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ORIGINAL RESEARCH

The clinicopathological significance and ethnic difference of *FHIT* hypermethylation in non-small-cell lung carcinoma: a meta-analysis and literature review

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Abstract: Emerging evidence indicates that FHIT is a candidate tumor suppressor in many types of tumors including non-small-cell lung carcinoma (NSCLC). However, the prognostic value and correlation between *FHIT* hypermethylation and clinicopathological characteristics of NSCLC remains unclear. In this report, we performed a meta-analysis to evaluate the effects of *FHIT* hypermethylation on the incidence of NSCLC and clinicopathological characteristics of human NSCLC patients. Final analysis of 1,801 NSCLC patients from 18 eligible studies was performed. *FHIT* hypermethylation was found to be significantly higher in NSCLC than in normal lung tissue. The pooled odds ratio (OR) from ten studies included 819 NSCLC and 792 normal lung tissues (OR =7.51, 95% confidence interval [CI] =2.98–18.91, $P<0.0001$). Subgroup analysis based on ethnicity implied that *FHIT* hypermethylation level was higher in NSCLC tissues than in normal tissues in both Caucasians ($P=0.02$) and Asians ($P<0.0001$), indicating that the difference in Asians was much more significant. *FHIT* hypermethylation was also correlated with sex status, smoking status, as well as pathological types. In addition, patients with *FHIT* hypermethylation had a lower survival rate than those without (hazard ratio =1.73, 95% CI =1.10–2.71, $P=0.02$). The results of this meta-analysis suggest that *FHIT* hypermethylation is associated with an increased risk and poor survival in NSCLC patients. *FHIT* hypermethylation, which induces the inactivation of *FHIT* gene, plays an important role in the carcinogenesis and clinical outcome and may serve as a potential diagnostic marker and drug target of NSCLC.

Keywords: FHIT, methylation, tumor suppressor gene, meta-analysis, odds ratio, hazard ratio

Introduction

Lung cancers consist of two major histological types, non-small-cell lung carcinoma (NSCLC) and small-cell lung carcinoma. NSCLC consists of squamous cell carcinoma (SCC), adenocarcinoma (AD), large-cell carcinoma, and others. NSCLC accounts for ~85% of all lung cancers, and there are ~80% of NSCLC cases in advanced stage where the prognosis remains poor.[1] Therefore, investigation of the mechanism of initiation, progression, and identification of prognostic markers is still needed for the selection of patients with NSCLC and to provide better individualized treatment. In recent years, a number of new tools, such as protein–protein interaction prediction approach,[2] pathway data integration,[3] and gene transcription analysis,[4] were widely used in the study of epigenetic regulation and modification. Epigenetic modification of gene expression plays an important role in carcinogenesis. Epigenetic alterations, particularly aberrant DNA methylation, one of the best-characterized epigenetic modifications,

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contribute to tumor initiation and progression.[5,6] Aberrant methylation of CpG dinucleotides is a commonly observed epigenetic modification in human cancer.[5–7] Thus, the analysis of specific gene promoter methylation as a tool for diagnosis of tumors or its use as prognostic marker has been widely used for many different cancers including NSCLC.[8]

FHIT, also known as bis(5′-adenosyl)-triphosphatase, is one of the histidine triad gene family members and is an enzyme encoded by the *FHIT* gene.[9,10] The *FHIT* gene locates the most common fragile site in the human genome, FRA3B (3p14.2), a region which frequently undergoes genomic rearrangement, biallelic loss, and cytogenetic abnormalities in tumors.[9,11,12] Previous reports showed that FHIT was inactivated by the loss of heterozygosity and methylation in cancer cells, which indicated that FHIT is a tumor suppressor protein.[13,14] Its precise function has been intensively studied in several tumors, by inducing cell cycle arrest and apoptosis, inhibition of cell proliferation, and increasing cell sensitivity to DNA-damaging agents.[15–17] Lack of expression of FHIT protein by promoter methylation (hypermethylation) has been found to play an important role in lung alveolar differentiation regulation and epithelial tumorigenesis.[18–21] Although previous studies indicated that inactivation of the *FHIT* is mainly induced by hypermethylation of *FHIT* gene, the reported rates of *FHIT* hypermethylation in NSCLC were remarkably diverse. Moreover, whether or not it is associated with the incidence and clinical characteristics of NSCLC is still unclear. The various results of these studies underpin the need for assessing the evidence of the relationship between *FHIT* inactivation and NSCLC. Hence, we conducted a systematic review and meta-analysis to quantitatively evaluate the effects of *FHIT* hypermethylation on the incidence and clinical characteristics of NSCLC.

Materials and methods

Search strategy and selection criteria

We searched PubMed, Embase, and ISI Web of Knowledge to identify studies from May 1, 1998 to October, 2015 using the search terms "lung" and "cancer or tumor or neoplasm or carcinoma", "methylation", and "FHIT or Fragile histidine triad protein or Bis (5′-adenosyl)-triphosphatase". We also searched manually for 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 language. After exclusion of nonrelevant 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 the same patient populations were reported in several publications.

Criteria that an eligible study had to meet were as follows: (1) *FHIT* hypermethylation evaluated in the primary NSCLC tissues, (2) research revealed the relationship between *FHIT* hypermethylation and NSCLC clinicopathological parameters and prognosis, (3) *FHIT* hypermethylation examined by polymerase chain reaction, 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 methodological assessment

Two authors (XW and GW) independently reviewed and extracted data from eligible studies. Disagreements were resolved by discussion and consensus. Two authors (XY and GH) reviewed all the articles that fit inclusion and exclusion criteria. The following information was recorded for each study: the first author name, year of publication, sample source, number of cases, clinicopathological parameters, cancer tumor node metastasis stage, methylation detection method, methylation rate and/or expression, and follow-up. Data for study characteristics and clinical responses were summarized and organized into a table format. Heterogeneity of investigation was evaluated to determine whether or not the data of the various studies could be analyzed for a meta-analysis.

