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RESEARCH ARTICLE

The clinicopathological significance and potential drug target of E-cadherin in NSCLC

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Abstract Human epithelial cadherin (E-cadherin), a member of transmembrane glycoprotein family, encoded by the *E-cadherin* gene, plays a key role in cell-cell adhesion, adherent junction in normal epithelial tissues, contributing to tissue differentiation and homeostasis. Although previous studies indicated that inactivation of the *E-cadherin* is mainly induced by hypermethylation of *E-cadherin* gene, evidence concerning *E-cadherin* hypermethylation in the carcinogenesis and development of non-small cell lung carcinoma (NSCLC) remains controversial. In this study, we conducted a meta-analysis to quantitatively evaluate the effects of *E-cadherin* hypermethylation on the incidence and clinicopathological characteristics of NSCLC. A comprehensive search of PubMed and Embase databases was performed up to October 2014. Analyses of pooled data were performed. Odds ratios (ORs) were calculated and summarized. Our meta-analysis combining 18 published articles demonstrated that the hypermethylation frequencies in NSCLC were significantly higher than those in normal control tissues, OR=3.55, 95 % confidence interval (CI)=1.98–6.36, $p<0.0001$. Further analysis showed that *E-cadherin* hypermethylation was not strongly associated with the sex or smoking status in NSCLC patients. In addition, *E-cadherin* hypermethylation was also not strongly associated with pathological types, differentiated status, clinical stages, or metastatic status in NSCLC patients. The results from the current study indicate that the hypermethylation frequency of *E-cadherin* in NSCLC is strongly associated with NSCLC incidence and it may be an early event in carcinogenesis of NSCLC. We also discussed the potential value of E-cadherin as a drug target that may bring new direction and hope for cancer treatment through gene-targeted therapy.

Keywords NSCLC · E-cadherin · Tumor suppressor gene · Methylation · Meta-analysis · Odds ratio

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Background

Lung cancer has been the most frequently diagnosed cancer and leading cause of cancer mortality in the USA and other developed countries [1]. Non-small cell lung carcinoma (NSCLC) consists of squamous cell carcinoma (SCC), adenocarcinoma (AC), large cell carcinoma, and others. NSCLC accounts for approximately 85 % of all lung cancers, and the prognosis remains poor [2]. Therefore, investigation on the mechanism of initiation and progression and identification of cancer risk marker are still needed for individualized treatment and better prognosis of NSCLC patients. Epigenetic regulation of tumor suppressor gene expression plays an important role in carcinogenesis. Aberrant methylation of tumor suppressor genes is a commonly observed epigenetic regulation in human tumors including NSCLC [3–5]. Patterns of DNA methylation can classify 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 [6]. Thus, measure of aberrant gene promoter methylation as a tool for diagnosis of tumors has been widely utilized for many different tumors including NSCLC [7].

Human epithelial cadherin (E-cadherin), a member of transmembrane glycoprotein family, also known as Cadherin-1 (CDH1), CAM 120/80, or uvomorulin, is encoded by the *E-cadherin* gene [8]. *E-cadherin* plays a key role in

cell-cell adhesion, adherent junction in normal epithelial tissues, contributing to tissue differentiation and homeostasis [9, 10]. Reduced *E-cadherin* expression was often detected and associated with cancer invasion and metastasis in a variety of epithelial tumors [11–13]. *E-cadherin* methylation which is associated with the low and absent E-cadherin expression was detected in several kinds of carcinoma including breast cancer, gastric cancer, and NSCLC [14–16]. Although previous studies indicated that inactivation of the *E-cadherin* is mainly induced by hypermethylation of *E-cadherin*, the reported *E-cadherin* hypermethylation rates in NSCLC were remarkably diverse. Evidence concerning *E-cadherin* hypermethylation in the carcinogenesis and development of NSCLC remains controversial. Numerous studies published in this field examined a small number of patients. In addition, its roles in NSCLC and clinicopathological significance have not been thoroughly investigated. Hence, we conducted a meta-analysis to quantitatively evaluate the effects of *E-cadherin* hypermethylation on the incidence and clinicopathological characteristics of NSCLC.

Methods

Publication selection

A systematical literature searching was performed by PubMed, Embase, and Web of Science up to October 15, 2014. We used the following search terms: "lung" and "cancer or tumor or neoplasm or carcinoma," "methylation," and "E-cadherin or CDH1 or CAM 120/80." We also searched manually for the reference lists of the retrieved articles and reviews for additional articles. After exclusion of non-relevant and/or redundant publications from 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.

Inclusion and exclusion criteria

Criteria that an eligible study has to meet were as follows: (1) *E-cadherin* hypermethylation evaluated in the primary NSCLC tissues, (2) researches revealed the relationship between *E-cadherin* hypermethylation and NSCLC clinicopathological parameters and prognosis, (3) *E-cadherin* hypermethylation examined by methylation-specific PCR (MSP) or quantitative MSP (QMSP), and (4) studies provided sufficient information to estimate hazard ratio (HR) about 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 (2) all publications regarding in vitro/ex vivo studies, cell lines, and human xenografts were also excluded.

