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ORIGINAL RESEARCH

Clinicopathological significance of DAPK promoter methylation in non-small-cell lung cancer: a systematic review and meta-analysis

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Background: Lung carcinogenesis is related to silencing of tumor suppressor genes and activation of oncogenes. The aim was to investigate the significance of death-associated protein kinase (DAPK) methylation in non-small-cell lung cancer (NSCLC) through a meta-analysis.

Methods: A detailed literature search was made in PubMed, Embase, and Web of Science databases. All analysis was performed with Review Manager 5.2.

Results: In total, 28 studies with a total of 2,148 patients were involved. The frequency of DAPK promoter hypermethylation was 40.50% in NSCLC, significantly higher than in non-malignant lung tissue; the pooled OR was 5.69, $P<0.00001$. Additionally, DAPK promoter hypermethylation was significantly correlated with poor overall survival in patients with NSCLC. However, there was no significant difference found while comparing the rate of DAPK promoter hypermethylation in adenocarcinoma and squamous cell cancer. The rate of DAPK promoter hypermethylation was similar between stage III/IV and stage I/II. In addition, the data showed that DAPK promoter hypermethylation was not associated with smoking behavior in patients with NSCLC.

Conclusion: DAPK promoter hypermethylation is correlated with risk of NSCLC and is a potential biomarker for prediction of poor prognosis in patients with NSCLC.

Keywords: DAPK, NSCLC, biomarker, methylation, adenocarcinoma, squamous cell cancer, drug target

Background

Lung cancer is the second most commonly diagnosed malignancy in men and the third most commonly diagnosed malignancy in women worldwide.[1] Lung cancer can be classified two major histological groups: small cell lung cancer and non-small-cell lung cancer (NSCLC). NSCLC accounts for more than 80% of all lung cancers, whereas 15%–20% is small cell lung cancer.[2,3] NSCLC includes adenocarcinoma (ADC), squamous cell carcinoma (SCC), and large-cell carcinoma, within them, ADC accounts for 40%, SCC for 25%–30%, and large-cell carcinoma for 10%–15%.[4,5] Lung carcinogenesis is related to silencing of tumor suppressor genes and activation of oncogenes. Accumulating data indicate that hypermethylation in CpG-rich promoter regions of many suppressor genes can contribute to the development and progression of a variety of cancers.[6,7]

Deiss and Kimchi discovered a large group of new genes by using "technical knock-out (TKO) and rescue" screen.[8] Those genes function as positive mediators

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of cell death pathways, therefore they were named "Death-Associated Protein or DAP genes.[9] One of the genes isolated by the TKO approach encoded a calcium calmodulin regulated serine/threonine kinase and was named DAP kinase (DAPk1 or DAPK).[10,11] Further investigation indicated that DAPK plays an important role in apoptotic and autophagic cell death,[12–14] tumor progression suppression, and metastasis suppression.[15–19] Last two decades, a number of studies showed that DAPK loss by its promoter hypermethylation was associated with the development and progression of NSCLC. However, the results from individual studies were inconsistent due to small size of samples. In the present study, 28 relevant studies were pooled, and a meta-analysis was performed to evaluate the clinicopathological significance of DAPK promoter hypermethylation in NSCLC.

Methods

Search strategy and selection criteria

The following electronic databases were screened for relevant articles without any language restrictions: PubMed (1966–2018), Embase (1980–2018), Web of Science (1945–2018), Cochrane Library Database (1972–2018). We used the following keywords: "DAPK methylation", "NSCLC", "Non-small-cell lung cancer", and "lung cancer". A search of PubMed yielded 65 articles, Embase yielded 101, and Web Science yielded 138 articles. The included criteria were as follows: (1) the association between DAPK methylation and the clinicopathological significance of NSCLC; (2) the association of DAPK and prognosis in patients with NSCLC. After screening by titles and abstracts, 38 relevant articles were included for full text review. The following exclusion criteria were used: (1) the same population or overlapping database; (2) conference abstracts containing insufficient data reviews, editorials, letters, case reports, and expert opinion; (3) the studies utilized cell lines. After evaluation, 28 articles fulfilled the entry criteria of this meta-analysis. The detailed information of 28 relevant articles was listed in Table 1.

