# Epigenetic Markers of Ovarian Cancer

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**Abstract.** In addition to genetic alterations, epigenetic DNA modifications regulate altered gene expression in cancer. In particular, genome-wide demethylation of normally methylated and silenced chromosomal regions, and hypermethylation and silencing of tumor suppressor genes are a common feature of cancer cells. The detection of cancer-specific methylation changes holds much promise in cancer diagnosis, prognosis, and therapeutic responsiveness. In this review, we outline the epigenetic changes identified in ovarian cancer, focusing on their potential as clinical markers for early detection and monitoring of disease progression.

## 1 Introduction

Ovarian cancer is the fourth leading cause of cancer death in women and has the highest mortality rate of the reproductive cancers (Jemal et al. 2005). 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 20% (Barnholtz-Sloan et al. 2003). In the absence of an early detection test, improved therapies for advanced disease are critical to improving the survival for women with ovarian cancer. Most patients 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. A better understanding of the molecular pathogenesis underlying ovarian cancer is key to identifying markers for early detection and novel therapeutics.

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 (Wolffe and Matzke 1999), 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; and chromatin remodeling via histone protein acetylation and methylation (Jones and Baylin 2002). The human genome is not methylated uniformly, containing regions of unmethylated segments interspersed with methylated regions (Bird 1986). 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 5kb that contain clusters of CpG dinucleotides called CpG islands (Gardiner-Garden and Frommer 1987). These CpG-rich regions are often located in the 5' region of genes and are associated with the promoters of genes. 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 (Clark and Melki 2002). The relationship between DNA methylation and post-translational modification of histones appears to be complex but they collectively result in transcriptional silencing through effects on transcription factor binding and repression of gene expression in normal cellular processes (Jones and Baylin 2002; Lund and van Lohuizen 2004; Stirzaker et al. 2004).

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 (Ehrlich 2002) as well as hypermethylation of CpG islands (Bird 1996; Egger et al. 2004; Esteller 2002; Herman and Baylin 2003; Jones and Laird 1999). 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 it originated (Feinberg and Tycko 2004; Walsh et al. 1998). Hypomethylation has been hypothesized to contribute to oncogenesis by transcriptional activation of oncogenes, activation of latent transposons, or by chromosome instability (Alves et al. 1996; Costello and Plass 2001; Eden et al. 2003; Gaudet et al. 2003; Tuck-Muller et al. 2000). At the same time, aberrant CpG island DNA methylation and histone modification, leading to transcriptional activation and gene silencing, is a common phenomenon in human cancer cells and an early event in carcinogenesis (Jones and Baylin 2002). In particular, hypermethylation of CpG islands in gene promoter regions is a frequent mechanism of inactivation of tumor suppressor genes (Ehrlich 2002; Herman and Baylin 2003; Jones and Baylin 2002; Jones and Laird 1999), and has been proposed as one of the two hits in Knudson's two hit hypothesis for oncogenic transformation (Jones and Laird 1999).

Epigenetic alterations, including CpG island DNA methylation, occur in ovarian cancer (Ahluwalia et al. 2001; Strathdee et al. 2001) and the identification of specific genes that are altered by these epigenetic events is an area of intense research. Although there are CpG islands that become methylated in multiple tumor types, differential patterns of methylation of specific genes can vary amongst neoplasms (Grady 2005), with certain CpG islands only methylated in specific tumor types (Costello et al. 2000; Esteller and Herman 2002; Melki and Clark 2002; Melki et al. 1999). Neoplasm-specific events may be useful as molecular biomarkers for early detection and prognostic significance. Large scale screening to identify ovarian cancer-specific epigenetic fingerprints is underway.

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

## 2 Epigenetically Regulated Genes in Ovarian Cancer

Most studies to date have focused on candidate gene approaches to identify epigenetically regulated genes in ovarian cancer, in particular methylated and silenced candidate tumor suppressor genes. Selected targets include genes with downregulated expression in ovarian cancer, genes in regions with known loss of heterozygosity (LOH) in ovarian cancer and thus where tumor suppressors likely reside; and genes that have been shown to be epigenetically regulated in other cancers. There is also a small but significant literature on hypomethylated genes in ovarian cancer. Although in their infancy, genome-wide array-based approaches to epigenetically regulated gene discovery are also beginning to emerge.

