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Application of DNA methylation biomarkers for endometrial cancer management

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Abstract

It has become clear that aberrant gene expression, via alterations in promoter methylation or histone acetylation, is a contributing factor for carcinogenesis, perhaps as important as genetic mutation. This is particularly evident in endometrial cancer, in which multiple genes are silenced through hypermethylation. In this review, we discuss the field of epigenetics and relevant techniques to characterize methylation and acetylation alterations. The CpG island methylator phenotype, epimutations and the effects of aging on methylation are also discussed. In endometrial cancer there is evidence that hypermethylation of relevant genes can be reversed using epigenetic inhibitors, resulting in re-expression of silenced genes. Preliminary data also suggest that a panel of methylation biomarkers could be useful for diagnosis and even screening in selected populations at high risk. This disease is particularly well suited for such a strategy given that the endometrium is readily accessible for testing and endometrial cancer precursors are well defined.

Keywords

biomarker; cancer diagnosis; cancer treatment; DNA methylation; endometrial cancer; epigenetics; intravaginal tampons

Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy and the fourth most common cancer in women, ranking only behind breast, lung and colorectal cancer. The American Cancer Society estimated that there will be 39,080 new cases of EC in the USA in 2007 (1). Although most women are diagnosed at an early stage and enjoy excellent survival, patients with advanced disease have a high rate of recurrence and a poor prognosis.

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Despite the intensive efforts for improvement of treatment, 5-year survival is only 25% for patients with extra-uterine disease. The most common treatment regimen includes the use of carboplatin or cisplatin, doxorubicin and paclitaxel, alone or in combination. While vaginal recurrences can be effectively treated with radiation therapy, the 5-year survival rate for nonvaginal recurrence is 13% or less with a median survival of 10 months (2,3). Therefore, novel methods for early detection and more effective treatment for patients with advanced or recurrent disease are paramount to improve long-term outcomes.

It has long been recognized that cancer cells undergo significant changes in 5-methylcytosine distribution including global hypomethylation and gene-specific hypermethylation of CpG islands. Epigenetic silencing leads to recessive changes that are often indistinguishable from those caused by genetic mutation or deletion. For example, germline mutations in DNA mismatch repair genes hMLH1 and hMSH2 result in hereditary nonpolyposis colorectal cancer (HNPCC), a familial syndrome associated with elevated risk of both colorectal cancer and EC (4). However, in sporadic ECs with MSI-H, hMLH1 and hMSH2 mutations are present in less than 10% of cases, but hMLH1 expression is reduced in the majority of tumors lacking detectable mutations. This silencing is mediated by hypermethylation of the hMLH1 promoter resulting in a loss of hMLH1 protein expression and the development of MSI-H phenotype. Further evidence for a causal link between hMLH1 epigenetic silencing and MSI has come from in vitro studies showing that demethylating agents are not only able to reactivate hMLH1 expression, but also restore the mismatch repair function (5,6). Many other epigenetically silenced genes have been described in EC, such as O(6)-methylguanine-DNA methyltransferase (MGMT) (7), phosphatase and tensin homolog (PTEN) (8,9), progesterone receptor (PR) (10–12), Homeobox gene (HOXA11), thrombospondin-2 (THBS2) (13), paternally expressed gene 3 (PEG3) (14) and glutathione S-transferase P1 (GSTP1) (15). It is noteworthy that interactions between genetic and epigenetic events have been observed in cancer pathogenesis. In such cases, one allele of a tumor suppressor gene can be disrupted by mutation while the other allele is transcriptionally inactivated by DNA hypermethylation (16,17). In addition, aberrant DNA methylation is a contributing factor for genetic aberrations including hypermutation (18), loss of heterozygosity (19,20) and aneuploidy (21,22).

Aberrant DNA methylation is an early carcinogenic event that has been identified in a variety of cancer precursors (23,24). With regards to EC, Esteller et al. reported that hMLH1 methylation is present in 7% of endometrial hyperplasias (25). This alteration appears to be restricted to atypical complex hyperplasia, a pathological change considered to be a direct precursor to endometrial carcinoma (26–28). Results from an independent study by Horowitz et al. confirmed these findings (29). DNA methylation alterations are also associated with later cancer stages, such as metastasis and development of drug resistance (30–32). These observations highlight the potential use of epigenetic aberrations as biomarkers for cancer detection. These pathways may also prove to be valuable targets for new treatment modalities. This review will focus on the application of DNA methylation assays for risk assessment/screening and epigenetic treatment of patients with EC.

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DNA methylation & its clinical detectionStudy population

The term 'CpG island' refers to conserved genomic regions containing more than 50% GC and enriched CpG dinucleotides with an observed versus predicted ratio exceeding 0.6 over a stretch of 200 base pairs. It is estimated that approximately 60% of protein-coding regions are associated with CpG islands in humans (33,34). These sequences are normally refractory to DNA methylation. However, in carcinomas and other diseases, specific CpG islands become partially or fully methylated, resulting in silencing of associated genes. Conversely, repetitive elements and retro–viral sequences that are normally suppressed by methylation may become demethylated and transcribed. The DNA methylation machinery is tightly regulated by cell signaling pathways and is composed of DNA methyltransferases (DNMT) and histone deacetylases. Although some in vitro data suggest that DNA demethylation can initiate gene transcription, it is debatable whether methylation of a native promoter is the cause or con–sequence of gene silencing. Despite this uncertainty, a close association between the presence of methylated CpG and silenced gene expression has been well established. Furthermore, numerous studies have demonstrated that pharmacological interference with DNMT inhibitors induces demethylation of methylated promoters and reverses the gene silencing process. This observation forms the foundation for intense efforts to identify and validate methylation target genes as cancer biomarkers (35–39). Because the role in DNA hypomethylation in cancer evolution is less well understood than hypermethylation and the detection of hypomethylation is less sensitive than hypermethylation due to the difficulty of achieving 100% bisulfite conversion, most biomarker research has concentrated on aberrantly hypermethylated DNA sequences.

DNA methylation biomarkers offer several advantages over RNA- or protein-based tests. First, DNA and its methylation modification is relatively stable and can withstand cell disruption, repeated freeze and thaw and paraffin fixation procedures performed for histopathological examination. In addition, PCR-based assays such as methylation-specific PCR (MSP) can be performed using partially degraded DNA templates, making it suitable for examination of DNA isolated from body fluids or exfoliated cells. Second, each cancer cell contains aberrant DNA methylation patterns involving multiple genes. These abnormalities can be measured in parallel using the same assay (e.g., MSP) with minor adjustments (e.g., different primers). This enables investigators to apply a panel of methylation markers with improved sensitivity and specificity. Simultaneous evaluation of multiple genes governing a wide variety of cell functions and pathways has the potential to provide information on diagnosis, predict chemo–sensitivity or direct targeted therapy. Third, unlike genetic mutations that often vary in genomic position over a wide region, aberrant DNA methylation is generally restricted to specific CpG islands associated with the target gene (40). Thus, when the MSP method is applied, a single pair of primers will be adequate for the entire population.

