Meta-analysis: Assocation between promoter hypermethylation of DAPK (Death-Associated Protein Kinase) and cervical cancer

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ABSTRACT

Purpose: Death-associated protein kinase (DAPK or DAPK1) is an important tumor suppressor protein that is involved in the regulation of cell activities. The aberrant methylation of *DAPK* promoter has been reported in patients with cervical cancer. However, the association between DAPK1 and cervical cancer was not always unification, in previous studies. Therefore, in the current study, a meta-analysis was performed for the association between *DAPK* gene's promoter hypermethylated and cervical cancer.

Methods: A systematic literature analysis was conducted based on the previous studies published in PubMed, PubMed Central (NCBI), Google by using the following keywords: cervical cancer, cervical carcinoma, Methylation, by the end of January 2018. The association between *DAPK* promoter methylation and cervical cancer was evaluated by odds ratio (ORs) and 95% confidence intervals (CI). To evaluate the potential sources of heterogeneity, the meta-regression analysis and subgroup analysis were conducted.

Results: A total of 21 case-control studies relevant to the association between *DAPK1* gene's promoter methylation frequency and cervical cancer, including 1600 cancer cases and 1011 control cases (non-cancerous cases). The analysis results indicated that the characteristic of candidate gene's promoter methylation increased the cervical cancer risk through the calculation of OR value (OR = 21.25; 95% CI = 8.73 - 52.97; $p < 0.001$; Random effect model). The association between *DAPK1* gene's promoter hypermethylation was confirmed in all the subgroups analyses, including materials and assays methods, ethnicity. Furthermore, this association is higher in cervical squamous cell carcinoma than cervical adenocarcinoma and is a characteristic of late-stage disease.

Conclusion: The hypermethylated *DAPK1* gene's promoter

was also one of the etiological factors, lead to cervical tumorigenesis.

1. Introduction

In addition to the infection with oncogenic human papillomavirus, which is the most significant risk factor in the etiology of cervical cancer, the epigenetic alterations could result in heritable gene silencing without changes to genetic sequences and are recognized as the important cause of human cervical cancer (Burd, 2003; Lu, Ma, & Zhao, 2012; Yang, 2013). The epigenetic molecular mechanisms associated with human cervical cancer comprise a variety of alterations, including the silencing of Tumor Suppressor Genes (TSGs) by hypermethylation of CpG islands (Lu et al., 2012; Yang, 2013). Many cellular pathways, including DNA repair, cell cycle, apoptosis, etc., are proven to be inactivated by the hypermethylation of CpG islands located in the promoter regions of many TSGs (Esteller, 2002; Le et al., 2017). Among the involved TSGs, the *Death-associated protein kinase 1* (*DAPK1*) gene, located at 9q21.33, encodes the 160-kD calmodulin dependent serine-threonine kinase involved in multiple cellular signaling pathways that trigger cell survival, apoptosis and autophagy (Cai, Xiao, Niu, & Zhong, 2017; Raveh & Kimchi, 2001). The decreased expression of *DAPK* is associated with the methylation of gene promoter has been frequently reported in various types of human cancers, including cancer of the cervix (Cai et al., 2017; Leung et al., 2008; Niklinska et al., 2009; Wang et al., 2016). However, there are significant differences in the frequency of *DAPK* promoter in patients with cervical cancer, due to different populations, sample sizes, methods for methylation analysis. Moreover, whether or not the methylation frequency of *DAPK* gene's promoter is correlated with clinicopathological characteristics, including sex, stages, and invasion remains debated. Thus, we performed the systematic review and meta-analysis to summarize the current previous studies and evaluate the relationship between the methylation status of *DAPK* promoter and cervical cancer as an epigenetic marker of cervical cancer risk.

