Ovarian Epithelial Carcinogenesis

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Abstract

The mortality rate of epithelial ovarian carcinoma (EOC) ranks the highest in all gynecological malignancies, although it is the third common cancer in the female reproductive system. In spite of the progress in reductive surgery and the extensive applications of platinum and paclitaxel and the other first-line chemotherapeutic drugs, the 5-year survival rate of EOC patient is improved merely from 36% in 1975 to 46% in 2011 [1]. The reason is that the definitions and carcinogenetic mechanisms closely related to EOC remain poorly understood. For over a decade, the rapid development of molecular genetics provides a new foundation for our understanding of ovarian epithelial carcinogenesis. In the current chapter, we will focus on the cell origin, pathogenesis, molecular genetics, and clinical applications of different EOC histological subtypes to improve our understanding of this deadly disease.

Keywords

Brenner tumors · cancer stem cell · carcinogenesis · clear cell carcinoma · endoreplication · endocycle · endometrioid carcinoma · endometriosis · endomitosis · epithelial ovarian carcinoma · epithelial-mesenchymal transition (EMT) · fere ex nihilo model · high-grade serous carcinoma · immune checkpoint · intraepithelial carcinoma · low-grade serous carcinoma · malignant mixed Müllerian tumors (MMMT) · mesenchymal-epithelial transition (MET) · mucinous carcinoma · ovarian epithelial inclusion · ovarian inclusion cyst · p53 signature · polyploid giant cancer cells (PGCCs) · secretory cell expansion · seromucinous carcinoma · serous tubal intraepithelial carcinoma · somatic blastomere · the dualistic model of ovarian carcinogenesis · the secondary Müllerian system · the somatic blastomere model · TP53 mutation · tumor progression · undifferentiated carcinoma · Walthard cell nests

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The mortality rate of epithelial ovarian carcinoma (EOC) ranks the highest in all gynecological malignancies, although it is the third common cancer in the female reproductive system. In spite of the progress in reductive surgery and the extensive applications of platinum and paclitaxel and the other first-line chemotherapeutic drugs, the 5-year survival rate of EOC patient is improved merely from 36% in 1975 to 46% in 2011 [1]. The reason is that the definitions and carcinogenetic mechanisms closely related to EOC remain poorly understood. For over a decade, the rapid development of molecular genetics provides a new foundation for our understanding of ovarian epithelial carcinogenesis. In the current chapter, we will focus on the cell origin, pathogenesis, molecular genetics, and clinical applications of different EOC histological subtypes to improve our understanding of this deadly disease.

4.1 The Models of Ovarian Carcinogenesis

Several theories have been proffered on the origins of ovarian cancer. Most intriguing are the theories on ovarian surface epithelial metaplasia, the secondary Müllerian system, the dualistic model of ovarian carcinogenesis, the *fere ex nihilo* model, and the recently described role of polyploid cells in tumor initiation and progression. Ovarian epithelial metaplasia is a classic theory of EOC, but it is difficult to find the transformation between ovarian surface epithelium (OSE) and carcinoma. Analogously, although the second Müllerian system theory is well-known, the progression process of EOC also cannot be confirmed.

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Therefore, these two theories have become less popular in recent years. On the other hand, the dualistic model of ovarian carcinogenesis is among most discussed model in past decade. However, ovarian cancer can be potentially have multiple cell origins, and the malignant transformation may be achieved via formation of tetraploidy or polyploidy as an intermediate stage and followed by amitosis rather mitosis to generate genetically aberrant stem cells for cancer initiation. In order to better understand the overall landscape of ovarian carcinogenesis, we will discuss each of cell origins and mechanisms involved in tumor progression in light of the most recent research progress.

4.1.1 The Ovarian Surface Epithelial Cells

This theory is based on the speculation that all ovarian epithelial tumors originate from OSE. The OSE represents mesothelial-like cells and is configured as a single layer of stable epithelium without prominent differentiated characteristics in the general state. They possess two potentials that differentiate into mesenchymal cells or epithelial cells, called epithelial-mesenchymal transition (EMT) or reversal of this process named mesenchymal-epithelial transition (MET). As one of the normal physiological functions, EMT/MET plays a vital role in regulating the OSE repair process after ovarian ovulation [2]. The imbalance of EMT/MET is suggested to be a possible mechanism in the initiation of EOC. In this hypothesis, ovarian epithelial inclusion (OEI)/inclusion cyst (OIC) is formed via OSE invagination. These OEIs may transform into Müllerian epithelial cells via metaplasia and give rise to different histological types (e.g., serous, endometrioid, clear cell, mucinous and transitional cells) under the influence of local factors (such as steroid hormones). Their morphologies are parallel to the mucosa of fallopian tube, endometrium, gastrointestinal tract or endocervix, and bladder, respectively. These OEIs with Müllerian phenotype further gain the ability for malignant transformation and to progress to corresponding EOCs (serous carcinoma, endometrioid carcinoma, mucinous carcinoma, or other subtype) with lineage infidelity via an abnormal regulation of homeobox (HOX) gene [3]. In this process of tumor transformation, both OEIs and cancer cells show the changes that cells gradually lose the characteristics of mesenchymal cells and obtain different Müllerian epithelial differentiated features, including the expression of specific epithelial marker (E-cadherin) in the differentiated stage [4].

