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Clinical significance of DAPK promoter hypermethylation in lung cancer: a meta-analysis

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Abstract: Death-associated protein kinase 1 (DAPK) is an important serine/threonine kinase involved in various cellular processes, including apoptosis, autophagy, and inflammation. DAPK expression and activity are deregulated in a variety of diseases including cancer. Methylation of the *DAPK* gene is common in many types of cancer and can lead to loss of DAPK expression. However, the association between *DAPK* promoter hypermethylation and the clinicopathological significance of lung cancer remains unclear. In this study, we searched the MEDLINE, PubMed, Web of Science, and Scopus databases, systematically investigated the studies of *DAPK* promoter hypermethylation in lung cancer and quantified the association between *DAPK* promoter hypermethylation and its clinicopathological significance by meta-analysis. We observed that the frequency of *DAPK* methylation was significantly higher in lung cancer than in non-malignant lung tissues (odds ratio 6.02, 95% confidence interval 3.17–11.42, $P<0.00001$). The pooled results also showed the presence of a prognostic impact of *DAPK* gene methylation in lung cancer patients (odds ratio 3.63, 95% confidence interval 1.09–12.06, $P=0.04$). In addition, we summarized these findings and discuss tumor suppressor function, clinicopathological significance, and potential drug targeting of DAPK in lung cancer.

Keywords: lung, adenocarcinoma, squamous cell carcinoma, non-small cell lung cancer, death-associated protein kinase gene, *DAPK*, methylation, meta-analysis

Introduction

Lung cancer remains the leading cause of cancer-related death among men and women worldwide.[1] It is estimated that 226,160 people in the USA have been diagnosed with lung cancer, and deaths for the year 2012 amounted to 160,340 people.[2] In spite of advances in diagnosis and treatment, the prognosis is still poor. The unsatisfactory outcome of lung cancer may be attributed to late diagnosis of the disease and inefficient therapy for advanced tumors. With the discovery of screening markers specific for lung cancer, it may be possible to achieve earlier diagnosis and develop more informed choices for treatment.

The two major forms of lung cancer are non-small cell lung cancer (NSCLC), which accounts for about 85% of lung cancer, and small cell lung cancer, which accounts for about 15%. NSCLC can be subdivided into adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma.[3] Like genetic mutations in cancer, epigenetic modification is frequently observed in human cancer. The aberrant mechanisms include increased methylation of DNA, deacetylation of core histone proteins, and RNA interference.[4] Hypermethylation in the promoter areas of tumor suppressor genes has been well established as a mechanism of transcriptional silencing in tumors, whereas tumors are also characterized by genome-wide hypomethylation.[5] Death-associated protein kinase (DAPK) is a serine/threonine kinase involved in a variety of cellular processes,

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including apoptosis initiated by interferon (IFN)-γ, tumor necrosis factor (TNF)-α and Fas ligand,[6,7] autophagy, and inflammation.[8] DAPK function is deregulated in a variety of diseases including cancer, neuronal death, and stroke.[8] As with many other tumor suppressor genes, the expression and function of *DAPK* is disrupted in various cancers. However, unlike genes such as p53,[9] DAPK is commonly inactivated in cancer as a result of hypermethylation at the promoter of the *DAPK* gene rather than mutation;[4,10] less frequently, loss of DAPK expression can also be due to homozygous deletion.[11,12]

Although methylation of the *DAPK* gene is found in various tumors, there is a big difference in frequency of methylation. It is logical to assume dysfunction of *DAPK* may be more crucial for the tumorigenesis of lung cancer because of the high frequency of methylation (on average 40.5%[13–24]) and prognostic effects.[18] The aim of this study was to review the available publications to summarize the data by meta-analysis and characterize the clinical significance of *DAPK* gene promoter methylation in tumorigenesis of the lung.

Methods

Search strategy

MEDLINE, PubMed, Web of Science, Scopus, and Embase were searched in October 2014 using the search terms: "death-associated protein kinase", "*DAPK*", "methylation", "lung cancer", "adenocarcinoma", and "squamous carcinoma". Research papers identified through the search approach as described above were screened by titles first, then by the abstracts of the publications. After exclusion of non-relevant publications and identification of duplicates from the 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 clinical studies except case reports were chosen, for instance, randomized controlled trials, cohort studies, case-control studies, and case series. The language of publication was restricted to English and Chinese. 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 multiple publications.

Selection criteria

We collected all eligible articles about the relationship between *DAPK* methylation and/or expression and the clinicopathological features and clinical outcomes in lung cancer patients in this meta-analysis. Studies meeting the following inclusion criteria were included: *DAPK* methylation and/or expression evaluated in lung tissues and other resources such as sputum, bronchial brush washing, and blood; research that revealed the relationship between *DAPK* methylation and/or expression and the clinicopathological parameters and prognosis of lung cancer; DAPK methylation and/or expression examined by methylation-specific polymerase chain reaction; articles published as full papers in English and Chinese; articles which provided sufficient information to estimate the hazard ratio for overall survival and 95% confidence interval (CI) and probabilities for overall survival where applicable. Exclusion criteria included the following: letters, reviews, case reports, conference abstracts, editorials, expert opinion, and non-English, non-Chinese language papers; articles having no information on overall survival or those for which the hazard ratio for overall survival could not be calculated from the given information; and all publications regarding in vitro/ex vivo studies, cell lines, and human xenografts.

