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Utility of Methylation Markers in Cervical Cancer Early Detection: Appraisal of the State-of-the-Science

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Abstract

Objective—We wanted to identify the most promising methylation marker candidates for cervical cancer early detection.

Methods—A systematic literature review was performed in Medline and weighted average frequencies for methylated genes stratified by tissue source and methods used were computed.

Results—51 studies were identified analyzing 68 different genes for methylation in 4376 specimens across all stages of cervical carcinogenesis. 15 genes, DAPK1, RASSF1, CDH1, CDKN2A, MGMT, RARB, APC, FHIT, MLH1, TIMP3, GSTP1, CADM1, CDH13, HIC1, and TERT have been analyzed in 5 or more studies. The published data on these genes is highly heterogeneous; 7 genes (CDH1, FHIT, TERT, CDH13, MGMT, TIMP3, and HIC1) had a reported range of methylation frequencies in cervical cancers of greater than 60% between studies. Stratification by analysis method did not resolve the heterogeneity. Three markers, DAPK1, CADM1, and RARB, showed elevated methylation in cervical cancers consistently across studies.

Conclusions—There is currently no methylation marker that can be readily translated for use in cervical cancer screening or triage settings. Large, well-conducted methylation profiling studies of cervical carcinogenesis could yield new candidates that are more specific for HPV-related carcinogenesis. New candidate markers need to be thoroughly validated in highly standardized assays.

Keywords

cervical cancer; biomarker; methylation; HPV; CIN

Introduction

Persistent infections with carcinogenic human papilloma virus (HPV) types are causally linked to the development of cervical cancer [1]. The development of invasive cancers from the initial viral infections takes decades, permitting detection and treatment of CIN2 and CIN3 (CIN2+). While cytological screening has substantially reduced cervical cancer incidence and mortality where it has been successfully implemented, it is limited by low

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Precis: Although methylation markers for cervical cancer screening have been analyzed in multiple studies, there are currently no convincing methylation marker candidates to be used in cervical cancer screening.

Conflict of interest statement: All authors declare that there are no conflicts of interest.

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single-test sensitivity and poor reproducibility for equivocal and minor abnormalities [1]. Despite the recently introduced preventive vaccines against HPV16 and HPV18, screening needs to continue, since only about 70% of cervical cancers will be prevented. However, HPV vaccination will further reduce the efficiency of cytological screening. Therefore, new screening modalities need to be evaluated and pursued [2].

Large randomized trials have shown that adding HPV DNA testing to cytology greatly increases the sensitivity of primary screening for CIN2+ [3–5] and HPV testing alone is considered by some to be a plausible primary screening method [6]. However, since a positive HPV DNA test almost always indicates a transient infection rather than risk of eventual invasive cervical cancer the positive predictive value (PPV) of HPV testing is low and a strategy needs to be developed to triage HPV DNA positive women.

Much effort has been put into identifying new biomarkers for CIN2+ to improve risk stratification, distinguishing women with benign infection from those requiring more intensive management [7].

Methylation of CpG islands within gene promoter regions can lead to silencing of gene expression. Methylation of tumor-relevant genes has been identified in many cancers: p16 methylation is the paradigm for epigenetic inactivation of a tumor suppressor gene, leading to abrogation of cell cycle control, escape from senescence, and induction of proliferation. MLH1 methylation has been identified as the first step in development of sporadic microsatellite unstable colorectal cancers. Likewise, many genes associated with tumor development have been found methylated in various cancer sites. Methylation has been detected already at precancerous stages, suggesting that methylation markers may have value in cervical cancer screening [8]. Furthermore, methylated DNA is a stable target and allows for flexibility of assay development.

Over the last decade, a growing number of studies evaluating methylation of host genes in cervical tissue have been published. Most of the candidate methylation markers analyzed in cervical tissues were selected because altered methylation was previously observed in other types of cancer. Reflecting the technical development in the methylation field over the past 20 years, many different technologies were used in these studies, analyzing both biopsy specimens as well as cytology samples.

The aims of this review are (1) to summarize the results of published methylation studies analyzing cervical tissues and cells, including the specimen types, markers and assays evaluated and (2) to assess the opportunities and challenges facing this line of research.

Material and Methods

A systematic literature research was performed of studies published in Medline until April 7, 2008, using the keywords: (methylation AND cervical),(methylation AND CIN), (methylation AND cervix). Only investigations that evaluated clinical specimens (either histologic or cytologic) were included and methylation frequencies for specific genes were considered; studies that analyzed methylation patterns for disease clustering without presenting individual frequency data by gene were not considered. Since we focused on host gene methylation, we did not include studies analyzing methylation of HPV genomes in cervical cancer progression.

The histologic diagnoses were cervical cancer, CIN3, CIN2, CIN1, or normal cervical tissue. The cytologic interpretations were high-grade squamous intraepithelial lesions (HSIL), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells- rule out high-grade lesion (ASC-H), atypical squamous cells of undetermined significance (ASC-US), or

negative for intraepithelial lesion or malignancy (NILM) [9]. Glandular lesions except for invasive adenocarcinoma were not included due to lack of research on these poorly-defined lesions. Several studies did not report exact cytologic and histologic categories, but grouped different categories (e.g. CIN1-3+ASC+SIL, CIN2-3+HSIL).

All gene names were checked on the homepage of the HUGO Gene Nomenclature Committee (www.genenames.org) and the official HUGO gene name was assigned. Multiple designations were found for several genes, including the following: CDH1 (cadherin, CDH1), CTNNB1 (b-catenin, CTNNB1), CADM1 (TSLC1, IGSF4, CADM1). Since the CDKN2A gene locus has 2 different promoters with CpG islands and encodes 2 different transcripts (p16^{INK4a}, p14^{ARF}), data on CDKN2A were used for the analysis of the p16^{INK4a} CpG island while data on p14 was used for the p14^{ARF} CpG island.