Data extraction and quality assessment

Two researchers (KZ and WC) independently collected the information and extracted the data regarding the authors, year, source of publication, inclusion criteria, *E-cadherin* methylation frequencies, sexual status, smoking history, pathological types, clinical staging, differentiation degree, lymph node metastasis, and prognostic conditions in patients and control groups. Any discrepancy was adjusted by discussion until they reach an agreement. Methodological evaluation was assessed by two independent researchers (NX and JZ) according to REMARK guidelines and ELCWP quality scale [17, 18].

Data analysis

Meta-analysis was performed by Reviewer Manager 5 (Cochrane Collaboration, Oxford, UK). The pooled odds ratios (ORs) and confidence intervals (CIs) were calculated to assess the correlation between *E-cadherin* methylation and NSCLC. Cochran's Q test and I^2 were adopted to assess heterogeneity among studies [19]. If Q test showed a $P<0.05$ or I^2 test was >50 %, it 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 attempt to identify potential sources of heterogeneity based on subgroup analyses [20, 21]. Publication bias was detected by Begge's test and funnel plots [22]. The analysis of meta-regression and publication bias was performed by using STATA version 10.0.

Results

Identification of relevant studies

We identified 97 publications by the search method as described above. Seventy-nine of those were excluded due to non-original articles (review), laboratory studies, or studies irrelevant to the current analysis. Finally, there were 18 studies included in the meta-analysis as shown in Fig. 1.

Study characteristics

Eighteen studies published from 2001 to 2012 were eligible for meta-analysis. A total of 1467 NSCLC patients from China, Singapore, South Korea, Japan, France, Chile,

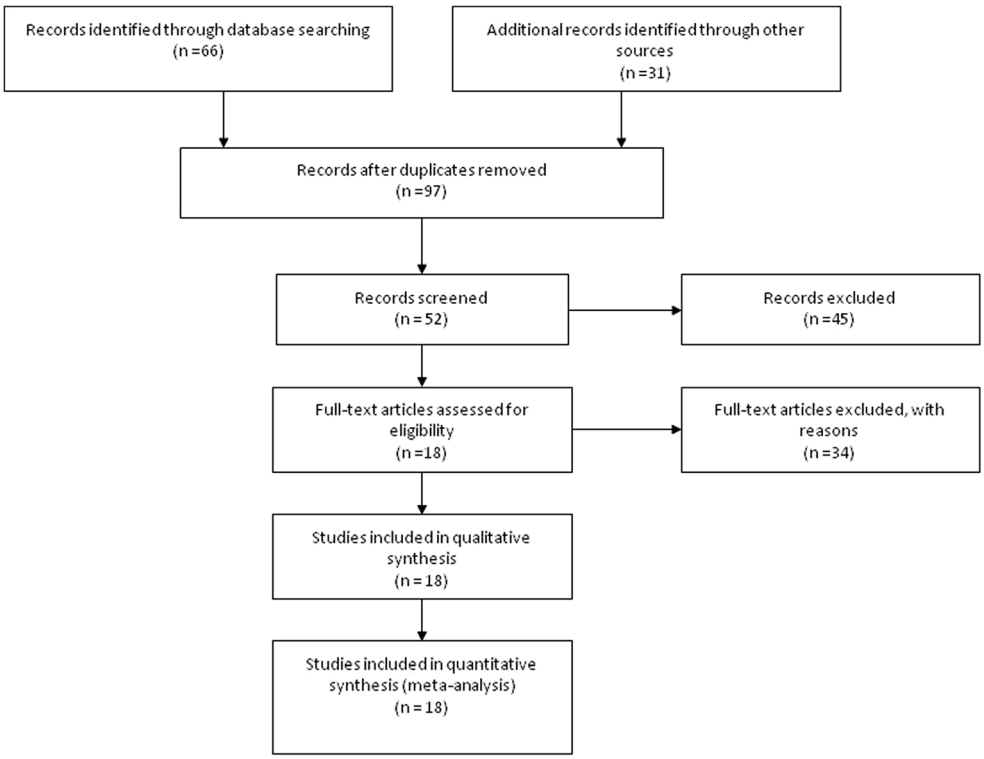

Fig. 1 Flow diagram of the literature search strategy and assessment of studies identified for meta-analysis

and USA were enrolled. Their basic characteristics are summarized in Table 1.

The correlation of E-cadherin hypermethylation with clinicopathological features

The inactivation of E-cadherin through hypermethylation in NSCLC

We first determined that *E-cadherin* hypermethylation was significantly higher in NSCLC than in normal lung tissues. The pooled OR from 13 studies including 731 NSCLC and 613 normal lung tissues is shown in Fig. 2 (OR=3.55, 95 % CI=1.98–6.36, $p<0.0001$), indicating that *E-cadherin* hypermethylation in NSCLC was significantly higher than that in normal lung tissues.

