—Review—

Clinicopathological Significance and Diagnostic Value of *DLEC1* Hypermethylation in Lung Cancer: A Meta-analysis

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Background: *DLEC1* is a tumor-suppressor gene which plays a role in carcinogenesis. The purpose of the current study was to help establish the diagnostic performance of *DLEC1* methylation in lung cancer.

Methods: PubMed, Embase, CNKI, and Wanfang databases were searched to obtain eligible studies. The pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the strength of the associations. The diagnostic value was assessed by the summary receiver operating characteristics test.

Results: A total of 7 articles, with 8 studies that included 673 lung cancer and 581 control samples, were collected in this meta-analysis. Our results showed a significant association of *DLEC1* hypermethylation with lung cancer ($P < 0.00001$, OR = 13.93, 95% CI = 9.44-20.55). The frequency of *DLEC1* methylation was significantly higher in squamous cell carcinoma (SCC) than adenocarcinoma (AC). Moreover, *DLEC1* was more frequently methylated in patients with lung cancer aged 60 years or over, patients with lymphatic metastasis, or patients with stage III/IV lung cancer. In addition, there was a sensitivity value of 0.90 (95% CI = 0.86-0.93) and a specificity value of 0.60 (95% CI = 0.56-0.63), a pooled positive-likelihood ratio (PLR) of 2.27 (95% CI = 2.08-2.48), a pooled negative-likelihood ratio (NLR) of 0.17 (95% CI = 0.12-0.23), a diagnostic odds ratio (DOR) of 14.72 (10.09-21) and an area under the curve (AUC) of 0.8146 using *DLEC1* methylation in the prediction of lung cancer risk.

Conclusion: This meta-analysis confirms that *DLEC1* methylation is a promising biomarker for lung cancer. (J Nippon Med Sch 2019; 86: 62–69)

Key words: *DLEC1*, methylation, lung cancer, meta-analysis

Introduction

Lung cancer is a common malignancy from epithelial cells, which has a very high death rate[1]. In China, an estimated 733,300 new cases (509,300 in men and 224,000 in women) of lung cancer could be diagnosed in 2015, and 610,200 deaths (432,400 in men and 177,800 in women) are estimated to occur from the disease[2]. Despite of the improvement in therapy, 5-year survival of lung cancer remains 18%[3]. Earlier diagnosis of lung cancer may play a significant role in easing the existing burden.

In order to search for more effective diagnostic strategies, molecular approaches have become the research focus to identify molecular biomarkers that can be utilized for early detection[4,5]. In molecular oncology, inactivation of tumor suppressor genes, overexpression of oncogenes, genetic/epigenetic mutations, and genomic instability are some of the widely investigated mechanisms[6].

As the bridge between the genetic and environmental aspects, epigenetic modification is proved to play an important role in carcinogenesis[7,8]. DNA methylation, the most studied epigenetic regulatory mechanism, could behave as a powerful biomarker for early detection of lung cancer[9,10]. Abnormal methylation of tumor suppressor genes (*RAR*β, *p16*, *DAPK*, *RASSF1A*, and *MGMT*) may be advantageous for the early diagnosis of non-small cell lung cancer (NSCLC)[11–13]. In addition, aberrant gene methylation is shown to be associated with smoking, a key risk factor for NSCLC[14].

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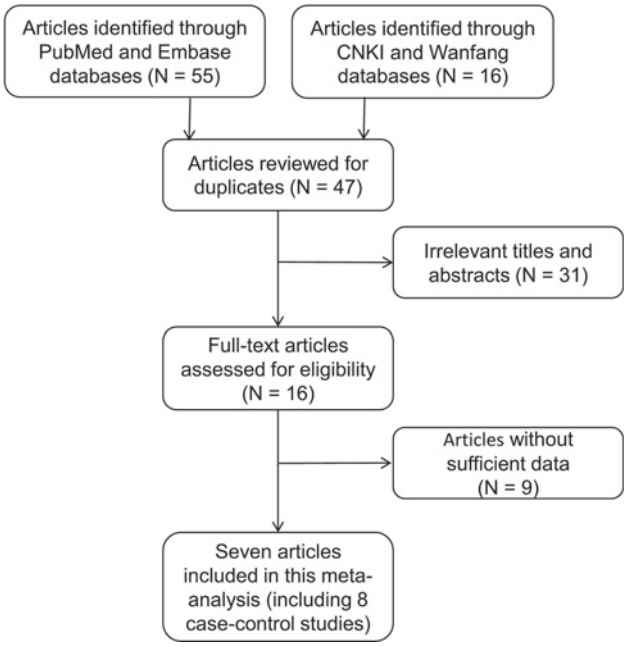

Fig. 1 Flow diagram of the stepwise selection from relevant studies.

