Ovarian Cancer Genome and Molecular Experimental Sciences

7

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Abstract

A large number of genomic studies have provided important insights into molecular pathogenesis of ovarian cancer. Ovarian cancer is divided into two types: type I and type II tumors. Type I ovarian tumors include clear cell, endometrioid, mucinous, and low-grade serous carcinomas, while type II tumors are mainly high-grade serous carcinomas. High-grade serous carcinomas are characterized by *TP53* gene mutations and extensive copy number alterations. Approximately half of high-grade serous ovarian carcinomas harbor homologous recombination pathway deficiency. Clear cell carcinomas are characterized by upregulation of *HNF1B* and *IL6* and mutations in *PIK3CA* and *ARID1A*. Alterations of HNF1B pathway, IL6 pathway, PI3K pathway, and SWI/SNF complex are influenced by copy number alterations and epigenetic regulation. Endometrioid carcinomas are divided into low-grade (G1–G2) and high-grade (G3) tumors, although some of high-grade serous carcinomas have been misclassified as high-grade endometrioid carcinomas. Low-grade endometrioid carcinomas harbor mutations in *CTNNB1, PTEN, KRAS, PIK3CA,* and *ARID1A*, while high-grade endometrioid carcinomas harbor *TP53* mutations. Mucinous carcinomas exhibit ERBB2/KRAS/BRAF pathway activation by *KRAS* or *BRAF* mutations or *ERBB2* amplifications. Unlike other type I tumors, half of mucinous carcinomas harbor *TP53* mutations. Low-grade serous carcinomas evolve from serous borderline tumor. *KRAS* and *BRAF* mutations are common in serous borderline tumors and low-grade serous carcinomas.

Keywords

Ovarian cancer • Genome • Genetic alterations

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7.1 Introduction

There are two types of epithelial ovarian cancer: type I and type II [1]. Type I tumors grow slowly, while type II tumors behave aggressively. Type I tumors contain low-grade serous, clear cell, endometrioid, and mucinous cancers, while type II tumors are mainly high-grade serous cancers. A large number of genomic studies have provided important insights into molecular pathogenesis of ovarian cancer. This chapter summarizes genomic alterations of epithelial ovarian cancer from histology to histology.

7.2 High-Grade Serous Ovarian Carcinoma

7.2.1 Germline Mutations in Ovarian Carcinoma

Ovarian carcinoma, mainly high-grade serous, can occur via germline gene mutations in DNA repair system. In a study of 1915 ovarian carcinoma cases, 347 (18%) carried pathogenic germline mutations, 280 (15%) had mutations in BRCA1 (n = 182) or BRCA2 (n = 98), and the remaining cases had mutations in other 5 homologous recombination (HR) pathway genes (*BARD1, BRIP1, PALB2, RAD51C, RAD51D*) and four mismatch repair (MMR) genes (*MSH2, MLH1, PMS2,* and *MSH6*) [2].

7.2.2 The Genomic Landscape of High-Grade Serous Ovarian Carcinoma

The Cancer Genome Atlas (TCGA) project analyzed more than 300 high-grade serous ovarian carcinoma cases with whole-exome sequencing, SNP array (to analyze copy number alterations), mRNA expression microarray, DNA methylation microarray, and microRNA microarray [3]. The TCGA analyses identified four ovarian cancer transcriptional subtypes, three miRNA subtypes, four promoter methylation subtypes, and a transcriptional signature correlated with prognosis.

Strikingly, nearly all the high-grade serous ovarian carcinoma cases harbored somatic mutations in *TP53* (96%). Furthermore, a study by five gynecologic pathologists who reviewed the negative TP53 cases from TCGA study found that all of the negative tumors except for one were histologically misclassified. The one exception contained a homozygous deletion of the gene, indicating that all high-grade serous ovarian carcinomas have a TP53 abnormality, which is almost always a mutation [4]. Somatic gene mutations other than *TP53* occurred in less than 5% of high-grade serous ovarian carcinomas.

Additional feature of high-grade serous ovarian carcinoma is the widespread copy number alterations. The TCGA analysis identified regional copy number aberrations including 8 recurrent gains and 22 losses [3], all of which have been reported previously [5]. Focal amplifications were observed in 63 regions. The most common focal amplifications encoded *CCNE1*, *MYC*, and *MECOM* in more than 20%

of tumors. The TCGA study also identified homozygous deletions of known tumor suppressor genes, such as *PTEN*, *RB1,* and *NF1*. A focal deletion at 10q23.31 that includes only *PTEN* has been found in approximately 7% of tumors, which is associated with downregulation of *PTEN* mRNA expression [3]. Another group confirmed that *PTEN* loss is a common event in high-grade serous ovarian cancer with significantly worse prognosis [6].