Relationship between the frequency of E-cadherin hypermethylation and sex status

Next, we determined whether or not *E-cadherin* hypermethylation rate was correlated with sex status. The pooled OR from eight studies included 723 males and 281 females with NSCLC, as shown in Fig. 3 (OR=0.81, 95 % CI=0.59–1.12, $p=0.21$), which indicates that *E-cadherin* hypermethylation was not significantly correlated with sex status.

Relationship between the frequency of E-cadherin hypermethylation and smoking status

Then, we determined whether or not *E-cadherin* hypermethylation rate was correlated with smoking status. The pooled OR from eight studies including 657 and 225 NSCLCs with and without smoking history is shown in Fig. 4 (OR=0.95, 95 % CI=0.65–1.38, $p=0.79$), which indicates that *E-cadherin* hypermethylation was not significantly correlated with smoking status in NSCLC patients.

Relationship between the frequency of E-cadherin hypermethylation and pathological types

We also determined whether or not *E-cadherin* hypermethylation was correlated with pathological types. The pooled OR from ten studies including 417 squamous cell carcinoma (SCC) and 474 adenocarcinoma (AD) is shown in Fig. 5 (OR=0.91, 95 % CI=0.65–1.25, $p=0.55$), which indicates that *E-cadherin* hypermethylation was not significantly correlated with pathological types.

The role of E-cadherin hypermethylation in NSCLC progression

We analyzed 437 NSCLC patients pooled from three studies to assess whether or not the aberrant *E-cadherin* hypermethylation in NSCLC was associated with the

Table 1 Basic characteristics of the included studies

Study	Country	Patients	Methods	Primary aim	Methylation site	E-cadherin expression
Zheng et al. [23]	China	37	Methylation-specific PCR (MSP)/RT-PCR	To investigate the promoter methylation status of the *E-cadherin* in NSCLC	Promoter, CpG islands	+
Guzman et al. [24]	Chile	26	MSP	Determine the frequency of *CDKN2A*, *CDH1*, and *MGMT* hypermethylation in NSCLC	Promoter, CpG islands	−
Begum et al. [25]	USA	76	MSP	Determine the methylation status of 15 genes in NSCLC	Promoter, CpG islands	−
Sasaki et al. [26]	Japan	116	MSP	Determine the methylation status of *DLEC1*, p16, and *CDH1* genes in NSCLC	Promoter, CpG islands	−
Vaissiere et al. [27]	France	209	MSP	Aims to determine the methylation status of five tumor suppressors in NSCLC	Promoter, CpG islands	−
Buckingham et al. [28]	USA	75	MSP/IHC	Determine the methylation status of eight tumor suppressors in NSCLC	Promoter, CpG islands	+
Wang et al. [29]	China	95	MSP/RT-PCR/IHC	Determine the inactivation of *E-cadherin* in NSCLC	Promoter, CpG islands	+
Feng et al. [30]	USA	49	MethyLight	Determine the methylation status of 27 tumor suppressors in NSCLC	Promoter, CpG islands	−
Wang et al. [31]	China	28	DNA microarray coupled with PCR	Determine the methylation status of 15 tumor suppressors in NSCLC	Promoter, CpG islands	−
Tan et al. [32]	Singapore	20	MSP	Determine the methylation status of four genes in NSCLC	Promoter, CpG islands	−
Kim et al. [33]	South Korea	88	MSP	Determine methylation patterns of *E-cadherin* and *H-cadherin* in NSCLC	Promoter, CpG islands	−
Gu et al. [34]	USA	155	MSP	The methylation profile of nine genes for NSCLC was analyzed and correlated with clinical data	Promoter, CpG islands	−
Nakata et al. [35]	Japan	224	MSP/IHC	Determine the inactivation of *CDH1*, *p16*, and *FHIT* in NSCLC	Promoter, CpG islands	+
Tsou et al. [36]	USA	7	MSP	Determine DNA methylation profiles in NSCLC	Promoter, CpG islands	−
Russo et al. [37]	USA	49	MSP	Determine the methylation status of six genes in NSCLC	Promoter, CpG islands	−
Topaloglu et al. [38]	USA	31	MSP	Examine methylation status of seven tumor suppressor genes in NSCLC	Promoter, CpG islands	−
Yanagawa et al. [39]	Japan	75	MSP	Examine methylation status of eight tumor suppressor genes in NSCLC	Promoter, CpG islands	−
Zochbauer et al. [40]	USA	107	MSP	Examine methylation status of eight tumor suppressor genes in NSCLC	Promoter, CpG islands	−

