Chapter

Epigenetic Events in Ovarian Cancer

Yanisa Rattanapan and Takol Chareonsirisuthigul

Abstract

Epigenetic aberrations are now well established in the development and progression of ovarian cancer, including DNA methylation, histone modifications, and microRNA dysregulation, and their progressive accumulation is correlated with the progression of the stage grade of disease. Epigenetic aberrations are relatively stable, linked to various subtypes of the disease, and present in circulating serum, representing promising diagnostic, prognostic, and pharmacodynamic biomarkers. Unlike DNA mutations and deletions, aberrant gene-repressive epigenetic changes, including DNA methylation inhibitors or histone-modifying enzymes, are theoretically reversible by epigenetic therapies. While no action against solid tumors, including ovarian cancer, has been shown in epigenetic monotherapies, preclinical studies indicate that they may be successful when used in conjunction with one another or with conventional chemotherapy, and combinatorial epigenetic therapy regiments are being investigated in cancer clinical trials. Improved interventions against this debilitating malignancy will provide a greater understanding of epigenetics' role in ovarian cancer.

Keywords: ovarian cancer, epigenetic, miRNA, DNA methylation, histone modification

1. Introduction

Among gynecological malignancies, ovarian cancer, a molecularly heterogeneous condition associated with poorest prognosis. The highest mortality rates are associated with ovarian cancer, reflecting the third most prevalent cancer in female carcinomas of the gynecologic system. While it accounts for just 3% of all female cancers, the worldwide annual prevalence of ovarian cancer is 220,000, with 21,750 reported new cases and 13,940 estimated deaths annually [1]. Specific diagnosis in more than 70% of OC cases is a potent factor for high fatality rates at an advanced disease stage and carries a poor prognosis with current therapies. In ovarian cancer, the median age of disease diagnosis is 60 years and its lifetime incidence is one in seventy with estimated lifetime mortality of one in ninety-five [2, 3]. Epithelial ovarian cancer (EOC) accounts for 90% of all types of OC cases distinguished at histopathological, clinical, and molecular levels by heterogeneity. The precise cause of the malignancy of the ovaries is still unclear. Significant risk factors associated with OC have been identified as a strong family history of either ovarian or breast cancer. More than one-fifth (approximately 23%) of ovarian carcinomas have inherited susceptibility and have BRCA1 and BRCA2 tumor suppressor gene mutations [4].

Rapid growth, unspecific clinical symptoms at the early stage of the disease, and the absence of earlier diagnosis methods make it challenging to diagnose promptly due to lack of effective screening. As a result, when the tumor has spread beyond the pelvis and is unlikely to be entirely removed by surgery, EOC is usually diagnosed at an advanced stage (FIGO III/IV). Long-term survival rates are poor (10–30 percent for women with disseminated malignancies. However, an ovarian cancer diagnosis at the localized level is considerable curable (over 95 percent five-year survival rate; [5]). Therefore, it is needed to explore cost effective and sensitive screening program for early detections and biomarkers to predict disease behaviors and responses to therapies. In identifying promising biomarkers of clinical utility for early diagnosis of OC, a better understanding of the EOC genome portrait would benefit.

Altered epigenetic states are closely associated with tumorigenesis of the ovaries. Epigenetics is characterized as a heritable alteration in gene expression without the DNA sequence itself being altered and involves DNA methylation, histone modification, nucleosome repositioning, and micro-RNAs (miRNAs) posttranscriptional gene regulation [6, 7]. Cancer vulnerability is inherited, but most of this inheritability remains unknown. Epigenetic changes in the parental germ line that do not require transmission of genetic variants from parent to offspring may mediate such missing heritability. DNA methylation, the addition of a methyl moiety to the cytosine-5 location within the sense of a CpG dinucleotide, mediated by DNA methyltransferases (DNMTs), is the most studied epigenetic shift [6]. While most CpG sites are methylated in the human genome, CpG-dense regions known as CpG islands (often gene-associated) are usually unmethylated in normal tissue. Also, histone proteins associated with DNA are subject to extensive modifications that mediate the assembly of chromatin that is transcriptionally permissive or restrictive (i.e., open or closed). DNA methylation and histone modifications are now recognized to be closely related [6]. The complete epigenetic state corresponding to a particular cell phenotype (e.g., DNA methylation, histone modification, and miRNA expression) is now referred to as the epigenome [8]. Though repressive epigenetic changes (including DNA methylation) control genes in normal tissues (e.g., imprinted genes and inactivation of female X-chromosomes), they are dramatically altered in cancer [6, 9]. Global DNA hypomethylation and localized hypermethylation of promoter-associated CpG islands occur primarily in cancer cells, with the latter acting as a replacement for point mutations or deletions to induce transcriptional silencing of tumor suppressor genes [6].

