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Review

DNA methylation changes in ovarian cancer: Implications for early diagnosis, prognosis and treatment

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Abstract

Objective. To review epigenetic changes identified in ovarian cancer, focusing on their potential as clinical markers for detection, monitoring of disease progression and as markers of therapeutic response.

Methods. A comprehensive review of English language scientific literature on the topics of methylation and ovarian cancer was conducted.

Results. Genome-wide demethylation of normally methylated and silenced chromosomal regions, and hypermethylation and silencing of genes including tumor suppressors are common features of cancer cells. Epigenetic alterations, including CpG island DNA methylation, occur in ovarian cancer and the identification of specific genes that are altered by epigenetic events is an area of intense research. Aberrant DNA methylation in ovarian cancer is observed in early cancer development, can be detected in DNA circulating in the blood and hence provides the promise of a non-invasive cancer detection test. In addition, identification of ovarian cancer-specific epigenetic changes has promise in molecular classification and disease stratification.

Conclusions. The detection of cancer-specific DNA methylation changes heralds an exciting new era in cancer diagnosis as well as evaluation of prognosis and therapeutic responsiveness and warrants further investigation.

Keywords: Methylation; Ovarian cancer; Cancer biomarkers

Contents

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Introduction

Ovarian cancer is the fifth leading cause of cancer death in women and has the highest mortality rate of cancers of the reproductive organs [1]. Ovarian cancer is often asymptomatic in its early stages and because of a lack of early detection strategies most patients are diagnosed with disseminated disease, for whom the 5-year overall survival rate is only approximately 20% [2]. In the absence of an early ovarian cancer detection test, improved therapies for advanced disease are paramount to improving the survival for women with ovarian cancer. A better understanding of the molecular pathogenesis of ovarian cancer is now required to identify biomarkers to enable early detection and novel therapeutic targets.

Both genetic (changes in DNA sequence such as deletions/amplifications and mutations) and epigenetic changes, defined as heritable changes in gene expression that occur without changes to the DNA sequence [3], contribute to malignant transformation and progression. Commonly occurring epigenetic events include DNA methylation, the addition of a methyl group to the 5'-carbon of cytosine in CpG sequences (Fig. 1A); and chromatin remodeling via histone protein acetylation and methylation [4]. In normal cells, the human genome is not methylated uniformly, containing unmethylated segments interspersed with methylated regions [5]. Although spontaneous deamination of methylated cytosine through evolution has decreased the proportion of CpG dinucleotides in the genome, there are regions ranging from 0.5 to 5 kb that contain clusters of CpG dinucleotides called CpG islands [6]. These CpG-rich regions are often located in the 5' region of genes and are associated with their promoters. In contrast to the bulk of DNA, the CpG sites within CpG islands are almost always methylation free. This appears to be a prerequisite for active transcription of the genes under their control [7]. The relationship between DNA methylation and post-translational modification of histones is complex but they collectively are associated with the regulation of gene transcription (Fig. 1B) [4,8,9].

The methylation patterns in cancer cells are significantly altered compared to those of normal cells. Cancer cells undergo changes in 5-methylcytosine distribution including global DNA hypomethylation [10,11] as well as hypermethylation of CpG islands (Fig. 1C) [12–17]. Genome hypomethylation, mainly due to hypomethylation of normally silenced repetitive sequences such as long interspersed nuclear elements, is present in most cancer cells compared to the normal tissue from which they originated [18,19]. Hypomethylation has been hypothesized to contribute to oncogenesis by transcriptional activation of oncogenes, activation of latent transposons, or by chromosome instability [20–24]. At the same time, aberrant CpG island DNA methylation and histone modification, leading to transcriptional inactivation and gene silencing, is a common phenomenon in human cancer cells and likely one of the earliest events in carcinogenesis [4]. In particular, hypermethylation of CpG islands in gene promoter regions is a frequent mechanism of inactivation of tumor suppressor genes [4,10,13,15], and has been proposed as one of the two hits in Knudson's two hit hypothesis for oncogenic transformation [13].