For each gene, we report the weighted average and the range of methylation frequencies observed in all included studies. To analyze host gene methylation in progression to cancer, we compared methylation frequencies of the 15 most frequently analyzed genes reported for normal tissue (including all studies reporting normal tissue, n=24 studies), cancer precursors (defined as high-grade cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesion, n=12 studies), and cancers (n=33 studies).

To analyze different methylation frequencies between squamous cell cancer (SCC) and adenocarcinoma (AC), we included 12 genes that were analyzed in at least two studies reporting frequencies for both differentiations.

Different methods were used to analyze gene methylation: Methylation specific PCR (MSP) is based on conversion of unmethylated Cytosin to Uracil by bisulfite treatment. Subsequently, amplification is performed using different primers designed for amplification of methylated or unmethylated CpGs. Quantitative MSP (Q-MSP, e.g. MethyLight) is based on the same principle but uses Real Time PCR amplification and increases specificity by adding a probe for the amplified sequence. Other methods used were bisulfite sequencing (analyzing a larger region for methylated CpGs than MSP/Q-MSP) and restriction based methylation detection (analyzing only single CpGs by methylation-sensitive restriction enzymes). Different sample types were analyzed in the studies, including fresh frozen tissue, exfoliated cells, and paraffin embedded tissue.

We stratified the reports on methylation frequencies by the following 9 combinations of assay and sample type: A) Fresh frozen tissue and MSP; B) Exfoliated tissue and MSP; C) Paraffin embedded tissue and MSP; D) Unspecified tissue and MSP; E) Fresh frozen tissue and Q-MSP; F) Exfoliated tissue and Q-MSP; G) Paraffin embedded tissue and Q-MSP; H) Unspecified tissue and Q-MSP; I) Fresh frozen tissue and other methods.

One study reported the highest frequencies for all 6 markers included in the study that belong to the 15 markers analyzed in detail [10]. In sensitivity analysis, the exclusion of this study did not substantially change the results.

Results

Studies and samples included, methods used

The initial Medline search yielded 3546 abstracts on methylation AND cervical/cervix/CIN. Fifty-one studies were identified that described methylation frequencies of human genes in cervical samples (Supplemental table 1).

The majority of the studies used MSP (32 of 51, 63%), followed by MethyLight (7 of 51, 14%), other quantitative MSP protocols (5 of 51, 10%), bisulfite sequencing (4 of 51, 8%),

and other methods (3 of 51, 6%). In 24 studies (47%), fresh frozen material was used for methylation analysis, followed by exfoliated cells (12 of 51, 24%), and paraffin embedded tissue (7 of 51, 14%). The most frequent combination of sample material and methods used in the studies was MSP with fresh frozen tissue samples (15 of 51, 29%) (Table 1). Eight studies reported the use of microdissected specimens [11–18].

47 of 51 studies (92%) analyzed cervical cancer samples, 26 of 51 (51%) normal cervical samples, and 16 of 51 (31%) included CIN or ASC/SIL (CIN1-3, ASCUS, LSIL, HSIL, in the following summarized as “CIN”). In total, 4376 samples covering 2836 cancer samples, 841 normal samples, and 709 CIN samples from the 51 studies are included in this review. Due to the heterogeneous CIN samples included in the different studies, no weighted mean and frequency ranges are presented for CIN.

Genes analyzed

In total, 68 different genes were analyzed in the studies, 31 genes were analyzed in more than 1 study, 15 in 5 or more studies, and 6 in ten or more studies (Table 2).

The genes analyzed in 5 or more studies (by descending frequency) were DAPK1, RASSF1, CDH1, CDKN2A, MGMT, RARB, APC, FHIT, MLH1, TIMP3, GSTP1, CADM1, CDH13, HIC1, and TERT. Gene names, gene ontology terms and data from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database on methylation in other cancer sites are summarized for these 15 genes in Supplemental Table 2.

Overall, there was a wide range of methylation frequencies in cervical cancers reported for most genes (Table 2, Figure 1). On the extremes, large variable frequencies were reported for TIMP3, MGMT, and CDH1 and somewhat less variation was observed for CADM1 and GSTP1. Based on mean percentages weighted by study size, the most frequently methylated genes were CDH1 (58%), DAPK1 (57%), CADM1 (55%), and TERT (55%). MGMT and RASSF1 showed methylation frequencies below 20% and GSTP1 and MLH1 were rarely if ever methylated in cervical cancers.

In normal cervix tissue, all genes except for HIC1 had weighted mean methylation frequencies below 30% (Table 2, Figure 2). However, some genes showed a wide range of reported frequencies, including FHIT, HIC1, and CDH1. These three genes showed a high variation in both cancer and normal tissue.

Methylation frequencies during cervical cancer progression

We analyzed the reported range and the weighted mean methylation frequencies for the above described 15 genes in studies that explicitly presented results for the CIN2/CIN3 and HSIL categories. All genes with high differences of methylation frequencies between normal tissue and cancers showed intermediate frequencies in CIN2/CIN3/HSIL (Supplemental Figure 1). Of these genes, the highest mean frequency was reported for CADM1 (33%), followed by CDH1 (29%), DAPK1 (29%), and TERT (29%). The highest weighted mean methylation frequencies in CIN2/CIN3/HSIL were reported for HIC1 (59%) and APC (34%), but both genes had lower methylation frequencies in cancer (Supplemental Figure 1).

