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Epigenetics in ovarian cancer

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Abstract

Ovarian cancer is a disease with a poor prognosis and little progress has been made to improve treatment. It is now recognized that there are several histotypes of ovarian cancer, each with distinct epidemiologic and genomic characteristics. Cancer therapy is moving beyond classical chemotherapy to include epigenetic approaches. Epigenetics is the dynamic regulation of gene expression by DNA methylation and histone post translational modification in response to environmental cues. Improvement in technology to study DNA methylation has enabled a more agnostic approach and, with larger samples sets, has begun to unravel how epigenetics contributes to the etiology, response to chemotherapy and prognosis in of ovarian cancer. Investigations into histone modifications in ovarian cancer are more nascent. Much more is needed to be done to fully realize the potential that epigenetics holds for ovarian cancer clinical care.

Keywords

Ovarian cancer; DNA methylation; Chromatin

1. Introduction

1.1. Ovarian cancer

Epithelial ovarian cancer (EOC) continues to confer a poor prognosis; diagnosis at a late stage and the high rate of resistance to the standard chemotherapy regimens contributing greatly to the mortality. EOC consists of several distinct diseases defined based on distinct histotypes with distinct genomic and epigenomic characteristics. High grade serous ovarian cancer (HGSOC) is the most common and one of the most aggressive histotypes, comprising some 70% of newly diagnosed cases. Endometrioid (ENDOC, 15%), clear cell carcinoma (CCOC, 12%), mucinous (MOC, 3%), low grade serous (LGSOC, < 10%) and are less frequently diagnosed histotypes. There are two histotypes of low malignant potential borderline disease (serous and mucinous) [1]. In addition to genomic characteristics, these EOC histotypes have different epidemiologic risk factors [2–4], expression signatures [5–7], clinical responses to therapy [8] and distinct precursor sites. HGSOC appears to originate from fallopian tube or ovarian surface epithelium [9], shows few mutational events compared with other histotypes [10] and generally have gross chromosomal instability

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exhibiting copy number variations [11,12]. LGSOC are considered to arise from borderline tumors [13] and commonly possess mutations in the *Ras* family of genes (*KRAS*, *BRAF*, *NRAS*, *ERBB2*, and *PTEN*) and are chromosomally stable [14]. EOC and CCOC are thought to emanate from endometrium or endometriosis and MOC from endocervix or intestinal mucosa [15]. The rarer histotypes, CCOC and MOC, also have distinct clinical, pathological and genetic characteristics [3,16–20]. ENDOC bears some similarity to CCOC, but unlike CCOC, can be high grade or low grade. High grade ENDOC shares some clinical features of HGSOC, while the low grade ENDOC are similar to CCOC. The work of many has led to identification of genetic differences between the different histotypes [21–27], and epigenetic changes are beginning to be characterized.

1.2. Epigenetics

Epigenetic regulatory elements are comprised of post-translationally modified cytosines, non-coding RNAs, and histone modifications [28]. These effects do not change the underlying DNA sequence, but are heritable and regulate the means by which the genome and environment interact [29]. DNA methylation (methyl CpG) is the most commonly studied epigenetic modification, whereby a methyl group is attached in the C⁵ position of cytosines (5mC), usually in a cytosine followed by a guanine (CpG) context. One percent of human genome is methylated and 5mC are spread throughout the genome with some aggregating into longer stretches of CpGs identified as CpG islands [30]. DNA methylation is necessary to silence retroviral elements that make up 5–8% of the human genome [31]. DNA methylation also plays a critical role in imprinting, cell differentiation during development and resultant phenotypic variability [32–34]. With advent of new sequencing methods, methylation of other nucleotide dyads are being described in mammalian genomes [35,36], widening the scope of epigenetic knowledge. DNA methylation may be associated with decreased expression of a gene; however, this does not directly imply a causal relationship. Showing an association between gene expression and DNA methylation is one means of inferring a possible functional consequence, however the application of methods such as CRISPR-based approaches in epigenome-editing offer a means to establish a causal function [37].

DNA methylation may be increased (hypermethylated) or decreased (hypomethylation) in disease settings compared to normal tissue. Hypermethylation of tumor suppressor gene promoters may lead to gene silencing and inactivation of pathways such as DNA mismatch repair in cancers with hypermethylation of *MLH1* [38]. Extensive hypermethylation, a CpG island methylator phenotype (CIMP), has been documented in multiple cancers [39]. Alternatively, hypomethylation may lead to expression of normally silenced oncogenes. There is also evidence that methylation within the gene body, counter to previous thought, may also regulate gene expression [40]; gene body hypomethylation also exists [41]. In EOC, as in cancer generally, global hypomethylation is seen across all histotypes and is associated with increasing stage, grade and mortality [33,42,43]. Hypermethylation on the other hand differs across the EOC histotypes. Aberrant expression of repeat elements, as well as oncogenes, is the result of hypomethylation, while hypermethylation silences genes that may regulate critical functions. Heterogeneity in DNA methylation underscores the

complexity of genomic underpinnings in cancer development in which mutations, structural aberrations and epigenetic dysfunction all play a role.