In this review we discuss genes currently identified as being deregulated by alterations in methylation status in ovarian cancer, with a focus on genes with clinical potential as diagnostic markers or markers of disease progression and therapeutic response.

Epigenetically regulated genes in ovarian cancer

As in other cancers, epigenetic alterations, including CpG island DNA methylation, are common in ovarian cancer. The identification of specific genes that are altered by these epigenetic events is an area of intense research. Most studies to date have focused on candidate gene approaches to identify hypermethylated and silenced candidate tumor suppressor genes but there is also a growing literature on specific regions of hypomethylation in ovarian cancer.

Hypermethylated and silenced genes

A large number of genes have been identified as hypermethylated and silenced in ovarian cancer, with the reported frequency of methylation often varying widely between independent studies (Table 1). Examples include genes that are downregulated in ovarian cancer that reside in regions with known loss of heterozygosity (LOH) in ovarian cancer and/or are epigenetically regulated in other cancers, eg. *OPCML* [25–27], *DLEC1* [28], *RASSF1A* [26,29–32], *ARLTS1* [33], *ARHI* [34,35] and *TCEAL7* [36]. In many cases, the functional effects of gene silencing on ovarian cancer pathogenesis are not known.

The most extensively studied gene is *BRCA1* (breast cancer susceptibility gene 1) due to its known role in inherited forms of ovarian cancer. *BRCA1* promoter methylation only occurs in breast and ovarian cancers [37,38] and mirrors the classical genetic mutation studies of familial cancers. In epithelial ovarian cancers *BRCA1* hypermethylation is associated with loss of BRCA1 expression [39–41] and is predominantly detected in cancers that exhibit LOH at the *BRCA1* locus [37,40]. In contrast, *BRCA2* promoter hypermethylation is rarely found in ovarian cancers [42,43]. *BRCA1* silencing is significantly associated with high grade tumors [44,45] and, although no correlation of methylation with histological stage has been found, methylation is detectable in early (stage 1A) tumors [41].

Interestingly, gene methylation patterns are often associated with molecular, clinical and pathological features of ovarian carcinomas. For example, aberrant methylation of the promoters of *SFN* (14-3-3sigma, an inhibitor of cell cycle progression), *TMS1* (target of methylation-induced silencing) and the *WT1* (Wilms tumor suppressor 1 gene) are more frequent events in clear cell ovarian tumors than in other histological types [26,46–49]. Methylation profiles can also differentiate between ovarian low malignant potential (LMP) tumors and invasive ovarian carcinoma, for example, *RASSF1A*, *APC*, *GSTP1* and *MGMT* show aberrant methylation exclusively in invasive ovarian carcinomas [50].

Hypomethylation in ovarian cancer

Global DNA hypomethylation increases with malignancy in ovarian epithelial neoplasms [51]. There are, however, limited

