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ORIGINAL RESEARCH

The association between methylated *CDKN2A* and cervical carcinogenesis, and its diagnostic value in cervical cancer: a meta-analysis

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Background: Cervical cancer is the second deadliest gynecologic malignancy, characterized by apparently precancerous lesions and cervical intraepithelial neoplasia (CIN), and having a long course from the development of CIN to cervical cancer. Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) is a well-documented tumor suppressor gene and is commonly methylated in cervical cancer. However, the relationship between methylated *CDKN2A* and carcinogenesis in cervical cancer is inconsistent, and the diagnostic accuracy of methylated *CDKN2A* is underinvestigated. In this study, we attempted to quantify the association between *CDKN2A* methylation and the carcinogenesis of cervical cancer, and its diagnostic power.

Methods: We systematically reviewed four electronic databases and identified 26 studies involving 1,490 cervical cancers, 1,291 CINs, and 964 controls. A pooled odds ratio (OR) with corresponding 95% confidence intervals (95% CI) was calculated to evaluate the association between methylated *CDKN2A* and the carcinogenesis of cervical cancer. Specificity, sensitivity, the area under the receiver operating characteristic curve, and the diagnostic odds ratio were computed to assess the effect of methylated *CDKN2A* in the diagnosis of cervical cancer.

Results: Our results indicated an upward trend in the methylation frequency of *CDKN2A* in the carcinogenesis of cervical cancer (cancer vs control: OR =23.67, 95% CI =15.54–36.06; cancer vs CIN: OR =2.53, 95% CI =1.79–3.5; CIN vs control: OR =9.68, 95% CI =5.82–16.02). The specificity, sensitivity, area under the receiver operating characteristic curve, and diagnostic odds ratio were 0.99 (95% CI: 0.97–0.99), 0.36 (95% CI: 0.28–0.45), 0.93 (95% CI: 0.91–0.95), and 43 (95% CI: 19–98), respectively.

Conclusion: Our findings indicate that abnormal *CDKN2A* methylation may be strongly correlated with the pathogenesis of cervical cancer. Our results also demonstrate that *CDKN2A* methylation might serve as an early detector of cervical cancer. These findings require further confirmation.

Keywords: p16, methylation, cervical cancer carcinogenesis

Introduction

Cervical cancer remains the second most common cancer in women worldwide. According to a report by the American Cancer Society, 12,990 new cervical cancers and 4,120 new deaths are projected to occur in the US in 2016,[1] although a substantially increasing incidence of cervical cancer has been seen in developing countries, which might be due to the inadequacy of the Pap screening test in these areas.[2]

The etiology of cervical cancer is related to interactions between host and environmental factors, such as contraceptive use,[3] infection with certain types of human papillomavirus (HPV),[4] having sex at an early age, and having many sexual partners.[5,6]

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Of all these related components, continuous infection with oncogenic HPV is an indisputable etiologic factor for cervical cancer.[7] However, most infections have no clinical syndrome and eventually resolve unaided. In a minority of infected women they may lead to the precancerous lesion of cervical cancer, cervical intraepithelial neoplasia (CIN). Typically, CIN expands slowly and takes several decades to progress into invasive cervical cancer, which is a good model for a multistage disease beginning with low-grade CIN and progressing to high-grade CIN, some of which develop into invasive cancers.[8] Most epidemiological studies have demonstrated that low-grade CIN is self-limiting, and that only a small subset with persistent HPV infection progress to high-grade CIN and eventually to invasive cervical cancer,[9] thereby attesting that HPV infection alone is not sufficient and that other factors that might accelerate the initiation of cervical cancer are required.[10]

Cancer is a group of disorders with different biological processes caused by a series of changes in tumor suppressor genes (TSGs),[11] including genetic changes and epigenetic alterations. In recent decades, great advances have been made in the identification of epigenetic changes in cancer, especially in characterizing the methylation of deoxyribonucleic acid (DNA).[12] Numerous data suggest that cancer is substantially affected by the methylation of multiple TSGs.[13,14]

Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) was first reported in early 1994;[15] it belongs to a family of cell cycle regulators[16] and is widely accepted as a TSG, owing to its ability to inhibit the catalytic activity of CDK4/cyclin D enzymes[17] and to block cell cycle progression at the G1/S checkpoint.[18] However, the loss of *CDKN2A* function due mainly to promoter hypermethylation is common in human cancers, including colorectal cancer,[19] hepatocellular carcinoma,[20] gastric carcinoma,[21] and breast cancer.[22] The diagnostic accuracy of methylated *CDKN2A* in discriminating cancer cells from normal tissues has also been investigated. The two requirements for a diagnostic biomarker, specificity and sensitivity, have been investigated in many cancers, demonstrating, for example, a 27% specificity and 70% sensitivity in colorectal cancer, and 100% specificity with 73% sensitivity in the serum of patients with liver cancer.[18,23,24]

As regards cervical cancer, most studies have demonstrated that the methylation frequency of *CDKN2A* was significantly higher in cervical cancer than in normal or benign tissue.[25,26] However, the changing trend in the methylation frequency of *CDKN2A* during the carcinogenesis of cervical cancer is contradictory. Some studies have shown that the upward methylation frequency of *CDKN2A* is observed during the carcinogenesis of cervical cancer,[27,28] whereas others have shown inconsistent results.[29] Also, the diagnostic accuracy of methylated *CDKN2A* in the differentiation of cervical cancer is underreported. Therefore, it is essential to combine these data in order to draw reliable conclusions and analyze its diagnostic power.

Meta-analysis is able to combine data from various studies and help establish relationships across studies[30] and can therefore predict a relatively reliable result through quantitative assessment. Therefore, we carried out a meta-analysis to improve our understanding of the role of methylated *CDKN2A* in the carcinogenesis of cervical cancer. The diagnostic accuracy of methylated *CDKN2A* in the discrimination of cervical cancer was also analyzed.

Materials and methods

Literature search

Four electronic databases, PubMed, Web of Science, Embase, and China National Knowledge Infrastructure, were searched for relevant studies until January 19, 2016 using the following keywords: ("*CDKN2A*" or "cyclin-dependent kinase inhibitor 2A" or "*p16*") and ("methylation" or "DNA methylation" or "promoter methylation") and ("cervical cancer" or "cervical carcinoma" or "cancer of uterine cervix").

