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DNA Methylation in Endometrial Cancer

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Abstract

Endometrial cancer is the most commonly diagnosed gynecological cancer, and it has been shown to be a complex disease driven by abnormal genetic, and epigenetic alterations, as well as environmental factors. Epigenetic changes resulting in aberrant gene expression are dynamic and modifiable features of many cancer types. A significant epigenetic change is aberrant DNA methylation. In this review, we review evidence on the role of aberrant DNA methylation, examining changes in relation to endometrial carcinogenesis, and report on recent advances in the understanding of the contribution of aberrant DNA methylation to endometrial cancer with the emphasis on the role of dietary/lifestyle and environmental factors, as well as opportunities and challenges of DNA methylation in endometrial cancer management and prevention.

Keywords

DNA methylation; endometrial cancer; epidemiology

Introduction

Endometrial cancer is the most commonly diagnosed gynecological cancer. It is the 4th most common cancer for women, with 42,160 new cases and 7,780 deaths occurring in the United States in 2009 [1]. Based on differences in clinicopathologic characteristics, there are two subtypes of endometrial carcinoma. Type I, endometrioid endometrial carcinoma, accounts for approximately 80% of cases, occurs frequently in pre- and peri-menopausal women, and is significantly related to a history of unopposed estrogen exposure or other hyperestrogenic risk factors. Type I tumors are usually well differentiated, and most patients present with early-stage of the disease and have a favorable prognosis. In contrast, type II, non-endometrioid endometrial carcinoma, is more common in older postmenopausal women, often poorly differentiated, and not associated with hyperestrogenic factors. Patients with type II tumors are more likely to have metastasis and are at high risk of relapse [2]. Type I tumors commonly have near-diploid karyotypes, microsatellite instability, and mutations in *PTEN*, *K-RAS*, and *CTNNB1* (*β*-catenin) genes; whereas type II tumors are characterized more often by *p53* mutation, overexpression of *Her-2/neu*, and an aneuploid karyotype [2].

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Although multiple risk factors, such as age, overweight/obesity, and postmenopausal hormone therapy, have been identified, the understanding of the etiologies of the two subtypes of endometrial cancer continues to evolve. Similar to other cancer sites, endometrial cancer has been shown to be a complex disease driven by abnormal genetic, and epigenetic alterations, as well as environmental factors. As a common molecular alteration in human neoplasia, epigenetics is defined as heritable changes in gene expression without alteration of the nucleotide sequence [3]. Epigenetic changes are dynamic and modifiable upon treatment with pharmacological agents. In the last ten years, it has become increasingly apparent that epigenetic regulation of gene expression is at least as important to carcinogenesis as more studied genetic disruptions including aneuploidy, point mutations, and variation in gene copy number, both gain or loss. While epigenetics refers to a broader class of changes, we focus here on the role of DNA methylation, a significant epigenetic change, examining those changes in relation to endometrial carcinogenesis. We report on recent advances in the understanding of the contribution of aberrant DNA methylation to endometrial cancer with the emphasis on the role of dietary/lifestyle and environmental factors, as well as opportunities and challenges of DNA methylation in endometrial cancer management and prevention.

Aberrant DNA Methylation in Endometrial Cancer

The best-known epigenetic event is aberrant DNA methylation. In humans, mediated by the three known active DNA cytosine methyltransferase (DNMT1, 3A, and 3B) [4], a methyl group ($-CH_3$) is covalently bonded to cytosine residues of the CpG dinucleotides, resulting in 5-methylcytosine. DNMT1 maintains attachment of methyl groups to hemimethylated DNA during replication, whereas DNMT3A and DNMT3B can catalyze *de novo* methylation of DNA [5–6]. These enzymes co-operatively regulate the dynamic methylation of DNA. CpG dinucleotides are frequently clustered in CpG islands, regions of DNA rich in CpG sites (60–70%) [7–8]. These island are usually in or around the promoter regions and often unmethylated in normal tissue [7–8], and most CpG sites in the human genome are methylated [9]. DNA methylation is found in normal tissue and contributes to control of transcription [10], affecting processes such as normal development [11–12], silencing of genes on the X-chromosome in females [10], [13], and gene imprinting[14]. Aberrant DNA methylation has been associated with most human tumors [15–16], as well as other non-neoplastic diseases and with aging [17–18].

Decreased DNA methylation (hypomethylation) is an early event in carcinogenesis, and one of the first epigenetic alterations [19]. Hypomethylation can occur at normally methylated DNA sequences as repeated sequences, as well as both encoding regions and introns of genes [15],[20]. It is associated with early stage genetic instability and up-regulation of gene expression [20–21]. Another form of aberrant DNA methylation, hypermethylation of CpG islands in the promoter region of particular genes, appears to be significant in carcinogenesis for a number of tumor sites. Promoter hypermethylation is associated with gene silencing, and can affect carcinogenesis particularly when the affected gene is a tumor suppressor genes or other genes involved in the cell cycle, DNA mismatch repair, cell-to-cell interaction, steroid receptor, apoptosis, and angiogenesis [22]. There is evidence that aberrant DNA methylation is an early and widespread alteration in endometrial tumorigenesis, which

is implicated in loss of expression of a variety of critical genes. A recent study of methylation profile in endometrial tumorigenesis showed that, among 24 tumor suppressor genes, the number of promoter methylated loci increased in the progression from normal endometrium to simple hyperplasia to complex hyperplasia (complex hyperplasia without atypia/complex hyperplasia with atypia) [23]. In addition, aberrant DNA methylation of some tumor suppressor genes was evident before endometrial carcinoma diagnosis in women with the DNA mismatch repair gene mutation [23]. This study provides important evidence into the timing and molecular alterations of the critical events in endometrial carcinogenesis, which may be useful to identify DNA methylation profile for early detection of endometrial cancer. In the following review, we summarize the genes that are frequently silenced by DNA methylation in endometrial cancer (Table 1) and discuss how this mechanism may contribute to endometrial carcinogenesis.

Promoter Hypermethylation of Genes in Endometrial Cancer

DNA mismatch repair gene in endometrial cancer

Germline mutations in DNA mismatch repair genes *hMLH1*, *hMSH2*, *hMSH3*, *hMSH6*, and *hPMS2* have been identified in majority hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch syndrome and in associated familial endometrial cancer [24]. A significant characteristic of HNPCC is the presence of microsatellite instability (MSI), changes in the lengths of repetitive genetic loci [24]. MSI has also been observed to be present in approximately 20% of sporadic endometrial carcinomas [25–30], while *hMLH1* and *hMSH2* mutations are rare (less than 10%) in sporadic endometrial cancers with the MSI + phenotype [31–34]. However, reduced protein expression of *hMLH1* and other mismatch repair genes is a common finding in endometrial cancers lacking detectable mutations [31, 35–38]. There is evidence that promoter methylation is associated with lack of expression of *hMLH1* in human cancers and in mismatch repair-defective human tumor cell lines [39–42]. A strong association between *hMLH1* promoter methylation and transcriptional silencing and MSI + phenotype was also reported in sporadic endometrial cancer, particularly in the endometroid type [27, 31, 38, 43–45]. In addition, the demethylation of the *hMLH1*gene in several cell lines, including one endometrial carcinoma cell line, using the agent 5-aza-2′-deoxycytidine resulted in reaction of *hMLH1*expression and restoration of the activity of mismatch repair genes [46]. *hMLH1* promoter methylation has also been shown to be an early event in the procession from normal endometrium to carcinoma, and as a feature of a subset of precursor lesion [47–51]. However, *hMLH2* methylation is very rare (1.4% of 138 studied cases) in endometrial cancer [44].

Methylation of steroid receptor genes in endometrial cancer

It is known that the endometrium is highly responsive to hormonal stimuli. The cyclic production of estrogen and progesterone during the menstrual cycle, and declining sex steroid hormone levels after menopause are directly correlated with endometrial proliferation and/or atrophic morphological changes. The majority of known risk factors for endometrial cancer are thought to be directly or indirectly related to exposure to hormones, particularly estrogen. A number of studies have evaluated the association of promoter

methylation of the estrogen receptor (ER) and progesterone receptor (PR) genes in endometrial cancer.