Methylation frequencies in squamous cell carcinomas and adenocarcinomas

We observed a high degree of heterogeneity in the methylation frequencies reported for cervical cancers in the different studies. To determine if the variation of methylation frequencies was related to cancer type, we compared methylation frequencies between SCC and AC. Several studies did not specify the differentiation of cancers; others reported only

SCCs. 19 studies reported methylation frequencies for SCCs and ACs. In total, 1062 SCCs and 396 ACs were included in these studies, indicating an oversampling of ACs compared with typical population diagnostic distributions. A total of 30 different genes have been compared between SCC and AC in these studies. 12 genes (RASSF1A, DAPK, CDKN2A, APC, CDH1, MGMT, TIMP3, HIC1, RARB, ESR1, FHIT, and MLH1) were analyzed in 2 or more studies (Table 2). Three genes, APC, TIMP3, and HIC1, showed >20% higher weighted mean methylation frequencies in AC as compared to SCC, while DAPK and CDH1 were more frequently found methylated in SCC. Still, the variation of frequencies reported for most of the 12 markers was similarly high in SCC and AC; only APC and TIMP3 had non-overlapping ranges of methylation frequencies in SCC and AC.

Influence of methods and tissue sources on methylation frequencies

To analyze the influence of specimen type and methylation detection methods on the methylation frequencies in cancers, we stratified the frequencies by the nine combinations of tissue source and assay described in the Methods section (Table 1, Supplemental Figure 2). Most combinations were used only in few studies. Though, based on limited numbers within each stratum, the analysis showed frequency ranges that were similar to the overall range. For example, CDH1 was analyzed with 4 different combinations in at least 2 studies and showed a wide variation among studies sharing each of the combinations. Similarly, CDH13 and FHIT showed a wide variation in the sample source/assay combinations with 2 or more studies. In contrast, DAPK1 was analyzed in 4 combinations in at least 2 studies and showed very consistent results irrespective of sample source and assay used (Supplemental Figure 2). In the strata D and E (MSP with unspecified tissue, Q-MSP with frozen tissue), CDH1, CDH13, and DAPK1 were analyzed in an identical set of studies, permitting direct comparison. While CDH1 and CDH13 showed a high frequency range between methods, the same studies produced much more precise results regardless of method for DAPK1. Thus, DAPK1 results are not demonstrably methods-dependent.

Discussion

The detection of methylated genes from cervical specimens is technically feasible and represents a source for detecting potential biomarkers of relevance to cervical carcinogenesis. In particular, there is the ultimate hope of finding methylation markers that, among HPV-infected women, would indicate the presence of CIN2+ and risk of cancer.

One striking conclusion of our survey of 51 studies is that methylation frequencies for the same gene vary widely between studies. This degree of heterogeneity combined with the low number of studies analyzing the same genes precluded us from performing a rigorous meta-analysis. In fact, we were unable to identify highly consistent results for most genes even when restricting analyses to studies of similar size or those that used common specimen sources or similar assays. These results suggest that the frequency of certain methylation markers may also vary for reasons related to differences in populations, specific features of assay protocols, chance or other unidentified factors. For example, CDH1 and FHIT, two genes with highly variable methylation frequencies in cancer between studies also show variable detection in normal tissue. In contrast, DAPK1 has been measured much more precisely over a wide range of heterogeneous studies, including those analyzing CDH1 and CDH13.

We described in detail the properties of 15 methylation markers that were analyzed in 5 or more studies. While we cannot discount the possibility of a promising biomarker among the remaining 53 candidates, there was insufficient information available to evaluate these candidates further.

The most important prerequisite for a potential biomarker is that it must be reliable in its measurement. Among the 15 genes analyzed in detail, 7 (CDH1, FHIT, TERT, CDH13, MGMT, TIMP3, HIC1) had a reported range of methylation frequencies in cervical cancers of greater than 60% between studies. Stratification for analysis method or specimen type used did not resolve the observed variations. However, since few studies used identical conditions to analyze the same markers, in most cases, numbers were insufficient to evaluate the influence of assay type and specimen on methylation frequencies. At the moment, assay reliability for these methylation markers therefore cannot be properly addressed since methods are poorly standardized. We acknowledge the possibility that the wide range of frequencies reported for some genes contrasting the more consistent measurement of few other genes in similar studies could be related to either unreliable assays for these specific genes or to biological variation.

Another prerequisite for a good biomarker is that it has high sensitivity and high specificity for disease detection, resulting in a high positive predictive value. Among the eight genes with limited variability, all but one (APC) had methylation frequencies below 5% in normal tissue. Of these, three had average frequencies of at least 30% in cancers (DAPK1, CADM1, and RARB). Based on these results, we conclude that there is currently no single methylation marker that has the appropriate performance to serve as a cervical cancer biomarker, especially since methylation frequencies in CIN2/CIN3/HSIL, the targets of most cervical cancer screening programs, were even lower for all genes.

Some groups have proposed the use of methylated gene panels to obtain an appropriate performance for cervical cancer screening. From the summary data in this review, the combination of DAPK1, CADM1, and RARB would appear the most promising. However, without a formal evaluation, it is unclear if methylation of these markers is mutually exclusive, entirely independent, or associated to some degree, which would affect the overall coverage of the marker panel and therefore has important impact on the sensitivity of marker combinations. We note that Feng et al. recently showed up to 74% sensitivity and 95% specificity for detecting cervical cancers using a panel of three candidate genes, DAPK1, RARB, and TWIST1 [19]. Our review would support further evaluation of two of these genes (DAPK1 and RARB).