2. Materials and methods

2.1. Search strategy, inclusion and exclusion criteria of literature

The systematic review of relevant literature was conducted by using many keywords for the literature search: methylation, *DAPK1*, *Death-associated protein kinase 1*, cervical cancer, etc. on four electronic databases: PUBMED, Web of Science, Embase database, updated to December 2018. The aim of the first selection was to identify studies that relevant to establish the association between *DAPK* promoter methylation and cervical cancer. Accordingly, the studies were included if they satisfied the following inclusion criteria: (1) The investigation/ identification of *DAPK* promoter methylation, which correlated with cervical cancer; (2) The correlation between *DAPK* promoter methylation and clinicopathological features of FDB; (3) cohort design studies of *DAPK* promoter methylation and cervical cancer; (4) the identification method was not excluded. Additionally, only studies written in English were included in the current study. Types of case reports, abstracts, reviews, and letters to the editor were eliminated.

2.2. Data extraction, quality assessment and statistical analysis

Two of the authors independently reviewed all the eligible studies. The data were

abstracted the following information in a standard format, including the name of the first author, year of publication, the region of study subjects, age of patients, source/type of samples, detection method, clinical features of cervical cancer, the sample size of case and control groups. Finally, the third reviewer independently reviewed the relevant extract data from the eligible studies.

In the current study, all data were analyzed using Medcalc®2018. The frequency of *DAPK* promoter methylation was observed in both case and control studies. The strength of the association between *DAPK* promoter methylation and cervical cancer was evaluated by Odds Ratio (OR) and Risk Relative (RR) with 95% confidence intervals (95% CIs). The degree of association between *DAPK* promoter methylation and clinical features was also evaluated by OR and RR with 95% CIs. Heterogeneity across studies was measured using Cochran's Q-test (Higgins & Thompson, 2002). The random-effects model was employed when the p-value was less than 0.05 in the Q-test, indicating the presence of substantial heterogeneity. The Cochran's Q-test only indicates the presence of heterogeneity, thus, we also reported I^2 statistic, which estimated the percentage of outcome variability that can be attributed to heterogeneity across studies (Higgins & Thompson, 2002). Furthermore, subgroups analyses were conducted by histological stage (Squamous Cell Carcinoma - SCC, Adenocarcinoma - AC), source/type of samples (Biopsy, scrape, and urine), detection methods (Methylation Specific PCR - MSP, Realtime quantitative MSP - QMSP), ethnicity (Asian, European, American, and African).

3. Results

3.1. Identified study and data characteristics

A total of 158 studies were initially identified by a systematic literature search. After the duplicates and non-relevant studies were excluded by considering the title and abstract of studies, 61 articles with potentially relevant studies were further investigated by examining the full text. Finally, 23 studies, including 21 case-control studies and 2 cohort studies, were included in current meta-analysis after excluding studied that not met the inclusion criteria. The detailed processes of the systematic review and selections are given as a PRISMA flow chart, illustrated in Figure 1.

