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

Yan Zhang 1,*
Jiang Wu 1,*
Gui Huang 1
Shouming Xu 2

1 Department of Pathology, Huaihe Hospital, Henan University; 2 School of Life Sciences, Henan University, Kaifeng 475004, People’s Republic of China

* These authors contributed equally to this work

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 into 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...
of cell death pathways, therefore they were named “Death-Associated Protein or DAP genes.” 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). Further investigation indicated that DAPK plays an important role in apoptotic and autophagic cell death, tumor progression suppression, and metastasis suppression. 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,
α encodes a proapoptotic protein involved in apoptosis initiated by inactivation. DAPK gene is located on chromosome 9q34.1. It has been associated with the methylation of the promoter region linked to carcinogenesis. Hypermethylation is the predominant mechanism to make tumor suppressor genes silent by promoter methylation 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. 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. Moreover, aggressiveness of malignant tumors has been associated with the methylation of the promoter region of the DAPK gene and loss of DAPK expression. 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. Moreover, aggressiveness of malignant tumors has been associated with the methylation of the promoter region of the DAPK gene and loss of DAPK expression.

### Table 1 Main characteristics of included studies

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

| 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. |
Records identified through PubMed searching (n=65)

Additional records identified through Embase (n=101), Web of Science (n=138)

Records screened (n=304)

Records excluded by titles and abstracts (n=266)

Full-text articles assessed for eligibility (n=38)

Full-text articles excluded, with reasons (insufficient data, DAPK protein expression, used serum) (n=10)

Studies included in qualitative synthesis (n=28)

Studies included in quantitative synthesis (meta-analysis) (n=28)

Figure 1 Schematic flow diagram for selection of included studies.

| Study or subgroup | NSCLC | Non-malignant lung tissue | OR | OR |
|-------------------|-------|---------------------------|----|----|
|                   | Events| Total | Events | Total | Weight | M–H, random, 95% CI | M–H, random, 95% CI |
| Ali 2017          | 133   | 160  | 49     | 70    | 10.4%  | 2.11 (1.09, 4.07)    |                |
| Belinsky 2007     | 22    | 72   | 5      | 25    | 7.8%   | 1.76 (0.59, 5.29)    |                |
| Fujii 2012        | 6     | 46   | 0      | 25    | 2.4%   | 8.19 (0.44, 151.58)  |                |
| Fujiiwara 2005    | 10    | 91   | 5      | 100   | 7.8%   | 2.35 (0.77, 7.14)    |                |
| Han 2009          | 8     | 14   | 4      | 20    | 5.8%   | 5.33 (1.16, 24.47)   |                |
| Jin 2010          | 88    | 150  | 15     | 150   | 10.6%  | 12.77 (6.84, 23.86)  |                |
| Kim 2005a         | 23    | 72   | 4      | 72    | 7.7%   | 7.98 (2.59, 24.54)   |                |
| Lichesl 2008      | 7     | 19   | 0      | 46    | 2.4%   | 55.80 (2.98, 1045.03)|                |
| Peng 2010         | 48    | 82   | 0      | 25    | 2.5%   | 71.70 (4.22, 1218.18)|                |
| Russo 2005        | 22    | 49   | 1      | 27    | 4.0%   | 21.19 (2.66, 168.75) |                |
| Safar 2005        | 12    | 32   | 6      | 32    | 7.6%   | 2.60 (0.83, 8.13)    |                |
| Toyooka 2003      | 14    | 38   | 1      | 15    | 3.8%   | 8.17 (0.97, 68.94)   |                |
| Yanagawa 2007     | 26    | 103  | 8      | 101   | 9.3%   | 3.93 (1.68, 9.17)    |                |
| Zhang 2010        | 11    | 78   | 3      | 78    | 6.7%   | 4.10 (1.10, 15.34)   |                |
| Zhang 2011        | 120   | 200  | 23     | 200   | 11.1%  | 11.54 (6.87, 19.39)  |                |
| Zöchbauer-Müller 2001 | 20 | 107 | 6      | 0     | Not estimable |                |                |
| Total (95% CI)    | 1313  |      | 986    |      | 100.0% | 5.69 (3.44, 9.39)    |                |
| Total events      | 570   |      | 130    |      |        |                 |                |

Heterogeneity: Tau^2=0.52, Chi^2=39.75, df=14 (P=0.0003); I^2=65%
Test for overall effect: Z=6.79 (P<0.00001)