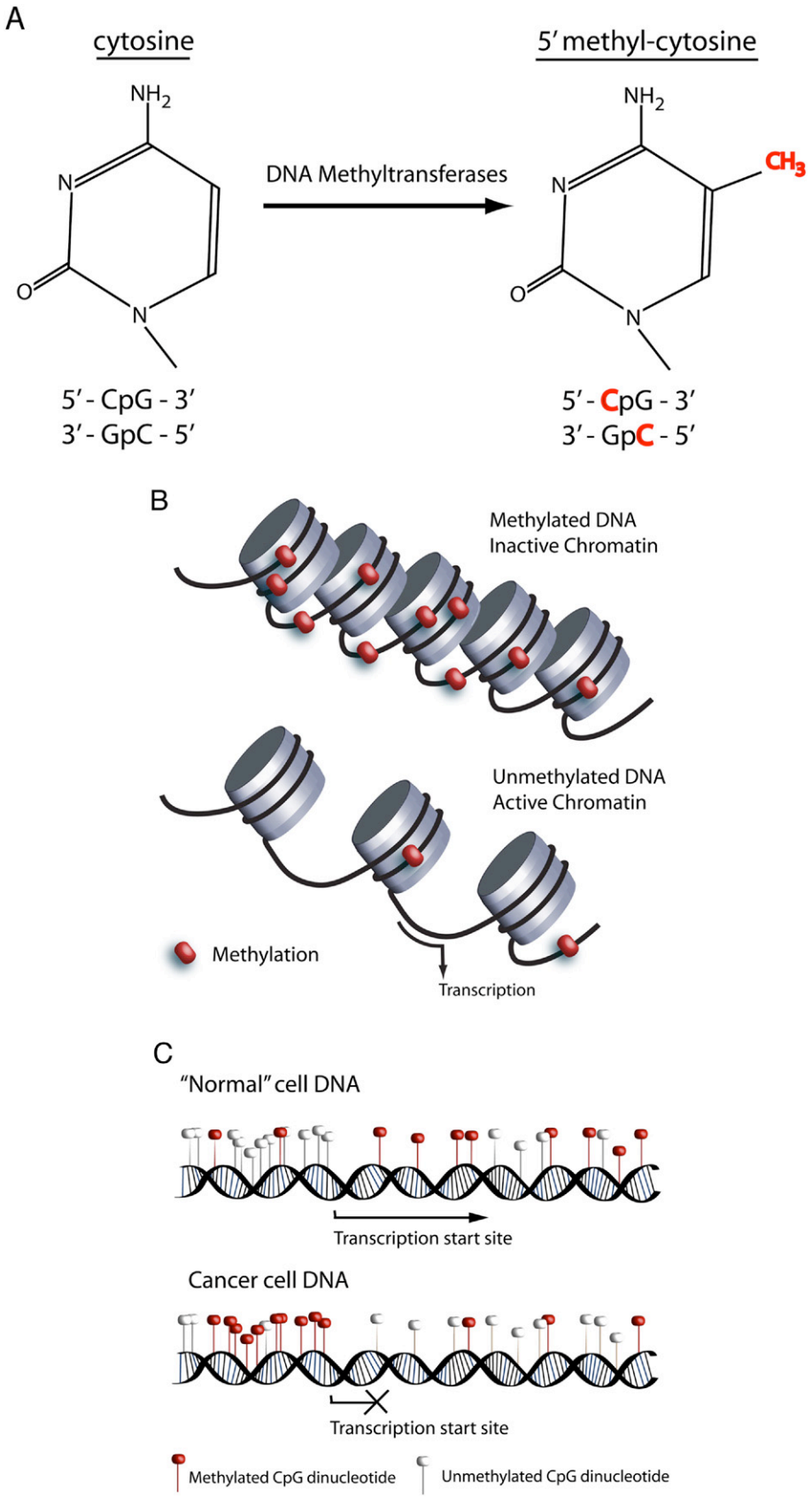

Fig. 1. Epigenetic processes involved in malignancy. A) DNA methylation: DNA methyltransferases add a methyl group to the 5'-carbon of cytosine in CpG dinucleotides. B) Chromatin structure: DNA methylation is associated with inactive chromatin; DNA demethylation relaxes chromatin structure allowing transcriptional complexes to bind and initiate gene transcription. C) In comparison to normal cells, tumor cells exhibit an altered distribution of 5-methylcytosine, in particular with hypermethylation of promoter-associated CpG islands resulting in gene inactivation.

Table 1
Selected genes that undergo CpG island hypermethylation in epithelial ovarian cancer

