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Review

# Genomic/Epigenomic Alterations in Ovarian Carcinoma: Translational Insight into Clinical Practice

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## Abstract

Ovarian carcinoma is the most lethal gynecological malignancy worldwide. Recent advance in genomic/epigenomic researches will impact on our prevention, detection and intervention on ovarian carcinoma. Detection of germline mutations in BRCA1/BRCA2, mismatch repair genes, and other genes in the homologous recombination/DNA repair pathway propelled the genetic surveillance of most hereditary ovarian carcinomas. Germline or somatic mutations in SMARCA4 in familial and sporadic small cell carcinoma of the ovary, hypercalcemia type, lead to our recognition on this rare aggressive tumor as a new entity of the atypical teratoma/rhaboid tumor family. Genome-wide association studies have identified many genetic variants that will contribute to the evaluation of ovarian carcinoma risk and prognostic prediction. Whole exome sequencing and whole genome sequencing discovered rare mutations in other drive mutations except p53, but demonstrated the presence of high genomic heterogeneity and adaptability in the genetic evolution of high grade ovarian serous carcinomas that occurs in cancer progression and chemotherapy. Gene mutations, copy number aberrations and DNA methylations provided promising biomarkers for the detection, diagnosis, prognosis, therapy response and targets of ovarian cancer. These findings underscore the necessity to translate these potential biomarkers into clinical practice.

Key words: ovarian carcinoma; mutation; hereditary; whole genome sequencing; whole exome sequencing; methylation.

## Background

Ovarian carcinoma (OC) is the most lethal gynecological malignancy worldwide. In the United States, OC is the fifth leading cause of cancer death in women, with estimated 14,240 deaths in 2016 [1]. Most OCs are high grade serous ovarian carcinomas (HGSOC). They are commonly diagnosed at an advanced stage with peritoneal dissemination and massive ascites. The 5-year survival rate in patients with advanced OC remains approximately 30% despite current standard combined therapy of debulking surgery and neoadjuvant chemotherapy of paclitaxel and carboplatin.

Our recent knowledge of molecular biology suggests that OCs can be divided into two groups, type I and type II [2]. Type I OC encompasses slow growing tumors and harbors mutations in KRAS, BRAF, PTEN, and CTNNB1, etc. Type II OC is clinically aggressive and pathologically high grade. They are characterized by TP53 mutations. Nearly one half of type II OCs have BRCA1/BRCA2 mutations, predisposing a considerable part of these women to hereditary breast and ovarian cancer (HBOC) [3]. Additional susceptibility genes in hereditary ovarian carcinomas are known to alter the homologous recombination (HR) /DNA repair pathway [3-5]. A recent surge in genomics/epigenomics studies has indicated that OC represents a genetically heterogeneous and complex group of diseases and does not conceive as a single entity [3-6]. The genetic heterogeneity may reflect the exerted clonal selections

in the progression of OCs, such as preserving cancer cells with chemoresistance and metastatic capacity. These findings indicate the urgent requirement of personalized regimen or target therapy in treating OCs. They provide useful biomarkers for the assessment of diagnosis, detection, intervention, and prognosis in OCs. This review will give a glimpse on the genomic advances in OC. Particular interest will be focused on the translating insight of these genomic alterations into clinical and pathological practice [Table 1].