Selection criteria

A study could be included if it met the following criteria: 1) it should be a case–control study; 2) it should analyze the methylation status of *CDKN2A* during the progression of cervical cancer; 3) it should test the methylation level of *CDKN2A* in human tissues; and 4) it should represent the available methylation data. No studies in cell lines or in animals have been included in the current study. In the current study, the CIN group consists of patients with CIN1, CIN2, and CIN3; normal individuals or patients with benign lesions were included in the control group.

Data extraction

Four authors (MY, HZ, JY, and CCZ) retrieved all the eligible studies and extracted the relevant data independently. The following information was extracted: the first author's name, the year of publication, the ethnic origins of study subjects, the number of participants, and the frequency of *CDKN2A* methylation.

Statistical analysis

The strength of association is represented as an overall odds ratio (OR) with corresponding 95% confidence interval (CI).

The heterogeneity of all eligible studies was quantified using the I^2 statistic and χ^2 tests, with corresponding *P*-value.[31] When there was heterogeneity in the meta-analysis a Dersimonian–Laird model (D+L) was applied to calculate a pooled OR ($I^2>50\%$, χ^2 test with $P<0.05$), otherwise, a Mantel–Haenszel (M–H) model was applied for the meta-analysis.[32] The source of heterogeneity was explored by meta-regression. If the source was uncertain, a sensitivity analysis was performed to assess the stability of the results by omitting a single study in the meta-analysis iteration to determine the effect of the individual data on the overall pooled OR. The stability of our results was also tested by switching the two models, D+L and M–H. Publication bias was quantitatively estimated with Begg's linear regression test. If a possible publication bias existed, the meta-trim method[33] and failsafe number (N_{fs}) were used to reestimate the effect.[34] Diagnostic meta-analyses were also performed. The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and their corresponding 95% CIs were calculated. Summary receiver operating characteristic curves with the areas under the receiver operating characteristic curve (AUC) were then generated. Data were analyzed mainly by the STATA-12.0 software (Stata Corporation, College Station, TX, USA). The N_{fs} was created using the Meta package in R (version 3.22, http://www.r-project.org/). All *P*-values are two sides and a *P*-value <0.05 was deemed statistically significant.

Results

Study characteristics

A total of 293 studies were identified using the search strategy described above, 267 of which were excluded after careful filtration. Of these, 116 studies were duplicates, 93 were without methylation data, 30 were abstracts or reviews, and 28 were irrelevant. Finally, 26 studies (ten published in English and 16 in Chinese) were included in the meta-analysis. The basic characteristics of all the included studies are shown in Table 1, and the selection process is illustrated in Figure 1.

Comparison of the methylation frequency of *CDKN2A* between cervical cancer and controls

Our study was quantitatively synthesized across 26 studies including 1,490 cervical cancers and 964 controls. For the

Table 1 The basic characteristics of all included studies

Author	Year	Country	Method	Cervical cancer patients (n)	CIN patients (n)	Control individuals (n)
Blanco-Luquin et al[51]	2015	Spain	MSP	67	95	13
Dong et al[10]	2001	Korea	MSP	53	NA	24
Narayan et al[52]	2003	Germany/Colombia	MSP	82	NA	8
Lea et al[53]	2004	USA	MSP	60	30	78
Kang et al[54]	2005	Korea	MSP	82	NA	17
Lin et al[55]	2005	Korea	MSP	67	30	20
Kim et al[56]	2005	Korea	MSP	41	30	11
Kim et al[57]	2010	Korea	MSP	69	99	41
Banzai et al[29]	2014	Japan	MSP	53	22	24
Carestiato et al[27]	2013	Brazil	MSP	29	84	28
Si et al[58]	2012	China	MSP	60	98	40
Xu et al[59]	2007	China	MSP	40	80	20
Yao et al[60]	2012	China	MSP	25	90	10
Ren et al[61]	2007	China	MSP	36	76	71
Li et al[62]	2015	China	MSP	100	NA	90
Liu et al[63]	2012	China	MSP	45	NA	10
Guan et al[64]	2008	China	MSP	60	NA	48
Wu et al[65]	2013	China	MSP	64	110	80
Ji et al[66]	2005	China	MSP	60	NA	70
Chen et al[67]	2008	China	MSP	40	NA	20
Wang[68]	2011	China	MSP	40	NA	10
Hao[69]	2011	China	MSP	80	105	53
Li[70]	2012	China	MSP	100	142	108
Lin et al[71]	2007	China	MSP	40	80	20
Jiao[72]	2012	China	MSP	30	90	30
Lin et al[73]	2007	Korea	MSP	67	30	20

Abbreviations: CIN, cervical intraepithelial neoplasia; NA, not available; MSP, methylation-specific PCR.