However, the theory has been challenged in the several aspects:

1. Different cells of origin: OSE belongs to the coelothelium (mesothelial cells), rather than Müllerian epithelium. The histotypes of EOCs primarily show differentiation toward Müllerian, rather than mesothelial cells.
2. Discrepancy in immunophenotype: OSE does not express common EOC markers (such as PAX-8), but highly express calretinin and the other mesothelial markers.
3. The unknown origin of OEI: Some epithelial cells in OEI exhibit the differentiation toward fallopian tube epithelium, rather than OSE.
4. Different histomorphology: There are no obvious histological transitions between mesothelial cells and Müllerian epithelium.

In addition, the histological observations on the ovaries from the carriers with hereditary *BRCA* mutation or contralateral normal ovary of sporadic EOC reveal that the morphological changes (hyperplastic papillae on the ovarian surface, the increase and dilatation of cortical inclusion cysts, and mild cell atypia) are not sufficient to achieve the diagnostic criteria for precancerous lesions. For all of the abovementioned reasons, this theory has become less popular in the past two decades.

4.1.2 The Secondary Müllerian System

At the early stage of embryogenesis, the somatic epithelium and its subepithelial mesenchyme are derived from the Müllerian ducts. During the embryonic development, the distal parts of the two Müllerian ducts (also known as the primary Müllerian system) fuse to form the uterus, cervix, and proximal one third of the vagina, while the proximal Müllerian ducts remain separated to become the two fallopian tubes [5]. The mucosal epithelium lining on those sites is called the Müllerian epithelium. Indeed, the ovaries do not belong to the Müllerian system because the gonads and reproductive tract are developed separately in embryonic stage.

In view of the similarity between ovarian epithelial tumors and Müllerian epithelium, Lauchlan [6] put forward the second Müllerian system theory in 1972, which is that the coelothelium has an ability to transform into the Müllerian epithelium. Taking this ability into consideration, he further suggested that OSE, OEI, and all extraovarian Müllerian-type epithelial tissues adjacent to fallopian tube and pelvic cavity are part of the second Müllerian system. The second Müllerian system includes endometriosis, endosalpingiosis, and endocervicosis, which are collectively referred to as Müllerianosis. With his comprehensive understanding of the morphologic features of these lesions, Dr. Lauchlan thought that these three lesions can change into each other through metaplasia and thus that all epithelial tumors in the ovary and pelvic cavity can be potentially derived from the second Müllerian system.

Similar to OSE metaplasia theory described above, the secondary Müllerian system theory cannot entirely account for other observations on ovarian carcinogenesis and accordingly has become less popular in recent years. However, it remains a potential cell of origin for extraovarian or pelvic Müllerian epithelial tumors, especially low-grade lesions (type I) as described below.

4.1.3 The Dualistic Model

In recent years, data have accumulated on the histological observation and molecular genetic levels demonstrated that EOCs are heterogeneous diseases with several histological subtypes with different cells of origin, pathogenesis, and clinical biological features. Kurman and colleagues proposed the "dualistic model" of ovarian carcinogenesis, i.e., EOC could be classified as type I and type II tumors based on their distinct set of clinicopathologic features [7–9].

Type I EOCs include low-grade serous carcinoma, endometrioid carcinoma, clear cell carcinoma, seromucinous carcinoma, mucinous carcinoma, and malignant Brenner tumors. In the genesis, these carcinomas often follow a sequential pattern of evolution from benign to borderline to malignant tumors. Clinically, these tumors grow slowly and most of them have an indolent biological behavior; most are confined to one ovary at presentation. All histological subtypes of type I EOCs are low-grade tumors, and the prognosis is relatively good, with the exception of clear cell carcinoma. The mortality rate accounts for only 10% of all EOCs. The tumor genomes are relatively stable at the molecular genetic level, although there are different genotypes in different histological types (see Table 4.1) [9].

Type II EOCs include high-grade serous carcinoma, undifferentiated carcinoma, and malignant mixed Müllerian tumors (MMMT, also called carcinosarcoma). Clinically, these tumors are highly aggressive and rapidly progressive. All histological subtypes of type II EOCs belong to high-grade tumors. More than 75% of cases are diagnosed at advanced stage (FIGO III and IV) with extensive dissemination. The prognosis is poor and the mortality rate accounts for 90% of all EOCs. In the molecular genetics, these tumors have highly unstable genomes and prone to have the amplification or deletion of DNA copy numbers. Among them, *TP53* gene mutation is most common (>95% of high-grade serous carcinoma) (Table 4.1) [9].