Exome sequencing has a limited ability to detect gene mutation by structural rearrangement. A whole-genome sequencing analysis for 92 cases of high-grade serous ovarian carcinoma was performed focusing on the mechanism of chemoresistance [7]. Although *NF1* and *RB1* were inactivated by truncating point mutations and indels in limited number of samples (*NF1*; $n = 3$, *RB1*; $n = 2$, out of 80), inclusion of gene breakage raised the frequency of inactivating mutations to 20% for *NF1* and 17.5% for *RB1*. Gene inactivation by breakage was also seen for *PTEN* (7.5%) and *RAD51B* (5%).

Homologous recombinant (HR) pathway-deficient tumors, having extensive copy number alterations and increased single nucleotide variants, are sensitive to platinum and PARP inhibitor. HR pathway-deficient tumors tend to use error-prone nonhomologous end joining to repair DNA, leading to extensive genome DNA variations. Approximately 50% of high-grade serous ovarian carcinomas exhibit genetic or epigenetic alterations in the FA-BRCA pathway (Fig. 7.1) [3, 8]. In TCGA analysis, germline *BRCA1/2* mutations are present in 14% [3], whereas somatic *BRCA1/2* mutations have been identified in 6% [3]. Importantly, 81% of *BRCA1* and 72% of

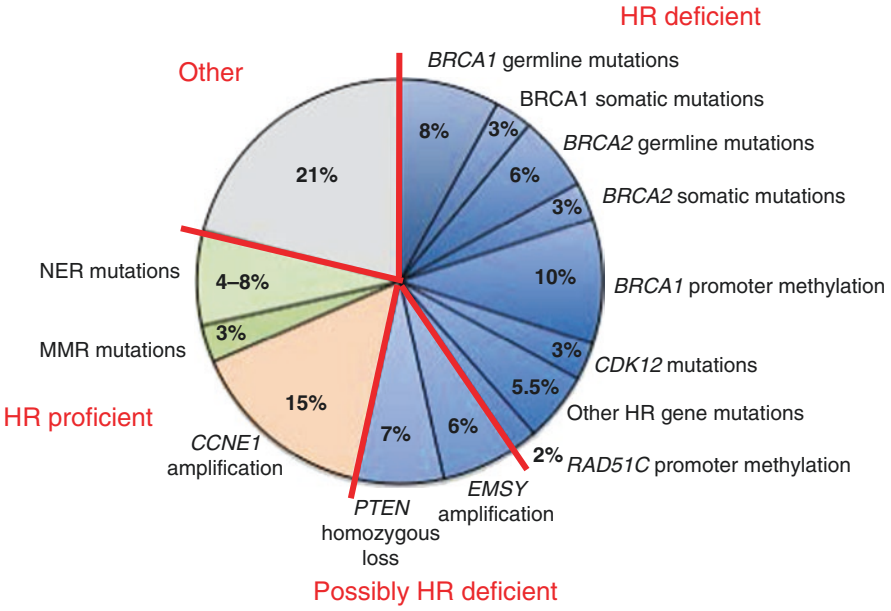

Fig. 7.1 HR-deficient and HR-proficient tumors of high-grade serous ovarian carcinoma [8]

BRCA2 mutations are accompanied by heterozygous loss [3]; thus, both alleles are inactivated. Epigenetic silencing via promoter hypermethylation occurs for *BRCA1*, but not *BRCA2*, in approximately 10% and is mutually exclusive of *BRCA1/2* mutations [3]. Other HR pathway alterations include mutations in FA genes (mainly *PALB2, FANCA, FANCI, FANCL,* and *FANCC*), in RAD genes (*RAD50, RAD51, RAD51C,* and *RAD54L*), and in DNA damage response genes (*ATM, ATR, CHEK1,* and *CHEK2*). *RAD51C* was also epigenetically silenced via promoter hypermethylation in about 2% of the cases [3]. CDK12 is known to promote the transcription of several HR pathway genes, including *BRCA1*. Inactivation mutation of *CDK12*, found in 3% of the cases [3], leads to downregulation of *BRCA1* and other HR genes [9, 10]. HR defect may also occur via indirect mechanism. PTEN inactivation has been reported to be synthetically lethal with PARP inhibition, and one of the proposed mechanisms is downregulation of *RAD51* [11, 12]. Additionally, overexpression and amplification of *EMSY*, which inhibits transcriptional activity of BRCA2 [13], is found in as high as 17% of high-grade serous ovarian carcinomas [3]. Furthermore, there may be other mechanisms of HR deficiency, such as miRNAs that target BRCA1/2 [14, 15].