The *ER* gene, located at chromosome 6q25.1, has a CpG island in its promoter and exon 1 regions. Reduced ER RNA and protein expression levels have been found in human endometrial cancer tissues and cell lines [52–53], while have not been consistently reported to be associated with aberrant promoter methylation of *ER* [53–57]. The expression of three isoforms of ERα (ERα-A, ERα-B, and ERα-C) and ERβ genes in endometrial cancer cell lines was investigated by Sasaki et al [53]. They found no ERα-C expression, as well as restoration of the expression with 5-aza-2′-deoxycytidine treatment. They further found promoter methylation of ERα-C isoform in 94% of endometrial cancer tissues [53]. However, Shiozawa et al [56] detected ER promoter methylation in only 24% of 25 endometrial cancer cases; methylation was correlated with ER-negative status of the tumors. Navari et al [55] also did not find any association between loss of ER expression and *de novo* methylation of the *ER* gene. Moreover, neither Shiozawa [56] nor Navari [55] detected alterations in the methylation patterns of different ER isoforms. Although the specific role of the three ERα isoforms in endometrial cancer is still not clear, it was hypothesized that each isoform has a specific character due to the existence of elaborate mechanisms regulating estrogenic effects. Further studies investigating promoter methylation of ERα isoforms in endometrial cancer are needed.

The *PR* gene, located at chromosome 11q13, also has a CpG island in its first exon. The *PR* gene encodes two distinct subtypes, PR-A (94 kDa) and PR-B (114 kDa), with distinct functions [58]. Previous studies showed that the ratio of PR-A to PR-B expression is abnormal in endometrial cancer, leading to an inappropriate response to progesterone [58–59]. Consistent with the transcriptional levels of the two PR isoforms, in one study, over 70% of the samples were promoter methylated for PR-B in endometrial cancer, primarily in tissues from type I cancers; however, PR-A was unmethylated in all cancer and normal endometrial samples [60]. It has been suggested that progesterone acts principally through PR-B to inhibit endometrial cancer cell invasiveness modulated by adhesion molecules [61]. Evidence from studies of endometrial cancer cell lines indicated that these changes may be the result of aberrant DNA methylation of these genes. Such cell culture studies have shown no PR-B expression, although PR-A expression was observed [60]. Other studies have shown a positive association between promoter hypermethylation of PR-B and reduced PR mRNA expression in these cancer cell lines [62–63]; DNMT inhibitor 5-aza-2′-deoxycytidine, as well as histone deacetylase (HDAC) inhibitor trichostatin A, led to demethylation and restoration of PR expression.

Methylation of tumor suppressor genes in endometrial cancer

Promoter hypermethylation of tumor suppressor genes is a major event in the origin of many cancers, and has been the focus of attention in the last decade. A number of studies established a list of tumor suppressor genes frequently hypermethylated in endometrial cancer. The phosphatase and tensin homolog (*PTEN*, also known as *MMAC1/TEP1*) gene on chromosome 10q23.3, responsible for Cowden syndrome [64] and BannyanZonana syndrome [65], encodes a dual-specificity phosphatase able to dephosphorylate both tyrosine phosphate

and serine/threonine phosphate residues [66]. It is reported to be important for the inhibition of cell migration and spreading and focal adhesion [66]. Inactivation of *PTEN* has been observed in a number of human cancers, including endometrial cancer. *PTEN* mutations occur in to 26–80% of endometrial cancers, making it the most commonly known genetic alteration associated with this disease [67–68]. Promoter hypermethylation, as the alternative mechanism of *PTEN* allelic inactivation, was first demonstrated as an occurrence in sporadic colorectal tumors with microsatellite instability, reported to be on the order of 19% of tumors [69]. A number of studies have also shown that *PTEN* promoter methylation in about 20% of sporadic type I endometrial carcinoma [70–72], and that this methylation is significantly associated with metastatic disease and with MSI phenotype [72]. However, results from Zysman et al [73] suggested that it is *PTEN* pseudogene, and not *PTEN* itself, which is predominantly methylated in endometrial cancer cell lines and in endometrial tumors. More studies with the ability to make distinction between promoter methylation of *PTEN* and its pseudogene are needed to understand better the mechanisms of *PTEN* inactivation in endometrial carcinogenesis.

Located on chromosome 9p21, the *p16* gene encodes a cyclin-dependent kinase inhibitor, which can block the cell cycle and arrest the growth of deregulated cancer cells [74]. Loss of p16 expression resulting from homozygous deletion, mutation or promoter methylation is a common feature of many human cancers and of cancer cell lines. Promoter methylation of *p16* gene has been observed in between 11% and 75% of sporadic endometrial cancers [51], [75–78]; however, other studies have reported much lower frequencies of *p16* methylation [50], [79–81]. This variability may be a reflection of variation in the sensitivity of the assays used to assess *p16* methylation, differences in primer design for the same assay, and differences in sample size or differences in the populations under study. Furthermore, there is limited and inconsistent data regarding the correlation between that *p16* promoter hypermethylation and clinicopathological features of endometrial cancer. Although Wong et al [75] reported that *p16* promoter methylation was associated with advanced stage and poorer survival of endometrial cancer, no correlation was observed between promoter methylation of *p16* and clinicpathological features and prognosis of endometrial cancer in another recent study [76]. In addition, some studies found that *p16* gene inactivation but rare *p16* methylation occurs in a subgroup of aggressive endometrial carcinomas with poor prognosis [80–81], which suggests that the molecular mechanisms of p16 inactivation in endometrial cancer remain unclear.

Another tumor suppressor gene, Ras-association domain gene family 1A (*RASSF1A*) is located at chromosome 3p21.3 and is known to induce cell cycle arrest through the Rb-mediated checkpoint by inhibiting the accumulation of cyclin D1 [82–83]. Alteration of this tumor suppressor gene is frequently found in tumors from a variety of sites [82–83]. *RASSF1A* promoter methylation has been reported to occur in 33–85% of endometrial cancers and is associated with reduced expression of RASSF1A [84–90]. Arafa et al [84] recently observed frequent hypermethylation of *RASSF1A* gene promoter (36%) in normal endometrium adjacent to endometrioid carcinoma, suggesting that *RASSF1A* promoter methylation is an early event in type I endometrial carcinogenesis. Hypermethylation of this gene has been found to be correlated with loss of heterozygosity (LOH); in a study of cervical cancer, 8 of 12 (67%) with hypermethylated *RASSF1A* gene showed concomitant LOH at 3p21 [91]. LOH

at 3p is a frequent occurrence in endometrial cancer [92]. Aberrant promoter methylation of *RASSF1A* gene combined with LOH at 3p may play a more important role in endometrial carcinogenesis. Moreover, promoter methylation of *RASSF1A* has also been inconsistently found to be associated with advanced stage, recurrence and survival for endometrial cancer [86–89, 93]. These inconsistent associations may be due to small sample size, different subtypes of endometrial cancer under study, and short follow-up period for recurrent and survival analyses.

Adenomatous polyposis coli (APC), a tumor suppressor gene, regulates *β*-catenin in the Wnt signaling pathway, and the aberrations in the Wnt pathway appear to impact the initiation and progression of several human cancers [94–95]. Alterations in the *APC* gene or in the *β*-catenin gene may affect the Wnt pathway and are thought to be associated with endometrial carcinogenesis [96–97]. However, mutations of the *APC* gene are not common events in endometrial cancers [96, 98–99]. *APC* gene promoter methylation has been demonstrated in around 20–45% of endometrial cancers [50, 78, 90, 93, 98–101], and more frequent *APC* promoter methylation in tumors with MSI than in those without MSI was reported in several studies [98–100]. No significant associations of *APC* promoter methylation with the cliniopathological factors or recurrence and distant metastases have been observed in endometrial cancers [50, 78].