For the methodological evaluation of the studies, three investigators (XW, GW, and XY) read through each publication independently, and they assessed and scored them according to the REMARK guidelines and ELCWP quality scale.[22,23] The three readers provided the quality scores and compared them, and then they reached a consensus value for each item.

Statistical analysis

Analysis was conducted using the STATA 12.0 (Stata Corporation, College Station, TX, USA) and Review Manager 5.2 (Cochrane Collaboration, Oxford, UK). The pooled

frequency of *FHIT* hypermethylation and 95% CIs were estimated. The frequency of *FHIT* hypermethylation was compared in different tumor characteristics. Heterogeneity among studies was evaluated with Cochran's *Q* test[24] and the I^2 statistic.[25,26] 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 ≥50%), a random-effect model was used to pool data and attempt to identify potential sources of heterogeneity based on subgroup analyses. The pooled OR was estimated for the association between *FHIT* hypermethylation and clinicopathological features. *P*-values tailed <0.05 were considered statistically significant.

Publication bias was assessed by using a method reported by Egger et al.[27] 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 by using STATA version 10.0.

Results

Seventy publications were identified by the search method as described in the "Materials and methods" section. Fifty-two of those were excluded as they were laboratory studies, non-original articles (review), or studies irrelevant to the current analysis. Eventually, there were 18 studies from 2001 to 2014 included in final meta-analysis[19,28–44] as shown in Figure 1. A total of 1,801 NSCLC patients from People's Republic of China, South Korea, Japan, Egypt, Italy, and USA were enrolled. Their basic characteristics are summarized in Table 1.

We first determined that *FHIT* hypermethylation was significantly higher in NSCLC than in normal lung tissues. The pooled odds ratio (OR) from ten studies including 819 NSCLC and 792 normal lung tissues is shown in Figure 2 (OR =7.51, 95% CI =2.98–18.91, *P*<0.0001), indicating that *FHIT* hypermethylation plays an important role in the carcinogenesis of NSCLC. Subgroup analysis based on ethnicity implied that *FHIT* hypermethylation level was higher in NSCLC tissues than in normal tissues in both Caucasians (*P*=0.02) and Asians (*P*<0.0001) as shown in Figure 3, indicating that the difference in Asians was much more significant. Next, we determined whether or not *FHIT* hypermethylation rate was correlated with sex status. The pooled OR from eight studies included NSCLC from 742 males and 298 females, as shown in Figure 4 (OR =1.44, 95% CI =1.07–1.94, *P*=0.02),

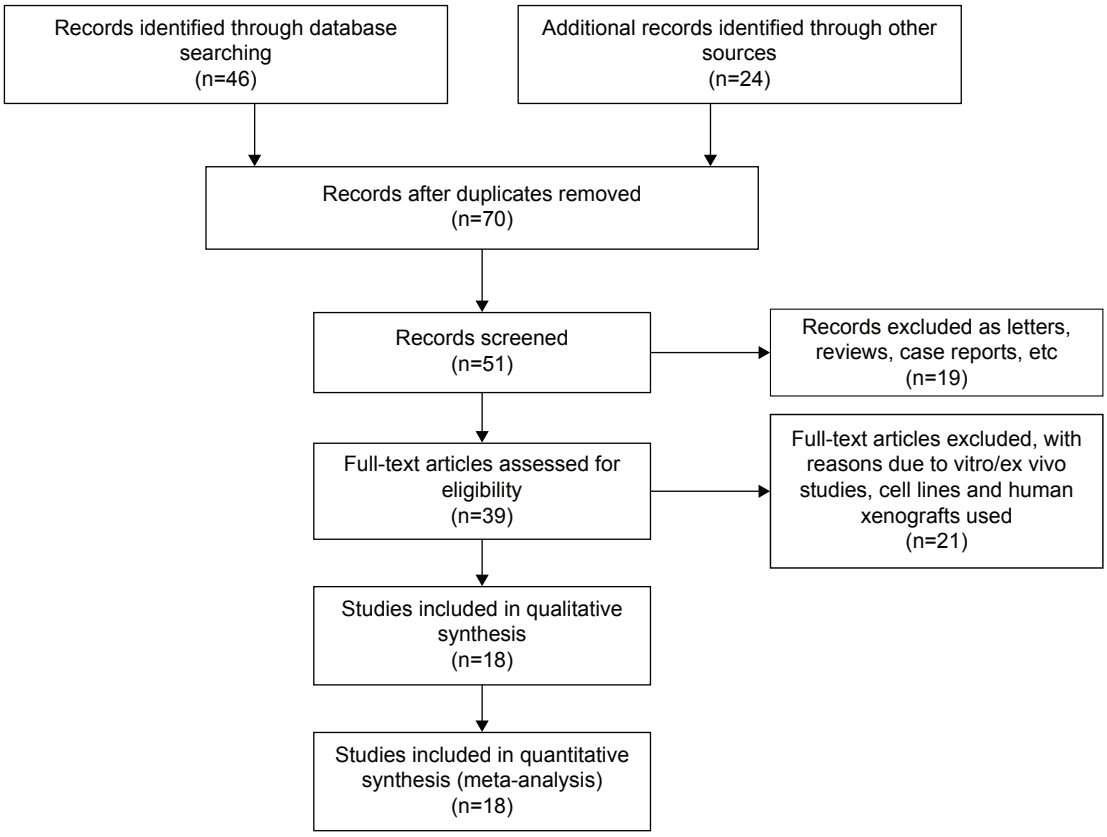

Figure 1 Flow chart of study selection.