HR pathway proficient tumors with *CCNE1* amplification were common in primary resistant and refractory cases [7]. Inactivation of the p53 pathway and activation of the CCNE1 pathway also contribute to chromosomal instability [16]. Alterations in nucleotide excision repair (NER) and mismatch repair (MMR) have been reported in up to 8% and 3% of high-grade serous ovarian carcinomas, which tumors are sensitive to platinum and resistant to PARP inhibitor [17].

Mechanism of acquired resistance to chemotherapy included breakage of tumor suppressor genes, reversion mutation of *BRCA1/2* mutated cases, and upregulation of *BRCA1* gene expression by demethylation of the methylated *BRCA1* promoter region in a primary tumor. Additionally, gene fusion of *ABCB1* with *SLC25A40* promoter caused upregulation of *ABCB1* expression, which can cause increased excretion of chemotherapeutic agents [7] (see also Sect. 3.4.1.1 in Chap. 3).

7.2.3 Experiments to Identify Origin of High-Grade Serous Ovarian Carcinoma

Recently, fallopian tubal epithelial cell has been thought as the origin of high-grade serous ovarian carcinoma [1]. Using a genetically engineered mouse that expresses Cre recombinase from a *Pax8* promoter, *Brca*, *Tp53*, and *Pten* genes were targeted in fallopian tubal secretory epithelial cells. This mouse model generated serous tubal intraepithelial carcinoma as the precursor lesion that gave rise to high-grade serous ovarian and peritoneal carcinomas [18]. In this model, tumor-bearing mice had higher serum CA125 levels than controls. Furthermore, the tumors had extensive copy number alterations similar to human high-grade serous ovarian carcinomas.

There is another idea regarding cell of origin of high-grade serous ovarian carcinoma. Cells of the hilum ovarian surface epithelium, the transitional area between

the ovarian surface epithelium, mesothelium, and tubal epithelium, express stem cell markers and display stem cell properties. The hilum cells show increased transformation potential after inactivation of tumor suppressor genes Tp53 and Rb1. Therefore, stem cell niches in those areas are susceptible to malignant transformation and could be the origin of high-grade serous ovarian carcinoma [19].

7.3 Ovarian Clear Cell Carcinoma

7.3.1 Gene Expression of Ovarian Clear Cell Carcinoma

Ovarian clear cell carcinoma shows unique clinical features including an association with endometriosis and poor prognosis. A gene expression microarray analysis identified genes commonly expressed in both ovarian clear cell carcinoma cell lines and clinical samples, which comprise an ovarian clear cell carcinoma gene signature. The gene signature contains known markers of ovarian clear cell carcinoma, such as *HNF1B*, *VCAN*, *IL6,* and other genes that reflect oxidative stress. Expression of ovarian clear cell carcinoma signature genes was induced by treatment of immortalized ovarian surface epithelial cells with the contents of endometriotic cysts, indicating that the ovarian clear cell carcinoma signature is largely dependent on the tumor microenvironment [20].

7.3.2 DNA Methylation Analysis of Ovarian Clear Cell Carcinoma

Recently, genome-wide methylation and expression data were generated for 14 ovarian clear cell carcinoma, 32 non-ovarian clear cell carcinoma, and four normal cell lines. Consensus clustering showed that ovarian clear cell carcinoma is epigenetically distinct. Inverse relationships between expression and methylation in ovarian clear cell carcinoma were identified, suggesting functional regulation by methylation, and included 22 hypomethylated genes and 276 hypermethylated genes. The ovarian clear cell carcinoma-specific hypomethylated genes were involved in response to stress and many contain HNF1-binding sites, while the ovarian clear cell carcinoma-specific hypermethylated genes included members of the ERα network and genes involved in tumor development [21].

