Association between Gene Promoter Methylation and Cervical Cancer Development: Global Distribution and A Meta-analysis

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ABSTRACT

DNA methylation is the main epigenetic event for gene silencing and is associated with carcinogenesis. In this meta-analysis, we evaluated the association between the methylation of the promoter regions of *APC, CADM1, CCNA1, CDH1, DAPK, FHIT, HIC1, MAL, MGMT, hMLH1, P16, PAX1, RAR-β*, and *RASSF1* genes and the risk of cervical cancer development and progression. Overall, 194 eligible studies were identified assessing the associations of promoter methylation status of aforementioned genes with low- and high-grade squamous intraepithelial lesions (LSIL and HSIL) and cervical cancer development. The majority of studies were conducted on Caucasian and Asian populations, whereas rare studies were available on the African population. Promoter methylation frequencies were shown to be significantly higher in LSIL and HSIL cervical cancer cases as compared to control specimens for *CADM1, CCNA1, CDH1, DAPK1, FHIT, MAL, P16, PAX1, RAR-β*, and *RASSF1* genes. A moderate association was found between *HIC* promoter methylation, whereas *APC, MGMT*, and *hMLH1* promoter methylation was not correlated with cervical cancer development. Promoter methylation could be considered as a noninvasive biomarker for early cervical lesions, making them highly promising targets for a personalized therapeutic approach.

Introduction

Cervical cancer is the second most frequent gynecologic malignancy in the world, ranking the fourth most frequently diagnosed cancer and the fourth leading cause of cancer-related deaths (1). According to GLOBOCAN, it accounted for approximately 570,000 new cases and more than 311,000 deaths in 2018, worldwide (1). Cervical cancer arises from the normal epithelium by distinct morphologic changes consisting of two major histotypes: squamous cell carcinoma and adenocarcinoma, accounting for 85%–90% and 10%–25% of cases, respectively (2). The progression to carcinoma is well documented and goes through well-defined preinvasive lesions (3). Cervical cancer epidemiologic and molecular studies have shown that the persistence of human papillomavirus (HPV) is the main etiologic factor for cervical carcinogenesis, but not exclusive (4). Additional genetic and epigenetic alterations are required for invasive cancer development from precancerous lesions (5). It is widely reported that epigenetic modifications, involving DNA methylation, histone modifications, nucleosome positioning, and noncoding RNAs, are associated with cancer development and progression (6, 7). Moreover, DNA methylation, considered as an important epigenetic mechanism for gene silencing, tends to accumulate with disease severity (8).

In the human genome, 60% of all promoters are associated with CpG islands and approximately 90% are unmethylated in the untransformed cells (9, 10). Conversely, in cancer, the methylation of CpG regions of many genes promoters is higher (11). Significantly, many of the inactivated genes are tumor suppressor genes and the inhibition of these genes expression by methylation is involved in cancer initiation, development, and progression (12).

Hypermethylation of the CpG islands of gene promoter is an important epigenetic mechanism for gene silencing, which may confer tumor cells of growth advantage (13). This epigenetic event leads to inhibition or inactivation of many cell signaling pathways. Indeed, hypermethylation of promoters may lead to the silencing of genes affecting important cellular signaling pathways, including apoptosis, cell cycle, DNA repair, cell adhesion, and detoxification (14). There is ample evidence indicating that hypermethylation of promoter regions of certain genes, such as *DAPK1* (15), *cyclins A1* (16), and *FHIT* (17), which are involved in cell cycle control and regulation, leads to apoptosis inhibition. Also, methylation of promoters of genes involved in cell adhesion, such as *CDH1* and *CADM1*, leads to downregulation, thus leading to an increased risk of cervical cancer (18). Likewise, this methylation also silences other genes, such as *RAR-β2, RASSF1*, and *APC*, involved in cell signaling (19–21).

Scientific evidence has clearly reported that epigenetic alterations linked to hypermethylation affect the CpG islands on the human genome and these alterations are not randomly distributed during carcinogenesis, but they affect specific genes involved in specific signaling pathways (22). Therefore, assessment of the methylation status of such genes is of a great interest to set up molecular signatures that could be used for diagnosis, prognosis, and/or as therapeutic targets.

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Thus, this study was planned to conduct a meta-analysis study to evaluate the hypermethylation status of some genes involved in cervical cancer development, to assess the correlation between genes hypermethylation and populations' origins and ethnicity, and to identify genes with the major impact on the onset of cervical cancer.

Materials and Methods

Publications search

Systematic search strategies were conducted via a range of online literature databases prior to February 1, 2019, including PubMed, EMBASE, EBSCO, Web of Science, and Cochrane Library. We systematically reviewed the studies of promoter methylation of 41 genes (*DcR1, DcR2, hTERT, P73, P16, PTEN, CDH1, APC, MGMT, Hmlh1, RASSF1, DAPK, FHIT, HIC1, RAR-β, TIMP2, TIMP3, CAV1, ESR1, miR-124, miR-34B, miR-203, ER1, ZNF582, ADCYAP1, SFRP1, SFRP2, SYK, CADM1, PRDM14, P53, CASP8, CCNA1, DOC2B, MAL, SOX1, PAX1, LMX1A, NKX6-1, WT1*, and *ONECUT1*) in cervical cancer.

The keywords combinations of Medical Subject Headings terms with all known genes name were ("gene name 1" or "gene name 2" or . . . or "gene name *n*") and ("methylation" or "DNA methylation" or "promoter methylation") and ("cervical cancer" or "cervical carcinoma" or "cancer of uterine cervix").

Meta-analysis

All the methodologies and procedures adopted in this work followed the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, which was published previously (23). The PRISMA flow diagram was used to include or exclude any publication found in the literature concerning the selected keywords (**Fig. 1**). Accordingly, studies were included in our meta-analysis if they met the following selection criteria: (i) studies assessing the association of the selected gene methylation with cervical cancer, (ii) detailed information about the frequency of the selected gene methylation for both the cancer group and the normal control group was reported, (iii) the methylation status was assessed on human samples, and (iv) only the most recent study with the most informative results was included in case of multiple publications for the same group and using duplicated sample data.