Table 1 Basic characteristics of the included studies

Study	Country	Patients	Methods	Primary aim
Haroun et al[28]	Egypt	28	MSP	Analyze the methylation status of three tumor suppressors in NSCLC
Li et al[29]	People's Republic of China	56	MSP	Analyze the methylation status of three tumor suppressors in NSCLC
Yanagawa et al[32]	Japan	62	MSP	Determine the methylation status of ten tumor suppressor genes in NSCLC
Song et al[33]	People's Republic of China	78	MSP/RT-PCR	Determine the methylation status of five tumor suppressors in NSCLC
Li et al[34]	People's Republic of China	123	MSP	Assess the methylation status of *FHIT* in NSCLC
Li et al[35]	People's Republic of China	52	MSP/RT-PCR	Determine methylation status and protein expression of *FHIT* in NSCLC
Verri et al[19]	Italy	187	MSP/immuno-histochemistry	Investigate the different molecular alterations leading to the inactivation of *FHIT* in NSCLC
Hsu et al[31]	People's Republic of China	63	MSP	Determine hypermethylation status of six genes in NSCLC
Yanagawa et al[36]	Japan	101	MSP	Determine the methylation status of ten genes in NSCLC
Kim et al[37]	South Korea	99	MSP	Determine methylation patterns of eight tumor suppressor genes in NSCLC
Kim et al[38]	South Korea	335	MSP	Determine methylation of five genes in NSCLC
Nakata et al[39]	Japan	139	MSP/immuno-histochemistry	Determine the inactivation of *p16*, *CDH1*, and *FHIT* in NSCLC
Iliopoulos et al[40]	USA	24	MSP/immuno-histochemistry	Determine the inactivation of *FHIT* and *WWOX* in breast cancer, bladder cancer, and NSCLC
Tomizawa et al[41]	Japan	54	MSP	Investigate the aberrant methylation of *RAR*β2, *RASSF1A*, and *FHIT* in NSCLC
Tzao et al[42]	People's Republic of China	44	MSP/RT-PCR	Determine protein and mRNA expression, and hypermethylation of the *FHIT* gene in NSCLC
Kim et al[43]	South Korea	125	MSP	Examine the clinicopathological and prognostic significance of *FHIT* methylation in NSCLC
Maruyama et al[44]	USA	124	MSP	Examine the correlation between the aberrant methylation of multiple genes and survival in patients with NSCLC
Zochbauer-Muller et al[30]	USA	107	MSP/northern blot analysis	Determine the correlation of protein and hypermethylation status of *FHIT* in breast cancer and NSCLC

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

indicating that *FHIT* hypermethylation was correlated with sex status and it was higher in male than female. Then, we determined whether or not *FHIT* hypermethylation rate was correlated with smoking status. The pooled OR from ten studies including 287 and 818 NSCLC patients with and without smoking history is shown in Figure 5 (OR =0.74, 95% CI =0.55–1.00, *P*=0.05), indicating that *FHIT* hypermethylation was correlated with smoking status in NSCLC patients. We also determined whether or not *FHIT* hypermethylation was correlated with pathological types. The pooled OR from ten studies including 528 SCC and 527 AD patients is shown in Figure 6 (OR =1.49, 95% CI =1.15–1.93, *P*=0.003), which indicates that *FHIT* hypermethylation plays a more important role in the pathogenesis of SCC.

We analyzed 366 NSCLC patients pooled from three studies to assess whether or not the aberrant *FHIT* hypermethylation in NSCLC was associated with the differentiated status. As shown in Figure 7A, aberrant *FHIT* hypermethylation was not significantly higher in poorly differentiated NSCLC than that in moderately or highly differentiated NSCLC (OR =1.30, 95% CI =0.80–2.09, *P*=0.29). In addition, aberrant *FHIT* hypermethylation was also not significantly higher in advanced NSCLC (III and IV) than that in early-staged NSCLC (I and II) (OR =1.17, 95% CI =0.75–1.83, *P*=0.50; Figure 7B). These results suggest that *FHIT* hypermethylation may not play an important role in NSCLC progression and differentiation stages. There are four studies estimating

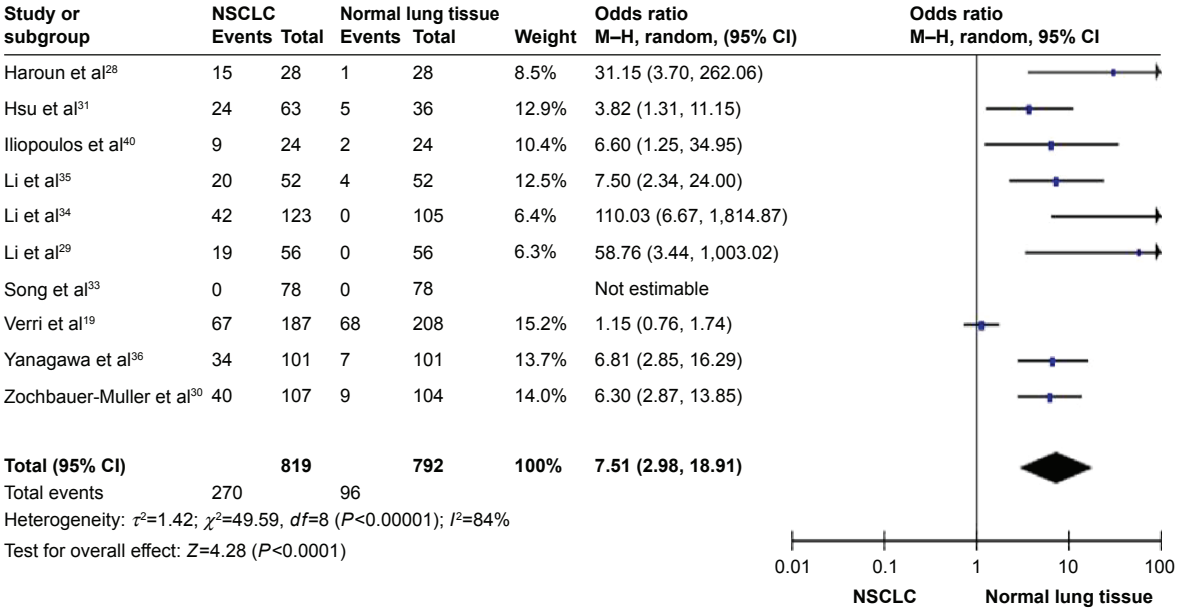

Figure 2 The pooled OR from ten studies included 819 NSCLC and 792 normal lung tissues (OR =7.51, 95% CI =2.98–18.91, $P<0.0001$).
Abbreviations: OR, odds ratio; NSCLC, non-small-cell lung carcinoma; CI, confidence interval; M–H, Mantel–Haenszel test.

the relationship between *FHIT* hypermethylation and OS in NSCLC patients. The pooled HR for OS showed that *FHIT* hypermethylation was associated with poor survival in NSCLC patients as shown in Figure 8 (HR =1.73, 95% CI =1.10–2.71, $P=0.02$).