### 2.1 Hypermethylated and Silenced Genes

Epigenetic regulation of BRCA1 (breast cancer susceptibility gene 1) has been studied extensively due to its known role in inherited forms of ovarian cancer. *BRCA1*, a breast and ovarian cancer susceptibility gene, is involved in the maintenance of genome integrity. Carriers of *BRCA1* germline mutations develop predominantly breast and ovarian tumors. *BRCA1* promoter methylation only occurs in breast and ovarian cancers (Esteller et al. 2001; Esteller et al. 2000) and mirrors the classical genetic mutation studies of familial cancers. Studies investigating *BRCA1* hypermethylation report methylation in 5-24% of epithelial ovarian cancers (Baldwin et al. 2000; Buller et al. 2002; Catteau et al. 1999; Geisler et al. 2002; Ibanez de Caceres et al. 2004; Rathi et al. 2002; Strathdee et al. 2001; Teodoridis et al. 2005) in association with loss of BRCA1 expression (Baldwin et al. 2000; Chan et al. 2002; Wilcox et al. 2005). No correlation of methylation with histological subtypes or grade or stage has been found (Wilcox et al. 2005); however, *BRCA1* silencing is detectable in early (stage 1A) tumors (Wilcox et al. 2005). LOH at the *BRCA1* locus occurs in a significant proportion of sporadic ovarian cancers (Esteller et al. 2000; Futreal et al. 1994); moreover, *BRCA1* hypermethylation is predominantly detected in cancers that exhibit LOH at the *BRCA1* locus (Chan et al. 2002; Esteller et al. 2000). Thus, silencing of *BRCA1* expression by methylation likely acts as the second hit required for tumor suppressor gene inactivation in Knudson's 2 hit hypothesis (Jones and Laird 1999).

Several other genes located at regions of LOH are methylated and silenced in ovarian cancer. *ARHI* (Ras homologue member 1), a maternally imprinted tumor suppressor of the ras superfamily expressed monoallelically from the paternal allele, maps to chromosome 1p31 which is associated with LOH in 40% of ovarian carcinomas. AHRI expression is lost in ovarian cancers (Yu et al. 1999; Yuan et al. 2003) compared to normal ovarian tissue including the ovarian surface epithelium (OSE).

The remaining paternal allele is silenced by methylation in 10-15% of cases (Yu et al. 2005).

*DLEC1* (deleted in lung and esophageal cancer 1) is located on 3p22.3 in a region of frequent LOH in cancer, and is a putative tumor suppressor in lung and other cancers. DLEC1 expression is downregulated in ovarian cancer cell lines and primary invasive epithelial ovarian cancers, where its expression is correlated with hypermethylation of the *DLEC1* promoter (Kwong et al. 2006).

p16 *(CDKN2A)* encodes a cyclin-dependant kinase inhibitor involved in the regulation of the cell cycle. p16 expression is frequently disrupted in cancer. The p16 locus at chromosome 9p21 is located in a region of LOH documented in a wide variety of cancers, including ovarian carcinoma (Campbell et al. 1995; Schultz et al. 1995). Hypermethylation of the *p16* promoter region is important in a subset of human carcinomas including lung, head and neck and pancreatic cancer (Esteller et al. 2001; Furonaka et al. 2004; Wong et al. 2003), but there is conflicting evidence as to whether *p16* methylation plays a role in ovarian carcinogenesis (Brown et al. 2001; Hashiguchi et al. 2001; Katsaros et al. 2004; Makarla et al. 2005; Niederacher et al. 1999; Rathi et al. 2002; Strathdee et al. 2001; Teodoridis et al. 2005).