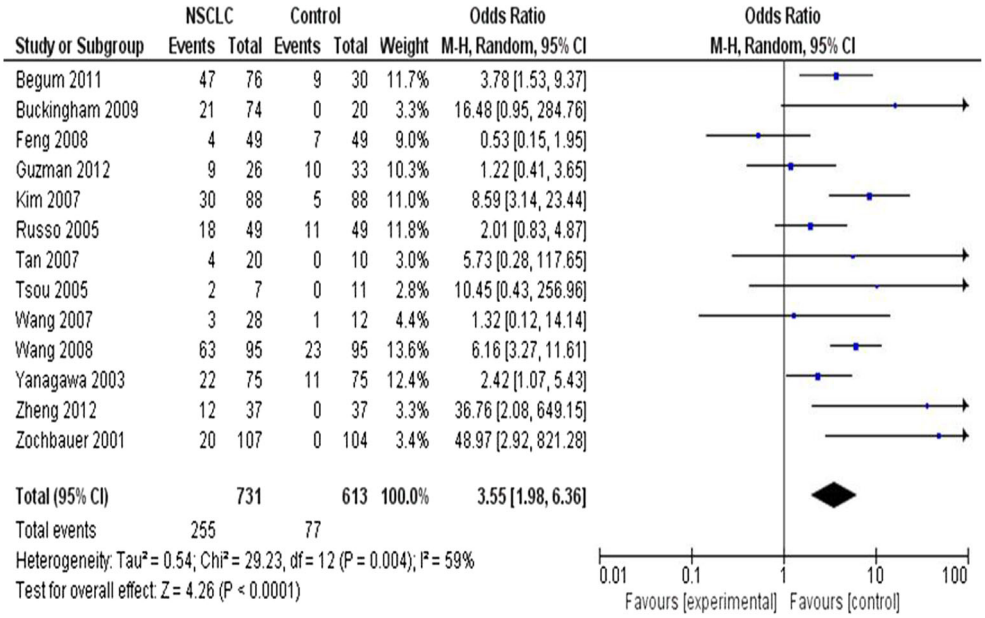

Fig. 2 The pooled OR from 13 studies including 731 NSCLC and 613 normal lung tissues, OR=3.55, 95 % CI=1.98–6.36, $p<0.0001$

differentiated status. As shown in Fig. 6a, aberrant *E-cadherin* hypermethylation was not significantly higher in poorly differentiated NSCLC than that in moderately or highly differentiated NSCLC, OR=0.4, 95 % CI=0.13–1.28, $p=0.12$. Aberrant *E-cadherin* hypermethylation was also not significantly higher in advanced NSCLC (III and IV) than that in early-stage NSCLC (I and II), OR=0.89, 95 % CI=0.63–1.26, $p=0.52$ (Fig. 6b). These results suggest that *E-cadherin* hypermethylation may not play an important role in NSCLC progression and different stages. In addition, aberrant *E-cadherin* hypermethylation was also not significantly higher in metastatic NSCLC than that in non-metastatic NSCLC, OR=1.10, 95 % CI=0.72–1.67, $p=0.67$ (Fig. 6c).

Sensitivity analyses and publication bias

A sensitivity analysis, in which one study was removed at a time, was conducted to assess the result stability. The pooled ORs were not significantly changed, indicating the stability of our analyses. The funnel plots were largely symmetric (Fig. 7a–g), suggesting that there were no publication biases

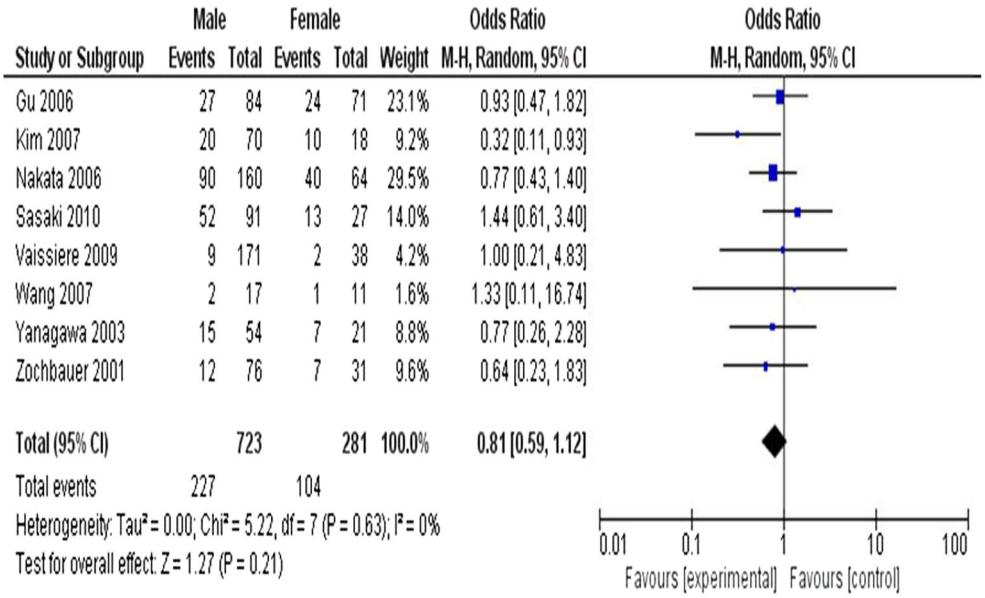

Fig. 3 The pooled OR from eight studies included 723 males and 281 females with NSCLC, OR=0.81, 95 % CI=0.59–1.12, $p=0.21$, which indicates that *E-cadherin* hypermethylation was not significantly correlated with sex status in NSCLC patients