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 9A–G), suggesting that there

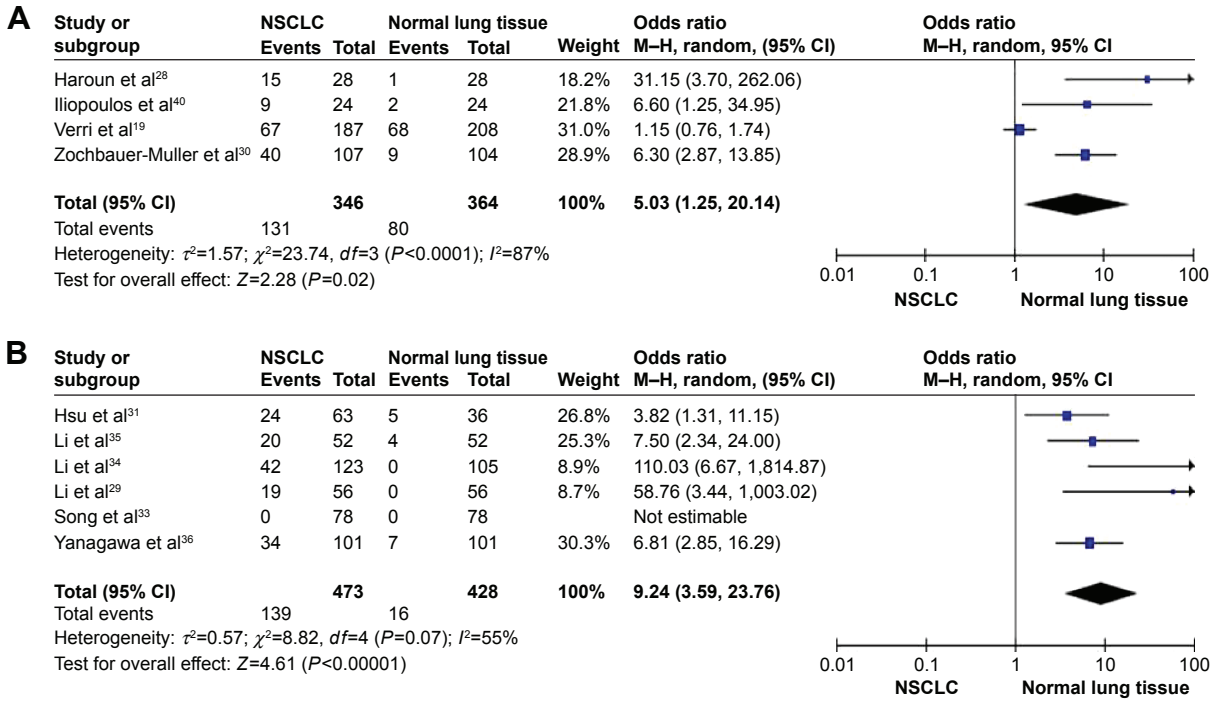

Figure 3 FHIT hypermethylation in NSCLC and normal lung tissues.
Notes: The pooled OR from four studies from Caucasians included 131 NSCLC and 80 normal lung tissues (OR =5.03, 95% CI =1.25–20.14, $P=0.02$) (**A**). The pooled OR from six studies from Asians included 473 NSCLC and 428 normal lung tissues (OR =9.24, 95% CI =3.59–23.76, $P<0.00001$) (**B**).
Abbreviations: OR, odds ratio; NSCLC, non-small-cell lung carcinoma; CI, confidence interval; M–H, Mantel–Haenszel test.