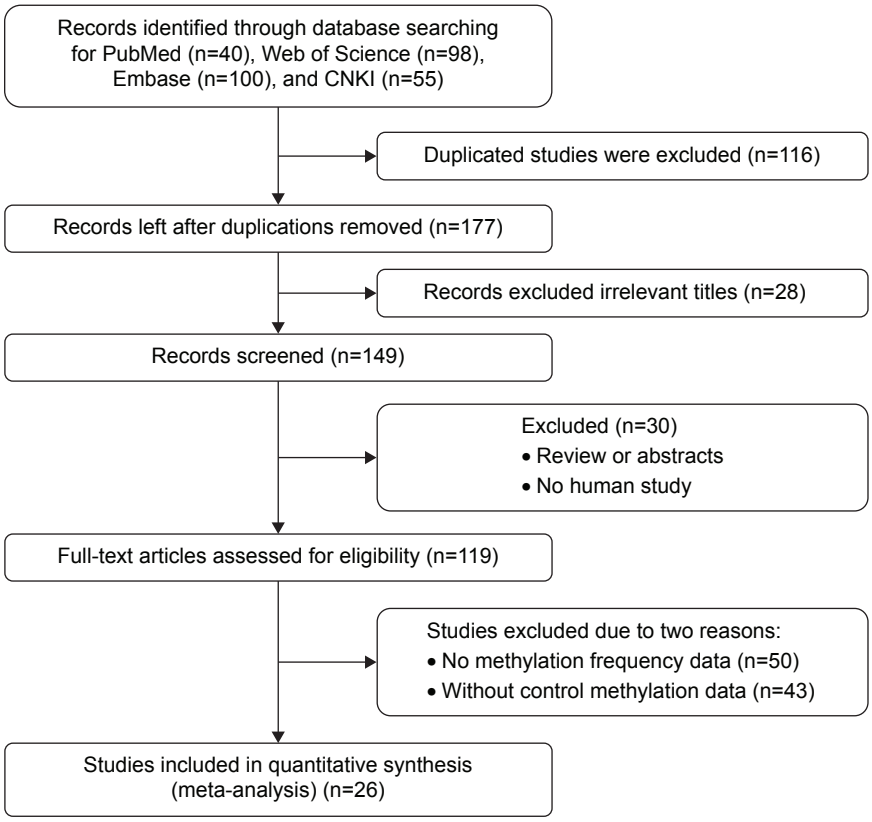


Figure 1 The flow diagram of the stepwise selection from relevant studies.
Abbreviation: CNKI, China National Knowledge Infrastructure.

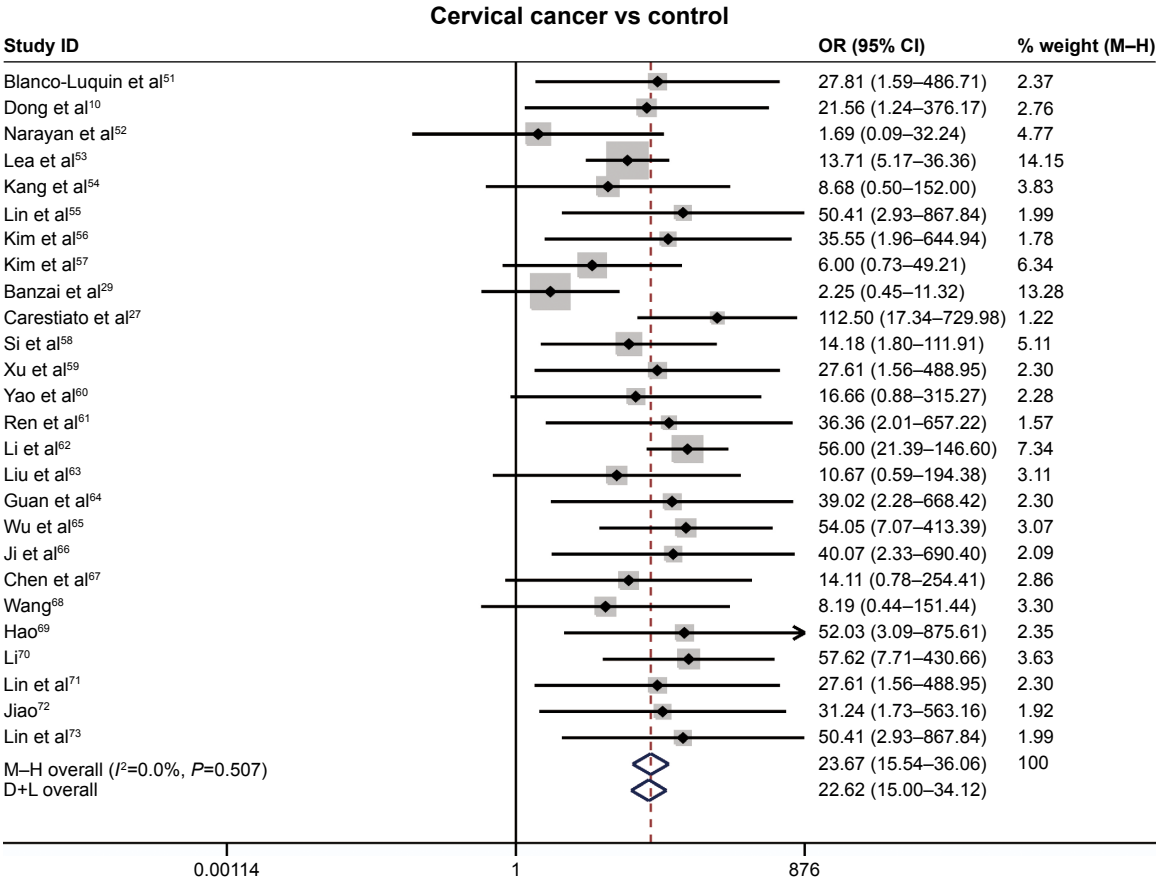
absence of heterogeneity (I^2=0%, χ^2=24.21, P=0.51), a D+L model was applied to calculate the association of methylated *CDKN2A* with cervical cancer. Our results indicated that the frequency of methylated *CDKN2A* in cervical cancers was significantly higher than in controls (OR =23.67, 95% CI =15.54–36.06, Figure 2). Similar results could be observed by switching to the M–H model to recalculate the pooled OR (Figure 2), and a sensitivity analysis was performed (Table 2), suggesting the stability and credibility of our results. However, the Begg's test implied the presence of publication bias (P=0.02, Figure 3). To adjust for this, a trim-and-fill method was implemented (Figure 4). After filling eight missing studies the pooled OR was similar to our previous results, indicating the stability of our results. Furthermore, we applied an N_{fs} to assess the efficacy of the meta-analysis ($N_{fs0.05}$=1,744, $N_{fs0.01}$=859), which indicated that our results were robust.

Comparison of the methylation frequency of *CDKN2A* between cervical cancer and CIN

Our analysis covered 17 studies involving 928 cervical cancers and 1,291 CINs. For the presence of heterogeneity (I^2=61.5%, χ^2=41.6, P<0.001), the M–H model was used. Our results showed that a higher frequency of methylated *CDKN2A* was observed in cervical cancers than in CINs (OR =2.53, 95% CI =1.79–3.5, Figure 2). To confirm the existence of heterogeneity among all relevant studies, a meta-regression was performed which showed that no single factor was responsible for the heterogeneity (Table 3). The pooled OR was not significantly changed by switching to the D+L model (Figure 2). The sensitivity analysis results further implied the stability and reliability of our results (Table 4). The Begg's test for publication bias was not statistically significant (P=0.23, Figure 3).