DLEC1 (deleted in lung and esophageal cancer), located on the 3p22-21.3, is a tumor-suppressor gene which plays a role in the cell-cycle control by inducing G1 arrest[15]. Previous studies showed that *DLEC1* promoter methylation was associated with downregulation or loss of *DLEC1* expression in lung cancer[16,17]. Recently, several studies reported that aberrant methylation of *DLEC1* had the potential to become a novel biomarker for patients with NSCLC[17–19]. However, the literature revealed that the characterization of *DLEC1* methylation in the diagnosis of patients with lung cancer was still debatable. Studies with a small number of samples might produce spurious results.

To present the correct conclusion by ruling out the wrong results, a meta-analysis was conducted to help establish the diagnostic performance of *DLEC1* methylation in lung cancer.

Methods and Materials

Identification of Eligible Studies for the Current Meta-analysis

A literature search was conducted using the combined keywords among the online literature libraries including PubMed, Enbase, CNKI, and Wanfang up to December of 2017. The keywords used were "(deleted in lung and esophageal cancer 1 OR *DLEC1*) AND (lung cancer OR lung neoplasm OR lung carcinoma OR pulmonary cancer) AND (methylation OR epigene*)".

We used the following criteria to select eligible studies from the literature: 1) all the studies were case-control based studies of *DLEC1* methylation; 2) all the cancer tissues in the cases were diagnosed by experienced physicians; 3) control tissues must have been non-cancerous ones from benign lung disease or healthy persons or the adjacent non-cancerous tissues of patients with lung cancer; 4) the studies must have contained sufficient information to infer *DLEC1* methylation frequency.

Extraction of *DLEC1* Methylation and Unmethylation Data

Among the retrieved full-text articles, we extracted the first author's name, published year, ethnicity, types of samples, detection method of methylation, the number of patients with lung cancer, the number of non-cancerous lung samples, and the number of methylations.

Statistical Analysis

All the statistical analyses were performed using Review Manager 5 and Meta-Disc 1.4 software. Odds ratio (OR) and 95% confidential interval (CI) values were calculated to evaluate the association of *DLEC1* methylation with lung cancer. Heterogeneity of meta-analysis was measured by Cochran's Q statistic and I^2 test[20]. We defined a significant heterogeneity in the meta-analysis if it had a $P < 0.05$ in the Q statistical test or $I^2 > 50\%$, and we applied a random-effect model for the meta-analysis with significant heterogeneity, otherwise a fixed-effect model was applied[20]. The diagnostic value of *DLEC1* methylation in the risk of lung cancer was evaluated using the pooled sensitivity and specificity, positive-likelihood ratio (PLR), negative-likelihood ratio (NLR), diagnostic odds ratio (DOR), and area under the curve (AUC) in the summary receiver operating characteristics (SROC) test.

Results

Study Characteristics

As shown in **Figure 1**, our initial literature search identified a total of 71 articles from the online databases including PubMed, Enbase, CNKI, and Wanfang. Among the articles, we excluded 24 overlapping articles among the various databases. A further check filtered out 31 irrelevant articles and 9 articles without sufficient data. At last, we identified 7 eligible articles (8 case-control studies) for the current meta-analysis.

Meta-analysis of *DLEC1* Methylation in Lung Cancer

DLEC1 methylation was assessed among a total of 673 cases of lung cancer and 581 control samples from 8 studies. Further analysis indicated no heterogeneity in the current meta-analysis ($I^2 = 0\%$). Therefore, a fixed-