Several methods are currently used to characterize DNA methylation. High-throughput assays including restriction landmark genomic scanning, differential methylation hybridization and gene chip hybridization following DNMT inhibitor-induced demethylation have been used to identify novel methylation targets (41–44). Chromatin immunoprecipitation (ChIP) and ChIP-on-chip methods are used to characterize DNA–

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protein interactions and chromatin changes. DNA sequencing and combined bisulfite-restriction analysis has also been used to analyze gene-specific methylation (45,46). Bisulfite sequencing and its quantitative version, pyrosequencing, provide detailed information regarding the methylation of each CpG dinucleotide (47,48). These two methods rely on DNA sequencing and thus require relatively large amounts of DNA and special equipment. Compared with bisulfite sequencing, MSP is faster, less expensive and can be performed in most laboratories. MSP also allows verification of specificity using electrophoretic examination of the final products. A new trend is to couple MSP to real-time measurement of amplification products (QMSP). Simultaneous quantification of both methylated and unmethylated DNA allows the calculation of a ratio, the methylation index, which enables quantitative comparisons (14,40,49). For these reasons, QMSP has been adopted by many laboratories as the assay of choice for translational studies and clinical applications.

Aberrantly methylated DNA can be detected in blood or tissues even in patients with nonmetastatic cancers. DNA isolated from serum has been used to detect lung (50–52) and prostate cancers (53). Thus, tests for DNA methylation represent a noninvasive means for cancer diagnosis. There is evidence that circulating nucleosomes associated with methylated DNA may be protected from DNAases by virtue of the compact configurations that are often found in association with silenced genes (54). If correct, this concept can help explain the better than expected success for methylation analysis using DNA purified from virtually all kinds of body fluids. For example, bronchial aspirates have been used for DNA methylation tests for APC, P16INK4a and RASSF1A. The results showed that QMSP could be applied as a reflex test in cases of suspected lung cancer that defy a definite diagnosis using conventional methods (55). Similar attempts were made for detection of pancreatic carcinoma using DNA isolated from pancreatic secretions and the results showed varied levels of success depending on the choice of marker genes (56,57). Quantitative promoter methylation analysis of nine genes was obtained from DNA purified in urine sediments. In a cohort of 175 patients with bladder cancer and 69 age-matched controls, a sensitivity of 82% and specificity of 94% was obtained (58). Methylation analysis of this panel of genes can be a useful tool for early detection and disease monitoring of patients with bladder cancer.

One report concerning aberrant DNA methylation and lung cancer risk deserves special attention. Belinsky et al. examined the methylation status of p16, MGMT, DAPK and RASSF1A in nonmalignant bronchial epithelium from smokers (59). Methylation of p16 was detected in 23 of 66 current and former smokers. Moreover, a high concordance (94%) of P16 methylation status was found between bronchial epithelium from lung lobes without cancer and the primary cancer tissues from the same patient. The authors concluded that aberrant DNA methylation, although not transforming by itself, presents an early surrogate for acquisition of additional genetic and epigenetic changes leading to lung cancer. As discussed below, a similar methylation panel may prove useful for the assessment of EC in patients at high risk.

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Rationale for clinical application of methylation biomarkers in EC management

The American Cancer Society published detailed analyses and guidelines for early detection of EC in 2001 (60). It was concluded that there was insufficient evidence to recommend screening for EC in the general population. This conclusion was partially due to an absence of detection assays with acceptable sensitivity and specificity that would justify the associated costs. However, there are many reasons to pursue a screening test for patients with EC. While patients with early-stage EC can be effectively treated with hysterectomy, few options exist for patients with advanced or recurrent disease. Most ECs are detected in elderly women, suggesting the presence of a relatively long 'incubation' time and, correspondingly, a wide window for early detection. Furthermore, the demographics of EC have been very well defined. Most EC occur in postmenopausal women with obesity and/or diabetes. In younger women EC is almost exclusively limited to patients with polycystic ovarian syndrome or who have been treated with estrogen unopposed by progesterone. Thus, screening women with these characteristics would be a logical strategy. While aberrant DNA methylation occurs in the development of virtually all cancers, it plays a particularly important role in EC. The methylation status and clinicopathological implications of hMLH1, PTEN, MGMT and steroid hormone receptors have been extensively characterized. Considered together with recent improvements in DNA isolation and methylation assays, the clinical application of epigenetic biomarkers for EC screening appears justified.

Tampons as a noninvasive approach for collecting DNA samples

Tampon devices are increasingly accepted by women because of their compatibility with a modern lifestyle characterized by increased social activities and exercise. This presents an opportunity for the application as a convenient tool for the collection of DNA samples in the clinical settings. In an early study, Bader et al. conducted a large trial to compare the efficacy of the tampon method with standard cervical smears (61). Patients cooperated readily in the tampon screening program and with proper guidance approximately 98% of patients successfully completed the trial. In total, 18 cervical and endometrial carcinomas were discovered by screening 2607 women with 97% overall agreement between tampon smear and vaginocervical smear, although low specificity was achieved using both methods. In an independent study, Brunschwig et al. examined the tampon smear method in patients with endometrial adenocarcinoma diagnosed by conventional biopsy. Sensitivity was 87% in a cohort of 23 patients (62). Although standard endometrial biopsy can be performed in the office, an approach utilizing a tampon would be expected to cause less discomfort. An additional advantage is that specimens could be self-collected by women at high risk (e.g., patients with HNPCC, obesity or diabetes). Use of the tampon as a collection device is noninvasive and associated with few side effects. The prototypic tampon designed by Draghi was a hand-made device composed of a compressed cotton cylinder covered with a nylon sheath (63,64). In the past, prolonged use (days to weeks) of high absorbency tampons was associated with vaginal ulceration (65,66) and staphylococcal toxic shock syndrome (67,68). Since the super−absorbent tampon made of carboxymethylcellulose was recalled in the 1980s, the risk of ulceration and infection with the normal absorbency tampons such as

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Tampax® (Procter & Gamble) should be minimal. Therefore, when used properly, the tampon can be a safe and convenient tool for collection of exfoliated DNA from the female genital tract.

Martin Widschwendter and his group from the University of Innsbruck, Austria, are among the first investigators who attempted to combine the advantages provided by tampon samples and methylation markers for EC detection. In an investigation of cervical cancer and dysplasia, cervicovaginal specimens were collected from patients with low-grade squamous intra–epithelial lesions (SIL), high-grade SIL and invasive cervical cancers, respectively (69). Patients who underwent hysterectomy for benign indications were included as controls. Comfort Mini Tampons (Johnson & Johnson) were inserted in the vagina by a physician after speculum examination the day before surgery and retained intravaginally for 30 min. The tampons were washed with 1.2 ml of phosphate buffered saline and supernatant collected by a brief centrifugation. Following DNA purification using an affinity column, DNA samples were subjected to real-time PCR methylation analysis of SOCS1, CDH1, TIMP3, GSPT1, DAPK1, hTERT, CDH13, HSPA2, MLH1, RASSFIA, SOCS2 and ACTB. For each target gene a ratio between the readings from SssI-untreated and SssI-treated DNA was calculated and the sample was considered methylation positive if the ratio reached more than 1%.