Comparison of the methylation frequency of *CDKN2A* between CINs and controls

The association between methylated *CDKN2A* and CIN was analyzed in 17 studies including 1,291 CINs and 667 controls. The pooled OR was computed by a D+L model, as no heterogeneity was observed (I^2=0.0%, χ^2=7.54, P=0.96). Our results demonstrated that the methylation frequency of *CDKN2A* was significantly elevated in CIN relative to the controls (OR =9.68, 95% CI =5.82–16.02, Figure 2) and the pooled OR was not significantly transformed by the M–H model



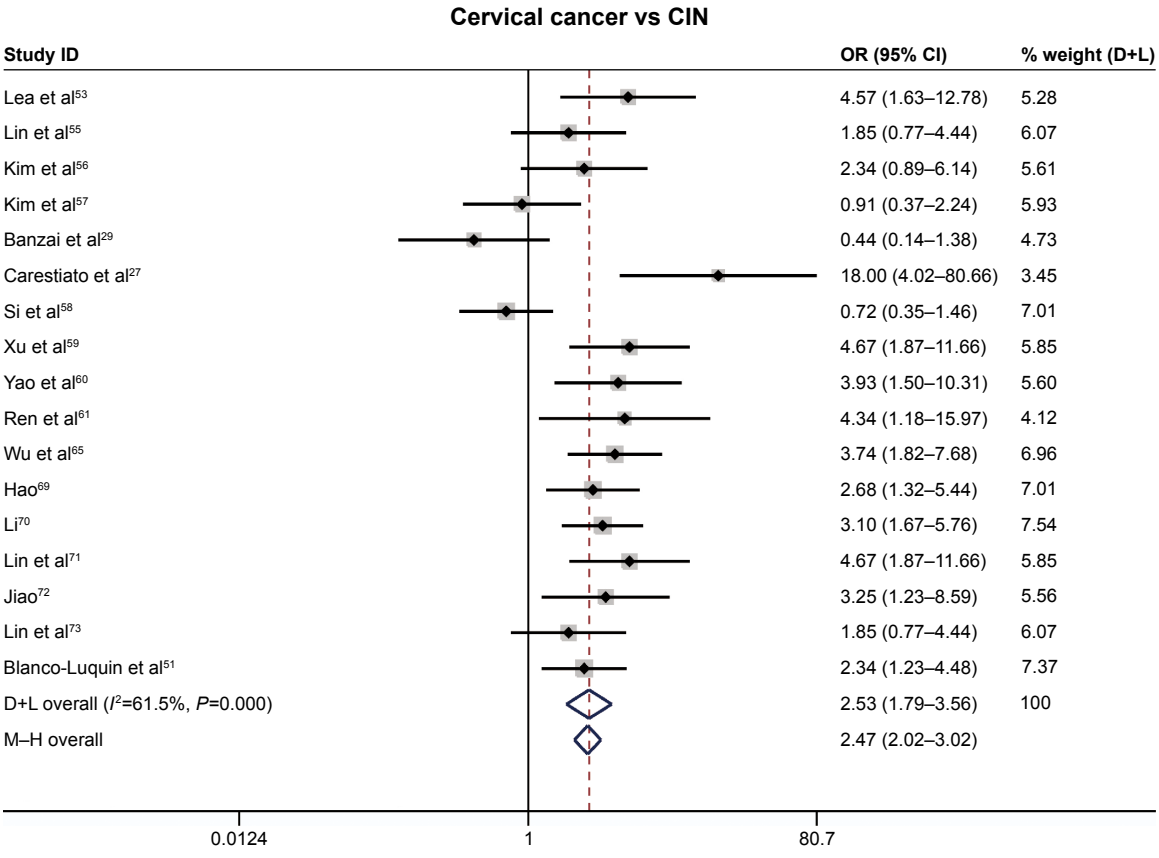


Figure 2 (*Continued*)