**Table 1.** Summary of selected genetic alterations and clinical significance in ovarian carcinomas.

| Catalogues | Major genetic alterations | Clinical implications | Ref(s). |
|---|---|---|---|
| *Detection/diagnosis* | | | |
| | Germline mutations in BRCA1 or BRCA2 | Increased lifetime risk of developing OC; routine surveillance for early OC; indications for risk-reducing salpino-oorphorectomy | 9, 10 |
| | Germline mutations in mismatch repair genes | A 8%-10% risk of OC (Lynch syndrome); routine clinical surveillance for early OC | 13 |
| | Novel germline mutations in BARD1, BRIP1, PALB2, RAD50, RAD51C, RAD51D, TP53, ASXL1, MAP3K1, and SETD2, etc | Conferred a subset of familial OCs with high and moderate penetrance or a moderate OC susceptibility that may warrant their use in routine clinical genetic testing | 15-19 |
| | An integrated analysis of germline and somatic exome variants in OC | The candidate variants and genes have important implications for OC susceptibility and the development of screening strategies. | 20 |
| | Germline mutation in SMARCA4 (BRG1) | Improvements in genetic counseling and early detection for SSCHOT | 32-34 |
| | Mutations/loss of expression in SMARCA4 (BRG1) | Essential for precision diagnosis and potential novel treatment for SSCHOT | 35-37 |
| | Mutations of PIK3CA, RB1, and MED1 in plasma of OC patients following therapy | Applicable to monitor OC patients with high systemic tumor burden, metastasis and therapy response | 61 |
| *Risk assessment* | | | |
| | rs7651446(3q25), rs9303542 (17q21), rs11782652 (8q21), rs1243180 (10p12), rs757210 (17q12) | Predicting OC risks | 41 |
| | rs8170 and rs2363956 at 19p13.11 | Predicting survival and genome-wide serous OC risks | 42 |
| | rs2072590 (2q31), rs2665390 (3q25), rs10088218 (8q24), rs9303542 (17q21) | Predicting OC risks | 43 |
| | rs3814113 (9p22.2) | OC risks, strongest for serous OC risks | 44 |
| | rs752590 (2q13), rs711830 (2q31.1), rs688187 (19q13.2) | Risk associations with mucinous OC | 45 |
| | Mutations in BRIP1 (c.2040_2041insTT, c.1702_1703del) | An increase in OC risks | 46 |
| | rs3814113 (9p22.2) | A reduced OC risk in BRCA1/BRCA2 mutation carriers | 49 |
| *Chemotherapy response/prognosis evaluation* | | | |
| | Germline/somatic mutations in BRCA1, BRCA2 and other genes in the HR pathway | 1) Predictive of platinum sensitivity and longer survival in women with HGSOC; 2) Benefit from PARP inhibitors. | 3, 29-31 |
| | rs7874043 in TTC39B | The minor allele is strongly associated with PFS in patients with serous carcinoma following first-line chemotherapy. | 50 |
| | rs4910232(11p15.3), rs2549714(16q23), and rs6674079 (1q22) | The rare alleles were significantly associated with poorer outcomes in OC patients who underwent first-line treatment of cytoreductive surgery and chemotherapy. | 51 |
| | rs1649942 | Associated with PFS and OS in OC patients with carboplatin-based chemotherapy | 52 |
| | Reactivation of HR genes in platinum-resistant versus primary OCs; Increased platinum score of 13 CNAs in recurrent tumors | Treatment options should be tailored to the changing genetic profiles. All primary platinum-sensitive HGSOCs are qualified for second-line PARP inhibitor treatment. | 56 |
| | Clonal escape in chemotherapy; Novel mutations in the Golgi and ECM pathways | Target therapy towards the persistent mutations may be effective for tumor relapse while novel mutations may offer new therapeutic targets for recurrent tumors. | 57 |
| | Mutations from 8 members of the ADAMTS family | Helpful molecular markers for predicting chemotherapy response and prognosis in OC. | 58 |
| | Gains on 1q, 5q14~q23, and 13q21~q32, and losses of 8p and 9q | Clinical carboplatin resistance | 62 |
| | Gains on 1q25.2 and 1q32.2 | Clinical carboplatin resistance | 63 |
| | Loss on 13q32.1 and 8p21.1 | Predictive markers of chemoresistant serous carcinoma | 64 |
| | Gain in 3q26.2, and losses in 6q11.2-12, 9p22.3, 9p22.2-22.1, 9p22.1-21.3, Xp22.2-22.12, Xp22.11-11.3, and Xp11.23-11.1. | Potential predictive markers of chemotherapy resistance | 65 |
| | Gains in 9p13.2-13.1, 9q21.2-21.32, 9q21.33, 9q22.2-22.31, 9q22.32-22.33 and 9q33.1-34.11 | Potential predictive markers of docetaxel/carboplatin resistance | 66 |
| | Losses of 4p, 4q31.1– qter, 5q12–q22, 8p, 16q, and X | Poor survival in stage III OC | 68 |
| | CCNE1 amplification | Poor prognosis in postoperative OCs | 4, 69 |
| | High-level amplifications at 8q24, loss of 5q | Favorable prognosis for serous OC | 70 |
| | Gain in 5p or gain in 1p and loss in 5q | A higher or significantly decrease risk of recurrence | 72 |
| | Two distinct hierarchical clusters of CNA | Patients from cluster-1 had a significantly shorter median PFS than those from cluster-2. | 73 |
| | Met amplification in ovarian clear cell carcinoma | Worse survival | 74 |
| *Target therapy/individualized therapy* | | | |
| | Various molecular subtypes of OC signature associated with survival | They provide an opportunity to improve OC outcomes through subtype-stratified care. | 3 |
| | Few point mutations in low grade serous carcinomas and borderline tumors | Target therapeutic agents against BRAF and KRAS might be particularly effective for the recurrent inoperative cases. | 53,54 |
| | Recurrent mutations in ELF3, RNF43, GNAS, ERBB3 and KLF5 in mucinous OC | Potential novel targeted therapy for some high grade mucinous carcinomas | 55 |
| | The heterogeneity in the genome of HGSOC under the selective pressure of chemotherapy | Overcoming resistance to conventional chemotherapy will require a diversity of approaches, such as use of new inhibitors MDR1 and PARP. | 4 |
| | PPM1D amplification | A potential therapeutic target for a subgroup of ovarian clear cell carcinomas | 75 |
| | HER2 amplification | A potential therapeutic target | 76-78 |

Abbreviations: OC=ovarian carcinoma; PFS= poor progression-free survival; HR= homologue recombinant; OS=overall survival; CNA=copy number aberration; HGSOC= high grade serous ovarian carcinoma.

## Germline Mutations in Hereditary Ovarian Carcinoma

Approximately 15% to 20% of OCs occur in a familial context with a highly penetrant, autosomal dominant genetic predisposition. These hereditary OCs have three major types including hereditary breast/OC (HBOC), site-specific OC, and Lynch syndrome II (hereditary non-polyposis colorectal cancer, HNPCC). The modified strategy for surveillance and clinical management of these hereditary OCs were listed in Figure 1. The detection of germline mutations in susceptibility genes remains the backbone in this protocol.

