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ORIGINAL RESEARCH

Clinicopathological significance and potential drug target of *T-cadherin* in NSCLC

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Background: Previous studies demonstrate that T-cadherin is a candidate tumor suppressor in several types of human tumors, including non-small cell lung cancer (NSCLC). Lack of protein expression of *T-cadherin* by hypermethylation has been found to play an important role in lung alveolar differentiation regulation and epithelial tumorigenesis. However, the correlation between *T-cadherin* hypermethylation and clinicopathological characteristics of NSCLC remains unclear. Here we conducted a systematic review and meta-analysis to quantitatively evaluate the effects of *T-cadherin* hypermethylation on the incidence of NSCLC and clinicopathological characteristics.

Methods: A detailed literature search was carried out for related research publications. Analyses of pooled data were performed. Odds ratio (OR) and hazard ratio (HR) were calculated and summarized, respectively.

Results: Final analysis of 1,172 NSCLC patients from 15 eligible studies was performed. *T-cadherin* hypermethylation was observed to be significantly higher in NSCLC than in normal lung tissue, based on the pooled OR from nine studies including 532 NSCLC and 372 normal lung tissue samples (OR=8.19, 95% confidence interval [CI]=5.41–12.39, $P<0.00001$). *T-cadherin* hypermethylation may also be associated with pathological types. The pooled OR was obtained from four studies including 111patients with squamous cell carcinoma and 106 with adenocarcinoma (OR=0.35, 95% CI=0.19–0.66, $P=0.001$), which indicated that *T-cadherin* hypermethylation plays a more important role in the pathogenesis of adenocarcinoma. We did not find that *T-cadherin* hypermethylation was correlated with the sex or smoking status, clinical stages, or epidermal growth factor receptor (EGFR) mutation status. However, *T-cadherin* hypermethylation was found to be significantly higher in poorly differentiated NSCLC than in moderately and highly differentiated NSCLC, and NSCLC patients with *T-cadherin* hypermethylation had a lower survival rate than those without *T-cadherin* hypermethylation.

Conclusion: The results of this meta-analysis suggest that *T-cadherin* hypermethylation is associated with an increased risk and worse survival in NSCLC. *T-cadherin* hypermethylation, which induces the inactivation of *T-cadherin* gene, plays an important role in the carcinogenesis, cancer progression, as well as clinical outcome.

Keywords: methylation, lung cancer, meta-analysis, EGFR, odds ratio, hazard ratio

Introduction

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer and is the leading cause of cancer-related deaths worldwide.[1,2] Despite the advances in early detection, radical surgical cure, and multimodal therapeutic modalities, at diagnosis, there are about 80% of NSCLC cases in advanced stage, and the prognosis remains poor.[3] Therefore, investigation of the mechanism of initiation, progression, as well as identification of prognostic markers is still needed for selection of patients with high chance of lung cancer recurrence and to provide better prognosis and

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individualized treatment. Epigenetic control of gene expression plays an important role in carcinogenesis. Aberrant methylation of CpG dinucleotides is a commonly observed epigenetic modification in human cancer.[4–6] Recently, Heller et al identified a large number of tumor-specific methylated genes in NSCLC patients and found that many of them were regulated by methylation and involved in the pathogenesis of NSCLC.[7] The DNA methylation signature of NSCLC affects the outcome of certain patients and can be practically determined by user-friendly polymerase chain reaction assays, and it has been shown that analysis of the best DNA methylation biomarkers improved prognostic accuracy beyond standard staging.[8] Interestingly, patterns of DNA methylation can divide NSCLC into two phenotypically distinct subtypes of tumors and provide proof of principle that differences in DNA methylation can be used as a platform for predictive biomarker discovery and development.[9] Detection of aberrant gene promoter methylation as a tool for the diagnosis of tumors or its use as prognostic marker has been widely described for many different cancers, including NSCLC.[10]

Cadherins function as membrane receptors mediating outside-in signals, activating small guanosine-5′-triphosphate (GTP)ases and the β-catenin/Wnt pathway, resulting in dynamic cytoskeleton reorganization and changes in the phenotype; as such, they are important determinants of tumor progression, serving as a suppressor of invasion and metastasis in many contexts.[11–13] T-cadherin, also known as cadherin 13 (CDH13) or H-cadherin (heart), is a unique member of the cadherin superfamily since it lacks the transmembrane and cytoplasmic domains and is anchored to the cell membrane through the glycosylphosphatidylinositol (GPI) anchor.[14–16] A number of studies demonstrated that T-cadherin is involved in low-density lipoproteins (LDL) hormone-like effects on Ca^{2+}-mobilization and increased cell migration, phenotype changes, as well as insulin-dependent signaling, eNOS activation, angiogenesis, as well as in the regulation of brain network development, plasticity, and function.[17–20] Abnormalities in the *T-cadherin* gene have been identified in several tumors, with upregulation of *T-cadherin* inducing cell cycle arrest, apoptosis, and inhibition of angiogenesis.[21–27] The introduction of *T-cadherin* in human breast carcinoma cells markedly reduced their invasive potential and growth rate; it also induced the reversion of morphology from an invasive type to a normal cell-like type.[28,29] *T-cadherin* methylation and/or gene deletion have been found to play an important role in lung alveolar differentiation regulation and epithelial tumorigenesis.[30–34] Although previous studies indicated that inactivation of the *T-cadherin* gene is mainly induced by hypermethylation of the gene, the reported *T-cadherin* hypermethylation rates in NSCLC were remarkably diverse. In addition, its roles in NSCLC and clinicopathological significance have not been thoroughly investigated. There were no previous meta-analyses in the literature that covered this research question. Hence, we conducted a systematic review and meta-analysis to quantitatively evaluate the effects of *T-cadherin* hypermethylation on the incidence and clinical characteristics of NSCLC.