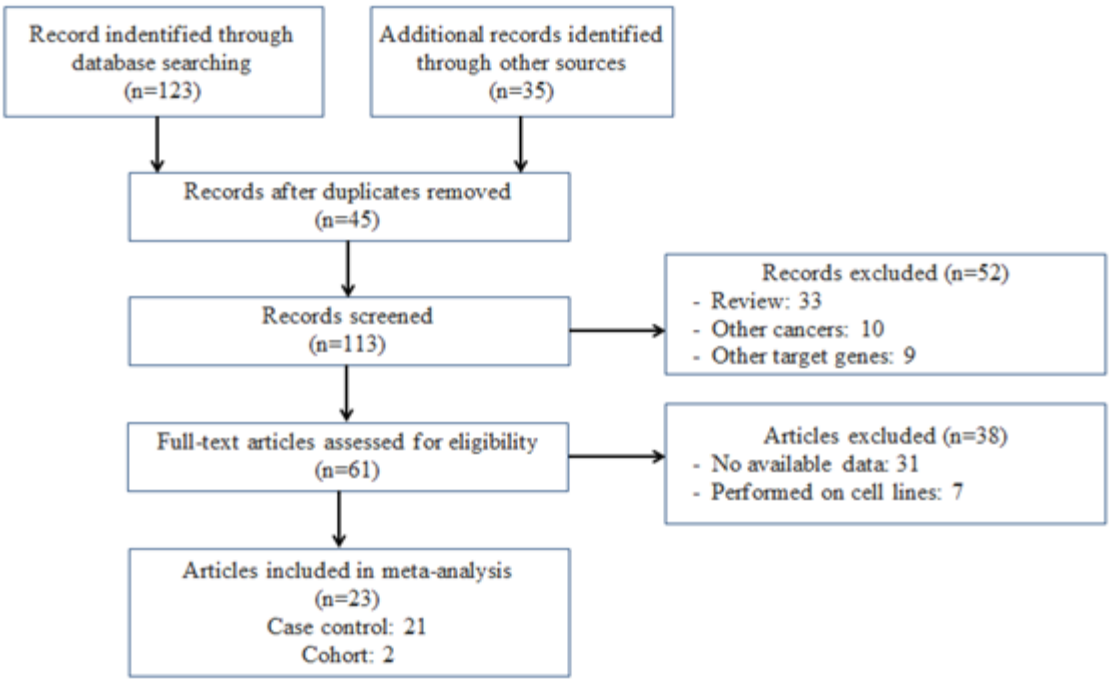

Figure 1. Flow chart of processes included in current meta-analysis

Out of the 23 studies included, 21 studies with 1600 cases and 1011 controls were combined to calculate the pooled OR between *DAPK* promoter methylation and cervical cancer. The 21 case - control studies encompassed the publication years from 2001-2017, 11 from Asian countries (counting for 52.38%), 5 from European countries (counting for 23.81%), 3 from American countries (counting for 14.29%), and 2 from Africa (counting for 9.52%), were included in the systematic review and analysis. The methylation detection methods consisted of 15 studies of using MSP (counting for 71.43%), 5 studies of using QMSP (counting for 23.81%), and 1 study of using Realtime PCR (counting for 4.76%), to explore *DAPK* promoter methylation in cervical cancer and corresponding to controls. The type of case samples consisted of 15 studies of using Biopsy - tumor tissues (counting for 71.43%), 5 studies of using scrape sample (counting for 23.81%), and 1 study of using urine (counting for 4.76%). The type of controls consisted of 20 studies using normal/benign biopsy sample (counting for 95.24%), and 1 study using healthy urine sample (counting for 4.76%). The detailed characteristic of the included studies was summarized in Table 1.

Table 1

Detail characteristic of studies included in the systematic review and meta-analysis of *DAPK* promoter methylation and cervical cancer

First Author	Year	Country	Ethnicity	Method	Types of case	Cases		Types of control	Control	
						Total	Events		Total	Events
Case - control studies										
Truong	2017	Vietnamese	Asian	MSP	Scrape	61	27	NCT	48	1
Banzai	2014	Japan	Asian	MSP	Biopsy	53	40	NCT	24	1
Niyaki	2012	China	Asian	MSP	Biopsy	30	19	NCT	30	1