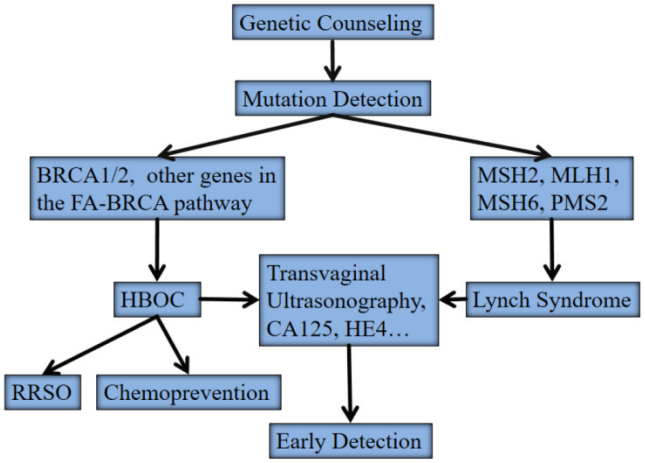


**Figure 1.** The modified strategy for surveillance and clinical management of hereditary ovarian cancers. Abbreviations: HBOC, hereditary breast/ovarian cancer; FA, Fanconi anemia; RRSO, risk-reducing salpino-oorphorectomy

HBOC is predominantly associated with germline mutations in two major susceptibility genes, BRCA1 and BRCA2 [7, 8]. Site-specific OC is believed to be an "ovarian-specific" variant of HBOC since no other susceptibility genes other than BRCA1 and BRCA2 have been identified yet. Women with a germline mutation in either BRCA1 or BRCA2 have a 45%-60% or 27% lifetime risk of developing OC by the age of 70 years, respectively [9, 10]. OC patients without familial history may also have germline BRCA1/BRCA2 mutations [3-5, 11]. A recent randomized controlled trial showed that population-based genetic testing on BRCA1/BRCA2 mutations in Ashkenazi Jews identified additional 56% carriers compared with the family history based testing [12]. Women with Lynch syndrome predispose to colorectal carcinoma, endometrial carcinoma, and OC, etc. The cumulative risk of OC is estimated to be 8%-10%. Germline mutations in mismatch repair genes, predominantly in MSH2, MLH1, MSH6 and PMS2, account for women with Lynch syndrome. These mutations have a much smaller contribution to hereditary OC than those of BRCA1/2. Germline mutation testing of mismatch repair genes can be considered directly for families that meet the Amsterdam criteria or tumors, and women with OC that harbor microsatellite instability with high frequency (MSI-H) or negative immunostaining for mismatch repair gene products in tumor tissues [12, 13]. A genome-wide array comparative genomic hybridization (aCGH) analysis showed that HBOC and Lynch syndrome associated OC had different genetic profiles and represented distinct genetic pathways [14]. Losses of 4q34, 13q12-q32 and 19p13 were overrepresented in the HBOC whereas gains on chromosomes 17 and 19 characterized the Lynch syndrome tumors.

Owing to the rapid development in DNA sequencing technology, a substantial number of novel germline mutations have also been identified in familial OCs or patients with early-onset OCs, which had no mutations in BRCA1/BRCA2 and mismatch repair genes. The reported genes included BARD1, BRIP1, CHEK2, MRE11A, NBN, PALB2, RAD50, RAD51C, RAD51D, TP53, NF1, MAP3K4, CDKN2B, and MLL3, etc [3-5, 15-19]. RAD51C and RAD51D are mutated in 1.5%-4% and 0.9% HBOC families with high and moderate penetrance, respectively [15, 16]. A recent case control study [19] in a large population found that the occurrence of RAD51C (0.41%) and RAD51D (0.35%) germline mutation was higher than that of RAD51B (0.06%). RAD51C and RAD51D mutations conferred a moderate OC susceptibility that may warrant their use alongside BRCA1 and BRCA2 in routine clinical genetic testing. PALB2 mutations have been found in 33 of 972 families (3.4%) with high risk of breast cancer, and 18 of these 33 families (55%) had a family member with OC [17]. A study of 1915 OC patients found that 347 (18%) carried pathogenic germline mutations in genes associated with OC risk, including PALB2, BARD1, BRIP1, RAD51C, RAD51D, BRCA1, BRCA2, MSH2, MLH1, PMS2, and MSH6[5]. Most mutations occur in BRCA1 (n=182) and BRCA2 (n=98). A whole exome sequencing (WES) study on 429 OCs identified that germline truncations and large deletions across Fanconi pathway genes occurred in 20% of cases [20]. The novel OC susceptibility genes included ASXL1, MAP3K1, and SETD2, etc. The targeted capture and massively parallel sequencing may simultaneously detect all these mutations with high coverage, sensitivity, and cost efficiency. This technique has been successfully found that 24% of 360 women with ovarian, fallopian tube, and peritoneal carcinoma carried germline loss-of-function mutations including

18% in BRCA1 or BRCA2, and 6% in BARD1, BRIP1, CHEK2, MRE11A, MSH6, NBN, PALB2, RAD50, RAD51C, or TP53[18]. These germline mutations will lead to significant improvements in genetic counseling and early detections of familiar OCs [Table 1].

The mutation spectrum in the susceptible genes is broad including small deletions, insertions, point mutations, and gene rearrangements that typically lead to prematurely truncated or non-functional protein products. Genetic tests on these germline mutations is critical for OC screening, but they were limited by the laborious and expensive traditional sequencing-based approach due to the large size, the wide distribution of the mutations, the absence of hotspots, and the lower prevalence of mutations in these genes. Genetic counseling has to be provided before genetic tests, such as the family cancer history for HBOC and the Amsterdam criteria for Lynch syndrome. The strong associations between morphology and genotype in OCs are also helpful in the identification of patients for genetic tests. Both BRCA1- and BRCA2- associated HGSOC had more frequent Solid, pseudoEndometrioid, and Transitional cell carcinoma-like morphology (SET features) [21]. OCs in Lynch syndrome are invariably associated the histotypes of endometrioid (in pure or mixed form) carcinoma and clear cell carcinoma [22, 23].