Methods

Search strategy and selection criteria

The following electronic databases were searched for relevant articles without any language restrictions: Web of Science™ (1945–2014), the Cochrane Library database, PubMed (1966–2014), Embase (1980–2014), Cumulative Index to Nursing and Allied Health Literature (CINAHL) (1982–2014), China National Knowledge Infrastructure (CNKI), Google Scholar, and the Chinese Biomedical Database (CBM) (1982–2014).

We searched articles using the search terms: "lung" and "cancer or tumor or neoplasm or carcinoma", "methylation", and "T-cadherin or CDH13 or cadherin 13". We also manually searched the reference lists of the retrieved articles and reviews for additional articles.

Although our search did not have language limits initially, for the full-text reading and final evaluation, we only performed the review of the studies published in English and Chinese language. After exclusion of nonrelevant and/or redundant publications from the different databases, the remaining papers were evaluated in the full-text version for inclusion and exclusion criteria and for relevant articles in the reference lists. All searched data were retrieved. Authors' bibliographies and references of selected studies were also searched for other relevant studies. The most complete study was chosen to avoid duplication if same patient populations were reported in several publications.

The criteria that an eligible study had to meet were as follows: 1) *T-cadherin* hypermethylation evaluated in the primary NSCLC tissues; 2) research revealed the relationship between *T-cadherin* hypermethylation and NSCLC clinicopathological parameters and prognosis; 3) *T-cadherin* hypermethylation examined by methylation-sensitive polymerase chain reaction; and 4) studies provided sufficient information to estimate hazard ratio (HR) for overall survival (OS) and 95% confidence interval (CI). The exclusion criteria included the following: 1) letters, reviews, case reports, conference abstracts, editorials, and expert opinion; and also,

2) all publications regarding in vitro/ex vivo studies, cell lines, and human xenograft.

Data extraction and methodological assessment

Two authors (ZW and BW) independently reviewed and extracted data from the eligible studies. Disagreements were resolved by discussion and consensus. Two authors (HG and GS) reviewed all of the articles that fit the inclusion and exclusion criteria. The following information was recorded for each study: first author name; year of publication; sample source; number of cases; clinicopathological parameter; cancer tumor/node/metastasis (TNM) stage; epidermal growth factor receptor (EGFR) mutation status; methylation detection method, methylation rate, and/or expression; and follow up. Data for study characteristics and clinical responses were summarized in table format. Heterogeneity of results was evaluated to determine whether the data of the various studies could be analyzed for a meta-analysis.

For the methodological evaluation of the studies, three investigators (ZW, BW, and XH) independently read through each publication and assessed and scored them according to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) guidelines and European Lung Cancer Working Party (ELCWP) quality scale.[35,36] The three readers provided the quality scores and compared them, and then reached a consensus value for each item.

Statistical analysis

Analysis was conducted using the STATA 12.0 (StataCorp LP, College Station, TX, USA) and Review Manager (RevMan), Version 5.2 (The Nordic Cochrane Centre, Copenhagen, Denmark). The pooled frequency of *T-cadherin* hypermethylation and 95% CIs were estimated. The frequency of *T-cadherin* hypermethylation was compared according to different tumor characteristics. Heterogeneity among studies was evaluated with Cochran's Q test[37] and the I^2 statistic.[38,39] When heterogeneity was not an issue (I^2 values $<50\%$), a fixed effect model was used to calculate parameters. If there was substantial heterogeneity (I^2 values $\geq 50\%$), a random effects model was used to pool data and to identify potential sources of heterogeneity, based on subgroup analyses. The pooled odds ratio (OR) was estimated for the association between *T-cadherin* hypermethylation and clinicopathological features. P-values tailed less than 0.05 were considered statistically significant.

Publication bias was assessed by using a method reported by Egger et al.[40] We also explored reasons for statistical heterogeneity using meta-regression, subgroup analysis, and sensitivity analysis. The analysis of meta-regression and publication bias was performed using STATA 10.0 (StataCorp LP).

Results

Forty-five publications were identified by the search method as described above. Thirty-two of those were excluded as they were laboratory studies, nonoriginal articles (review), or studies irrelevant to the current analysis. Eventually, there were 15 studies included in final meta-analysis,[30,33,41–53] as shown in Figure 1. Fifteen studies published from 2001 to 2012 were eligible for meta-analysis. A total of 1,172 NSCLC patients from the People's Republic of China, Japan, South Korea, the Czech Republic, Italy, the Republic of Serbia, and the USA were enrolled. Their basic characteristics are summarized in Table 1.