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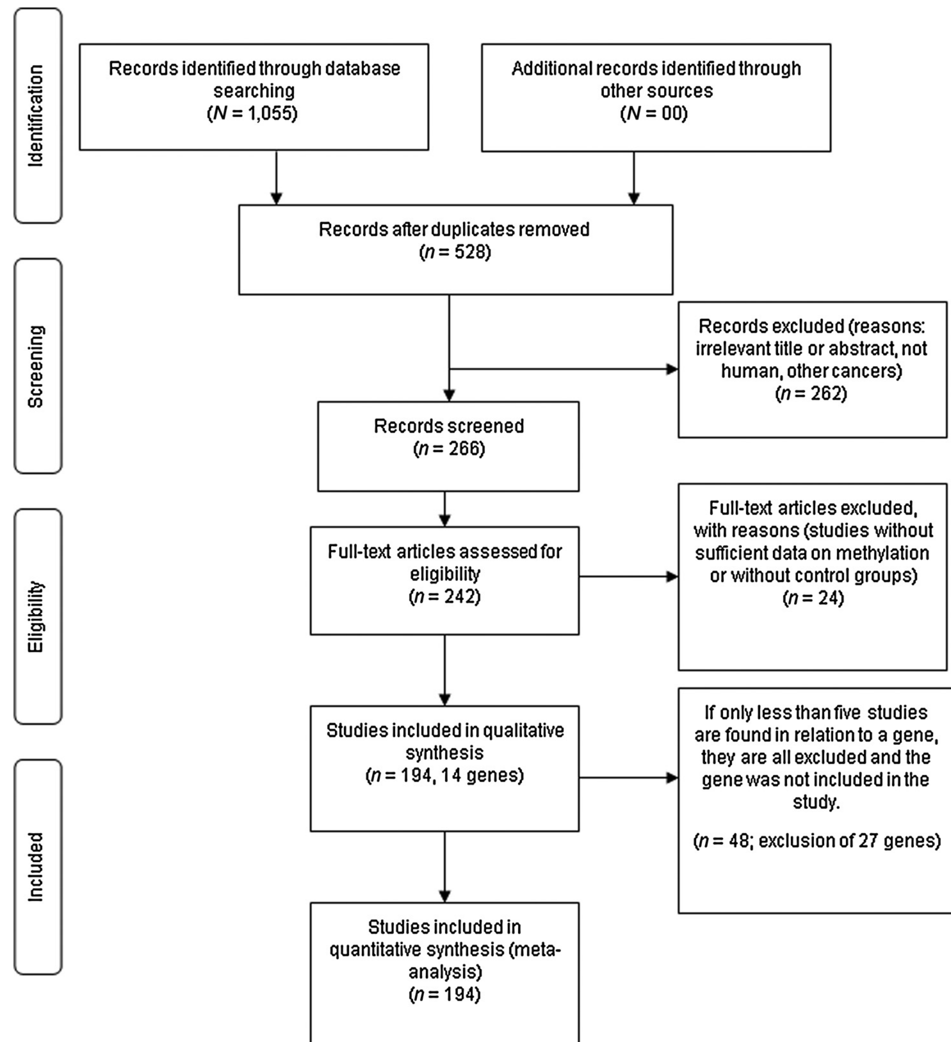

Figure 1.
Flowchart diagram of relevant studies for meta-analysis.

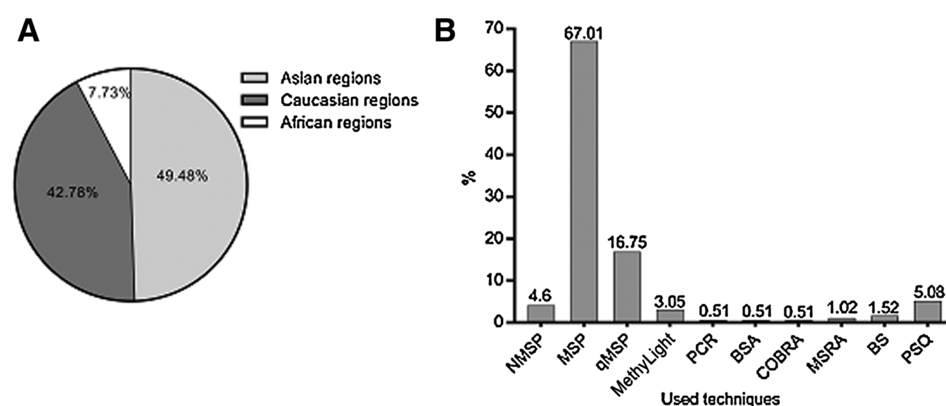

Figure 2.
Worldwide distribution of selected studies: according to geographic area (**A**) and according to the techniques used for promoter methylation assessment (**B**).

Data extraction

Available data were extracted for the selected studies by two authors independently. For each gene, all information regarding author's name, publication year, patients' ethnicities, methods used in the methylation assessment, and the number of participants in the case and control groups was extracted (Supplementary Tables S1–S16). In case of disagreements, differences were discussed by the two authors. All the selected studies used in this meta-analysis are cited in the "References" section in the Supplementary Table S17.

Data synthesis and publication bias

The strength of association is represented as an overall 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. A Mantel–Haenszel model was applied for the meta-analysis. When there was a source of heterogeneity in the meta-analysis, 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 using one of the two models, DerSimonian–Laird or Mantel–Haenszel (24). Publication bias was quantitatively estimated with Begg linear regression test. Data were analyzed by the Meta-Essentials tool (version 1.4). Graphs were generated using GraphPad Prism 8.0 (GraphPad Software, Inc.).

Results

Flow and characteristics of the included studies

A total of 194 studies were included in this meta-analysis to assess the methylation status of selected genes. In this meta-analysis, the interest was given to 14 genes known for their implication in affecting important cellular signaling pathways, including apoptosis, cell cycle, DNA repair, cell adhesion, and detoxification (14). Accordingly, apoptosis, considered as an important life process, is blocked through the hypermethylation of the promoters regions of some genes involved in cell-cycle control and regulation, including the *DAPK1* (15), *A1 cyclins* (16), and the *FHIT* (17). Other genes have been reported to be likely involved in the development of cervical cancer when they are methylated. Among them, genes involved in cell adhesion, such as *CDH1* and *CADM1*, are downregulated in cervical cancer cases following methylation of promoters (18). In addition, the *RAR-β2, RASSF1*, and *APC*, reported to play an important role in cell signaling, are affected during carcinogenesis by a genetic silencing event following methylation (19–21).

Likewise, the triggering of cervical cancer is also influenced by the methylation of the promoters of *hypermethylated in cancer 1* gene (*HIC1*), *T-lymphocyte maturation associated* gene (*MAL*), *p16*, and paired box 1 (*PAX1*), which contribute mainly as zinc-finger transcription factor (25), in apical transport of membrane (26), cell-cycle regulation (27), and embryogenesis (28), respectively. Cervical carcinogenesis was also linked to the hypermethylation of O(6)-methylguanine-DNA methyltransferase (*MGMT*) and human mutL homolog 1 (*hMLH1*), which participate in DNA repair functions (27). The exclusion of other genes from the meta-analysis was considered as an essential criterion because of the lack and scarcity of studies on them.

The distribution of selected studies according to their geographic area is reported in **Fig. 2**. Results clearly showed that most studies were conducted on Asian populations, with a total of 96 studies (49.48%), and Caucasian populations, with 83 studies (42.78%). However, only 15 studies were conducted on African populations (7.73%).

Regarding the method of promoter methylation assessment, methylation-specific PCR (MSP), considered as the "gold standard method," was used in most studies (67.01%), whereas 16.75% of studies used the quantitative methylation-specific PCR (qMSP). However, BSA and PCR were rarely used by researchers.

Distribution of methylated genes in the world population

The distribution of methylation rate in genes promoters in cancer cases and control samples is reported in **Fig. 3**. Accordingly, the methylation status of the promoter region of *PAX1* gene took interest exclusively in Asian population and was not reported in other regions. In contrast, the population of this region did not present methylation of *hMLH1* gene promoter, which was methylated only on Caucasian and African populations. Quantitively, high methylation levels were observed for *PAX1, CCNA1*, and *CADM1* genes and were reported in Asian and Caucasian populations, which also presented significative high methylation of *MAL*. *DAPK1* gene exhibited a similar methylation rate between Asian, Caucasian, and African populations, but overall, the methylation status of selected genes was variable according to the study population. Of particular interest, the methylation status was mostly higher in cancer cases as compared with control samples, excepted for *HIC1* and *APC* genes, assessed in African population, where cancer cases and normal samples shared the same methylation status (Supplementary Table S18).