The investigators found no differences in methylation between the control and low-grade SIL groups. However, methylation in these two groups was significantly less frequent than in patients with high-grade SIL and invasive cancer. The elevation of methylation in high-grade SIL was found in all the genes examined except GSTP1 and SOCS2. Each sample from cervical cancer patients contains three or more methylated genes, with hTERT found to be methylated only in cancer samples (80% positivity). This suggests that hTERT could be used to differentiate high-grade SIL from invasive carcinoma.

The same group performed a parallel study for the early detection of EC in 124 patients (70). This study included 15 cases of EC, five cases of invasive cervical cancer, 35 cases of cervical intraepithelial neoplasia (CIN) and 69 patients with benign uterine diseases. Applying the same sample collection procedure, 38 genes were examined in DNA samples from five EC and four control patients to determine the appropriate genes with the greatest difference between cancer and control samples. Five genes including RASSF1A, hMLH1, CDH13, HSPA2 and SOCS2, were chosen for further analysis. The sample was considered methylation positive if three or more genes were found to be hypermethylated. Using this standard, tampon collected samples from all of the 15 EC patients were found to be positive whereas 99 of 109 from the other cohort were negative. Of the ten patients who were methylation positive but did not have EC, one had CIN and four had invasive cervical cancer.

The investigators also analyzed 16 patient samples collected between the primary (curettage or punch biopsy of the cervix) and secondary surgery (hysterectomy). All nine EC patients diagnosed with residual cancer were confirmed to have three or more genes methylated. The three CIN III and one benign patient were methylation negative. Combining all the data from patients aged between 50 and 75 and excluding CIN III or cervical cancers, the sensitivity

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for EC detection was 100% and specificity was 97%, with only one false positive. It should be pointed out that these preliminary studies were based on a relatively small group of patients readily diagnosed using conventional methods. Despite this limitation, these results strongly suggest that a DNA methylation assay on tampon-collected cervicovaginal secretions is a useful tool for detection of EC. The authors of the above studies felt that, at this time, the sensitivity and specificity of the DNA methylation assay is still too low to be used as a screening test. However, the panel of genes utilized has a huge impact on sensitivity and specificity. The two investigations were based on a small number of methylation targets selected by literature review. Selection of marker genes based, for example, on comprehensive profiling data should lead to a panel with improved positive and negative predictive values.

The discriminating power of MSP relies on the differential sequences of methylated and unmethylated cytosines following bisulfite conversion. Bisulfite conversion causes considerable DNA damage and additional DNA is lost in the purification step, such that DNA quantity becomes a limiting factor in the clinical setting. Nonetheless, the studies described above demonstrate that a 30-min tampon retention time yielded sufficient DNA for methylation analysis. In the above studies, tampon samples were subjected to centrifugation and only supernatants were stored for DNA isolation. Thus, it is not clear if additional DNA could be recovered from cells/tissues that are presumably present in the sediments. More rigorous studies are required to assess the integrity, purity, stability following storage and final quantity of DNA recovered using this method.

It has been reported that hypermethylation of tumor suppressor genes is readily detectable in atypical complex hyperplasia. Although considered a precancerous condition, when subjected to vigorous review there is little agreement between pathologists and approximately 30% of patients with this diagnosis will ultimately be proven to have an invasive EC when subjected to hysterectomy. The sensitivity and specificity of a DNA methylation assay as a tool to direct management of this disorder in women who wish to preserve fertility has not been systematically evaluated, but may prove useful.

Despite their related Müllerian origins, ovarian, cervical and ECs have differing DNA methylation profiles (71). Using pooled DNA from primary cancers, Yang et al. found that DAPK is selectively methylated in cervical cancer (41). On the other hand, BRCA1 methylation was only detected in ovarian cancers. Cervical squamous cell carcinoma (CSCC), cervical adenocarcinoma (CA) and endometrial adenocarcinoma (EA) represent the malignancies most likely to be detected by examination of the tampon collection method. Kang et al. compared the DNA methylation patterns of nine tumor suppressor genes in EC (72). Hypermethylation of CDH1 and DAPK were found more frequently in CSCC than CA (86.6 vs 43.3%; 77.4 vs 46.7%), whereas HLTF and TIMP3 were methylated more often in CA than CSCC (3.2% vs 43.3; 8.1 vs 53.3%). Most EA (81.0%) contain hypermethylated RASSF1A, but a smaller percentage of CA (33.3%) carries the same epigenetic abnormality. These differences were highly statistically significant and suggest that a well-designed panel of methylation markers may not only indicate the presence of a malignancy, but also suggest histological subtype.

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Postoperative monitoring for EC recurrence &/or therapeutic response

The CpG island methylator phenotype (CIMP) with con–cordant methylation of multiple promoters defines a distinct class of cancers associated with several important clinicopathological characteristics and chemoresistance (73,74). In colorectal cancers, CIMP is related to microsatellite instability-high (MSI-high), BRAF mutation, loss of heterozygosity (75–77) and polymorphisms and dietary pattern affecting one-carbon metabolism (78,79). Studies have linked CIMP with reduced survival in patients with advanced colorectal cancer treated with 5-fluorouracil-based chemotherapy (80). Ogino et al. reported that CIMP predicts poor survival in microsatellite stable metastatic colorectal carcinoma treated with combination chemotherapy (81). These studies support the application of epigenetic markers for improved management of cancer patients. It is important to point out that the clinicopathological implication of CIMP is cancer-type dependent. For example, in contrast to the findings in colorectal cancer, Shaw et al. observed that in oral cancers CIMP is associated with an upregulated host inflammatory response and less aggressive tumor behavior (82). Thus, CIMP could be associated with divergent or even opposite effects in cancers from different cell types, underscoring the need for a detailed evaluation in each individual cancer type.

Several investigators have proposed that the clustering of methylated genes in a subset of ECs qualifies as CIMP (13,71). This group of cancers has a three- to fivefold elevated frequency of aberrant gene methylation. Whitcomb et al. conducted an extensive methylation analysis in primary and recurrent EC and EC cell lines (13). A high rate of HOXA11 and THBS2 methylation was observed in cancer tissues and cell lines. Additionally, HOXA11 promoter methylation occurred in 90 and 51% of recurrent and primary, nonrecurrent ECs, respectively. Furthermore, HOXA11 methylation in early-stage cancers was found to be associated with poor outcome. Unlike colorectal and gastric cancers, more evidence is required for confirmation of the presence of CIMP and to define the clinicopathological implication of CIMP for ECs.