Finally, we consider the biological relevance to further inform our evaluation of candidate genes. Notably, a clear role of methylation in carcinogenesis has been demonstrated only for 6 genes (DAPK1, RASSF1, CDKN2A, RARB, MLH1, and GSTP1, see supplemental table 2). Two of the 15 genes, TERT and GSTP1, encode for proteins with oncogenic functions, so their methylation is not likely to drive carcinogenesis. CDKN2A (encoding p16), is methylated in several tumors early in carcinogenesis. In cervical cancer, however, p16 is found strongly overexpressed due to HP-oncogene mediated release of E2F from RB. Recently, it has been demonstrated that p16 methylation does not affect protein expression in cervical cancer [20]. Thus, most of the 15 genes analyzed in this study have no strong a-priori for potentially being important in cervical carcinogenesis. Furthermore, none of the 7 genes with the highest variation of methylation frequencies is among the genes with a clearly defined role of methylation in the carcinogenesis of other tumors. This evaluation suggests that the current genes may not be optimal candidates for cervical carcinogenesis. To date, only few agnostic profiling studies for aberrant methylation in cervical carcinogenesis have been performed [21–23]. We therefore believe that, if methylation markers for cervical cancer screening are to be further pursued, future large well-conducted investigations should also incorporate a discovery effort specifically for methylated genes in cervical cancer. During the last years, several new platforms (e.g. microarray format, bead array format, 454 sequencing format) have been developed that allow for accurate high-throughput genome-wide DNA methylation profiling [24]. Markers or marker panels

identified in these approaches could be translated to smaller scaled assays such as Methylight to be used in cervical cancer screening.

In summary, to identify promising methylation candidates for cervical carcinogenesis, further tissue-based profiling studies using reliable and validated assays are needed. In addition, analyses of exfoliated tissue (e.g. stored in liquid based cytology media) will be critical as this would represent the most likely specimen used for cervical cancer screening and triage. Both efforts will require large well-powered epidemiologic studies designed to properly identify and then validate candidate methylation markers and panels of markers for utility in the early detection of cervical cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. *Lancet* 2007 Sep 8;370(9590):890–907. [PubMed: 17826171]
2. Franco EL, Cuzick J. Cervical cancer screening following prophylactic human papillomavirus vaccination. *Vaccine* Jan 3;2008 26(S1):A16–A23. [PubMed: 18642468]
3. Bulkman NW, Berkhof J, Rozendaal L, van Kemenade FJ, Boeke AJ, Bulk S, Voorhorst FJ, Verheijen RH, van GK, Boon ME, Ruitinga W, van BM, Snijders PJ, Meijer CJ. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. *Lancet* 2007 Nov 24;370(9601):1764–72. [PubMed: 17919718]
4. Mayrand MH, Duarte-Franco E, Rodrigues I, Walter SD, Hanley J, Ferenczy A, Ratnam S, Coutlee F, Franco EL. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. *N Engl J Med* 2007 Oct 18;357(16):1579–88. [PubMed: 17942871]
5. Naucier P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgrén K, Radberg T, Strander B, Forslund O, Hansson BG, Rylander E, Dillner J. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. *N Engl J Med* 2007 Oct 18;357(16):1589–97. [PubMed: 17942872]
6. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2005. Lyon F. Human Papillomaviruses. IARC Monographs; 2007.
7. Wentzensen N, von Knebel Doeberitz M. Biomarkers in cervical cancer screening. *Dis Markers* 2007;23(4):315–30. [PubMed: 17627065]
8. Esteller M. Epigenetics in cancer. *N Engl J Med* 2008 Mar 13;358(11):1148–59. [PubMed: 18337604]
9. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, Raab S, Sherman M, Wilbur D, Wright T Jr, Young N. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* 2002 Apr 24;287(16):2114–9. [PubMed: 11966386]
10. Widschwendter A, Gatringer C, Ivarsson L, Fiegl H, Schneitter A, Ramoni A, Muller HM, Wiedemair A, Jerabek S, Muller-Holzner E, Goebel G, Marth C, Widschwendter M. Analysis of aberrant DNA methylation and human papillomavirus DNA in cervicovaginal specimens to detect invasive cervical cancer and its precursors. *Clin Cancer Res* 2004 May 15;10(10):3396–400. [PubMed: 15161694]
11. Cheung TH, Lo KW, Yim SF, Chan LK, Heung MS, Chan CS, Cheung AY, Chung TK, Wong YF. Epigenetic and genetic alternation of PTEN in cervical neoplasm. *Gynecol Oncol* 2004 Jun;93(3):621–7. [PubMed: 15196854]
12. 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 Aug 1;9(8):2981–4. [PubMed: 12912945]