Data extraction

The data extraction followed the procedure as described by the published literature.[25] Two investigators independently extracted data from eligible studies. Disagreements were resolved by discussion and consensus. Two investigators reviewed all of the articles that met the 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, stage, *DAPK* methylation and/or expression, and patient survival. The study characteristics and clinical responses were summarized and turned into table format. Heterogeneity of investigation was evaluated to determine whether the data from the various studies could be analyzed in a meta-analysis.

Statistical analysis

The analysis was conducted using Stata version 12.0 (Stata Corp, College Station, TX, USA) and Review Manager version 5.2 (Cochrane Collaboration, Oxford, UK). Comparisons of dichotomous measures were done by pooled estimates of odds ratios (ORs) and their 95% CIs. A P-value <0.05 was considered to be statistically significant. Heterogeneity was examined by a Chi-square test with significance set at $P<0.10$; the total variation among the studies was estimated by I^2. We used I^2 statistic to assess heterogeneity. The I^2 value is an estimate of the amount of variance due to

between-study heterogeneity rather than chance (Cochran statistics). Substantial heterogeneity exists when I^2 exceeds 50%. If there was heterogeneity among studies, we used a random effect model to pool the ORs; otherwise, a fixed effect model was selected.

The database search generated 47 articles from MEDLINE, PubMed, Web of Science, Scopus, and Embase. After initial screening of all titles, abstracts, and eligibility, 18 full-text studies were selected for more detailed assessment. The search of the article references did not produce additional publications. Eventually, 18 publications met the inclusion criteria for qualitative study and meta-analysis. The article search and study selection is shown in Figure 1.

Results

Identification of relevant studies

Forty-seven publications were identified by the search method as described above. Twenty-eight of those were excluded for being laboratory studies, non-original articles (review), lacking of matched controls, or studies irrelevant to the current analysis. Eventually, there were 18 studies included in final meta-analysis (Figure 1).

Study characteristics

Eighteen studies published from 2000 to 2014 were eligible for meta-analysis.[13–23,26–32] One paper[31] was written in Chinese with an abstract in English available on the PubMed website. A total of 1,270 lung cancer patients were enrolled, and 694 non-malignant lung tissues as controls were included, from the People's Republic of China, Japan, Korea, Poland, Germany, and the USA. Their basic characteristics are summarized in Table 1. As described above, the database search generated 47 articles from MEDLINE, PubMed, Web of Science, Scopus, and Embase. The other 29 publications were excluded due to lack of full text, for being vitro/ex vivo studies, using cell lines or human xenografts, or being irrelevant studies.[6,8,25,32–57]

DAPK methylation and clinicopathological features

Inactivation of *DAPK* through methylation in lung cancer

In Figure 2, the first column has the study name; the second column is the proportion of *DAPK* methylation in lung cancers, and the third column is the proportion of *DAPK* methylation in normal controls. The weight in the fourth

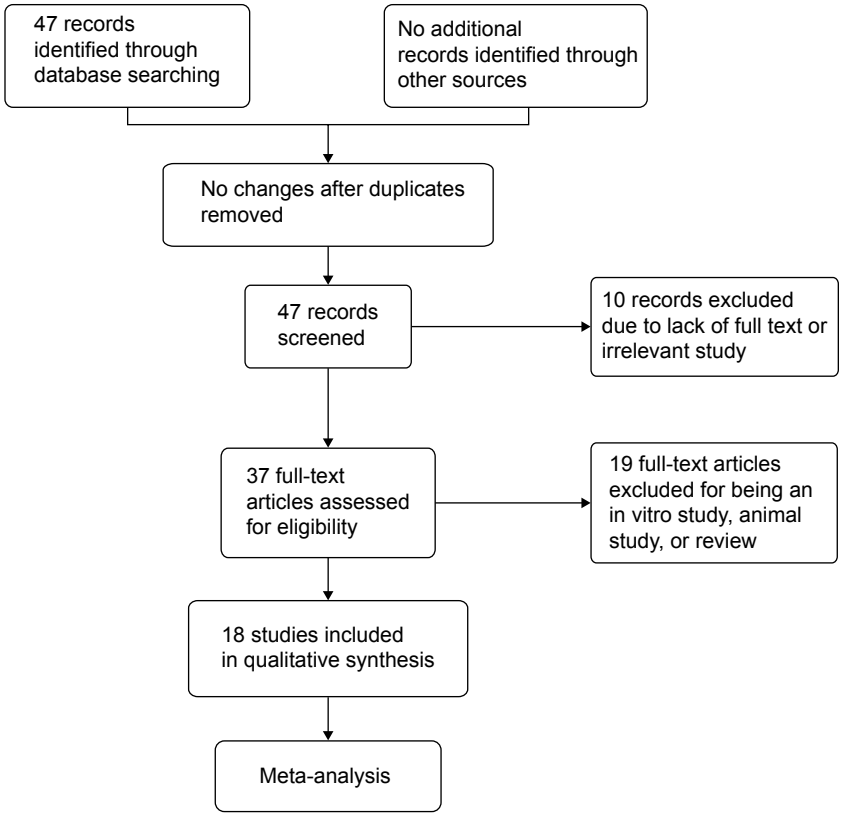

Figure 1 Flow chart of study selection.