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.
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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.

| Study or subgroup | Stage III–IV Events Total | Stage I–II Events Total | Weight | OR M–H, fixed, 95% CI | OR M–H, fixed, 95% CI |
|-------------------|---------------------------|-------------------------|--------|-----------------------|-----------------------|
| Guo 2015          | 20 91                     | 15 111                  | 13.8%  | 1.80 (0.86, 3.77)     | 1.80 (0.86, 3.77)     |
| Jin 2016          | 65 104                    | 55 95                   | 28.2%  | 1.21 (0.69, 2.14)     | 1.21 (0.69, 2.14)     |
| Kim 2005b         | 7 17                      | 13 42                   | 5.8%   | 1.56 (0.49, 5.01)     | 1.56 (0.49, 5.01)     |
| Kontic 2012       | 7 29                      | 4 18                    | 4.9%   | 1.11 (0.27, 4.51)     | 1.11 (0.27, 4.51)     |
| Liu 2007          | 15 50                     | 25 72                   | 18.8%  | 0.81 (0.37, 1.75)     | 0.81 (0.37, 1.75)     |
| Nikitinska 2009   | 17 41                     | 5 20                    | 5.1%   | 2.13 (0.65, 6.97)     | 2.13 (0.65, 6.97)     |
| Toyooka 2003      | 6 18                      | 8 20                    | 6.6%   | 0.75 (0.20, 2.83)     | 0.75 (0.20, 2.83)     |
| Yanagawa 2007     | 12 39                     | 14 62                   | 9.8%   | 1.52 (0.62, 3.76)     | 1.52 (0.62, 3.76)     |
| Zöchbauer-Müller 2001 | 9 43                  | 7 45                    | 7.1%   | 1.44 (0.48, 4.28)     | 1.44 (0.48, 4.28)     |
| Total (95% CI)    | 291                       | 496                     | 100.0% | 1.30 (0.96, 1.74)     | 1.30 (0.96, 1.74)     |

Total events: 158

Heterogeneity: Chi²=3.89, df=8 (P=0.87); I²=0%

Test for overall effect: Z=1.71 (P=0.09)

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.

| Study or subgroup | Smoking Events Total | Non smoking Events Total | Weight | OR M–H, fixed, 95% CI | OR M–H, fixed, 95% CI |
|-------------------|----------------------|--------------------------|--------|-----------------------|-----------------------|
| de Fraipont 2005  | 18 121               | 1 4                      | 2.4%   | 0.52 (0.05, 5.32)     | 0.52 (0.05, 5.32)     |
| Divine 2005      | 16 45                 | 45 125                   | 22.4%  | 0.98 (0.48, 2.00)     | 0.98 (0.48, 2.00)     |
| Fujiwara 2005    | 9 43                  | 10 38                    | 12.3%  | 0.74 (0.26, 2.08)     | 0.74 (0.26, 2.08)     |
| Jin 2016         | 94 145                | 26 54                    | 19.5%  | 1.98 (1.05, 3.74)     | 1.98 (1.05, 3.74)     |
| Kontic 2012      | 1 11                  | 11 44                    | 5.8%   | 0.30 (0.03, 2.62)     | 0.30 (0.03, 2.62)     |
| Liu 2007         | 28 81                 | 12 41                    | 15.2%  | 1.28 (0.57, 2.88)     | 1.28 (0.57, 2.88)     |
| Soria 2002       | 13 89                 | 4 11                     | 8.9%   | 0.30 (0.08, 1.17)     | 0.30 (0.08, 1.17)     |
| Yanagawa 2007    | 20 73                 | 6 28                     | 9.2%   | 1.38 (0.49, 3.91)     | 1.38 (0.49, 3.91)     |
| Zöchbauer-Müller 2001 | 18 98              | 2 9                      | 4.4%   | 0.79 (0.15, 4.11)     | 0.79 (0.15, 4.11)     |
| Total (95% CI)   | 706                   | 354                      | 100.0% | 1.11 (0.80, 1.54)     | 1.11 (0.80, 1.54)     |

Total events: 217

Heterogeneity: Chi²=9.75, df=8 (P=0.47); I²=0%

Test for overall effect: Z=1.29 (P=0.20)

Figure 6
Forest plot for DAPK promoter hypermethylation in NSCLC 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.