Histones play a fundamental role in chromatin organization, and along with DNA methylation, are an active area of research in cancer [44]. Histones are the most abundant of proteins bound to DNA, regulating gene expression and influencing how DNA is packaged around the nucleosome. Histone post-translational modifications include acetylation, methylation, phosphorylation, sumoylation, deamination, ADP ribosylation, proline isomerization, ubiquitination all playing key roles in regulation of chromatin function. Histone acetylation of lysines is generally associated with euchromatin, a gene transcription-ready state, as are H3K4 mono-di and tri-methylation, mono methylation in H3K27, H3K79, H4K20 and H3BK5. Heterochromatin, on the other hand, is generally not permissive and contains repressive histone marks (H3K27me₂, me₃; H3K9me₂, me₃). Bivalent marks, “poised”, contain both repressive (e.g. H3K27me₃) and active (e.g. H3K4me₃) marks, are commonly encountered in embryonic stem cells [45].

Histone acetylation is dynamic with histone acetyltransferases (HATs) and deacetyltransferases (HDACs) playing a role [46]. HDACs, which are aberrantly expressed in a number of cancers including ovarian cancer [47], are comprised of eighteen proteins grouped into four classes; classes I, II and IV HDACs are zinc dependent enzymes, while class III are NAD⁺ dependent [48]. Class III HDACs include the sirtuin enzymes, products of the *SIRT1-SIRT7* gene family [49]. Class I HDAC enzymes are expressed in all cell types, while class II enzymes show tissue-specific expression. Bromodomain and extra-terminal domain (BET) proteins (BRD2, BRD3, BRD4 and testis-specific BRDT proteins) act as “readers” of the histone marks by binding to the acetylated lysine on histone tails to promote transcription [50]. This review will examine DNA methylation changes in EOC considering the different histotypes, histone modifications and application of epigenetics in the clinical setting.

2. DNA methylation analyses in EOC

DNA methylation analyses have either focused on the methylation state of specific sets of genes or have assessed genome-wide DNA methylation comparing EOC histotypes or cancer and normal ovarian tissue. These studies vary widely in the number of samples analyzed, consideration of histotypes, and analysis methods.

2.1. Targeted analyses

Historically, early efforts to analyze DNA methylation in EOC were targeted to genes commonly mutated in cancer such as genes involved in DNA repair, cell cycle and growth regulation (*BRCA1*, *PTEN*, *CDKN2A*, *MLH1*, *RASSF1A*, *CDH1* and others) [51–64]. Fig. 1 shows the current estimates for frequency of histotype specific DNA methylation for the above set of genes. DNA methylation of candidate genes is most commonly studied and reported in HGSOC; whereas the small number of studies including rarer histotypes highlights the difficulty in acquiring large enough sample numbers. The majority of analyses used methylation-specific PCR (MSP), which evaluates the presence of a PCR fragment amplified with primers that target the preserved methylated cytosines in bisulfite modified DNA. The presence of a methylated fragment is typically reported as a frequency of

methylated samples per study. Frequencies of methylation vary widely between studies, due in part to small numbers of samples when considering grade or histotypes. Thus, determining whether DNA methylation of tumor suppressor genes is common in EOC from these studies is quite difficult.

2.2. Genome-wide analyses

Methods genome-wide DNA methylation assessment came into use over a decade ago in EOC studies. Using an early array-based method three groups profiled 1505 CpGs (Illumina GoldenGate) [65–67] considering tumor grade and histotype in their analyses (Table 1). Several studies used normal ovarian or fallopian tissue as the control [11,12,66,68–70] while the other studies used hierarchical clustering to discern the methylation patterns within EOC cases [20,65,67], making comparison between analyses difficult. However, significant histotypespecific methylation was noted both between histotypes and when compared to a normal tissue. HGSOC, while showing some hypermethylation, is more hypomethylated than ENDOC or CCOC. In addition, HGSOC appeared distinct from LGSOC based on methylation patterns [67]. Importantly, Houshdaran et al. reported that commonly used EOC cell lines formed a separate cluster from EOC tissue, and there was no association between cell lines and any reported histotype lineage. Notably, following gene expression analysis revealed only a minority of methylated genes to have decreased expression [65].