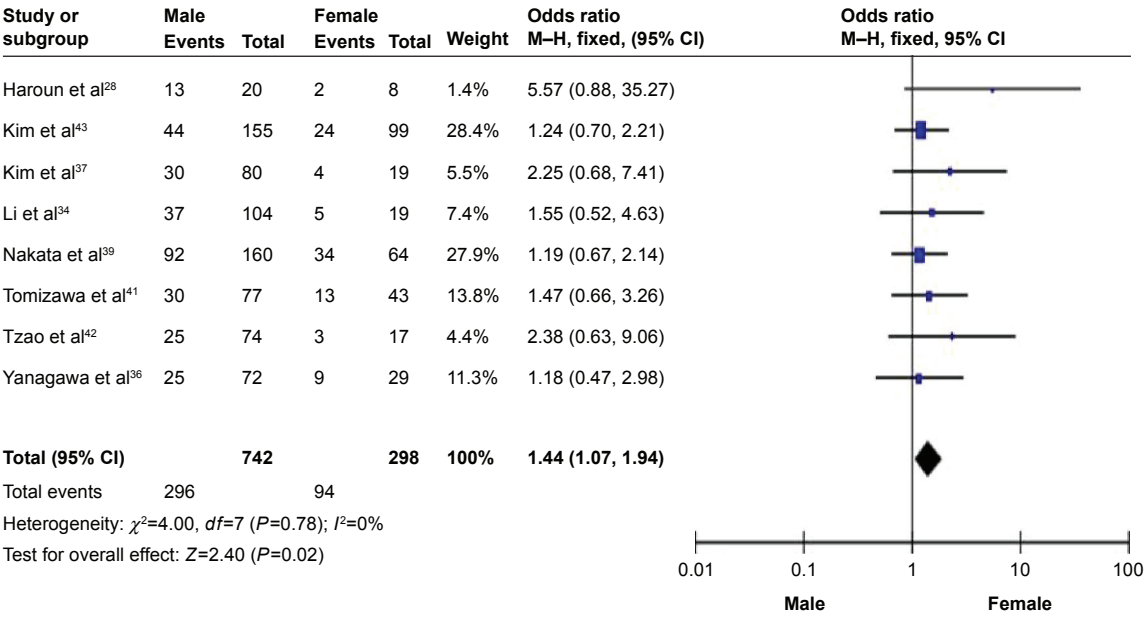

Figure 4 The pooled OR from eight studies included NSCLC tissue from 742 males and 298 females (OR =1.44, 95% CI =1.07–1.94, *P*=0.02), which indicates that *FHIT* hypermethylation was correlated with sex status in NSCLC patients.
Abbreviations: OR, odds ratio; NSCLC, non-small-cell lung carcinoma; CI, confidence interval; M–H, Mantel–Haenszel test.

were no publication biases in the meta-analysis of *FHIT* hypermethylation and clinicopathological features.

Discussion

FHIT is genetically or epigenetically altered in many different kinds of primary and advanced carcinomas. Inactivation of *FHIT* by promoter hypermethylation plays an important role in tumorigenesis in several types of tumors including NSCLC.[38,45–53] To date, there have been some studies describing the methylation status of *FHIT* in NSCLC; however, the roles of methylation of *FHIT* in NSCLC and clinical significance have not been thoroughly investigated. We conducted

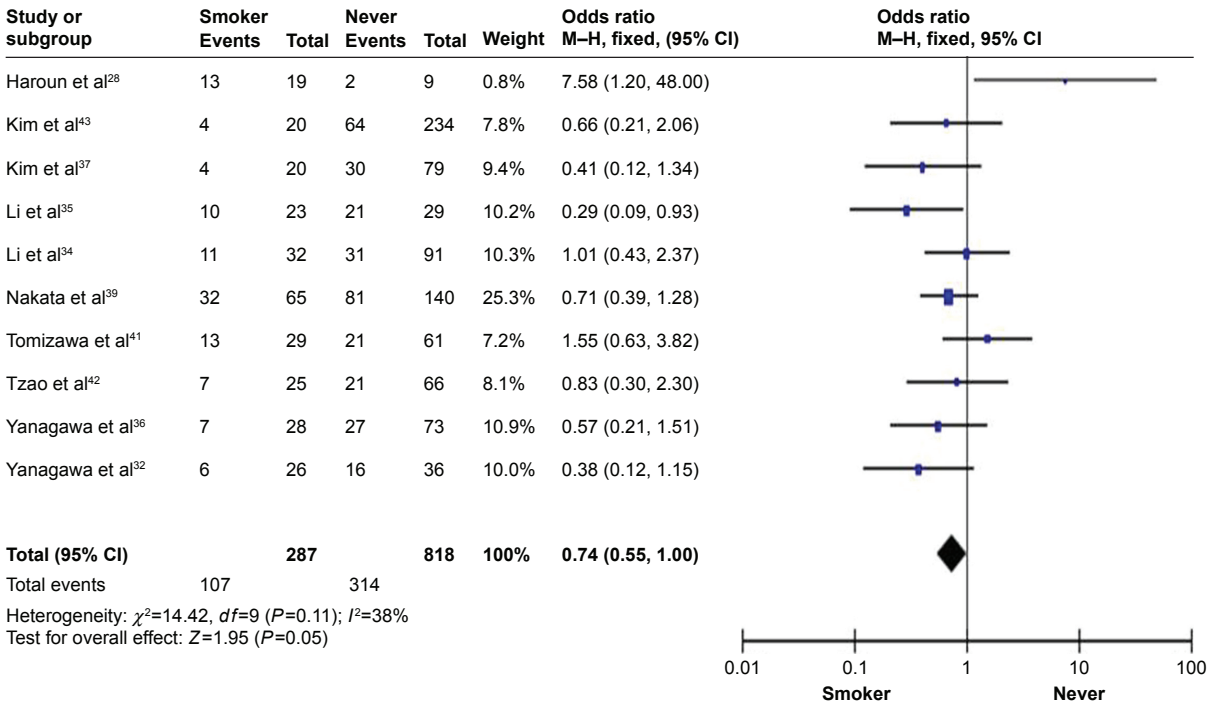

Figure 5 One thousand one hundred and five NSCLC patients with the smoking status pooled from ten studies.
Note: Aberrant *FHIT* hypermethylation was correlated with the smoking status in NSCLC patients (OR =0.74, 95% CI =0.55–1.00, *P*=0.05).
Abbreviations: NSCLC, non-small-cell lung carcinoma; OR, odds ratio; CI, confidence interval; M–H, Mantel–Haenszel test.