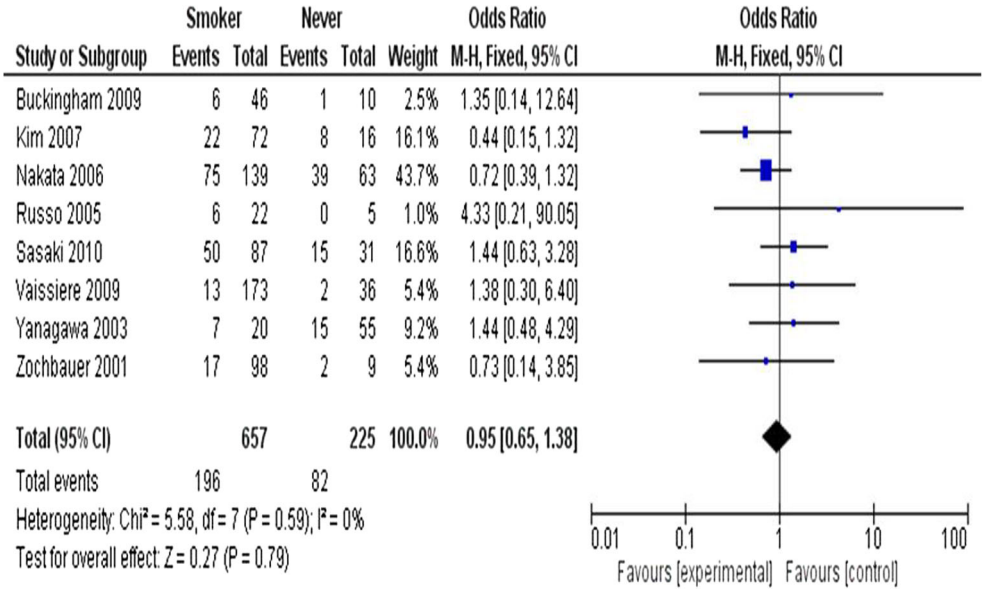

Fig. 4 Nine hundred eighty-two NSCLC patients with the smoking status pooled in eight studies. Aberrant *E-cadherin* hypermethylation was not significantly correlated with the smoking status in NSCLC patients, OR=0.95, 95 % CI=0.65–1.38, p=0.79

in the meta-analysis of *E-cadherin* hypermethylation and clinicopathological features.

Discussion

The hypermethylation of tumor suppressor gene is an essential component of the molecular mechanism in the gene epigenomic regulation for cancer initiation and progression [41]. Inactivation of *E-cadherin* by promoter hypermethylation plays an important role in tumorigenesis in several types of tumors including NSCLC [14, 16, 42–44]. Although there have been some studies describing the methylation status of *E-cadherin* in NSCLC, the roles of inactivation of *E-cadherin* by hypermethylation in NSCLC and clinicopathological significance have not been thoroughly investigated. Our meta-analysis combining 18 published articles demonstrated that the hypermethylation frequencies in NSCLC were significantly higher than those in normal control tissues, OR=3.55, 95 % CI=1.98–6.36, $p<0.0001$. Further analysis showed that *E-cadherin* hypermethylation was not strongly associated with the sex or smoking status in NSCLC patients. In addition, *E-cadherin* hypermethylation was also not strongly associated with pathological types, differentiated status, clinical stages, or metastatic status in NSCLC patients. The results from the current study indicate that the hypermethylation frequency of *E-cadherin* in NSCLC is strongly associated with NSCLC incidence; however, *E-cadherin* hypermethylation may be an early event in carcinogenesis of NSCLC. In support of this conclusion, Ceteci F et al. observed that postnatal inactivation