Data extraction and study assessment

Two reviewers (YZ and JW) extracted data from selected studies independently by using a standardized data extraction form including the following items: first author's name, year of publication, country, number of patients, histology, stage of NSCLC, smoking status of patients with NSCLC, method for methylation detection. Any disagreement was discussed and reached a consensus for all issues.

Statistical analysis

ORs with 95% confidence intervals (CIs) were calculated by using a fixed or random effect model depending on heterogeneity (a fixed effect model for I^2<50%, a random effect model for I^2>50%). The analysis was performed to compare DAPK promoter hypermethylation between NSCLC and normal tissue, DAPK promoter hypermethylation in different stage of NSCLC, DAPK promoter hypermethylation in different histology type of NSCLC, as well as in smoker and non-smoker patients with NSCLC. All *P*-values were two sided. *P*-value less than 0.05 was considered statistically significant. Funnel plots were used for detection of publication bias. All analysis was performed with Review Manager 5.2.

Results

In total, 28 studies were included in the present study after screening 304 articles by two reviewers (Figure 1). The following items were collected from each study: first author, published year, country, histology of NSCLC, and DAPK hypermethylation status, smoking status as well as patient prognosis (Table 1).

The total number of NSCLC tumor from 28 studies is 2148, 870 of them were with DAPK promoter hypermethylated, the rate was 40.50%. Whereas the promoter hypermethylation rate from individual study ranged from 10.99% to 83.13% (Table 1). The frequency of DAPK promoter hypermethylation was significantly higher in NSCLC than in non-malignant lung; and the pooled OR was 5.69 with 95% CI 3.44–9.39, Z=6.79, P<0.00001 (Figure 2). DAPK promoter methylation was similar between SCC and ADC; the pooled OR was 1.30 with 95% CI 0.96–1.74, Z=1.71, P=0.09, I^2=0% (Figure 3). In addition, DAPK methylation was not significantly correlated with stages of NSCLC; OR was 0.78 with 95% CI 0.54–1.13, Z=1.29, P=0.20, I^2=0% (Figure 4). The rate of DAPK methylation was not associated with smoking behavior in patients with NSCLC; OR was 1.11 with 95% CI 0.80–1.54, Z=0.62, P=0.53, I^2=18% (Figure 5). However, DAPK promoter hypermethylation was significantly associated with poor prognosis in patients with NSCLC; HR was 1.25 with 95% CI 1.06–1.46, Z=2.68, P=0.007, I^2=0% (Figure 6).

The quality of each study was assessed using the Newcastle Ottawa Quality Assessment Scale (NOQAS). This scale for non-randomized case controlled studies and cohort studies was used to allocate a maximum of nine points for the quality of selection, comparability, exposure, and outcomes for study participants. Of the studies, 15 scored eight points,