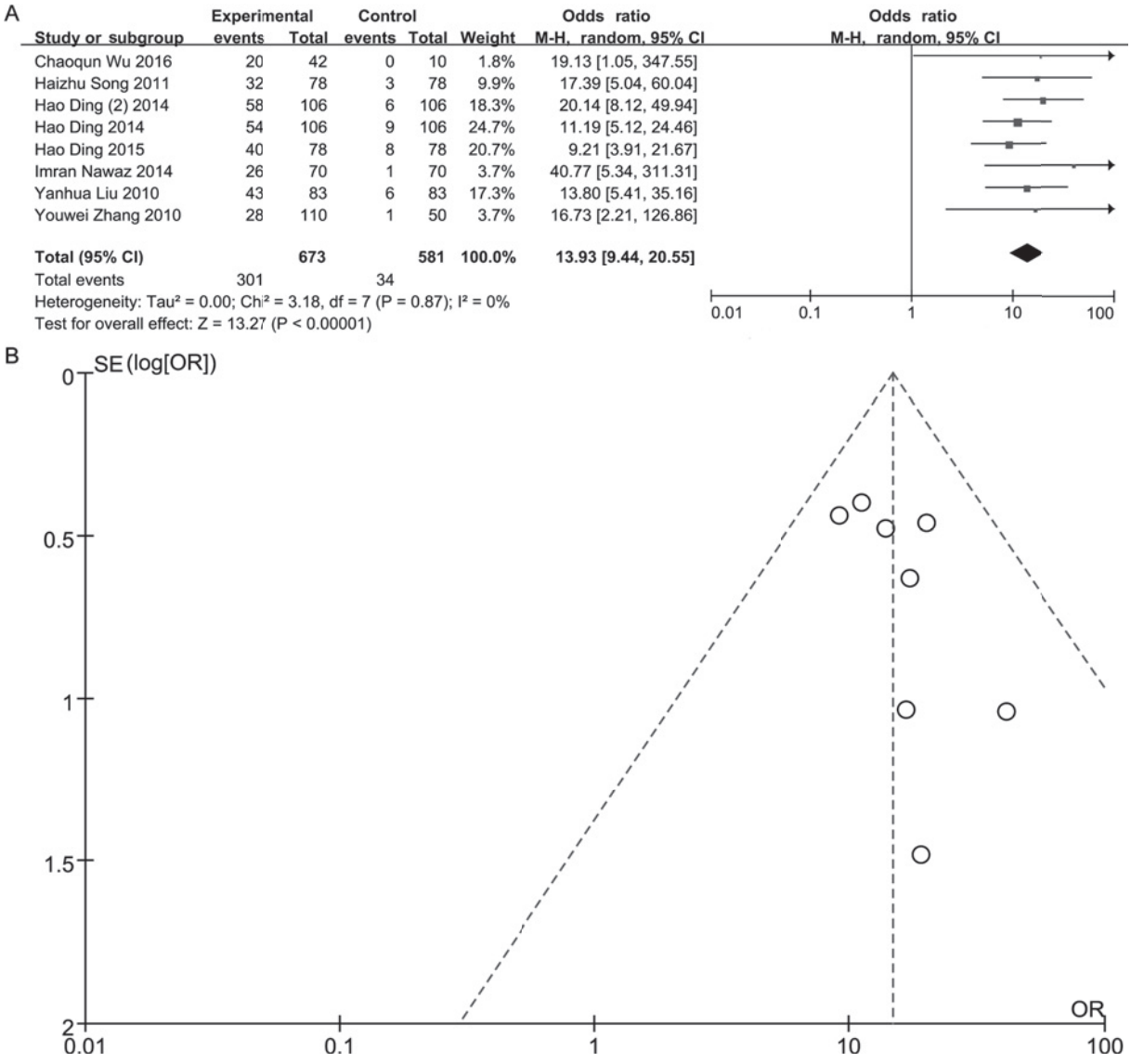

Fig. 2 Forest plot (A) and funnel plot (B) of *DLEC1* methylation in lung cancer.

effect model was applied for the current meta-analysis. Our results showed a significant association of *DLEC1* hypermethylation with lung cancer ($P < 0.00001$, OR = 13.93, 95% CI = 9.44-20.55, **Fig. 2A**). The funnel plots were largely symmetrical, suggesting that there was no publication bias in the current meta-analysis (**Fig. 2B**).

Subgroup Meta-analysis in CRC Samples

Among the 8 studies, *DLEC1* methylation was detected by an MSP method in Asian patients with lung cancer (**Table 1**). Moreover, *DLEC1* methylation was detected in CRC tissues (n = 4) and plasma (n = 4), therefore, we performed a subgroup meta-analysis by sample type. *DLEC1* methylation was associated with risk of lung cancer regardless of the tissue-based studies ($P < 0.00001$, OR = 18.86, 95% CI = 10.83-32.84) and plasma-based studies ($P < 0.00001$, OR = 11.32, 95% CI = 6.52-19.67).