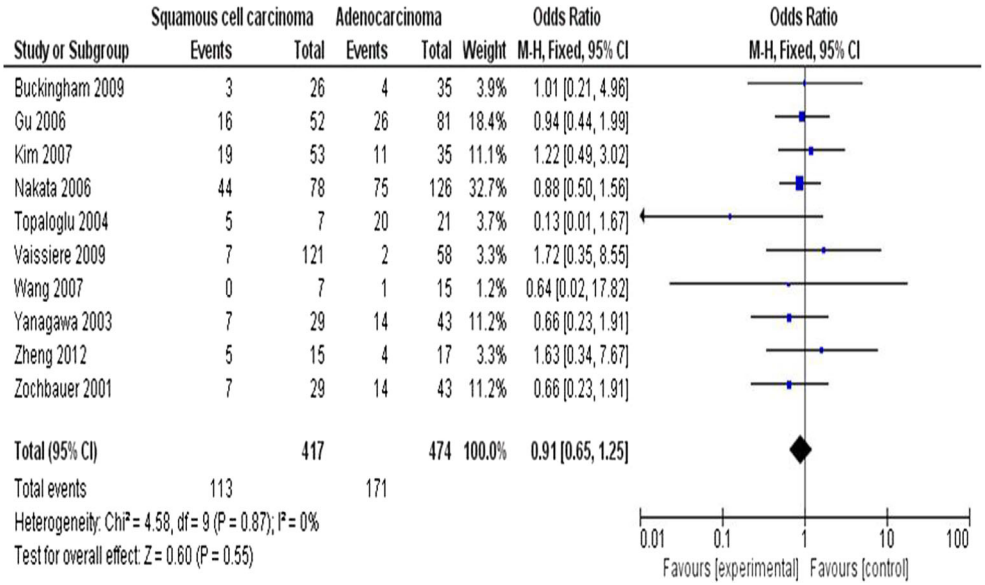

Fig. 5 The pooled OR from ten studies including 417 squamous cell carcinoma (SCC) and 474 adenocarcinoma (AD), OR=0.91, 95 % CI=0.65–1.25, p=0.55, indicating that *E-cadherin* hypermethylation was not significantly correlated with pathological types