Table 1 Main characteristics of included studies

Study	Year	Country	Sample size (M/T)	DAPK methylation rate (%)	Histology			Stage (TNM)		Smoking status		Method
					NCT	AC	SCC	I+II	III+IV	+	–	
Ali et al[28]	2017	India	133/160	83.13	49/70	–	–	–	–	–	–	MSP
Jin et al[29]	2016	China	120/199	60.30	–	55/95	65/104	–	–	94/145	26/54	MSP
Guo et al[30]	2015	China	35/202	17.33	–	15/111	20/91	16/100	5/27	–	–	MSP
Kontic et al[31]	2012	Serbia	11/47	23.40	–	4/18	7/29	10/35	2/18	1/11	11/44	MSP
Fujii et al[32]	2012	Japan	6/46	13.04	0/25	–	–	–	–	–	–	MSP
Zhang et al[33]	2011	China	120/200	61.00	23/200	–	–	–	–	–	–	MSP
Zhang et al[34]	2010	China	11/78	14.10	3/78	–	–	–	–	–	–	MSP
Jin et al[35]	2010	China	88/150	58.67	15/150	–	–	–	–	–	–	MSP
Peng et al[36]	2010	China	48/82	58.54	0/25	–	–	–	–	–	–	MSP
Niklinska et al[37]	2009	Japan	22/61	36.07	–	–	–	–	–	–	–	MSP
Han et al[38]	2009	USA	8/14	57.14	4/20	–	–	–	–	–	–	MSP
Licchesi et al[39]	2008	USA	7/19	36.8	0/46							MSP
Katayama et al[40]	2007	Japan	–		–	–	–	2/8	8/26	–	–	MSP
Yanagawa et al[41]	2007	Japan	26/101	25.74	8/101	14/62	12/39	20/75	6/26	20/73	6/28	MSP
Liu et al[42]	2007	China	40/122	32.79	–	25/72	15/50	17/54	17/54	28/81	12/41	MSP
Belinsky et al[43]	2007	USA	22/72	30.56	5/25	–	–	–	–	–	–	MSP
Fischer et al[44]	2007	Germany	–		–	–	–	–	–	–	–	MSP
Kim et al[45]	2005a	Korea	23/72	31.94	4/72	–	–	–	–	–	–	MSP
de Fraipont et al[46]	2005	France	–		–	–	–	–	–	18/121	1/4	MSP
Safar et al[47]	2005	USA	12/32	37.50	6/32	–	–	16/48	8/57	–	–	MSP
Russo et al[48]	2005	USA	22/49	44.90	1/27	–	–	–	–	–	–	MSP
Kim et al[49]	2005b	Korea	–		–	13/42	7/17	9/34	9/27	–	–	MSP
Fujiwara et al[50]	2005	Japan	10/91	10.99	5/100	–	–	6/60	4/31	9/43	10/38	MSP
Divine et al[51]	2005	USA	72/206	34.95	–	–	–	–	–	16/45	45/125	MSP
Lu et al[19]	2004	USA	–		–	–	–	–	–	–	–	MSP
Toyooka et al[52]	2003	USA	14/38	36.84	1/15	8/20	6/18	–	–	–	–	COBRA
Soria et al[53]	2002	USA	–		–	–	–	–	–	13/89	4/11	MSP
Zöchbauer-Müller et al[54]	2001	Australia	20/107	18.69	6/104	7/45	9/43	17/82	3/25	18/98	2/9	MSP

Abbreviations: ADC, adenocarcinoma; COBRA, combined bisulfite restriction analysis; DAPK, death-associated protein kinase; M, number of NSCLC with methylation; MSP, methylation-specific PCR; NCT, normal control tissue; NSCLC, non-small-cell lung cancer; SCC, squamous cell cancer; T, total number of NSCLC.

11 scored seven points, and two scored six points (data not shown). Hence, the studies were of a relatively high quality. 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 (data not shown). The funnel plots were largely symmetric (Figure 7), suggesting there were no publication biases in the meta-analysis of DAPK promoter hypermethylation and clinicopathological features.

Discussion

Aberrant methylation in tumor suppressor genes has been linked to carcinogenesis. Hypermethylation is the predominant mechanism to make tumor suppressor genes silent by promoter inactivation. DAPK gene is located on chromosome 9q34.1. It encodes a proapoptotic protein involved in apoptosis initiated by THN-α, IFN-γ, Fas, and TRAIL. DAPK promoter methylation has been observed in about 30 types of tumor including NSCLC. Moreover, aggressiveness of malignant tumors has been associated with the methylation of the promoter region of the DAPK gene and loss of DAPK expression.[20] A number of studies evaluated the methylation rate in NSCLC, which ranged from 10.99% to 83.33% due to small size of samples. We pooled 28 studies including 2,148 NSCLC patients, 870 of them were with DAPK gene promoter hypermethylated; hypermethylation rate was 40.50%, 5.69 times higher than the one in non-malignant lung tissue. Therefore, DAPK promoter hypermethylation was correlated with the risk of NSCLC. Previous evidence indicated that the expression of DAPK was partially restored by treatment with a demethylation agent, 5′-aza-2′-deoxycitidine.[15] DAPK could be a potential target for development of new strategy of treatment.