Six studies investigated *DLEC1* methylation in a total of 176 cases of adenocarcinoma (AC) and 217 cases of squamous cell carcinoma (SCC) respectively. *DLEC1* methylation was identified in 128 out of 217 cases of SCC (59.0%) and 67 out of 176 cases of AC (38.1%). The frequency of *DLEC1* methylation was significantly higher in cases of SCC than AC ($P < 0.0001$, OR = 0.42, 95% CI = 0.28-0.64, **Fig. 3A**). Subgroup meta-analysis by age showed that a significant difference in *DLEC1* methylation was found between patients with CRC aged 60 years or older and patients with CRC who were younger than 60 ($P = 0.03$, OR = 1.82, 95% CI = 1.06-3.11, **Fig. 3B**). *DLEC1* methylation appeared more frequently in patients with lung cancer aged 60 years or older.

A significant relationship was found in studies of *DLEC1* methylation in lung cancer related to the lymph node status and clinical stage. Four studies investigated the relationship between *DLEC1* methylation in lung can-

Table 1 The main characteristics of all available studies

First author	Year	Sample	Method	Case		Control	
				M+	Total	M+	Total
Yanhua Liu	2010	Tissue	MSP	43	83	6	83
Youwei Zhang	2010	Plasma	MSP	28	110	1	50
Haizhu Song	2011	Tissue	MSP	32	78	3	78
Hao Ding	2014	Tissue	nMSP	58	106	6	106
Hao Ding	2014	Plasma	nMSP	54	106	9	106
Imran Nawaz	2014	Tissue	MSP	26	70	1	70
Hao Ding	2015	Plasma	nMSP	40	78	8	78
Chaoqun Wu	2016	Plasma	nMSP	20	42	0	10

M+: the number of methylation; Total: the number of cases or controls. nMSP: nested methylation-specific polymerase chain reaction.