Higher resolution methylation arrays: Illumina's Human Methylation 27 K, Human Methylation 450 K, and custom microarrays, have been utilized in the past seven years in studies with larger sample numbers [11,12,20,69–71] (Table 1). The Cancer Genome Atlas (TCGA), collaboration between the National Cancer Institute and National Human Genome Research Institute, has profiled the genomes of 33 types of cancer, including ovarian cancer. TCGA EOC study included mostly HGSOC, while the other large studies included additional histotypes. EOC methylation was compared with normal fallopian tube DNA methylation in four studies [11,12,69,70] while one compared methylation differences between the histotypes [20]. TCGA identified 168 genes with altered gene expression in HGSOC compared to normal fallopian tube, with 11.5% hypermethylated at *BRCA1*, similar to frequencies noted in HGSOC in the targeted analyses [12]. Four methylation subtypes were revealed using clustering analysis, but were not as stable as gene expression clustering subtypes identified in EOC. In another analysis of HGSOC [11], 543 hypermethylated genes with decreased gene expression were reported, and five of those genes overlapped with those identified in the TCGA dataset. Differences in statistical analysis techniques may account for the relatively low consistency of results across studies [72]. Sánchez-Vega et al. conducted a study comparing DNA methylation between both HGSOC and ENDOC and normal fallopian tissue. Of the 12 CpG loci that were differentially methylated in both HGSOC and ENDOC compared to normal tissue all but one CpG had reduced methylation in tumors compared to fallopian tube DNA [70]. Hypermethylation was noted in a CpG island downstream of the transcription start site for *ZNF154*; however not all ENDOC in this analysis showed hypermethylation at *ZNF154* with some ENDOC cases having a low-intensity methylator phenotype, as also shown in TCGA study. Seven of the 12 loci that were found to be differentially methylated in the majority of cancer types in the TCGA, where not found by Sánchez-Vega et al. possibly to the relatively

small number of samples in the original study [70]. Overall, ENDOC appears to have a significantly different methylation profile compared to HGSOC [69,70], with most having a CIMP phenotype [73].

A CCOC-specific methylation profile was identified in one study [20]. What is notable in CCOC genome-wide studies is the paucity of tumor suppressor gene hypermethylation. CCOC gene expression studies support a specific dysregulation of genes that are involved in oxidative stress response, including *HNFI1B* and *VCAN* [17], supporting a role for microenvironment in tumor development. Interestingly, while *HNFI1B* is overexpressed in CCOC, it is methylated in ~50% of HGSOC [12,19]. A variant in *HNFI1B* was identified as an HGSOC susceptibility locus [74] and recently Ross-Adams et al. [75] reported that the susceptibility allele was associated with *HNFI1B* promoter methylation. This methylation, upstream of the transcription start site, also bears hallmarks of poised and active enhancers (H3K27Ac, H3K4me1 and H3K3Me3 histone marks). *HNFI1B* plays a critical role in the epithelial-mesenchymal transition (EMT), in which cells lose cellular adhesion and polarity acquiring an invasive phenotype [76]. EMT properties are also seen in cells under stress imbued by tumor microenvironment resulting in methylation dysregulation potentially contributing to a tumor promoting role. Thus, unmethylated *HNFI1B* appears to act as an oncogene in CCOC, but when hypermethylated, acts like a tumor suppressor in the more aggressive HGSOC histotype [75]. This study provides an excellent example the complexity of genomic and epigenetics in EOC.

These studies demonstrate the growing consensus that the epigenome is altered in EOC. Both CCOC and ENDOC are more likely to have a hypermethylated phenotype compared with HSGOC which are overall hypomethylated and has fewer hypermethylation events. CCOC and ENDOC are thought to arise from endometrial epithelium and endometriosis, suggesting that inflammation may play a role.

3. Chromatin alterations in ovarian cancer

Regulation of gene expression is closely related to the chromatin state. Mutations in genes involved in chromatin remodeling (*ARID1A*, *SPOP*, *KMT2D*) have been reported in CCOC [77,78], implicating aberrant chromatin remodeling in this histotype. Assessing the chromatin state is classically achieved through the histone marks described earlier. These histone post-translational modifications and HDAC expression have been evaluated in EOC using immunohistochemistry (IHC) and next generation sequencing (NGS) approaches (Table 2). SIRT1, a class II HADC, was found to have higher expression in invasive serous EOC, compared to benign or borderline cases, and was higher in serous compared with mucinous tumors [79]. IHC-evaluated reduced expression of the repressive histone mark H3K27me3 was noted in 55% of EOC cases, 33% of borderline OC cases and cystadenomas, and 16% of normal ovary tissue. A decrease in H3K27me3 expression was associated with increasing stage, but not grade, patient, age, or histotype [80].