Association of *p16* methylation status with cervical cancer risk

A total of 21 studies, including 13 on Asian, six on Caucasian, and two on African populations, involving 1,141 cancer cases and 1,158

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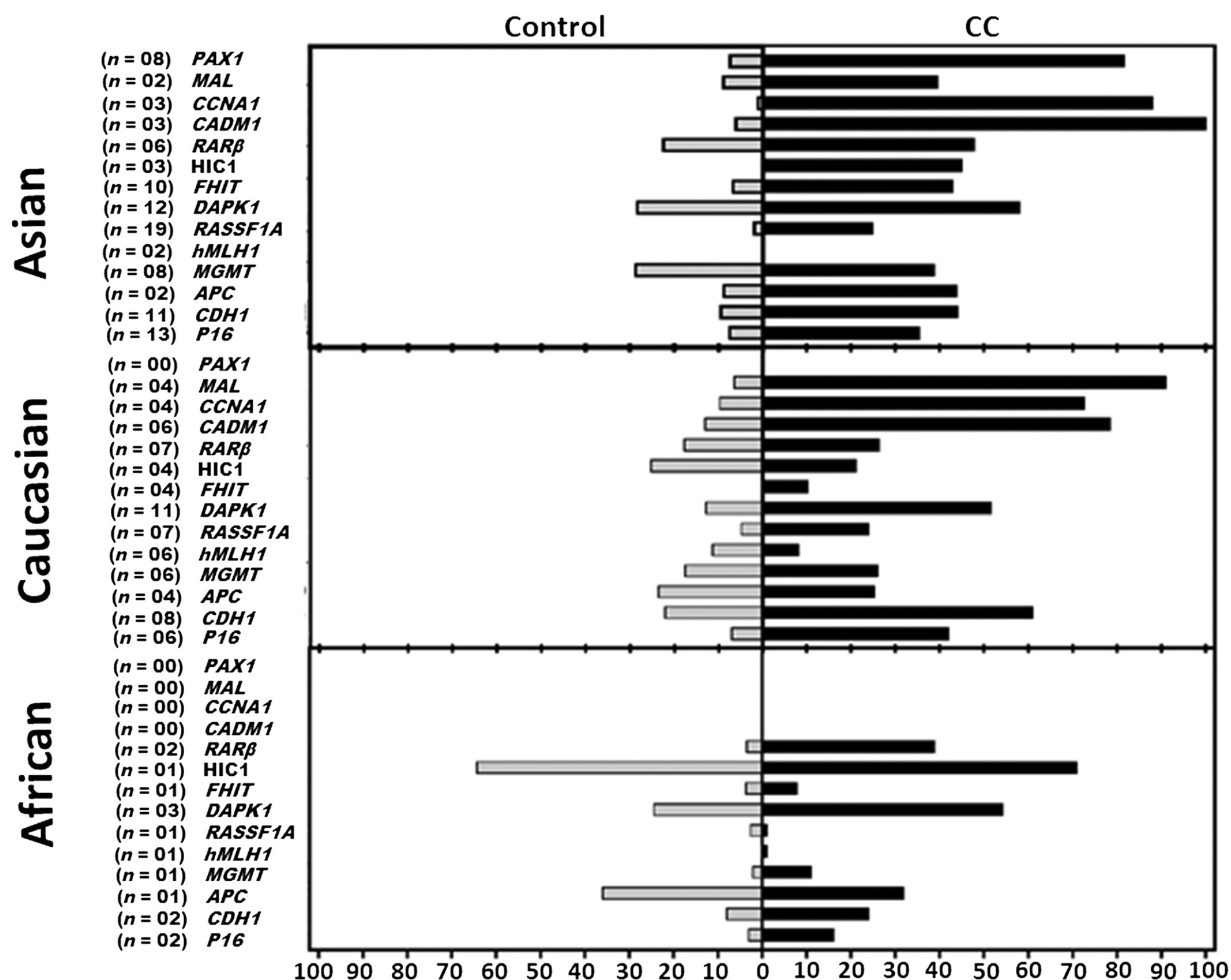

Figure 3.
Distribution of methylation rate of cancer cases and controls in different genes according to the population origin. CC, cervical cancer.

controls, were included in this analysis to assess the effect of *p16* promoter hypermethylation on cervical cancer development. These studies were mostly conducted by using MSP techniques, except two that were done with nested methylation-specific polymerase chain reaction and pyrosequencing. There was a significant association between *p16* promoter hypermethylation and increased cervical cancer risk, with an OR of 12.24 (95% CI, 6.38–23.46; Supplementary Fig. S1).

Association of *PAX1* methylation status with cervical cancer risk

The study of *PAX1* promoter gene was quantitatively synthesized across eight studies, including 271 cervical cancer cases and 355 controls. The result indicated that the frequency of methylated *PAX1* in cervical cancer samples was significantly higher than that in controls (OR, 157.99; 95% CI, 24.83–1,005.47; Supplementary Fig. S2). A statistically significant heterogeneity across the included studies was observed ($I^2 = 65.40\%$; $P < 0.001$). Thus, to confirm the existence of heterogeneity among all relevant studies, a meta-regression was performed showing that no single factor was responsible for the heterogeneity.

Association of *RAR-β* methylation status with cervical cancer risk

Overall, 15 studies, including 971 patients with cervical cancer and 1,126 controls, were performed to evaluate the relationship between *RAR-β* promoter methylation and cervical cancer development. These studies were conducted on Caucasian (7/15), Asian (6/15), and African (2/15) populations. In these studies, MSP technique was used in most studies (80%), qMSP, pyrosequencing, and MethyLight were used in one study each. Data analysis showed that *RAR-β* promoter methylation was associated with an increased cervical cancer risk, with a pooled OR of 5.36 (95% CI, 2.71–10.58), with a statistically significant heterogeneity across the included studies ($I^2 = 73.63\%$; $P < 0.000$; Supplementary Fig. S3).

Association of *RASSF1* methylation status with cervical cancer risk

In this analysis, *RASSF1* methylation status was quantitatively synthesized across 27 studies (19 on Asian population, seven on Caucasians, and one from Africa) including 1,775 cervical cancer samples and 997 controls. Random effects model was applied to

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calculate the association between *RASSF1* gene promoter methylation and cervical cancer risk. Data analysis indicated that the frequency of methylated *RASSF1* in cervical cancer samples was significantly higher than that in controls (OR, 7.23; 95% CI, 3.73–13.82). Moreover, a statistically significant heterogeneity across the included studies was reported (I^2 = 64.35%; P < 0.000; Supplementary Fig. S4).

Association of *APC* methylation status with cervical cancer risk

Overall, only seven studies were reported to have evaluated the relationship between *APC* promoter methylation status and cervical cancer risk, including 441 patients with cervical cancer and 260 controls. Studies were mainly done on Asian populations; MSP was used in four studies and qMSP in three studies. These studies highlighted a nonsignificant association between *APC* promoter hypermethylation and cervical cancer risk, with an OR of 2.07 (95% CI, 0.84–5.14). Moreover, there was statistically significant heterogeneity across the included studies (I^2 = 46.29%; Supplementary Fig. S5).