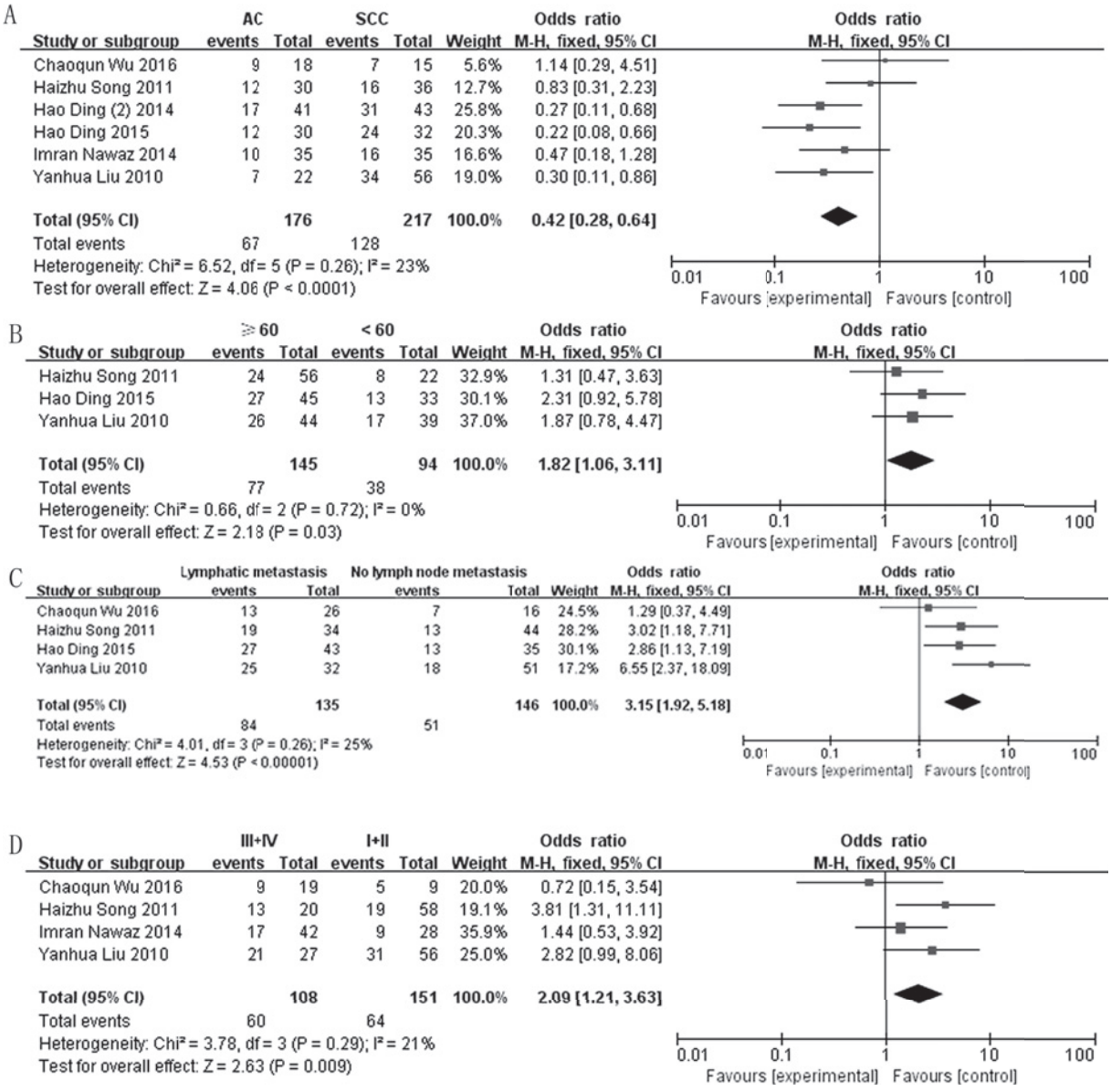

Fig. 3 Subgroup meta-analysis by tissue subtype (A), age (B), lymph node status (C), and clinical stage (D) of *DLEC1* methylation in lung cancer.

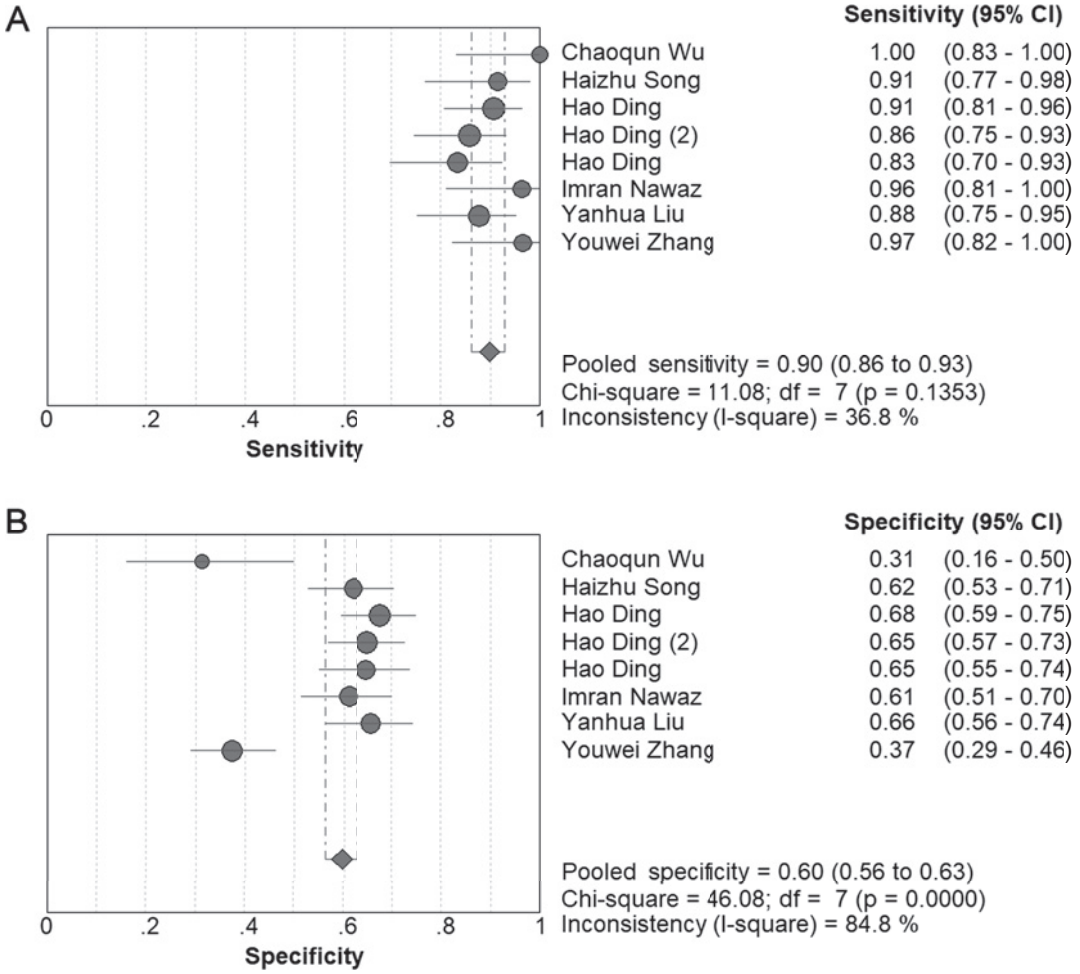

Fig. 4 Forest plots of sensitivities (A) and specificities (B) for *DLEC1* hypermethylation in the diagnosis of lung cancer.