β-catenin can complex with molecules including both APC and E-cadherin and is also involved in cell adhesion, together with E-cadherin, *α*-catenin and *γ*-catenin [102]. A few studies have evaluated promoter methylation of *E-cadherin*, a possible tumor suppressor gene, in endometrial cancer [50, 99, 103–104]. Results of these studies have not been consistent. Sito et al [104] reported that the aberrant methylation in promoter region of *E-cadherin* gene is associated with poor differentiation and myometrial invasion in endometrial carcinomas, suggesting a possible role of *E-cadherin* in endometrial cancer progression. However, no association between *E-cadherin* hypermethylation and clinicopathological or immunohistochemical features of endometrial cancer was found in other studies [50, 99, 103]. Pijnenborg et al [99] did not find *E-cadherin* gene promoter methylation in the tested endometrial tumors, although the absence of E-cadherin expression was detected and found to be associated with the development of distant metastases.

Aberrant MGMT methylation in endometrial cancer

O-6-methyguanine-DNA-methyltransferase (*MGMT*), a DNA repair gene, reverses DNA damage via alkylation by removing the methyl group from the O6-guanine and hence protects against DNA mutations. *MGMT* promoter methylation has been found for a number of cancer sites and precancerous lesions, including colorectal, gastric, lung, and glioblastoma; however, it has only been examined in a few studies of endometrial cancer [77, 84, 101, 105]. Methylation of *MGMT* was detected in 48% of synchronous carcinomas of the uterine corpus and ovary [77]; however, low frequency or absence of *MGMT* promoter methylation in singly occurring endometrial cancer was reported in several other studies [84, 101, 105].

Methylation and inactivation of other genes in endometrial cancer

Retinoic acid receptor (RAR) α, -β and –γ and retinoid X receptor are members of the intercellular receptor superfamily. The *RARβ*$_2$ gene has been found to be silenced by promoter methylation in both type I endometrial cancers and endometrial hyperplasia [84], [106], suggesting aberrant methylation of *RARβ*$_2$ gene as an early epigenetic alteration of endometrial carcinogenesis. Other genes, such as cell cycle inhibitor gene *14-3-3σ* [107], paternally expressed gene 3 (*PEG3*) involved in apoptosis [108], the detoxifying enzyme glutathione S-transferase P1 (*GSTP1*) [109], homeobox genes *HOPX*, *HOXA10* and *HOXA11* [110–112], *RUNX3* tumor suppressor gene [113], and metallothionein 1E gene (*MT-1E*) [114], have been found to be inactivated by aberrant methylation in promoter region in endometrial cancer; however, the impacts of promoter methylation of these genes on endometrial carcinogenesis have not been well known and need to be further investigated.

Difference in DNA methylation in type I and type II endometrial cancers

There is evidence that type I and II endometrial cancers also contain distinct profiles of aberrant DNA methylation. Promoter hypermethylation of genes including *MLH1*, *APC*, *MGMT*, *PTEN*, and *RASSF1A* is more frequently detected in type I than type II tumors [72], [86], [98], [115]. In addition, promoter hypermethylation of PR-B has been found as a mechanism of loss of PR-B expression in endometrial cancer; and tumors and cell lines applied in those studies included predominantly type I cancers [60], [62], although PR negative is more common in type II cancer [116]. These findings suggest that promoter hypermethylation may play less of a role in the tumorigenesis of type II cancers.

Some recent studies investigated the expression levels of DNMT1 and DNMT3B in normal endometrium and type I and type II endometrial carcinomas. Compared to normal endometrium, expression levels of both DNMT1 and DNMT3B were significantly increased in type I cancers but down-regulated in type II cancers [86], [117]. Although DNMT1 is known to function as a maintenance methyltraferase, there is evidence that DNMT1 and DNMT3B cooperatively catalyze *de novo* methylation [6]. The lower expression of DNMT1 and DNMT3B may result in global hypomethylation in type II endometrial cancers and contribute to histological differences. More studies are needed to characterize the aberrant DNA methylation profiles in type I and II cancers, to identify different mechanisms for different phenotypes, and to elucidate appropriate prevention approaches.

Environmental Factors, DNA Methylation and Endometrial Cancer

Epidemiology and experimental studies have found a number of agents in diet and environment involved the epigenetic alterations in the process of a variety of human cancers, including endometrial cancer. Those agents are considered “epigenetic carcinogens” (epimutagens) [118].

Diet

Diet is an important modifier of DNA methylation profile. The most studied and among the best understood is the relationship between micronutrients involved in one-carbon metabolism and DNA methylation [119–121]. Folate plays a central role in one carbon

metabolism that provides a methyl group for a variety of biological process including methylation of DNA, RNA and protein, as well as the synthesis of purines and pyrimidines for DNA synthesis [119–121]. Vitamins B_2, B_6, and B_{12}, the essential amino acid, methionine, are also involved in one carbon metabolism, potentially affecting genomic DNA methylation and synthesis and thereby causing dysregulation of gene expression [119–121]. Low levels of folate, vitamins B_2, B_6, B_{12} and methionine have been shown to be associated with cancer and cardiovascular disease risk [122–123]. Deficiencies of methyl donor intake and excess alcohol consumption have been found to induce promoter hypermethylation and global hypomethylation in animal models [124–126]. However, there is limited evidence from epidemiological studies of different cancer sites that these nutrients intake affect DNA methylation [127–131]; and very little known for the potential effect of low level of methyl donor intake on endometrial DNA methylation. One concern with interpretation of these inconsistent findings is that each study examined methylation status of a limited number of genes, it cannot be ruled out that methyl donor intake may have impact on promoter methylation of other excluded genes. There is evidence that the folate status may be tissue specific [121], which may explain at least in part the inconsistency of study results. Moreover, diet can also alter histone modification [118]. It is possible that diet may impact development of cancer through the mechanisms of both DNA methylation and other epigenetic alterations.

Phytoestrogens are naturally occurring compounds in many foods such as soy and soy products, and associated with reduced endometrial cancer risk in Asian populations and Western vegetarian populations. Genistein, one of many phytoestrogens presented in soy, can inhibit cell growth, angiognesis and induces apoptosis in cancer cell lines and animal models [132–134]; and exhibit mixed estrogenic and anti-estrogenic properties [135]. The underlying molecular mechanisms for these effects of genistein are not well established, but recent studies suggest that genistein may regulate gene activity by modulating epigenetic events. While there are no data on this possible association for endometrial cancer, there is some evidence from other cancer sites that phytoestrogen intake may affect methylation. Day et al [136] reported gene-specific increases in prostate DNA methylation patterns among mice treated with a diet high in genistein compared to mice on a control diet. Several studies also have shown that genistein, lignans, and other related soy isoflavones can reverse hypermethylation and reactivate the silenced genes, including *RAR*β_2, *p16*, *MGMT*, *GSTP*1 and *BTG3* genes, in esophageal, breast and renal cancer cell lines [137–140]. These *in vitro* data showed that genistein can have a dose-dependent inhibition of DNMTA and HDAC activities [137], and increase active chromatin modifications near the transcription start site [140]. However, in a recent study conducted in healthy premenopausal women, promoter methylation of *RAR*β_2 and *CCND2* genes in mammary tissue was lower for women with low blood genistein and higher among those with higher circulating genistein [141]. These *in vitro* studies used higher concentrations of genistein than those found in women consuming soy products [137, 141]. Further studies are needed to evaluate the impact and potential mechanism of dietary phytoestrogens on DNA methylation in endometrial tissue, and identify the proper administration level of phytoestrogens recommended for chemoprevention of endometrial cancer.

Other lifestyle factors

Physical activity has been shown to be associated with modestly reduced risk of endometrial cancer. A recent cross-sectional study in women without breast cancer found that both lifetime and recent physical activity were inversely related with promoter hypermethylation of *APC* gene in nonmalignant breast tissue [142]. No similar studies on endometrial cancer have been reported, but the finding for breast tissue suggests that the protective effect of physical activity on endometrium may be associated with epigenetic changes including DNA methylation.