There are two main clinical options for germline mutation carriers: increased surveillance for the detection of stage I OC, or risk-reducing salpino-oophorectomy (RRSO). Currently transvaginal sonography and serum CA125 remains the mainstay for OC detection, but lack sensitivity and specificity for early stage OC. In addition to CA125, other serum biomarkers such as human epididymis protein 4 (HE4) may improve the screening efficiency for early detection of OC to a certain degree [24]. RRSO has increasingly been shown to be an effective strategy for reducing cancer risk by up to 96%, but it has the disadvantages of hormonal deprivation and potential peritoneal carcinoma. Occult carcinomas are frequently found in the fallopian tube (57%-100%) at the time of RRSO [25]. Tubal epitheliums in RRSO samples have increased proliferating index, the likelihood of p53 mutation, and progression into serous tubal intraepithelial carcinoma [26]. These findings support that tumor cells from the fallopian tube are one of the major origin of OC [2]. Therefore, the removal of both fallopian tubes can theoretically replace RRSO to reduce the OC risk although more compelling evidence should be provided in the future. Oral contraceptive provides an alternative for chemoprevention of OC in women with high OC risks who do not accept RRSO.

Several investigations have indicated that gene mutations may have therapeutic significance [Table 1]. BRCA1- or BRCA2-functionally deficient cell lines are more sensitive to platinum [27, 28]. BRCA1/BRCA2 mutations are predictive of platinum sensitivity and longer survival in HGSOC patients [29]. A recent study also showed that OC patients with a BRCA2 mutation had longer progression-free survival and overall survival compared with those without mutations [30]. For breast and OC patients with pathogenic BRCA mutations, PARP inhibitors might be one of the most promising targeted substances since the efficacy of this monotherapy reached a response rate of approximately 40% over an average of six months. A phase II clinical trial of Olaparib (AZD2281), an oral PARP inhibitor, showed an objective responsive rate of 33% (14/42) in OCs who received multiple prior treatments. Most of these responsive cases (11/14) had a germline BRCA1/BRCA2 mutation [31].

Small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is the most common undifferentiated malignancy in women under 40 years. The diagnosis of SCCOHT is challenging even for the expert pathologists due to its overlapping morphological features with juvenile granulosa cell tumor and other malignancies in the ovary. Recent advance on genomic alterations has substantially changed our recognition on this rare ovarian cancer. Familial cases have been suggested to show an autosomal dominant transmission. A WES study on six individuals from three families discovered germline mutations in SMARCA4 (BRG1) [32]. Germline or somatic mutations were frequently found and correlated with loss of protein expression in sporadic SCCOHT cases [33, 34]. Subsequent immumohistochemical studies have indicated that loss of SMARCA4 and SMARCA2 protein expression is sensitive and specific for SCCOHT, that make them valuable in the differential diagnosis of SCCOHT [35-37]. These findings suggest that SCCOHT is a new entity of the atypical teratoma/rhabdoid tumor (AT/RT) family. Genetic testing and consideration of the novel chemotherapeutic regimens used to AT/RT will help to improve the prognosis of SCCOHT.

## Genetic Variants and Somatic Mutations in OC

It is well established that two distinct pathways of pathogenesis exist in OC, type I and type II [2]. Type I OC encompasses histotypes of low-grade serous carcinoma, endometrioid carcinoma, mucinous carcinoma and some clear cell carcinomas while type II contains HGSOC and other poorly differentiated carcinomas. Both types of OC are known to harbor

mutations in different genes [2, 3]. ARIDA1 mutations are present in 46% and 30% of endometrioid carcinoma and clear cell carcinoma, respectively [38]. Moreover, consistent ARIDA1and PIK3CA mutations in ovarian clear cell carcinoma and concurrent endometriosis support that benign endometriotic lesions are clonally related to clear cell carcinomas [39]. The next-generation sequencing technology and bioinformatics approaches enable new high-throughput genomic screening, such as genome-wide association studies (GWAS), whole genome sequencing (WGS), and whole exome sequencing (WES), RNA-sequencing and other targeted methods. So far, these methods have been widely applied in cancer research. The Cancer Genome Atlas (TCGA) has been developed to generate and analyze molecular profiles at the DNA, mRNA, protein and epigenetic levels for various cancers and their subtypes including OC [40]. The surge of deep sequencing uncovered a substantial number of cancer susceptibility genetic variants and somatic mutations in OC tissues. These genetic data give us more significant insights on the pathogenesis and clinical intervention on OCs.

## Genome-wide association studies (GWAS)

GWAS is designed to compare genotype frequencies of the common variants throughout the genome between cases and controls. GWAS studies identified many genetic variants, most single nucleotide polymorphisms (SNP), that show strong associations with OC risks [Table 1, 41-45]. A study on 457 Icelanders using SNP chips found a rare (0.41% allelic frequency) frameshift mutation, c.2040_2041insTT, in the BRIP1 (FANCJ) gene that confers an increase in OC risk with a moderate penetrance [46]. Recent investigations have been focused on the association between OC risks and SNPs in susceptible chromosomal regions (such as 2q31, 3q25, 5p15, 8q21, 8q24, 10p12, 17q12, 17q21.31, and 19p13) or in a specific functional group (such as epithelial-mesenchymal transition genes) in a large sample size [47, 48]. However, these variants only showed weak effects on OC risk with the odds ratios around 0.8 or 1.2 in general. In the evaluation of cancer risk, these low-penetrant loci have little clinical utility in the general population, but may be useful for counseling women with BRCA1/BRCA2 mutations [Fig. 1]. BRCA1 mutation carriers with the TT genotype at SNP rs3814113 (at the 9p22.2 locus) had a predictive OC risk to age at 80 years of 48% while those with the CC genotype had a risk of 33% [49].