Table 4.1 The comparison between type I and type II epithelial ovarian carcinomas [9]

	Type I epithelial ovarian carcinomas	Type II epithelial ovarian carcinomas
Histologic features		
Histological types	Low-grade serous carcinoma, endometrioid carcinoma, clear cell carcinoma, seromucinous carcinoma, mucinous carcinoma, malignant Brenner tumors	High-grade serous carcinoma, undifferentiated carcinoma, malignant mixed Müllerian tumors
Tumor grade	Low-grade (except clear cell carcinoma)	High-grade
Proliferation activity	Usually low	Usually high
Clinical features		
FIGO stage	Usually early stage (FIGO I)	Usually advanced stage (FIGO III and FIGO IV)
Clinical process	Slow and indolent	Rapid and aggressive
Response to chemotherapy	General	Good (but late recurrence)
Progress course	Benign to borderline to malignant tumors	Mostly from serous tubal intraepithelial carcinoma
Early screening	Feasible	Difficult
Prognosis	Relatively good	Relatively poor
Genetic features		
Chromosomal instability	Low	High
Common gene mutation	*KRAS*, *BRAF*, *PTEN*, *ARID1A*, *PIK3CA*, *CTNNB1*, *ERBB2*, *PPP2R1A*	*TP53, BRCA1/2*
Deficiency of homologous recombination repair proteins	Rare	Common

4.1.4 The *Fere Ex Nihilo* Model

The dualistic model of ovarian carcinogenesis helps in our understanding of EOCs and may even provide a frame work to guide clinical decision-making. However, this model may be too simplified for such a highly heterogeneous group of diseases. For example, even among high-grade serous carcinomas, the tumors have different clinical biological behaviors [10]. In particular, Silva raised several questions that argue against fallopian tube theory for pelvic serous carcinoma (see Sect. 4.2.1.2). Toward this end, Silva put forward the *fere ex nihilo* model (or out of nothing) from unremarkable primitive or early epithelial or mesenchymal stem cells: this model hypothesizes that uncommitted or stem cells from the mesenchyme could be a potential source of transformation for both benign and malignant tumors. During this process, stromal fibroblasts, via MET, could be the origin of high-grade serous carcinoma, and both stromal-epithelial interaction and steroid hormones play critical roles in tumorigenesis and progression [11].

The stem cells are defined as a subgroup of cells with differentiation potential and the ability for self-renewal. Under certain conditions, these cells can differentiate into a variety of functional cells. It has been reported that both OSE and ovarian cancer cell are capable of expressing stem cell markers such as SOX2 [12], CD133 [13], and NANOG [14]. The small populations of stem cells, which possess the features of stem cell or mimic stem cells, are predominantly located in hilar OSE in mouse model. The hilum OSE are cycling slowly and express stem cell markers ALDH1, LGR5, LEF1, CD133, and CK6B [15]. Therefore, those OSE cells or other stromal cell types that express stem cell markers can potentially be the cell of origin of benign and malignant ovarian tumors [16, 17].

Evidence supporting this view includes the development of benign epithelial neoplasms of the ovaries from guinea

pigs following treatment of testosterone and estrogenic hormones [18, 19]. Inflammation is a well-known risk factor for ovarian cancer, as may occur in ovulation, which causes the rupture of OSE and a repair process, resulting in pelvic foci of inflammatory microenvironment. Using SV40 T/t antigen to disable to ovarian surface epithelial cells together with oncogene *RAS*, Liu's laboratory has successfully been able to transform the normal ovarian epithelial cells into high-grade Müllerian carcinoma, which is associated with massive upregulation of inflammatory cytokine (e.g., interleukin-1β, interleukin-6, and interleukin-8) [20]. Through the chemokines and cytokines secreted by cancer cells, for example, Gro-1 can induce stromal fibroblast senescence [21]. CXCR2 and interleukin-1β promote tumor cell proliferation [22, 23]. The close interaction among tumor cells, stromal cells, and inflammatory tumor microenvironment provides a favorable condition for tumor cell recruitment, adhesion, migration, survival, and colonization [24, 25]. The fact that OSE can be transformed into high-grade Müllerian carcinoma provides further support that the OSEs can be also the potential cells of origin for high-grade carcinoma.

4.1.5 Somatic Blastomere Model

The above theories described the cell of origin for ovarian cancer. However, it remains unknown how the cells are transformed into carcinoma. The most accepted paradigm in carcinogenesis is an accumulation of genetic mutations or aneuploidy [26, 27]. However, these theories at the individual gene or individual chromosomal levels cannot entirely account for enormous genomic and epigenetic changes detected by The Cancer Genome Atlas (TCGA) projects in high-grade carcinomas [28]; it has been argued that somatic mutation theory may be wrong for most cancer [29]. In addition, all of the four models described above fail to consider the level of differentiation as reflected in the level of malignancy in different histotypes. Recently, we have proposed a unified somatic blastomere model to explain the origin of all cancers and disease relapse [30]. This model is based on long-term and puzzling observation that early blastomere stage embryo is highly chaotic [31] with high frequency of polyploidy [32, 33]. The endoreplication and cell fusion are required for development from blastomere to compaction/morula stage embryo, which is required for the first differentiation event to become trophoblasts and inner cell mass following fertilization [34]. Unlike the mitotic cell cycle, which involves several distinct phases including DNA synthesis (S) and distribution of replicated DNAs to two identical daughter cells via mitosis (M) with the intervening gap phase (G), endoreplication represents a specific process in which nuclear membrane does not break down while the genome is replicated twice or multiple times without cell division and subsequently separated into daughter cells without formation of mitotic spindle. There are two kinds of endoreplication. The first form is called the endocycle, which consists of alternating DNA synthesis (S) phases and gap (G) phases without chromosome segregation during a mitotic (M) phase or cell division (cytokinesis). The developmentally controlled endocycle results in cells with a single polyploid nucleus and no feature of mitosis to support specific need of development [35–39]. Another form of endoreplication is known as endomitosis, in which cells execute an abortive mitosis that does not result in fully separate sister chromatids or cell division, followed by subsequent re-entering of S phase to generate multinucleated cells [36–39]. Endocycle and endomitosis can be mixed, and the distinction between the two forms may be context development depending on specific type of development.