Gene name	Percentage methylated	Fraction methylated	Histological type analyzed	Significant associations with histological type	References
High methylation					
OPCML	33–83%	57/69; 25/75; 20/43	S+non-S		[25,26,113]
HSulf-1	75%	12/16	Unspecified		[86]
GATA4	60%	9/15	CC, E		[114]
DAPK	0–67%	0/106; 3/16; 0/80; 20/30	S, CC, E, M, U/m		[26,32,47,76]
CDH13	13–67%	9/49; 6/46; 10/51; 4/6	S, M, CC, E, U, A	CC [115]	[31,50,115,116]
TES	70%	7/10	Unspecified		[117]
Moderate methylation					
BRCA1	5–31%	13/106; 5/49; 12/50; 4/31; 12/98; 8/50; 44/215; 8/49; 11/88; 20/64; 2/43; 18/221; 2/20	S, M, CC, E, U/m, A	S [45,118]	[26,31,32,37,39,41, 44,45,89,118–122]
HIC1	16–52%	13/75; 17/49; 14/88; 46/89	S, M, CC, E, A, m, other		[26,31,89,123]
DLEC1	50%	7/14	S		[28]
CDKN2A (p16)	0–41%	1/26; 5/49; 5/16; 89/215; 9/49; 12/46; 0/88; 17/89; 100/249; 0/57; 7/46; 6/23	S, M, CC, E, U/m	S+M [45] or U [124]	[26,31,32,44,45,50, 89,123–127]
CTGF	59%	39/66	Unspecified		[128]
HOXA9	51%	26/51	S, E, M, CC	CC	[115]
GATA5	33%	5/15	CC, S, E		[114]
DCR1	31–43%	23/75; 10/23	S+non-S		[26,129]
SFN	41–55%	22/54; 11/20	S, E, M, CC	S [130] and CC [49]	[49,130]
ESR1	56%	117/215	CC, E, M, S, U, other		[44]
RASSF1A	10–50%	28/106; 2/21; 8/20; 20/49; 25/50; 7/46; 23/47	S, M, CC, E, U, A		[26,29–32,50,115]
IGFBP3	44%	104/235	CC, E, M, S, U, other		[79]
PYCARD	5–40%	4/77; 8/20; 15/80; 14/89	S, E, CC, M, U, other	CC [47]	[26,46,47,123]
WT1	50%	21/42	S, CC	88% CC, 24% S	[48]
APC	11–47%	9/49; 5/16; 5/46; 12/51; 42/89	S, M, CC, E, U, A, other		[31,32,50,115,123]
MINT31	50–52%	46/88; 45/89	S, M, CC, E, m, other		[89,123]
CDH1	22–42%	14/49; 10/46; 34/80	S, M, CC, E, U, A		[31,50,131]
Low methylation					
FANCF	0–28%	0/106; 4/19; 5/18	S+non-S		[26,95,132]
ING1	24%	21/88	S, M, E, CC, U		[133]
SOCS1	23%	10/43	Unspecified		[134]
SOCS2	14%	6/42	Unspecified		[134]
TCF2	26%	26/98	S, M, E, CC, U/m		[135]
MLH1	2–13%	6/106; 19/215; 6/88; 3/24; 1/51; 7/75; 5/68	S, M, CC, E, U/m		[26,44,89,91,115,136,137]
HOXB5	12%	6/52	S, E, M, CC		[115]
THRA	24%	20/88	S, M, CC, E, m		[89]
TP73	0–10%	0/106; 7/88	S, M, CC, E, m		[26,89]
GPR150	27%	4/15	S, E, M, CC, m	S and CC	[138]
ITGA8	13%	2/15	S, E, M, CC, m	S, m	[138]
SCGB3A1	10%	5/52	S, E, M, CC	CC and M	[115]
CRABP1	4%	2/48	S, E, M, CC	CC	[115]
PRTFDC1	7%	1/15	S, E, M, CC, m	S	[138]
HOXD11	7%	1/15	S, E, M, CC, m	S	[138]
CCND2	23%	16/71	S, E, CC, M		[139]
RIZ1	23%	20/89	S, E, M, CC, other		[123]
CDKN2B	0–19%	0/88; 17/89	S, M, CC, E, m, other		[89,123]
PTEN	8–17%	5/58; 4/49; 15/89;	S, M, E, CC	M [45]	[26,45,123]
RARB	2–17%	1/49; 5/46; 15/89	S, M, CC, E, U, A, other		[31,50,123]
SFRP1	5–12%	4/76; 2/17	S, E, M		[26,140]
MGMT	4–9%	1/26; 2/46	S, M, CC, E, U		[26,50]
UCHL1	6%	1/17	Unspecified		[141]
CDKN2A (p14)	9–24%	3/16; 12/49; 4/46	S, M, E, CC, other		[32,45,126]
MYO18B	13%	2/15	S, E, M, CC, m		[142]
DR4	28%	10/36	Unspecified		[107]
MINT25	12–16%	9/75; 13/88	S, M, CC, E, m		[26,89]