2. DNA methylation in ovarian cancer

The substantial shortcomings of the therapies examined above in the treatment of ovarian cancer have set the stage for the use, either alone or in combination with other therapies, of novel epigenetic therapies to treat this disease. By adding a methyl group to stimulate regions of DNA to silence gene expression, the epigenetic alteration of DNA methylation controls gene expression. This mechanism is critical during sensitive cellular processes, such as embryonic development, inactivation of X-chromosomes, and genomic imprinting. The organ's normal development and maturation are determined by a precise balance of active and silenced genes [10]. On the other hand, cancer promotes global hypermethylation of CpG islands associated with promoters, which are typically unmethylated in normal tissue, silencing genes essential for cellular homeostases, such as genes suppress tumors. To promote tumorigenesis, aberrant DNA methylation and structural chromatin changes will alter gene

expression [11]. A repressive and tightly woven chromatin structure is caused by DNA methylation, which can minimize gene expression in DNA repair, apoptosis, differentiation, drug resistance, angiogenesis, and metastasis. In cancer, gene promoters' hypermethylation causes the genes involved in cell cycle control, including BRCA1, CDKN2A, RASSF1A, LOTI, DAPK, ICAM-l, PALB2, RAD51C, and BRIP1 to be downregulated. Therefore, substantial loss of CpG hypermethylation in ovarian cancer is correlated with cancer cell growth inhibition [12].

In ovarian cancers, hypermethylation of particular gene promoters has been established. Compared to non-neoplastic tissues, promoter hypermethylation of tumor suppressors BRCA1 and RASSF1A were significantly higher in ovarian cancers [13]. This hypermethylation silences expression to suppress BRCA1 activity, driving genomic instability in ovarian cancers, analogous to the mutations in BRCA1 discussed earlier. RASSF1A encodes a protein controlling the cell cycle; silencing this gene promotes cell-cycle progression and unregulated cell development. Compared to benign cases, tissues from patients with serous and non-serous EOC display significantly higher RASSF1A promoter methylation. [14]. In clear-cell ovarian cancer, hypermethylation is also observed. 22 separate CpG loci associated with nine genes (VWA1, FOXP1, FGFRL1, LINC00340, KCNH2, ANK1, ATXN2, NDRG21 and SLC16A11) were hypermethylated. Inversely associated with tumor gene expression, multiple loci methylation, most notably KCNH22 (HERG, a potassium channel). Loss of KCNH2 (HERG) expression by methylation may be a good prognostic marker, provided that overexpression of the Eag family members of the potassium channel promotes increased proliferation and results in poor prognosis [15]. However, superficial cell carcinomas also suppress methylation of the gene encoding the HNF1B transcription factor, while this gene is methylated in high-grade serous ovarian cancers [16]. In invasive carcinomas, Makarla et al. found hypermethylation of eight cancer-related genes (p16, RARβ, E-cadherin, H-cadherin, APC, GSTP1, MGMT, and RASSF1A) was significantly higher compared to non-invasive cancers and benign cystadenomas [17].