We did not find significant association of DAPK promoter hypermethylation with tumor histology, smoking,

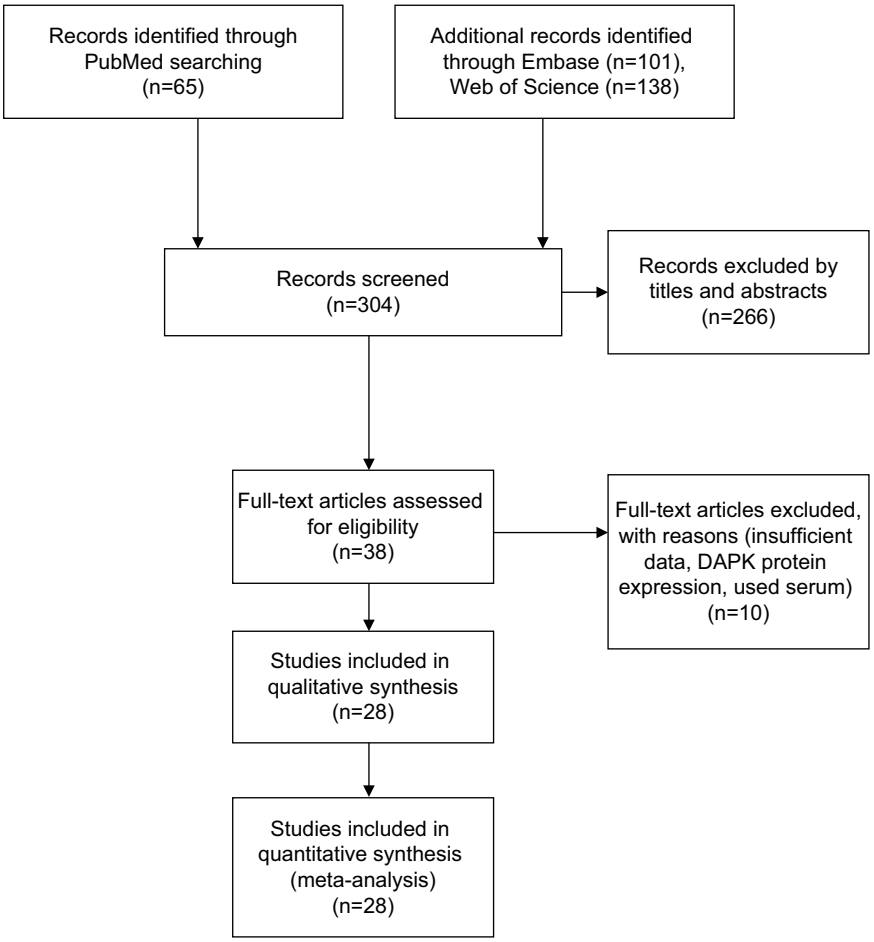

Figure 1 Schematic flow diagram for selection of included studies.