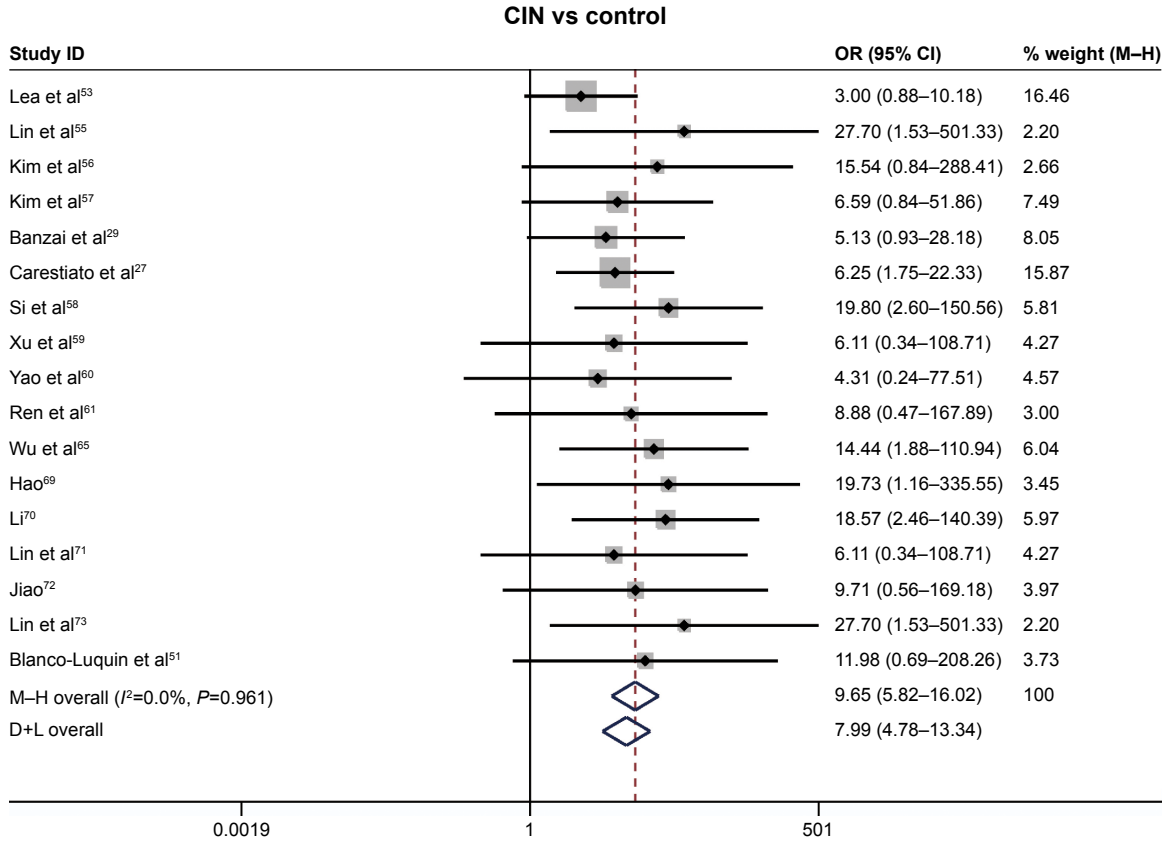


Figure 2 Forest plot of *CDKN2A* methylation status during the carcinogenesis of cervical cancer.
Note: Weights are from random effects analysis.
Abbreviations: *CDKN2A*, cyclin-dependent kinase inhibitor 2A; CI, confidence interval; CIN, cervical intraepithelial neoplasia; OR, odds ratio.

Table 2 Sensitivity analysis of pooled OR for *CDKN2A* methylation between cervical cancer and control

Study excluded	Year	Estimated OR (95% CI)
Blanco-Luquin et al[51]	2015	23.57 (15.40–36.07)
Dong et al[10]	2001	23.73 (15.51–36.31)
Narayan et al[52]	2003	24.77 (16.19–37.89)
Lea et al[53]	2004	25.31 (15.92–40.22)
Kang et al[54]	2005	24.26 (15.86–37.12)
Lin et al[55]	2005	23.12 (15.11–35.39)
Kim et al[56]	2005	23.45 (15.32–35.89)
Kim et al[57]	2010	24.86 (16.16–38.26)
Banzai et al[29]	2014	26.95 (17.32–41.93)
Carestiato et al[27]	2013	22.56 (14.69–34.65)
Si et al[58]	2012	24.18 (15.72–37.19)
Xu et al[59]	2007	23.58 (15.41–36.08)
Yao et al[60]	2012	23.83 (15.57–36.48)
Ren et al[61]	2007	23.47 (15.34–35.91)
Li et al[62]	2015	21.11 (13.39–33.26)
Liu et al[63]	2012	24.09 (15.74–36.87)
Guan et al[64]	2008	23.31 (15.23–35.67)
Wu et al[65]	2013	22.71 (14.77–34.92)
Ji et al[66]	2005	23.32 (15.24–35.69)
Chen et al[67]	2008	23.95 (15.65–36.66)
Wang[68]	2011	24.20 (15.81–37.04)
Hao[69]	2011	22.98 (15.02–35.17)
Li[70]	2012	22.39 (14.55–34.44)
Lin et al[71]	2007	23.58 (15.41–36.08)
Jiao[72]	2012	23.52 (15.37–35.99)
Lin et al[73]	2007	23.13 (15.11–35.39)

Abbreviations: *CDKN2A*, cyclin-dependent kinase inhibitor 2A; CI, confidence interval; OR, odds ratio.

(Figure 2). The sensitivity analysis confirmed the stability and credibility of our results (Table 5). No publication bias was observed by Begg's test (P=0.39, Figure 3).

Diagnostic accuracy of methylated *CDKN2A* in distinguishing cervical cancer from controls

The diagnostic accuracy of methylated *CDKN2A* in the detection of cervical cancer was analyzed from 26 studies involving 1,490 cervical cancers and 964 controls. The summary specificity and sensitivity of methylated *CDKN2A* for distinguishing cervical cancer from controls were 0.99 (95% CI: 0.97–0.99) and 0.36 (95% CI: 0.28–0.45), respectively (Figure 5). The summary receiver operating characteristic curves based on the specificity and sensitivity is shown in Figure 6, and the AUC for methylated *CDKN2A*-diagnosed cervical cancer was 0.93 (95% CI: 0.91–0.95). The summary diagnostic OR was 43 (95% CI: 19–98). The PLR and NLR were 27.9 (95% CI: 12.5–62.2) and 0.64 (95% CI: 0.57–0.73), respectively. As indicated by the value of PLR, cervical cancer patients have a nearly 28 times higher chance of having methylated *CDKN2A* than normal controls. As indicated by the value of NLR, noncancer controls have a

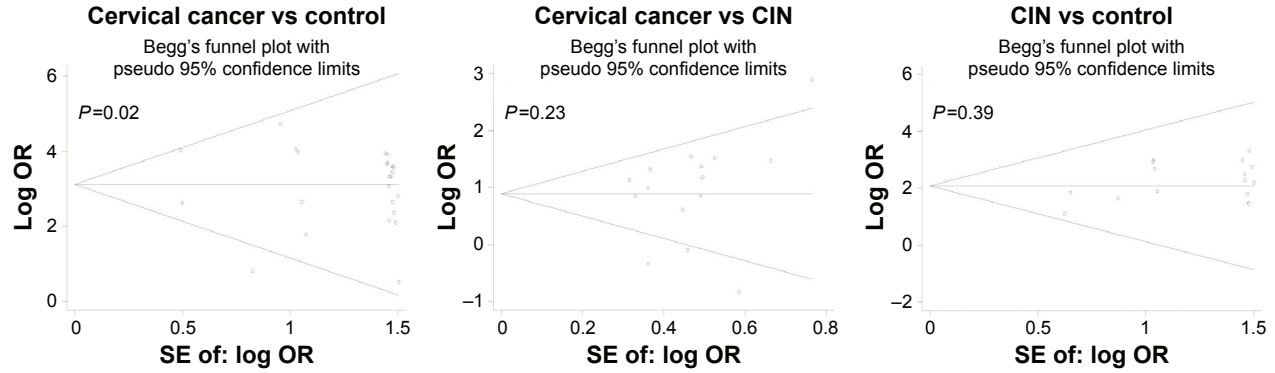


Figure 3 Funnel plot of publication biases on the relationships between abnormal *CDKN2A* promoter methylation and the pathogenesis of cervical cancer.
Abbreviations: *CDKN2A*, cyclin-dependent kinase inhibitor 2A; CIN, cervical intraepithelial neoplasia; SE, standard error; OR, odds ratio.