First Author	Year	Country	Ethnicity	Method	Types of case	Cases		Types of control	Control	
						Total	Events		Total	Events
Sun	2012	China	Asian	MSP	Biopsy	331	181	NCT	336	157
Huang	2011	Taiwan	Asian	MSP	Scrape	26	13	NCT	15	3
Missaoui	2011	Tunisia	African	MSP	Biopsy	42	22	NCT	8	0
Kim	2010	Korea	Asian	MSP	Biopsy	69	50	NCT	41	11
Yang	2010	Netherlands	European	QMSP	Biopsy	60	31	BCT	20	5
Flatley	2009	UK	European	MSP	Scrape	42	17	NCT	40	0
Iliopoulos	2009	Greece	European	RMethyLight	Biopsy	61	41	NCT	15	0
Leung	2008	China	Asian	MSP	Biopsy	107	60	NCT	27	0
Zhao	2008	China	Asian	MSP	Biopsy	112	45	NCT	20	0
Feng	2007	Senegalease	African	QMSP	Urine	63	31	U	16	1
Shivapurkar	2007	USA	American	QMSP	Biopsy	45	24	NCT	12	0
Jeong	2006	Korea	Asian	MSP	Biopsy	78	35	NCT	24	1
Wisman	2006	Netherland	European	QMSP	Scrape	30	19	NCT	19	0
Feng	2005	USA	American	MSP	Biopsy	176	69	NCT	140	3
Gustafson	2004	USA	American	MSP	Biopsy	28	7	NCT	11	0
Reesink-Peters	2004	Netherlands	European	QMSP	Scrape	48	35	NCT	41	2
Yang	2004	HK	Asian	MSP	Biopsy	85	51	NCT	100	0
Dong	2001	Korea	Asian	MSP	Biopsy	53	27	NCT	24	0
Cohort studies										
Kalantari	2014	USA	American	MSP	Scrape	408	317	NA	NA	NA
Henken	2007	Netherlands	European	MS-MLPA	Biopsy	24	15	NA	NA	NA

Note: Note: MSP: Methylation specific PCR; QMSP: Realtime quantitative MSP - QMSP; RMethyLight: Real-time MethyLight; NCT: normal cervical tissue; BCT: benign cervical tissue; U: Urine of healthy woman; NA: non-analysis

Source: The researcher's data analysis

Among the 2 included cohort studies consisted 432 cases, from American countries (1 of 2 studies, counting for 50%) and European countries (1 of 2 studies, counting for 50%), to estimate the frequency of *DAPK* promoter methylation by using MSP (1 of 2 studies, counting for 50%) and MS-MLPA (1 of 2 studies, counting for 50%). The type of case samples consisted of 1 studies using Scrape samples (counting for 50%), and 1 study using Biopsy - tumor tissues (counting for 50%). The detailed characterization of the included studies was summarized in Table 1.

3.2. Meta-analysis

Association between *DAPK1* promoter methylation and cervical cancer

In the current meta-analysis, the heterogeneity among included 21 case-control studies was significant for Cochran's Q-test ($p < 0.001$), thus, the random effect model was applied to evaluate the strength of the association between *DAPK* promoter methylation and cervical cancer. The association was estimated by calculation of OR value with 95% confidence intervals (95% CIs). As the results, we found that the frequency of DAPK promoter methylation was 52.75% (844 of 1600 cases), 18.40% (186 of 1011 controls) for cases and controls, respectively. Moreover, we found that *DAPK* promoter methylation was significantly associated with an increased cervical risk with a pooled OR of 21.51 (95% CI = 8.73-52.97) due to the significant heterogeneity ($I^2 = 85.64\%$, $p < 0.001$), based on the random effect model (Figure 2).

Subgroup analysis

In the current study, subgroups analyses were performed by the source of sample types, methylation detection method and ethnicity. The association between *DAPK1* promoter methylation and cervical cancer was observed in each subgroup (Table 2). The significant association between *DAPK* promoter methylation and cervical cancer was identified including MSP and QMSP methods. The ORs were 21.41 (95% CI = 7.03-65.19) in MSP subgroup and 20.45 (95% CI = 5.61-74.51) in QMSP subgroup, based on the random effect model, while I^2 were high with 87.60% and 58.34%, respectively. The subgroup analysis by the source of samples particularly focused on biopsy and scrape samples, reported that ORs were 20.30 (95% CI = 6.87-60.00) in case of using a biopsy, based on the random effect model, and 25.65 (95% CI = 11.25-58.44) in case of using Scrape sample, based on the fixed-effect model. According to subgroup analysis by ethnicity, a significant association between methylation status and cervical cancer was found among the Asian region and the non-Asian region, including American, European and African regions. In detail, the ORs were 20.93 (95% CI = 5.77-75.97) in Asian region, 26.34 (95% CI = 5.08-136.62) in European region, based on the random effect model, 25.48 (95% CI = 9.16-70.92) in American region, and 15.88 (95% CI = 2.92-86.41) in African region while I^2 were high with 85.50%, moderate with 68.91%, and low with both 0%, respectively.