Table 1 Basic characteristics of the included studies

Study (country)	Patients (samples)	Methods	Primary aim	Methylation site
Tang et al[18] (USA)	135/tissue	MSP	*DAPK* hypermethylation in primary NSCLC	Promoter, CpG islands
Kim et al[13] (USA)	185/tissue	MSP	Associations of tobacco carcinogen and asbestos as well as demographic and clinical factors with *DAPK* hypermethylation in NSCLC	Promoter, CpG islands
Toyooka et al[19] (USA)	38/tissue, 15/control	MSP	A new methodology development with gene silencing in lung cancers	Promoter, CpG islands
Yanagawa et al[23] (Japan)	75/tissue paired with 75/non-neoplastic lung tissue	MSP	Clinicopathological significance of gene hypermethylation in NSCLC	Promoter, CpG islands
Guo et al[26] (USA)	20/tissue, 20/non-neoplastic lung tissue	MSP	DNA hypermethylation at bronchial margins as early epigenetic events in the primary tumor	Promoter, CpG islands
Kim et al[14] (Korea)	61/tissue, 61/non-neoplastic lung tissue	MSP	Role of DNA hypermethylation in prediction of clinical outcomes in primary NSCLC	Promoter, CpG islands
Kim et al[27] (Korea)	72/tissue, 72/non-neoplastic lung tissue	MSP	Role of methylation status in prediction of long-term survival in lung cancer	Promoter, CpG islands
Russo et al[28] (USA)	49/tissue, 49/non-neoplastic lung tissue	MSP	Pattern of gene methylation status at distinct stages of NSCLC as early diagnostic and therapeutic markers	Promoter, CpG islands
Safar et al[16] (USA)	31/tissue, 31/non-neoplastic lung tissue	MSP	Prognostic potential of multigene hypermethylation profiling in NSCLC	Promoter, CpG islands
Vallbohmer et al[20] (Germany)	91/tissue, 91/non-neoplastic lung tissue	MSP	Role and prognosis of multiple genes in NSCLC	Promoter, CpG islands
Shivapurkar et al[17] (USA)	40/tissue, 40/non-neoplastic lung tissue	MSP	A methylation gene panel in lung cancers	Promoter, CpG islands
Yanagawa et al[22] (Japan)	101/tissue, 101/non-neoplastic lung tissue	MSP	TSG methylation status and the clinicopathologic characteristics	Promoter, CpG islands
Feng et al[29] (USA)	49/tissue, 49/non-neoplastic lung tissue	MethyLight assays	Comparison of DNA methylation (cancerous versus noncancerous) in NSCLC by MethyLight assays	Promoter, CpG islands
Wang et al[21] (People's Republic of China)	28/tissue, 12/nonmalignant tissue	Three-dimensional polyacrylamide gel microarray coupled with linker-PCR	DNA hypermethylation of multiple genes in NSCLC using a three-dimensional polyacrylamide gel microarray	Promoter, CpG islands
Niklinska et al[15] (Poland)	70/tissue	MSP	Role of *DAPK* and *RASSF1A* hypermethylation in prognosis of primary NSCLC	Promoter, CpG islands
Peng et al[31] (People's Republic of China)	82/tissue	MSP	Aberrant hypermethylation of *RASSF1A*, *p16 in NSCLC* and in sputum	Promoter, CpG islands
Zhang et al[30] (People's Republic of China)	78/ tissue, 78/non-neoplastic lung tissue	MSP	Methylation profiles of NSCLC in the Chinese population	Promoter, CpG islands
Kontic et al[32] (USA)	65/tissue	Bisulfite pyrosequencing	Associations between DNA methylation and clinicopathological features	Promoter, CpG islands

Abbreviations: NSCLC, non-small cell lung cancer; tissue, lung tissue; MSP, methylation-specific PCR; PCR, polymerase chain reaction; TSG, tumor suppressor gene.

column is proportional to the inverse of the variance of the study (high variance, associated with a small study, meaning less weight is given to that study, and vice versa). The OR is shown numerically in the fifth column. In this case, the CI of the summary OR does not include 1.0 (it is 3.17–11.42), suggesting that the association is statistically significant. In the light of the "OR diagram" in the final column, the vertical line indicates an OR of 1.0. If *DAPK* methylation occurred more frequently in cancer tissue than in normal tissue, that would make the OR less than 1.0. The horizontal dots and bars represent the relative risk and 95% CI for each study. In summary, we observed that the frequency of *DAPK* methylation was significantly higher in lung cancer than in non-malignant lung tissues used as controls. The pooled OR from 13 studies including 733 lung cancer patients and 694 non-malignant lung tissues as controls shown in Figure 2 (OR 6.02, 95% CI 3.17–11.42, $P<0.00001$) indicates that inactivation of *DAPK* through methylation plays an