GWAS is also helpful to identify genetic predictors of treatment outcome in OC patients [Table 1, 50-52]. A recent GWAS study on 1244 patients with ovarian serous carcinoma given carboplatin- and paclitaxel-based chemotherapy, identified that the minor alleles of two SNPs (rs7874043 and rs72700653) were associated with progression-free survival [50]. Functional analysis showed that both SNPs act as a transcriptional enhancer on PSIP1 and CCDC171 promoters. PSIP1 is a known oncogene that controls a caspase-independent lysosomal cell death pathway. Successful inhibition of PSIP1 may provide a novel approach to target OC. A GWAS study from Ovarian Cancer Association Consortium identified five SNPs significantly associated with poorer outcomes in OC patients underwent first-line treatment of cytoreductive surgery and chemotherapy [51]. Three of them, rs4910232 (11p15.3), rs2549714 (16q23), and rs6674079 (1q22), were located in long noncoding RNAs (lncRNAs) RP11-179A10.1, RP11-314O13.1, and RP11-284F21.8, respectively. Huang RS et al. [52] also found that SNP rs1649942 was significantly associated with decreased progression free survival and overall survival in OC patients received at least four cycles of carboplatin-based chemotherapy. The potential mechanism of this SNP may be through its association with the expression of 18 target genes, ten of which are correlated with carboplatin sensitivity in lymphoblastoid cell lines. Taken together, genetic variations in chemotherapy response appears to be partly responsible for the different clinical outcomes in OC patients following chemotherapy.

## Whole-exome sequencing (WES)

WES allowed the discovery of low-frequency variants in individuals with familial, highly penetrant diseases, or those with certain complex quantitative traits by using exome array-captured sequencing. In 2011, TCGA Network provided a large-scale integrative view of mRNA expression, miRNA expression, promoter methylation, DNA copy number and exome sequencing in HGSOCs [3]. That study found frequent TP53 mutation (96%), infrequent but statistically recurrent mutations in other genes including BRCA1, CSMD3, NF1, CDK12, FAT3, GABRA6, GRCA2 and RB1, and additionally 113 significant focal DNA copy number aberrations (CNAs) and promoter methylations of 168 genes. The integrative analysis of various genetic alterations generated four transcriptional subtypes, three microRNA subtypes, four promoter methylation subtypes and a 193-gene transcriptional signature, which were correlated with overall survival in OC. A later WES study with 429 OC cases from TCGA [20] observed novel significantly mutated genes including NRAS, NF2, ATR, ATP, and APC, etc.. Moreover, the combined analysis of germline-somatic changes revealed several pathways with significant

enrichment of variants, such as Fanconi/DNA repair pathway, MAPK pathway and histone methyltransferases in HGSOCs[20]. These discoveries from the TCGA project provided molecular targets and related pathways, for potential therapeutic interventions on OC in the future.

Two WES studies with a small sample size demonstrated that point mutations were very few in low grade serous carcinoma (70 somatic mutations in 64 genes in 7 cases) and serous borderline tumors of the ovary (16 somatic mutations in 2 cases) [53, 54]. These data indicates that low grade serous carcinoma and serous borderline tumor do not require many mutations to achieve malignancy or malignant potential. Therefore, target therapeutic agents, such as those against BRAF and KRAS, might be particularly effective for the recurrent inoperative cases. Ryland GL, e al. [55] performed WES on 24 mucinous tumors including 5 benign cystadenomas, 8 borderline tumors, and 11 carcinomas. They found a high percentage of TP53 mutations and recurrent mutations in RNF43, ELF3, GNAS, ERBB3 and KLF5. These diversified mutations suggest genetic heterogeneity in the pathogenesis of ovarian mucinous carcinoma and confer to a novel targeted therapy for some high grade mucinous carcinomas considering of the limited success in treating advanced mucinous carcinomas.

Most HGSOCs benefit from platinum- and taxol-based chemotherapy initially, but progressive chemoresistance will occur subsequently and result in tumor recurrence and metastasis eventually. Little is known about the underlying mechanisms of chemoresistance. Lanbrechts S et al. [56] investigated on the WES and SNP profiling in 31 before and after first-line chemotherapy-paired OC tissues. They found frequent HR-deficiency (loss-of-heterozygosity and mutation in HR genes) in primary tumors, relapse tumors and tumors resistant to second-line platinum, implicating the persistent qualification for second-line PARP inhibitor treatment in recurrent and chemoresistant tumors. A platinum score of 13 regions with copy number alterations including MECOM, CCNE1 and ERBB2 increased in recurrent tumors. The copy number alterations contribute to the acquired resistance after a single line of platinum therapy. About 58% (623/1074) of somatic mutations were identical between primary and recurrent tumors. Consistent with this finding, another WES study showed that most mutations in primary tumors persisted in tumor cells from ascites at three time points (primary, first recurrence and second recurrence) [57]. Both studies suggested that most clones could escape current chemotherapy treatments. In addition, novel mutations in the Golgi and ECM pathways were also found in recurrent tumors [57]. Both WES studies suggested the genome evolution in the progression of HGSOC from primary to recurrent or chemoresistant diseases. Biopsies at relapse are prerequisite for personalized genomic evaluation to tailor treatment. The persistent mutations in the recurrent tumors indicate that improved target therapy towards these mutations may be ultimately effective for the prevention and intervention of tumor relapse while novel mutations may offer new therapeutic targets for recurrent tumors. Recently, Liu, et al. [58] analyzed WES data from 512 OC patients and found that mutations from 8 members of the ADAMTS family were significantly associated with a higher chemotherapy sensitivity, a longer platinum-free duration, a better overall survival and progression-free survival than ADAMTS wild-type cases, even after adjustment by BRCA1/BRCA2 mutations, surgical stage, residual tumor, and patient age. ADAMTS mutations appeared to be helpful molecular markers for predicting chemotherapy response in OC patients.