The loss of *T-cadherin* messenger (m)RNA and/or protein expression was strongly correlated with the promoter hypermethylation in several types of cancer, including NSCLC.[32,33,54–62] *T-cadherin* hypermethylation was significantly higher in NSCLC than in normal lung tissue. The pooled OR from nine studies including 532 NSCLC and 372 normal lung tissue samples is shown in Figure 2 (OR=8.19, 95% CI=5.41–12.39, $P<0.00001$), and indicates that *T-cadherin* inactivation through hypermethylation plays an important role in the carcinogenesis of NSCLC.

It was previously reported that *T-cadherin* hypermethylation rate in female NSCLC patients was significantly higher than that in male patients.[41,42] However, the pooled OR from seven studies including 388 male and 222 female NSCLC patients, as shown in Figure 3A (OR=0.87, 95% CI=0.46–1.66, $P=0.68$), indicates that *T-cadherin* hypermethylation is not strongly associated with sex status in NSCLC patients. Pesek et al reported that the *T-cadherin* hypermethylation rate in NSCLC patients without a smoking history was significantly higher than that in patients with a smoking history (80% vs 43%; 16/20 vs 42/98) (chi-square =0.96, $P=0.002$).[42] The pooled OR from six studies including 447 NSCLC patients with and 237 patients without smoking history is shown in Figure 3B (OR=1.07, 95% CI=0.56–2.04, $P=0.85$) and indicates that *T-cadherin* hypermethylation is not strongly associated with the smoking status in NSCLC patients. Histology was associated with *T-cadherin* hypermethylation, which was observed more frequently in patients with adenocarcinoma (AD).[41] The pooled OR from four studies including 111 squamous cell carcinoma patients and 106 AD patients is shown in Figure 3C (OR=0.35, 95% CI=0.19–0.66, $P=0.001$)

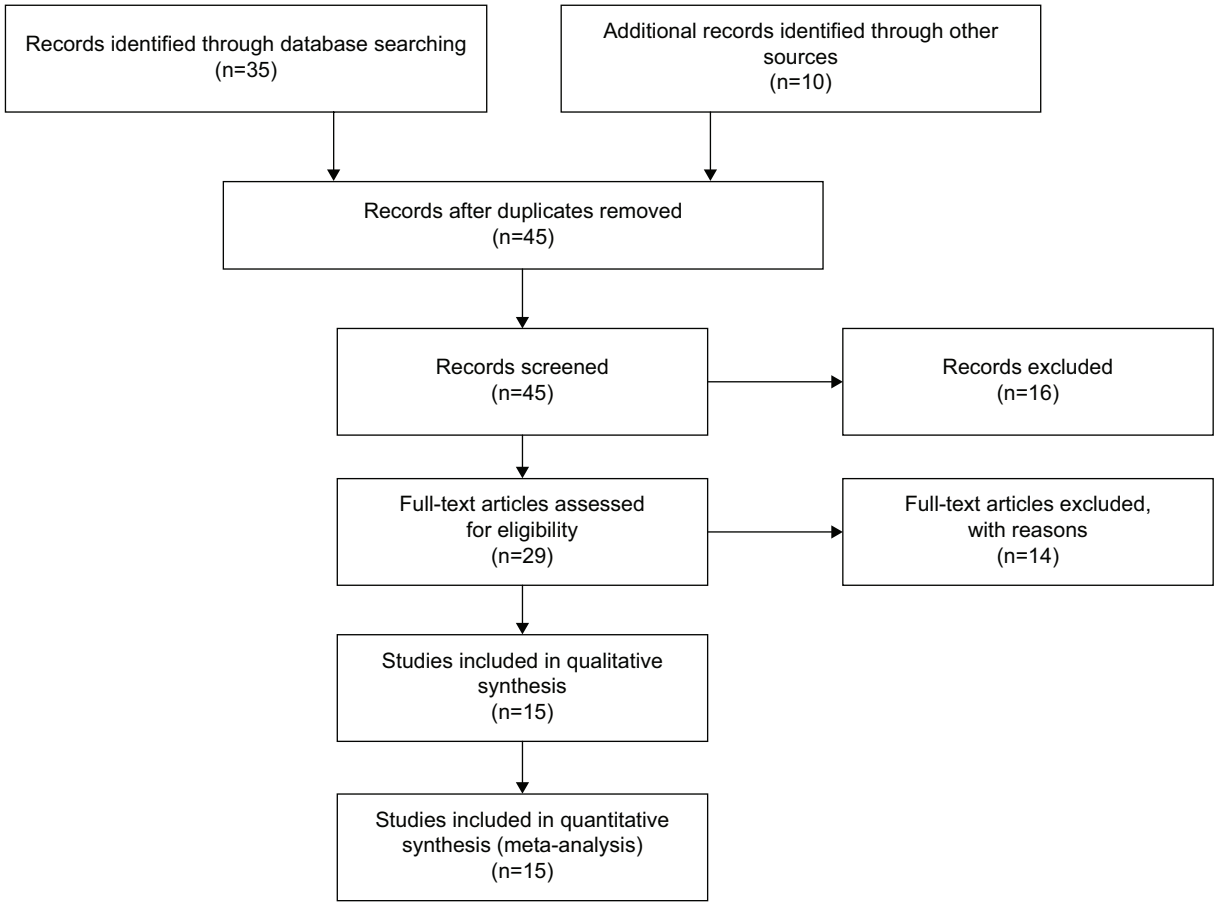


Figure 1 Flow chart of study selection.

and indicates that *T-cadherin* hypermethylation plays a more important role in the pathogenesis of AD.