| Study or subgroup | SCC Events Total | ADC Events Total | Weight | OR M–H, fixed, 95% CI | OR M–H, fixed, 95% CI |
|-------------------|------------------|-----------------|--------|-----------------------|-----------------------|
| Guo 2015          | 20 91            | 15 111          | 13.8%  | 1.80 (0.86, 3.77)     | 1.80 (0.86, 3.77)     |
| Jin 2016          | 65 104           | 55 95           | 28.2%  | 1.21 (0.69, 2.14)     | 1.21 (0.69, 2.14)     |
| Kim 2005b         | 7 17             | 13 42           | 5.8%   | 1.56 (0.49, 5.01)     | 1.56 (0.49, 5.01)     |
| Kontic 2012       | 7 29             | 4 18            | 4.9%   | 1.11 (0.27, 4.51)     | 1.11 (0.27, 4.51)     |
| Liu 2007          | 15 50            | 25 72           | 18.8%  | 0.81 (0.37, 1.75)     | 0.81 (0.37, 1.75)     |
| Nikitinska 2009   | 17 41            | 5 20            | 5.1%   | 2.13 (0.65, 6.97)     | 2.13 (0.65, 6.97)     |
| Toyooka 2003      | 6 18             | 8 20            | 6.6%   | 0.75 (0.20, 2.83)     | 0.75 (0.20, 2.83)     |
| Yanagawa 2007     | 12 39            | 14 62           | 9.8%   | 1.52 (0.62, 3.76)     | 1.52 (0.62, 3.76)     |
| Zöchbauer-Müller 2001 | 9 43         | 7 45            | 7.1%   | 1.44 (0.48, 4.28)     | 1.44 (0.48, 4.28)     |
| Total (95% CI)    | 432              | 485             | 100.0% | 1.30 (0.96, 1.74)     | 1.30 (0.96, 1.74)     |

Total events: 158

Heterogeneity: Chi²=3.89, df=8 (P=0.87); I²=0%

Test for overall effect: Z=1.29 (P=0.20)
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%, 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. 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, 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. 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.

References
1. Global Burden of Disease Cancer Collaboration; Fitzmaurice C, Allen C, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. JAMA Oncol. 2017;3(4):524–548.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7–30.
3. Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med. 2008;359(13):1367–1380.
4. Reck M, Popat S, Reimnuth N, et al. Metastatic non-small-cell lung cancer (NSCLC): ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2014;25 Suppl 3:iii27–iii39.
5. Thakur MK, Wozniak AJ. Spotlight on necitumumab in the treatment of non-small-cell lung carcinoma. Lung Cancer. 2017;8:13–19.
6. Baylin SB, Esteller M, Rountree MR, Bachman KE, Schuebel K, Herman JG. Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer. Hum Mol Genet. 2001;10(7):687–692.
7. Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP. Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res. 1998;72:141–196.
8. Deiss LP, Kimchi A. A genetic tool used to identify thioredoxin as a mediator of a growth inhibitory signal. Science. 1991;252(5002):117–120.
9. Kimchi A. DAP genes: novel apoptotic genes isolated by a functional approach to gene cloning. Biochim Biophys Acta. 1998;1377(2):F13–F33.
10. Deiss LP, Feinstein E, Berissi H, Cohen O, Kimchi A. Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the gamma interferon-induced cell death. Genes Dev. 1995;9(1):15–30.
11. Gozuacik D, Kimchi A. DAPK protein family and cancer. Autophagy. 2006;2(2):74–79.
12. Kögel D, Reimert C, Düssmann H, Mech P, Scheidtmann KH, Prehn JH. The death associated protein (DAP) kinase homologue Dlk/ZIP kinase induces p19ARF and p53-independent apoptosis. Eur J Cancer. 2003;39(2):249–256.
13. Inbal B, Bialik S, Sabanay I, Shani G, Kimchi A. DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. J Cell Biol. 2002;157(3):455–468.
14. Pattingre S, Tassa A, Xu Q, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell. 2005;122(6):927–939.
15. Kissil JL, Feinstein E, Cohen O, et al. DAP-kinase loss of expression is a new marker for breast cancer prognosis. Clin Cancer Res. 2004;10(9):3124–3130.
16. Lehmann U, Celikkaya G, Hasenheimer B, Länger F, Kreipe H. Promoter hypermethylation of the death-associated protein kinase gene in breast cancer is associated with the invasive lobular subtype. Cancer Res. 2002;62(22):6634–6638.
17. Lévy D, Plu-Bureau G, Decroix Y, et al. Death-associated protein kinase loss of expression is a new marker for breast cancer prognosis. Clin Cancer Res. 2004;10(9):3124–3130.
18. Kim DH, Nelson HH, Wienecke JK, et al. Promoter methylation of DAP-kinase: association with advanced stage in non-small cell lung cancer. Oncogene. 2001;20(14):1765–1770.
19. Lu C, Soria JC, Tang X, et al. Prognostic factors in resected stage I non-small-cell lung cancer: a multivariate analysis of six molecular markers. J Clin Oncol. 2004;22(22):4575–4583.
20. Tang X, Khuri FR, Lee JJ, et al. Hypermethylation of the death-associated protein (DAP) kinase promoter and aggressiveness in stage I non-small-cell lung cancer. J Natl Cancer Inst. 2000;92(18):1511–1516.
21. Bains MS. Surgical treatment of lung cancer. Chest. 1991;100(3):826–837.
22. Buckingham L, Penfield Faber L, Kim A, et al. PTEN, RASSF1 and DAPK site-specific hypermethylation and outcome in surgically treated stage I and II non-small cell lung cancer patients. Int J Cancer. 2010;126(7):1630–1639.
23. Feng Q, Hawes SE, Stern JE, et al. DNA methylation in tumor and matched normal tissues from non-small cell lung cancer patients. *Cancer Epidemiol Biomarkers Prev.* 2008;17(3):645–654.