Polyploid giant cancer cells (PGCCs), characterized by a single, giant nucleus or multinucleated cells, are commonly found in tumor tissues with high-grade carcinoma or after treatment (such as chemotherapy, radiotherapy, or targeted therapy) [40–42]. PGCCs have a distinct advantage over regular cancer cells in dealing with stresses (e.g., hypoxia, starvation, temperature, pH, and diet conditions in physiologic stresses; drugs and radiation in pathologic stresses) and reproduction [35–39]. Increasing DNA content by endoreplication is a widely utilized effective mechanism to sustain the mass production of proteins and high metabolic activity necessary for tumor growth. Following endoreplication, cancer cells may thus arrest the mitotic cell cycle and allow the cell to survive during mitotic catastrophe or genotoxic stresses and enter endoreplication cell cycle to form PGCCs [30, 43–45].

Accumulating evidence suggests that PGCCs may have played a fundamental role in tumor initiation. They may have hijacked normal embryonic developmental program to facilitate the generation of new diploid cancer initiating cells in response to oncogenic and therapeutic stress [30, 45]. Our laboratory has provided the first experimental evidence that normal ovarian or fallopian tubal epithelial cells and cancer cells can undergo endoreplication [42, 46]. This process can lead to genomic instability and dedifferentiation into more primitive state, to facilitate the reprogramming and emergence of new cancer initiating cells. We have shown that this multistep reprogramming process includes four distinct but overlapping process including initiation, self-renewal, termination, and stability and facilitating normal or cancer cells to be reprogrammed to cancer cells or resistant cancer cells [45]. The mitotic apparatus for well-known mitotic division is shut down with activation of senescence program in the giant cell cycle; emergence of new tumor initiating cells is largely from amitotic separation from giant mother cells including budding, splitting, and branching, the more primitive mode of cell division used in fungi [42, 45]. Unexpectedly, endoreplication of ovarian cancer cells recapitulates the division and growth pattern of blastomere stage embryo to form compaction and morulae-like embryonic cell types, which is

associated with massive mitotic and cytokinesis failure and genomic instability [30]. Moreover, the endoreplicating cells recapitulate the morphology and spatial and time-dependent activation of embryonic reprogramming factor OCT4/NANOG/SOX2, capable of differentiation into three germ layers and develop into malignant germ cell tumors [30]. Formation of tetraploidy or polyploidy is a common feature at the border of normal epithelial cells and mesenchymal and high grade carcinoma, which is associated with activation of senescence and dedifferentiation program and stem cell activation, supporting the generality of our model to other types of cancer [47]. Further, formation of polyploidy appears to be a major mechanism in response to starvation or mitotic insult in Drosophila [48]. Subsequent generation of intestinal stem cells from polyploidy cells is associated amitosis, a primitive form of cell division without using mitotic spindle [48]. Taken together, the above data together provide a previously appreciated mechanism via formation of polyploid cells for generating genetically altered daughter stem cells in response to acute or chronic insults using primitive mode of cell division for cancer initiation [47].

Our model also explains the level of differentiation observed in the ovarian cancer. Depending on the level of stem cell arrested at the specific developmental hierarchy during organ development, the tumors could behave in benign or low-grade type of malignancy such as cystadenoma or borderline tumor or high-grade carcinoma (type II tumor). The closer toward the embryonic stage, the higher developmental potential to allow the tumor to behave in malignant manner [30]. Thus, it is possible that high-grade serous carcinoma or the other EOCs, particularly those cancers with marked nuclear atypia, may be achieved via the giant cell cycle-mediated reprogramming following the redifferentiation and followed by developmental arrest [30, 42, 45]. This model also offers a sensible explanation why high-grade serous carcinoma is usually detected in late stage with wide dissemination in the peritoneal cavity. The schematic diagram on how epithelial or mesenchymal cell is transformed into cancer cells via the giant cell cycle is shown in Fig. 4.1. The details on the role of the PGCCs and the giant cell cycle in tumor initiation and disease relapse can be found in recent review by Liu [47].

4.2 The Cell Origin and Molecular Genetic Profiles

During the past decade, it has become clear that some high-grade serous carcinomas arise from the mucosal epithelium of fallopian tube fimbria [49–51]. In addition, significant progress has also been made in the cell origin of the other subtypes of EOCs. These progresses will help out our understanding on the origin of EOC and offer a new potential strategy for treatment and early screening.