We analyzed 363 NSCLC patients pooled from four studies to assess whether the aberrant *T-cadherin* hypermethylation in NSCLC was associated with advanced stage. As shown in Figure 4A, aberrant *T-cadherin* hypermethylation was not significantly higher in advanced NSCLC (stage III and IV) than that in early stage NSCLC (stage I and II), OR=0.93, 95% CI=0.57–1.53, *P*=0.79. However, as shown in Figure 4B, aberrant *T-cadherin* hypermethylation was significantly higher in poorly differentiated NSCLC than in moderately and highly differentiated NSCLC (OR=3.61, 95% CI=1.76–7.39, *P*=0.0004). These results suggest that *T-cadherin* hypermethylation may play an important role in NSCLC progression and development. There were four studies that estimated the relationship between *T-cadherin* hypermethylation and OS in NSCLC. The pooled HR for OS showed that *T-cadherin* hypermethylation was associated with worse survival in NSCLC patients, as shown in Figure 4C (HR=3.21, 95% CI=1.41–7.31, *P*=0.005). In addition, there were three studies that determined the relationship between *T-cadherin* hypermethylation and EGFR mutation status in NSCLC. The pooled OR from three studies including 137 EGFR mutation-positive patients and 245 EGFR mutation-negative patients is shown in Figure 5 (OR=1.03, 95% CI=0.27–3.99, *P*=0.96) and indicates that *T-cadherin* hypermethylation has no significant correlation with EGFR mutation status in NSCLC.

A sensitivity analysis, in which one study was removed at a time, was conducted to assess the result stability. The pooled ORs and HRs were not significantly changed, indicating the stability of our analyses. The funnel plots were largely symmetric (Figure 6), suggesting there were no publication biases in the meta-analysis of *T-cadherin* hypermethylation and clinicopathological features.

Discussion

Inactivation of *T-cadherin* by promoter hypermethylation plays an important role during normal development and in the tumorigenesis of several types of tumors, including NSCLC.[41,47,56,63–69] To date, there have been some studies describing the precise expression, prognostic impact, and methylation status of *T-cadherin* in NSCLC; however, the role of *T-cadherin* inactivation by hypermethylation in

Table 1 Basic characteristics of the included studies

Study	Country	Patients	Methods	Primary aim	Methylation site	T-cadherin expression
Kontic et al[41]	Republic of Serbia	65	MSP	Determine the frequency of *T-cadherin* hypermethylation in NSCLC	Promoter, CpG islands	–
Sui et al[53]	People's Republic of China	40	MSP/RT-PCR	Determine the significance of *T-cadherin* methylation in NSCLC	Promoter, CpG islands	+
Pesek et al[42]	Czech Republic	121	Methylation-specific, multiplex ligation-dependent probe amplification	Determine the clinical significance of hypermethylation status in NSCLC	Promoter, CpG islands	–
Liao et al[52]	People's Republic of China	44	MSP	Determine the status of *T-cadherin* methylation in NSCLC	Promoter, CpG islands	–
Zhang et al[44]	People's Republic of China	78	MSP	Determine the methylation status of multiple genes in NSCLC	Promoter, CpG islands	–
Kubo et al[43]	Japan	100	MSP	Aims to determine the methylation status of five tumor suppressors in NSCLC	Promoter, CpG islands	–
Feng et al[45]	United States	49	DNA methylation (MethyLight) analysis	Determine the clinical significance of seven tumor suppressors in NSCLC	Promoter, CpG islands	–
Brock et al[30]	United States	79	MSP	Determine the association between gene methylation and recurrence in NSCLC	Promoter, CpG islands	–
Wang et al[48]	People's Republic of China	28	DNA microarray-coupled PCR	Determine status of methylation of 15 genes in NSCLC	Promoter, CpG islands	–
Kim et al[47]	South Korea	88	MSP	Determine the role of *T-cadherin* in pathogenesis of NSCLC	Promoter, CpG islands	–
Hsu et al[46]	People's Republic of China	63	MSP	Determine methylation patterns of six tumor suppressor genes in NSCLC	Promoter, CpG islands	–
Suzuki et al[49]	Japan	150	MSP	Analyze the methylation profile of nine genes for NSCLC, and correlate with clinical data	Promoter, CpG islands	–
Toyooka et al[50]	Japan	164	MSP	Determine the relationship between genetic and epigenetic alterations in NSCLC	Promoter, CpG islands	–
Ulivi et al[51]	Italy	61	MSP	Detect promoter methylation of $p16^{INK4A}$ and *T-cadherin* genes in NSCLC	Promoter, CpG islands	–
Toyooka et al[33]	United States	42	MSP	Analyze DNA methylation status of *T-cadherin* gene in NSCLC patients	Promoter, CpG islands	+

Abbreviations: MSP, methylation-specific PCR; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; RT-PCR, reverse-transcription PCR.