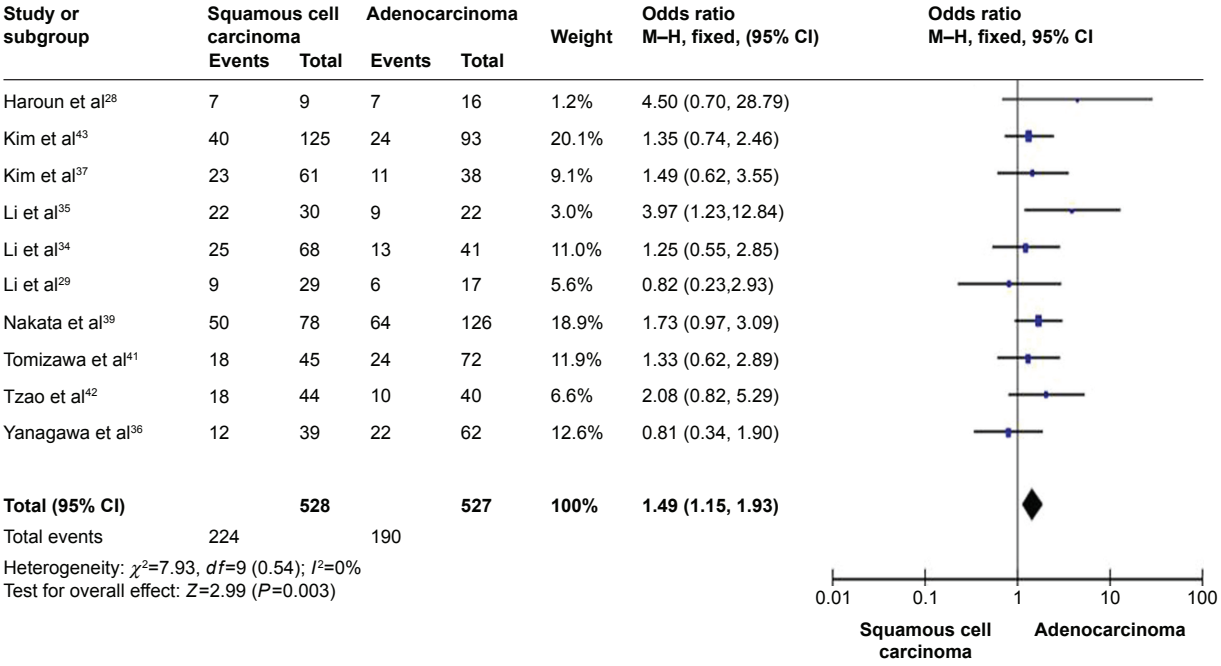

Figure 6 The pooled OR from ten studies included 528 SCC and 527 AD patients (OR =1.49, 95% CI =1.15–1.93, *P*=0.003), indicating that *FHIT* hypermethylation plays a more important role in the pathogenesis of SCC.
Abbreviations: OR, odds ratio; SCC, squamous cell carcinoma; AD, adenocarcinoma; CI, confidence interval; M–H, Mantel–Haenszel test.

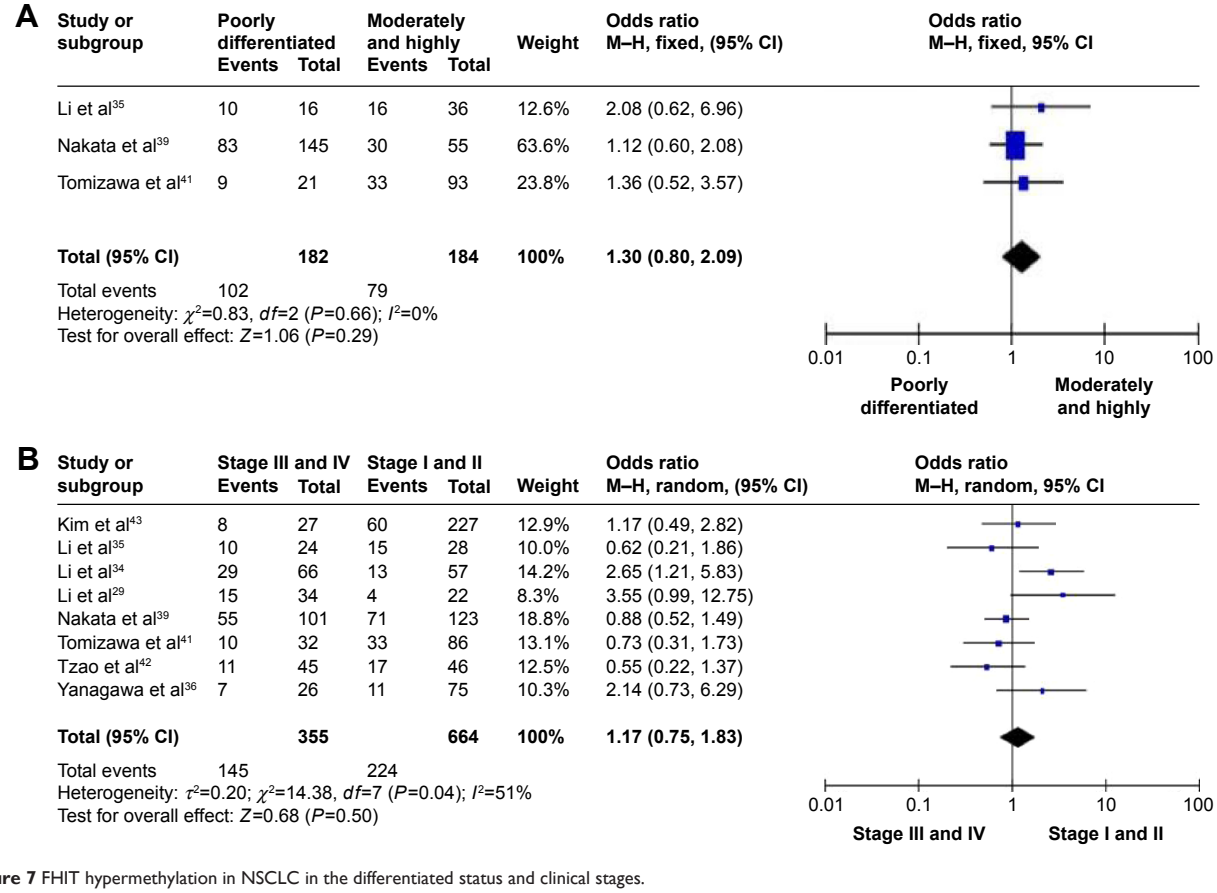

Figure 7 FHIT hypermethylation in NSCLC in the differentiated status and clinical stages.
Notes: Three hundred and sixty-six NSCLC patients were pooled from three studies to assess whether or not the aberrant *FHIT* hypermethylation in NSCLC was associated with the differentiated status. Aberrant *FHIT* hypermethylation was not significantly higher in poorly differentiated NSCLC than that in moderately and highly differentiated NSCLC (OR =1.30, 95% CI =0.80–2.09, *P*=0.29) (**A**). Aberrant *FHIT* hypermethylation was also not significantly higher in advanced NSCLC (III and IV) than that in early-staged NSCLC (I and II) (OR =1.17, 95% CI =0.75–1.83, *P*=0.50) (**B**).
Abbreviations: NSCLC, non-small-cell lung carcinoma; OR, odds ratio; CI, confidence interval; M–H, Mantel–Haenszel test.