13. Kang S, Kim HS, Seo SS, Park SY, Sidransky D, Dong SM. Inverse correlation between RASSF1A hypermethylation, KRAS and BRAF mutations in cervical adenocarcinoma. *Gynecol Oncol* 2007 Jun;105(3):662–6. [PubMed: 17360030]
14. Li J, Zhang Z, Bidder M, Funk MC, Nguyen L, Goodfellow PJ, Rader JS. IGSF4 promoter methylation and expression silencing in human cervical cancer. *Gynecol Oncol* 2005 Jan;96(1):150–8. [PubMed: 15589594]
15. Singh RK, Indra D, Mitra S, Mondal RK, Basu PS, Roy A, Roychowdhury S, Panda CK. Deletions in chromosome 4 differentially associated with the development of cervical cancer: evidence of slit2 as a candidate tumor suppressor gene. *Hum Genet* 2007 Aug;122(1):71–81. [PubMed: 17609981]
16. Steenbergen RD, Kramer D, Braakhuis BJ, Stern PL, Verheijen RH, Meijer CJ, Snijders PJ. TSLC1 gene silencing in cervical cancer cell lines and cervical neoplasia. *J Natl Cancer Inst* 2004 Feb 18;96(4):294–305. [PubMed: 14970278]
17. Yu MY, Tong JH, Chan PK, Lee TL, Chan MW, Chan AW, Lo KW, To KF. Hypermethylation of the tumor suppressor gene RASSF1A and frequent concomitant loss of heterozygosity at 3p21 in cervical cancers. *Int J Cancer* 2003 Jun 10;105(2):204–9. [PubMed: 12673680]
18. Zhang Z, Huettner PC, Nguyen L, Bidder M, Funk MC, Li J, Rader JS. Aberrant promoter methylation and silencing of the POU2F3 gene in cervical cancer. *Oncogene* 2006 Aug 31;25(39):5436–45. [PubMed: 16607278]
19. Feng Q, Balasubramanian A, Hawes SE, Toure P, Sow PS, Dem A, Dembele B, Critchlow CW, Xi L, Lu H, McIntosh MW, Young AM, Kiviat NB. Detection of hypermethylated genes in women with and without cervical neoplasia. *J Natl Cancer Inst* 2005 Feb 16;97(4):273–82. [PubMed: 15713962]
20. Nehls K, Vinokurova S, Schmidt D, Kommos F, Reuschenbach M, Kisseljov F, Einenkel J, von Knebel DM, Wentzensen N. p16 methylation does not affect protein expression in cervical carcinogenesis. *Eur J Cancer*. 2008 Aug 20;
21. Lai HC, Lin YW, Huang TH, Yan P, Huang RL, Wang HC, Liu J, Chan MW, Chu TY, Sun CA, Chang CC, Yu MH. Identification of novel DNA methylation markers in cervical cancer. *Int J Cancer* 2008 Jul 1;123(1):161–7. [PubMed: 18398837]
22. Sova P, Feng Q, Geiss G, Wood T, Strauss R, Rudolf V, Lieber A, Kiviat N. Discovery of novel methylation biomarkers in cervical carcinoma by global demethylation and microarray analysis. *Cancer Epidemiol Biomarkers Prev* 2006 Jan;15(1):114–23. [PubMed: 16434596]
23. Wang SS, Smiraglia DJ, Wu YZ, Ghosh S, Rader JS, Cho KR, Bonfiglio TA, Nayar R, Plass C, Sherman ME. Identification of novel methylation markers in cervical cancer using restriction landmark genomic scanning. *Cancer Res* 2008 Apr 1;68(7):2489–97. [PubMed: 18381458]
24. Gargiulo G, Minucci S. Epigenomic profiling of cancer cells. *Int J Biochem Cell Biol*. 2008 Aug 13;
25. Kahn SL, Ronnett BM, Gravitt PE, Gustafson KS. Quantitative methylation-specific PCR for the detection of aberrant DNA methylation in liquid-based Pap tests. *Cancer* 2008 Feb 25;114(1):57–64. [PubMed: 18181097]
26. Kuzmin I, Liu L, Dammann R, Geil L, Stanbridge EJ, Wilczynski SP, Lerman MI, Pfeifer GP. Inactivation of RAS association domain family 1A gene in cervical carcinomas and the role of human papillomavirus infection. *Cancer Res* 2003 Apr 15;63(8):1888–93. [PubMed: 12702579]
27. Choi CH, Lee KM, Choi JJ, Kim TJ, Kim WY, Lee JW, Lee SJ, Lee JH, Bae DS, Kim BG. Hypermethylation and loss of heterozygosity of tumor suppressor genes on chromosome 3p in cervical cancer. *Cancer Lett* 2007 Sep 18;255(1):26–33. [PubMed: 17467893]
28. Feng Q, Hawes SE, Stern JE, Dem A, Sow PS, Dembele B, Toure P, Sova P, Laird PW, Kiviat NB. Promoter hypermethylation of tumor suppressor genes in urine from patients with cervical neoplasia. *Cancer Epidemiol Biomarkers Prev* 2007 Jun;16(6):1178–84. [PubMed: 17548682]
29. Henken FE, Wilting SM, Overmeer RM, van Rietschoten JG, Nygren AO, Errami A, Schouten JP, Meijer CJ, Snijders PJ, Steenbergen RD. Sequential gene promoter methylation during HPV-induced cervical carcinogenesis. *Br J Cancer* 2007 Nov 19;97(10):1457–64. [PubMed: 17971771]