Despite the uncertainty regarding the significance of CIMP in EC, several studies have demonstrated that the methylation status of individual genes is predictive of clinical manifestations and response to treatment. Methylation of E-cadherin was found in 82% of EC and associated with tumor dedifferentiation, deep myometrial invasion and lymph node metastasis (83). While PTEN mutation correlated with low International Federation of Gynecology and Obstetrics (FIGO)-stage, endometrioid subtype, low grade and favorable prognosis (84), PTEN hypermethylation appeared to be associated with metastatic disease and MSI (9). These findings suggest that characterization of methylation in specific genes can provide useful prognostic information and guide postsurgical treatment of EC.

DNA methylation aberrations and gene silencing can be modified by the use of epigenetic modification reagents. DNMT and histone deacetylase (HDAC) inhibitors are small hydrophobic compounds that can easily reach their nuclear targets. Because of their power to reactivate many tumor suppressors and to restore the normal regulatory pathways disrupted by the epigenetic silencing mechanism, these reagents have shown antiproliferative and/or cell killing effects in various cancers. In vitro studies using cancer cell lines have

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suggested that DNA demethylation, histone modification and reactivation of expression can be achieved using one-tenth the IC50 concentration. Therefore, relatively low concentrations of epigenetic modification reagents may be sufficient to restore the cell cycle and/or cell apoptosis pathways. Conceivably, this re-sensitization scheme could be enhanced by the use of additional chemotherapeutic agents. If lower doses are required, this will also lead to a reduction in the side effects of chemotherapy. Our in vitro studies in EC cell lines have shown synergism between HDAC inhibitors and several conventional chemotherapy drugs including paclitaxel, doxorubicin and carboplatin (85). Studies from this and other laboratories have suggested that both cell cycle and apoptotic regulatory pathways are involved (86–88). Monitoring DNA methylation changes before and during drug administration will guide rational design for the most effective combination treatment regimen. In this context, reversal of epigenetic silencing of the PR using DNMT and HDAC inhibitors provides an example. Progestational therapy is effective against endometrial hyperplasia and well-differentiated invasive EC (89,90) and most of the anti-cancer effects are mediated by PR-B (91,92). Advanced and poorly differentiated ECs are frequently PR-B negative and therefore resistant to progestational therapy (10,93). It has been shown that PR-B is silenced by the hypermethylation mechanism in EC and DNMT inhibitors in combination with HDAC inhibitors can reverse the silencing process and induce PR-B expression (11,12). Preliminary studies also suggest that re-activation of PR-B restores EC response to progestins [Unpublished Data, Jiang et al.].

DNA methylation assay for risk assessment

Germline errors in DNA methylation and associated changes in expression patterns in high eukaryotes are also called ‘epi−mutations’. Several studies indicate the presence of germline epi−mutations in humans. Suter et al. documented two individuals with heterozygous, hypermethylated hMLH1 promoters in various normal tissues as well as spermatozoa (94). The immediate importance of this discovery is that it helps to explain the origin of the epigenetic background and provides a potential mechanism for inheritance of epigenetic-related diseases. Follow-up investigations by the same group have confirmed the inheritance of hMLH1 epimutations from a mother to her son (95). Individuals carrying germline aberrant methylation of hMLH1 develop multiple carcinomas with mismatch repair deficiency. The spectrum and pathology of malignancies in colorectal and ECs in these patients are very similar to those seen in HNPCC (94,96,97). More recently, a heritable germline epimutation of MSH2 in a family with HNPCC was identified (98). In this case, three siblings were found to carry the epimutation and developed early-onset colorectal cancer or EC. It was proposed that a mosaic state of methylation in different tissues could act as the first hit for tumorigenesis.

Germline epimutations do not follow the usual rules for stable genetic transmission of sequences to produce a Mendelian trait. Rather, germline epimutation is often stochastic, mosaic and reversible. It has been estimated that the rate of primary epi−mutations is one or two orders of magnitude greater than somatic DNA mutations (99). Some investigators argue that epimutations may constitute a previously overlooked basis for many common diseases (96). It remains to be investigated how frequently epi−mutations are inherited, what percentage of the population is affected and what the associated risk is to carriers. The clear

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tendency for increased cancer risk seems to suggest the need for more aggressive screening in MLH1 and MSH2 epimutation carriers and their descendants. Also, hypermethylation of hMLH1 in somatic cells suggests that these individuals may suffer defects in one or more methylation pathways and are therefore predisposed to aberrant DNA methylation in additional genes as observed in CIMP.

DNA methylation patterns are also affected by the aging process. Early studies examining the total content of methylated cytosine indicated a tendency for decreased global DNA methylation levels with age in most vertebrate tissues (100). However, these studies did not investigate changes in transcription. Subsequent studies examined the effects of aging on methylation in or near specific genes. Demethylation with age in β-actin was observed in rat spleen, but not brain or liver. The c-myc gene was demethylated with age in murine spleen, but this did not affect the steady state of its mRNA levels. Age-related changes in methylation also occur outside of coding regions. Repetitive DNA sequences in the brain (101) of aged rats contain greater amounts of methylated cytosines than identical sequences from younger rats. However, an opposite trend was found in other tissues, including the thymus and heart (102). It was suggested that this hypomethylation may promote chromosomal translocation with aging (103).

Age-dependent changes in methylation have been found in several genes relevant to cancer. For example, CpG islands associated with ER and IGF become increasingly methylated with age in human colonic mucosa (104,105). The same group also reported that 19 of 30 genes known to be abnormally methylated in colo–rectal carcinoma became hypermethylated with age in normal colorectal mucosa (106). Thus, age-related methylation change can occur in a clonal or cell-type-specific manner, a pattern consistent with the clonal expansion process during cancer development. These observations suggest that methylation changes could be one of many factors predisposing the elderly to an increased risk of malignancy (100). Toyota and Issa proposed a model based on global profiling of DNA methylation in colorectal cancer (73). According to this model, methylated cytosines are classified into two categories, type A (age-related) and type C (cancer-related). Initially, type A methylation arises as a function of age in normal colorectal epithelial cells. The role of this type of methylation in tumorigenesis in not clear. However, by affecting genes regulating growth and/or differentiation, such methylation events result in a predisposition to colon carcinoma. In contrast, type C methylation was found exclusively in a subset of cancers that display the CpG island methylator phenotype. This type of aberrant methylation affects a number of tumor suppressor genes and directly contributes to cancer development. Since age is a significant risk factor for EC, comparative studies on methylation profiles in normal endometrium from young and old women will provide insight into the pathogenesis of EC.

Expert commentary

Until recently, the majority of cancer research has focused on genetic mutation and downstream effects of these alterations. The epigenetic regulation of gene expression is understudied and our understanding of these changes must evolve in order to develop the next generation of therapeutics. At the present time, the first generation of epigenetic inhibitors is US FDA approved and has been demonstrated to have a low toxicity profile in

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comparison with traditional cytotoxic agents. New HDAC inhibitors, now in Phase I and II clinical trials, appear to be even better tolerated.