Study or subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds ratio M–H, random, 95% CI
Feng et al[29]	8	49	0	49	3.6%	20.28 (1.14, 361.89)
Guo et al[26]	10	20	1	20	5.2%	19.00 (2.12, 170.38)
Kim et al[14]	18	61	15	61	11.2%	1.28 (0.58, 2.86)
Kim et al[27]	23	72	7	72	10.6%	4.36 (1.73, 10.98)
Russo et al[28]	22	49	2	49	7.7%	19.15 (4.18, 87.81)
Shivapurkar et al[17]	17	40	1	40	5.6%	28.83 (3.60, 231.10)
Safar et al[16]	12	31	5	31	9.2%	3.28 (0.99, 10.90)
Toyooka et al[19]	14	38	1	15	5.4%	8.17 (0.97, 68.94)
Vallbohmer et al[20]	60	91	0	91	3.8%	351.48 (21.11, 5,852.80)
Wang et al[21]	14	28	2	12	7.0%	5.00 (0.92, 27.08)
Yanagawa et al[23]	21	75	10	75	11.1%	2.53 (1.10, 5.83)
Yanagawa et al[22]	26	101	8	101	11.0%	4.03 (1.72, 9.42)
Zhang et al[30]	11	78	3	78	8.6%	4.10 (1.10, 15.34)
Total (95% CI)		**733**		**694**	**100.0%**	**6.02 (3.17, 11.42)**
Total events	256		55			

Heterogeneity: τ^2=0.78; χ^2=34.40, *df*=12 (*P*=0.0006); I^2=65%

Test for overall effect: *Z*=5.50 (*P*<0.00001)

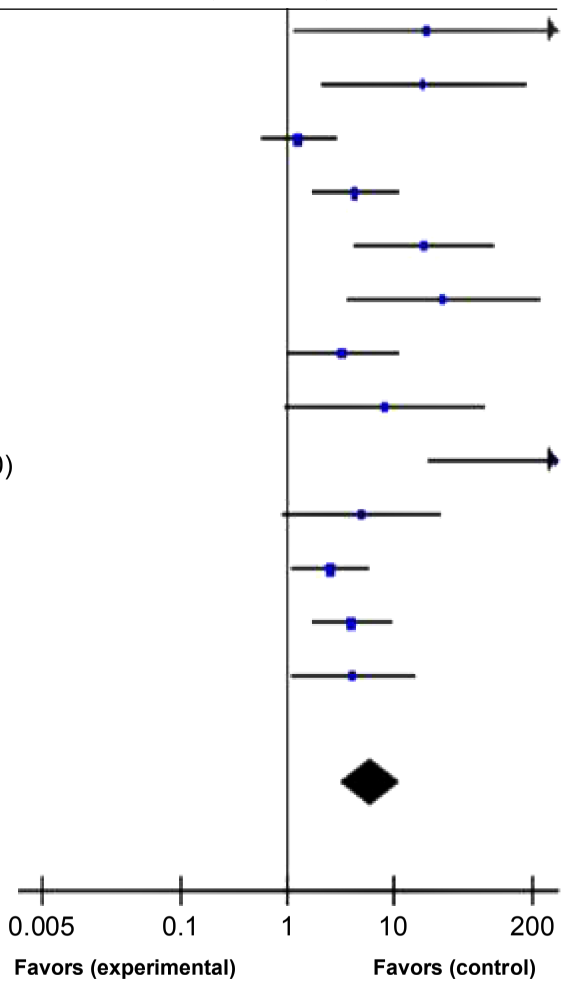

Figure 2 The included studies investigated *DAPK* methylation status between 733 lung cancer patients and 694 nonmalignant controls with the pooled odds ratio being 6.02 (95% CI 3.17–11.42, *Z*=5.50, *P*<0.00001).
Abbreviations: CI, confidence interval; M–H, Mantel–Haenszel test.

important role in the pathogenesis of lung cancer. The overall methylation frequency of *DAPK* in lung cancer tissues was higher than that in normal controls, suggesting a potential role of *DAPK* methylation analysis in diagnosing lung cancer.

Methylation of *DAPK*, histologic types, and disease stage

We further determined the possible associations between *DAPK* hypermethylation and clinicopathological features. The two major forms of lung cancer are NSCLC, which accounts for about 85% of lung cancer, and small cell lung cancer, which accounts for about 15%. NSCLC can be subdivided into adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma.[3] To determine whether methylation of DAPK is associated with histological subtypes, we analyzed these relationships in adenocarcinoma and squamous cell carcinoma separately. When the data were stratified by histology, adenocarcinomas showed the same frequent methylation of *DAPK* in tumors with squamous carcinoma histology as shown in Figure 3 (OR 0.91, 95% CI 0.64–1.29) from ten studies including 401 lung cancer patients and 263 non-malignant lung tissues. The OR is shown numerically in the fifth column, and the CI of the summary of OR does include 1.0 (it is 0.64–1.29), suggesting that the association is not statistically significant. The meta-analysis was performed to determine whether methylation of the promoter region of the *DAPK* gene is independently associated with tumor stage. When the data were stratified by disease stage using the tumor node metastasis staging system (TNM) (TNM I + II versus TNM III + IV), methylation of *DAPK* did not increase with increasing stage as shown in Figure 4 (OR 0.95, 95% CI 0.53–1.73). The OR is shown numerically in the fifth column, and the CI of the summary of OR does include 1.0 (it is 0.53–1.73), suggesting that the association is not statistically significant.