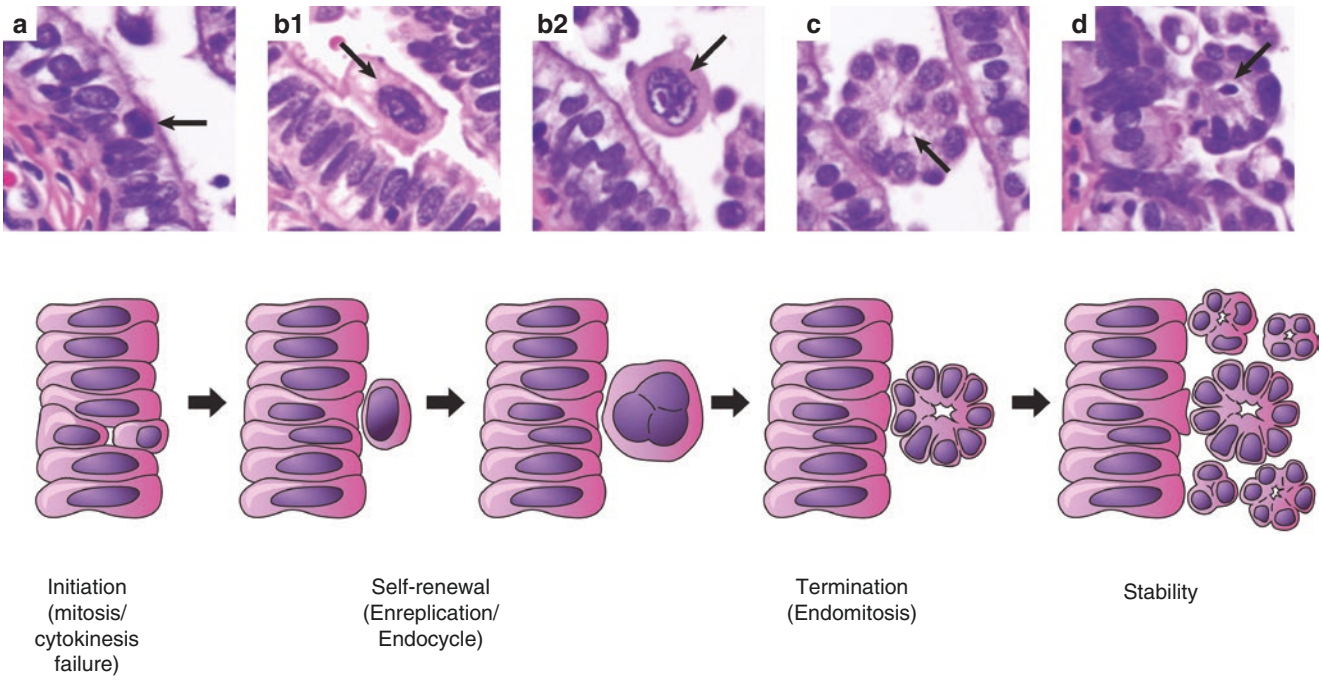

Fig. 4.1 A schematic diagram of somatic blastomere-like model for how normal fallopian tubal and ovarian epithelial cells are transformed into high-grade serous carcinoma. Normal fallopian tubal cells (or ovarian epithelial cells or stromal fibroblasts) (**a**) the nucleus starts endoreplication due to mitotic/cytokinesis failure; (**b**) endoreplicating cells grow autonomously (self-renewal, b1 and b2) and lead to genomic chaos and facilitate the genomic reorganization and reprogramming. With the termination of the giant cell cycle via endomitosis to form early tumor papillae (**c**), multiple tumor papilla with different genetic and epigenetic changes together generated via amitotic endoreplication division. In this process, the clone(s) with advantageous p53 mutations achieve the stable tumor expansion and develop into high-grade serous carcinoma (**d**)

4.2.1 Ovarian Serous Carcinoma

Serous carcinoma is the most common histological subtype of ovarian epithelial tumors. It represents a heterogeneous group of tumors at both morphology and molecular genetics and exhibits a typical dualistic model of ovarian carcinogenesis. In 2004, based on the systematic analysis of histology and clinical behavior with more than 10-year follow-up, Malpica et al. [40] at MD Anderson Cancer Center first proposed a two-tier system for grading ovarian serous carcinoma. Namely, ovarian serous carcinoma is classified as low-grade and high-grade. This grading system is currently widely used and is accepted by the WHO classification of gynecological tumors [52, 53]. Although they are in the same category of serous carcinoma, low-grade serous carcinoma is significantly different from high-grade serous carcinoma in morphology, genotyping, and biological behavior.

4.2.1.1 Low-Grade Serous Carcinoma

Low-grade serous carcinoma has typical features of type I EOCs. The progression from benign to borderline to malignant process can be observed histologically. The epithelial cells lining on the serous cystadenoma have the same histological and immunophenotype as OEI cells. The areas of benign serous components present in almost all borderline tumors. Likewise, the transition from borderline to malignant components also exists in the majority of low-grade serous carcinoma. As the precursor lesion represents an important clue for tumor origin, the abovementioned pathological findings suggest that the ovarian serous cystadenoma, borderline tumor, or low-grade carcinoma may develop via a sequential progression process from OEIs.