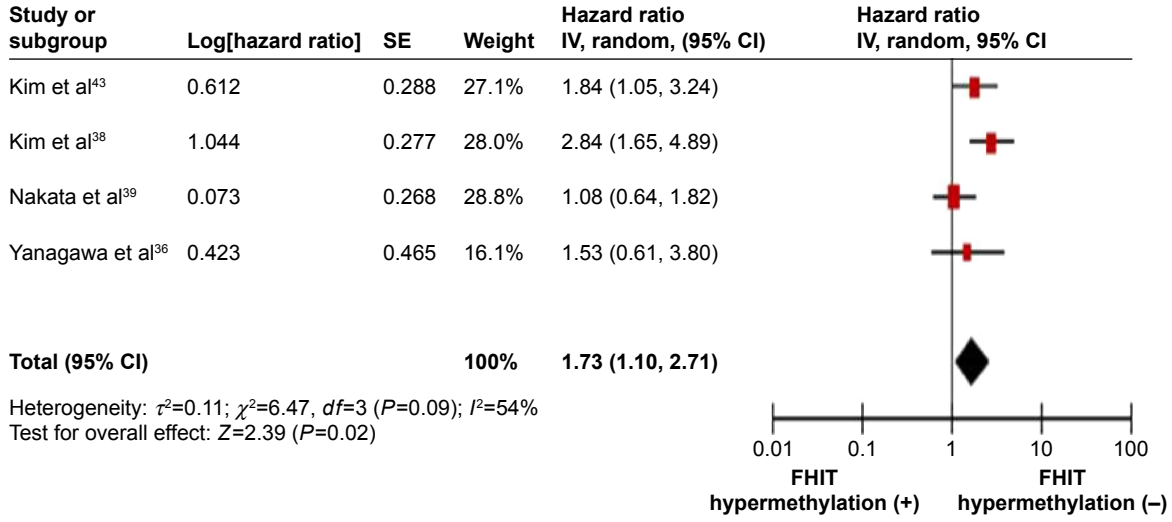

Figure 8 Four studies included were investigated for the relationship between OS and *FHIT* hypermethylation.
Note: The pooled HR for OS showed that *FHIT* hypermethylation was associated with poor survival in NSCLC (HR =1.73, 95% CI =1.10–2.71, *P*=0.02).
Abbreviations: OS, overall survival; HR, hazard ratio; NSCLC, non-small-cell lung carcinoma; CI, confidence interval; IV, independent variable.

the meta-analysis to determine the correlation between *FHIT* hypermethylation and clinicopathological characteristics of NSCLC. Analysis of the pooled data showed that NSCLC had a higher hypermethylation than normal lung tissue, indicating that *FHIT* hypermethylation plays an important role in the carcinogenesis of NSCLC. Interestingly, subgroup analysis based on ethnicity implied that *FHIT* hypermethylation level was higher in NSCLC tissues than in normal tissues in both Caucasians (P=0.02) and Asians ($P<0.0001$) as shown in Figure 3, indicating that the difference in Asians was much more significant. Additional interesting findings included that *FHIT* hypermethylation was also correlated with sex status, smoking status, as well as pathological types. The results from the current study demonstrated that the hypermethylation rate of *FHIT* gene in NSCLC was significantly higher than that in the normal lung tissues, indicating that *FHIT* promoter hypermethylation was common in NSCLC. Therefore, detection of *FHIT* promoter hypermethylation may provide an invaluable diagnostic marker for NSCLC patients. Since changes in *FHIT* promoter hypermethylation are reversible, drug treatment through demethylation may be useful to delay carcinogenesis and progression and to improve prognosis. Lung cancer cell clones carrying conditional FHIT transgenes showed significant suppression of xenograft tumor growth after induction of expression of the FHIT transgene, suggesting that treatments to restore endogenous FHIT expression in lung cancers would result in decreased tumorigenicity.[20] In addition, injection of 5-aza-2-deoxycytidine and trichostatin A in nude mice with established H1299 tumors showed suppressed growth of small tumors without apparent toxicity and responding tumors showed restoration of FHIT.[20] These preclinical studies show the therapeutic potential of restoration of tumor suppressor expression through epigenetic modulation. This approach may bring new direction and hope for cancer treatment through gene-targeted therapy.

FHIT is thought to affect cellular function and behavior largely through its signaling properties. FHIT also activates caspase-8 and caspase-2, which causes the release of cytochrome c and finally induces apoptosis.[54] FHIT and p53, the two most commonly altered tumor suppressor genes, might rely on common mediators and cross talk between these proteins in regulation of growth-related pathways; thus, the inactivation of both genes results in prominent deregulation of cell proliferation and tumor progression in lung cancer.[55] A number of studies showed that inactivation of FHIT can cause tumor aberrant progression and link to clinicopathological characteristics.[28,38,56–58] Therefore, FHIT can be considered as a tumor suppressor, and its inactivation could contribute to tumor progression and poor prognosis. Although only four studies evaluated the relationship between OS and *FHIT* hypermethylation in NSCLC, they showed very similar results.[36,38,39,43] Based on this meta-analysis, the pooled HR for OS showed that *FHIT* hypermethylation was associated with poor survival in NSCLC patients (HR =1.73, 95% CI =1.10–2.71, P=0.02). Therefore, we may consider that *FHIT* hypermethylation in NSCLC tends to indicate a poor prognosis.