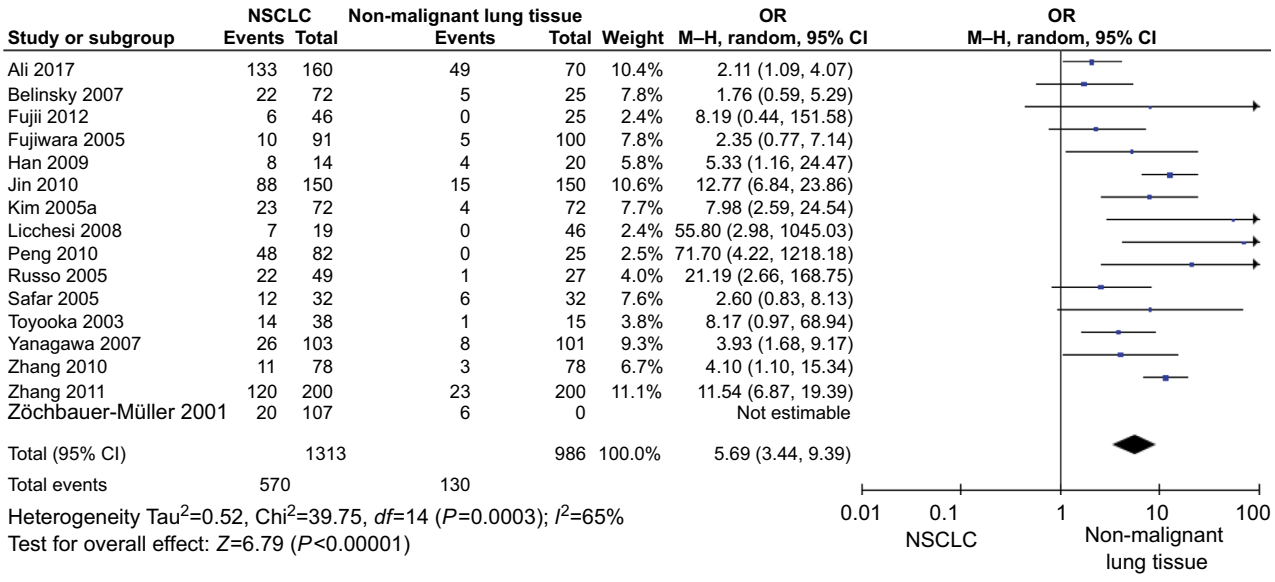

Figure 2 Forest plot for DAPK promoter hypermethylation in NSCLC and non-malignant lung tissue.

Notes: The squares represent the weight of individual study in the meta-analysis, the line width indicates the corresponding 95% CI, the diamond represents the pooled OR, and the width of diamond indicates 95% CI.

Abbreviations: DAPK, death-associated protein kinase; M–H, Mantel–Haenszel; NSCLC, non-small-cell lung cancer.

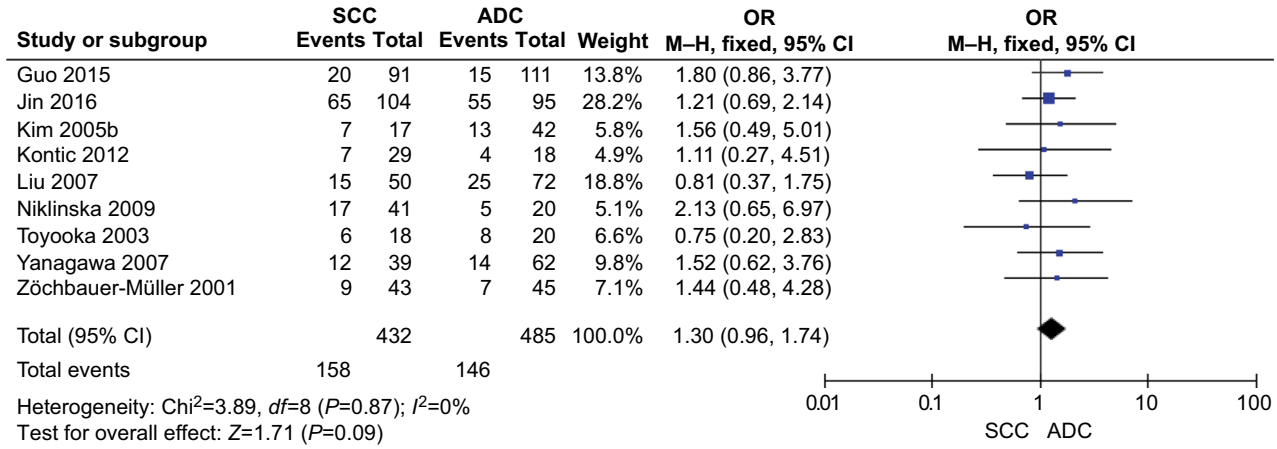

Figure 3 Forest plot for DAPK promoter hypermethylation in SCC and ADC.
Notes: The squares represent the weight of individual study in the meta-analysis, the line width indicates the corresponding 95% CI, the diamond represents the pooled OR, and the width of diamond indicates 95% CI.
Abbreviations: ADC, adenocarcinoma; DAPK, death-associated protein kinase; M–H, Mantel–Haenszel; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma.