Study or subgroup	NSCLC Events	Total	Normal lung tissue Events	Total	Weight	Odds ratio M–H, fixed, 95% CI
Feng et al[45]	28	49	1	49	2.2%	64.00 (8.16–501.90)
Hsu et al[46]	28	63	9	63	25.4%	4.80 (2.02–11.38)
Kim et al[47]	26	88	7	88	25.0%	4.85 (1.98–11.91)
Kubo et al[43]	33	100	0	30	2.6%	30.27 (1.80–510.43)
Liao et al[52]	24	44	0	12	1.8%	29.88 (1.67–535.96)
Sui et al[53]	13	40	1	15	5.0%	6.74 (0.80–56.95)
Toyooka et al[33]	18	42	2	25	7.3%	8.63 (1.80–41.41)
Wang et al[48]	15	28	3	12	9.9%	3.46 (0.77–15.56)
Zhang et al[44]	38	78	8	78	20.8%	8.31 (3.53–19.56)
Total (95% CI)		532		372	100.0%	8.19 (5.41–12.39)
Total events	223		31			

Heterogeneity: χ^2=9.50, *df*=8 (*P*=0.30); I^2=16%
Test for overall effect: *Z*=9.95 (*P*<0.00001)

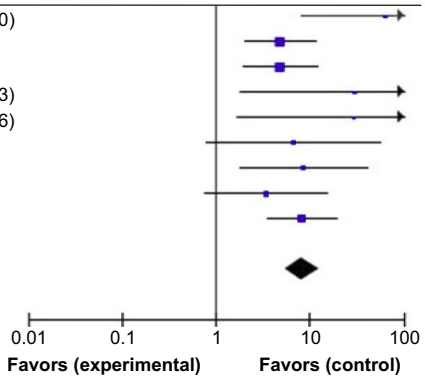
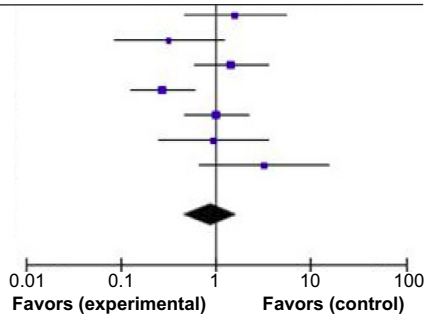


Figure 2 *T-cadherin* hypermethylation in NSCLC and normal lung tissue.
Notes: The pooled OR from nine studies including 532 NSCLC and 372 normal lung tissue samples (OR=8.19, 95% CI=5.41–12.39, *P*<0.00001).
Abbreviations: CI, confidence interval; M–H, Mantel–Haenszel; NSCLC, non-small cell lung cancer; OR, odds ratio.

A

Study or subgroup	Male Events	Total	Female Events	Total	Weight	Odds ratio M–H, random, 95% CI
Kim et al[47]	22	70	4	18	13.1%	1.60 (0.47–5.44)
Kontic et al[41]	5	44	6	21	12.0%	0.32 (0.08–1.21)
Kubo et al[43]	12	38	15	62	16.7%	1.45 (0.59–3.55)
Pesek et al[42]	25	69	33	49	18.2%	0.28 (0.13–0.60)
Suzuki et al[49]	27	101	13	49	18.2%	1.01 (0.47–2.19)
Ulivi et al[51]	32	49	8	12	12.0%	0.94 (0.25–3.58)
Wang et al[48]	11	17	4	11	9.9%	3.21 (0.66–15.59)
Total (95% CI)		388		222	100.0%	0.87 (0.46–1.66)
Total events	134		83			

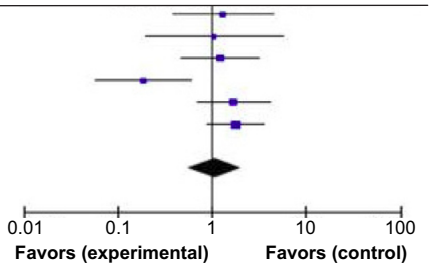
Heterogeneity: τ^2=0.44; χ^2=15.42, *df*=6 (*P*=0.02); I^2=61%
Test for overall effect: *Z*=0.42 (*P*=0.68)



B

Study or subgroup	Smoker Events	Total	Never Events	Total	Weight	Odds ratio M–H, random, 95% CI
Kim et al[47]	22	72	4	16	14.4%	1.32 (0.38–4.55)
Kontic et al[41]	9	52	2	12	10.0%	1.05 (0.20–5.61)
Kubo et al[43]	9	30	18	70	18.4%	1.24 (0.48–3.19)
Pesek et al[42]	42	98	16	20	15.3%	0.19 (0.06–0.60)
Suzuki et al[49]	32	109	8	41	19.5%	1.71 (0.71–4.11)
Toyooka et al[50]	30	86	18	78	22.6%	1.79 (0.90–3.56)
Total (95% CI)		447		237	100.0%	1.07 (0.56–2.04)
Total events	144		66			

Heterogeneity: τ^2=0.36; χ^2=11.78, *df*=5 (*P*=0.04); I^2=58%
Test for overall effect: *Z*=0.19 (*P*=0.85)



C

Study or subgroup	Squamous cell carcinoma Events	Total	Adenocarcinoma Events	Total	Weight	Odds ratio M–H, random, 95% CI
Kim et al[47]	12	53	14	35	39.6%	0.44 (0.17–1.12)
Kontic et al[41]	1	35	8	20	30.0%	0.04 (0.00–0.39)
Ulivi et al[51]	9	16	25	36	20.4%	0.57 (0.17–1.91)
Wang et al[48]	3	7	9	15	9.9%	0.50 (0.08–3.08)
Total (95% CI)		111		106	100.0%	0.35 (0.19–0.66)
Total events	25		56			