Prognostic value of *DAPK* gene methylation in lung cancer

We analyzed the relationship between methylation of the *DAPK* gene and patient survival. Three included studies[14,15,18] estimated the relationship between overall survival in lung cancers and *DAPK* methylation. The pooled results (Figure 5) showed the presence of a prognostic impact of *DAPK* gene methylation in lung cancer patients (OR 3.63, 95% CI 1.09–12.06, Z=2.10, P=0.04). The hazard ratio is shown numerically in the fifth column, and the CI of the summary of hazard ratios does not include 1.0 (it is 1.09–12.06), suggesting that the association is statistically significant.

Sensitivity analyses and publication bias

A sensitivity analysis, in which one study was removed at a time, was conducted to assess the stability of the results. The pooled ORs were not significantly changed, indicating

Study or subgroup	Adenocarcinoma Events	Total	Squamous carcinoma Events	Total	Weight	Odds ratio M–H, fixed, 95% CI
Kim et al[13]	23	94	15	59	20.9%	0.95 (0.45, 2.01)
Kim et al[14]	13	42	5	17	7.4%	1.08 (0.31, 3.69)
Kontic et al[32]	4	18	7	29	6.3%	0.90 (0.22, 3.64)
Niklinksa et al[15]	5	20	15	30	13.5%	0.33 (0.10, 1.15)
Peng et al[31]	23	35	0	0		Not estimable
Tang et al[18]	34	71	16	51	14.6%	2.01 (0.95, 4.27)
Toyooka et al[19]	8	20	4	12	4.5%	1.33 (0.30, 5.96)
Wang et al[21]	7	15	5	7	5.5%	0.35 (0.05, 2.41)
Yanagawa et al[23]	10	43	10	29	13.7%	0.58 (0.20, 1.63)
Yanagawa et al[22]	10	43	10	29	13.7%	0.58 (0.20, 1.63)
Total (95% CI)		**401**		**263**	**100.0%**	**0.91 (0.64, 1.29)**
Total events	137		87			

Heterogeneity: χ^2=9.54, df=8 (P=0.30); I^2=16%
Test (for overall effect: Z=0.54 (P=0.59)

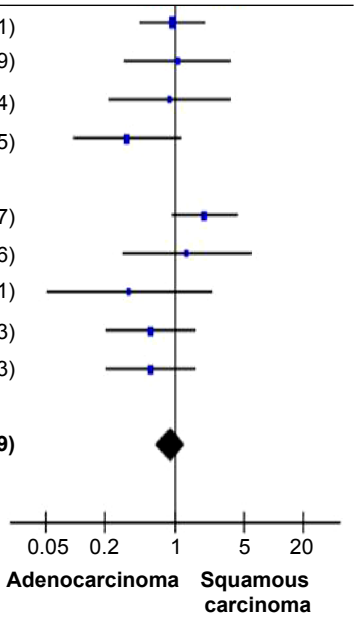

Figure 3 The included studies investigated *DAPK* methylation status between 401 tumors of adenocarcinoma and 263 of squamous carcinomas. The combined odds ratio was 0.91 (95% CI 0.64–1.29, Z=0.54, P=0.59).
Abbreviations: CI, confidence interval; M–H, Mantel–Haenszel test.