Association of *CADM1* methylation status with cervical cancer risk

A total of nine selected studies, including 339 cervical cancer specimens and 285 controls, were analyzed to evaluate the association between *CADM1* promoter methylation and cervical cancer development. On the basis of the fixed effects model, the results showed that *CADM1* promoter methylation was associated with an increased cervical cancer risk with a pooled OR of 21.73 (95% CI, 5.70–82.89; Supplementary Fig. S6). There was statistically significant heterogeneity across the included studies (I^2 = 51.01%).

Association of *CCNA1* methylation status with cervical cancer risk

Overall, seven studies have reported results on *CCNA1* methylation status and cervical cancer risk. These studies were conducted on 565 samples, including 287 cervical cancer specimens and 278 controls. The heterogeneity of the selected studies was evaluated using the Mantel–Haenszel model (I^2 = 43.30%; χ^2 = 10.58; P < 0.000) and was statistically significant. On the basis of the fixed effects model, the results showed that *CCNA1* promoter methylation was not significantly associated with cervical cancer status (OR, 71.10; 95% CI, 18.66–270.96; Supplementary Fig. S7).

Association of *CDH1* methylation status with cervical cancer risk

The association of *CDH1* methylation status with cervical cancer risk was performed in 21 studies involving 917 cervical cancer samples and 907 controls. Statistical analysis showed a significant heterogeneity across the included studies (I^2 = 66.51%; P < 0.000). Results indicated that the frequency of methylated *CDH1* in cervical cancer samples was significantly higher than that in controls (OR, 8.76; 95% CI, 3.68–20.82; Supplementary Fig. S8).

Association of *DAPK1* methylation status with cervical cancer risk

A total of 1,836 cervical cancer specimens and 1,180 controls from 26 selected articles were used to evaluate the association between *DAPK1* methylation status and cervical cancer development. On the basis of the fixed effects model, the results showed that *DAPK1* promoter methylation was associated with an increased cervical cancer risk, with a pooled OR of 21.74 (95% CI, 12.29–38.46). Moreover, there was statistically significant heterogeneity across the included studies (I^2, 86.03%; P < 0.000; Supplementary Fig. S9).

Association of *FHIT* methylation status with cervical cancer risk

To assess the association between *FHIT* promoter methylation status and cervical cancer risk, 15 studies were selected, with respect to inclusion and exclusion criteria, involving 980 cervical cancer cases and 679 controls. On the basis of the fixed effects model, results showed that *FHIT* promoter methylation was significantly associated with an increased cervical cancer risk, with a pooled OR of 7.42 (95% CI, 3.12–17.65; Supplementary Fig. S10). There was statistically significant heterogeneity across the included studies (I^2, 52.84%; P < 0.000).

Association of *HIC1* methylation status with cervical cancer risk

In this meta-analysis, only eight studies were selected to assess the association between *HIC1* methylation status and cervical cancer risk. These studies were conducted on 404 patients with cervical cancer and 336 controls, and were basically conducted using the MSP approach. Statistical analysis showed a significant heterogeneity across the included studies (I^2 = 53.78%; P < 0.000). Data analysis showed a great association between *HIC1* promoter hypermethylation and increased cervical cancer risk, with an OR of 4.09 (95% CI, 1.60–10.42; Supplementary Fig. S11).

Association of *MAL* methylation status with cervical cancer risk

In this meta-analysis, only six studies satisfied the inclusion/exclusion criteria. These studies were conducted on 253 cancer cases and 225 controls and exhibited a good heterogeneity by using the Mantel–Haenszel model (I^2 = 62.37%; χ^2 = 13.29; P < 0.000). On the basis of the fixed effects model, results clearly showed that *MAL* promoter methylation status was not significantly associated with cervical cancer development (OR, 40.95; 95% CI, 5.07–330.94; Supplementary Fig. S12).

Association of *MGMT* methylation status with cervical cancer risk

The association between *MGMT* methylation status and cervical cancer risk was evaluated in 1,145 cancer cases and 938 controls, from 15 selected studies published from 2001 to 2015. On the basis of the fixed effects model, results showed that *MGMT* promoter methylation was highly associated with an increased cervical cancer risk, with a pooled OR of 3.34 (95% CI, 1.58–7.03). Moreover, a statistically significant heterogeneity across the included studies was observed (I^2 = 68.04%; P < 0.000; Supplementary Fig. S13).

Association of *hMLH1* methylation status with cervical cancer risk

A total of nine studies, involving 441 cancer cases and 361 controls, were included in this meta-analysis. Random effects model was applied to calculate the association of methylated *hMLH1* promoter with cervical cancer risk. The results clearly showed that the frequency of methylation of *hMLH1* gene promoter in cervical cancer samples was significantly the same as that obtained with controls (OR, 1.19; 95% CI, 0.49–2.88). Moreover, there was lower

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heterogeneity across the included studies ($I^2 = 4.72\%$; $P < 0.324$; Supplementary Fig. S14).

Correlation of genes promoter methylation with clinicopathologic features of the cervix

In this meta-analysis, some of the selected articles also included data on precancerous lesions, including low- and high-grade squamous intraepithelial lesions. Therefore, we analyzed the relationships between all promoter genes methylation status and clinicopathologic features [low-grade squamous intraepithelial lesion (LSIL)/high-grade squamous intraepithelial lesion (HSIL)], taking into consideration the geographic distribution and the methylation techniques.

For instance, and to have homogeneity between studies, some articles were omitted in the data analysis. This omission was not made in a random or targeted manner to avoid any influence on the results. Indeed, all the studies were compared and only the studies presenting unique data and those that do not correlate with global literature were omitted. In addition, we selected for this omission only studies with conclusions not highlighting the importance of methylation as a cervical cancer marker [e.g., studies showing that methylation is also overexpressed in controls (patients without cervical cancer)]. In addition, the method used was also taken into consideration, if it was classic and used exclusively by one team compared with what was used by other groups, the study may be subject to omission.

Accordingly, the study conducted by Gasperove and colleagues on Caucasian population was omitted (Supplementary Table S17). Indeed, in this study the methylation rate of the *CDH1, HIC1*, and *DAPK1* promoters was abnormally higher in controls as compared with the other studies and showed no significant difference between the different clinicopathologic grades. The study conducted by Pathak and colleagues was also omitted because the *CDH1* promoter methylation frequency was significantly higher in LSIL and HSIL than in cancer cases and control tissues (Supplementary Table S18). With the omission of the study conducted by Sun and colleagues (which highlighted the overexpression of methylation of *DAPK1* and *MGMT* in controls), studies exploring the methylation status of *DAPK1* and *MGMT* promoters regions in precancerous and cancerous cervical lesions were highly homogenous (Supplementary Table S18). Moreover, by deleting the Bai and colleagues' study, the results showed that *FHIT* promoter methylation was significantly correlated with histologic subtype (LSIL and HSIL; Supplementary Table S18).

Deletion of the study by Widschwendter and colleagues made the other studies, evaluating the methylation status of *RASSF1* in precancerous lesions, cancer cases, and control tissues, highly homogenous (Supplementary Table S18).

Finally, for *MAL* gene promoter methylation, the omission of the study by Kim and colleagues (who concluded that methylation of *MAL* gene promoter was lower in cervical cancer, 4/48), made the studies analyzing LSIL cases highly homogenous (Supplementary Table S18).