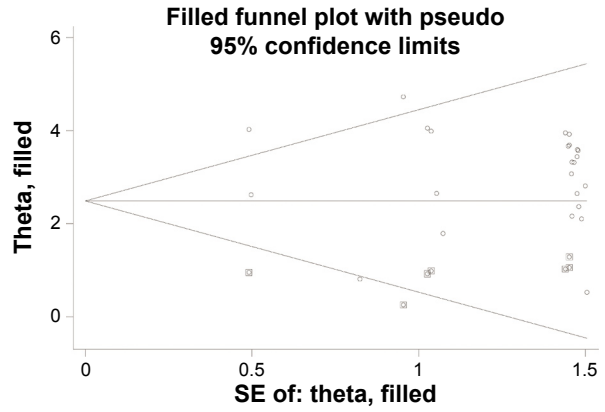


Figure 4 Begg's funnel plot of publication bias test after trim-and-fill method.
Abbreviation: SE, standard error.

1.5-fold greater chance (the reciprocal of the value of NLR) of having unmethylated *CDKN2A* than patients with cervical cancer. The Fagan plot analyses based on the PLR and NLR demonstrated that the probability of a patient being diagnosed with cervical cancer was respectively 90%, 97%, and 99% following a positive methylated *CDKN2A* result, whereas the pretest probability of being diagnosed with cervical cancer was 25%, 50%, and 75%, respectively. However, the probability of an exclusion diagnosis of cervical cancer was 82%, 51%, and 34% following a negative methylated *CDKN2A* result, namely unmethylation of *CDKN2A*. The Fagan plots are shown in Figure 7.

Table 3 Mixed effects of meta-regression analysis to identify heterogeneity source

Heterogeneity sources	Coefficient	t	*P*-value	95% CI	
				Lower	Upper
Publication year	0.043	0.65	0.521	–0.093	0.178
Case size	0.005	0.38	0.704	–0.02	0.03
Ethnicity	–0.002	0	0.998	–1.359	1.356

Abbreviation: CI, confidence interval.

Table 4 Sensitivity analysis of pooled OR for *CDKN2A* methylation between cervical cancer and CIN

Study excluded	Year	Estimated OR (95% CI)
Lea et al[53]	2004	2.41 (1.96–2.95)
Lin et al[55]	2005	2.51 (2.04–3.09)
Kim et al[56]	2005	2.48 (2.02–3.05)
Kim et al[57]	2010	2.61 (2.12–3.22)
Banzai et al[29]	2014	2.62 (2.14–3.22)
Carestiato et al[27]	2013	2.30 (1.88–2.83)
Si et al[58]	2012	2.79 (2.26–3.45)
Xu et al[59]	2007	2.39 (1.95–2.95)
Yao et al[60]	2012	2.43 (1.97–2.98)
Ren et al[61]	2007	2.44 (1.99–2.99)
Wu et al[65]	2013	2.39 (1.93–2.95)
Hao[69]	2011	2.45 (1.99–3.03)
Li[70]	2012	2.41 (1.94–2.98)
Lin[71]	2007	2.39 (1.95–2.95)
Jiao[72]	2012	2.44 (1.99–3.01)
Lin et al[73]	2007	2.51 (2.04–3.09)
Blanco-Luquin et al[51]	2015	2.49 (2.01–3.07)

Abbreviations: *CDKN2A*, cyclin-dependent kinase inhibitor 2A; CI, confidence interval; CIN, cervical intraepithelial neoplasia; OR, odds ratio.

Table 5 Sensitivity analysis of pooled OR for *CDKN2A* methylation between CIN and control

Study excluded	Year	Estimated OR (95% CI)
Lea et al[53]	2004	10.96 (6.24–19.25)
Lin et al[55]	2005	9.25 (5.52–15.48)
Kim et al[56]	2005	9.49 (5.68–15.88)
Kim et al[57]	2010	9.90 (5.87–16.69)
Banzai et al[29]	2014	10.04 (5.91–17.08)
Carestiato et al[27]	2013	10.29 (5.92–17.89)
Si et al[58]	2012	9.02 (5.34–15.24)
Xu et al[59]	2007	9.81 (5.87–16.40)
Yao et al[60]	2012	9.91 (5.93–6.56)
Ren et al[61]	2007	9.67 (5.78–16.18)
Wu et al[65]	2013	9.34 (5.54–15.77)
Hao[69]	2011	9.29 (5.55–15.55)
Li[70]	2012	9.09 (5.39–15.34)
Lin[71]	2007	9.81 (5.87–16.40)
Jiao[72]	2012	9.65 (5.77–16.13)
Lin et al[73]	2007	9.25 (5.52–15.48)
Blanco-Luquin et al[51]	2015	9.56 (5.72–15.99)

Abbreviations: *CDKN2A*, cyclin-dependent kinase inhibitor 2A; CI, confidence interval; CIN, cervical intraepithelial neoplasia; OR, odds ratio.