The necrotic or apoptotic cells can release DNA into the bloodstream [59]. The term, cell-free DNA (cfDNA), is referred to the dead cell-derived DNA that is selectively isolated from the plasma. In cancer patients, such cfDNA from tumor cells is called circulating tumor DNA (ctDNA) [60].The majority of plasma cfDNA in cancer patients come from cancer cells. ctDNA is a reflection of genomic alterations in solid cancers and offer a unique non-invasive "liquid biopsy" for serially monitoring cancers. Theoretically, all genomic alterations in cancer tissues, such as gene mutations, microsatellite instability, gene amplifications, and promoter hypermethylation, etc., can be detected in ctDNA. The potential application of high-throughput sequencing, such as WES, in plasma ctDNA constitutes a new paradigm for the detection and monitoring of various cancers. Murtaza M [61] undertook exome sequencing to track genomic evolution of metastatic cancers in response to therapy in serial plasma samples from six patients with advanced breast, ovarian and lung cancers over 1-2 years. They found a functional mutation in PIK3CA following treatment with paclitaxel, a truncating mutation in RB1 following treatment with cisplatin, a truncating mutation in MED1 following treatment with tamoxifen and trastuzumab, and following subsequent treatment with lapatinib, a splicing mutation in GAS6 in the same patient, and a resistance-conferring mutation in EGFR following treatment with gefitinib. This non-invasive approach characterized plasma cancer exomes is applicable to monitor OC patients with high systemic tumor burden, metastasis and therapy response.

### Whole-genome sequencing (WGS)

WGS is technically propelled by next generation sequencing. It can detect rare and novel genetic variations, and may become the most promising approach in precision (personalized) medicine with the decreasing sequencing cost and expanding bioinformatics resources in the future. The first WGS analysis of tumor and germline DNA samples from 92 patients with HGSOC was reported recently [4]. The tumors included primary refractory, resistant, sensitive and matched acquired resistant disease. This study confirmed previous findings including prevalent TP53 mutations, infrequent somatic mutations in other driver genes, deficiency in HR pathway (50% cases including germline and somatic mutations of related genes and BRCA1 methylation), and CCNE1 gain/amplification (19% cases). CCNE1 gain/amplification, which was largely exclusive of HR pathway dysfunction, was common in primary resistant and refractory tumors. Tumors with BRCA1 mutations had more structural variants and genome-wide coding small mutations compared to HR-intact samples. They had a favorable response to treatment. Disruption of transcription unit (gene breakage), and commonly inactivated tumor suppressor genes NF1, RB1, PTEN, RAD51B, FOXO1, and BCL2L11, also contributed to the acquired chemoresistance. Other molecular events associated with acquired chemoresistance included reversions of germline mutations in BRCA1/BRCA2 in individual patients, loss of promotor methylation in BRCA1, transition of C2/immune molecular subtype to C1 subtype, and recurrent promoter fusions involving ABCB1, resulting in overexpression of the drug efflux pump MDR1. These findings clearly demonstrate the heterogeneity and adaptability in the genome of HGSOC with the pressure of chemotherapy. Personalized genomic evaluation should be urgently prompted for patients with recurrent HGSOC. A diversity of novel precision solutions should critically be encouraged to overcome the resistance to current chemotherapy.

## DNA CNAs in OC

Genomic instability is a hallmark of cancer, and genomic aberrations in the form of DNA copy number alterations are important in tumorigenesis of OC [4]. HGSOC is genetically unstable by showing highly rearranged karyotypes with numerical and structural chromosome aberrations. Conventional comparative genome hybridization (CGH) and aCGH have been used to scan genome-wide genetic alterations. Knowledge about the acquired genomic CNAs of OCs remains unsatisfactory in general because of small sample sizes in most studies and the insufficient genomic mapping resolution (5~10 Mb by CGH and 0.2~2Mb by aCGH) to delineate precise patterns of gene amplifications and losses. The identification of these chromosomal aberrations and the genes rearranged behind the aberrations may ultimately have a clinical application in predicting chemotherapy response, prognosis, and recurrence risks, etc.