Results are summarized in **Table 1** and showed that many methylation statuses of promoter genes were correlated with clinicopathologic features. Of particular interest, methylation of *p16, CDH1, RASSF1, RAR-β, DAPK1, FHIT, CADM1, MAL, CCNA1*, and *PAX1* genes promoters was significantly higher in cervical cases than in controls, and total events increased according to the lesion progression. Indeed, for these genes, total methylation events were lower in low grades and significantly higher in advanced stages (**Table 1**). For *hMLH1* and *APC* genes, no significant difference was observed between cervical cases, including LSIL, HSIL, and cancer cases, with corresponding controls. Methylation events were similar between low grade, high grade, and cancer cases and lower as compared with corresponding controls. Finally, methylation statuses of *MGMT* and *HIC1* genes promoters were slightly higher than corresponding controls, but no significant difference was observed between low grade, high grade, and cancer cases for both of them.

Publication bias

Begg funnel plot with pseudo 95% confidence limits and Egger test were applied to estimate the publication bias of the included literature. Begg and Egger tests revealed an obvious asymmetry in the overall meta-analysis for the most included genes (Supplementary Figs. S15–S17).

Begg funnel plot with pseudo 95% confidence limits and Egger test were also applied to estimate the publication bias with regard to clinicopathologic features (Supplementary Figs. S18–S27).

Discussion

During cervical carcinogenesis, different genetic and epigenetic alterations occur in both the HPV and the host genomes (29). The main epigenetic alterations are the global DNA hypomethylation and hypermethylation of tumor suppressor genes involved in different cell signaling pathways: DNA repair, cell adhesion, nuclear receptors cell cycle, and checkpoint controls (30, 31). The great interest given to the evaluation of the methylation status of some genes was started with the discovery of demethylating agents able to reactivate the expression of hypermethylated and silenced tumor suppressor genes in tumor tissues (32). Since then, detection of epigenetic changes in cytologic smears had presented a potential interest for assessment of new molecular biomarkers that could be used for early detection, prognosis, and/or as vaccine or therapeutic targets.

In this meta-analysis, we have tried to provide a general and global picture about the methylation profile of genes involved in cervical cancer development and progression. A total of 194 worldwide studies were included in this study and analyzed using a model considering that promoter methylation could be a tissue-specific event, providing a subgroup analysis by histologic tumor type. In this study, the analysis has focused on the methylation status of the promoters of *APC, CADM1, CCNA1, CDH1, DAPK, FHIT, HIC1, MAL, MGMT, hMLH1, p16, PAX1, RAR-β*, and *RASSF1* genes, assessed on 11,201 samples from patients with cancer and 10,184 samples from healthy controls.

Scientific evidence has shown that *APC, FHIT, CDH1*, and *HIC1* are associated with Wnt signaling pathway, playing an important role in carcinogenesis, in particular for the development of uterine cancer. In normal cases, *APC* encodes for a tumor suppressor antagonist protein that interacts with the Wnt signaling pathway by inactivating β-catenin, which is known by its ability to bind to the TCF–LEF complex, inducing the expression of genes involved in carcinogenesis, such as *VEGF, c-myc*, and *matrix metalloproteinase-7* (*MMP-7*; ref. 33). β-catenin–TCF complex can be also inactivated by E-cadherin or HIC1 protein, which are coded by *CDH1* and *HIC1* genes, respectively (34, 35). The methylation rate of the *APC* and *HIC1* genes was almost the same when comparing between cancer samples and normal samples in African and Caucasian regions. On the contrary, in Asia, these genes were only hypermethylated in cervical cancer cases. The overmethylation of *APC* and *HIC1* in normal cells was already reported by Reesink-Peters and colleagues and Waki and colleagues, showing that age has an effect on the methylation of certain tumor suppressor genes, such as *APC* (36, 37).

Moreover, they indicated that this related methylation is not immediately tumorigenic, but gradually participates in the functional

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Table 1. Association between methylation events and clinicopathologic features.

	Comparison	Studies (*N*)	Methylation events in cases	Total cancer cases	%	Methylation events in controls	Total controls	%	Heterogeneity I^2 (%)	Heterogeneity *P*	Effect size OR, Mantel–Haenszel random (95% CI)
p16	LSIL/CIN1	10	66	368	17.93	62	602	10.30	0	0.446	2.25 (1.29–3.94)
	HSIL/CIN2–3	10	146	487	29.98			10.30	23.7	0.225	5.72 (2.92–11.2)
	CC	21	390	1,141	34.18	78	1,158	6.74	53.05	0.002	12.24 (6.38–23.46)
CDH1	LSIL/CIN1	9	53	304	17.43	58	639	9.08	0	0.566	1.34 (0.52–3.47)
	HSIL/CIN2–3	9	133	471	28.24	57	616	9.25	5.8	0.385	3.74 (1.66–8.43)
	CC	21	427	917	46.56	105	907	11.58	66.51	0.000	8.76 (3.68–20.82)
APC	LSIL/CIN1	1	5	20	25.00	8	20	40.00	Nd	Nd	Nd
	HSIL/CIN2–3	1	5	20	25.00			40.00	Nd	Nd	Nd
	CC	7	132	441	29.93	71	260	27.31	46.29	0.083	2.07 (0.84–5.14)
MGMT	LSIL/CIN1	6	114	346	32.95	50	233	21.46	0	0.554	2.14 (3.13–4.06)
	HSIL/CIN2–3	6	144	410	35.12			21.46	56.73	0.041	2.21 (0.70–6.96)
	CC	15	366	1,145	31.97	190	938	20.26	68.04	0.000	3.34 (1.58–7.03)
hMLH1	LSIL/CIN1	5	37	281	13.17	19	180	10.56	0	0.838	1.28 (0.76–2.14)
	HSIL/CIN2–3	5	39	310	12.58			10.56	0	0.933	1.08 (0.74–1.58)
	CC	9	20	441	4.54	19	361	5.26	4.72	0.396	1.19 (0.49–2.88)
HIC1	LSIL/CIN1	4	58	151	38.41	39	156	25.00	0	0.476	3.89 (0.83–18.24)
	HSIL/CIN2–3	3	99	241	41.08			25.00	0	0.714	4.81 (0.67–34.35)
	CC	8	170	404	42.08	122	336	36.31	53.78	0.034	4.09 (1.6–10.42)
RASSF1	LSIL/CIN1	5	13	143	9.09	3	131	2.29	0	0.502	2.87 (0.62–13.33)
	HSIL/CIN2–3	6	60	315	19.05	3	139	2.16	0	0.976	8.06 (4.84–13.43)
	CC	27	431	1,775	24.28	24	997	2.41	64.35	0.000	7.23 (3.79–13.82)
RAR-β	LSIL/CIN1	9	88	314	28.03	79	579	13.64	67.48	0.003	1.97 (0.62–6.24)
	HSIL/CIN2–3	8	130	466	27.90	75	544	13.79	58.49	0.018	1.07 (0.31–3.90)
	CC	15	427	971	43.98	224	1,126	19.89	73.63	0.000	5.36 (2.71–10.58)
DAPK1	LSIL/CIN1	11	103	428	24.07	63	351	17.95	0	0.454	1.64 (1.04–2.57)
	HSIL/CIN2–3	13	277	879	31.51	63	383	16.45	0	0.698	4.98 (3.23–7.70)
	CC	29	1,066	1,836	58.06	223	1,180	18.90	86.03	0.000	21.74 (12.29–38.46)
FHIT	LSIL/CIN1	3	20	164	12.20	8	210	3.81	0	0.898	3.76 (2.76–6.85)
	HSIL/CIN2–3	4	32	243	13.17	11	319	3.45	0	0.719	6.34 (2.30–17.47)
	CC	15	328	980	33.47	38	679	5.60	52.84	0.008	7.42 (3.12–17.65)
CADM1	LSIL/CIN1	9	44	234	18.80	24	285	8.42	0.3	0.421	0.19 (0.45–3.12)
	HSIL/CIN2–3	9	101	377	26.79			8.42	42.95	0.081	4.01 (1.73–9.29)
	CC	9	197	339	58.11			8.42	51.01	0.038	21.73 (5.70–82.89)
MAL	LSIL/CIN1	6	18	209	8.61	16	225	7.11	0	0.588	1.91 (0.67–5.41)
	HSIL/CIN2–3	6	72	249	28.92			7.11	7.41	0.340	2.31 (0.22–24.76)
	CC	6	181	253	71.54			7.11	62.37	0.021	40.95 (5.07–330.94)
CCNA1	LSIL/CIN1	5	15	117	12.82	6	155	3.87	0	0.983	2.84 (1.86–4.34)
	HSIL/CIN2–3	5	59	162	36.42			3.87	0	0.542	45.99 (7.26–291.26)
	CC	7	212	287	73.87	18	278	6.47	43.30	0.102	71.10 (18.66–270.96)
PAX1	LSIL/CIN1	7	22	308	7.14	23	314	7.32	0	0.472	1 (0.45–2.21)
	HSIL/CIN2–3	7	101	295	34.24			7.32	57.91	0.027	8.20 (2.27–29.70)
	CC	8	219	271	80.81	23	355	6.48	65.40	0.005	157.99 (24.83–1005.47)