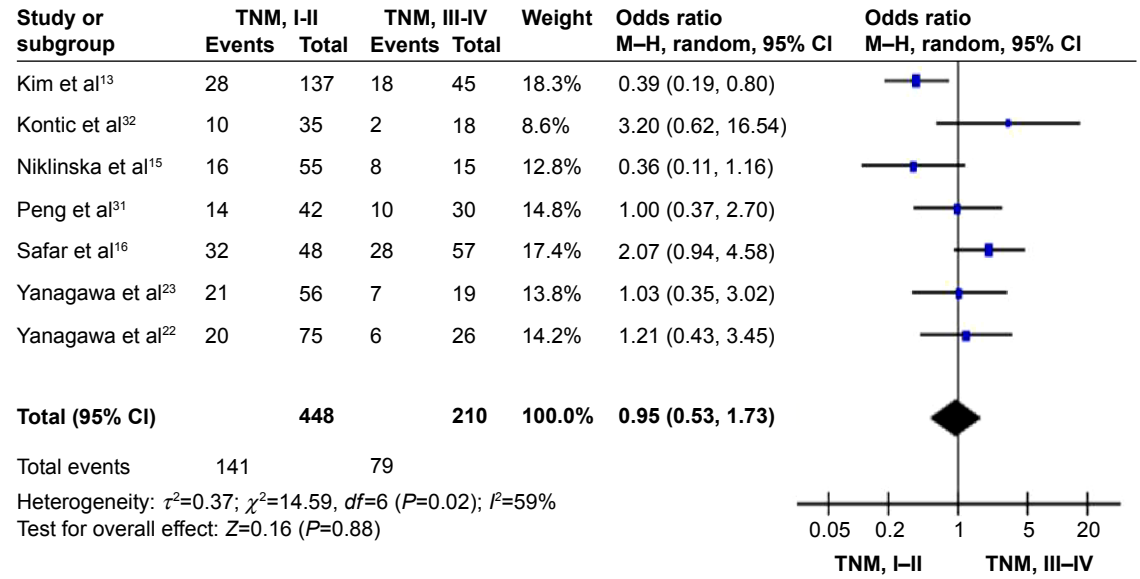

Figure 4 Pooled results of methylation analysis of *DAPK* gene in different disease stages (TNM I + II versus TNM III + IV) in lung cancer. The pooled odds ratio is 0.95, 95% CI 0.53–1.73, *Z*=0.16, *P*=0.88).
Abbreviations: CI, confidence interval; M–H, Mantel–Haenszel test; TNM, tumor node metastasis staging system.

the stability of our analyses. The funnel plots were largely symmetrical, suggesting there was no publication bias in the meta-analysis of *DAPK* gene methylation/expression and clinicopathological features (Figure 6).

Discussion

DAPK is a Ca^{2+}/calmodulin-regulated, 160 kDa serine/threonine, microfilament-bound kinase known to be activated by IFN-γ and TNF-α or Fas ligand-induced, and subsequently results in apoptosis.[58–60] Aggressiveness of cancers has been associated with loss of DAPK expression[61] mediated by methylation of the promoter region of the *DAPK* gene.[10,62–64] From the present meta-analysis, we concluded that: *DAPK* gene inactivation through methylation plays an important role in the pathogenesis of lung cancer, and could be one of the determinants for its malignancy, as supported by higher *DAPK* methylation frequency in malignant lesions than in normal controls; overall survival tends to be shorter in lung cancer patients with epigenetic abnormalities of *DAPK* than in those with normal expression of *DAPK* gene; and no correlation exists between histological subtypes/disease stages (TNM I + II versus TNM III + IV) and hypermethylation status of the *DAPK* gene.

A meta-analysis is a more rigorous and methodologically complicated review than an overview or systematic review. There are two major reasons to do a meta-analysis; one is to quantitatively combine the results of previous studies to reach a summary estimate, and the other is to help guide

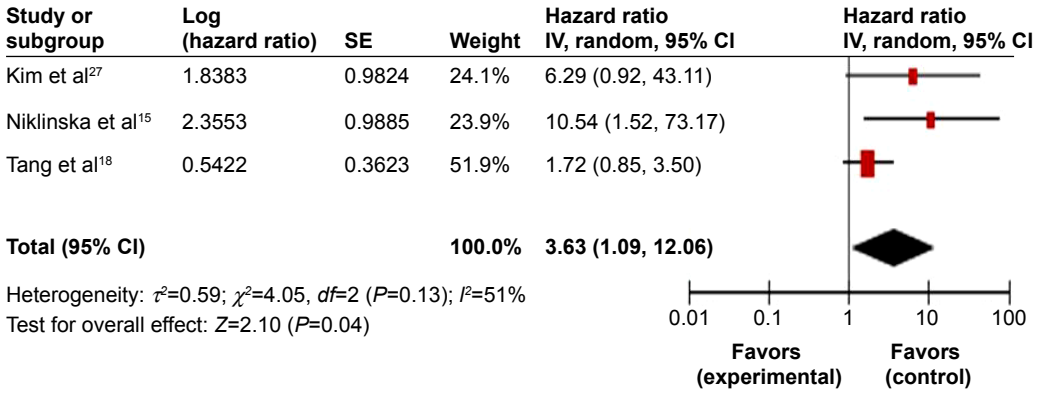

Figure 5 All three included studies estimated the relationship between overall survival and *DAPK* methylation. The pooled hazard ratio for overall survival showed that *DAPK* hypermethylation was associated with worse survival in lung cancer (hazard ratio 3.63, 95% CI 1.09–12.06, *P*=0.04).
Abbreviations: CI, confidence interval; IV, inverse variance; SE, standard error.