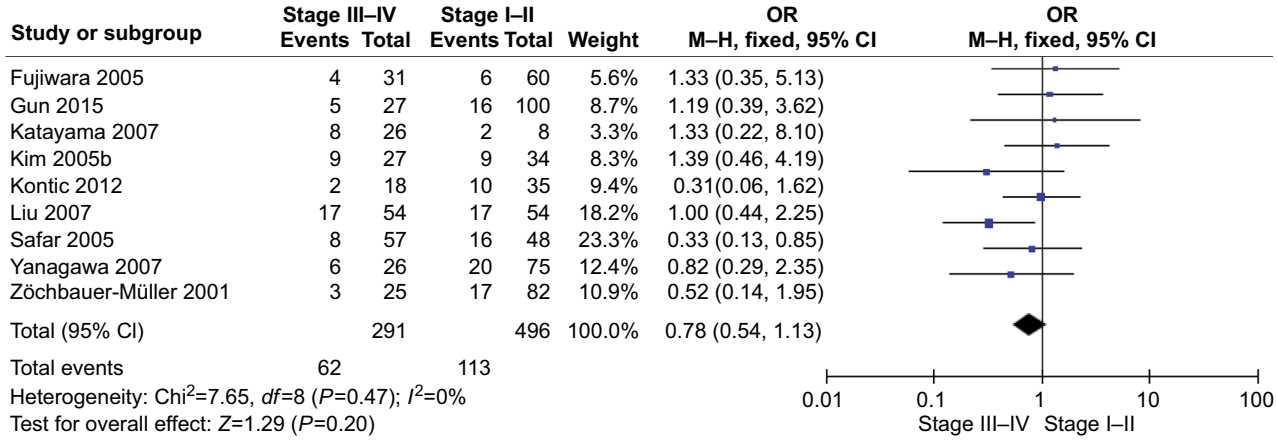

Figure 4 Forest plot for DAPK promoter hypermethylation in NSCLC stage III/IV and stage I/II.
Notes: The squares represent the weight of individual study in the meta-analysis, the line width indicates the corresponding 95% CI, the diamond represents the pooled OR, and the width of diamond indicates 95% CI.
Abbreviations: DAPK, death-associated protein kinase; M–H, Mantel–Haenszel; NSCLC, non-small-cell lung cancer.

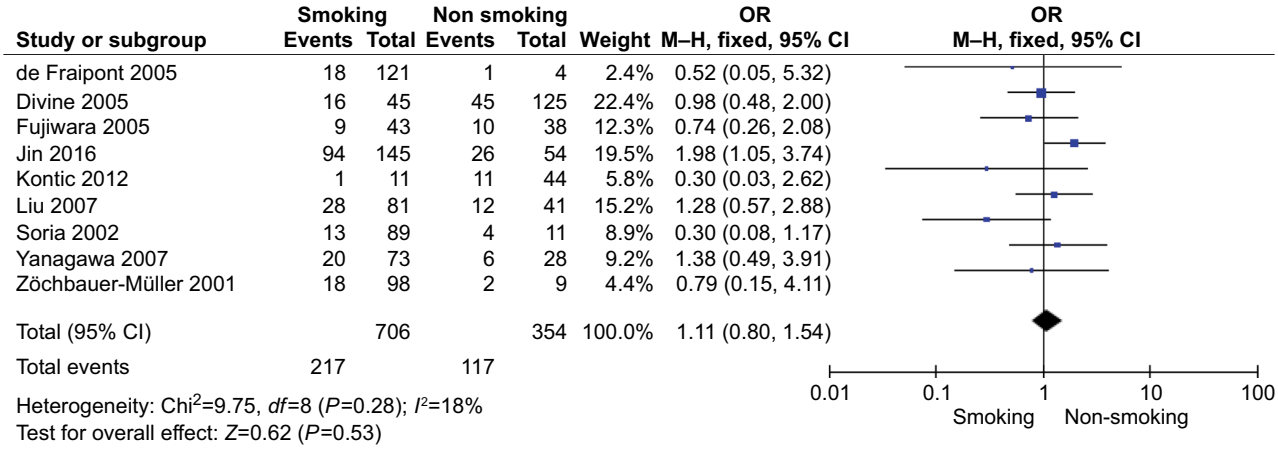

Figure 5 Forest plot for DAPK promoter hypermethylation in NSCLC patients with smoking and non-smoking behavior.
Notes: The squares represent the weight of individual study in the meta-analysis, the line width indicates the corresponding 95% CI, the diamond represents the pooled OR, and the width of diamond indicates 95% CI.
Abbreviations: M–H, Mantel–Haenszel; NSCLC, non-small-cell lung cancer.