7.3.3 Genetic Analyses of Ovarian Clear Cell Carcinoma

ARID1A mutations were reported in 46–57% and *PIK3CA* mutations in 31–33% of ovarian clear cell carcinoma samples [22–24]. A whole-exome sequencing of 39 ovarian clear cell carcinoma samples identified recurrent somatic mutations in 426 genes [25]. In these 39 samples, *ARID1A* (62%) and *PIK3CA* (51%) were frequently mutated, and known key ovarian clear cell carcinoma-related genes such as *KRAS* (10%), *PPP2R1A* (10%), and *PTEN* (5%), as well as novel genes *MLL3* (15%),

ARID1B (10%), and *PIK3R1* (8%) were also mutated. Gene interaction analysis and functional assessment revealed that mutated genes were clustered into groups pertaining to chromatin remodeling, cell proliferation, DNA repair and cell cycle checkpointing, and cytoskeletal organization.

A copy number variation analysis based on the above exome sequencing identified frequent amplification of *MYC* (chr8q, 64%), *ZNF217* (chr20q, 54%), and *ERBB2, STAT3, HNF1B, PPM1D* (chr17q, 46%) loci as well as deletion in *SMARCA4* (chr19p, 41%), *RB1* (chr13q, 28%), *NOTCH1* (chr9q, 21%), and *SMAD4* (chr18q, 21%) loci. Other copy number alterations included amplification of *IL6*, *IL6R*, *KRAS*, *PIK3CA*, *PIK3C2B, CDK2, CDK4*, and *CCNE1*, as well as deletion of *ARID1A, SMARCC1, SMARCA2, ARID1B, CDKN1A, CDKN2A, CDKN2B*, and *TP53*. Integration of the analyses discovered that frequently mutated or amplified/deleted genes were involved in the KRAS/PI3K signaling (82%) and MYC/RB signaling (75%) pathways as well as the critical chromatin remodeling complex SWI/SNF (85%) [25] (see also Sect. 3.4.3 in Chap. 3).

7.3.4 Role of ARID1A, PIK3CA, and IL6 in the Carcinogenesis of Ovarian Clear Cell Carcinoma

Concurrent *Arid1a* inactivation and *Pik3ca* activation in mouse ovaries generated adenocarcinomas similar to human ovarian clear cell carcinomas. These tumors expressed Hnf1b, a marker of ovarian clear cell carcinoma. Furthermore, in this model, the tumor growth was promoted through sustained IL6 overproduction [26].

Ovarian clear cell carcinoma was generated in vitro by introducing *ARID1A* knockdown and mutant *PIK3CA* into a normal human ovarian epithelial cell line. Loss of *ARID1A* impairs the recruitment of the Sin3A-HDAC complex, while the *PIK3CA* mutation releases RelA from IκB, leading to NF-kB pathway activation resulting in IL6 overexpression [27].

Collectively, these findings indicate that *ARID1A* and *PIK3CA* mutations, frequently seen in ovarian clear cell carcinoma, are sufficient to generate ovarian clear cell carcinoma, associated with the specific gene expression including *HNF1B* and *IL6* (see also Sect. 3.4.3 in Chap. 3).

7.4 Ovarian Endometrioid Carcinoma

7.4.1 Genetic Analysis of Ovarian Endometrioid Carcinoma

Gene mutations in ovarian endometrioid carcinoma samples with different grades (grade 1, $n = 20$; grade 2, $n = 26$; grade 3, $n = 26$) were analyzed, and mutations in *CTNNB1* (13%, 5%, 0%), *APC* (5%, 0%, 0%), *KRAS* (10%, 12%, 0%), *PTEN* (20%, 8%, 0%), *PIK3CA* (20%, 8%, 0%), and *TP53* (15%, 46%, 65%) were found [28]. Therefore, high-grade ovarian endometrioid carcinomas are likely to harbor *TP53* mutations, while low-grade ovarian endometrioid carcinomas frequently harbor

mutations of Wnt/β-catenin pathway and/or KRAS/PI3K pathway genes. In another study, *ARID1A* mutations were reported in 10 of 33 ovarian endometrioid carcinomas (30%) [23]. Another group reported mutations of *CTNNB1* (53%), *PIK3CA* (40%), *ARID1A* (30%), *PTEN* (17%), *KRAS* (33%), *PPP2R1A* (17%), and *TP53* (7%) in low-grade (grade 1 and 2) ovarian endometrioid carcinomas ($n = 30$) [29]. Activating mutations of the *CTNNB1* gene is associated with squamous differentiation [30].