External steroid hormones

In the past several decades, external sources of steroid hormones, which influence cell proliferation and therefore the risk of endometrial cancer, have been widely used and contribute to endometrial cancer development, particularly type I tumors. Epigenetic events may play an important role in the physiological response to external steroid hormones and undergo continuous modification and alteration. Progestrone has been given in combination with estrogen in hormone therapy to prevent the increasedrisk of endometrial cancer associated with unopposed estrogentherapy. The use of combined oral contraceptives (COCs) has generally been seen to reduce the risk of endometrial cancer [143–145], which may be due to the progestrone component of COCs. Recent studies showed that DNMT3A and DNMT3B in human endometrium are under the regulation of both progesterone and estrogen, suggesting DNA methylation may be influenced by sex steroid hormones [146]. It is possible the external progesterone mediates a protective effect on endometrium through impacting DNMT activities or down-regulation of an epimutagen effect of estrogen by epigenetic modifications. However, more studies are needed to clarify the molecular mechanisms of external progestin on endometrium.

Conclusion

Recent developments in the field of epigenetics, especially studies of DNA methylation, have provided valuable insights for understanding the role of epigenetic alterations in normal cellular processes and abnormal changes leading to endometrial carcinogenesis. These new insights hold tremendous potential in the diagnosis, treatment and prevention of endometrial cancer.

There is accumulating evidence that DNA methylation changes may contribute to carcinogenesis in the endometrium, although evidence regarding these changes induced by dietary/lifestyle and environmental factors in endometrial cancer is quite limited. There is still much to explore regarding the target genes of aberrant methylation, how they contribute to the carcinogenic process and what factors affect that methylation. The emerging powerful technologies [147] can be used to quickly identify DNA methylation profiles for different subtypes of endometrial cancer. In addition, epigenetic-epidemiological studies provide opportunities not only to study the contribution of epigenetics to endometrial cancer but to understand the joint impact of genetic, epigenetic and environmental exposures on the risk of endometrial cancer.

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References

1. American Cancer Society. 2009Cancer Facts and Figures 2009. www.cancer.org
2. Prat J, Gallardo A, Cuatrecasas M, Catasús L. Endometrial carcinoma: pathology and genetics. Pathology. 2007; 39:72–87. [PubMed: 17365824]
3. Holliday R. The inheritance of epigenetic defects. Science. 1987; 238:163–70. [PubMed: 3310230]
4. Robertson KD, Uzvolgyi E, Liang G, Talmadge C, Sumegi J, Gonzales FA, et al. The human DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors. Nucleic Acids Res. 1999; 27:2291–8. [PubMed: 10325416]
5. Turek-Plewa J, Jagodziński PP. The role of mammalian DNA methyltransferases in the regulation of gene expression. Cell Mol Biol Lett. 2005; 10:631–47. [PubMed: 16341272]
6. Brenner C, Fuks F. DNA methyltransferases: facts, clues, mysteries. Curr Top Microbiol Immunol. 2006; 301:45–66.
7. Cross SH, Bird AP. CpG islands and genes. Curr Opin Genet Dev. 1995; 5:309–14. [PubMed: 7549424]
8. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med. 2003; 349:2042–54. [PubMed: 14627790]
9. Bird AP. Gene number, noise reduction and biological complexity. Trends Genet. 1995; 11:94–100. [PubMed: 7732579]
10. Robertson KD. DNA methylation and human disease. Nat Rev Genet. 2005; 6:597–610. [PubMed: 16136652]
11. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. Science. 2001; 293:1089–93. [PubMed: 11498579]
12. Gopalakrishnan S, Van Emburgh BO, Robertson KD. DNA methylation in development and human disease. Mutat Res. 2008; 647:30–8. [PubMed: 18778722]
13. Heard E, Clerc P, Avner P. X-chromosome inactivation in mammals. Annu Rev Genet. 1997; 31:571–610. [PubMed: 9442908]
14. Ideraabdullah FY, Vigneau S, Bartolomei MS. Genomic imprinting mechanisms in mammals. Mutat Res. 2008; 647:77–85. [PubMed: 18778719]
15. Esteller M. Epigenetics in cancer. N Engl J Med. 2008; 358:1148–59. [PubMed: 18337604]
16. Wajed SA, Laird PW, DeMeester TR. DNA methylation: an alternative pathway to cancer. Ann Surg. 2001; 234:10–20. [PubMed: 11420478]
17. Wilson AG. Epigenetic regulation of gene expressio in the inflammatory response and relevance to common disease. J Periodontol. 2008; 79 (8 Suppl):1514–9. [PubMed: 18673005]
18. Liu L, van Groen T, Kadish I, Tollefsbol TO. DNA methylation impacts on learning and memory in aging. Neurobiol Aging. 2009; 30:549–60. [PubMed: 17850924]
19. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature. 1983; 301:89–92. [PubMed: 6185846]
20. Kisseljova NP, Kisseljova FL. DNA demethylation and carcinogenesis. Biochemistry. 2005; 70:743–52. [PubMed: 16097937]
21. Wilson AS, Power BE, Molloy PL. DNA hypomethylation and human diseases. Biochim Biophs Acta. 2007; 1775:138–62.
22. Widschwendter M, Jones PA. DNA methylation and breast carcinogenesis. Oncogene. 2002; 21:5462–82. [PubMed: 12154408]
23. Nieminen TT, Gylling A, Abdel-Rahman WM, Nuorva K, Aarnio M, Renkonen-Sinisalo L, et al. Molecular analysis of endometrial tumorigenesis: importance of complex hyperplasia regardless of atypia. Clin Cancer Res. 2009; 15:5772–83. [PubMed: 19723644]
24. Wheeler JMD. Epigenetics, mismatch repair genes and colorectal cancer. Ann R Coll Surg Engl. 2005; 87:15–20. [PubMed: 15720901]