*OPCML*, located at 11q25, is hypermethylated in 33-83% of epithelial ovarian cancers (Sellar et al. 2003; Teodoridis et al. 2005; Zhang et al. 2006). *SFRP1*, a Wnt antagonist located at chromosome 8p11.2, is methylated in 5-12% of primary ovarian cancers (Takada et al. 2004; Teodoridis et al. 2005). *MYO18B*, located at 22q121.1, is methylated and silenced in primary ovarian cancer (Yanaihara et al. 2004). The TRAIL receptor *DR4*, located at 8p21.1, is methylated in 28% of ovarian cancers and is associated with loss of expression (Horak et al. 2005). Each of these genes are located in chromosomal regions associated with LOH in ovarian cancer.

Certain epigenetically-regulated genes postulated to act as tumor suppressors in other carcinomas are also methylated and transcriptionally silenced in ovarian cancer. For example, *de novo* methylation and inactivation of the ras homologue *RASSF1A* tumor suppressor gene is one of the most frequently detected epigenetic events in human cancer (Pfeifer et al. 2002). *RASSF1A* is methylated and silenced in 10-50% of primary ovarian cancers (Agathanggelou et al. 2001; Ibanez de Caceres et al. 2004; Rathi et al. 2002; Teodoridis et al. 2005; Yoon et al. 2001). Hypermethylation and loss of expression of insulin-like growth factor binding protein 3 (IGFBP3), a member of the IGFBP family which regulates mitogenic and apoptotic effects of insulin-like growth factors, occurs in non-small cell lung cancer and hepatocellular carcinoma (Chang et al. 2002; Hanafusa et al. 2002). *IGFBP3* promoter methylation is detected in 44% of samples from epithelial ovarian cancer patients (Wiley et al. 2006). *ARLTS1*, a tumor suppressor previously described as a low penetrance cancer gene, shows downregulated expression in ovarian carcinomas due to DNA methylation in its promoter region (Petrocca et al. 2006).

Gene methylation patterns can also represent molecular characterizations of pathological and clinical features of ovarian carcinomas. For example, 14-3-3sigma (*SFN*), an inhibitor of cell cycle progression that is epigenetically deregulated in other cancers (Akahira et al. 2004b; Mhawech et al. 2005), is methylated at a higher frequency in ovarian clear cell carcinomas than in other histological types of ovarian cancer (Kaneuchi et al. 2004). Similarly, aberrant methylation of *TMS1* (target of

methylation-induced silencing) and the *WT1* (Wilms tumor suppressor 1 gene) sense and antisense promoters is more frequent in clear cell ovarian tumors than in other histological types (Akahira et al. 2004a; Kaneuchi et al. 2005; Teodoridis et al. 2005; Terasawa et al. 2004). 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 (Makarla et al. 2005).

There are numerous other genes that exhibit promoter methylation and decreased expression in ovarian cancer. For example, loss of expression of TCEAL7 (Bex4) in primary cancers correlates with methylation of CpG sites in the promoter (Chien et al. 2005). Decreased levels of FANCF expression found in most ovarian cancers is in part due to promoter hypermethylation (28%) (Wang et al. 2006). Promoter methylation of $p33^{ING1b}$, the inhibitor of growth *1b* gene, is found in 24% of ovarian cancers and is significantly correlated with loss of mRNA expression (Shen et al. 2005). The CpG islands of the suppressor of cytokine signaling genes *SOCS1* and *SOCS2* are hypermethylated in 23% and 14% primary ovarian cancers respectively (Sutherland et al. 2004). *TCF2*, which encodes the transcription factor HNF1β, is methylated in 26% of primary ovarian cancers (Terasawa et al. 2006). Finally, methylation of *MLH1* (6-13%) (Helleman et al. 2006; Strathdee et al. 2001; Strathdee et al. 1999; Teodoridis et al. 2005), *HIC1* (16-35%) (Rathi et al. 2002; Strathdee et al. 2001; Teodoridis et al. 2005), *hTR* (24%) (Strathdee et al. 2001), *p73* (10%) (Strathdee et al. 2001) and *MINT25* (12-16%) (Strathdee et al. 2001; Teodoridis et al. 2005) have been reported in primary ovarian cancers.