Cells of origin: While it is well-known that the fallopian tube can serve as a precursor lesion for high-grade serous carcinoma, recent evidence suggests that these epithelial cells can be also potential source of low-grade lesions including cystadenoma, serous borderline tumor, and low-grade serous carcinoma. Due to close anatomic relationship between the fallopian tubal fimbria and ovaries, ovarian rupture caused by periodic ovulation may provide an opportunity for implantation of the epithelial cells of fallopian tube on the ovary. Through morphological and immunohistochemical comparisons among OSEs, OEIs, tubal epithelium, serous cystadenomas, serous borderline tumors, and low-grade serous carcinomas, Zheng and Kong et al. [23, 54, 55] found two types of OEIs including mesothelial type (calretinin+/PAX8−/tubulin–) and tubal type (calretinin–/PAX8+/tubulin+). The tubal OEIs account to 78% of all OEIs and have a significantly higher proliferative index, which may further proliferate and progress to ovarian serous cystadenoma or borderline tumors. Conversely, mesothelial OEIs may not develop to tumor because of its low proliferative rate. As the mucosal epithelium of fallopian tubal fimbria can directly detach and adhere to the ovarian surface, it is possible that the tubal OEIs may be formed via the shedding and invagination of tubal mucosal epithelium into the ovarian cortex. Interestingly, the different proportional distribution of mixed ciliated and secretory cells has been found in the tubal OSEs, tube OEIs, serous cystadenomas, and borderline tumors, with a remarkably increasing secretory/ciliated cell ratio in the low-grade serous carcinoma, suggesting that tubal epithelial cells could be the potential origin of these tumors.

In the 2014 WHO classification, ovarian borderline serous tumors are further divided into two types, namely, serous borderline tumor/atypical proliferative serous tumor and borderline serous tumor-micropapillary variant/noninvasive low-grade serous carcinoma. Both of them have the same *KRAS* mutation rate (about 50% of cases); however, there is a more analogous genetic profile between borderline serous tumor-micropapillary variants and low-grade serous carcinoma in comparison with serous borderline tumor, which suggests that borderline serous tumor-micropapillary variant may be an intermediate lesion in the development of borderline serous tumor to low-grade serous carcinoma [52]. On the other hand, development of low-grade serous carcinoma from serous borderline tumor, regardless conventional type or micropapillary variants, is time-dependent [56]. With increasing follow-up time, conventional serous borderline tumor will also develop into low-grade serous carcinoma. It remains to be determined whether such further subclassification is clinically meaningful or potentially misleading as the term of atypical proliferative tumor neglects the low malignant potential of conventional serous borderline tumor.

Genetic and genomic profiles: The most common molecular genetic alterations in low-grade serous carcinoma are *KRAS*, *BRAF*, or *ERBB2* mutations. These three gene mutations can promote the transduction of growth signals into the nucleus, resulting in uncontrollable cell proliferation and malignant transformation via sustained activation of the downstream MAPK kinase signaling pathway [57, 58]. Accumulating evidence points toward that there are mutually exclusive relationships among *KRAS*, *BRAF*, or *ERBB2* mutations with only one gene mutation exists at an individual tumor in most cases. The mutation rate accounts for about 2/3 of the borderline tumors and low-grade serous carcinoma, of which 33.3% of the borderline tumors and low-grade serous carcinomas have *KRAS* mutations at codon 12 and 13, 33.3% of serous borderline tumors and a small number of low-grade serous carcinomas show *BRAF* mutation at codon 600, whereas *ERBB*2 mutation is less than 5% of entire tumors. *KRAS* or *BRAF* mutation is considered to be an early event in low-grade serous carcinomas because they also exist in serous cystadenomas adjacent to borderline serous tumors [59]. Compared with *BRAF* mutation, serous borderline tumors with *KRAS* mutation have a greater potential to progress to low-grade serous carcinoma. In fact, *BRAF* mutation in advanced low-grade serous carcinoma is