25. Caduff RF, Johnston CM, Svoboda-Newman SM, Poy EL, Merajver SD, Frank TS. Clinical and pathological significance of microsatellite instability in sporadic endometrial carcinoma. Am J Pathol. 1996; 148:1671–8. [PubMed: 8623934]
26. Duggan BD, Felix JC, Muderspach LI, Tourgeman D, Zheng J, Shibata D. Microsatellite instability in sporadic endometrial carcinoma. J Natl Cancer Inst. 1994; 86:1216–21. [PubMed: 8040889]
27. Gurin CC, Federici MG, Kang L, Boyd J. Causes and consequences of microsatellite instability in endometrial carcinoma. Cancer Res. 1999; 59:462–6. [PubMed: 9927063]
28. Helland A, Børresen-Dale AL, Peltomäki P, Hektoen M, Kristensen GB, Nesland JM, et al. Microsatellite instability in cervical and endometrial carcinomas. Int J Cancer. 1997; 70:499–501. [PubMed: 9052745]
29. Kobayashi K, Sagae S, Kudo R, Saito H, Koi S, Nakamura Y. Microsatellite instability in endometrial carcinomas: frequent replication errors in tumors of early onset and/or of poorly differentiated type. Genes Chromosomes Cancer. 1995; 14:128–32. [PubMed: 8527394]
30. Muresu R, Sini MC, Cossu A, Tore S, Baldinu P, Manca A, et al. Chromosomal abnormalities and microsatellite instability in sporadic endometrial cancer. Eur J Cancer. 2002; 38:1802–9. [PubMed: 12175698]
31. Esteller M, Levine R, Baylin SB, Ellenson LH, Herman JG. MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. Oncogene. 1998; 17:2413–7. [PubMed: 9811473]
32. Katabuchi H, van Rees B, Lambers AR, Ronnett BM, Blazes MS, Leach FS, et al. Mutations in DNA mismatch repair genes are not responsible for microsatellite instability in most sporadic endometrial carcinomas. Cancer Res. 1995; 155:5556–60. [PubMed: 7585634]
33. Kobayashi K, Matsushima M, Koi S, Saito H, Sagae S, Kudo R, et al. Mutational analysis of mismatch repair genes, hMLH1 and hMSH2, in sporadic endometrial carcinomas with microsatellite instability. Jpn J Cancer Res. 1996; 87:141–5. [PubMed: 8609062]
34. Lim PC, Tester D, Cliby W, Ziesmer SC, Roche PC, Hartmann L, et al. Absence of mutations in DNA mismatch repair genes in sporadic endometrial tumors with microsatellite instability. Clin Cancer Res. 1996; 2:1907–11. [PubMed: 9816148]
35. Staebler A, Lax SF, Ellenson LH. Altered expression of hMLH1 and hMSH2 protein in endometrial carcinomas with microsatellite instability. Hum Pathol. 2000; 31:354–8. [PubMed: 10746679]
36. Stefansson I, Akslen LA, MacDonald N, Ryan A, Das S, Jacobs IJ, et al. Loss of hMSH2 and hMSH6 expression is frequent in sporadic endometrial carcinomas with microsatellite instability: a population-based study. Clin Cancer Res. 2002; 8:138–43. [PubMed: 11801550]
37. Baldinu P, Cossu A, Manca A, Satta MP, Pisano M, Casula M, et al. Microsatellite instability and mutation analysis of candidate genes in unselected sardinian patients with endometrial carcinoma. Cancer. 2002; 94:3157–68. [PubMed: 12115348]
38. Kondo E, Furukawa T, Yoshinaga K, Kijima H, Semba S, Yatsuoka T, et al. Not hMSH2 but hMLH1 is frequently silenced by hypermethylation in endometrial cancer but rarely silenced in pancreatic cancer with microsatellite instability. Int J Cancer. 2000; 17:535–41.
39. Kane MF, Loda M, Gaida GM, Lipman J, Mishra R, Goldman H, et al. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res. 1997; 57:808–11. [PubMed: 9041175]
40. Cunningham JM, Christensen ERTD, Kim CY, Roche PC, Burgart LJ, Thibodeau SN. Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. Cancer Res. 1998; 58:3455–60. [PubMed: 9699680]
41. Veigl ML, Kasturi L, Olechnowicz J, Ma A, Lutterbaugh JD, Periyasamy S, et al. Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. Proc natl Acad Sci USA. 1998; 95:8698–702. [PubMed: 9671741]
42. Fleisher AS, Esteller M, Wang S, Tamura G, Suzuki H, Yin J, et al. Hypermethylation of the hMLH1 gene promoter in human gastric cancers with microsatellite instability. Cancer Res. 1999; 59:1090–5. [PubMed: 10070967]

43. Simpkins SB, Bocker T, Swisher EM, Mutch DG, Gersell DJ, Kovatich AJ, et al. MLH1 promoter methylation and gene silencing is the primary cause of microsatellite instability in sporadic endometrial cancers. Hum Mol Genet. 1999; 8:661–6. [PubMed: 10072435]
44. Salvesen HB, MacDonald N, ryan A, Iversen OE, Jocobs IJ, akslen LA, et al. Methylation of hMLH1 in a population-based series of endometrial carcinomas. Clin Cancer Res. 2000; 6:3607–13. [PubMed: 10999752]
45. Zighelboim I, Goodfellow PJ, Gao F, Gibb RK, Powell MA, Rader JS, et al. Microsatellite instability and epigenetic inactivation of MLH1 and outcome of patients with endometrial carcinomas of the endometrioid type. J Clin Oncol. 2007; 25:2042–8. [PubMed: 17513808]
46. Herman JG, Uma A, Polyak K, Graff JR, Ahuja N, Issa JP, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc natl Acad Sci USA. 1998; 95:6870–5. [PubMed: 9618505]
47. Esteller M, Catasus L, Matias-Guiu X, Mutter GL, Prat J, Baylin SB, et al. hMLH1 promoter hypermethylation is an early event in human endometrial tumorigenesis. Am J Pathol. 1999; 155:1767–72. [PubMed: 10550333]
48. Horowitz N, Pinto K, Mutch DG, Herzog TJ, Rader JS, Gibb R, et al. Microsatellite instability, MLH1 promoter methylation, and loss of mismatch repair in endometrial cancer and concomitant atypical hyperplasia. Gynecol Oncol. 2002; 86:62–8. [PubMed: 12079302]
49. Kanaya T, Kyo S, Sakaguchi J, Maida Y, Nakamura M, Takakura M, et al. Association of mismatch repair deficiency with PTEN frameshift mutations in endometrial cancers and the precursors in a Japanese population. Am J Clin Pathol. 2005; 124:89–96. [PubMed: 15923161]
50. Banno K, Yanokura M, Susumu N, Kawaguchi M, Hirao N, Hirasawa A, et al. Relationship of the aberrant DNA hypermethylation of cancer-related genes with carcinogenesis of endometrial cancer. Oncol Rep. 2006; 16:1189–96. [PubMed: 17089036]
51. Guida M, Sanguedolce F, Bufo P, Di Spiezio Sardo A, Bifulco G, Nappi C, et al. Aberrant DNA hypermethylation of hMLH-1 and CDKN2A/p16 genes in benign, premalignant and malignant endometrial lesions. Eur J Gynaecol Oncol. 2009; 30:267–70. [PubMed: 19697618]
52. Piva R, Kumar VL, Hanau S, Rimondi AP, Pansini S, Mollica G, et al. Abnormal methylation of estrogen receptor gene and reduced estrogen receptor RNA levels in human endometrial carcinomas. J Steroid Biochem. 1989; 32:1–4. [PubMed: 2913392]
53. Sasaki M, Kotcherguina L, Dharia A, Fujimoto S, Dahiya R. Cytosine-phosphoguanine methylation of estrogen receptors in endometrial cancer. Cancer Res. 2001; 61:3262–6. [PubMed: 11309276]
54. Hori M, Iwasaki M, Shimazaki J, Inagawa S, Itabashi M. Assessment of hypermethylated DNA in two promoter regions of the estrogen receptor alpha gene in human endometrial diseases. Gynecol Oncol. 2000; 76:89–96. [PubMed: 10620447]
55. Navari JR, Roland PY, Keh P, Salvesen HB, Akslen LA, Iversen OE, et al. Loss of estrogen receptor (ER) expression in endometrial tumors is not associated with de novo methylation of the 5′ end of the ER gene. Clin Cancer Res. 2000; 6:4026–32. [PubMed: 11051252]
56. Shiozawa T, Itoh K, Horiuchi A, Konishi I, Fujii S, Nikaido T. Down-regulation of estrogen receptor by the methylation of the estrogen receptor gene in endometrial carcinoma. Anticancer Res. 2002; 22:139–43. [PubMed: 12017277]
57. Maeda K, Tsuda H, Hashiguchi Y, Yamamoto K, Inoue T, Ishiko O, et al. Relationship between p53 pathway and estrogen receptor status in endometrioid-type endometrial cancer. Hum Pathol. 2002; 33:386–91. [PubMed: 12055672]
58. Sasaki M, Kaneuchi M, Fujimoto S, Tanaka Y, Dahiya R. Hypermethylation can selectively silence multiple promoters of steroid receptors in cancers. Mol Cell Endocrinol. 2003; 202:201–7. [PubMed: 12770752]
59. Kumar NS, Richer J, Owen G, Litman E, Horwitz KB, Leslie KK. Selective down-regulation of progesterone receptor isoform B in poorly differentiated human endometrial cancer cells: implications for unopposed estrogen action. Cancer Res. 1998; 58:1860–5. [PubMed: 9581825]
60. Sasaki M, Dharia A, Oh BR, Tanaka Y, Fujimoto S, Dahiya R. Progesterone receptor B gene inactivation and CpG hypermethylation in human uterine endometrial cancer. Cancer Res. 2001; 61:97–102. [PubMed: 11196205]