Resistance to chemotherapy in OC is not well understood yet. Specific CNAs are potential biomarkers for chemoresistance in OC [Table 1] [56, 62-66]. Osterberg L, et al.[62] found that gains of 1q, 5q14~q23, and 13q21~q32, and losses of 8p and 9q were clinically associated with carboplatin resistance in 63 early-stage OCs. Their later study using high solution aCGH further specified the genomic regions of carboplatin resistance [63]. The regions with copy number changes might contain candidate genes involved in carboplatin resistance, which are essential for improving the prognosis and treatment of women with OC. The candidate genes in these regions include RASAL2, GPR52, RGL1, FAIM3, RAP2A, and EXTL3, etc [63, 64]. The extensive non-linear divergence of chemo-sensitive and resistant sub-clones on cell line series before and after platinum resistance supported the presence of a profound intra-tumor genetic heterogeneity in genomically unstable HGSOCs [67]. Identifying different genetic sub-clones has important clinical implications for developing novel therapy targeting a common or specific genetic alteration, and personalized, combined therapies targeting different subpopulations.

Some CNAs may be associated with tumor relapse and survival in OCs [Table 1, 68-75].Amplifications of some candidate genes, such as CCNE1 and PPM1D, were potential indicators for poor outcomes in ovarian serous carcinomas and clear cell carcinomas [4, 69, 75]. These genes are possible therapeutic targets for a specific subgroup of OCs.

Trastuzumab, a humanized monoclonal antibody targeting the human epidermal growth factor receptor 2 (HER2) has impacted on the treatment of breast cancer. Effort has been taken to investigate HER2 amplification and related target therapy in OCs. The observed rates of HER2 overexpression and/or amplification in OCs ranged from 2% to 66%. Tuefferd et al. [76] found that the rate of HER2 overexpression was 12.8% (42/320) in patients with advanced ovarian or primary peritoneal carcinomas. HER2 overexpression did not demonstrate a prognostic value in this study. In contrast, a tissue microarray study on 401 serous OCs showed that HER2 amplification was detected in 7% of the cancers, and was associated with a poor prognosis, a shorter disease-free survival and overall

survival [77]. HER2 amplification was observed in about 19% ovarian mucinous carcinomas, but was not indicative for the assessment of overall survival [78]. Whether HER2 amplification is of prognostic significance is yet to be determined, HER2-directed therapies remain a potential target considering the dismal prognosis of patients in late stages of OCs.

## Epigenetic alterations in OC

Epigenetic modifications, which unlike genetic alterations, does not alter the primary DNA sequence, but influence the transcriptional process by DNA methylation, histone modifications and dysregulations of nucleosomes. DNA hypermethylation typically occurs in gene-associated CpG islands and hyomethylation in repetitive genomic DNA. Core histone proteins (H2A,-2B, -3 and -4) can be modified by methylation, acetylation, and phosphorylation, etc. Many tumor suppressor genes can by silenced by DNA hypermethylation. The well characterized genes include BRCA1, hMLH1, MGMT, HOXA9, RASSF1A, OPCML and CCBE1, etc [79, 80]. Emerging data have implicated that the inactivation of tumor suppressor genes by epigenetic alterations should be regarded as "the second hit" in the Knudson's two-hit hypothesis in cancer biology. Novel epigenetic therapies, such as DNA methyltransferase inhibitors and histone deacetylase inhibitors, have become potential therapeutic options for various cancers including OC.

Epigenetic biomarkers, particularly DNA methylations, have potential clinical utility for detection/diagnosis, chemotherapy response and prognosis in OC [Table 2, 81-95]. Epigenetic regulation of Wnt and Akt/mTOR pathways may serve as biomarkers for prognosis and/or treatment response in OC [91, 92, 96, 97]. Dai et al. [92] examined promoter methylation at 302 loci in a panel of 137 Wnt pathway genes in 111 screening cases and 61 validation cases. They demonstrated that methylations at 7 loci (FZD4, DVL1, NFATC3, ROCK1, LRP5, AXIN1, and NKD1) were associated with poor progression-free survival. Furthermore, hypermethylations of DVL1 and NFATC3 were involved in poor response to platinum chemotherapy. Patients with progressive or stable disease had increased methylation than those with partial or complete response. The authors recently analyzed the progression free survival-related DNA methylation at the CpG island promoter of genes in other pathways including Akt/mTOR, p53, redox, and HR DNA repair [97]. They identified that methylations of promoter regions of VEGFB, VEGFA, HDAC11, FANCA, E2F1, GPX4, PRDX2, RAD54L and RECQL4 were associated with increased hazard of disease progression, independently from conventional clinical prognostic factors both in the screening cohort (n=150) and the TCGA validation cohort (n=311). Moreover, methylations at VEGFB and GPX4 were more likely to have poor response to chemotherapy. In combination with methylation profile in the previous Wnt pathway [92], they constructed a diagnostic model including the methylations of NKD1, VEGFB and PRDX2, from which, methylation index was calculated to identify two distinct prognostic groups. The patients with increased methylation index were more likely to have poor response to chemotherapy.

**Table 2.** Selected genes with promotor hypermethylation and their clinical correlations in ovarian carcinomas.

| Genes | Clinical correlations | Ref(s). |
|---|---|---|
| RASSF1A | Detection of OC | 81, 82 |
| BRCA1 | Detection of OC; poor prognosis; improved chemotherapy response | 81,83,84 |
| APC | Serum/ascites diagnosis of OC | 81,83 |
| MGMT | Detection of OC; improved chemotherapy response | 83,85 |
| hMLH1 | Poor prognosis; improved chemotherapy response | 86-88 |
| HOXA9 | Detection of OC | 89 |
| OPCML | Detection of OC | 90 |
| SFRP-1, -2, -4, -5 | Detection of OC; Cancer recurrence; Poor prognosis | 91 |
| FZD4, DVL1, NFATC3, ROCK1, LRP5, AXIN1, and NKD1 | Poor prognosis | 92 |
| FBXO32 | Poor prognosis | 93 |
| HOXA11 | Poor clinical outcome. | 94 |
| FANCF | Cisplatin resistance | 95 |

Abbreviations: OC=ovarian carcinoma.