24. Guo H, Zou S, Tan L, Wu X, Wu Z, Ran R. Clinicopathological significance of WIF1 hypermethylation in NSCLC, a meta-analysis and literature review. *Oncotarget.* 2017;8(2):2550–2557.

25. Jin G, Kim MJ, Jeon HS, et al. PTEN mutations and relationship to EGRF, ERBB2, KRAS, and TP53 mutations in non-small cell lung cancers. *Lung Cancer.* 2010;69(3):279–283.

26. Tammenmagi MC, McLaughlin JR, Bull SB. Meta-analyses of p53 tumor suppressor gene alterations and clinicopathological features in resected lung cancers. *Cancer Epidemiol Biomarkers Prev.* 1999;8(7):625–634.

27. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of diffuse large B-cell lymphoma. *Science.* 2012;337(6097):889–900.

28. Ali A, Kumar S, Kakaria VK, et al. Detection of promoter DNA methylation of APC, DAPK, and GSTP1 genes in tissue biopsy and matched serum of advanced-stage lung cancer patients. *Cancer Invest.* 2017;35(6):423–430.

29. Jin Y, Xu P, Liu X, et al. Cigarette smoking, BPDE-DNA adducts, and aberrant promoter methylation of tumor suppressor genes (TSGs) in NSCLC from Chinese population. *Cancer Invest.* 2016;34(4):173–180.

30. Guo M, Alumkal J, Drachova T, et al. CHFR methylation strongly correlates with methylation of DNA damage repair and apoptotic pathway genes in non-small cell lung cancer. *Disco Med.* 2015;19(104):151–158.

31. Kontic M, Stojisic J, Jovanovic D, et al. Aberrant promoter methylation of CDH13 and MGMT genes is associated with clinicopathological characteristics of primary non-small-cell lung carcinoma. *Clin Lung Cancer.* 2012;13(4):297–303.

32. Fuji M, Fujimoto N, Hiraki A, et al. Aberrant DNA methylation analysis in pleural fluid for differential diagnosis of malignant pleural mesothelioma. *Cancer Sci.* 2012;103(3):510–514.

33. Zhang CY, Jin YT, Xu HY, et al. Relationship between promoter methylation of p16, DAPK and RAR beta genes and the clinical data of non-small cell lung cancer. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi.* 2011;28(1):23–28.

34. Zhang Y, Wang R, Song H, et al. Methylation of multiple genes as a candidate biomarker in non-small cell lung cancer. *Cancer Lett.* 2011;303(1):21–28.

35. Jin Y, Xu H, Zhang C, et al. Combined effects of cigarette smoking, gene polymorphisms and methylation of tumor suppressor genes on non small cell lung cancer: a hospital-based case-control study in China. *BMC Cancer.* 2010;10:422.

36. Peng Z, Shan C, Wang H. Value of promoter methylation of RASSF1A, p16, and DAPK genes in induced sputum diagnosis in patients with lung cancer. *Zhong Nan Da Xue Xue Bao Yi Xue Ban.* 2010;35(3):247–253.