30. Jo H, Kang S, Kim JW, Kang GH, Park NH, Song YS, Park SY, Kang SB, Lee HP. Hypermethylation of the COX-2 gene is a potential prognostic marker for cervical cancer. *J Obstet Gynaecol Res* 2007 Jun;33(3):236–41. [PubMed: 17578348]
31. Lai HC, Lin YW, Chang CC, Wang HC, Chu TW, Yu MH, Chu TY. Hypermethylation of two consecutive tumor suppressor genes, BLU and RASSF1A, located at 3p21.3 in cervical neoplasias. *Gynecol Oncol* 2007 Mar;104(3):629–35. [PubMed: 17097722]
32. Lattario F, Furtado YL, Silveira FA, do VI, Almeida G, da Gloria da Costa Carvalho. Evaluation of DAPK gene methylation and HPV and EBV infection in cervical cells from patients with normal cytology and colposcopy. *Arch Gynecol Obstet.* 2007 Nov 20;
33. Oikonomou P, Messinis I, Tsezou A. DNA methylation is not likely to be responsible for hTERT expression in premalignant cervical lesions. *Exp Biol Med (Maywood)* 2007 Jul;232(7):881–6. [PubMed: 17609503]
34. Seng TJ, Low JS, Li H, Cui Y, Goh HK, Wong ML, Srivastava G, Sidransky D, Califano J, Steenbergen RD, Rha SY, Tan J, Hsieh WS, Ambinder RF, Lin X, Chan AT, Tao Q. The major 8p22 tumor suppressor DLC1 is frequently silenced by methylation in both endemic and sporadic nasopharyngeal, esophageal, and cervical carcinomas, and inhibits tumor cell colony formation. *Oncogene* 2007 Feb 8;26(6):934–44. [PubMed: 16862168]
35. Shivapurkar N, Sherman ME, Stastny V, Echebiri C, Rader JS, Nayar R, Bonfiglio TA, Gazdar AF, Wang SS. Evaluation of candidate methylation markers to detect cervical neoplasia. *Gynecol Oncol* 2007 Dec;107(3):549–53. [PubMed: 17894941]
36. Jeong DH, Youm MY, Kim YN, Lee KB, Sung MS, Yoon HK, Kim KT. Promoter methylation of p16, DAPK, CDH1, and TIMP-3 genes in cervical cancer: correlation with clinicopathologic characteristics. *Int J Gynecol Cancer* 2006 May;16(3):1234–40. [PubMed: 16803511]
37. Kang S, Kim JW, Kang GH, Lee S, Park NH, Song YS, Park SY, Kang SB, Lee HP. Comparison of DNA hypermethylation patterns in different types of uterine cancer: cervical squamous cell carcinoma, cervical adenocarcinoma and endometrial adenocarcinoma. *Int J Cancer* 2006 May 1;118(9):2168–71. [PubMed: 16331610]
38. Kitkumthorn N, Yanatatsanajit P, Kiatpongsan S, Phokaew C, Triratanachatt S, Trivijitsilp P, Termrungruanglert W, Tresukosol D, Niruthisard S, Mutirangura A. Cyclin A1 promoter hypermethylation in human papillomavirus-associated cervical cancer. *BMC Cancer* 2006;6:55. [PubMed: 16524460]
39. Narayan G, Goparaju C, rias-Pulido H, Kaufmann AM, Schneider A, Durst M, Mansukhani M, Pothuri B, Murty VV. Promoter hypermethylation-mediated inactivation of multiple Slit-Robo pathway genes in cervical cancer progression. *Mol Cancer* 2006;5:16. [PubMed: 16700909]
40. Ren CC, Miao XH, Yang B, Zhao L, Sun R, Song WQ. Methylation status of the fragile histidine triad and E-cadherin genes in plasma of cervical cancer patients. *Int J Gynecol Cancer* 2006 Sep; 16(5):1862–7. [PubMed: 17009983]
41. Wisman GB, Nijhuis ER, Hoque MO, Reesink-Peters N, Koning AJ, Volders HH, Buikema HJ, Boezen HM, Hollema H, Schuurin E, Sidransky D, van der Zee AG. Assessment of gene promoter hypermethylation for detection of cervical neoplasia. *Int J Cancer* 2006 Oct 15;119(8): 1908–14. [PubMed: 16736496]
42. Kang S, Kim JW, Kang GH, Park NH, Song YS, Kang SB, Lee HP. Polymorphism in folate- and methionine-metabolizing enzyme and aberrant CpG island hypermethylation in uterine cervical cancer. *Gynecol Oncol* 2005 Jan;96(1):173–80. [PubMed: 15589597]
43. Shigematsu H, Suzuki M, Takahashi T, Miyajima K, Toyooka S, Shivapurkar N, Tomlinson GE, Mastrangelo D, Pass HI, Brambilla E, Sathyanarayana UG, Czerniak B, Fujisawa T, Shimizu N, Gazdar AF. Aberrant methylation of HIN-1 (high in normal-1) is a frequent event in many human malignancies. *Int J Cancer* 2005 Feb 10;113(4):600–4. [PubMed: 15472908]
44. Takahashi T, Suzuki M, Shigematsu H, Shivapurkar N, Echebiri C, Nomura M, Stastny V, Augustus M, Wu CW, Wistuba II, Meltzer SJ, Gazdar AF. Aberrant methylation of Reprimin in human malignancies. *Int J Cancer* 2005 Jul 1;115(4):503–10. [PubMed: 15700311]
45. Gustafson KS, Furth EE, Heitjan DF, Fansler ZB, Clark DP. DNA methylation profiling of cervical squamous intraepithelial lesions using liquid-based cytology specimens: an approach that utilizes receiver-operating characteristic analysis. *Cancer* 2004 Aug 25;102(4):259–68. [PubMed: 15368319]