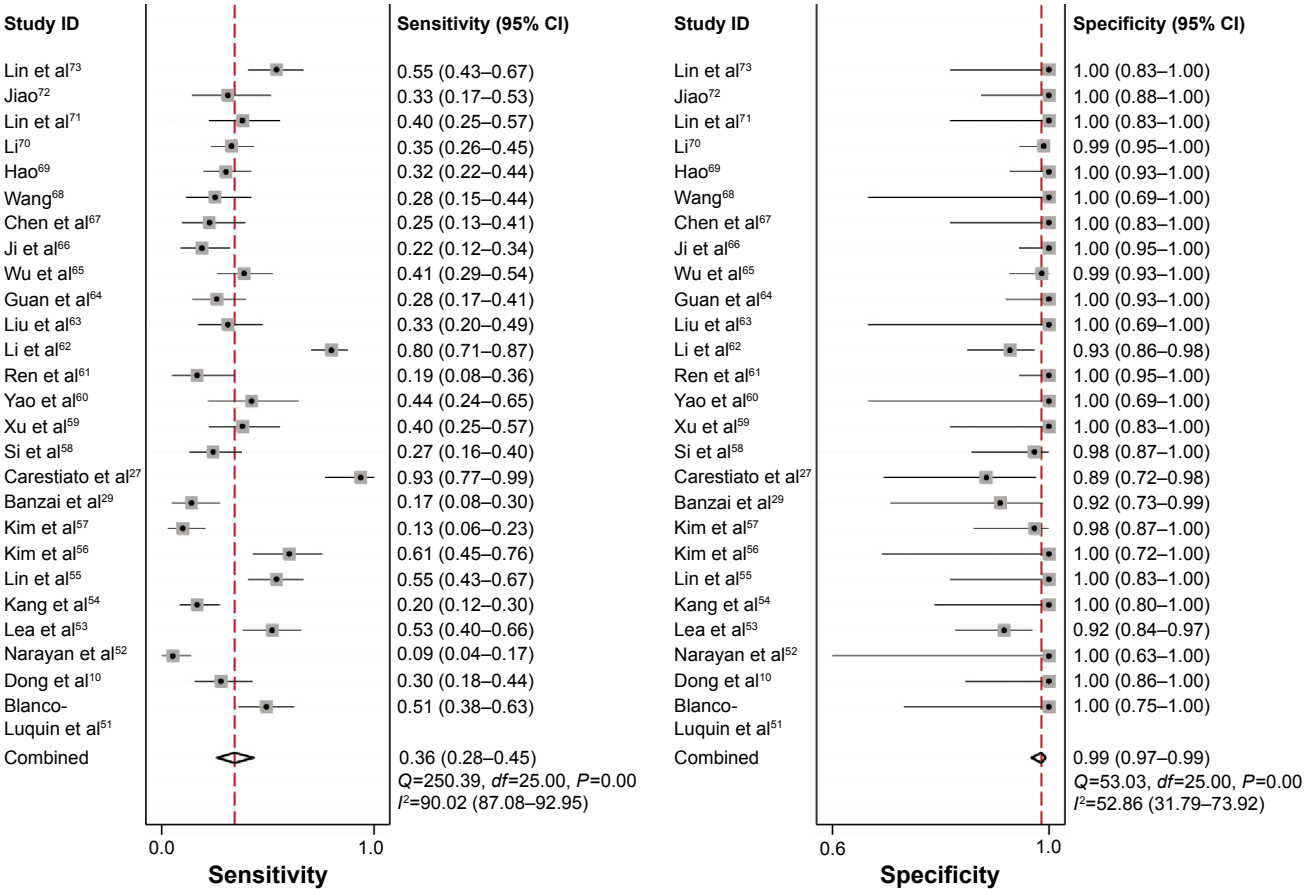


Figure 5 Forest sensitivity and specificity of methylated *CDKN2A* for differentiation of pancreatic mass.
Abbreviations: *CDKN2A*, cyclin-dependent kinase inhibitor 2A; CI, confidence interval; *df*, degrees of freedom.

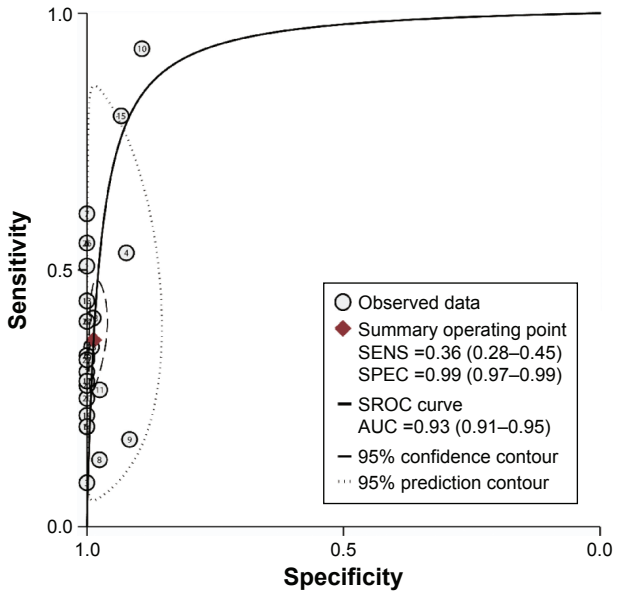


Figure 6 SROC plot with best-fitting asymmetric curve of methylated *CDKN2A* for cervical cancer diagnosis.
Abbreviations: AUC, area under the receiver operating characteristic curve; SROC, summary of receiver operating characteristic; SENS, sensitivity; SPEC, specificity.

Discussion

CDKN2A has been proved to bear a striking resemblance to classic TSGs such as *p53*, and to be an important negative regulator of cell growth and proliferation.[35] It is largely reported that a critical mechanism for silencing *CDKN2A* is hypermethylation of its regulatory region.[36] Abnormal methylation of the *CDKN2A* gene is a common event in many types of human cancer, as well as in cervical cancer.[27,37–40] However, conclusions regarding the role of methylated *CDKN2A* during the carcinogenesis of cervical cancer are inconsistent. Thus, in order to address inconsistent conclusions and to provide a better understanding of the relationship between the aberrant methylation of *CDKN2A* and the progression of cervical cancer, we performed a comprehensively quantitatively synthesized analysis across all relevant studies. Our results show that the abnormal methylation of *CDKN2A* is significantly higher in cervical cancer than in control tissues (including both normal individual tissues and benign tissues), as well as in precancerous lesions

