH1* methylation was detected much more frequently in stages III and IV than in stages I and II. However, *CDH1* methylation itself does not correlate with pathological type, sex status, lymph node metastasis, or degree of differentiation. Possible reasons for this finding might be the widely heterogeneous results for the subgroups or the lack of cases and controls in the subgroups. Other potentially significant factors may need to be investigated, such as patient age, tumor size, and biopsy sample control.[42]

Conclusion

CDH1 promoter methylation is associated with lung cancer based on this meta-analysis. *CDH1* methylation might be a biomarker of lung cancer, with potential value in predicting the prognosis of the disease, and warrants further studies involving more clinical cases for meta-analysis in the future. In addition, the potential variables on *CDH1* methylation from different group database are still not clear due to the limitation of the statistical power of meta-analysis.

Disclosure

The authors have no financial involvement with any organization or entity with a financial interest in the subject matter or materials discussed in this paper.

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