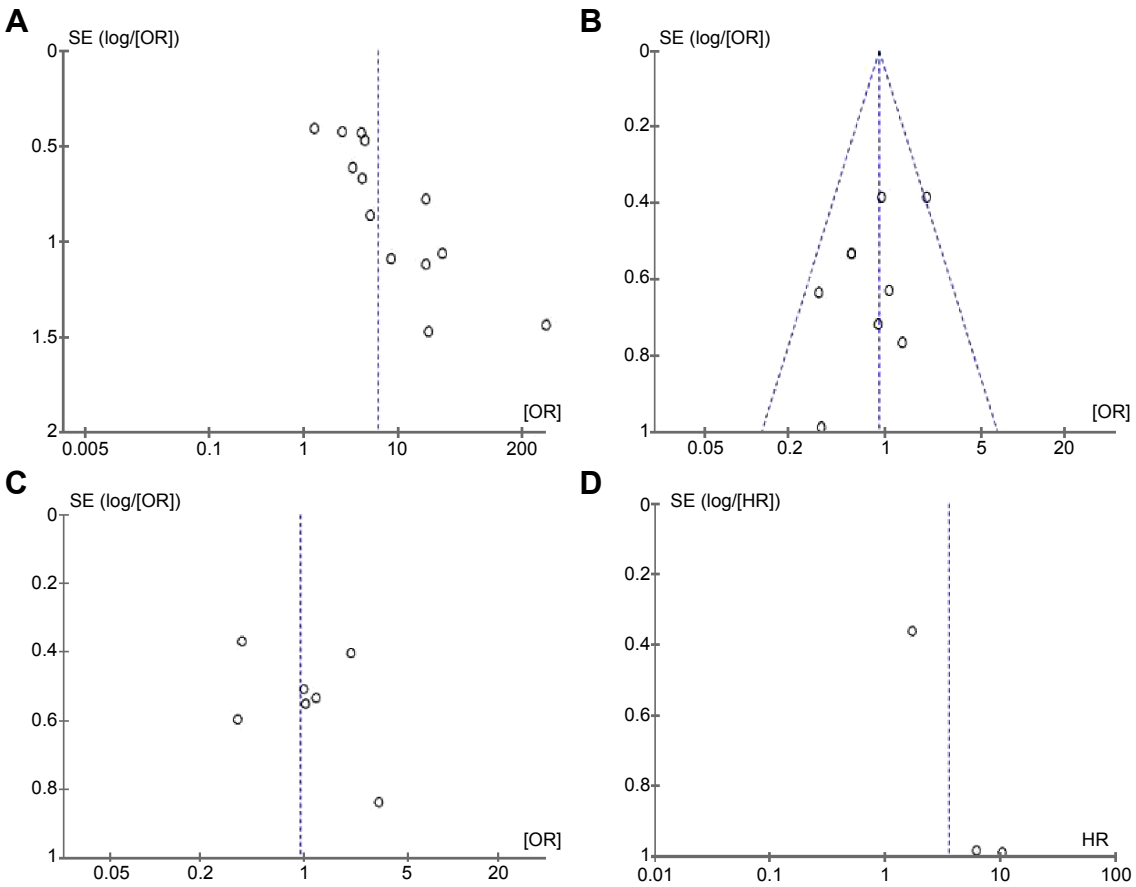

Figure 6 Funnel plot of publication bias in the meta-analysis of *DAPK* hypermethylation and clinicopathological features.
Notes: *DAPK* methylation in lung cancer (**A**), subtypes of histology (**B**), disease stages, TNM I + II versus III + IV (**C**), and overall survival (**D**). The x axis indicates the value of OR or HR and the y axis gives the SE multiplied by log scale of OR or HR.
Abbreviations: HR, hazard ratio; OR, odds ratio; SE, standard error; TNM, tumor node metastasis staging system.

further research. Based on the meta-analysis results, we suggest that *DAPK* hypermethylation should be regarded an early diagnostic marker for lung cancer and also a predictor for the prognosis of lung cancer. DAPK inhibitors, which are still in the early stages of evaluation, really need to be explored and developed.

Wild-type *DAPK* functions as an apoptosis inducer in a variety of cancers. However, some newly discovered splice variants of *DAPK* in cancer can antagonize the action of wild-type DAPK,[65] which is similar to the situation occurring in p53 and p73 gene in cancer.[66,67] In addition, in certain specific tissues or genetic environments, *DAPK* may be a survival promoter and promote tumorigenicity.[68–71]

Recent studies have revealed the mechanisms of DAPK-mediated cell death induced by TNF-α and IFN-γ,[72] suggesting that DAPK can mediate the proapoptotic activity of TNF-α and IFN-γ via nuclear factor kappa B (NF-κB) signaling pathways. In the presence of DAPK, apoptosis induced by TNF-α or IFN-γ was additively increased, while the NF-κB activity induced by TNF-α or IFN-γ was inhibited. The activity of NF-κB was dependent on the level of DAPK, indicating the requirement of DAPK for activation of NF-κB.[72]

The mechanism for the role of *DAPK* has been largely attributed to promoter hypermethylation, which leads to gene silencing. However, recent studies indicate that *DAPK* expression can be detected in some types of cancer, but its function is still repressed, suggesting that regulation of *DAPK* function occurs in different ways. Homozygous deletions, allelic deletion, and point mutations have been found to attenuate *DAPK* expression.[4] A positive correlation was also found between hyperphosphorylation of DAPK and activated Src kinase in colon cancer cell lines and primary tissue,[73] suggesting a mechanism for inactivation of *DAPK* at the post-translational level in cancer cells.

The two major forms of lung cancer are NSCLC, which accounts for about 85% of lung cancers, and small cell lung cancer, which accounts for about 15%. NSCLC can be subdivided into adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma.[3] Our results are consistent with a

previous publication reporting that the prevalence of *DAPK* gene hypermethylation was indistinguishable among the major histological subtypes of lung cancer.[74]

In addition to primary tumors, *DAPK* gene hypermethylation status has been detected in other sample sources. However, we were unable to undertake a meta-analysis due to the limited number of studies performed, so we summarize their data as follows. Two studies compared hypermethylation of *DAPK* in tumors, bronchial margins, and bronchoalveolar fluid.[26,75,76] They found that there was significant concordance of the *DAPK* gene with regard to epigenetic changes between tumors, bronchial margin, and bronchial washings. Their data suggest that the frequencies of methylation of the *DAPK* gene found in bronchoalveolar fluid and in primary tumors were overall comparable. Their results can be interpreted as the bronchoalveolar fluid not reflecting the presence of tumor cells per se but as the bronchoalveolar fluid detecting a field effect present in the bronchial mucosa in close proximity to the tumor.