Recent results have raised the possibility of applying DNA methylation assays for EC management. Although these studies are in the early stages of development, DNA samples sufficient for methylation analysis are readily accessible via minimally invasive measures in patients at high risk. Genome-wide profiling of DNA methylation targets is necessary to identify a panel of markers with the highest specificity and sensitivity. Ultimately, prospective clinical studies must be performed for validation. Additional research is needed in order to elucidate the mechanisms by which methylation is regulated and determine how risk factors such as hormonal use or diabetes impact methylation in EC patients. Establishment of the causal relationship among the hormonal alteration, DNMT regulation and dysfunction in precancerous cells and the hypermethylation of tumor suppressor genes will provide a theoretical foundation for the clinical application of epigenetic biomarkers.

Five-year view

Given that germline genetic mutation as a cause of cancer constitutes a small minority of cases and that gene therapy has been slow to materialize in the clinic, epigenetic research will command an ever increasing presence in the field of cancer research and therapeutics. An epigenome will be established, in a similar way that the genome was sequenced. This will facilitate comparisons between normal and diseased subjects, not just in cancer research, but in the study of medical disorders as well. Technical innovation will lead to further streamlining and/or automation of DNA methylation assays. Faster, more cost-effective kits requiring smaller amounts of DNA samples for bisulfite conversion and methylation detection will be commercially available in a few years. These developments will significantly speed up the process to establish standards for DNA methylation analysis.

It is particularly exciting that, in contrast to genetic mutation, changes in gene expression are reversible. DNMT inhibitor Decitabine and HDAC inhibitor vorinostat have been approved by FDA. Multiple epigenetic inhibitors are currently being investigated in both preclinical and clinical settings. The generation of epigenetic inhibitors will continue to grow. The regulation and function of DNA methylation enzymes and their interaction with other chromatin components and cellular pathways will be intensively investigated. Development of drugs with specificity to subclasses of acetylases and methyltransferases will also occur in the near future. More research will uncover important differences in splice variants which will also be exploited by drug development. Preliminary data support synergy between these inhibitors and traditional cytotoxic agents. Future drug regimens will include combinations of these agents with improved efficacy and lower toxicity.

References

Papers of special note have been highlighted as: • of interest •• of considerable interest

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. CA Cancer J Clin. 2008; 58:71–96. [PubMed: 18287387]
2. Creutzberg CL, van Putten WL, Koper PC, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial.

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PORTEC Study Group. Post Operative Radiation Therapy in Endometrial Carcinoma. Lancet. 2000; 355:1404–1411. [PubMed: 10791524]

3. Jereczek-Fossa B, Badzio A, Jassem J. Surgery followed by radiotherapy in endometrial cancer. Analysis of survival and patterns of failure. Int J Gynecol Cancer. 1999; 9:285–294. [PubMed: 11240781]
4. Bocker T, Ruschoff J, Fishel R. Molecular diagnostics of cancer predisposition. Hereditary non-polyposis colorectal carcinoma and mismatch repair defects. Biochim Biophys Acta. 1999; 1423:O1–O10. [PubMed: 10382540]

5•. Esteller M. Epigenetic lesions causing genetic lesions in human cancer. Promoter hypermethylation of DNA repair genes. Eur J Cancer. 2000; 36:2294–2300. Detailed review on hypermethylation of DNA repair genes in cancer as well as the intricate connection between epigenetic events and genetic lesions. [PubMed: 11094302]

6•. Herman JG, Umar A, Polyak K, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci USA. 1998; 95:6870–6875. hMLH1 promoter hypermethylation/silencing is associated with MSI+ phenotype and restoration of hMLH1 expression rescues the mismatch repair function. [PubMed: 9618505]

7. Furlan D, Carnevali I, Marcomini B, et al. The high frequency of de novo promoter methylation in synchronous primary endometrial and ovarian carcinomas. Clin Cancer Res. 2006; 12:3329–3336. [PubMed: 16740754]
8. Macdonald ND, Salvesen HB, Ryan A, et al. Molecular differences between RER+ and RER-sporadic endometrial carcinomas in a large population-based series. Int J Gynecol Cancer. 2004; 14:957–965. [PubMed: 15361209]
9. Salvesen HB, MacDonald N, Ryan A, et al. PTEN methylation is associated with advanced stage and microsatellite instability in endometrial carcinoma. Int J Cancer. 2001; 91:22–26. [PubMed: 11149415]
10. Ehrlich CE, Young PC, Stehman FB, Sutton GP, Alford WM. Steroid receptors and clinical outcome in patients with adenocarcinoma of the endometrium. Am J Obstet Gynecol. 1988; 158:796–807. [PubMed: 2966586]
11. Sasaki M, Dharia A, Oh BR, et al. Progesterone receptor B gene inactivation and CpG hypermethylation in human uterine endometrial cancer. Cancer Res. 2001; 61:97–102. [PubMed: 11196205]
12. Xiong Y, Dowdy SC, Gonzalez Bosquet J, et al. Epigenetic-mediated upregulation of progesterone receptor B gene in endometrial cancer cell lines. Gynecol Oncol. 2005; 99:135–141. [PubMed: 16024066]
13. Whitcomb BP, Mutch DG, Herzog TJ, et al. Frequent HOXA11 and THBS2 promoter methylation, and a methylator phenotype in endometrial adenocarcinoma. Clin Cancer Res. 2003; 9:2277–2287. [PubMed: 12796396]
14. Dowdy SC, Gostout BS, Shridhar V, et al. Biallelic methylation and silencing of paternally expressed gene 3 (PEG3) in gynecologic cancer cell lines. Gynecol Oncol. 2005; 99:126–134. [PubMed: 16023706]
15. Chan QK, Khoo US, Chan KY, 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]
16. Grady WM, Willis J, Guilford PJ, et al. Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer. Nat Genet. 2000; 26:16–17. [PubMed: 10973239]
17. Esteller M, Silva JM, Dominguez G, et al. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst. 2000; 92:564–569. [PubMed: 10749912]
18. Jolly CJ, Neuberger MS. Somatic hypermutation of immunoglobulin kappa transgenes. Association of mutability with demethylation. Immunol Cell Biol. 2001; 79:18–22. [PubMed: 11168618]
19. Florl AR, Franke KH, Niederacher D, et al. DNA methylation and the mechanisms of CDKN2A inactivation in transitional cell carcinoma of the urinary bladder. Lab Invest. 2000; 80:1513–1522. [PubMed: 11045568]
20. Matias-Guiu X, Catasus L, Bussaglia E, et al. Molecular pathology of endometrial hyperplasia and carcinoma. Hum Pathol. 2001; 32:569–577. [PubMed: 11431710]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