Three studies used NGS approaches to examine histone marks. Two EOC cell lines developed from an HGSOC patient tumor ascites, defined as pre-transformed (A4 clone) and transformed (A4T, demonstrating expression of markers associated with a progenitor state)

[81], were profiled for DNA methylation, histone marks, and gene expression [82]. Comparing A4 with LGSOC and A4T with TCGA HGSOC samples, a modest overlap of shared genes was noted. This may be due to the small number of LGSOC cases in TCGA or the definition of A4 cell line as pre-transformed EOC. Chapman-Rothe et al. [83] profiled a single primary HGSOC case for H3K27me3 (active) and H3K4me3 (repressive) marks and examined the histone-marked gene sets in two gene expression profiles sets, eight benign ovarian lesions, and the TCGA dataset of eight fallopian tube samples and 499 HGSOC. Bivalent (presence of both active and repressive marks) and H3K27me3 marked genes were associated with significantly lower expression in the HGSOC compared to eight benign serous ovarian lesions. Of the 580 bivalent marks in the HGSOC, 215 are similarly bivalent in embryonic stem cells, but 365 appear to be tumor-specific. These bivalent genes were enriched for the PI3K and TGF- β pathways. Methylation of H3K27 is mediated by the polycomb repressor complex 2 (*PRC2*) [84] and expression of *PRC2*-complex genes (*EZH2*, *SUZ12*, *EED*, *RBBP7*) were negatively correlated with H3K27me3, supporting H3K27 methylation role in mediating gene silencing in HGSOC. Another study examined DNA methylation and gene expression in a cisplatin-resistant ovarian cancer cell line with an altered H3K27 protein, in which the H3 lysine was mutated to arginine and thus not modifiable by methylation [82,85]. Loss of DNA methylation and altered gene expression was observed in this cell line as well as a sensitization to cisplatin. Tumor suppressor genes, *MLH1*, *ARH1* and *RASSF1A* were upregulated, while *NKX2* was down regulated. The mechanism underlying the sensitization may be due increased tumor suppressor gene expression, accessibility to DNA, or an undefined role for H3K27 methylation in adduct repair. These studies indicate that chromatin state is altered in at least some EOC, and the presence of tumor-specific bivalent histone marks may play a role in tumor progression and chemoresistance. As these data are based on limited sample numbers, further work is need to determine whether these findings extend to other HGSOC cases and the other histotypes.

4. Clinical aspects of epigenetics in EOC

4.1. Chemoresistance

Acquisition of resistance to therapy is common in EOC and contributes to the high mortality from the disease. Alterations in DNA methylation have been hypothesized to play a role, because DNA methylation is one mechanism cells employ to respond to environmental stimuli [29]. Platinum-based chemotherapy is the primary line of drug therapy for all advanced stage EOC cases; carboplatin combined with paclitaxel is the standard of care, irrespective of histotype [86,87]. Early studies on breast and ovarian cancer cell lines reported sensitivity to platinum-based chemotherapy in cells with either mutant or hypermethylated *BRCA1* [88–90]. More recently, several studies have examined DNA methylation and chemoresistance [11,91,92] or survival [93–95] using genome-wide approaches (Table 3). One study, using a custom microarray [92], identified 749 CpG loci that differed in methylation between resistant and sensitive HGSOC, 509 were within promoter regions. They used a short hairpin RNA screen (which silences target genes via RNA interference) to examine the effect on carboplatin sensitivity in ovarian cancer cell lines (a normal human surface epithelial line and two EOC lines resistant to carboplatin), and identified 19 genes associated with carboplatin resistance. De Leon et al. [91] examined

methylation changes in A2780 (ovarian carcinoma) cell line xenografts, treated with carboplatin or control diluent. Six genes had altered methylation of which two hypomethylated genes, *TMEM88* and *DAXX*, had an associated increase in gene expression in platinum resistant xenografts. *TMEM88* protein expression was evaluated in an ovarian tissue microarray with ~50% of the EOC showing decreased expression. *TMEM88* is a Wnt signaling inhibitor, and was inversely correlated with c-MYC gene expression in the TCGA dataset. In a study of acquired resistance, Patch et al. [11] examined methylation changes in 13 paired resistant and sensitive HGSOC and noted 94 CpG probes with > 10% methylation difference between the sensitive and resistant HGSOC, affecting *COLA1A1*, *SPTBN4* and *MYO1D* genes. Longer survival, using a cutoff of > 28 months, was associated with hypomethylation in *GREB1*, *TGIF*, *TOB1* and hypermethylation in *TMCO5*, *PTPRN*, *GUCY2C* in a study of 20 EOC patients [93]. Methylation in peripheral blood leukocytes of EOC patients was compared between cancer presentation and relapse in 146 patients [94]. Nine CpG sites were validated in an independent set of cancer presentation and relapse samples (n = 45 each). Eight CpG were significantly associated with survival and consensus clustering revealed two classes of relapse samples, class 1 with a poorer survival than class 2. Notably, methylation at presentation of the eight CpG did not predict time to recurrence or overall survival. Two reports used methylation-capture sequencing (MethylCap-seq) to assess DNA methylation and survival. Huang et al. [95] identified differential methylated regions (DMR) associated with progression-free survival (PFS), and validated the DMR in platinum resistant and sensitive cell lines. Hafner et al. [71] evaluated DNA methylation difference between pools of HGSOC DNA with poor or good survival followed by validation in an independent set of samples revealing two genes, *RUNX3* and *CAM21*, to be associated with PFS. In cell lines, increased methylation of these two genes was associated with reduced gene expression.