46. Ivanova T, Vinokurova S, Petrenko A, Eshilev E, Solovyova N, Kisseljov F, Kisseljova N. Frequent hypermethylation of 5' flanking region of TIMP-2 gene in cervical cancer. *Int J Cancer* 2004 Mar 1;108(6):882–6. [PubMed: 14712492]
47. Kim TY, Lee HJ, Hwang KS, Lee M, Kim JW, Bang YJ, Kang GH. Methylation of RUNX3 in various types of human cancers and premalignant stages of gastric carcinoma. *Lab Invest* 2004 Apr;84(4):479–84. [PubMed: 14968123]
48. Lea JS, Coleman R, Kurien A, Schorge JO, Miller DS, Minna JD, Muller CY. Aberrant p16 methylation is a biomarker for tobacco exposure in cervical squamous cell carcinogenesis. *Am J Obstet Gynecol* 2004 Mar;190(3):674–9. [PubMed: 15041998]
49. Narayan G, rias-Pulido H, Nandula SV, Basso K, Sugirtharaj DD, Vargas H, Mansukhani M, Villella J, Meyer L, Schneider A, Gissmann L, Durst M, Pothuri B, Murty VV. Promoter hypermethylation of FANCF: disruption of Fanconi Anemia-BRCA pathway in cervical cancer. *Cancer Res* 2004 May 1;64(9):2994–7. [PubMed: 15126331]
50. Reesink-Peters N, Wisman GB, Jeronimo C, Tokumaru CY, Cohen Y, Dong SM, Klip HG, Buikema HJ, Suurmeijer AJ, Hollema H, Boezen HM, Sidransky D, van der Zee AG. Detecting cervical cancer by quantitative promoter hypermethylation assay on cervical scrapings: a feasibility study. *Mol Cancer Res* 2004 May;2(5):289–95. [PubMed: 15192122]
51. Shivapurkar N, Toyooka S, Toyooka KO, Reddy J, Miyajima K, Suzuki M, Shigematsu H, Takahashi T, Parikh G, Pass HI, Chaudhary PM, Gazdar AF. Aberrant methylation of trail decoy receptor genes is frequent in multiple tumor types. *Int J Cancer* 2004 May 1;109(5):786–92. [PubMed: 14999791]
52. Widschwendter A, Muller HM, Hubalek MM, Wiedemair A, Fiegl H, Goebel G, Mueller-Holzner E, Marth C, Widschwendter M. Methylation status and expression of human telomerase reverse transcriptase in ovarian and cervical cancer. *Gynecol Oncol* 2004 May;93(2):407–16. [PubMed: 15099954]
53. Widschwendter A, Muller HM, Fiegl H, Ivarsson L, Wiedemair A, Muller-Holzner E, Goebel G, Marth C, Widschwendter M. DNA methylation in serum and tumors of cervical cancer patients. *Clin Cancer Res* 2004 Jan 15;10(2):565–71. [PubMed: 14760078]
54. Yang HJ, Liu VW, Wang Y, Chan KY, Tsang PC, Khoo US, Cheung AN, Ngan HY. Detection of hypermethylated genes in tumor and plasma of cervical cancer patients. *Gynecol Oncol* 2004 May;93(2):435–40. [PubMed: 15099958]
55. Chan TF, Su TH, Yeh KT, Chang JY, Lin TH, Chen JC, Yuang SS, Chang JG. Mutational, epigenetic and expressional analyses of caveolin-1 gene in cervical cancers. *Int J Oncol* 2003 Sep;23(3):599–604. [PubMed: 12888893]
56. Chen CL, Liu SS, Ip SM, Wong LC, Ng TY, Ngan HY. E-cadherin expression is silenced by DNA methylation in cervical cancer cell lines and tumours. *Eur J Cancer* 2003 Mar;39(4):517–23. [PubMed: 12751384]
57. Narayan G, rias-Pulido H, Koul S, Vargas H, Zhang FF, Villella J, Schneider A, Terry MB, Mansukhani M, Murty VV. Frequent promoter methylation of CDH1, DAPK, RARB, and HIC1 genes in carcinoma of cervix uteri: its relationship to clinical outcome. *Mol Cancer* 2003 May 13;2:24. [PubMed: 12773202]
58. Wu Q, Shi H, Suo Z, Nesland JM. 5'-CpG island methylation of the FHIT gene is associated with reduced protein expression and higher clinical stage in cervical carcinomas. *Ultrastruct Pathol* 2003 Nov;27(6):417–22. [PubMed: 14660280]
59. Ivanova T, Petrenko A, Gritsko T, Vinokourova S, Eshilev E, Kobzeva V, Kisseljov F, Kisseljova N. Methylation and silencing of the retinoic acid receptor-beta 2 gene in cervical cancer. *BMC Cancer* 2002 Mar 21;2:4. [PubMed: 11945179]
60. Dong SM, Kim HS, Rha SH, Sidransky D. Promoter hypermethylation of multiple genes in carcinoma of the uterine cervix. *Clin Cancer Res* 2001 Jul;7(7):1982–6. [PubMed: 11448914]
61. Virmani AK, Muller C, Rathi A, Zochbauer-Mueller S, Mathis M, Gazdar AF. Aberrant methylation during cervical carcinogenesis. *Clin Cancer Res* 2001 Mar;7(3):584–9. [PubMed: 11297252]

62. Wong YF, Chung TK, Cheung TH, Nobori T, Yu AL, Yu J, Batova A, Lai KW, Chang AM. Methylation of p16INK4A in primary gynecologic malignancy. *Cancer Lett* 1999 Mar 1;136(2): 231–5. [PubMed: 10355753]

Figure 1.

Methylation frequencies reported in cervical cancers for 15 genes. The center of the circle indicates the reported frequency. The size of the circle indicates the size of the study. Horizontal lines indicate the weighted average methylation frequency for that gene based on all studies in cancers.

Figure 2.

Methylation frequencies reported in normal cervical tissue for 15 genes. The center of the circle indicates the reported frequency. The size of the circle indicates the size of the study. Horizontal lines indicate the weighted average methylation frequency for that gene based on all studies in normal tissue.

Table 1

Summary of Materials and Methods Included in 51 studies

	Fresh frozen	Exfoliated cells	Paraffin embedded tissue	Not specified	Total
MSP	15	5	4	8	32
QMSP	3	7	2	0	12
Other	6	0	1	0	7
Total	24	12	7	8	51

MSP=methylation specific PCR; QMSP=quantitative MSP (including Methylight)