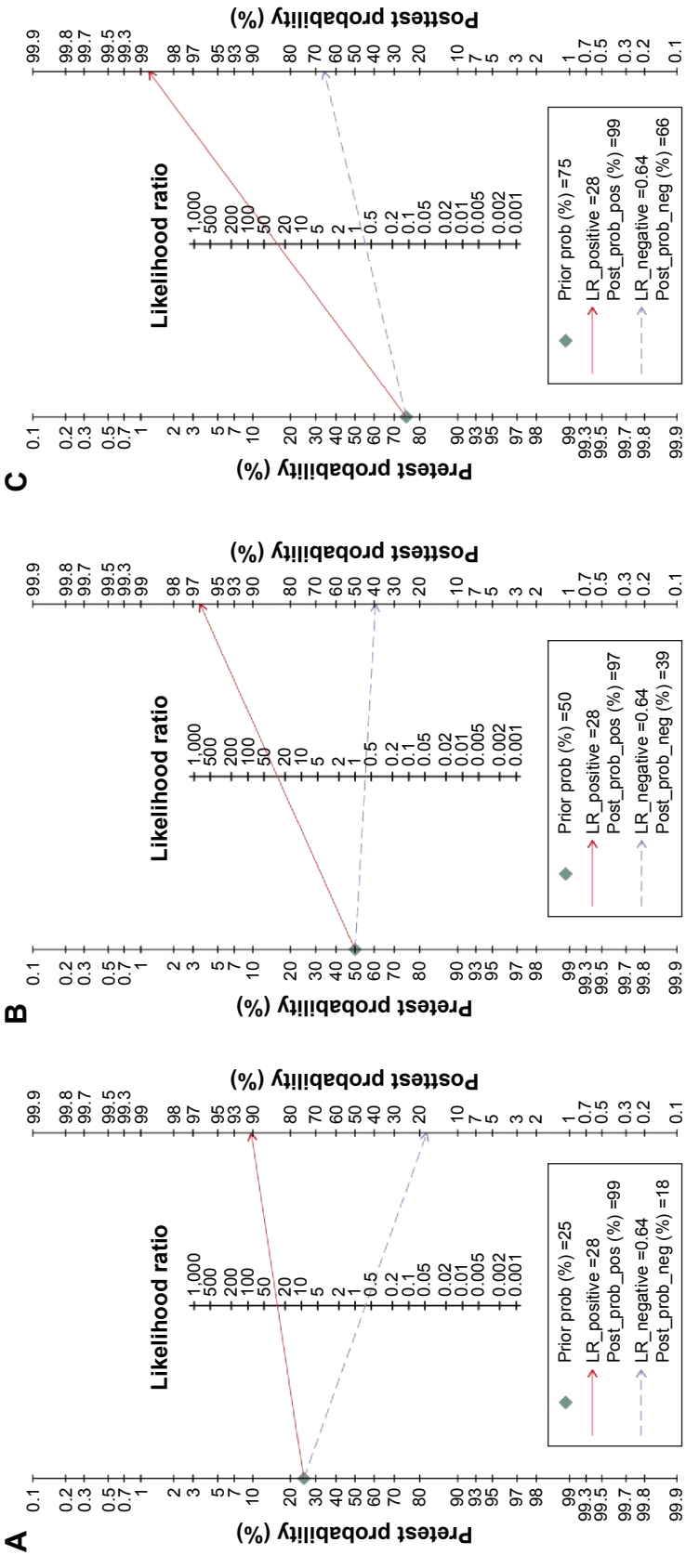


Figure 7 Fagan plot analysis to evaluate the clinical utility of methylated *CDKN2A* for identification of cervical cancer.
Note: With a pretest probability of cervical cancer of 25% (**A**), 50% (**B**), and (**C**) 75% the post-test probability of cervical cancer.
Abbreviations: *CDKN2A*, cyclin-dependent kinase inhibitor 2A; LR, likelihood ratio.

(including CIN1–CIN3), and that the frequency of *CDKN2A* methylation is higher in CINs than in control tissues. Our results suggest that abnormal *CDKN2A* methylation might be involved in the initiation and progression of cervical cancer. 5-Azacytidine was the first hypomethylating agent to be approved by the US Food and Drug Administration for the treatment of myelodysplastic syndrome,[41] and subsequent studies further confirmed its anticancer effectiveness.[42,43] Advanced studies have also demonstrated that the methylation status of cell cycle regulators is associated with the sensitivity of chemotherapeutic agents in breast cancer.[44] From this perspective, hypomethylation of methylated *CDKN2A* using a hypomethylating agent might be a whole new approach to cervical cancer therapy.

The overall 5-year survival rate of cervical cancer is 68%, whereas if patients are diagnosed when the cervical cancer is localized, the 5-year survival rate is 92%.[1] This implies that early diagnosis is of practical significance in the evaluation of survival rate. Advanced research has reported that DNA methylation is considered a vigorous tool for the diagnosis of cancers such as lung cancer,[45] and that methylation of *CDKN2A* is a diagnostic biomarker for many human cancers, including oral cancer and lung cancer.[46,47] However, the diagnostic value of methylated *CDKN2A* in cervical cancer is not so well investigated. Therefore, we performed diagnostic meta-analyses to evaluate the diagnostic performance of *CDKN2A* in detecting cervical cancer. The most commonly used terms to estimate diagnostic accuracy are specificity and sensitivity, which in our study were 0.99 and 0.36, respectively. As an independent indicator of prevalence combining the strengths of specificity and sensitivity, the DOR is the ratio of the odds of true positivity to the odds of negative positivity, which ranges from zero to infinity. A higher DOR value represents better diagnostic accuracy.[48] In our study the value of DOR was 43, supporting the good performance of methylated *CDKN2A* in distinguishing cervical cancer tissues from controls. The ROC plot is an index of diagnostic accuracy and an AUC >0.7 is deemed a good risk predictor.[49,50] In the current study the AUC is 0.93, suggesting that methylated *CDKN2A* could be an extremely useful biomarker for the detection of cervical cancer. Further, the clinical performance of *CDKN2A* methylation was explored using Fagan plots. The Fagan plot analysis results showed that a positive result for methylated *CDKN2A* could be used to distinguish cervical cancer tissue from normal tissue, with a 90% probability of diagnosing cervical cancer. Also, if the pretest probability was low there was a $>80\%$ probability that a diagnosis of cervical cancer could be ruled out following a negative result for methylated *CDKN2A*, such as unmethylated *CDKN2A*. Altogether, methylated *CDKN2A* has good diagnostic power to discriminate cervical cancer from benign or normal tissues.

However, some limitations of this study should be taken into consideration. First, studies on *CDKN2A* methylation in cervical cancer with statistical significance were more likely to be published, and unpublished studies were not included in our study. Second, relevant studies in other languages were also not included. Third, a significant heterogeneity was observed; therefore, our conclusions should be interpreted with caution.

In summary, *CDKN2A* methylation has been shown to be involved in the carcinogenesis of cervical cancer and is regarded as a useful biomarker for the identification of cervical cancer. Future large-scale studies, especially regarding the accurate evaluation of *CDKN2A* methylation, are required to verify our conclusions.

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Author contributions

JL, CCZ, HJZ, MY: Conceived and designed the experiments. JL, CCZ, TLB, TJG: Performed the experiments. JL CCZ: Analyzed the data. CCZ: Contributed analysis tools. JL, CCZ, MY: Wrote the manuscript. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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