Abbreviations: CC, cervical cancer; Nd, not determined.

inactivation of the gene. Therefore, we can easily assume that detection of *APC* and *HIC1* methylation may help predict future risk of developing cancer in the patient, but will in no way confirm a positive diagnosis. Moreover, taken together, these results suggest that the use of methylation of the promoters of these genes may not be reliable for the diagnosis of cervical cancer, specifically in African and Caucasian patients. In addition, their use as a therapeutic target in these two populations can lead to undesirable effects.

FHIT also binds to β-catenin causing the transcription repression of certain genes, such as *cyclin D1* (38). Dysfunction of *FHIT* induces overexpression of cyclin D1, which in turn leads to abnormalities in the cell cycle and an increased risk of cancer initiation (38). Although the HPV E6 protein induces an increase in the signaling response of the Wnt/β-catenin/TCF pathway (39), methylations occurring in this pathway, especially for *APC, FHIT, CDH1*, and *HIC1*, have been reported as essential factors which can influence the development of cervical cancer (17, 40, 41). In fact, cells can escape apoptosis with the overexpression of the Wnt/β-catenin pathway (42). The cells may also benefit from the inactivation of apoptosis when *DAPK* gene (apoptosis mediator) is methylated and gradually leads to the development of cervical cancer and metastasis (43).

Other genes associated with cell-cycle functions and apoptosis proprieties were also explored, including *CCNA1, p16*, and *RASSF1* encoding for cyclin A1, $p16^{INK4A}$, and RASSF1 proteins, respectively. These proteins are essential for cells to regulate cell-cycle progression and to induce apoptosis (44, 45). Hypermethylation of the promoters of these genes is widely reported and occurs in both precancerous lesions and cancers. This epigenetic silencing of

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CCNA1, p16, and *RASSF1* genes is associated with dysfunction of the cell-cycle progression and apoptosis avoidance, thereby promoting cervical cancer (46–50).

RAR-β is a member of the thyroid-steroid hormone receptor superfamily of nuclear transcriptional regulators that binds retinoic acid (the biologically active form of vitamin A) and also mediates cellular signaling during embryonic morphogenesis, cell growth, and differentiation (32, 51). Hypermethylation of *RAR-β* in many cancer types, including cervical cancer, is widely discussed (52, 53) to limit retinoic acids production, widely known for their involvement in tumor suppressor activity due to their antiproliferative and apoptosis-inducing effects (54).

In this meta-analysis, results clearly showed that methylation of *p16, CDH1, RASSF1, RAR-β, DAPK1, FHIT, CADM1, MAL, CCNA1*, and *PAX1* genes promoters was significantly higher in cervical cases than in controls, and was also present in precancerous lesions. For the others, the methylation status of their promoters regions showed no significant difference between cervical features and was mostly similar to that reported in control specimens.

In this meta-analysis, the promoters of *CADM1, MAL*, and *PAX1* genes were reported to be highly hypermethylated in low-grade and high-grade lesions, and cancer cases. These genes play an important role during cervical cancer development, they are involved in cell adhesion, apical membrane proteins transport, and cell differentiation, respectively (55–57). Hypermethylation of the promoters regions of these genes is widely reported to induce loss of their functions during cervical carcinogenesis (58–60). On the other hand, the interest was also given to *MGMT* and *hMLH1* genes involved in DNA repair (61, 62). Hypermethylation of the promoters regions of these genes often results in epigenetic silencing which, in turn, suppresses the repair function, leading to an accumulation of genomic abnormalities and thereby, promotion of many kinds of cancers, including cervical cancer (62). In our study, it was shown that *MGMT* and *hMLH1* genes' methylation is rarely studied in the world and showed no significant difference between cases and controls, limiting the interest on these genes as biomarkers for cervical cancer development and progression.

By analyzing all generated data in this meta-analysis, ethnical origin seems to play an important role in determining the methylation profile of investigated genes. Most studies were conducted on Caucasian (43%) and Asian (49%) populations, and limited studies have explored the association between epigenetic alteration and cervical cancer development in African population, which represents the main limitation of this study. Moreover, the methylation of some genes has not been reported in certain regions of the world. For instance, the methylation status of the *PAX1* and *hMLH1* genes is not explored in African/Caucasian populations and in Asia, respectively. These imply, subject to taking only the data analyzed in this study, the acceptability of the hypothesis that we have issued forward. The impact of ethnicity on epigenetic diversity was already discussed by Ahmad and colleagues, highlighting that epigenetic events are closely related to ethnical/racial differences affecting the overall cancer incidence and mortality. Moreover, it can be assumed that cultural and socio-economic inequalities, notably linked to low income or stress conditions, mainly influence the epigenome while favoring epigenetic modifications in the individual (63).

Of particular interest, some studies done in African populations are conducted on exfoliated cells, suggesting that difference in methylation events could be due to cell sampling rather than a difference between populations.

By omitting the heterogeneous studies, we performed subgroup analysis by histologic types. On the basis of the random effects model, results showed that the heterogeneity totally disappeared in LISL and HISL subgroups (**Table 1**). Thereby, we found that the hypermethylation rates in LSIL, HSIL, and cervical cancer specimens were gradually increased in some promoter genes, resulting in a growing trend in effects of *p16, CDH1, RASSF1, DAPK1, FHIT, CADM1, MAL, CCNA1*, and *PAX1* hypermethylation on LSIL, HSIL, and cervical cancer development. These results suggest that *p16, CDH1, RASSF1, DAPK1, FHIT, CADM1, MAL, CCNA1*, and *PAX1* promoters hypermethylation may be a promising epigenetic marker for the progression of cervical carcinogenesis. Hence, detecting hypermethylation in these genes' promoters may help clinicians to determine whether patients with cervical neoplasia are in disease regression, persistence, or progression. In this field, evaluation of the hypermethylation of these genes in LSIL cases will be of a great interest for a more effective clinical management of these patients.