61. Dai D, Wolf DM, Litman ES, White MJ, Leslie KK. Progesterone inhibits human endometrial cancer cell growth and invasiveness: down-regulation of cellular adhesion molecules through progesterone B receptors. Cancer Res. 2002; 62:881–6. [PubMed: 11830547]
62. Xiong Y, Dowdy SC, Gonzalez Bosquet J, Zhao Y, Eberhardt NL, Podratz KC, et al. Epigenetic-mediated upregulation of progesterone receptor B gene in endometrial cancer cell lines. Gynecol Oncol. 2005; 99:135–41. [PubMed: 16024066]
63. Ren Y, Liu X, Ma D, Feng Y, Zhong N. Down-regulation of the progesterone receptor by the methylation of progesterone receptor gene in endometrial cancer cells. Cancer Genet Cytogenet. 2007; 175:107–16. [PubMed: 17556066]
64. Nelen MR, Padberg GW, Peeters EA, Lin AY, van den Helm B, Frants RR, et al. Localization of the gene for Cowden disease to chromosome 10q22–23. Nat Genet. 1996; 13:114–6. [PubMed: 8673088]
65. Marsh DJ, Dahia PL, Zheng Z, Liaw D, Parsons R, Gorlin RJ, et al. Germ-line mutations in PTEN are present in Bannayan-Zonana syndrome. Nat Genet. 1997; 16:333–4. [PubMed: 9241266]
66. Waite KA, Eng C. Protean PTEN: Form and Function. Am J Hum Genet. 2002; 70:829–44. [PubMed: 11875759]
67. Tashiro H, Blazes MS, Wu R, Cho KR, Bose S, Wang SI, et al. Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. Cancer Res. 1997; 27:3935–40. [PubMed: 9307275]
68. Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Baak JP, Lees JA, et al. Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst. 2000; 92:924–30. [PubMed: 10841828]
69. Goel A, Arnold CN, Niedzwiecki D, Carethers JM, Dowell JM, Wasserman L, et al. Frequent inactivation of PTEN by promoter hypermethylation in microsatellite instability-high sporadic colorectal cancers. Cancer Res. 2004; 64:3014–21. [PubMed: 15126336]
70. Macdonald ND, Salvesen HB, Ryan A, Malatos S, Stefansson I, Iversen OE, et al. Molecular differences between RER+ and RER− sporadic endometrial carcinomas in a large population-based series. Int J Gynecol Cancer. 2004; 14:957–65. [PubMed: 15361209]
71. Salvesen HB, Stefansson I, Kretzschmar EI, Gruber P, MacDonald ND, Ryan A, et al. Significance of PTEN alterations in endometrial carcinoma: a population-based study of mutations, promoter methylation and PTEN protein expression. Int J Oncol. 2004:25. [PubMed: 14654937]
72. Salvesen HB, MacDonald N, Ryan A, Jacobs IJ, Lynch ED, Akslen LA, et al. PTEN methylation is associated with advanced stage and microsatellite instability in endometrial carcinoma. Int J Cancer. 2001; 91:22–6. [PubMed: 11149415]
73. Zysman MA, Chapman WB, Bapat B. Considerations when analyzing the methylation status of PTEN tumor suppressor gene. Am J Pathol. 2002; 160:795–800. [PubMed: 11891178]
74. Liggett WH Jr, Sidransky D. Role of the p16 tumor suppressor gene in cancer. J Clin Oncol. 1998; 16:1197–206. [PubMed: 9508208]
75. Wong YF, Chung TK, Cheung TH, Nobori T, Yu AL, Yu J, et al. Methylation of p16INK4A in primary gynecologic malignancy. Cancer Lett. 1999; 136:231–5. [PubMed: 10355753]
76. Ignatov A, Bischoff J, Schwarzenau C, Krebs T, Kuester D, Herrmann K, et al. P16 alterations increase the metastatic potential of endometrial carcinoma. Gynecol Oncol. 2008; 111:365–71. [PubMed: 18790522]
77. Furlan D, Carnevali I, Marcomini B, Cerutti R, Dainese E, Capella C, et al. The high frequency of de novo promoter methylation in synchronous primary endometrial and ovarian carcinomas. Clin Cancer Res. 2006; 12:3329–36. [PubMed: 16740754]
78. Yang HJ, Liu VW, Wang Y, Tsang PC, Ngan HY. Differential DNA methylation profiles in gynecological cancers and correlation with clinicopathological data. BMC Cancer. 2006; 23:212. [PubMed: 16928264]
79. Nakashima R, Fujita M, Enomoto T, Haba T, Yoshino K, Wada H, et al. Alteration of p16 and p15 genes in human uterine tumours. Br J Cancer. 1999; 80:458–67. [PubMed: 10408854]
80. Salesen HB, Das S, Akslen LA. Loss of nuclear p16 protein expression is not associated with promoter methylation but defines a subgroup of aggressive endometrial carcinomas with poor prognosis. Clin Cancer Res. 2000:153–9. [PubMed: 10656444]

81. Semczuk A, Boltze C, Marzec B, Szczygielska A, Roessner A, Schneider-Stock R. p16INK4A alterations are accompanied by aberrant protein immunostaining in endometrial carcinomas. J Cancer Res Clin Oncol. 2003; 129:589–96. [PubMed: 12920579]
82. Agathanggelou A, Cooper WN, Latif F. Role of the Ras-association domain family 1 tumor suppressor gene in human cancers. Cancer Res. 2005; 65:3497–508. [PubMed: 15867337]
83. Donninger H, Vos MD, Clark GJ. The RASSF1A tumor suppressor. J Cell Sci. 2007; 120 (Pt 18): 3163–72. [PubMed: 17878233]
84. Arafa M, Kridelka F, Mathias V, Vanbellinghen JF, Renard I, Foidart JM, et al. High frequency of RASSF1A and RARb2 gene promoter methylation in morphologically normal endometrium adjacent to endometrioid adenocarcinoma. Histopathology. 2008; 53:525–32. [PubMed: 18783461]
85. Pallarés J, Velasco A, Eritja N, Santacana M, Dolcet X, Cuatrecasas M, et al. Promoter hypermethylation and reduced expression of RASSF1A are frequent molecular alterations of endometrial carcinoma. Mod Pathol. 2008; 21:691–9. [PubMed: 18469797]
86. Liao X, Siu MK, Chan KY, Wong ES, Ngan HY, Chan QK, et al. Hypermethylation of RAS effector related genes and DNA methyltransferase 1 expression in endometrial carcinogenesis. Int J Cancer. 2008; 123:296–302. [PubMed: 18404674]
87. Pijnenborg JM, Dam-de Veen GC, Kisters N, Delvoux B, van Engeland M, Herman JG, et al. RASSF1A methylation and K-ras and B-raf mutations and recurrent endometrial cancer. Ann Oncol. 2007; 18:491–7. [PubMed: 17170014]
88. Jo H, Kim JW, Kang GH, Park NH, Song YS, Kang SB, et al. Association of promoter hypermethylation of the RASSF1A gene with prognostic parameters in endometrial cancer. Oncol Res. 2006; 16:205–9. [PubMed: 17120618]
89. Kang S, Lee JM, Jeon ES, Lee S, Kim H, Kim HS, et al. RASSF1A hypermethylation and its inverse correlation with BRAF and/or KRAS mutations in MSI-associated endometrial carcinoma. Int J Cancer. 2006; 119:1316–21. [PubMed: 16619251]
90. Joensuu EI, Abdel-Rahman WM, Ollikainen M, Ruosaari S, Knuutila S, Peltomäki P. Epigenetic signatures of familial cancer are characteristic of tumor type and family category. Cancer Res. 2008; 68:4597–605. [PubMed: 18559504]
91. Yu MY, Tong JH, Chan PK, Lee TL, Chan MW, Chan AW, et al. Hypermethylation of the tumor suppressor gene RASSFIA and frequent concomitant loss of heterozygosity at 3p21 in cervical cancers. Int J Cancer. 2003; 105:204–9. [PubMed: 12673680]
92. Jones MH, Nakamura Y, Koi S, Fujimoto L, Hasumi K, Kato K. Allelotype of uterine cancer by analysis of RFLP and microsatellite polymorphisms: Frequent loss of heterozygosity on chromosome arms 3p, 9q, 10q, and 17p. Genes Chromosomes Cancer. 1994; 9:119–23. [PubMed: 7513541]
93. Kang S, Kim JW, Kang GH, Lee S, Park NH, Song YS, et al. Comparison of DNA hypermethylation patterns in different types of uterine cancer: cervical squamous cell carcinoma, cervical adenocarcinoma and endometrial adenocarcinoma. Int J Cancer. 2006; 118:2168–71. [PubMed: 16331610]
94. Fodde R. The APC gene in colorectal cancer. Eur J Cancer. 2002; 38:867–71. [PubMed: 11978510]
95. Barker N, Clevers H. Catenins, Wnt signaling and cancer. Bioessays. 2000; 22:961–5. [PubMed: 11056471]
96. Schlosshauer PW, Pirog EC, Levine RL, Ellenson LH. Mutational analysis of the CTNNB1 and APC genes in uterine endometrioid carcinoma. Mod Pathol. 2000; 13:1066–71. [PubMed: 11048799]
97. Kobayashi K, Sagae S, Nishioka Y, Tokino T, Kudo R. Mutations of the beta-catenin gene in endometrial carcinomas. Jpn J Cancer Res. 1999; 90:55–9. [PubMed: 10076565]
98. Moreno-Bueno G, Hardisson D, Sánchez C, Sarrió D, Cassia R, García-Rostán G, et al. Abnormalities of the APC/beta-catenin pathway in endometrial cancer. Oncogene. 2002; 21:7981–90. [PubMed: 12439748]