cer and the lymph node status, and the pooled data indicated that *DLEC1* was more frequently methylated in patients with lung cancer with lymphatic metastasis than those without lymphatic metastasis ($P < 0.00001$, OR = 3.15, 95% CI = 1.92-5.18, **Fig. 3C**). Interestingly, the frequency of *DLEC1* methylation in stage III/IV lung cancer (60/108, 55.6%) was significantly increased compared to stage I/II lung cancer (64/151, 42.4%) ($P = 0.009$, OR = 2.09, 95% CI = 1.21-3.63, **Fig. 3D**).

No association was found between *DLEC1* methylation and smoking behavior ($P = 0.32$) in a study including 361 patients with lung cancer. Subgroup meta-analysis by gender indicated no significant correlation between *DLEC1* methylation and gender ($P = 0.40$) in studies that included 338 men and 119 women with lung cancer.

Diagnostic Value of *DLEC1* Methylation in the Prediction of Lung Cancer

We estimated the diagnostic value of *DLEC1* methylation in lung cancer. Our results showed there was a sensitivity value of 0.90 (95% CI = 0.86-0.93, **Fig. 4A**) and a specificity value of 0.60 (95% CI = 0.56-0.63, **Fig. 4B**), a pooled PLR of 2.27 (95% CI = 2.08-2.48, **Fig. 5A**), a pooled NLR of 0.17 (95% CI = 0.12-0.23, **Fig. 5B**), a DOR of 14.72 (10.09-21), and an AUC of 0.8146 using *DLEC1* methylation in the prediction of lung cancer risk (**Fig. 6**). This suggests a potential usage of *DLEC1* methylation in the diagnosis of lung cancer.

Discussion

The poor outcome of patients with lung cancer is partly because more than one-half of cases are diagnosed at a late stage[3]. Currently, the best way to solve this problem is earlier diagnosis with successful surgical intervention[21]. Thus, developing new ways for early diagnosis may help to improve the quality of life of patients with lung cancer. Nowadays, a number of potential biomarkers have been reported, but very few have reached clinical standards to be an efficient index due to small study sizes and lack of assay optimization[22,23]. The aberrant of *DLEC1* methylation had been reported in several cancers, such