High-grade endometrioid carcinoma tumors with TP53 mutations have expression profiles similar to those of high-grade serous carcinoma [31]. However, these tumors may have been misclassified, as suggested by more recent studies reporting a subset of high-grade serous carcinomas that display a pseudoendometrioid pattern [32] (see also Sect. 3.4.2 in Chap. 3).

7.4.2 Mouse Models of Ovarian Endometrioid Carcinoma

Like ovarian clear cell carcinomas, ovarian endometrioid carcinomas are frequently associated with endometriosis. Peritoneal endometriosis occurs in mice by the activation of an oncogenic K-ras. Additionally, expression of oncogenic K-ras and Pten deletion within the ovarian surface epithelium leads to the induction of adenocarcinomas similar to human ovarian endometrioid carcinomas [33]. In another study, inactivation of the *Pten* and *Apc* in murine ovaries resulted in the formation of endometrioid adenocarcinomas [28]. More recently, codeletion of *Arid1a* and *Pten* resulted in ovarian endometrioid carcinoma [34].

7.4.3 Microsatellite Instability (MSI) in Ovarian Endometrioid Carcinoma

Ovarian cancer, particularly endometrioid adenocarcinoma, is associated with Lynch syndrome, although the risk is much smaller than for uterine cancer. Among 71 cases with ovarian endometrioid adenocarcinoma, 7 (10%) tumors had abnormal mismatch repair (MMR) protein status, defined as complete loss of expression of MLH1, MSH2, MSH6, and/or PMS2. Each of these tumors with abnormal MMR status demonstrated MSI. Importantly, concurrent uterine tumor was present in 5/7 patients whose ovarian tumor had abnormal MMR/MSI [35].

7.4.4 Genetic Analysis of Synchronous Endometrial and Ovarian Carcinoma

Five to ten percent of women with ovarian endometrioid carcinomas present with concurrent endometrial carcinoma. Based on both targeted and exome sequencing of 18 synchronous endometrial and ovarian tumors, most (17/18) cases showed evidence of clonality. Importantly, 10 of 11 cases that fulfilled clinicopathological

criteria that would lead to classification as independent endometrial and ovarian primary carcinomas showed evidence of clonality [36]. Therefore, the genome-wide analysis demonstrated that most synchronous endometrial and ovarian carcinoma tumors develop from a clonal origin.

7.5 Mucinous Ovarian Tumors

7.5.1 Origin of Mucinous Ovarian Tumors

Mucinous ovarian carcinomas typically display heterogeneity, with lesion of mucinous cystadenoma admixed with borderline tumor and carcinoma. The identical KRAS mutation in these components provides strong evidence that mucinous cystadenomas are the precursor lesions of mucinous carcinoma [37, 38].

In terms of the origin of mucinous cystadenoma, a subset develops from mucinous epithelium in mature teratomas. A microsatellite genotyping analysis of mucinous tumors associated with a teratoma revealed five of six pairs of tumors with teratoma showed a high or complete degree of allelotype matching, which differed from the somatic allelotypes of the normal control tissue [39].

It has been proposed that many of nongerm cell mucinous tumors are derived from Brenner tumors. In a study of 40 mucinous cystadenomas, 67 Brenner tumors, and 13 combined tumors, a total of 25% of tumors with a mucinous component contained a Brenner component, and 16% of tumors with a Brenner component contained a mucinous component. Mucinous tumors are typically large, whereas Brenner tumors tend to be smaller. Accordingly, the Brenner tumor is compressed by the large mucinous cystadenoma and may be overlooked [40]. This hypothesis was supported by a recent study showing that, in combined Brenner and mucinous tumors, the Brenner and mucinous components are clonally related [41] (see also Sect. 3.4.4 in Chap. 3).