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

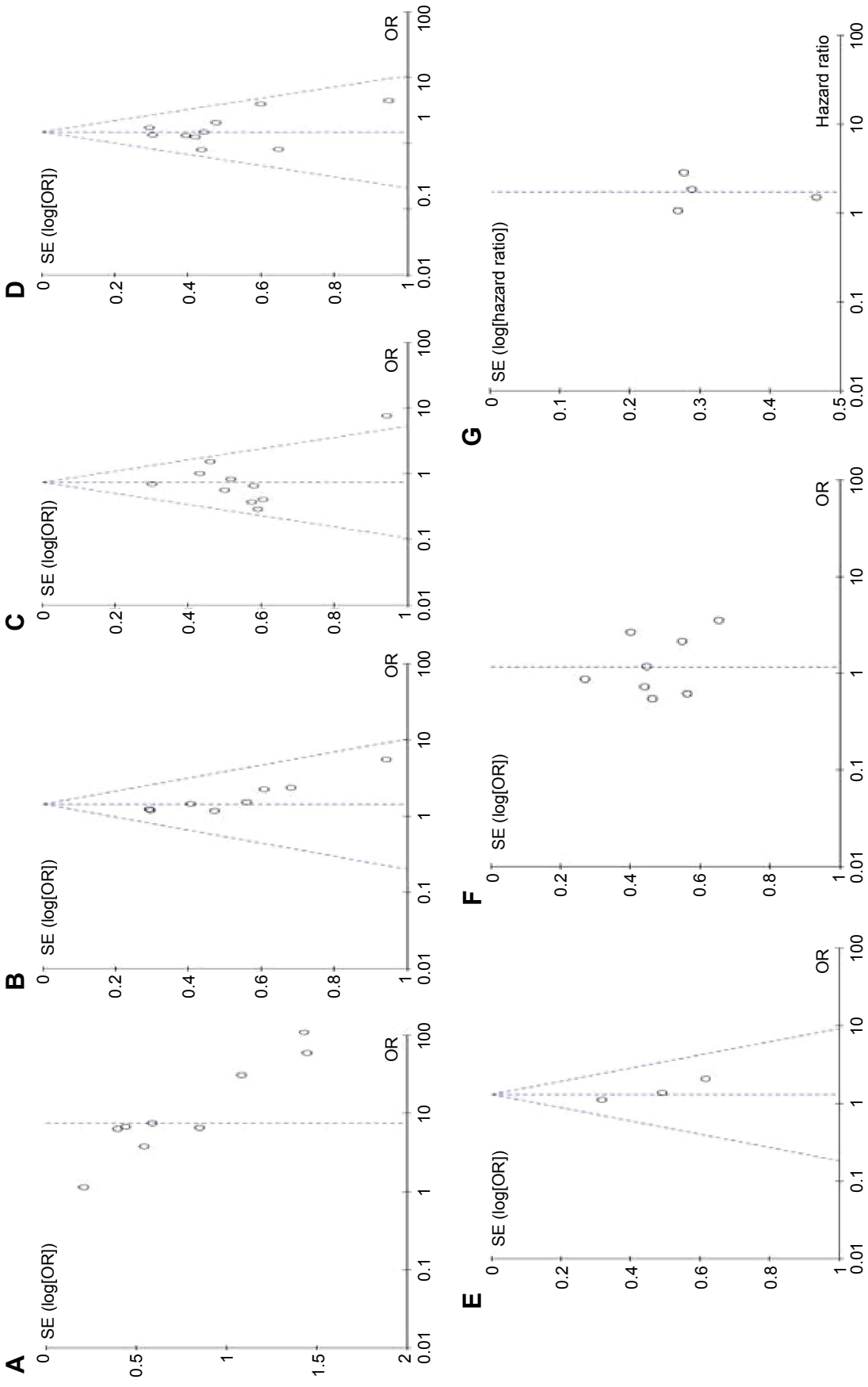

Figure 9 The funnel plots were largely symmetric, which suggests that there were no publication biases in the meta-analysis of *FHIT* hypermethylation and clinicopathological features.
Notes: The funnel plot from ten studies comparing NSCLC and normal lung tissue (**A**). The funnel plot from eight studies determined the relationship between *FHIT* hypermethylation and the sex status in NSCLC patients (**B**). The funnel plot from ten studies determined the relationship between *FHIT* hypermethylation and the smoking status in NSCLC patients (**C**). The funnel plot from ten studies comparing *FHIT* hypermethylation between squamous cell carcinoma and adenocarcinoma (**D**). The funnel plot from three studies determined *FHIT* hypermethylation in different differentiated NSCLCs (**E**). The funnel plot from eight studies determined *FHIT* hypermethylation in different-staged NSCLC (**F**). The funnel plot from four studies determined the relationship between *FHIT* hypermethylation and overall survival in NSCLC (**G**).
Abbreviations: NSCLC, non-small-cell lung carcinoma; SE, standard error; OR, odds ratio.

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. 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, caution should be taken when our findings are interpreted among the general population.

In conclusion, our meta-analysis showed that NSCLC tissue had a higher *FHIT* hypermethylation than normal lung tissue, higher in male than female, higher in nonsmoker than smoker, and higher in SCC than AD. In addition, *FHIT* hypermethylation is associated with an increased risk and poor survival in NSCLC. *FHIT* hypermethylation, which induces the inactivation of *FHIT* gene, may play an important role in the carcinogenesis and clinical outcome and may serve as a potential diagnostic marker and drug target of NSCLC. Further large-scale studies, especially multicenter and well-matched cohort research, will provide more insight into the role of *FHIT* in the prognosis and clinical implementation of NSCLC patients.

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Disclosure

The authors report no conflicts of interest in this work.

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