These studies reveal little overlap in the genes identified for chemoresistance or survival in part because of the use of cell line xenografts which may not be reflective of the original lineage, and the small number of patient derived xenografts or samples used. Only one study, from those reviewed, had > 100 patients and assessed blood leukocytes methylation rather than tumor methylation. Most studies evaluated HGSOC histotype, so there are no data available on rarer histotypes with a more methylated genome. As expressed in Patch et al. [11], development of chemoresistance is likely a complex process and involves more than a single gene. Much more needs to be done to understand how epigenetics, DNA methylation in particular, influences chemoresistance.

4.2. Epigenetic inhibitors in EOC

DNA demethylation agents such as 5-aza-cytidine (AZA) and decitabine have been used to express genes silenced by DNA methylation [96], and have been used with success in patients with acute myeloid leukemia (AML) and other malignancies [97]. These agents inhibit DNA methylation by binding covalently to DNA methyltransferase 1 (*DNMT1*) [98]. The current histone deacetylase (HDACi) inhibitors used clinically target zinc ion dependency of HDAC's catalytic activity, which affects HDACs I, II and IV but not III. The proposed mechanism for HDACi in cancer treatment is based on histone deacetylases allowing for expression of tumor suppressor genes, however these agents also acetylate non-

histone proteins which not part of epigenetic regulation [99]. These inhibitors have been shown to be effective cancer treatments in a variety of cancer types, and have been associated with tumor growth inhibition, apoptosis and cell differentiation [100].

The potential for epigenetic therapy for EOC was first elucidated by examining the effect of demethylating agents on platinum resistant ovarian cancer cell lines. AZA enhanced the sensitivity of platinum resistant ovarian cancer cell lines and [101,102]. Similarly, HDACi and BET inhibitors which work to alter histone tail state have been evaluated in cell lines [11,103–108], and have generally shown efficacy alone or when used in combination with platinum chemotherapy [103,109]. HDACs may also promote DNMT1 protein degradation, thereby inhibiting DNA methylation and allowing re-expression of repressed genes. HDACs are expressed at higher levels in all EOC histotypes compared to normal ovarian tissues [110,111].

Decitabine has been used in studies of DNA methylation and gene expression [112]. In EOC patients with previous chemotherapy and either resistant or refractory disease, treatment with decitabine has had varied efficacy, producing antitumor responses in 18–30% of cases [113,114], or having no effect [115]. Several phase I clinical trials using AZA or decitabine with paclitaxel/carboplatin in several cancers, including EOC patients, have been reported [113,115–118]. It should also be noted that these phase I–II studies had small sample numbers and a mix of EOC histotypes.

BET inhibitors, such as JQ1 and I-BET inhibit the binding of histone's acetylated lysines, displacing BET proteins from chromatin and preventing gene transcription, have been shown to be variably effective in a number of cancers [119,120]. JQ1 represses the growth of cisplatin treated ovarian cancer cell lines and xenografts via suppression of *BRD4*-mediated *ALDH1A1* expression, a gene associated with chemoresistance in ovarian cancer [107]. However, resistance to BET inhibitors has been reported [121].

HDACis have been reported to sensitize previously platinum resistant EOC cell lines [103,104], noting that different doses were required for at least two cell lines[103], suggesting histotype specific differences[122]. A response to an HDACi, suberoylamilide hydroxamic acid (SAHA), in combination with paclitaxel was reported in ascitesderived HGSOC cells from four patients initially resistant to paclitaxel [123]. Several clinical trials involving HDACi are ongoing or due are pending. A pan-HDACi, Quisinostat, in combination with paclitaxel and carboplatin is currently being evaluated for safety and efficacy in advanced platinum therapy resistant EOC [124]. A phase 1b/2 study of avelumab with and without entinostat (class I HDACi) in advanced EOC is planned [125]. This is an emerging therapy in EOC, and with development of more selective HDACi and perhaps targeting histotypes most likely to respond, this approach may find a way into clinical care.