21••. Dodge JE, Okano M, Dick F, et al. Inactivation of Dnmt3b in mouse embryonic fibroblasts results in DNA hypomethylation, chromosomal instability, and spontaneous immortalization. J Biol Chem. 2005; 280:17986–17991. DNA methylation mechanism and potential role of DNMT3B for the establishment of DNA methylation pattern in embryonic fibroblasts. [PubMed: 15757890]

22. Pradhan M, Abeler VM, Danielsen HE, et al. Image cytometry DNA ploidy correlates with histological subtypes in endometrial carcinomas. Mod Pathol. 2006; 19:1227–1235. [PubMed: 16729014]

23. Umbricht CB, Evron E, Gabrielson E, et al. Hypermethylation of 14-3-3 sigma (stratifin) is an early event in breast cancer. Oncogene. 2001; 20:3348–3353. [PubMed: 11423985]

24. Ibanez de Caceres I, Battagli C, Esteller M, et al. Tumor cell-specific BRCA1 and RASSF1A hypermethylation in serum, plasma, and peritoneal fluid from ovarian cancer patients. Cancer Res. 2004; 64:6476–6481. [PubMed: 15374957]

25. Esteller M, Catasus L, Matias-Guiu X, et al. hMLH1 promoter hypermethylation is an early event in human endometrial tumorigenesis. Am J Pathol. 1999; 155:1767–1772. [PubMed: 10550333]

26. Norris HJ, Connor MP, Kurman RJ. Preinvasive lesions of the endometrium. Clin Obstet Gynaecol. 1986; 13:725–738. [PubMed: 3791827]

27. Fox H, Buckley CH. The endometrial hyperplasias and their relationship to endometrial neoplasia. Histopathology. 1982; 6:493–510. [PubMed: 6754573]

28. Nasir A, Boulware D, Kaiser HE, et al. Cyclooxygenase-2 (COX-2) expression in human endometrial carcinoma and precursor lesions and its possible use in cancer chemoprevention and therapy. In Vivo. 2007; 21:35–43. [PubMed: 17354612]

29. Horowitz N, Pinto K, Mutch DG, et al. Microsatellite instability, MLH1 promoter methylation, and loss of mismatch repair in endometrial cancer and concomitant atypical hyperplasia. Gynecol Oncol. 2002; 86:62–68. [PubMed: 12079302]

30. Wang C, Mirkin BL, Dwivedi RS. DNA (cytosine) methyltransferase overexpression is associated with acquired drug resistance of murine neuroblastoma cells. Int J Oncol. 2001; 18:323–329. [PubMed: 11172599]

31. 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; 97:1002–1009. [PubMed: 12569599]

32. Nakayama H, Sano T, Motegi A, et al. Increasing 14-3-3 sigma expression with declining estrogen receptor a and estrogen-responsive finger protein expression defines malignant progression of endometrial carcinoma. Pathol Int. 2005; 55:707–715. [PubMed: 16271083]

33. Shames DS, Minna JD, Gazdar AF. Methods for detecting DNA methylation in tumors. From bench to bedside. Cancer Lett. 2007; 251:187–198. [PubMed: 17166656]

34. Shi H, Wang MX, Caldwell CW. CpG islands. Their potential as biomarkers for cancer. Expert Rev Mol Diagn. 2007; 7:519–531. [PubMed: 17892361]

35. Baylin SB. DNA methylation and gene silencing in cancer. Nat Clin Pract Oncol. 2005; 2:S4–S11. [PubMed: 16341240]

36••. Laird PW. Cancer epigenetics. Hum Mol Genet. 2005; 14:R65–R76. Broad review covering the significance of DNA methylation in cancer progression as well as implication of epigenetic markers for cancer detection and assessment. [PubMed: 15809275]

37. Issa JP. DNA methylation as a therapeutic target in cancer. Clin Cancer Res. 2007; 13:1634–1637. [PubMed: 17363514]

38. Fiegl H, Elmasry K. Cancer diagnosis, risk assessment and prediction of therapeutic response by means of DNA methylation markers. Dis Markers. 2007; 23:89–96. [PubMed: 17325428]

39. Herranz M, Esteller M. DNA methylation and histone modifications in patients with cancer. Potential prognostic and therapeutic targets. Methods Mol Biol. 2007; 361:25–62. [PubMed: 17172706]

40. Liu ZJ, Maekawa M. Polymerase chain reaction-based methods of DNA methylation analysis. Anal Biochem. 2003; 317:259–265. [PubMed: 12758266]

41. Yang HJ, Liu VW, Wang Y, et al. Differential DNA methylation profiles in gynecological cancers and correlation with clinico-pathological data. BMC Cancer. 2006; 6:212. [PubMed: 16928264]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