3. Histone modification in ovarian cancer

Chromatin modifying enzymes are altered in ovarian cancers beyond DNA methylation. High levels of H3K9 methyltransferase G9a, which adds histone methyl groups (H3K9) to promote the compact structure of chromatin and silence genes, have been associated with late-stage high-grade and serous ovarian cancer, as well as shorter survival in patients with ovarian cancer [18]. Genes marked by the chromatin modifications of activating H3K4me3 and silencing H3K27me3 are identified as "poised" or bivalent; these are not transcribed into embryonic stem cells but resolved as differentiated stem cells into active and transcribed (H3K4me3) or silenced and not transcribed (H3K27me3). In 499 high-grade serous ovarian cancers, compared to eight normal fallopian tube samples, these bivalent chromatin loci were silenced and included genes in the PI3K and TGF-beta signaling pathways. Stem-like cells of ovarian cancer and chemo-resistant cells of ovarian cancer have demonstrated increased silencing of these genes [19]. As previously mentioned, the gene encoding the ARID1A chromatin remodeler is mutated in over 50 percent of ovarian clear cell carcinomas. In a mouse model of ovarian cancer, Bitler et al. showed inhibiting EZH2 methyltransferase, which adds the H3K27me3 mark to silence gene expression, induced regression of ARID1A-mutated tumors. This occurred via PIK3IPI upregulation, an ARID1A and EZH2 target increased by EZH2 inhibition and inhibits PI3K/Akt oncogenic signaling [20].

4. MicroRNA dysregulation in ovarian cancer

The most recently discovered epigenetic miRNAs represent ovarian tumors have recently become a phenomenon, and it was found to substantially up-regulate miR-199a, miR-200a, miR-200a, miR-214, and down-regulate miR-100 and, precisely, miR-100 and miR-214 to target the tumor suppressor, miR-214 was shown to PTEN and is associated with resistance to platinum [21, 22]. Let-7 miRNA family as one of the regulator of the MYCN pathway that linking to the platinum-resistant trait. It was recently discovered that miRNA let-7i was a tumor substantially down-regulated suppressor in platinum-resistant ovarian tumors, and restored let-7i gain-of-function chemoresistant ovarian cancer drug sensitivity cells, thus representing a biomarker and therapeutic candidate goal [23]. MiR-429, miR-200a, and miR-200b, respectively a single primary transcript was found to be clustered on epithelial-to-mesenchymal transition-regulated (EMT, a metastatic phenotype) ZEB1/SIP1 repressor, with negative regulation of miR-200a and miR-200b ZEB1/SIP1 and the development of a loop of double-negative feedback [24]. In another study, 27 miRNAs were substantially correlated with chemotherapy response, indicating a chemotherapy response miRNA (similar to DNA methylation) represent potential biomarkers for ovarian prognosis and diagnosis [25]. Regarding the regulation of miRNA genes, a group of six chromosomes, 19 miRNAs clustered on chromosome 19, and seven clusters were up-regulated on chromosome 14, DNMT-inhibitor decitabine inhibitor, showing that miRNAs can be controlled by DNA methylation [26]. What's more, an overall, collective tumor—MiRNAs' suppressive effect has been suggested by Drosha and Dicer down-regulation, involving two enzymes in the processing of miRNA, being significantly connected with an early stage of ovarian cancer and poor prognosis [27, 28].

5. Epigenetic biomarkers for ovarian cancer

As mentioned above, the development of ovarian cancer is well characterized by a range of combinatorial epigenetic aberrations distinct from this malignancy, including but not limited to RASSF1A, DAPK, H-Sulf-1, BRCA1, and HOXA10 DNA methylation. As a result, these methylated DNA sequences represent potential diagnostic, staging, prognostic, and therapy response monitoring (predictive biomarkers) biomarkers [29]. DNA methylation biomarkers have several advantages over other types of biomarkers, such as proteins, gene expression, and DNA mutations, including their stability, ability to amplify (thus greatly enhancing detection sensitivity), relatively low cost of the assessment, and restriction to small DNA regions (CpG islands) [30]. It also acts as a biomarker to predict response to platinum-based chemotherapy regimen and the poly-ADP ribose polymerase inhibitor (PARPi). In the future, DNA methylation tests of resected ovarian tumors are highly likely to be used to customize care individually, similar to the recently discovered predictive markers of non-small cell lung cancer in stage I [31]. While single-gene methylation evaluation lacks adequate specificity for ovarian cancer diagnosis, it is believed that multiple methylation biomarker panels will achieve the precision needed for widespread population screening [30, 32]. To that end, a panel of 112 methylated DNA markers was found to correlate progression-free survival with ovarian cancer [33].