Heterogeneity: χ^2=4.43, *df*=3 (*P*=0.22); I^2=32%
Test for overall effect: *Z*=3.29 (*P*=0.001)

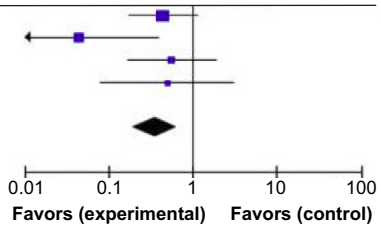


Figure 3 *T-cadherin* hypermethylation rate in NSCLC patients.
Notes: (**A**) The pooled OR from seven studies including 388 male and 222 female NSCLC patients (OR=0.87, 95% CI=0.46–1.66, *P*=0.68), which indicates that *T-cadherin* hypermethylation is not strongly associated with sex, in NSCLC patients. (**B**) The pooled OR from six studies of 684 NSCLC patients. Aberrant *T-cadherin* hypermethylation was not strongly associated with the smoking status in NSCLC patients (OR=1.07, 95% CI=0.56–2.04, *P*=0.85). (**C**) The pooled OR from four studies including 111 SCC and 106 AD patients (OR=0.35, 95% CI=0.19–0.66, *P*=0.001), which indicated that *T-cadherin* hypermethylation plays more important role in the pathogenesis of AD.
Abbreviations: AD, adenocarcinoma; CI, confidence interval; M–H, Mantel–Haenszel; NSCLC, non-small cell lung cancer; OR, odds ratio; SCC, squamous cell carcinoma.