Legend: S serous, M mucinous, CC clear cell, E endometriod, U undifferentiated, A adenocarcinoma.
Highly methylated ≥60%; Moderately methylated 30>60%; Lowly methylated <30%.

examples to date of specific gene activation by hypomethylation in ovarian cancer and demethylation appears to be important in the abnormal overexpression of maspin (*SERPINB5*) [52], synuclein-γ (*SNCG*) [53,54] and claudin 4 (*CLDN4*) [55,56] in ovarian carcinomas. In addition, demethylation of the CpG dinucleotides associated with the L1 and HERV-W retrotransposons, repetitive sequences that are widely distributed throughout the genome, occurs in malignant relative to non-malignant ovarian tissue consistent with an elevation in expression levels [57]. It is hypothesized that an increase in hypomethylation promotes recombination among homologous elements leading to chromosomal aberrations which are associated with cancer [58,59].

Hypomethylation and rearrangements in heterochromatin in the vicinity of the centromeres of chromosomes 1 and 16 are frequent in ovarian cancers [60]. The degree of malignancy correlates significantly with the extent of hypomethylation of satellite 2 (Sat2) DNA, the main sequence in the heterochromatin region adjacent to the centromere of these chromosomes. Furthermore, there is a highly significant difference in levels of satellite hypomethylation in the major DNA component of all the human centromeres, satellite α (Satα), in ovarian cancer [61]. Advanced stage of disease and tumor grade is associated significantly with extensive hypomethylation of Chr1 Sat2 or Chr1 Satα; and serous and endometriod ovarian cancers have significantly higher hypomethylation levels than LMP or mucinous tumors [61]. In addition, the methylation status of NBL2, a complex tandem DNA repeat in acrocentric chromosomes, is significantly related to degree of malignancy of ovarian epithelial carcinomas, with hypomethylation seen only in the carcinomas [62].

Clinical epigenetic markers

Alterations in epigenetic patterns, including changes in DNA methylation, have several advantages as a means to detect and classify cancer. Firstly, methylation analysis utilizes DNA, a more chemically stable molecule than RNA or protein. Secondly, aberrant DNA methylation is a binary signal, where the presence of methylation indicates the presence of malignant cells. This can be detected at low concentration in a background of excess normal DNA molecules by sensitive assays that depend on signal amplification by PCR. Such assays include methylation-specific PCR (MSP) [63] and quantitative MSP [64] including the fluorescence-based real-time PCR-based MethyLight technique and headloop suppression PCR [65], which allows the detection of a single methylated allele in 10 000 unmethylated alleles [66]. These universal assays have sufficient signal-to-noise ratio and throughput capacity to sensitively analyze a broad spectrum of markers in multiple samples and thus may be useful for diagnostic purposes in the clinical setting [67–69]. The third advantage of using DNA methylation to detect cancer is that assay design can focus on a single amplifiable region (eg CpG island) rather than scanning an entire gene for mutations. Moreover, the detection of methylation of multiple genes can be combined in a high-throughput manner to improve the specificity of cancer detection. In addition, methylation biomarkers are detectable in patient serum/plasma and other bodily fluids draining or surrounding a tumor site [70], and hence have potential application as the basis of non-invasive detection tests. To this end, a number of studies have shown the feasibility of detecting altered methylation patterns in circulating tumor DNA [71] from patients with a broad spectrum of tumors [72–74]. A recent workshop report on Standards and Metrology for Cancer Detection and Diagnostics focusing upon DNA methylation [75] reviews the current challenges in the field and provides recommendations towards the future clinical application of methylated DNA sequences as cancer biomarkers.