6. Clinical trials of epigenetic therapeutic in ovarian cancer

DNA methylation inhibitor and HDAC inhibitor are cancer therapeutics, begins primarily as a treatment for hematological disorders in the early 2000s. The FDA

approved 5-Azacytidine (AZA) and 5-aza-2′-deoxycytidine (decitabine) for myelodysplastic syndrome (MDS) in 2004 and 2006, while the HDAC suberoylanilide hydroxamic acid (SAHA) inhibitor was approved in 2006 for the treatment of persistent or cutaneous T cell lymphoma [34]. Epigenetic therapy was initially performed in a clinical trial. It was setting either alone or with the standard in combination care to resensitize either the tumor to anticancer treatment or avoid therapy resistance production. Ultimately, these medications have been tested against resilient OC tumors, like both SAHA and AZA clinical trials, ovarian cancer, which is platinum-resistant. Although there are no antitumors, the behavior was detected after SAHA treatment, and AZA demonstrated the partial reaction. Still, it was correlated with significant adverse effects such as tiredness and myelosuppression [35]. The HDAC inhibitor, in a related analysis Belinostat, was given to platinum-resistant patients with ovarian tumors. Still, they have similarly caused significant adverse reaction events such as thrombocytopenia, neutropenia, and vomiting, leading to the end of the analysis with no clinical advantage over conventional therapy [34]. Similarly, in a phase I study, the vorinostat pan-HDACi carboplatin or gemcitabine was administered despite the extreme hematological toxicities caused and caused observed partial response, leading to the termination of the study [36].

7. Future prospects

Exponential advancement in DNA methylation-based biomarker growth has been observed over the last decade. A variety of cfDNA and tissue-dependent screening assays have paved their way into clinics due to the consistency of DNA and methylation patterns. Several tests for early detection of colon, lung, and prostate cancer are commercial success based on DNA methylation biomarkers. New technologies that enable the rapid identification of methylation signatures directly from the blood can promote sample-to-respond solutions, allowing molecular diagnostics for the next-generation point of care. Besides, ongoing work on liquid biopsies together with the latest advanced technologies such as digital PCR, bisulfite sequencing, methyl immune precipitation coupled with next-generation sequencing, and methylation arrays together with advanced statistical data analysis will mitigate the complicated problems of non-invasive system creation by overcoming the existing challenges to precision medicine.

8. Conclusion

Ovarian cancer causes substantial morbidity and mortality. Owing to unspecific signs at the early stage of the disease, their appearance at an advanced stage, and poor survival, the difficulty of promptly diagnosing ovarian cancer at its early stage remains difficult. Improved methods of detection are, therefore, urgently needed. This chapter identifies the possible clinical usefulness of epigenetic signatures such as DNA methylation, modifications of histones, and microRNA dysregulation, which play an essential role in ovarian carcinogenesis and its use in the development of diagnosis, prognosis, and biomarkers for prediction. New treatment options separate from conventional treatment options chemotherapy that benefits from developments in the understanding of ovarian cancer pathophysiology to enhance performance, they are required. Recent work has shown that mutations in epigenetic regulator-encoding genes are mutated in ovarian cancer, driving tumorigenesis and drug resistance. Several of these modifiers of epigenetics for ovarian cancer treatment have emerged as promising drug targets.