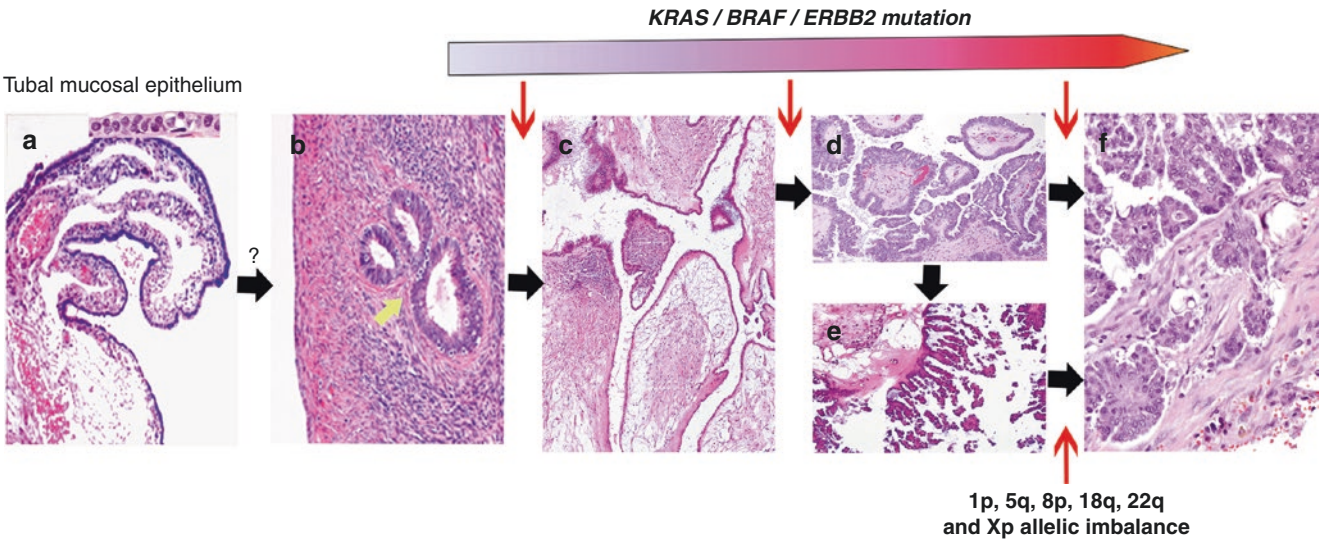

Fig. 4.2 The illustration of progression of ovarian low-grade serous carcinoma and associated changes in molecular genetics. The mucosal epithelial cells (**a**) of fallopian tubal fimbria may shed and adhere to the ovarian surface and then form the tubal OEIs (**b**, yellow arrow). The progression process is from serous cystadenoma (**c**) to borderline serous tumor/atypical proliferative serous tumor (**d**) and eventually to low-grade serous carcinoma (**f**), with/without the stage of borderline serous tumor-micropapillary variants/noninvasive low-grade serous carcinoma (**e**). During the period of tumorigenesis and progression, the *KRAS/BRAF/ERBB2* mutation rate gradually increases. And the multiple chromosomal allelic imbalances also promote the formation of low-grade serous carcinoma simultaneously

very rare [60]. In addition, the low-grade serous carcinomas are more likely to have an allele imbalance of 1p, 5q, 8p, 18q, 22q, and Xp chromosomes than borderline tumors [61]. The heterozygosity deletion of ch1p36 and the heterozygosity/homozygous deletion of ch9p21 often are found in low-grade serous carcinoma. Given that there are tumor suppressor genes (such as miR-34a) in ch1p36 region and *CDKN2A/B* (encoding tumor suppressor protein p14, p16, and p15) in ch9p21 region, these deletions may lead to the uncontrollable cell growth in borderline tumors and eventually progress to low-grade serous carcinomas [62] (Fig. 4.2).

4.2.1.2 High-Grade Serous Carcinoma

High-grade serous carcinoma is the most common EOC. Its incidence is about 60–80% of all EOCs. More than 75% of tumors are in advance stage with extensive abdominopelvic dissemination at the diagnosis [63]. However, there are usually no morphologically recognizable precursor lesions in ovarian tissues. Recent studies showed that some ovarian high-grade serous carcinomas may not originate in ovarian tissues but in the seeding from serous epithelial tumor cells in the mucosal epithelium of fallopian tubal fimbria as secondary tumors. The spectrum of tumorigenesis and progression is from tubal secretory cell expansion [64] or secretory cell outgrowths to tubal p53 signature, to tubal serous tubal intraepithelial carcinoma (STIC)/noninvasive high-grade serous carcinoma, to shedding and implantation on the ovary, and eventually to ovarian high-grade serous carcinoma.

Evidence supporting the fallopian tubal epithelial origin as the main source of pelvic serous carcinoma: The following evidence accumulated in the literatures that provide the support for the origin of high-grade serous carcinoma. (1) In the specimens of prophylactic bilateral salpingectomy from the patients with hereditary *BRCA1/2* mutation carriers, there are the occult precancerous lesions that are p53 signature and STICs, but no malignant ovarian tumors. The p53 signature is arbitrarily defined as more than 12 successive secretory cells with benign morphological features exhibiting strongly positive expression of p53 immunohistochemical staining and less than 10% of Ki-67 cell proliferation index [65]. As for STIC, its cytological features are enlarged, polymorphic, and hyperchromatic nuclei with nucleoli and mitotic figures, high ratio of nucleus to cytoplasm, multilayered cells, or the lack of cellular polarity. The immunohistochemical staining of p53 exhibits strongly positive or totally negative expression, and Ki-67 cell proliferation index is more than 10% [51]. (2) There are tubal p53 signature and/or STICs in 50–60% of cases with the sporadic ovarian and/or peritoneal high-grade serous carcinoma [62]. (3) Similar to ovarian high-grade serous carcinomas, there are the overexpression of p53 protein and the mutation of *TP53* gene both in p53 signature and STICs. The *TP53* mutation rate gradually increases with the process of tumor progression from p53 signature to STICs to high-grade serous carcinoma. (4) The evidence that there is the same *TP53* mutation site in concurrent p53 signature, STICs, and ovarian high-grade serous carcinoma supports the notion that high-grade serous carcinoma arises from the clonal proliferation of p53 signature cells [66, 67]. (5) The genetic profile of high-grade serous carcinoma is close to tubal epithelial

cells [68]. (6) STICs have shorter telomeres compare with concurrent ovarian high-grade serous carcinoma, while telomere shortening is known to be an early molecular event of tumorigenesis [69]. (7) In the models of genetically modified mice, the secretory cells in the mucous of fallopian tube can transform to malignant lesion due to *Tp53*, *Pten*, and *Brca* mutations, resulting in STIC and ovarian high-grade serous carcinoma [70, 71]. (8) A recent study confirmed that the salpingectomy is an effective method to reduce EOC risk in the general population based on the analysis of large clinical database between 1973 and 2009 [72].