99. Pijnenborg JM, Kisters N, van Engeland M, Dunselman GA, de Haan J, de Goeij AF, et al. APC, beta-catenin, and E-cadherin and the development of recurrent endometrial carcinoma. Int J Gynecol Cancer. 2004; 14:947–56. [PubMed: 15361208]
100. Zysman M, Saka A, Millar A, Knight J, Chapman W, Bapat B. Methylation of adenomatous polyposis coli in endometrial cancer occurs more frequently in tumors with microsatellite instability phenotype. Cancer Res. 2002; 62:3663–6. [PubMed: 12097272]
101. Suehiro Y, Okada T, Okada T, Anno K, Okayama N, Ueno K, et al. Aneuploidy predicts outcome in patients with endometrial carcinoma and is related to lack of CDH13 hypermethylation. Clin Cancer Res. 2008; 14:3354–61. [PubMed: 18519763]
102. Ilyas M, Tomlinson IPM. The interatcions of APC, E-cadherin and β-catenin in tumor development and progression. J Pathol. 1997; 182:128–37. [PubMed: 9274521]
103. Moreno-Bueno G, Hardisson D, Sarrió D, Sánchez C, Cassia R, Prat J, et al. Abnormalities of E- and P-cadherin and catenin (beta-, gamma-catenin, and p120ctn) expression in endometrial cancer and endometrial atypical hyperplasia. J Pathol. 2003; 199:471–8. [PubMed: 12635138]
104. Saito T, Nishimura M, Yamasaki H, Kudo R. Hypermethylation in promoter region of E-cadherin gene is associated with tumor dedifferention and myometrial invasion in endometrial carcinoma. Cancer. 2003; 97:1002–9. [PubMed: 12569599]
105. Esteller M, Hamilton SR, Burger PC, Baylin SB, Herman JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. Cancer Res. 1999; 59:793–7. [PubMed: 10029064]
106. Li R, Saito T, Tanaka R, Satohisa S, Adachi K, Horie M, et al. Hypermethylation in promoter region of retinoic acid receptor-beta gene and immunohistochemical findings on retinoic acid receptors in carcinogenesis of endometrium. Cancer Lett. 2005; 219:33–40. [PubMed: 15694662]
107. Mhawech P, Benz A, Cerato C, Greloz V, Assaly M, Desmond JC, et al. Downregulation of 14–3–3sigma in ovary, prostate and endometrial carcinomas is associated with CpG island methylation. Mod Pathol. 2005; 18:340–8. [PubMed: 15257317]
108. Dowdy SC, Gostout BS, Shridhar V, Wu X, Smith DI, Podratz KC, et al. Biallelic methylation and silencing of paternally expressed gene 3 (PEG3) in gynecologic cancer cell lines. Gynecol Oncol. 2005; 99:126–34. [PubMed: 16023706]
109. Chan QK, Khoo US, Chan KY, Ngan HY, Li SS, Chiu PM, et al. Promoter methylation and differential expression of pi-class glutathione S-transferase in endometrial carcinoma. J Mol Diagn. 2005; 7:8–16. [PubMed: 15681469]
110. Yamaguchi S, Asanoma K, Takao T, Kato K, Wake N. Homeobox gene HOPX is epigenetically silenced in human uterine endometrial cancer and suppresses estrogen-stimulated proliferation of cancer cells by inhibiting serum response factor. Int J Cancer. 2009; 124:2577–88. [PubMed: 19173292]
111. Yoshida H, Broaddus R, Cheng W, Xie S, Naora H. Deregulation of the HOXA10 homeobox gene in endometrial carcinoma: role in epithelial-mesenchymal transition. Cancer Res. 2006; 66:889–97. [PubMed: 16424022]
112. Whitcomb BP, Mutch DG, Herzog TJ, Rader JS, Gibb RK, Goodfellow PJ. Frequent HOXA11 and THBS2 promoter methylation, and a methylator phenotype in endometrial adenocarcinoma. Clin Cancer Res. 2003; 9:2277–87. [PubMed: 12796396]
113. Yoshizaki T, Enomoto T, Fujita M, Ueda Y, Miyatake T, Fujiwara K, et al. Frequent inactivation of RUNX3 in endometrial carcinoma. Gynecol Oncol. 2008; 110:439–44. [PubMed: 18572225]
114. Tse KY, Liu VW, Chan DW, Chiu PM, Tam KF, Chan KK, et al. Epigenetic alteration of the metallothionein 1E gene in human endometrial carcinomas. Tumour Biol. 2009; 30:93–9. [PubMed: 19420986]
115. Risinger JI, Maxwell GL, Berchuck A, Barrett JC. Promoter hypermethylation as an epigenetic component in Type I and Type II endometrial cancers. Ann N Y Acad Sci. 2003; 983:208–12. [PubMed: 12724225]
116. Reid-Nicholson M, Iyengar P, Hummer AJ, Linkov I, Asher M, Soslow RA. Immunophenotypic diversity of endometrial adenocarcinomas: implications for differential diagnosis. Mod Pathol. 2006; 19:1091–100. [PubMed: 16648864]