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References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30. doi:10.3322/caac.21590

[2] Cannistra SA. Cancer of the ovary [published correction appears in N Engl J Med. 2005 Jan 6;352(1):104]. N Engl J Med. 2004;351(24):2519-2529. doi:10.1056/NEJMra041842

[3] Howe HL, Wu X, Ries LA, et al. Annual report to the nation on the status of cancer, 1975-2003, featuring cancer among U.S. Hispanic/Latino populations. Cancer. 2006;107(8):1711-1742. doi:10.1002/cncr.22193

[4] Ramus SJ, Harrington PA, Pye C, et al. Contribution of BRCA1 and BRCA2 mutations to inherited ovarian cancer. Hum Mutat. 2007;28(12):1207-1215. doi:10.1002/humu.20599

[5] Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. 2011;61(4):212-236. doi:10.3322/caac.20121

[6] Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007;128(4):683-692. doi:10.1016/j.cell.2007.01.029

[7] Lopez J, Percharde M, Coley HM, Webb A, Crook T. The context and potential of epigenetics in oncology. Br J Cancer. 2009;100(4):571-577. doi:10.1038/sj.bjc.6604930

[8] Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell. 2007 Feb 23;128(4):669-81. doi: 10.1016/j.cell.2007.01.033. PMID: 17320505.

[9] Esteller M. Epigenetics in cancer. N Engl J Med. 2008;358(11):1148-1159. doi:10.1056/NEJMra072067

[10] Jones PA, Issa J-PJ, Baylin S. Targeting the cancer epigenome for therapy. Nat Rev Genet. 2016;15(17(10)):630-41.

[11] Nervi C, De Marinis E, Codacci-Pisanelli G. Epigenetic treatment of solid tumours: a review of clinical trials. Clin Epigenetics. 2015;7:127. Published 2015 Dec 10. doi:10.1186/s13148-015-0157-2

[12] Ahluwalia A, Yan P, Hurteau JA, et al. DNA methylation and ovarian cancer. I. Analysis of CpG island hypermethylation in human ovarian cancer using differential methylation hybridization. Gynecol Oncol. 2001;82(2):261-268. doi:10.1006/gyno.2001.6291

[13] Ibanez de Caceres I, Battagli C, Esteller M, et al. Tumor cell-specific BRCA1 and RASSF1A hypermethylation in serum, plasma, and peritoneal fluid from ovarian cancer patients. Cancer Res. 2004;64(18):6476-6481. doi:10.1158/0008-5472.CAN-04-1529

[14] Rattanapan Y, Korkiatsakul V, Kongruang A, et al. EGFL7 and RASSF1 promoter hypermethylation in epithelial ovarian cancer. Cancer Genet. 2018;224-225:37-40. doi:10.1016/j.cancergen.2018.04.117

[15] Cicek MS, Koestler DC, Fridley BL, et al. Epigenome-wide ovarian cancer analysis identifies a methylation profile differentiating clear-cell histology with epigenetic silencing of the HERG K+ channel. Hum Mol Genet. 2013;22(15):3038-3047. doi:10.1093/hmg/ddt160

[16] Shen H, Fridley BL, Song H, et al. Epigenetic analysis leads to identification of HNF1B as a subtype-specific susceptibility gene for ovarian cancer. Nat Commun. 2013;4:1628. doi:10.1038/ncomms2629

[17] Makarla PB, Saboorian MH, Ashfaq R, et al. Promoter hypermethylation profile of ovarian epithelial neoplasms. Clin Cancer Res. 2005;11(15):5365-5369. doi:10.1158/1078-0432.CCR-04-2455

[18] Hua KT, Wang MY, Chen MW, et al. The H3K9 methyltransferase G9a is a marker of aggressive ovarian cancer that promotes peritoneal metastasis. Mol Cancer. 2014;13:189. Published 2014 Aug 12. doi:10.1186/1476-4598-13-189

[19] Chapman-Rothe N, Curry E, Zeller C, et al. Chromatin H3K27me3/H3K4me3 histone marks define gene sets in high-grade serous ovarian cancer that distinguish malignant, tumour-sustaining and chemo-resistant ovarian tumour cells. Oncogene. 2013;32(38):4586-4592. doi:10.1038/onc.2012.477

[20] Bitler BG, Aird KM, Garipov A, et al. Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. Nat Med. 2015;21(3):231-238. doi:10.1038/nm.3799