Early diagnostic markers

Early diagnosis is critical for the successful treatment of many types of cancer, including ovarian cancer. As aberrant methylation is frequently observed in cancer development and is thought to be one of the earliest molecular changes in carcinogenesis [4], the detection of alterations in DNA methylation patterns has applicability to the detection of early-stage or potentially pre-malignant disease. Surprisingly, despite little blood-borne spread, specific methylated DNA markers can be detected in the serum, plasma and peritoneal fluid of ovarian cancer patients, as demonstrated by a recent feasibility study [32]. Tumor-specific hypermethylation of at least one of a panel of six tumor suppressor gene promoters, including *RASSF1A*, *BRCA1*, *APC*, *CDKN2A* and *DAPK*, could be detected in the serum or plasma of ovarian cancer patients with 100% specificity and 82% sensitivity, including 13/17 cases of stage I (confined to the ovary) disease. Methylation was observed in only 1 peritoneal fluid sample from 15 stage IA or B patients, but 11/15 paired sera were positive for methylation [32]. In addition to proof of principle, these data indicate that circulating ovarian tumor DNA is more readily accessible in the bloodstream than in the peritoneum, consistent with previous studies [71]. *DAPK* methylation in whole peripheral blood DNA of ovarian cancer patients has also been independently examined [76]. *DAPK* methylation could be detected in 14/16 peripheral bloods when the primary tumor was positive for *DAPK* methylation, with 10/10 peripheral bloods being negative when the primary tumor was negative for *DAPK* methylation.

Hence, despite several limitations including the sensitivity of methylation assays relative to the amount of circulating tumor DNA which may lead to stochastic sampling issues [75], in principal, these studies demonstrate that detection of specific epigenetic markers in the circulation of patients is a promising new approach as a screening method for the detection of early-stage ovarian cancer. The challenge remains to identify methylated markers that are commonly found in patients with ovarian cancer that would be suitable for diagnostic purposes. Unlike prostate cancer, in which *GSTP1* is methylated in over 90% of cancers [77,78], no single gene in ovarian cancer has been identified as being methylated in more than a relatively small proportion of cancers (Table 1). Although new genome-wide approaches may discover such a gene(s), it is likely that a panel of methylated genes will be required to detect ovarian cancer at sufficient specificity and sensitivity. A combination of

genes that are commonly methylated in cancer and genes that are methylated specifically in ovarian cancer is the most likely methylation signature capable of distinguishing ovarian cancers from neoplasms of other organs and from benign disease.

Prognostic markers

Several epigenetically regulated genes have been assessed for their positive prognostic potential in ovarian cancer. For example, *IGFBP-3* hypermethylation is associated with disease progression and death in ovarian cancer, particularly in patients with early-stage disease, where methylation is associated with a 3-fold higher risk of disease progression and a 4-fold higher risk of death [79]. When *IGFBP-3* methylation was combined with methylation in the promoter regions of *CDKN2A*, *BRCA1* or *MLH1*, the risk of disease progression in patients with ≥ 3 methylated genes was increased 7-fold [44]. Conversely, hypermethylation of 18S and 28S rDNA is associated with prolonged progression-free survival of ovarian cancer patients [80]. Hypomethylation of certain chromosomal regions also appears to have prognostic power; patients who demonstrated little or no hypomethylation of Chr1 Sat2 or Chr1 Satα had a significantly longer relapse-free survival compared with patients with strong hypomethylation of these regions [61]. However, the small sample sizes used in these studies require their validity be assessed in large independent studies.

It is likely that determining the methylation status of multiple genes simultaneously, rather than individual genes, will provide a more sensitive and specific assay for molecular classification and prognosis of ovarian cancer patients. To this end, genome-wide array-based approaches are being utilized to identify prognostic "methylation signatures" that can predict patient outcome. For example, using differential methylation hybridization [81], Wei et al. could stratify late-stage ovarian tumors into 2 distinct groups with significantly different outcome based on methylation profiling of 956 CpG island-containing loci [82]. This study was recently extended to identify 112 discriminatory methylated gene loci capable of predicting progression-free survival with 95% accuracy using rigorous classifying algorithms [83]. Hence, although in its infancy and awaiting validation in randomized controlled trails, the identification of a prognostic panel of hypermethylated DNA markers for ovarian cancer remains a realistic possibility.