This meta-analysis is very informative and showed that some studied genes exhibit a significant association between the methylation of their promoters and the risk of cervical cancer development. However, the main limitation of this investigation is the technique used for methylation status assessment. Indeed, most studies were performed using MSP, considered as the gold standard method (70.30%), in which PCR products are run on a gel and results are reported as methylated or unmethylated at the target DNA sequence. Moreover, this technique lacks to identify partial levels of methylation, which is considered as highly relevant feature both biologically and clinically. To overcome the limitation of this conventional method, qMSP was developed in recent years and was reported to be more specific and more sensitive, and allows for high-throughput analysis, making it more suitable as a screening tool.

In conclusion, this meta-analysis gave evidence that ethnicity plays a crucial role in the distribution of promoters' methylation across human genomes and highlights the association between the methylation status of the promoter region of some genes and promotion of cervical cancer in some cases. However, the technique used in the investigation can also generate questions related to the effectiveness of the detection of this methylation. Other analyses are, therefore, necessary to generate other data, making it possible to validate the highly promoter epigenetic markers for the diagnosis and development of therapeutic targets.

Authors' Disclosures

No disclosures were reported.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68: 394–424.

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2. Chaturvedi AK, Kleinerman RA, Hildesheim A, Gilbert ES, Storm H, Lynch CF, et al. Second cancers after squamous cell carcinoma and adenocarcinoma of the cervix. J Clin Oncol 2009;27:967–73.
3. Zaino RJ. Glandular lesions of the uterine cervix. Mod Pathol 2000;13:261–74.
4. Bosch FX, Lorincz A, Munoz N, Meijer CJLM, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol 2015;55: 244–65.
5. Steenbergen RDM, Snijders PJF, Heideman DAM, Meijer CJLM. Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. Nat Rev Cancer 2014;14:395–405.
6. Kanwal R, Gupta S. Epigenetic modifications in cancer. Clin Genet 2012;81: 303–11.
7. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis 2009;31: 27–36.
8. Feng C, Dong J, Chang W, Cui M, Xu T. The progress of methylation regulation in gene expression of cervical cancer. Int J Genomics 2018;2018: 8260652.
9. Ezkurdia I, Juan D, Rodriguez JM, Frankish A, Diekhans M, Harrow J, et al. Multiple evidence strands suggest that there may be as few as 19,000 human protein-coding genes. Hum Mol Genet 2014;23:5866–78.
10. Rauch T, Pfeifer GP. Methylated-CpG island recovery assay: a new technique for the rapid detection of methylated-CpG islands in cancer. Lab Investig 2005;85: 117280.
11. Moarii M, Boeva V, Vert J-P, Reyal F. Changes in correlation between promoter methylation and gene expression in cancer. BMC Genomics 2015;16:873.
12. Lee E, Muller WJ. Oncogenes and tumor suppressor genes. Cold Spring Harb Perspect Biol 2010;2:a003236.
13. Esteller M. CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. Oncogene 2002;21:5427–40.
14. Nowacka-Zawisza M, Wisnik E. DNA methylation and histone modifications as epigenetic regulation in prostate cancer. Oncol Rep 2017;38:2587–96.
15. Michie AM, McCaig AM, Nakagawa R, Vukovic M. Death-associated protein kinase (DAPK) and signal transduction: regulation in cancer. FEBS J 2010;277: 74–80.
16. Weiss D, Basel T, Sachse F, Braeuninger A, Rudack C. Promoter methylation of cyclin A1 is associated with human papillomavirus 16 induced head and neck squamous cell carcinoma independently of p53 mutation. Mol Carcinog 2011;50: 680–8.
17. Ki K-Do, Lee S-K, Tong S-Y, Lee J-M, Song D-H, Chi S-G. Role of 5'-CpG island hypermethylation of the FHIT gene in cervical carcinoma. J Gynecol Oncol 2008; 19:117–22.
18. Wentzensen N, Sherman ME, Schiffman M, Wang SS. Utility of methylation markers in cervical cancer early detection: appraisal of the state-of-the-science. Gynecol Oncol 2009;112:293–9.
19. Zhang Z, Joh K, Yatsuki H, Zhao W, Soejima H, Higashimoto K, et al. Retinoic acid receptor β2 is epigenetically silenced either by DNA methylation or repressive histone modifications at the promoter in cervical cancer cells. Cancer Lett 2007;247:318–27.
20. Kausar Neyaz M, Suresh Kumar R, Hussain S, Naqvi S, Kohaar I, Thakur N, et al. Effect of aberrant promoter methylation of FHIT and RASSF1A genes on susceptibility to cervical cancer in a North Indian population. Biomarkers 2008;13:597–606.
21. van der Meide WF, Snellenberg S, Meijer CJLM, Baalbergen A, Helmerhorst TJM, van der Sluis WB, et al. Promoter methylation analysis of WNT/beta-catenin signaling pathway regulators to detect adenocarcinoma or its precursor lesion of the cervix. Gynecol Oncol 2011;123:116–22.
22. Pfeifer GP. Defining driver DNA methylation changes in human cancer. Int J Mol Sci 2018;19:1166.
23. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.
24. Suurmond R, van Rhee H, Hak T. Introduction, comparison and validation of meta-essentials: a free and simple tool for meta-analysis. Res Synth Methods 2017;8:537–53.
25. Wales MM, Biel MA, Deiry WEl, Nelkin BD, Issa J-P, Cavenee WK, et al. p53 activates expression of HIC-1, a new candidate tumour suppressor gene on 17p13.3. Nat Med 1995;1:570–7.
26. Martin-Belmonte F, Puertollano R, Millan J, Alonso MA. The MAL proteolipid is necessary for the overall apical delivery of membrane proteins in the polarized epithelial Madin-Darby canine kidney and Fischer rat thyroid cell lines. Mol Biol Cell 2000;11:2033–45.
27. Spathis A, Aga E, Alepaki M, Chranioti A, Meristoudis C, Panayiotides I, et al. Promoter methylation of p16(INK4A), hMLH1, and MGMT in liquid-based cervical cytology samples compared with clinicopathological findings and HPV presence. Infect Dis Obstet Gynecol 2011;2011:927861.
28. McGaughran JM, Oates A, Donnai D, Read AP, Tassabehji M. Mutations in PAX1 may be associated with Klippel-Feil syndrome. Eur J Hum Genet 2003;11: 468–74.
29. Soto D, Song C, McLaughlin-Drubin ME. Epigenetic alterations in human papillomavirus-associated cancers. Viruses 2017;9:248.
30. Lu Q, Ma D, Zhao S. DNA methylation changes in cervical cancers. Methods Mol Biol 2012;863:155–76.
31. Plesa A, Iancu IV, Botezatu A, Huica I, Stoian M, Anton G. The involvement of epigenetic mechanisms in HPV-induced cervical cancer. In: Rajkumar R, editor. Human papillomavirus – research in a global perspective. IntechOpen; 2016.
32. Dueñas-González A, Lizano M, Candelaria M, Cetina L, Arce C, Cervera E. Epigenetics of cervical cancer. An overview and therapeutic perspectives. Mol Cancer 2005;4:38.
33. Ayala-Calvillo E, Mojica-Vazquez LH, Garcia-Carranca A, González-Maya L. Wnt/β-catenin pathway activation and silencing of the APC gene in HPV-positive human cervical cancer-derived cells. Mol Med Rep 2018;17:200–8.
34. Thievessen I, Seifert H-H, Swiatkowski S, Florl AR, Schulz WA. E-cadherin involved in inactivation of WNT/β-catenin signalling in urothelial carcinoma and normal urothelial cells. Br J Cancer 2003;88:1932–8.
35. Valenta T, Lukas J, Doubravska L, Fafilek B, Korinek V. HIC1 attenuates Wnt signaling by recruitment of TCF-4 and β-catenin to the nuclear bodies. EMBO J 2006;25:2326–37.
36. Reesink-Peters N, Wisman GBA, Jéronimo C, Tokumaru CY, Cohen Y, Dong SM, et al. Detecting cervical cancer by quantitative promoter hypermethylation assay on cervical scrapings: a feasibility study. Mol Cancer Res 2004;2: 289–95.
37. Waki T, Tamura G, Sato M, Motoyama T. Age-related methylation of tumor suppressor and tumor-related genes: an analysis of autopsy samples. Oncogene 2003;22:4128–33.
38. Weiske J, Albring KF, Huber O. The tumor suppressor Fhit acts as a repressor of β-catenin transcriptional activity. Proc Natl Acad Sci U S A 2007;104: 20344–9.
39. Sominsky S, Kuslansky Y, Shapiro B, Jackman A, Haupt Y, Rosin-Arbesfeld R, et al. HPV16 E6 and E6AP differentially cooperate to stimulate or augment Wnt signaling. Virology 2014;468–470:510–23.
40. Liu G. CDH1 promoter methylation in patients with cervical carcinoma: a systematic meta-analysis with trial sequential analysis. Futur Oncol 2017;14: 51–63.
41. Narayan G, Arias-Pulido H, Koul S, Vargas H, Zhang FF, Villella J, et al. Frequent promoter methylation of CDH1, DAPK, RARB, and HIC1 genes in carcinoma of cervix uteri: its relationship to clinical outcome. Mol Cancer 2003;2:24.
42. Saavedra KP, Brebi PM, Roa JCS. Epigenetic alterations in preneoplastic and neoplastic lesions of the cervix. Clin Epigenetics 2012;4:13.
43. Agodi A, Barchitta M, Quattrocchi A, Maugeri A, Vinciguerra M. DAPK1 promoter methylation and cervical cancer risk: a systematic review and a meta-analysis. PLoS One 2015;10:e0135078.
44. Lapenna S, Giordano A. Cell cycle kinases as therapeutic targets for cancer. Nat Rev Drug Discov 2009;8:547–66.
45. Lau WM, Ho TH, Hui KM. p16INK4A-silencing augments DNA damage-induced apoptosis in cervical cancer cells. Oncogene 2007;26:6050–60.
46. Cohen Y, Singer G, Lavie O, Dong SM, Beller U, Sidransky D. The RASSF1A tumor suppressor gene is commonly inactivated in adenocarcinoma of the uterine cervix. Clin Cancer Res 2003;9:2981–4.
47. Donninger H, Vos MD, Clark GJ. The RASSF1A tumor suppressor. J Cell Sci 2007;120:3163–72.
48. Huang L-W, Pan H-S, Lin Yu-H, Seow K-M, Chen H-Ju, Hwang J-L. P16 methylation is an early event in cervical carcinogenesis. Int J Gynecol Cancer 2011;21:452–6.
49. Kuzmin I, Liu L, Dammann R, Geil L, Stanbridge EJ, Wilczynski SP, et al. Inactivation of RAS association domain family 1A gene in cervical carcinomas and the role of human papillomavirus infection. Cancer Res 2003;63:1888–93.
50. Yang B, Miao S, Zhang LN, Sun H-B, Xu Z-N, Han C-S. Correlation of CCNA1 promoter methylation with malignant tumors: a meta-analysis introduction. Biomed Res Int 2015;2015:134027.
51. Niederreither K, Dolle P. Retinoic acid in development: towards an integrated view. Nat Rev Genet 2008;9:541–53.