[21] Iorio MV, Visone R, Di Leva G, et al. MicroRNA signatures in human ovarian cancer. Cancer Res. 2007;67(18):8699-8707. doi:10.1158/0008-5472.CAN-07-1936

[22] Yang H, Kong W, He L, et al. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN [published correction appears in Cancer Res. 2008 Mar 1;68(5):1609]. Cancer Res. 2008;68(2):425-433. doi:10.1158/0008-5472.CAN-07-2488

[23] Yang N, Kaur S, Volinia S, et al. MicroRNA microarray identifies Let-7i as a novel biomarker and therapeutic target in human epithelial ovarian cancer. Cancer Res. 2008;68(24):10307-10314. doi:10.1158/0008-5472.CAN-08-1954

[24] Bracken CP, Gregory PA, Kolesnikoff N, et al. A double-negative feedback loop between ZEB1-SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition. Cancer Res. 2008;68(19):7846-7854. doi:10.1158/0008-5472.CAN-08-1942

[25] Boren T, Xiong Y, Hakam A, et al. MicroRNAs and their target messenger RNAs associated with ovarian cancer response to chemotherapy. Gynecol Oncol. 2009;113(2):249-255. doi:10.1016/j.ygyno.2009.01.014

[26] Zhang L, Volinia S, Bonome T, et al. Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. Proc Natl Acad Sci U S A. 2008;105(19):7004-7009. doi:10.1073/pnas.0801615105

[27] Merritt WM, Lin YG, Han LY, et al. Dicer, Drosha, and outcomes in patients with ovarian cancer [published correction appears in N Engl J Med. 2010 Nov 4;363(19):1877]. N Engl J Med. 2008;359(25):2641-2650. doi:10.1056/NEJMoa0803785

[28] Rattanapan Y, Korkiatsakul V, Kongruang A, Siriboonpiputtana T, Rerkamnuaychoke B, Chareonsirisuthigul T. MicroRNA Expression Profiling of Epithelial Ovarian Cancer Identifies New Markers of Tumor Subtype [published online ahead of print, 2020 Jul 22]. Microrna. 2020;10.2174/2211536609666200722125737. doi:10.2174/2211536609666200722125737

[29] Balch C, Huang TH, Brown R, Nephew KP. The epigenetics of ovarian cancer drug resistance and resensitization. Am J Obstet Gynecol. 2004;191(5):1552-1572. doi:10.1016/j.ajog.2004.05.025

[30] Laird PW. The power and the promise of DNA methylation markers. Nat Rev Cancer. 2003;3(4):253-266. doi:10.1038/nrc1045

[31] Brock MV, Hooker CM, Ota-Machida E, et al. DNA methylation markers and early recurrence in stage I lung cancer. N Engl J Med. 2008;358(11):1118-1128. doi:10.1056/NEJMoa0706550

[32] Balch C, Montgomery JS, Paik HI, Kim S, Kim S, Huang TH, Nephew KP 2005 New anti-cancer strategies: epigenetic therapies and biomarkers. Front Biosci 10:1897-1931

[33] Wei SH, Balch C, Paik HH, et al. Prognostic DNA methylation biomarkers in ovarian cancer. Clin Cancer Res. 2006;12(9):2788-2794. doi:10.1158/1078-0432.CCR-05-1551

[34] Nervi C, De Marinis E, Codacci-Pisanelli G. Epigenetic treatment of solid tumours: a review of clinical trials. Clin Epigenetics. 2015;7:127. Published 2015 Dec 10. doi:10.1186/s13148-015-0157-2

[35] Fu S, Hu W, Iyer R, et al. Phase 1b-2a study to reverse platinum resistance through use of a hypomethylating agent, azacitidine, in patients with platinum-resistant or platinum-refractory epithelial ovarian cancer. Cancer. 2011;117(8):1661-1669. doi:10.1002/cncr.25701

[36] Matulonis UA, Oza AM, Ho TW, Ledermann JA. Intermediate clinical endpoints: a bridge between progression-free survival and overall survival in ovarian cancer trials. Cancer. 2015;121(11):1737-1746. doi:10.1002/cncr.29082