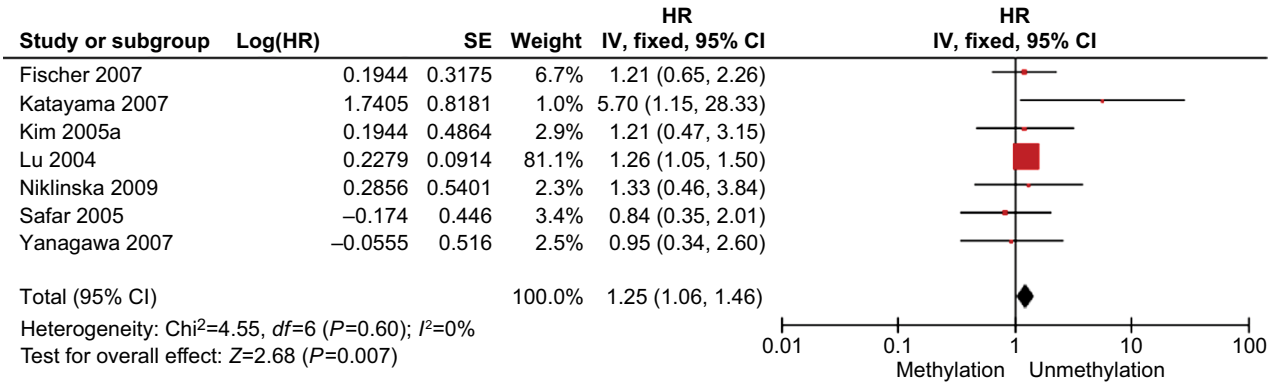

Figure 6 Forest plot for the association of DAPK promoter hypermethylation and the overall survival of NSCLC patients.
Notes: The squares represent the weight of individual study in the meta-analysis, the line width indicates the corresponding 95% CI, the diamond represents the pooled OR, and the width of diamond indicates 95% CI.
Abbreviations: DAPK, death-associated protein kinase; NSCLC, non-small-cell lung cancer; SE, standard error.