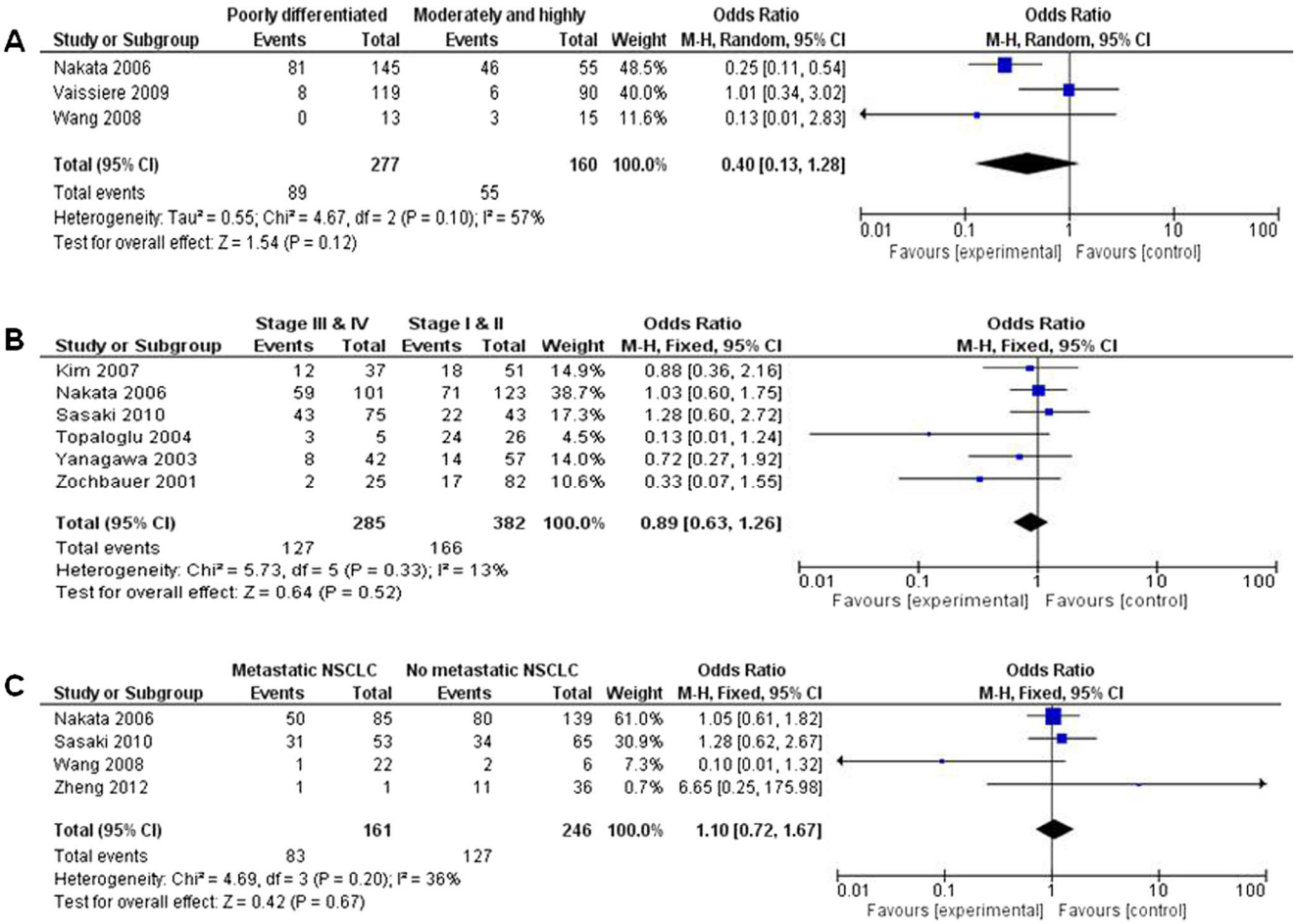

Fig. 6 Four hundred thirty-seven NSCLC patients pooled from three studies to assess whether or not the aberrant *E-cadherin* hypermethylation in NSCLC was associated with the differentiated status. Aberrant *E-cadherin* hypermethylation was not significantly higher in poorly differentiated NSCLC than that in moderately and highly differentiated NSCLCs, OR=0.4, 95 % CI=0.13–1.28, p=0.12 (**a**). Aberrant *E-cadherin* hypermethylation was not significantly higher in advanced NSCLC (III and IV) than that in early-stage NSCLC (I and II), OR=0.89, 95 % CI= 0.63–1.26, p=0.52 (**b**). Aberrant *E-cadherin* hypermethylation was also not significantly higher in metastatic NSCLC than that in non-metastatic NSCLC, OR=1.10, 95 % CI=0.72–1.67, p=0.67 (**c**)

of E-cadherin affected Clara cell differentiation and compromised airway regeneration under injury conditions, and the loss of E-cadherin function leads to tumor formation when additional mutations are sustained [45]. Their results indicate that E-cadherin plays a critical role in the regulation of proliferation and homeostasis of the epithelial cells lining the conducting airways.

Since changes in *E-cadherin* promoter hypermethylation are reversible, drug treatment through demethylation may be useful to delay carcinogenesis and progression. In fact, treatment of *E-cadherin*-negative tumor cells with the demethylating agent, 5-aza-2′-deoxycytidine, induced re-expression of *E-cadherin* mRNA and/or protein in several types of tumor cells including colorectal cancer [46], esophageal cancer [47], prostate cancer [48], and NSCLC [49]. 1α, 25(OH)(2)D(3) promoted differentiation of breast cancer MDA-MB-231 cells by inducing de novo E-cadherin expression, an effect that was time- and dose-dependent [50]. Transfection of *E-cadherin* cDNA into R-HepG2 cells, in which *E-cadherin* promoter was hypermethylated in drug resistance of a doxorubicin-induced multidrug-resistant hepatocellular carcinoma cell line, led to increased amount of doxorubicin uptake, decreased cell viability, decreased P-glycoprotein expression, and increased apoptotic population of cells exposed to doxorubicin [51]. Interestingly, a combination of histone deacetylase inhibitors and DNA methyltransferase inhibitors suppresses the growth of endometrial cancer, which is likely mediated by upregulation of *E-cadherin* and downregulation of Bcl-2 [52]. Therefore, the approaches targeting E-cadherin to reverse epigenetic silencing, reactivate gene expression, and, finally, induce a therapeutic effect such as differentiation, growth arrest, or apoptosis may bring new direction and hope for cancer treatment through gene-targeted therapy.

E-cadherin as a tumor suppressor gene functionally keeps cell-cell adhesion and controls epithelial cell arrangement in