37. Niklinska W, Naumnik W, Sulewska A, Kozłowski M, Pankiewicz W, Milewski R. Prognostic significance of DAPK and RASSF1A promoter methylation in non-small cell lung cancer (NSCLC). *Folia Histochim Cytobiol.* 2009;47(2):275–280.

38. Han W, Wang T, Reilly AA, Keller SM, Spivack SD. Gene promoter methylation assessed by exhaled breath, with differences in smokers and lung cancer patients. *Respir Physiol Neurobiol.* 2009;168(3):233–238.

39. Licchesi JD, Westra WH, Hooker CM, Herman JG. Promoter hypermethylation of hallmark cancer genes in atypical adenomatous hyperplasia of the lung. *Clin Cancer Res.* 2008;14(9):2570–2578.

40. Katayama H, Hiraki A, Fujiwara K, et al. Aberrant promoter methylation profile in pleural fluid DNA and clinicopathological factors in patients with non-small cell lung cancer. *Asian Pac J Cancer Prev.* 2007;8(2):221–224.

41. Yanagawa N, Tamura G, Oizumi H, et al. Promoter hypermethylation of RASSF1A and RUNX3 genes as an independent prognostic prediction marker in surgically resected non-small cell lung cancers. *Lung Cancer.* 2007;58(1):131–138.

42. Liu Y, Gao W, Siegfried JM, Weissfeld JL, Luecketh JD, Keohavong P. Promoter methylation of RASSF1A and DAPK and mutations of K-ras, p53, and EGFR in lung tumors from smokers and never-smokers. *BMC Cancer.* 2007;7:74.

43. Belinsky SA, Grimes MJ, Casas E, et al. Predicting gene promoter methylation in non-small-cell lung cancer by evaluating sputum and serum. *Br J Cancer.* 2007;96(8):1278–1283.

44. Fischer JR, Ohmacht U, Rieger N, et al. Prognostic significance of RASSF1A promoter methylation on survival of non-small cell lung cancer patients treated with gemcitabine. *Lung Cancer.* 2007;56(1):115–123.

45. Kim YT, Park SJ, Lee SH, et al. Prognostic implication of aberrant promoter hypermethylation of Cpg islands in adenocarcinoma of the lung. *J Thorac Cardiovasc Surg.* 2005;130(5):1378–1378.

46. de Fraipont F, Moro-Sibilot D, Michelland S, Brambilla E, Brambilla C, Fayot MC. Promoter methylation of genes in bronchial lavages: a marker for early diagnosis of primary and relapsing non-small cell lung cancer? *Lung Cancer.* 2005;50(2):199–209.

47. Safar AM, Spencer H, Su X, et al. Methylation profiling of archived non-small cell lung cancer: a promising prognostic system. *Clin Cancer Res.* 2005;11(12):4400–4405.

48. Russo AL, Thiagalingam A, Pan H, et al. Differential DNA hypermethylation of critical genes mediates the stage-specific tobacco smoke-induced neoplastic progression of lung cancer. *Clin Cancer Res.* 2005;11(7):2466–2470.

49. Kim YT, Lee SH, Sung SW, Kim JH. Can aberrant promoter hypermethylation of Cpg islands predict the clinical outcome of non-small cell lung cancer after curative resection? *Ann Thorac Surg.* 2005;79(4):1180–1188; discussion 1180–1188.

50. Fujiwara K, Fujimoto N, Tabata M, et al. Identification of epigenetic aberrant promoter methylation in serum DNA is useful for early detection of lung cancer. *Clin Cancer Res.* 2005;11(3):1219–1225.

51. Divine KK, Pulling LC, Marron-Tenada PG, et al. Multiplicity of abnormal promoter methylation in lung adenocarcinomas from smokers and never smokers. *Int J Cancer.* 2005;114(3):400–405.

52. Toyooka S, Toyooka KO, Miyajima K, et al. Epigenetic down-regulation of WIF1 gene expression in lung adenocarcinoma. *Clin Cancer Res.* 2009;15(8):2506–2515.

53. Yano K, Kato R, Inazu Y, et al. Epigenetic down-regulation of the p16 gene in NSCLC. *Jpn J Cancer Res.* 2009;99(1):15–20.

54. Tooyooka S, Toyooka KO, Miyajima K, et al. Epigenetic down-regulation of WIF1 gene expression in lung adenocarcinoma. *Clin Cancer Res.* 2009;15(8):2506–2515.

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