## 2.2 Chromatin Modifications Influencing Gene Expression

Histone modification leads to changes in chromatin structure and results in alteration of transcriptional activity of a gene locus (Berger 2002; Gregory et al. 2001; Rice and Allis 2001). Multiple acetylations at both histone H3 and H4 subunits are associated with transcriptionally active sequences and a lack of histone acetylation (hypoacetylation) correlates with transcriptional silencing (Rice and Allis 2001). The removal of acetyl groups can lead to chromatin condensation and result in repression of transcription. Additionally, the unmodified histone lysine residues can be mono-, di- or tri-methylated. Histone H3 di- or tri-methylation at lysine K4 is also associated with active transcription (Rice and Allis 2001). In contrast, histone H3 di- and tri-methylation at lysine K9 are enriched in transcriptionally silenced, densely packed, heterochromatin and are thus associated with gene silencing (Fischle et al. 2003; Lachner et al. 2001; Rice and Allis 2001).

The emergence of chromatin immunoprecipitation (ChIP) analysis as a method to identify histone modifications has aided the identification of genes silenced by chromatin remodeling in ovarian cancer. For example, there is an enhanced association between acetylated histone H3 and H4 and the *DLEC1* promoter in cells that have lost DLEC1 expression (Kwong et al. 2006), indicating that histone hypoacetylation is used to suppress DLEC1 expression in ovarian cancers. Acetylation and methylation of chromatin in the promoter region of *ARHI* is associated with loss of expression in ovarian cancer cells (Yu et al. 2003). *GATA4* and *GATA6* gene silenc-

ing, via an alteration of chromatin conformation, correlates with hypoacetylation of histones H3 and H4 and loss of histone H3 lysine K4 tri-methylation at their promoters in ovarian cancer cell lines (Caslini et al. 2006). In contrast to *DLEC1* where gene silencing is also associated with DNA hypermethylation (Kwong et al. 2006), *GATA4* and *GATA6* silencing is independent of promoter methylation (Caslini et al. 2006).

In addition to gene silencing, increased histone acetylation can lead to re-expression of genes in ovarian cancer cells. For example, high histone H3 acetylation and an open chromatin conformation, in addition to reduced DNA methylation, are important in claudin-4 (CLDN4) overexpression in ovarian cancer (Hibbs et al. 2004; Honda et al. 2006; Hough et al. 2000; Lu et al. 2004; Rangel et al. 2003; Zhu et al. 2006).

## 2.3 Hypomethylated Genes

Global DNA hypomethylation increases with malignancy in ovarian epithelial neoplasms (Cheng et al. 1997). There are, however, limited examples of specific gene activation by hypomethylation in ovarian cancer. Demethylation of the maspin (*SERPINB5*) promoter in ovarian cancer cells is associated with a gain of maspin mRNA expression (Rose et al. 2006). Demethylation is also important in the abnormal expression of the metastasis-related gene synuclein-γ *(SNCG)*, a member of a family of small cytoplasmic proteins. Synuclein-γ is not normally expressed in OSE due to dense methylation in the promoter region but is hypomethylated and re-expressed in aggressive ovarian cancer cell lines (Gupta et al. 2003) and in a substantial proportion of malignant ovarian carcinoma samples (Czekierdowski et al. 2006).

Similarly, little is known about activation of latent retrotransposons, though hypomethylation of the CpG dinucleotides associated with the L1 and HERV-W retrotransposons occurs in malignant relative to non-malignant ovarian tissue consistent with an elevation in expression levels (Menendez et al. 2004).