7.5.2 Genetic Features of Mucinous Ovarian Tumors

KRAS-activating mutation is the most common single molecular genetic alteration in mucinous carcinomas, occurring in 65% of cases [42]. Another study identified mutations in a novel gene, *RNF43*, a zinc finger-dependent E3 ubiquitin protein ligase. *RNF43* mutations were observed with a frequency of 2/22 (9%) in mucinous ovarian borderline tumors and 6/29 (21%) in mucinous ovarian carcinomas [43]. In contrast to other type I ovarian carcinomas, *TP53* mutation is frequent in mucinous carcinomas, being present in approximately one-half of cases [42, 43]. In a genetic analysis of a total of 82 mucinous ovarian tumors, which included exome sequencing of 24 tumors and a validation cohort of benign 58 tumors for specific gene regions, benign, borderline, and carcinoma samples harbored mutations in *BRAF* (0%, 10%, 23%), *TP53* (9%, 14%, 52%), and *RNF43* (0%, 7%, 20%), respectively, which mutations were associated with progression of the disease. Other recurrent,

but not associated with progression, mutations were found in *KRAS* (54%), *CDKN2A* (16%), *ARID1A* (8%), *ELF3* (6%), *GNAS* (6%), *ERBB3* (5%), and *KLF5* (5%) [44].

Overexpression and amplification of *ERRB2* was observed in 11/176 (6%) mucinous borderline tumors and 29/154 (19%) mucinous cancers. *KRAS* mutations and *ERRB2* amplification are near mutually exclusive (#41#). Thus, mutations in *KRAS*, *BRAF*, and/or *ERRB2* amplification are present in the majority of mucinous neoplasms, indicating RAS/RAF pathway activation is frequent in this tumor. (See also Sect. 3.4.4 in Chap. 3).

7.6 Serous Borderline Tumor and Low-Grade Serous Ovarian Carcinoma

It has been well established that low-grade serous ovarian carcinomas can develop from serous borderline tumor. Deletions of ch1p36 and ch9p21 are much more common in low-grade serous ovarian carcinomas than in serous borderline tumors [45]. The ch1p36 region contains several candidate tumor suppressor genes including miR-34a. Then, the ch9p21 region including the *CDKN2A/B* locus encodes three tumor suppressor proteins, p14 (Arf), p16, and p15. Thus, deletions of ch1p36 or ch9p21 may cause progression of some serous borderline tumors to low-grade serous carcinomas.

KRAS mutations occur in one-third of serous borderline tumors and log-grade serous ovarian carcinomas, and *BRAF* mutations occur in another one-third of serous borderline tumors but less commonly in low-grade serous ovarian carcinomas [46, 47]. *BRAF*-mutated advanced-stage low-grade serous ovarian carcinomas are much less common than are *BRAF*-mutated advanced-stage serous borderline tumors [48–50]. *ERBB2* and *NRAS* mutations are also detected in a small percentage of low-grade serous ovarian carcinomas [47, 51]. These mutations result in activation of the MAP kinase signal transduction pathway. Exome sequencing analyses also identified *BRAF* and *KRAS* as the most frequently mutated genes (#43#, #44#).

A better outcome has been reported for women whose tumors contain *BRAF* mutations than for women with *KRAS* mutations or wild-type *BRAF* and *KRAS* [48, 49, 52]. *BRAF* mutations correlate with the presence of cells with abundant eosinophilic cytoplasm, which may suggest cellular senescence caused by BRAF activation [53, 54] (see also Sect. 3.4.1.2 in Chap. 3).

Conclusion

Type I tumors, containing low-grade serous, clear cell, endometrioid, and mucinous cancers, are characterized by activating mutations in the ERRB2/KRAS/BRAF/MEK pathway, PI3K/AKT pathway, and Wnt pathway and inactivation mutations in the *PTEN*- and ARID1A-related chromatin remodeling. In contrast, type II tumors, mainly high-grade serous cancers, are characterized by inactivation of the TP53, deficiency of the HR pathway, and extensive copy number alterations. Representative genetic alterations are summarized in Table 7.1. These findings would lead to discovery of effective molecularly targeted drugs and their biomarkers.

Table 7.1 Representative genetic alterations in epithelial ovarian cancer

	Gene mutation	Copy number amplification	Copy number loss
High-grade serous	*TP53, BRCA1/2*	*CCNE1, MYC, MECOM*	*PTEN, RB1, NF1*
Clear cell	*ARID1A, PIK3CA*	*MYC, ZNF217, ERBB2, STAT3, HNF1B, PPM1D*	*SMARCA4, RB1, SMAD4*
Endometrioid (low-grade)	*CTNNB1, PIK3CA, KRAS, PTEN*		
Mucinous	*KRAS, TP53, BRAF, RNF43, CDKN2A*	*ERBB2*	
Low-grade serous	*KRAS, BRAF*		*CDKN2A/2B,* miR-34a

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