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52. Ivanova T, Petrenko A, Gritsko T, Vinokourova S, Eshilev E, Kobzeva V, et al. Methylation and silencing of the retinoic acid receptor-β2 gene in cervical cancer. BMC Cancer 2002;2:4.
53. Jha AK, Nikbakht M, Parashar G, Shrivastava A, Capalash N, Kaur J. Reversal of hypermethylation and reactivation of the RARβ2 gene by natural compounds in cervical cancer cell lines. Folia Biol 2010; 56:195–200.
54. Brtko J. Role of retinoids and their cognate nuclear receptors in breast cancer chemoprevention. Cent Eur J Public Health 2007;15:3–6.
55. Hartsough EJ, Weiss MB, Heilman SA, Purwin TJ, Kugel CH, Rosenbaum SR, et al. CADM1 is a TWIST1-regulated suppressor of invasion and survival. Cell Death Dis 2019;10:281.
56. Hatta M, Nagai H, Okino K, Onda M, Yoneyama K, Ohta Y, et al. Down-regulation of members of glycolipid-enriched membrane raft gene family, MAL and BENE, in cervical squamous cell cancers. J Obstet Gynaecol Res 2004;30: 53–8.
57. Robson EJD, He SJ, Eccles MR. A panorama of PAX genes in cancer and development. Nat Rev Cancer 2006;6:52–62.
58. Overmeer RM, Henken FE, Bierkens M, Wilting SM, Timmerman I, Meijer CJ, et al. Repression of MAL tumour suppressor activity by promoter methylation during cervical carcinogenesis. J Pathol 2009;219:327–36.
59. Su Po-H, Lai H-C, Huang R-L, Chen L-Yu, Wang Yu-C, Wu T-I, et al. Paired box-1 (PAX1) activates multiple phosphatases and inhibits kinase cascades in cervical cancer. Sci Rep 2019;9:9195.
60. Woo J, Kim SJ, Song K-J, Kim SS, Yoon C-H, Choi B-S, et al. Hypermethylation of the tumor-suppressor cell adhesion molecule 1 in human papillomavirus-transformed cervical carcinoma cells. Int J Oncol 2015;46:2656–62.
61. Li GM. Mechanisms and functions of DNA mismatch repair. Cell Res 2008;18: 85–98.
62. Lin Z, Gao M, Zhang X, Kim Y-S, Lee E-S, Kim H-K, et al. The hypermethylation and protein expression of p16 INK4Aand DNA repair gene O 6-methylguanine-DNA methyltransferase in various uterine cervical lesions. J Cancer Res Clin Oncol 2005;131:364–70.
63. Ahmad A, Azim S, Zubair H, Khan MA, Singh S, Carter JE, et al. Epigenetic basis of cancer health disparities: looking beyond genetic differences. Biochim Biophys Acta Rev Cancer 2017;1868:16–28.

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