Hypomethylation and rearrangements in heterochromatin in the vicinity of the centromeres of chromosomes 1 and 16 are frequent in many types of cancer, including ovarian epithelial carcinomas (Narayan et al. 1998). Satellite 2 (Sat2) DNA is the main sequence in the heterochromatin region adjacent to the centromere of these chromosomes. In all normal tissues, Sat 2 DNA is highly methylated but in ovarian carcinomas there is significantly more hypomethylation in Sat2 DNA sequences in the juxtacentromeric heterochromatin of chromosome 1 (Chr1 Sat2) and chromosome 16 compared with borderline (LMP) ovarian tumors and cystadenomas (Qu et al. 1999). Thus degree of malignancy significantly correlates with the extent of Sat2 DNA hypermethylation (Qu et al. 1999). In addition, the study by Widschwendter *et al.* (2004) of 115 ovarian cancers and 26 non-neoplastic ovarian specimens demonstrated 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. Advanced stage of disease and tumor grade were associated significantly with frequent hypomethylation of Chr1 Sat2 or Chr1 Satα; and serous and endometriod ovarian cancers had significantly higher hypomethylation levels than LMP or mucinous tumors (Widschwendter et al. 2004a). Finally, 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 (Nishiyama et al. 2005).

## 3 Clinical Epigenetic Markers

Alterations in epigenetic patterns, including changes in DNA methylation, have several advantages as a means to detect and classify cancer: 1) methylation analysis utilizes DNA, a more chemically stable molecule than RNA or protein; 2) aberrant DNA methylation is a 'positive' signal that can be detected 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) (Herman et al. 1996) and quantitative MSP (Laird 2003) including the fluorescence-based real-time PCR-based MethyLight technique (Eads et al. 2000) and headloop suppression PCR, which allows the detection of a single methylated allele in 10 000 unmethylated alleles (Rand et al. 2005). These assays have sufficient signal-to-noise ratio and throughput capacity to sensitively analyze a broad spectrum of markers and thus may be useful in the clinical setting (Muller et al. 2004; Muller et al. 2003; Widschwendter et al. 2004b); 3) 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; 4) aberrant methylation is frequently observed in early cancer development, and hence has applicability to the detection of early stage disease; and 5) methylation biomarkers are present in patient serum/plasma and other bodily fluids (Cottrell and Laird 2003), and hence may have application as the basis of non-invasive detection tests. To this end, a number of studies have shown the feasibility of detecting hypermethylation of multiple genes in circulating DNA from patients with a broad spectrum of tumors (Hoque et al. 2004; Hoque et al. 2006; Leung et al. 2005).

### 3.1 Prognostic Markers

Several epigenetically regulated genes have been assessed for their positive prognostic potential in ovarian cancer. For example, *IGFBP-3* methylation is associated with disease progression and death in ovarian cancer, particularly in patients with early-stage disease, where methylation was associated with a 3-fold higher risk of disease progression and a 4-fold higher risk of death (Wiley et al. 2006). Similarly, hypermethylation of 18S and 28S rDNA is associated with prolonged progression-free survival of ovarian cancer patients (Chan et al. 2005). 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 (Widschwendter et al. 2004a). However, the small sample sizes used in these studies require these results to be confirmed by 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 (Huang et al. 1999), Wei *et al*. (2002) could stratify late-stage ovarian tumors into 2 distinct groups with significantly different outcome based on methylation profiling of 956 CpG island-containing loci (Wei et al. 2002). 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 (Wei et al. 2006). Hence, although in its infancy, the identification of a prognostic panel of hypermethylated DNA markers for ovarian cancer remains a realistic possibility.

## 3.2 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. Epigenetic gene regulation plays a prominent role in both intrinsic and acquired drug resistance in cancer (Balch et al. 2004), 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 (Teodoridis et al. 2004). For example, Teodoridis *et al*. (2005) 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 (Teodoridis et al. 2005).

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 common patterns of CpG island methylation can be identified as being selected for by chemotherapy *in vitro* (Wei et al. 2003). There is an increasing volume of evidence from clinical studies that supports this hypothesis. In the study by Wei *et al*. (2002) discussed in Section 3.1 above, patients stratified as having a short progression-free survival (with a high degree of CpG island methylation) have 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 more readily acquire resistance to chemotherapy.