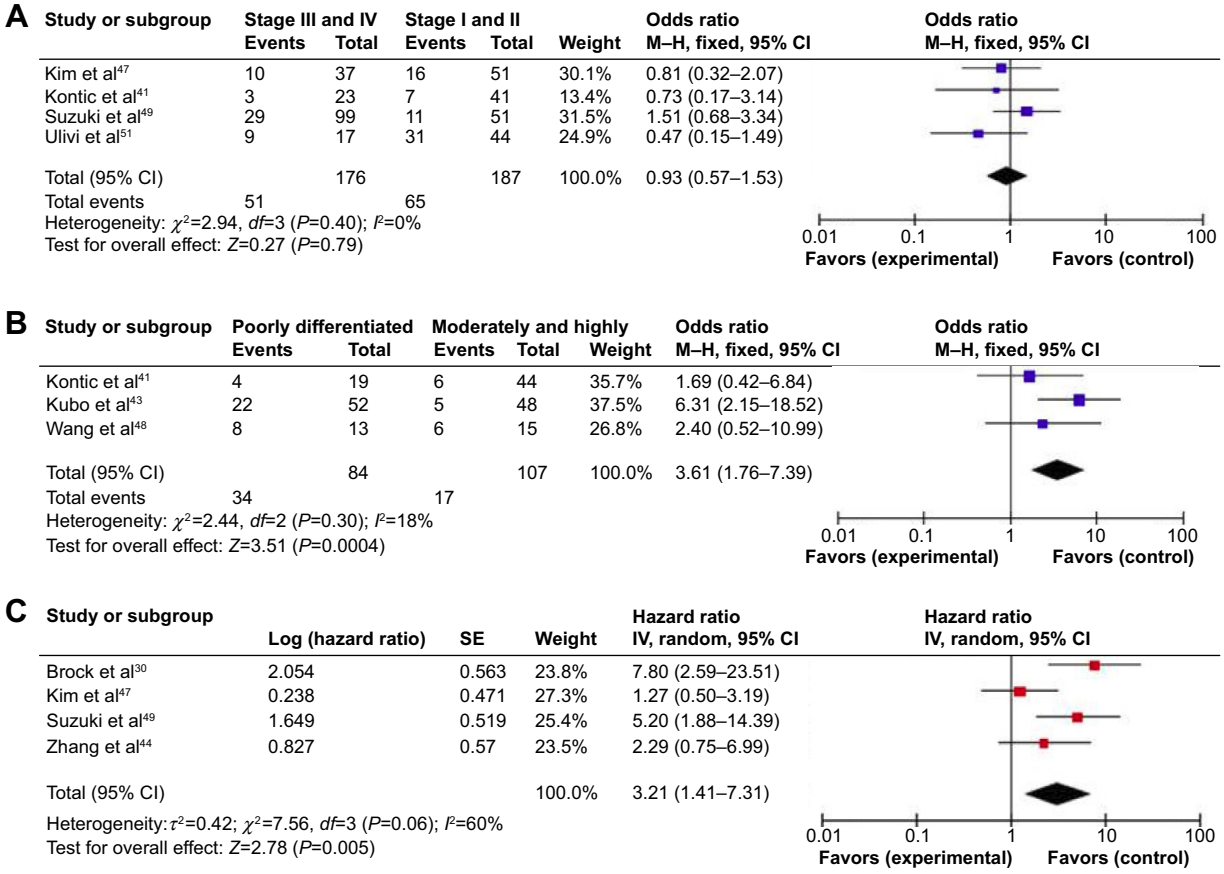


Figure 4 The clinicopathological significance of T-cadherin hypermethylation rate in NSCLC patients.
Notes: (**A**) 363 NSCLC patients pooled from four studies to assess whether the aberrant *T-cadherin* hypermethylation in NSCLC was associated with advanced stage. Aberrant *T-cadherin* hypermethylation was not significantly higher in advanced NSCLC (stage III and IV) than that in early stage NSCLC (stage I and II) (OR=0.93, 95% CI=0.57–1.53, *P*=0.79). (**B**) However, aberrant *T-cadherin* hypermethylation was significantly higher in poorly differentiated NSCLC than that in moderately and highly differentiated NSCLC (OR=3.61, 95% CI=1.76–7.39, *P*=0.0004). (**C**) Four of the included studies investigated the relationship between OS and *T-cadherin* hypermethylation. The pooled HR for OS showed that *T-cadherin* hypermethylation was associated with worse survival in NSCLC (HR=3.21, 95 % CI=1.41–7.31, *P*=0.005).
Abbreviations: CI, confidence interval; HR, hazard ratio; M–H, Mantel–Haenszel; NSCLC, non-small cell lung cancer; OR, odds ratio; OS, overall survival.

NSCLC and its clinical significance have not been thoroughly investigated. Therefore, we conducted the meta-analysis to determine the correlation between *T-cadherin* hypermethylation and clinicopathological characteristics in NSCLC. Analysis of the pooled data showed that 1) NSCLC had higher hypermethylation than did normal lung tissue; 2) *T-cadherin* hypermethylation is not strongly associated with sex or smoking status in NSCLC patients; 3) *T-cadherin* hypermethylation plays a more important role in the pathogenesis of AD than in squamous cell carcinoma; 4) aberrant *T-cadherin* hypermethylation was not significantly higher in advanced NSCLC (stage III and IV) than that in early stage NSCLC (stage I and II); 5) aberrant *T-cadherin* hypermethylation was not significantly correlated with EGFR mutation status

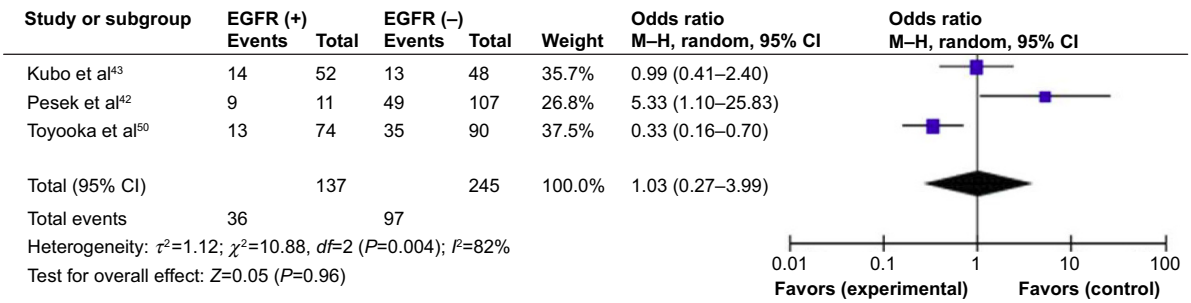


Figure 5 *T-cadherin* hypermethylation and EGFR mutation status in NSCLC.
Notes: The pooled OR from three studies including 137 EGFR(+) patients and 245 EGFR(–) patients (OR=1.03, 95% CI=0.27–3.99, *P*=0.96).
Abbreviations: CI, confidence interval; EGFR, epidermal growth factor receptor; EGFR (+), EGFR mutation-positive; EGFR (–), EGFR mutation-negative; M–H, Mantel–Haenszel; OR, odds ratio.