Some recent studies have focused on genome-wide identification of methylated biomarkers in OCs. The methylated DNA immunoprecipitation microarray (MeDIP-chip) identified 367 CpG islands specifically methylated in OC versus normal ovaries [98]. A publication from the TCGA data found 168 epigenetically silenced genes [3]. AMT, CCL21, and SPARCL1 showed promtor hypermethylation in most cancers and may serve as biomarkers for the presence of OC. Consensus clustering of these methylations across the tumors generated four subtypes that were associated with prognostic differences. Other genome-wide studies in OC have produced methylation signatures associated with progression-free survival [99, 100], and response to platinum-based chemotherapy [101]. Genome-wide methylation analyses can reliably identify markers of potentially prognostic value. Häfner et al. [102] detected 220 differentially methylated regions in tumor tissue of patients with short vs. long progression-free survival (106 hypo- and 114 hypermethylated regions) by genome-wide array analyses. Validation experiments proved that patients

harboring methylation at the CpG island of RUNX3/CAMK2N1 had a significantly lower progression free survival. These potential biomarkers identified by genome screens merits further investigation in large cohorts with well clinical documentation.

Non-invasive assay has been successfully applied to detect specific methylation profiles in the blood or ascites from OC patients in several studies [81, 82, 90, 103]. Liggett TE, et al. [82] found that different methylation patterns of three promoters (RASSF1A, CALCA, and EP300) in cell-free plasma DNA can discriminate between OC vs. healthy controls with a sensitivity of 90.0% and a specificity of 86.7%. Similarly, a combined analysis of methylations at five genes (BRCA1, HIC1,PAX5, PGR and THBS1) in patient plasma DNA generated 85% sensitivity and 61% specificity for cancer detection [103]. A recent study from China [90] showed that OPCML hypermethylation for serum samples was detected in 80.0% OC patients and not in healthy individuals. Tumor-specific hypermethylations in serum DNA appeared to be valuable biomarkers for the early detection of OC.

## Conclusions and future perspectives

A rich resource of information has been generated from molecular and genetic studies on OCs. Identification of germline mutations of BRCA1/BRCA2 and other genes have substantially propelled the surveillance and prevention of hereditary OCs. Germline/somatic mutations of SMARCA4 are now essential for the precision diagnosis of SCCOHT, and provide potential novel therapy for this rare aggressive ovarian cancer. The application of new high-throughput genomic techniques has uncovered a substantial number of genetic susceptible variants, somatic mutations, copy number alterations and epigenetic modifications in OC. Theoretically, the tumor specific genetic alterations correlate with the presence of OC. As a collar, they are precious biomarkers with potential clinical utility for risk assessment, diagnosis/detection, prognosis and response to chemotherapy, and target/precision therapy in OC patients [Table 1; Table 2]. Genetic susceptible variants are important tools for molecular test in high-risk populations. Biomarkers from early staging OC cases are helpful for early cancer detection and that from recurrent tumors for monitoring systemic tumor burden, metastasis and therapy response. Fortunately, it is clear that some genetic changes, such as gene mutations and DNA methylations, have been detected by non-invasive methods in the blood and ascites from women with OC. Moreover, even detection of most genomic and epigenomic alterations in tumor tissue can provide valuable information for precision medicine and prognostic prediction at present. Finally, the presence of great heterogeneity and apparent chemotherapy-exerted clonal evolution in OC underscore the necessity of genomic evaluation for different tumor tissues, such as primary and metastasis/recurrence, or low and high grade, and adjust therapy accordingly.

Before translating these genomic and epigenomic alterations into clinical utility, several key points should be considered: 1) Large, well annotated prospective cohorts are required to further validate the clinical significance of the potential biomarkers. The performance of these biomarkers should be reproducible and consistent among various laboratories. 2) The biological samples for research should be obtained, stored and processed properly following the required protocols and standards. The related clinicopathological documentations should be clear and precise including longitudinal follow up and other details. 3) Novel non-invasive assays should be prompted for early detection, diagnosis, prognosis and therapy response. Cell free plasma/serum DNA provides a hopeful source for these molecular tests, but more compelling evidence should be accumulated to support the identical genetic/epigenetic patterns between samples from blood and tumor tissues. 4) For target/precision therapy, extensive molecular biological experiments should be performed to clarify the functions of potential targets, and more robust and stable models should be developed to identify molecular signatures with various clinical phenotypes. A combination of target therapy, epigenetic therapy and conventional therapy, will holds great promise for the treatment of OCs in the future.

## Abbreviations

OC: ovarian carcinoma; HGSOC: high grade serous ovarian carcinomas; HBOC: hereditary breast/ovarian cancer; RRSO: risk-reducing salpino-oorphorectomy; GWAS: genome-wide association studies; WGS: whole genome sequencing; WES: whole exome sequencing; TCGA: The Cancer Genome Atlas; cfDNA: cell-free DNA; CGH: comparative genomic hybridization; aCGH: array comparative genomic hybridization; CNA: copy number aberration; SNP: single nucleotide polymorphism; HR: homologous recombination; SCCOHT: Small cell carcinoma of the ovary, hypercalcemic type

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## Competing interests

The authors have declared that no competing interest exists.

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