Silencing of *hMLH1*, a DNA mismatch repair gene, by hypermethylation of its promoter CpG island (Strathdee et al. 2001) has been linked with acquired resistance to platinum-based drugs in ovarian cell line models (Brown et al. 1997; Plumb et al. 2000; Strathdee et al. 1999). 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, consistent with acquisition of methylation after che-

motherapy. Moreover, acquisition of *MLH1* methylation at relapse predicts poor overall patient survival, and is associated with drug resistance (Gifford et al. 2004).

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 (Taniguchi et al. 2003). 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 (Taniguchi et al. 2003). Methylation of *FANCF* has been observed in primary ovarian cancers (Taniguchi et al. 2003) but its relevance to clinical outcome following chemotherapy has yet to be established.

Although no functional role has yet been assigned, methylation-controlled DNAJ (*MCJ*) was identified as a gene that rendered epithelial cells more sensitive to cisplatin and paclitaxel, the mainstay of chemotherapy for ovarian cancer patients (Shridhar et al. 2001). Unusually for a CpG island-associated gene, cell type specific DNA methylation and gene silencing of *MCJ* are observed in normal cells, including OSE (Strathdee et al. 2004). 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 (Strathdee et al. 2004; Strathdee et al. 2005). 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 (Gifford et al. 2004; Teodoridis et al. 2004).

### 3.3 Early Diagnostic Markers

Early diagnosis is critical for the successful treatment of many types of cancer, including ovarian cancer. The detection of cancer at early stages by noninvasive methods may be aided by the identification of cancer-specific biomarkers detectable in bodily fluids. It has been known for many years that tumors appear to "shed" DNA into the circulation (Hickey et al. 1999). Moreover, specific methylated DNA markers can be detected in the serum/plasma and peritoneal fluid of ovarian cancer patients (Ibanez de Caceres et al. 2004). 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 (Lee et al. 1994; Song et al. 2002), no single gene in ovarian cancer has been identified as being methylated in more than a relatively small proportion of cancers. 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.

The detection of *RASSF1A* methylation in bodily fluids promises to be a useful marker for early cancer detection (Pfeifer et al. 2002). In a recent feasibility study, tumor-specific hypermethylation of at least one of a panel of six tumor suppressor gene promoters, including *RASSF1A*, *BRCA1*, *APC*, *p14*, *p16* and *DAPK* could be detected in the serum or plasma of ovarian cancer patients with 100% specificity and 82% sensitivity (Ibanez de Caceres et al. 2004), including 13/17 cases of stage I 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 (Ibanez de Caceres et al. 2004). 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 (Hickey et al. 1999).

Although several limitations still exist, including the sensitivity of methylation assays relative to the amount of circulating tumor DNA, in principal, detection of specific epigenetic markers in the circulation of patients appears a promising candidate for the detection of early stage ovarian cancer.

## 4 Conclusions

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, DNA integrity, and assay platform and design. 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 is critical in determining the true methylation frequency of a given chromosomal region.

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 have been examined. It is likely that a shift from candidate gene to genome-wide array-based approaches will aid in the discovery of methylated genes (Ahluwalia et al. 2001; Horak et al. 2005; Ushijima 2005). 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 can-

cer, which will in part be aided by the establishment of the Human Epigenomic Project (Jones and Martienssen 2005).

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 (Wei et al. 2006). Precise functional and genetic studies will be necessary to determine which epigenetic events are critical to tumorigenesis and thus have biological consequences, 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 (Frigola et al. 2006), despite having no immediate effect on tumor phenotype (Teodoridis et al. 2004).

Finally, the identification of epigenetic changes that correlate with clinicopathological parameters 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. Moreover, epigenetically silenced cancer genes offer new targets for therapeutic approaches based on re-expression of tumor suppressor genes via demethylation and deacetylating drugs (Yoo and Jones 2006). The next decade will determine whether the promise of epigenetic markers holds true.

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