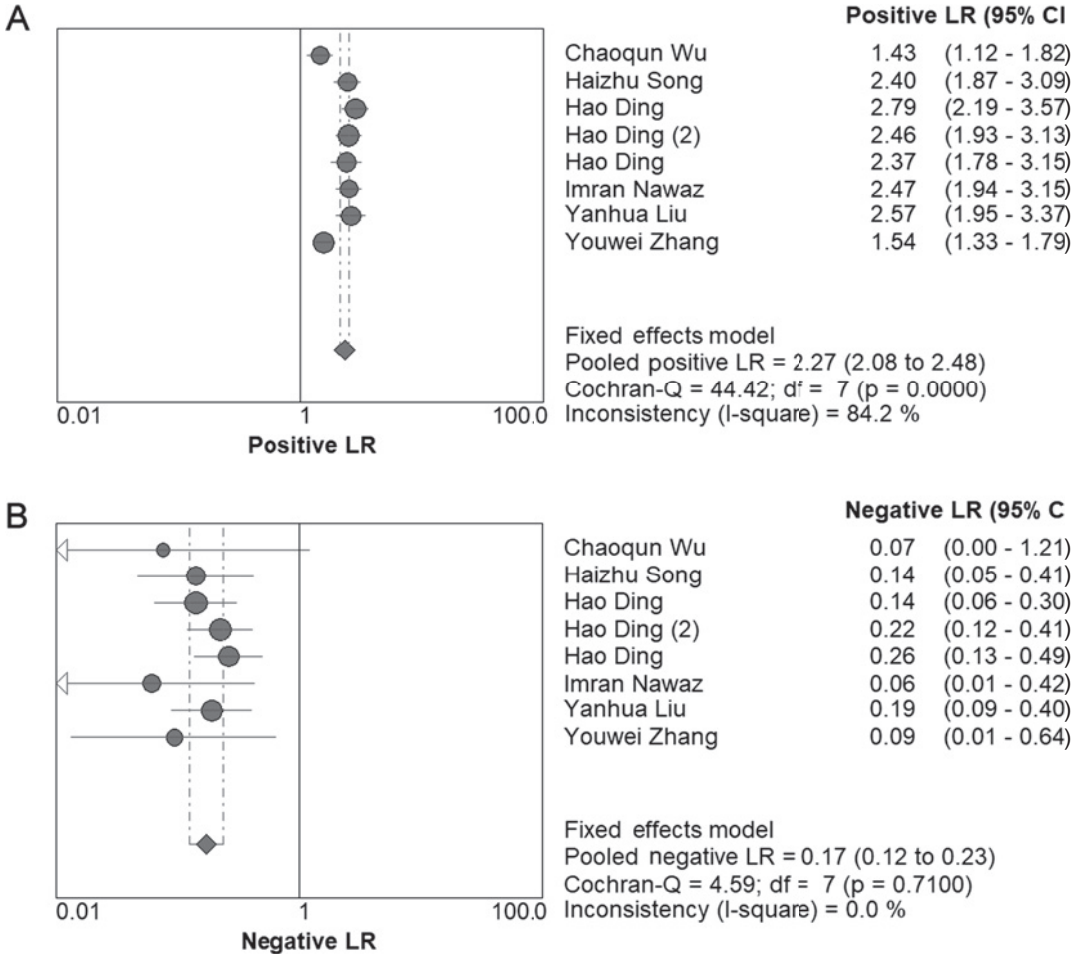

Fig. 5 Forest plots of positive-likelihood ratios (PLR) (A) and negative-likelihood ratios (NLR) (B) of *DLEC1* hypermethylation as a diagnostic biomarker for lung cancer.

as prostate cancer[24], gastric cancer[25], and lung cancer[18]. However, the diagnostic role of the methylation status of the *DLEC1* gene in lung cancer lacks comprehensive assessment. We therefore performed a meta-analysis to evaluate the diagnostic ability for the *DLEC1* methylation in lung cancer.

In the meta-analysis, the results revealed that *DLEC1* methylation was significantly associated with lung cancer risk. In addition, we further confirmed the diagnostic role of *DLEC1* hypermethylation for lung cancer. *DLEC1* methylation detection in patients with lung cancer exhibited a potential diagnostic utility given a high sensitivity of 90% and a poor specificity of 60%. Furthermore, the PLR was 2.27, NLR was 0.17, and DOR value was 14.72, indicating a high-level of accuracy. Compared with the conventional cancer markers (AUC: 0.755 for CYFRA21-1; 0.684 for CEA and 0.776 for NSE)[26], *DLEC1* methylation status is a good biomarker in lung cancer diagnosis with a moderate-to high AUC of 0.8146. In addition, the way to improve sensitivity and specificity is to combine *DLEC1* methylation with other biomarkers.

Previous studies demonstrated that the rate of *WIF1*[27] and *CHFR*[28] hypermethylation was higher in SCC than in AC. Our subgroup analysis revealed that the frequency of *DLEC1* methylation was also significantly higher in SCC than AC, suggesting the usefulness of *DLEC1* methylation as a biomarker in differentiating SCC and AC. And the molecular mechanism may be different between SCC and AC. However, the definitive mechanism should be confirmed in future studies.

It was reported that there was a novel mechanistic link between aberrant hypermethylation in cancer and aging[29,30]. In the current study, *DLEC1* hypermethylation was found in the older population (age $\geq$ 60 years old), which provided a potentially age-specific biomarker of lung cancer. Unfortunately, only 3 studies were included in this subgroup meta-analysis. More studies are needed to broadly establish this association in lung cancer.

DLEC1 was more frequently methylated in patients with lung cancer with lymphatic metastasis than those