Table 2

Subgroup analysis in the meta-analysis of *DAPK* promoter methylation and cervical cancer

Variables	N	Test of association OR (95% CI)	Z	P-value	Test of heterogeneity Model	P*h*	I^2 (%)
Materials							
Biopsy	15	20.30 [6.87-60.00]	5.44	< 0.001	R	<0.001	87.11
Scrape	5	25.65 [11.25-58.44]	7.72	< 0.001	F	<0.001	47.96
Urine	1	14.53 [18.07-116.74]	2.52	0.01	-	-	-
Methods							
MSP	15	21.41 [7.03-65.19]	5.39	< 0.001	R	<0.0001	87.60

Variables	N	Test of association OR (95% CI)	Z	P-value	Test of heterogeneity Model	P*h*	I^2 (%)
QMSP	6	20.45 [5.61-74.51]	4.57	< 0.001	R	0.03	58.34
Ethnicity							
Asian	11	20.934 [5.77-75.97]	4.63	< 0.001	R	<0.001	85.50
European	5	26.34 [5.08-136.62]	3.90	< 0.001	R	0.001	68.91
American	3	25.48 [9.16-70.92]	6.20	< 0.001	F	0.72	0.00
Africa	2	15.88 [2.92-86.41]	3.2	0.001	F	0.89	0.00

Source: The researcher's data analysis

4. Discussion

DNA hypermethylation of CpG islands located in the promoter of TSGs resulting in silencing the expression of TSGs that plays a crucial role in the carcinogenesis of the tumor. The *DAPK* promoter methylation mainly induced the loss of *DAPK* functions provides a unique mechanism that links suppression of apoptosis to metastasis of tumor, including cervical cancer (Cai et al., 2017; Inbal et al., 1997; Leung et al., 2008; Niklinska et al., 2009; Wang et al., 2016). The current results suggested that individuals with the *DAPK* methylation are associated with cervical cancer by calculating pooled OR of 21.51 (95% CI = 8.73-52.97).

Study	Cervical cancer Events	Cervical cancer Total	Cervical cancer Events	Cervical cancer Total	Weight (%)	Odds Ratio M-H, Random, 95% CI
Truong 2017	27	61	1	48	4.94	37.32 [4.83 - 288.23]
Banzai 2014	40	53	1	24	4.87	70.77 [8.68 - 576.56]
Niyaki 2012	19	30	1	30	4.84	50.09 [5.97 - 420.381
Sun 2012	181	331	157	336	6.57	1.37 [1.02 - 1.87]
Huang 2011	13	26	3	15	5.62	4.00 [0.91 - 17.58]
Missaoui 2011	22	42	0	8	3.91	18.66 [1.01 - 343.99]
Kim 2010	50	69	11	41	6.24	7.18 [3.01 - 17.12]
Yang 2010	31	60	5	20	6	3.21 [1.03 - 9.95]
Flatley 2009	17	42	0	40	3.98	55.59 [3.20 - 965.34]
Iliopoulos 2009	41	61	0	15	3.97	62.76 [3.57 - 1101.85]
Leung 2008	60	107	0	27	4.01	70.05 [4.16 - 1178.36]
Zhao 2008	45	112	0	20	4.01	27.64 [1.63 - 468.57]
Feng 2007	31	63	1	16	4.89	14.53 [1.81 - 116.74]
Shivapurkar 2007	24	45	0	12	3.94	28.49 [1.59 - 510.29]
Jeong 2006	35	78	1	24	4.93	18.72 [2.41 - 145.61]
Wisman 2006	19	30	0	19	3.93	66.13 [3.63 - 1202.13]
Feng 2005	69	176	3	140	5.94	29.45 [9.02 - 96.15]
Gustafson 2004	7	28	0	11	3.87	8.02 [0.42 - 153.44]
Reesink-Peters 2004	35	48	2	41	5.53	52.5 [11.064 - 249.115]
Yang 2004	51	85	0	100	4.03	300.04 [18.028 - 4993.69
Dong 2001	27	53	0	24	3.98	50.85 [2.94 - 879.40]
Total (95% CI)		1600		1011	1	**21.51 [8.73-52.97]**
Total events	844		186			