4.3. Cell free DNA

Liquid biopsies, intended to detect circulating cell-free tumor DNA (ctDNA) and circulating tumor cells found in most cases of advanced malignancy [126,127]. though less frequently in patients with localized disease [128–130], are an active area of development [128]. DNA methylation based ctDNA liquid biopsies may be used to detect aberrant levels of

methylation in genes or DNA regulatory elements previously detected in patient's primary cancer or developed due to chemoresistance. Alternatively, liquid biopsies may be used to screen high risk patients for the presence of cancer. In EOC, most reports have focused on specific gene targets, such as tumor suppressor genes, evaluating methylation in matched EOC tumor tissues and plasma [131–133]. Notably, assessment of methylation in healthy populations, vital to improvement in sensitivity and specificity of liquid biopsies, has not been adequate. The presence of methylated *RASSF1A* and *BRCA1* has been reported in plasma of EOC patients [54,132,134] and also in healthy controls [135–138], highlighting the need for larger sample numbers of healthy tissue. DNA methylation for seven genes (*APC*, *RASSF1A*, *CDH1*, *RUNX3*, *TFP12*, *SRFP5*, *OPCML*) was evaluated in 202 plasma samples from healthy individuals and patients with benign, early or late stage EOC. Including health controls in the study was reported to improve sensitivity and specificity of early stage EOC [138], however, no validation of this has been published. A phase III clinical trial using carboplatin/taxoid in EOC evaluated *MLH1* methylation in plasma and found increased methylation at relapse which was associated with poor overall survival [131]. This non-invasive holds great promise and as methods for genome-wide methylation profiling of small amounts of degraded DNA are developed, and as we will learn more about the role of DNA methylation in EOC, the hope is to apply this to clinical care.

4.4. Molecular methylation profiling in EOC

Molecular methylation profiling can be helpful in understanding underlying differences in survival and response to therapy of previously homogenous ovarian cancer histotypes. Zhang et al. evaluated whether genome-wide DNA methylation profiles are associated with differential survival within HGSOC cases by clustering CpGs in 568 of the 583 TCGA HGSOC cases with both available methylation and survival data [139]. With 201 CpGs, those significantly associated with patient survival out of the 14,877 available, Zhang et al. was able to identify four methylation profiles with significantly different survival times. Two of the four TCGA methylation profiles (C1 and C3) showed higher global methylation and longer survival. The importance of this type of analysis is that it shows that histological subtyping of ovarian cancer can be made better defined with the addition of epigenetic information. In the clinic, molecular methylation profiling can eventually be used to build predictive models to communicate personalized response to therapy.

Abbreviations

5mC	5hydroxymethylcytosine
AML	acute myeloid leukemia
BET	bromodomain inhibitors
CCOC	clear cell ovarian cancer
ctDNA	cell-free tumor DNA
ChIP-onchip	chromatin immunoprecipitation with DNA microarray
CIMP	CpG island methylator phenotype

CpG	cytosine followed by guanine
DMR	differentially methylated regions
EMT	epithelial-mesenchymal transition
ENDOC	endometrioid ovarian cancer
EOC	epithelial ovarian cancer
HGSOC	high grade serous ovarian cancer
HDAC	histone deacetylase
HDACi	histone deacetylase inhibitor
IHC	immunohistochemistry
LGSOC	low grade serous ovarian cancer
MeDIP	Methylated DNA immunoprecipitation
MethylCap-seq	methylation-capture sequencing
MOC	mucinous ovarian cancer
MSP	methylation-specific PCR
NGS	next generation sequencing
PBL	peripheral blood leukocytes
PFS	progression free survival
PTM	post-translation modification
SAHA	suberoylamilide hydroxamic acid
SOC	serous ovarian cancer
TCGA	the cancer genome atlas

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Summary

Epigenetics is making its way into the clinic, thus understanding the role it plays in ovarian cancer is highly relevant. Whereas early studies of DNA methylation treated all ovarian cancer as one group, later studies have built histotype classification although the rarer ones have made it difficult to generate conclusive histotype-specific data. Nonetheless, the development of genome-wide approaches, and collaborative efforts to study the rarer histotypes, will yield more comprehensive data. Studies of histone modification proteins are more nascent, yet clinical trials in inhibitors of these proteins are underway. Much more needed to be done to fully realize the potential that epigenetics holds for ovarian cancer clinical care.

Fig. 1.
DNA methylation of selected genes in histotype specific epithelial ovarian cancer reported in targeted analysis studies.

The histogram indicates the percentage of samples showing methylation of the target genes (by colour for each gene) on the Y axis, with the number of samples analyzed above each bar, and the reference number for the studies on the X axis for each of the EOC histotypes, when reported: SOC/EOC serous ovarian cancer, grade not specified or EOC, histotype not specified; HGSOC high grade serous ovarian cancer; LGSOC low grade serous ovarian cancer; ENDOC endometrioid ovarian cancer; CCOC clear cell ovarian cancer; MOC mucinous ovarian cancer.