Overview of methylation markers analyzed in cervical specimens

Table 2

Marker	Studies	Total Samples	Total CA	Freq CA	CA-low	CA-high	Total NL	Freq NL	NL-low	NL-high	Total CIN	Method s
DAPK1	18	1528	881	0.57	0.38	0.82	362	0.03	0	0.3	295	4
RASSF1	15	1595	1109	0.12	0	0.46	259	0.03	0	0.41	193	5
CDH1	13	1014	767	0.58	0	0.91	148	0.24	0	0.58	109	3
CDKN2A	13	1331	829	0.21	0.05	0.57	330	0.04	0	0.09	172	2
MGMT	10	973	565	0.17	0.05	0.92	278	0.07	0	0.15	130	3
RARB	10	780	380	0.33	0.13	0.53	248	0.03	0	0.11	113	5
APC	9	896	581	0.23	0.11	0.54	240	0.26	0	0.39	75	3
FHIT	9	983	613	0.41	0	0.79	268	0.08	0	0.94	102	2
MLH1	9	881	488	0.02	0	0.36	229	0	0	0.01	174	3
TIMP3	9	540	433	0.19	0.01	1	51	0			56	4
GSTP1	6	697	330	0.02	0	0.21	235	0.03	0	0.11	142	3
CADM1	5	285	99	0.55	0.42	0.65	57	0.05	0	0.1	129	3
CDH13	5	483	202	0.39	0.1	0.82	141	0.18	0	0.2	150	3
HIC1	5	539	244	0.39	0.04	0.71	191	0.43	0	0.64	104	2
TERT	5	260	110	0.55	0.2	0.82	40	0.18	0	0.27	76	1
HLTF	3	273	256	0.08	0.04	0.16	17	0				1
PI4	3	211	164	0.02	0	0.05	19	0			28	1
THBS1	3	273	256	0.37	0.35	0.38	17	0				1
TP73	3	153	106	0.11	0	0.5	19	0.05	0	0.18	28	2
CALCA	2	68	49	0.8	0.68	1	19	0				2
CDKN2B	2	322	106	0.04	0.04	0.08	131	0.04			85	2
COX2	2	181	164	0.15	0.15	0.15	17	0				1
ESR1	2	73	54	0.17			19	0				2
RUNX3	2	122	122	0.02	0.02	0.03						1
SLIT2	2	382	200	0.62	0.6	0.64	51	0			131	2
SOCs2	2	82	26	0.54	0.53	0.64					56	1
SPARC	2	142	22	0.91			51	0.14	0.05	0.23	69	2
TPP2	2	142	22	0.82			51	0.14	0	0.36	69	2
TNFRSF10	2	130	95	0.76			12	0			23	2

Marker	Studies	Total Samples	Total CA	Freq CA	CA-low	CA-high	Total NL	Freq NL	NL-low	NL-high	Total CIN	Method s
C												
TWIST1	2	299	119	0.37			101	0.01				2
VHL	2	313	99	0.01	0	0.02	136	0.01	0	0.01	78	1
ASC	1	289	89	0.07			130	0.03			70	1
BRCA1	1	90	82	0.06			8	0				1
BRCA2	1	109	91	0			18	0				1
C15orf48	1	43	22	0.36			21	0				1
CAV1	1	144	72	0.06			72	0				1
CCNA1	1	84	35	0.94			25	0			24	1
CCND2	1	189	54	0.07			84	0.04			51	1
CHFR	1	24	24	0.38								1
CTNNB1	1	49	30	0			19	0				1
DLC1	1	8	8	0.88								1
FANCF	1	109	91	0.3			18	0				1
HS3ST2	1	80	45	0.93			12	0			23	1
HSPA2	1	55	11	0.73							44	1
LHFPL4	1	27	15	0.67							12	1
MTIG	1	43	22	0.55			21	0.05				1
MYOD1	1	19	19	1								1
NOL4	1	27	15	0.53							12	1
PGR	1	19	19	1								1
POU2F3	1	53	46	0.39			7	0				1
PRDM2	1	216	77	0.03			100	0.02			39	1
PTEN	1	72	62	0.58							10	1
RB1	1	90	82	0			8	0				1
ROBO1	1	280	119	0.46			51	0			110	1
ROBO3	1	245	118	0.36			41	0			86	1
RPRM	1	49	49	0.18								1
RRAD	1	43	22	0.68			21	0.43				1
SCGB3A1	1	44	44	0.18								1
SFN	1	239	70	0.99			104	0.91			65	1

Marker	Studies	Total Samples	Total CA	Freq CA	CA-low	CA-high	Total NL	Freq NL	NL-low	NL-high	Total CIN	Method s
SFRP1	1	43	22	0.58			21	0.05				1
SLIT1	1	246	119	0.53			40	0			87	1
SLIT3	1	248	118	0.49			40	0			90	1
SOC31	1	55	11	0.5							44	1
SOC33	1	80	45	0.27			12	0			23	1
SYK	1	198	73	0.15			83	0.06			42	1
TIMP2	1	36	36	0.47								1
TNFRSF10	1	50	50	0.18								1
D												
ZMYND10	1	273	127	0.77			40	0.13			106	1

Studies: Number of studies that analyzed the respective gene. Total: Total number of samples analyzed. Freq: Average frequency in samples, weighted by study size. Freq-low/high: Range of reported frequencies. Methods: Total number of different methylation detection methods applied for that gene.

Table 3

Methylation frequencies in squamous cell cancers and adenocarcinomas

Gene	Studies	SCC	SCC-Pos	freq	low	high	AC	AC-pos	freq	low	high
RASSF1A	9	531	44	0.08	0	0.3	260	68	0.26	0.12	0.45
DAPK	8	368	217	0.59	0.47	0.77	119	41	0.34	0	0.59
CDKN2A	7	428	138	0.32	0.08	0.61	119	31	0.26	0.07	0.75
APC	5	206	20	0.1	0.05	0.15	73	37	0.51	0.4	0.8
CDHI	5	291	175	0.6	0	0.89	87	29	0.33	0	0.9
MGMT	4	185	33	0.18	0.05	0.94	59	13	0.22	0	0.88
TIMP3	4	164	10	0.06	0.03	0.13	58	31	0.53	0.23	0.63
HIC1	3	128	23	0.18	0.05	0.32	35	17	0.49	0	0.63
RARB	3	113	29	0.26	0.13	0.31	21	3	0.14	0	0.25
ESR1	2	36	11	0.31	0.25	0.38	16	5	0.31	0.13	0.5
FHIT	2	97	9	0.09	0	0.17	13	0	0		
MLH1	2	82	2	0.02	0.01	0.05	38	3	0.08	0.03	0.25

Studies: Number of studies that analyzed the respective gene. SCC: Total number of squamous cell cancer samples analyzed. Freq: Average frequency in cancer samples weighted by study size. Low/high: Range of reported frequencies. AC: Total number of adenocarcinoma samples analyzed.