42. Zighelboim I, Goodfellow PJ, Schmidt AP, et al. Differential methylation hybridization array of endometrial cancers reveals two novel cancer-specific methylation markers. Clin Cancer Res. 2007; 13:2882–2889. [PubMed: 17504987]
43. Paz MF, Wei S, Cigudosa JC, et al. Genetic unmasking of epigenetically silenced tumor suppressor genes in colon cancer cells deficient in DNA methyltransferases. Hum Mol Genet. 2003; 12:2209–2219. [PubMed: 12915469]
44. Hoque MO, Kim MS, Ostrow KL, et al. Genome-wide promoter analysis uncovers portions of the cancer methylome. Cancer Res. 2008; 68:2661–2670. [PubMed: 18413733]
45. Suzuki H, Itoh F, Toyota M, et al. Distinct methylation pattern and microsatellite instability in sporadic gastric cancer. Int J Cancer. 1999; 83:309–313. [PubMed: 10495421]
46. Eads CA, Laird PW. Combined bisulfite restriction analysis (COBRA). Methods Mol Biol. 2002; 200:71–85. [PubMed: 11951656]
47. Uhlmann K, Brinckmann A, Toliat MR, et al. Evaluation of a potential epigenetic biomarker by quantitative methyl-single nucleotide polymorphism analysis. Electrophoresis. 2002; 23:4072–4079. [PubMed: 12481262]
48. Colella S, Shen L, Baggerly KA, Issa JP, Krahe R. Sensitive and quantitative universal pyrosequencing methylation analysis of CpG sites. Biotechniques. 2003; 35:146–150. [PubMed: 12866414]
49•. Brena RM, Huang TH, Plass C. Quantitative assessment of DNA methylation. Potential applications for disease diagnosis, classification, and prognosis in clinical settings. J Mol Med. 2006; 84:365–377. Comprehensive review on different methods used for quantitative analysis of DNA methylation. [PubMed: 16416310]
50. Bearzatto A, Conte D, Frattini M, et al. p16(INK4A) hypermethylation detected by fluorescent methylation-specific PCR in plasmas from non-small cell lung cancer. Clin Cancer Res. 2002; 8:3782–3787. [PubMed: 12473590]
51. Ulivi P, Zoli W, Calistri D, et al. p16INK4A and CDH13 hypermethylation in tumor and serum of non-small cell lung cancer patients. J Cell Physiol. 2006; 206:611–615. [PubMed: 16222700]
52. Xie GS, Hou AR, Li LY, et al. Aberrant p16 promoter hypermethylation in bronchial mucosae as a biomarker for the early detection of lung cancer. Chin Med J. 2006; 119:1469–1472. [PubMed: 16989749]
53. Chuang CK, Chu DC, Tzou RD, et al. Hypermethylation of the CpG islands in the promoter region flanking GSTP1 gene is a potential plasma DNA biomarker for detecting prostate carcinoma. Cancer Detect Prev. 2007; 31:59–63. [PubMed: 17291695]
54. Herman JG. Circulating methylated DNA. Ann NY Acad Sci. 2004; 1022:33–39. [PubMed: 15251936]
55. Schmiemann V, Bocking A, Kazimirek M, et al. Methylation assay for the diagnosis of lung cancer on bronchial aspirates. A cohort study. Clin Cancer Res. 2005; 11:7728–7734. [PubMed: 16278393]
56. Jiang P, Watanabe H, Okada G, et al. Diagnostic utility of aberrant methylation of tissue factor pathway inhibitor 2 in pure pancreatic juice for pancreatic carcinoma. Cancer Sci. 2006; 97:1267–1273. [PubMed: 16965396]
57. Watanabe H, Okada G, Ohtsubo K, et al. Aberrant methylation of secreted apoptosis-related protein 2 (SARP2) in pure pancreatic juice in diagnosis of pancreatic neoplasms. Pancreas. 2006; 32:382–389. [PubMed: 16670620]
58. Hoque MO, Begum S, Topaloglu O, et al. Quantitation of promoter methylation of multiple genes in urine DNA and bladder cancer detection. J Natl Cancer Inst. 2006; 98:996–1004. [PubMed: 16849682]
59•. Belinsky SA, Palmisano WA, Gilliland FD, et al. Aberrant promoter methylation in bronchial epithelium and sputum from current and former smokers. Cancer Res. 2002; 6:2370–2377. Identification of multiple hypermethylated genes in sputum from former and current smokers.
60. Smith RA, von Eschenbach AC, Wender R, et al. American Cancer Society guidelines for the early detection of cancer. Update of early detection guidelines for prostate, colorectal, and endometrial cancers. Also Update 2001 – testing for early lung cancer detection. CA Cancer J Clin. 2001; 51:38–75. quiz 77–80. [PubMed: 11577479]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

61. Bader GM, Simon TR, Koss LG, Day E. A study of the detection-tampon method as a screening device for uterine cancer. Cancer. 1957; 10:332–337. [PubMed: 13426989]
62. Brunschwig A. Detection of endometrial adenocarcinoma by tampon-smear method. Cancer. 1957; 10:120–123. [PubMed: 13413807]
63. Brunschwig A. A method for mass screening for cytological detection of carcinoma of the cervix uteri. Cancer. 1954; 7:1182–1184. [PubMed: 13209517]
64. Papanicolaou GN. Cytological evaluation of smears prepared by the tampon method for the detection of carcinoma of the uterine cervix. Cancer. 1954; 7:1185–1190. [PubMed: 13209518]
65. Barrett KF, Bledsoe S, Greer BE, et al. Tampon-induced vaginal or cervical ulceration. Am J Obstet Gynecol. 1977; 127:332–333. [PubMed: 835631]
66. Weissberg SM, Dodson MG. Recurrent vaginal and cervical ulcers associated with tampon use. JAMA. 1983; 250:1430–1431. [PubMed: 6887466]
67. Kehrberg MW, Latham RH, Haslam BT, et al. Risk factors for staphylococcal toxic-shock syndrome. Am J Epidemiol. 1981; 114:873–879. [PubMed: 7315834]
68. Fox H. The pathology of tampon usage and of the toxic shock syndrome. Postgrad Med J. 1985; 61(Suppl 1):31–33.
69••. Widschwendter A, Gattringer C, Ivarsson L, et al. Analysis of aberrant DNA methylation and human papillomavirus DNA in cervicovaginal specimens to detect invasive cervical cancer and its precursors. Clin Cancer Res. 2004; 10:3396–3400. Methylation patterns of multiple methylation target genes are documented and found to be useful for prediction of invasive cervical cancers. [PubMed: 15161694]
70. Fiegl H, Gattringer C, Widschwendter A, et al. Methylated DNA collected by tampons – a new tool to detect endometrial cancer. Cancer Epidemiol Biomarkers Prev. 2004; 13:882–888. [PubMed: 15159323]
71. Campan M, Weisenberger DJ, Laird PW. DNA methylation profiles of female steroid hormone-driven human malignancies. Curr Top Microbiol Immunol. 2006; 310:141–178. [PubMed: 16909910]
72. Kang S, Kim JW, Kang GH, 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; 118:2168–2171. [PubMed: 16331610]
73. Toyota M, Issa JP. CpG island methylator phenotypes in aging and cancer. Semin Cancer Biol. 1999; 9:349–357. [PubMed: 10547343]
74. Issa JP. CpG island methylator phenotype in cancer. Nat Rev Cancer. 2004; 4:988–993. [PubMed: 15573120]
75. Ogino S, Kawasaki T, Kirkner GJ, et al. CpG island methylator phenotype-low (CIMP-low) in colorectal cancer. Possible associations with male sex and KRAS mutations. J Mol Diagn. 2006; 8:582–588. [PubMed: 17065427]
76. Ogino S, Kawasaki T, Kirkner GJ, et al. 18q loss of heterozygosity in microsatellite stable colorectal cancer is correlated with CpG island methylator phenotype-negative (CIMP-0) and inversely with CIMP-low and CIMP-high. BMC Cancer. 2007; 7:72. [PubMed: 17474983]
77. Goel A, Nagasaka T, Arnold CN, et al. The CpG island methylator phenotype and chromosomal instability are inversely correlated in sporadic colorectal cancer. Gastroenterology. 2007; 132:127–138. [PubMed: 17087942]
78. Curtin K, Slattery ML, Ulrich CM, et al. Genetic polymorphisms in one-carbon metabolism. associations with CpG island methylator phenotype (CIMP) in colon cancer and the modifying effects of diet. Carcinogenesis. 2007; 28:1672–1679. [PubMed: 17449906]
79. Slattery ML, Curtin K, Sweeney C, et al. Diet and lifestyle factor associations with CpG island methylator phenotype and BRAF mutations in colon cancer. Int J Cancer. 2007; 120:656–563. [PubMed: 17096326]
80. Shen L, Catalano PJ, Benson AB III, et al. Association between DNA methylation and shortened survival in patients with advanced colorectal cancer treated with 5-fluorouracil based chemotherapy. Clin Cancer Res. 2007; 13:6093–6098. [PubMed: 17947473]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