Markers of therapeutic responsiveness

Variations in patterns of methylation can occur within the same tumor types, and in addition to providing prognostic information, methylation patterns are associated with response to chemotherapy. Most patients with ovarian cancer receive cytotoxic chemotherapy following surgical resection of their tumor; however, although the majority of patients are initially responsive to chemotherapy, most eventually develop drug-resistant disease, which is essentially incurable. Epigenetic gene regulation plays a prominent role in both intrinsic and acquired drug resistance in cancer [84], and therefore epigenetic markers may prove useful in predicting chemotherapy response and outcome in patients with ovarian cancer. Methylated genes implicated in drug resistance are those involved in processes known to influence chemosensitivity, such as DNA repair and damage response pathways, cell cycle control, and apoptosis [85]. For example, Teodoridis et al. showed that methylation of at least one of three genes involved in DNA repair/drug detoxification (*BRCA1*, *GSTP1* and *MGMT*) is associated with improved response to chemotherapy of patients with late-stage epithelial ovarian tumors [26]. More recently, HSulf-1 expression has been shown to influence response to chemotherapy; patients with advanced stage primary epithelial ovarian tumors that express high levels of HSulf-1 show an increased response rate to chemotherapy compared to patients whose tumors express low or moderate levels of HSulf-1 [86]. *HSulf-1* is often downregulated in ovarian cancer by methylation-associated silencing, and downregulation of HSulf-1 in ovarian cancer cell lines *in vitro* leads to the attenuation of cisplatin-induced cytotoxicity [86,87].

Chemotherapy itself can exert a positive selective pressure on subpopulations of cells in an initially chemoresponsive tumor. A number of recent studies suggest a direct role for epigenetic inactivation of genes underlying acquired chemoresistance at disease relapse. For example, matched cell line models of acquired resistance have shown that chemotherapy can select for common patterns of CpG island methylation *in vitro* [88]. There is an increasing volume of evidence from clinical studies that supports this hypothesis. In the study by Wei et al. [82] discussed above, patients stratified as having a short progression-free survival (with a high degree of CpG island methylation) had a worse response to second-line cytotoxic therapies compared to patients with a longer progression-free survival (and low CpG island methylation), suggesting that patients with high CpG island methylation acquire resistance to chemotherapy more readily.

Silencing of *hMLH1*, a DNA mismatch repair gene, by hypermethylation of its promoter CpG island [89,90] has been linked with acquired resistance to platinum-based drugs in ovarian cell line models [91–93]. Moreover, methylation of *MLH1* is increased at relapse in epithelial ovarian cancer patients, with 25% (34/138) of plasma samples from relapsed patients showing methylation of *MLHI* which is not evident in matched pre-chemotherapy plasma samples. The acquisition of *MLH1* methylation at relapse predicts poor overall patient survival, and is associated with drug resistance [94].

FANCF is crucial for the activation of the DNA repair complex containing BRCA1 and BRCA2. Methylation-induced inactivation of *FANCF* is observed in ovarian cancer cells with a defective BRCA2 pathway, associated with increased sensitivity to cisplatin. Demethylation and re-expression of *FANCF* is associated with acquisition of cisplatin resistance in ovarian cancer cell lines [95]. It has been proposed that inactivation of *FANCF* occurs early in tumor progression but chemotherapy selects for cells in which *FANCF* methylation has been reversed and therefore display higher resistance to platinum-based chemotherapy [95]. Methylation of *FANCF* has been observed in primary ovarian cancers [95] but its relevance to clinical outcome following chemotherapy has yet to be established.

Methylation-controlled J protein (*MCJ*), a member of the cochaperones which is required to repress the expression of the drug transporter *ABCB1* (P-glycoprotein) [96], was identified as a gene that, when active, rendered epithelial cells more sensitive to cisplatin and paclitaxel, the mainstay of chemotherapy for ovarian cancer patients [97]. Unusually for a CpG island-associated gene, cell type specific DNA methylation and gene silencing of *MCJ* are observed in normal cells, including normal ovarian surface epithelium, the likely cells of origin of ovarian carcinomas [98]. The majority of late-stage ovarian cancers also exhibit *MCJ* methylation; however, many of these have undergone a partial demethylation of the *MCJ* gene promoter, with only 17% of cancers maintaining very high (>90%) methylation, which is correlated with a poor response to chemotherapy and decreased survival [98,99]. Hence, *MCJ* methylation may be a useful marker of response to chemotherapy in ovarian cancer.