Table 1

Genome-wide DNA methylation analyses in EOC

Method	Histotype and number of samples used ^a	Analysis	Key findings	Ref
GoldenGate	5 SOC/EOC 5 ENDOC 4 CCOC 3MOC	Differential methylation normal vs cancer	Hypermethylated CpG: SOC 20; MOC 37; 43% CpG unique ENDOC 15; CCOC 5	[68] ^b
GoldenGate	1 SOC/EOC 14 HGSOC 9 ENDOC 3 CCOC EOC cell lines	Differential methylation within histotype Association with gene expression	90 CpG in 68 genes had significant histotype specific methylation CCOC more likely hypermethylated. 10% of 492 CpGs are hypermethylation and associated with decreased gene expression. EOC cell lines distinct from tumors.	[65]
GoldenGate	46 HGSOC 8 LGSOC	Differential methylation Within histotype	HGSOC more hypomethylated Hypermethylated at <i>GFAP</i> , <i>TAL1</i> , <i>IPF1</i> , <i>AREG</i> , <i>HOXA9</i>	[67]
27 K	489 HGSOC	Differential methylation normal vs cancer Association with gene expression	168 genes methylated with altered gene expression. <i>AMT</i> , <i>CCL21</i> , <i>SPARCL1</i> methylated majority of sample <i>RAB25</i> methylated in a subset; <i>BRCA1</i> methylated in 11.5% of sample. Four methylation subtypes identified	[12]
27 K	11 ENDOC ^b 34 HGSOC 4 LGSOC	Methylation cluster analysis using multiple algorithms	Three clusters: normal, EODOC and HGSOC Serous generally hypomethylated ENDOC very hypermethylated, with hypomethylation of usually methylated sites	[69]
27 K	fallopian tube 53 ENDOC 83 HGSOC	Differential methylation normal vs cancer within histotype Association with gene expression	12 CpG loci: <i>ZNF154*</i> ; <i>ZNF11</i> ; <i>TUBGCP2</i> ; <i>C19orf19</i> ; <i>BTNL2*</i> ; <i>KRTAP11-1*</i> ; <i>TMC6</i> ; <i>TMC8</i> ; <i>DEFB118*</i> ; <i>FLJ44674*</i> ; <i>SNTB1</i> ; <i>VHL*</i> ; <i>LMLN</i> ; <i>IQCG</i> ; <i>CASP8*</i> * seen in TCGA multiple cancers data	[70]
450 K	344 HGSOC 80 ENDOC 16 LGSOC 20 CCOC 12 MOC	Methylation cluster analysis using Gaussian distributed recursive portioned mixture model	CCOC methylation profile identified including <i>KCNH2</i> , <i>VWA1</i> , <i>NRDG2</i> , <i>SLC25A9</i>	[20]
450 K	80 HGSOC 7 fallopian tube	Differential methylation normal vs cancer	433 genes hypermethylated correlated with decreased expression. <i>ALDH1A3</i> , <i>AMT</i> , <i>LONRF2</i> , <i>NPDC1</i> , <i>SLC16A5</i> also hypermethylated in TCGA dataset	[11]

HGSOC: High grade serous ovarian cancer; LGSOC: Low grade serous ovarian cancer; ENDOC: Endometrioid ovarian cancer; CCOC: Clear cell ovarian cancer; MOC: Mucinous ovarian cancer.

^a SOC/EOC: Serous ovarian cancer, grade not specified or ovarian cancer, histology not specified.

^b 5 ovarian endometrioid and 6 endometrial endometrioid tumors.

Table 2

HDACs and histone alterations in EOC.

Targets	Samples used ^a	Analyses	Key findings	Ref
<i>SIRT1</i> (HDAC)	TMA of 113 SOC 21 MOC 34 EOC 12 normal ovaries 31 borderline 26 cystadenomas	IHC composite score of intensity × area	Higher <i>SIRT1</i> expression in malignant SOC compared with benign or borderline cases and MOC	[79]
H3K27me3	164 EOC/SOC 21 MOC 34 ENDOC	IHC composite score of intensity × area	Decreased expression in 55.3%, 38% borderline, 39% cystadenomas and 16% normal ovaries.	[80,78]
H3K4	HGSOC Cell lines: A4- P;A4-T	MeDIPb	76 genes hypomethylated and 31 genes hypermethylated with associated gene expression change	[82]
H3K9		ChIP-on-chipc	5 genes hypomethylated and 2 hypermethylated seen TCGA, in the following comparisons: A4-P with LGSOC and A4-T with HGSOC.	
H3K27		Gene expression	Histone changes: bivalent histone marks noted	
H3K4me3	1 HGSOC	MeDIPb	Gene set 1: n = 580, bivalent signature (M3K37me3 and H3K4me3)	[83]
H3K27me3	8 fallopian tube 499 HGSOC (TCGA)	ChIP-on-chipc Gene expression	Gene set 2: n = 913 H3K27me3 Significant decrease in expression of genes with bivalent or repressive Histone PTM	
H3K27	CP70 cell line	H3K27R mutant Methylation	Global methylation decrease Upregulation of tumor suppressor genes Resensitizes CP70 to cisplatin	[85]