81. Ogino S, Meyerhardt JA, Kawasaki T, et al. CpG island methylation, response to combination chemotherapy, and patient survival in advanced microsatellite stable colorectal carcinoma. Virchows Arch. 2007; 450:529–537. [PubMed: 17372756]

82. Shaw RJ, Hall GL, Lowe D, et al. CpG island methylation phenotype (CIMP) in oral cancer. associated with a marked inflammatory response and less aggressive tumour biology. Oral Oncol. 2007; 43:878–886. [PubMed: 17257884]

83. Mell LK, Meyer JJ, Tretiakova M, et al. Prognostic significance of E-cadherin protein expression in pathological stage I–III endometrial cancer. Clin Cancer Res. 2004; 10:5546–5553. [PubMed: 15328195]

84. Salvesen HB, Stefansson I, Kretzschmar EI, 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; 25:1615–1623. [PubMed: 15547698]

85. Dowdy SC, Jiang S, Zhou XC, et al. Histone deacetylase inhibitors and paclitaxel cause synergistic effects on apoptosis and microtubule stabilization in papillary serous endometrial cancer cells. Mol Cancer Ther. 2006; 5:2767–2776. [PubMed: 17121923]

86. Takai N, Desmond JC, Kumagai T, et al. Histone deacetylase inhibitors have a profound antigrowth activity in endometrial cancer cells. Clin Cancer Res. 2004; 10:1141–1149. [PubMed: 14871994]

87. Ahn MY, Jung JH, Na YJ, Kim HS. A natural histone deacetylase inhibitor, Psammaplin A, induces cell cycle arrest and apoptosis in human endometrial cancer cells. Gynecol Oncol. 2008; 108:27–33. [PubMed: 17920664]

88. Jiang S, Dowdy SC, Meng XW, et al. Histone deacetylase inhibitors induce apoptosis in both type I and type II endometrial cancer cells. Gynecol Oncol. 2007; 105:493–500. [PubMed: 17303224]

89. Podratz KC. Hormonal therapy in endometrial carcinoma. Recent Results Cancer Res. 1990; 118:242–251. [PubMed: 2236840]

90. Lentz SS. Endocrine therapy of endometrial cancer. Cancer Treat Res. 1998; 94:89–106. [PubMed: 9587684]

91. Dai D, Wolf DM, Litman ES, et al. Progesterone inhibits human endometrial cancer cell growth and invasiveness. down-regulation of cellular adhesion molecules through progesterone B receptors. Cancer Res. 2002; 62:881–886. [PubMed: 11830547]

92. Dai D, Albitar L, Nguyen T, et al. A therapeutic model for advanced endometrial cancer. Systemic progestin in combination with local adenoviral-mediated progesterone receptor expression. Mol Cancer Ther. 2005; 4:169–175. [PubMed: 15657363]

93. Lentz SS, Brady MF, Major FJ, et al. High-dose megestrol acetate in advanced or recurrent endometrial carcinoma. A Gynecologic Oncology Group Study. J Clin Oncol. 1996; 14:357–361. [PubMed: 8636744]

94. Suter CM, Martin DI, Ward RL. Germline epimutation of MLH1 in individuals with multiple cancers. Nat Genet. 2004; 36:497–501. [PubMed: 15064764]

95. Hitchins MP, Wong JJ, Suthers G, et al. Inheritance of a cancer-associated MLH1 germ-line epimutation. N Engl J Med. 2007; 356:697–705. [PubMed: 17301300]

96. Martin DI, Ward R, Suter CM. Germline epimutation. A basis for epigenetic disease in humans. Ann NY Acad Sci. 2005; 1054:68–77. [PubMed: 16339653]

97. Valle L, Carbonell P, Fernandez V, et al. MLH1 germline epimutations in selected patients with early-onset non-polyposis colorectal cancer. Clin Genet. 2007; 71:232–237. [PubMed: 17309645]

98. Chan TL, Yuen ST, Kong CK, et al. Heritable germline epimutation of MSH2 in a family with hereditary nonpolyposis colorectal cancer. Nat Genet. 2006; 38:1178–1183. [PubMed: 16951683]

99. Horsthemke B. Epimutations in human disease. Curr Top Microbiol Immunol. 2006; 310:45–59. [PubMed: 16909906]

100. Richardson B. Impact of aging on DNA methylation. Ageing Res Rev. 2003; 2:245–261. [PubMed: 12726774]

101. Rath PC, Kanungo MS. Methylation of repetitive DNA sequences in the brain during aging of the rat. FEBS Lett. 1989; 244:193–198. [PubMed: 2466694]

102. Romanov GA, Vanyushin BF. Methylation of reiterated sequences in mammalian DNAs. Effects of the tissue type, age, malignancy and hormonal induction. Biochim Biophys Acta. 1981; 653:204–218. [PubMed: 7225396]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

103•. Lengauer C, Kinzler KW, Vogelstein B. DNA methylation and genetic instability in colorectal cancer cells. Proc Natl Acad Sci USA. 1997; 94:2545–2550. Mechanistic investigation on how DNA methylation changes may lead to alterations in gene expression and genomic instability. [PubMed: 9122232]

104. Issa JP, Vertino PM, Boehm CD, et al. Switch from monoallelic to biallelic human IGF2 promoter methylation during aging and carcinogenesis. Proc Natl Acad Sci USA. 1996; 93:11757–11762. [PubMed: 8876210]

105. Ahuja N, Li Q, Mohan AL, et al. Aging and DNA methylation in colorectal mucosa and cancer. Cancer Res. 1998; 58:5489–5494. [PubMed: 9850084]

106. Toyota M, Ahuja N, Ohe-Toyota M, et al. CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci USA. 1999; 96:8681–8686. [PubMed: 10411935]

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Key issues

- Cancer cells undergo changes in 5-methylcytosine distribution that may lead to recessive changes indistinguishable from those caused by genetic mutation or deletion.
- Epigenetic silencing can be reversed using small molecule inhibitors of methylation and deacetylation, some of which are either US FDA approved or currently in clinical trials.
- Preclinical studies suggest that epigenetic inhibitors may synergize with other cytotoxic agents for cancer treatment.
- Methylation-specific PCR is the fastest, most economical and most convenient technique to characterize epigenetic changes at specific promoter sites or throughout the entire genome; chromatin immunoprecipitation, DNA sequencing combined bisulfite-restriction analysis and pyrosequencing are also useful modalities.
- DNA methylation analysis offers several practical advantages over protein or RNA-based indices of gene expression in the clinic.
- Tissue suitable for DNA methylation assays is readily accessible from patients for endometrial cancer and may prove useful for screening, diagnosis, prognostication or direct therapy.
- Genome-wide profiling efforts may lead to identification of more and better epigenetic markers useful for cancer management with improved sensitivity and specificity.
- Age-dependent changes in methylation have been found in several genes relevant to cancer and suggest one explanation for the rising prevalence of cancer in the elderly.