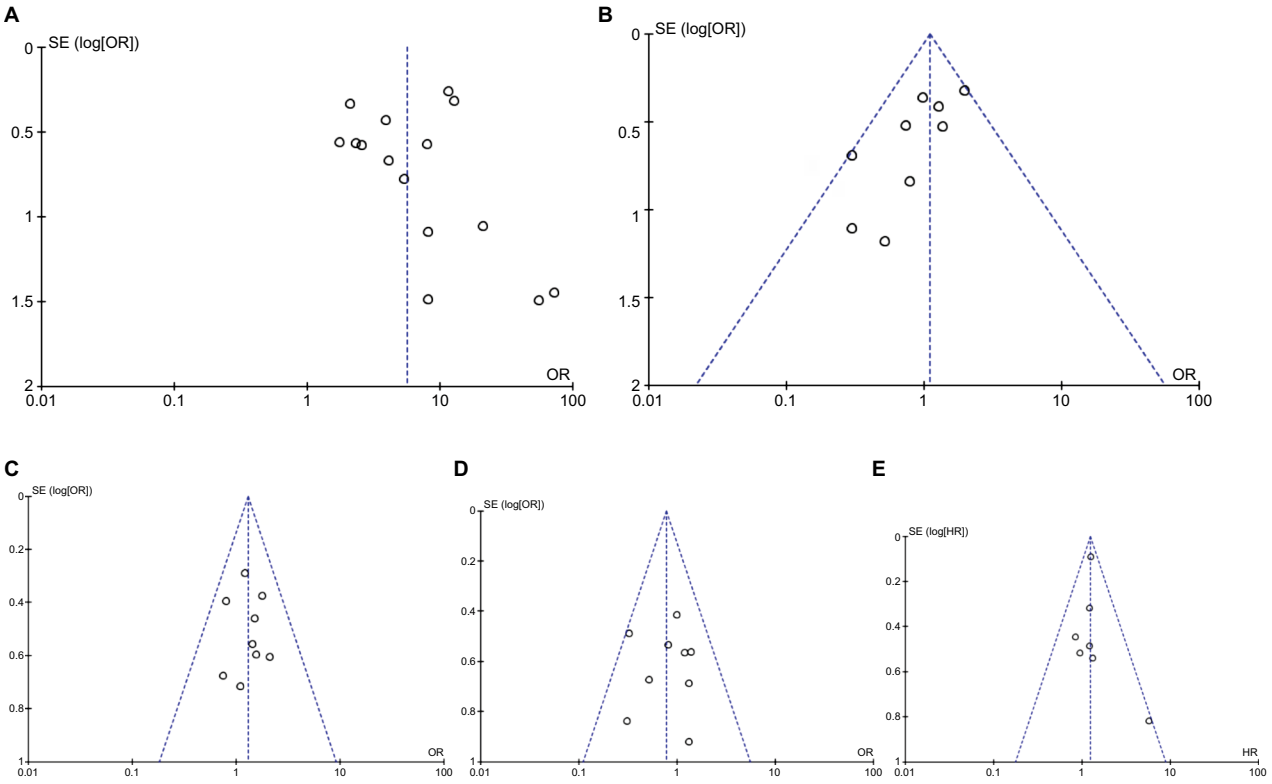

Figure 7 Funnel plot for publication bias.
Notes: (**A**) DAPK promoter hypermethylation in NSCLC and non-malignant lung tissue; (**B**) DAPK promoter hypermethylation in SCC and ADC; (**C**) DAPK promoter hypermethylation in NSCLC stage III/IV and stage I/II. (**D**) DAPK promoter hypermethylation in NSCLC patients with smoking and non-smoking behavior; (**E**) the association of DAPK promoter hypermethylation and the overall survival of NSCLC patients. Area of the circle represents the weight of individual study.
Abbreviations: ADC, adenocarcinoma; DAPK, death-associated protein kinase; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma; SE, standard error.

and stage. Although TNM staging system still remained the most powerful tool for medical decision making, it is difficult to accurately predict the prognosis for individual patient. The 5-year survival rate for patients with stage I NSCLC is about 65%–80%,[21] therefore a more accurate tool, independent from TNM stage, is very important to predict prognosis in those patients. Our finding indicated that DAPK was correlated to worse survival in our meta-analysis, supporting the importance of epigenetic gene regulation in NSCLC progression and prognosis. Loss of apoptotic functions would compromise cell death induced by unrepaired DNA damage.[22] In addition, DAPK

promoter hypermethylation is associated with metastatic status. Taken together, DAPK promoter hypermethylation leads to worse prognosis in patients with NSCLC. DAPK hypermethylation is a potential predictor of survival in patients with NSCLC.

Given the important role of smoking in the development of lung cancer and the fact that DNA methylation is an early event in carcinogenesis,[23] several biomarker such as Wnt inhibitory factor-1 (Wif1), Phosphatase and tensin homologue deleted on chromosome 10 (PTEN), and TP53 were associated with smoking behavior.[24–27] However, no correlation was found between DAPK promoter hypermethylation and the smoking behavior in the present study. Further confirmation needs to be finished in future when more relative studies are available.

Our findings should be interpreted in view of certain limitations. First, most of the included studies were retrospective, 26 out of 28 were of sufficient quality (NOQAS ≥ 7). Hence, the studies were of a relatively high quality. Although the possibility of selection, sample, and publication bias could not be excluded, no obvious bias was detected by the funnel plots. Second, present findings were based on individual unadjusted ORs and further confirmation needs to be finished by evaluation adjusted with other potential risk factors.

Conclusion

In summary, present findings suggested that DAPK promoter hypermethylation was correlated with the risk of NSCLC; and DAPK is a promising drug target for development of new therapy strategy. Additionally, DAPK promoter hypermethylation was a potential predictor of poor prognosis in patients with NSCLC.

Data sharing statement

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work. The corresponding author had full access to all data and the final responsibility for the decision to submit the article for publication. All authors read and approved the final manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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