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^aSOC/EOC: Serous ovarian cancer, grade not specified or ovarian cancer, histology not specified.

Table 3

DNA methylation: therapy and prognosis.

Method	Samples used ^a	Analyses/Comparisons	Key findings	Ref
<i>Chemoresistance</i>				
280 K custom	36 HGSOC	15 refractory/resistant;	749 CpG (~60% hypermethylated and ~40% hypomethylated in resistant HGSOC)	[92]
	EOC cell lines: HOSE 6-3, SKOV3, CAO V3	21 sensitive HGSOC; 9 normal ovaries shRNA screen of 296 candidate genes (within 2KB of TSS)	19 genes: <i>GSK3B</i> , <i>DOK2</i> , <i>APRT</i> , <i>OXSR1</i> , <i>CENBO</i> , <i>FZD1</i> , <i>ESRRA</i> , <i>HIRIP3</i> , <i>GTF2b</i> , <i>SGPL1</i> , <i>GABPA</i> , <i>TMIST1</i> , <i>MDH1</i> , <i>NR2E</i> , <i>NR3C2</i> , <i>SOX9</i> , <i>TOBI</i> , <i>UNG</i> , <i>ZIC1</i>	
27 K	20 EOC (mostly HGSOC);	Survival: 28, 47 month cutoff (note, 6 month cutoff didn't reveal any significant findings)	82 genes with > 10% difference, including hypomethylated: <i>GREB1</i> , <i>TGIF</i> , <i>TOBI</i> hypermethylated: <i>TMC05</i> , <i>PTPRN</i> , <i>GUCY2C</i>	[93]
27 K	3 normal ovarian tissue EOC cell lines: A2780 xenografts EOC cell lines: A2780, CP70, PE1, PEO3	Xenograft treated with carboplatin or diluent: resistant vs control, mRNA Resistant vs sensitive EOC cell lines	Hypomethylated: <i>SSH3</i> , <i>SLC2A4</i> , <i>TMEM88</i> , <i>DAXX</i> , <i>MEST</i> mRNA changed: <i>TMEM88</i> , <i>DAXX</i> Cell line study platinum resistant vs sensitive: only <i>TMEM88</i> <i>TMEM88</i> expressed in 24/47 EOC	[91]
<i>Prognosis</i>				
450 K	IHC EOC TMA Peripheral blood lymphocytes from 146 EOC ^b 45 EOC	Presentation/relapse PFS median < 10.8 Presentation/relapse months (n = 54) PFS median 48.5 months (n = 54)	333 CpG significantly changed between presentation and relapse. Nine selected to validate Eight of the 333 CpG were significantly associated with survival, but in cluster analysis did not predict time of progression or overall survival	[94]
MethylCap-seq	50 SOC 20 EOC (benign) 6 normal ovaries Cell lines: SKOV3 sensitive CP70 resistant IOSE normal	Differentially methylated regions and PFS Validation of 16 DMR in cell lines	63 DMR associated with PFS Pathway analysis: 23/63 genes connected to mTOR, <i>MAPK</i> , <i>VEGF</i> , <i>ErbB</i> , <i>NOTCH</i> , <i>Wnt</i> , <i>TGF-β</i> , hedgehog, cell cycle, adherens junction,	[95]
MethylCap-seq	Two pools each with 3 HGSOC and 3 HGSOC C with good and poor survival respectively.		106 hypomethylated and 114 hypermethylated genes.	[71]
MSP	Validation in 18 HGSOC good survival and 30 with poor survival <i>CAMK2I</i> methylation		37 CpG: <i>RUNX3/CAMK2I</i> associated with PFS	
Cell lines: SKOV3 (sensitive) SKOV3-12.8 (resistant) 6 platinum resistant and 42 sensitive EOC			Increased methylation in resistant cells, and lower gene expression 2/6 resistant and 3/42 sensitive with <i>CAMK2I</i> hypermethylation	

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SOC/EOC: Serous ovarian cancer, grade not specified or ovarian cancer, histology not specified.
DNA form Peripheral blood leukocytes from EOC patients.