These data remain to be validated in large prospective studies, nonetheless the identification of ovarian cancer-specific epigenetic changes clearly has promise in disease stratification and treatment individualization [85,94].

Future challenges

There is an ever-increasing literature detailing epigenetically regulated genes in ovarian cancer, in particular hypermethylated and silenced genes. However, many of these reported changes remain unverified in independent studies. Moreover, the frequency of methylation detection for individual genes can vary widely between studies. This variability in detection is likely a result of disparate tumor cohorts, sample processing methodologies and DNA integrity, and assay platform and design [75]. There are now several validated assays to assess DNA methylation, including high-throughput quantitative approaches; however it should be emphasized that regardless of the approach chosen, careful assay design and correct interpretation of the results are critical in determining the true methylation frequency of a given chromosomal region.

Although there are CpG islands that are commonly methylated in multiple tumor types, methylation patterns specific for individual cancer types also occur [100–104]. To date, no gene (s) have been identified that are methylated and silenced in a high proportion of ovarian cancer cells. However, only a fraction of potential methylation targets has been examined. It is likely that a shift from candidate gene to genome-wide array-based approaches (reviewed in [105]), will aid in the discovery of an ovarian cancer-specific epigenetic fingerprint, including highly methylated genes specific for ovarian cancer [106–108]. For example, a recent study which correlated genes with down-regulated expression in ovarian cancer relative to normal controls to gene re-expressed in response to a demethylating agent [109] identified many novel methylation-responsive genes in ovarian cancer. In addition, the experimental validation of a computational approach to genomic "sweeping" for potentially hypermethylated genes in ovarian cancer has highlighted the ability of algorithms to accurately identify novel genes for future study [110]. We would anticipate from earlier studies that some of these methylation targets will be specific to the majority of cells in a particular stratified group of ovarian cancers, such as histological phenotype or cancers with acquired resistance to chemotherapy. Like other array-based data discovery platforms, methylated genes identified by high-throughput screening approaches will require careful analysis and validation. In particular it will be important to concurrently analyze expression and DNA sequence changes in matched clinical samples to allow accurate analysis of methylation profiling data. Data analysis will also rely on prior knowledge of normal levels of DNA methylation in the ovary to establish a baseline from which to identify alterations in ovarian cancer, which will in part be aided by the establishment of the Human Epigenomic Project [111]. In addition to a well characterized set of profiles for normal human cells with which cancer epigenomes can be compared, there is also a need for comprehensive epigenetic profiling across a wider range of stages of the tumorigenic process. If a gene(s) is subject to silencing in the normal ovary or in peripheral blood there are obvious implications as to whether it can be used as part of a diagnostic screen.

In combination with genetic changes, it is clear that a distinct set of epigenetic changes underlie ovarian cancer initiation and development. Identifying the methylation signature of ovarian cancer cells will likely lead to a greater understanding of the molecular pathways causing ovarian cancer progression [83]. Precise functional and genetic studies will be necessary to determine which epigenetic events are critical to tumorigenesis and thus have biological consequences offering selective clonal advantage, as compared to "bystander" genes that are methylated and selected during tumor development, perhaps due to epigenetic silencing of large chromosomal regions containing tumor suppressor genes [17], despite having no immediate effect on tumor phenotype [85].

Conclusions

The identification of epigenetic changes in ovarian cancer that correlate with high specificity and sensitivity to the clinicopathological features of the disease and patient outcome may provide new markers of clinical benefit. There is now accumulating evidence that epigenetic biomarkers offer great potential in the detection of cancer in its earliest stages, and to accurately assess individual risk. As more methylated genes are identified algorithms could be developed to score the specificity of a particular gene hypermethylation panel for detection of ovarian cancer compared with other cancer types. Moreover, epigenetically silenced cancer genes offer new targets for therapeutic approaches based on re-expression of tumor suppressor genes via demethylation and deacetylating drugs [112]. The next decade will determine whether the promise of epigenetic markers holds true.

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