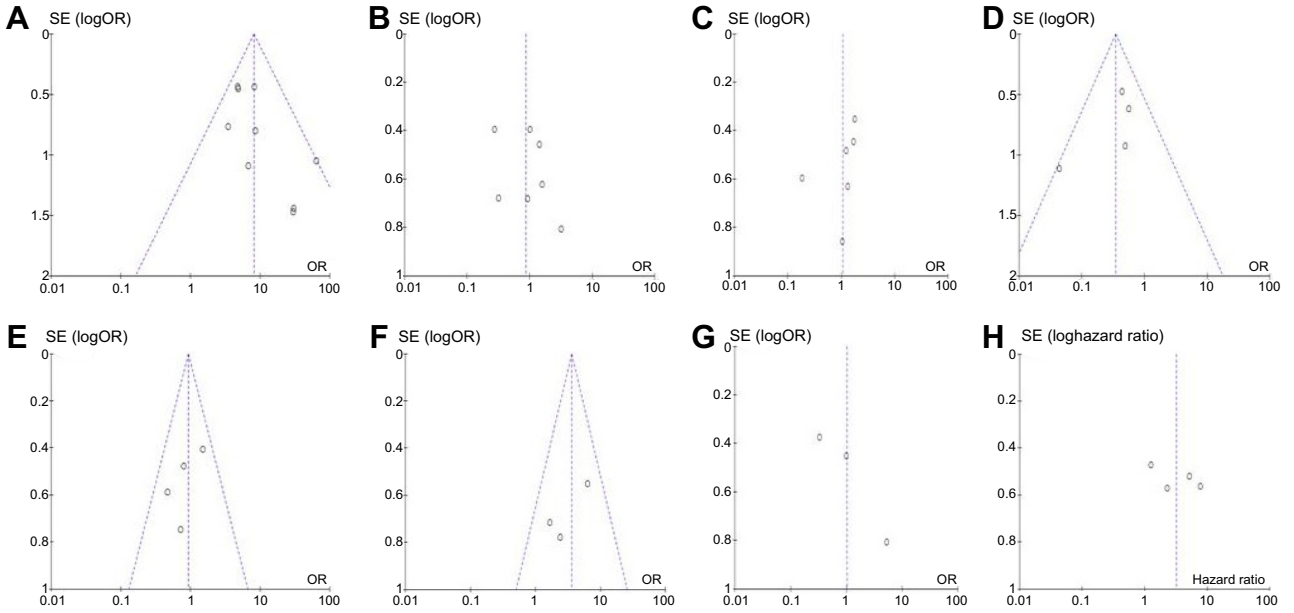


Figure 6 The funnel plot analysis.

Notes: The funnel plots were largely symmetric, suggesting there were no publication biases in the meta-analysis of *T-cadherin* hypermethylation and clinicopathological features. The funnel plot from nine studies comparing NSCLC and normal lung tissue (**A**). The funnel plot from seven studies determining *T-cadherin* hypermethylation and sex, in NSCLC patients (**B**). The funnel plot from six studies determining *T-cadherin* hypermethylation and smoking status in NSCLC patients (**C**). The funnel plot from four studies comparing *T-cadherin* hypermethylation between SCC and AD (**D**). The funnel plot from four studies determining *T-cadherin* hypermethylation in different stages of NSCLC (**E**). The funnel plot from three studies determining *T-cadherin* hypermethylation in differently differentiated NSCLC (**F**). The funnel plot from three studies determining *T-cadherin* hypermethylation in EGFR mutation-positive and EGFR mutation-negative NSCLC (**G**). The funnel plot from four studies determining the relationship between *T-cadherin* hypermethylation and OS in NSCLC (**H**).

Abbreviations: AD, adenocarcinoma; CI, confidence interval; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; OR, odds ratio; OS, overall survival; SCC, squamous cell carcinoma; SE, standard error of the mean.

in NSCLC. However, aberrant *T-cadherin* hypermethylation was significantly higher in poorly differentiated NSCLC than in moderately and highly differentiated NSCLC; and finally, 6) NSCLC patients with *T-cadherin* hypermethylation had a lower OS that those without *T-cadherin* hypermethylation. The results from the current study demonstrated that the hypermethylation rate of the *T-cadherin* gene promoter in NSCLC was significantly higher than that in the normal lung tissues, indicating that *T-cadherin* promoter hypermethylation was common in NSCLC. Since changes in *T-cadherin* promoter hypermethylation are reversible, drug treatment targeting demethylation may be useful to delay carcinogenesis and progression, and to improve prognosis. Treatment of *T-cadherin*-negative tumor cells with the demethylating agent 5-aza-2′-deoxycytidine induced reexpression of this gene in several types of tumor cells, including colorectal cancer,[54,55,60] pancreatic cancer,[62] esophageal cancer,[56] hepatocellular carcinoma,[70,71] as well as B cell lymphoma.[58] Therefore, this approach may bring new direction and hope for cancer treatment through gene-targeted therapy.

Epigenetic alterations, particularly aberrant DNA methylation, one of the best-characterized epigenetic modifications, contribute to tumor initiation and progression.[5,6] *T-cadherin* has been reported to encode *T-cadherin*, an adiponectin receptor discovered after adipoR1 and adipoR2.[72] T-cadherin is thought to affect cellular function and behavior, largely through its signaling properties.[26] *T-cadherin* reexpression in most cancer cell lines inhibits cell proliferation and invasiveness, increases susceptibility to apoptosis, and reduces tumor growth in vivo models.[55,73,74] Therefore, *T-cadherin* can be considered as tumor suppressor and its inactivation could contribute to tumor progression and poor prognosis. Although only four studies evaluated the relationship between OS and *T-cadherin* hypermethylation in NSCLC, they showed very similar results.[30,44,47,49] Based on this meta-analysis, we may consider that *T-cadherin* hypermethylation in NSCLC tends to indicate a poor prognosis. Inconsistent with this finding, it was also found, using the kmplot tool (http://kmplot.com/analysis/index.php?p=service&cancer=lung), that low *T-cadherin* mRNA expression also indicates a poor prognosis in NSCLC patients.

Consistent results were shown in sensitivity analyses, and no evidence of publication bias was found. This study has several potential limitations. First, the possibility of information and selection biases and unidentified confounders could not be completely excluded because all of the included studies were observational. Second, the searching strategy was restricted to articles published in English or Chinese; articles with potentially high-quality data that were published in other languages were

not included because of anticipated difficulties in obtaining accurate medical translation. Hence, cautions should be taken when our findings are interpreted among the general population.

In conclusion, our meta-analysis showed *T-cadherin* may play an important role in NSCLC initiation and progression. In addition, *T-cadherin* hypermethylation is associated with an increased risk and worse OS in NSCLC. Further large-scale studies, especially multicenter and well-matched cohort research, will provide more insight into the role of *T-cadherin* in the prognosis and clinical intervention for NSCLC patients.

Disclosure

The authors report no conflicts of interest in this work

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