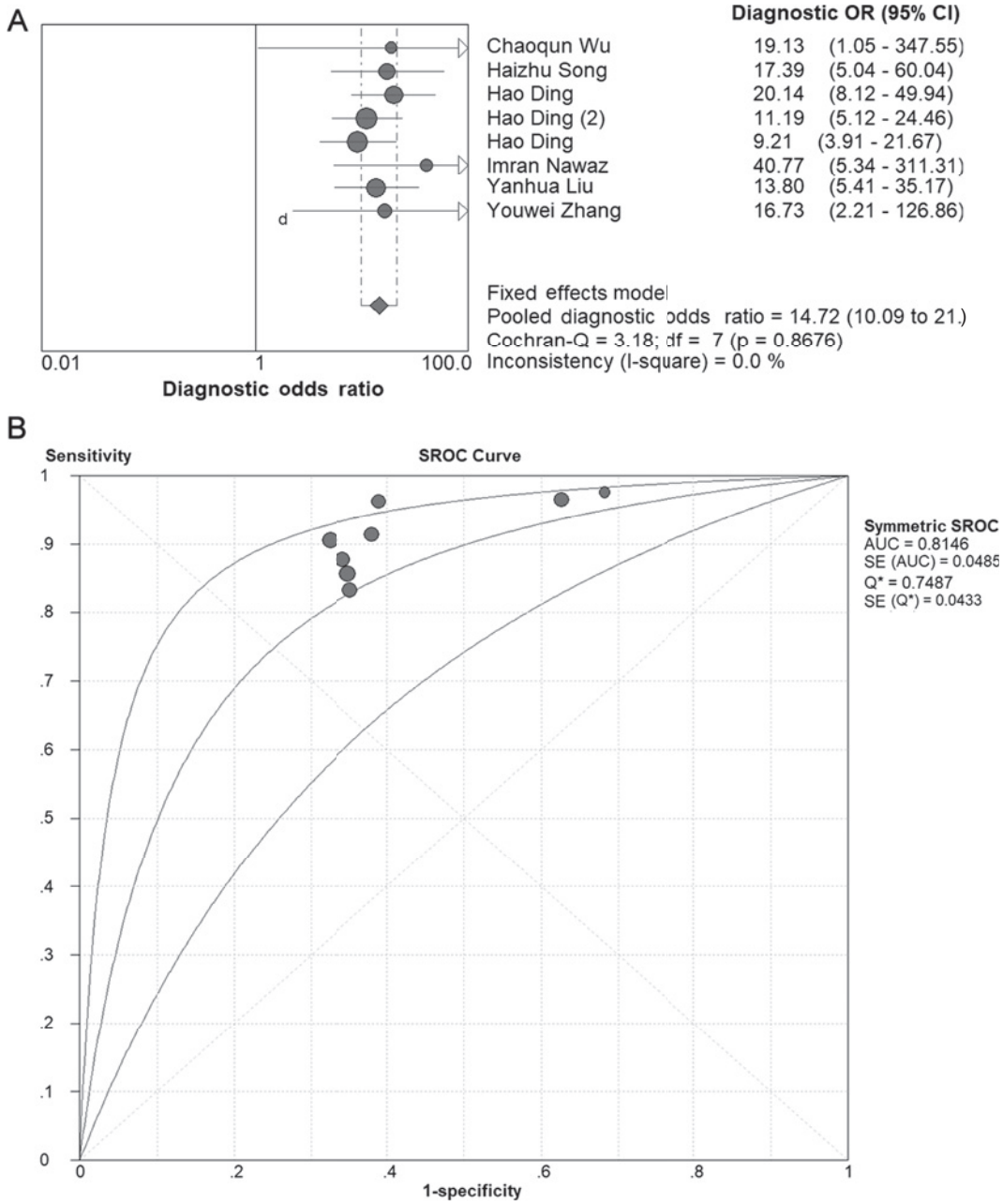

Fig. 6 Forest plot of diagnostic odds ratio (DOR) (A) and summary receiver operating characteristic (SROC) curves (B) of *DLEC1* hypermethylation as a diagnostic biomarker for lung cancer.

without lymphatic metastasis. *DLEC1* methylation appeared more frequently in patients with stage III/IV lung cancer compared to patients with stage I/II lung cancer. These results demonstrated that *DLEC1* methylation may play an important role in the occurrence and development of lung cancer.

There were several limitations in this meta-analysis. Firstly, all the eligible studies were performed in Asian patients. There were only a few studies from Caucasians and Africans. Therefore, more studies with a larger number of participants are needed to assess the association in Caucasians and Africans. Secondly, the methylation evaluation of *DLEC1* was based on one region, which might not be representative of the whole gene. Moreover, the great diversity of the primers used in each individual article might be one of the explanations for the discrepancy of *DLEC1* methylation in the detection of lung cancer risk.

In conclusion, this study indicated that *DLEC1* methylation might be a valuable diagnostic biomarker for lung cancer.

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and DG contributed to the interpretation of data and completion of figures and tables. All authors read and approved the final manuscript.

Conflict of Interest: The authors declare no competing financial interests.

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