Heterogeneity: Tau^2=0.006, Chi^2= 139.26, df= 20 (P<0.0001), I^2=85.64%

Test for overall effect: Z=6.67 (P<0.001)

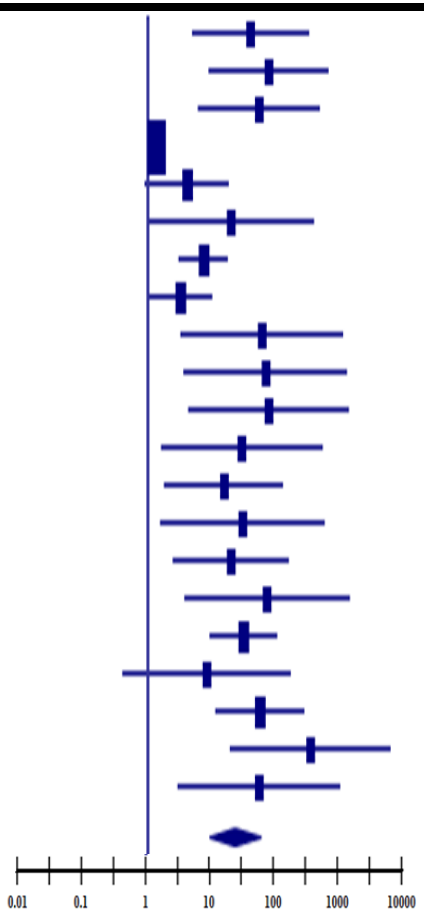

Figure 2. Forest plot of *DAPK1* promoter methylation and cervical cancer by calculating of OR value based on random effect model

In our study reported that a meta-analysis of 21 published studies on 2611 samples, including 1600 cancerous samples and 1011 non-cancerous samples. As the results, we reported that the *DAPK* would appear the promising methylated gene for cervical screening, which was similar to the previous study of Agodi, Barchitta, Quattrocchi, Maugeri, and Vinciguerra (2015) summarized the results of 20 published studies relevant to *DAPK* methylation, from 2001 to 2004, on 1929 samples. Because of the moderate heterogeneity between studies, the subgroup analyses by methylation detection method, sources of cancerous samples used to detect the *DAPK* methylation were performed. In the methylation detection method, a significant association between *DAPK* methylation and cervical cancer was observed between the MSP and QMSP in the random-effect method. The higher OR was observed in MSP method (MSP: OR = 21.41, 95% CI = 7.03-65.19; QMSP: OR = 20.45, 95% CI = 5.61-74.51). It could be explained that MSP method was considered to be "gold standard" method for methylation detection within reported results as methylated and/or unmethylated on the DNA sequence. The subgroup analysis by sources of samples revealed a significant association in subgroups, in detail, the OR was highly observed in both biopsy and scrape samples and the heterogeneity in the scrape subgroup was moderately low (I^2 = 47.96%). Finally, the *DAPK* methylation was the significant association revealed to ethnicity towards European country.

5. Conclusion

The methylation of *DAPK* was reported as the epigenetic characteristic in many human cancers, including cervical cancer. The present meta-analysis provides evidence to conclude that the a significantly strong correlation between *DAPK* tumor suppressor gene hypermethylation in cervical cancer.

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