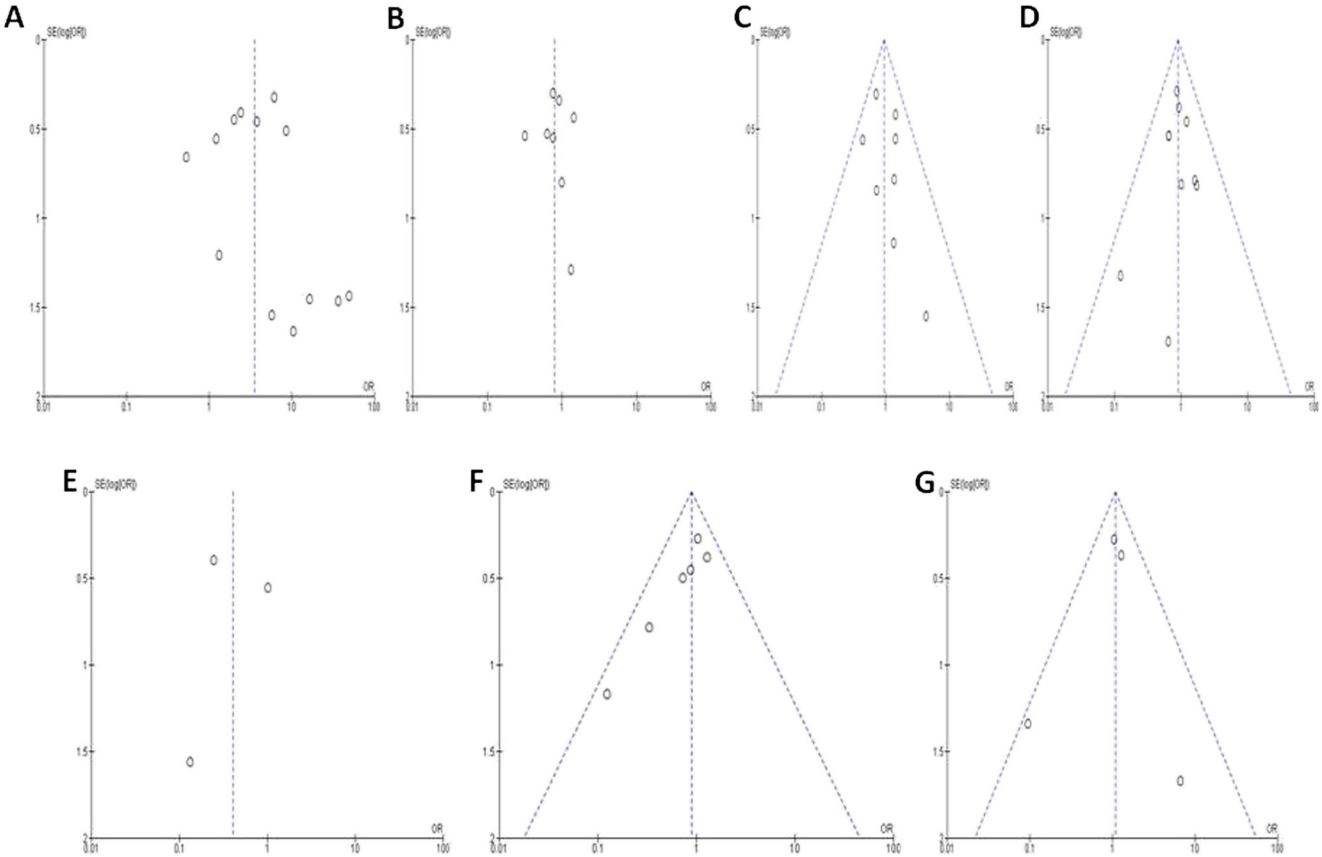

Fig. 7 The funnel plots were largely symmetric, which suggests that there were no publication biases in the meta-analysis of *E-cadherin* hypermethylation and clinicopathological features. The funnel plot from 13 studies comparing NSCLC and normal lung tissue (**a**). The funnel plot from eight studies determined the relationship between *E-cadherin* hypermethylation and the sex status in NSCLC patients (**b**). The funnel plot from eight studies determined the relationship between *E-cadherin* hypermethylation and the smoking status in NSCLC patients (**c**). The funnel plot from ten studies comparing *E-cadherin* hypermethylation between squamous cell carcinoma (SCC) and adenocarcinoma (AD) (**d**). The funnel plot from three studies determined *E-cadherin* hypermethylation in different differentiated NSCLCs (**e**). The funnel plot from six studies determined *E-cadherin* hypermethylation in different staged NSCLCs (**f**). The funnel plot from four studies comparing *E-cadherin* hypermethylation in metastatic and non-metastatic NSCLCs (**g**)

normal order and layer. A number of studies demonstrate that loss of the expression or function of *E-cadherin* can initiate the activation of several signaling pathways including the canonical Wnt and Rho family GTPase-mediated modulation of the actin cytoskeleton which are associated with epithelial-mesenchymal transition, finally leading to cancer cell metastasis [53, 54]. To better understand the correlation between *E-cadherin* methylation and NSCLC, comprehensive evaluation on the methylation markers in NSCLC should be further addressed. Although a large number of studies have demonstrated the potential relationship between *E-cadherin* methylation and NSCLC, a meta-analysis can summarize the studies and compare different subgroup characters.

A sensitivity analysis was used to assess the result stability, and the pooled ORs were not significantly changed, indicating the stability of our analyses. In addition, the funnel plots were largely symmetric, indicating that there were no publication biases in the meta-analysis of *E-cadherin* hypermethylation and clinicopathological features. However, there are several potential limitations in this meta-analysis. First, we only selected articles published in English; thus, other articles that were published in other languages were not selected, due to anticipated difficulties in obtaining accurate medical translation. Second, most selected articles are from Asia; hence, cautions should be taken when our findings are interpreted among the general populations. Third, DNA methylation is influenced by several clinicopathological parameters that are not taken into account in the study (i.e., age, ethnics, previous treatments, etc.).

In summary, this meta-analysis shows that *E-cadherin* hypermethylation is strongly associated with NSCLC incidence; however, *E-cadherin* hypermethylation is also not significantly associated with pathological types, differentiated status, clinical stages, or metastatic status in NSCLC patients. These results indicate that *E-cadherin* methylation might be an early biomarker of carcinogenesis of NSCLC, with potential value for predicting the diagnosis of NSCLC patients. In addition, the potential value of E-cadherin as a drug target

may bring new direction and hope for cancer treatment through gene-targeted therapy.

Conflicts of interest None

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