Peng et al[31] determined the aberrant methylation status of *DAPK* along with *RASSF1A* and *p16* gene promoter region in induced sputum from lung cancer patients, and found that joint detection for promoter hypermethylation of *DAPK* gene along with *RASSF1A* and *p16* gene in induced sputum may be used as a simple and effective index of the diagnosis and prognosis of lung cancers, and can improve the positive rate. On the contrary, when *DAPK*, *RASSF1A* and *CDH13* were observed to be significantly hypermethylated in lung tumors compared with matched normal tissue, however, these changes could not be detected in patients' blood samples by Kontic et al[32] suggesting these three genes are not suitable markers for the early detection of NSCLC, although they seem to play a role in the pathogenesis of NSCLC.

Given the central role of smoking in the development of lung cancer and the fact that DNA methylation is an early event in tumorigenesis,[29] some biomarkers such as CCND2 and APC were investigated and demonstrated to be frequently hypermethylated in both cancerous and non-cancerous lung tissues from smokers with NSCLC, indicating that hypermethylation of these genes may be associated with chronic smoking status.[29] However, no correlation was found between *DAPK* methylation and the smoking habits (smoking history) of individuals with NSCLC.[76]

DNA methylation and histone acetylation are the two most studied epigenetic changes in cancer. DAPK I inhibitors are still in the early stages of evaluation. DNA methyltransferase inhibitors, ie, azacitidine (5-azacytidine, 5AC), 5-aza-2′-deoxycytidine (decitabine) and pyrimidin-2-one β-ribofuranoside (zebularine) have been studied in the preclinical setting, and even in clinical trials.[77,78] Under equivalent and clinically relevant treatment schemes, the three DNA methyltransferase inhibitors left a distinct gene expression profile in cells, indicating that these drugs are not identical in function. Differences in structure and cellular pharmacology between the three DNA methyltransferase inhibitors may account for the diversity observed. 5AC and decitabine have a nitrogen at position 5 of the pyrimidine ring instead of a carbon, but zebularine does not. 5AC is a ribonucleoside that is incorporated mainly into RNA, whereas decitabine and zebularine are incorporated only into DNA.[78] Application of decitabine is limited by its high toxicity (myelosuppression and neurological toxicity) and instability in physiological solutions,[79,80] while zebularine is less toxic and more stable, and can be taken orally.[5,81] A Phase I study in advanced solid malignancies has found that a combination of erlotinib and 5AC was well tolerated, with promising clinical activity in lung, head and neck, and ovarian cancer. The recommended dose for Phase II study is erlotinib 150 mg daily and 5AC 75 mg/m^2 daily on days 1–4 and 15–18 of a 28-day cycle.[82] Another Phase I study involving lung, esophageal, or pleural cancer revealed that prolonged decitabine infusions can regulate gene expression in these primary thoracic malignancies.[83] With respect to decitabine, Phase I, II, and III studies have been conducted. Chu et al[84] observed that decitabine and valproic acid are an effective combination in reactivating silent genes by hypermethylation, as demonstrated by re-expression of fetal hemoglobin in patients with advanced stage IV NSCLC in a Phase I study; however, the continuous study was hampered by remarkably high neurological toxicity at a relatively low dosage. In another Phase I–II clinical trial, decitabine was reported to exert a remarkable chemotherapeutic action for NSCLC, but this action is delayed and prolonged, with the major toxicity being myelosuppression.[85] In hematological malignancy, a randomized Phase II study demonstrated that addition of valproic acid to decitabine was not associated with an improved outcome in patients with myelodysplastic syndrome or elderly patients with acute myeloid leukemia.[86] A post hoc analysis of a randomized Phase III study[87] evaluated the impact of decitabine on transfusion dependence and survival in 485 elderly patients with newly diagnosed acute myeloid leukemia. More red blood cell and platelet transfusion-dependent patients at baseline became transfusion-independent with decitabine than did corresponding controls. In addition, patients who achieved transfusion independence with decitabine had increased treatment continuation, even in the absence of

complete remission. Zebularine is now being tested in preclinical animal tumor or human cancer cell lines,[88] but there is no phase study available yet.

DAPK was first identified in 1995,[58] and *DAPK* gene methylation has been discovered in more than 30 types of cancers although the methylation rates vary.[8] Re-expression of *DAPK* by demethylation in tumors may possibly bring clinical benefits as a potential drug target.[8] Taken together, DAPK is an interesting therapeutic target in multiple human diseases including cancer. The development of specific compounds for activation of DAPK will be a promising strategy for targeting DAPK in the clinic.

Disclosure

The authors report no conflicts of interest in this work.

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