117. Xiong Y, Dowdy SC, Xue A, Shujuan J, Eberhardt NL, Podratz KC, et al. Opposite alterations of DNA methyltransferase gene expression in endometrioid and serous endometrial cancers. Gynecol Oncol. 2005; 96:601–9. [PubMed: 15721400]
118. Herceg Z. Epigenetics and cancer: towards an evaluation of the impact of enironmental and dietary factors. Mutagenesis. 2007; 22:91–103. [PubMed: 17284773]
119. Mason JB. Biomarkers of nutrient exposure and status in one-carbon (methyl) metabolism. J Nutr. 2003; 133:941S–7S. [PubMed: 12612180]
120. Choi SW, Mason JB. Folate status: effects on pathways of colorectal carcinogenesis. J Nutr. 2002; 132:2413S–8S. [PubMed: 12163703]
121. Kim YI. Nutritional epigenetics: impact of folate deficiency on DNA methylation and colon cancer susceptibility. J Nutr. 2005; 135:2703–9. [PubMed: 16251634]
122. Mason JB, Choi SW, Liu Z. Other one-carbon micronutrients and age modulate the effects of folate on colorectal carcinogenesis. Nutr Rev. 2008; 66 (Suppl 1):S15–7. [PubMed: 18673480]
123. Thompson J. Vitamins and minerals 4: overview of folate and the B vitamins. Community Pract. 2006; 79:197–8. [PubMed: 16780289]
124. Pufulete M, Emery PW, Sanders TA. Folate, DNA methylation and colo-rectal cancer. Proc Nutr Soc. 2003; 62:437–45. [PubMed: 14506892]
125. Kim YI, Pogribny IP, Basnakian AG, Miller JW, Selhub J, James SJ, et al. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. Am J Clin Nutr. 1997; 65:46–52. [PubMed: 8988912]
126. Pogribny IP, James SJ. De novo methylation of the p16INK4A gene in early preneoplastic liver and tumors induced by folate/methyl deficiency in rats. Cancer Lett. 2002; 187:69–75. [PubMed: 12359353]
127. de Vogel S, Bongaerts BW, Wouters KA, Kester AD, Schouten LJ, de Goeij AF, et al. Associations of dietary methyl donor intake with MLH1 promoter hypermethylation and related molecular phenotypes in sporadic colorectal cancer. Carcinogenesis. 2008; 29:1765–73. [PubMed: 18339680]
128. Kraunz KS, Hsiung D, McClean MD, Liu M, Osanyingbemi JO, Nelson HH, et al. Dietary folate is associated with *p16*^{INK4A} methylation in head and neck squamous cell carcinoma. Int J Cancer. 2006; 119:1553–7. [PubMed: 16646054]
129. Slattery ML, Curtin K, Sweeney C, Levin TR, Potter J, Wolff RK, et al. Diet and lifestyle factor associations with CpG island methylator phenotype and BRAF mutations in colon cancer. Int J Cancer. 2006; 120:656–63. [PubMed: 17096326]
130. van Engeland M, Weijenberg MP, Roemen GM, Brink M, de Bruïne AP, Goldbohm RA, et al. Effects of dietary folate and alcohol intake on promoter methylation in sporadic colorectal cancer: the Netherlands cohort study on diet and cancer. Cancer Res. 2003; 63:3133–7. [PubMed: 12810640]
131. Zhu K, Davidson NE, Hunter S, Yang X, Payne-Wilks K, Roland CL, et al. Methyl-group dietary intake and risk of breast cancer among African-American women: a case-control study by methylation status of the estrogen receptor alpha genes. Cancer Causes Control. 2003; 14:827–36. [PubMed: 14682440]
132. Li Y, Upadhyay S, Bhuiyan M, Sarkar FH. Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein. Oncogene. 1999; 18:3166–72. [PubMed: 10340389]
133. Wietrzyk J, Boratynski J, Grynkiewicz G, Ryczynski A, Radzikowski C, Opolski A. Antiangiogenic and antitumour effects in vivo of genistein applied alone or combined with cyclophosphamide. Anticancer Res. 2001; 21:3893–6. [PubMed: 11911265]
134. Adlercreutz H. Phyto-oestrogens and cancer. Lancet Oncol. 2002; 3:364–73. [PubMed: 12107024]
135. Price KR, Fenwick GR. Naturally occurring oestrogens in foods-a review. Food Addit Contam. 1985; 2:73–106. [PubMed: 4018320]
136. Day JK, Bauer AM, DesBordes C, Zhuang Y, Kim BE, Newton LG, et al. Genistein alters methylation patterns in mice. J Nutr. 2002; 132 (8 Suppl):2419S–23S. [PubMed: 12163704]

137. Fang MZ, Chen D, Sun Y, Jin Z, Christman JK, Yang CS. Reversal of hypermethylation and reactivation of p16INK4a, RARbeta, and MGMT genes by genistein and ther isoflavones from soy. Clin Cancer Res. 2005; 11:7034–41.

138. Cui Y, Lu C, Liu L, Sun D, Yao N, Tan S, et al. Reactivation of methylation-silenced tumor suppressor gene p16INK4a by nordihydroguaiaretic acid and its implication in G1 cell cycle arrest. Life Sci. 2008; 82:247–55. [PubMed: 18164318]

139. King-Batoon A, Leszczynska JM, Klein CB. Modulation of gene methylation by genistein or lycopene in breast cancer cells. Environ Mol Mutagen. 2008; 49:36–45. [PubMed: 18181168]

140. Majid S, Dar AA, Ahmad AE, Hirata H, Kawakami K, Shahryari V, et al. BTG3 tumor suppressor gene promoter demethylation, histone modification and cell cycle arrest by genistein in renal cancer. Carcinogenesis. 2009; 30:662–70. [PubMed: 19221000]

141. Qin W, Zhu W, Shi H, Hewett JE, Ruhlen RL, MacDonald RS, et al. Soy isoflavones have an antiestrogenic effect and alter mammary promoter hypermethylation in healthy premenopausal women. Nutr Cancer. 2009; 61:238–44. [PubMed: 19235040]

142. Coyle YM, Xie XJ, Lewis CM, Bu D, Milchgrub S, Euhus DM. Role of physical activity in modulating breast cancer risk as defined by APC and RASSF1A promoter hypermethylation in nonmalignant breast tissue. Cancer Epidemiol Biomarkers Prev. 2007; 16:192–6. [PubMed: 17301249]

143. Vessey MP, Painter R. Endometrial and ovarian cancer and oral contraceptives-findings in a large cohort study. Br J Cancer. 1995; 71:1340–2. [PubMed: 7779735]

144. Tao MH, Xu WH, Zheng W, Zhang ZF, Gao YT, Ruan ZX, et al. Oral contraceptive and IUD use and endometrial cancer: a population-based case-control study in Shanghai, China. Int J Cancer. 2006; 119:2142–7. [PubMed: 16823853]

145. Maxwell GL, Schildkraut JM, Calingaert B, Risinger JI, Dainty L, Marchbanks PA, et al. Progestin and estrogen potency of combination oral contraceptives and endometrial cancer risk. Gynecol Oncol. 2006; 103:535–40. [PubMed: 16740300]

146. Yamagata Y, Asada H, Tamura I, Lee L, Maekawa R, Taniguchi K, et al. DNA methyltransferase expression in the human endometrium: down-regulation by progesterone and estrogen. Hum Reprod. 2009; 24:1126–32. [PubMed: 19202141]

147. Foley DL, Craig JM, Morley R, Olsson CJ, Dwyer T, Smith K, et al. Prospects for epigenetic epidemiology. Am J Epidemiol. 2009; 169:389–400. [PubMed: 19139055]

Table 1

Selected genes frequently silenced by DNA promoter methylation in endometrial carcinoma

Gene	Alternate gene name	Function
APC	Adenomatous polyposis coli	Tumor suppressor gene, cell adhesion, signal transduction, stabilization of the cytoskeleton, regulation of cell cycle and apoptosis
CDH1 (E-cad)	E-cadherin	Epithelial cell-cell adhesion, suppresses invasion and metastasis
ERα	Estrogen receptor α	Steroid receptor, regulation of cell proliferation
MGMT	O-6-methylguanine-DNA methyltransferase	DNA repair gene
hMLH1	Human Mut-L homolog 1	DNA mismatch repair gene
p16	Cyclin-dependent kinase inhibitor 2A	Tumor suppressor gene, cell cycle regulation, involved in senescence
PR-B	Progesterone receptor	Steroid receptor, growth regulation
PTEN	Phosphatase and tensin homolog	Tumor suppressor gene, controls proliferation and apoptosis
RASSF1A	Ras association domain family protein 1	Tumor suppressor gene, cell cycle regulation, microtubule stabilization, cellular adhesion and inhibits tumor formation, apoptosis
RARβ$_2$	Retinoic acid receptor	Apoptosis, involved in senescence, inhibition of proliferation