Based on above data, the secretory cells in the mucosa of fallopian tube have been proposed to be the cell origin of high-grade serous carcinoma via the following process [73]: the secretory cells have DNA damages that cannot be normally repaired due to the stimulation of a variety of DNA toxicity factors, resulting in the accumulation of DNA damages. Because of the pressure of survival, there are a series of molecular genetic alterations in the damaged cells, such as adaptive *TP53* mutations, which lead to uncontrollable cell growth, over-proliferation, secretory cell outgrowths, and then p53 signature. Some cells with p53 signature can develop to STIC directly or through tubal dysplasia to form STIC [65, 74]. Due to the close contact between the fallopian tubal fimbria and the ovarian surface, and the loose adhesion between STIC cells, it is possible the tumor cells shed and implant on the ovarian surface and ultimately form "ovarian" high-grade serous carcinoma, a possibility that is more plausible than ovarian metastasis via lymphovascular invasion. Recent studies that there are tumor cells in the intraperitoneal washings in some patients with STIC also provide a direct evidence for this disseminated implantation [51].

Evidence against fallopian tubal epithelial cells as main source of pelvic serous carcinoma: Despite of prevalent view of fimbria of fallopian tube as a major source of high-grade serous carcinoma discussed above, there are several important clinical and pathological observations of pelvic serous carcinoma that cannot be clearly explained by fallopian tube origin [11]:

1. Most of high-grade serous carcinomas are at stages III and IV, while 70% the fallopian tubal carcinoma are stage I or II.
2. There is no direct evidence that the cells from small tubal intraepithelial carcinoma can travel all the way against gravity to the abdomen rather to the pelvis, which is against the law of gravity.
3. Most STICs are noninvasive, while most high-grade serous carcinomas are highly invasive; it is difficult to rationalize that the noninvasive precursor carcinomas give rise to 70% invasive carcinoma in peritoneal cavity.
4. Many high-grade serous carcinomas are located intraovarian stromal, not on the surface.
5. No STICs in the fallopian tube are identified in 50% serous carcinoma; it will be very difficult to unify a theory while no precursor lesions are identified in 50% of cases of the same cancer.
6. The main molecular evidence of clonality of the same *TP53* mutation should be treated with caution as it has been shown to be an unreliable indicator of metastasis in many other tumors.

Ovarian serous carcinoma as multicellular cells of origin: As mentioned above, there are approximately half of the cases of ovarian high-grade serous carcinoma without concurrent STIC, suggesting that these tumors may be derived from other cell origins [75, 76]. One possible source of cell origin is the OEIs within ovarian cortex; some OEIs may be formed by direct shedding and implantation of normal fallopian tubal epithelial cells into the ovarian cortex [55, 77], which undergo a series of molecular genetics and morphological change including *TP53* mutations, and eventually develop into high-grade serous carcinomas [78]. In addition, it is documented that a small number of low-grade ovarian serous carcinoma can progress into high-grade serous carcinoma or that borderline serous tumors can progress to high-grade serous carcinoma after tumor recurrence. The incidence by this pathway is likely to be low, since less than 3% of high-grade serous carcinomas show concurrent serous borderline tumor, low-grade carcinoma, and high-grade components in same tumor. These low-grade serous carcinomas may progress to high-grade carcinoma possibly via *TP53* mutations [79]. In addition, some high-grade serous carcinoma has been observed to arise from adenofibroma; and the fibroma components cannot be explained by tubal implantation theory. Based on the clinical and pathologic observation and arguable evidence for fallopian tube theory, in his alternative view article, Silva proposed that the *fere ex nihilo* model including the multicentric cell origin under the influence of environmental factors (such as hormones) may be the responsible for development of various ovarian carcinomas (see Sect. 1.1.4). However, regardless of the cells of origin, the transformation process can be achieved via the giant cell cycle described above (Fig. 4.1).

Subclassification of high-grade serous carcinoma. Through the morphological analysis of high-grade serous carcinoma-associated *BRCA1/2* mutation, Soslow et al. [80] found that these tumors usually present with solid pattern, endometrioid carcinoma- and transitional cell carcinoma-like feature, which is characterized by "SET" (solid, pseudo endometrioid, transitional cell carcinoma-like). *BRCA1*-associated cancer is associated with highly cellular proliferative activity, tumor-infiltrating lymphocytes, and geographic or comedo necrosis. Compared with the classic high-grade serous carcinoma whose histological patterns are papillary, glandular, cribriform, and solid area with slit-like space, the

SET subtype is more common in younger women, and the tumor cells are more sensitive to chemotherapy due to deficiencies of homologous recombination. Moreover, the patients have better prognosis [80, 81]. In view of the above-mentioned different clinicopathological characteristics, it is suggested that high-grade serous carcinoma should be divided into classic subtype and SET subtype [9].

Based on the results of TCGA genome analysis, in 2013 Verhaak et al. [82] presented a molecular subtype of high-grade serous carcinoma associated with the prognosis of patients. That is classification of ovarian cancer (CLOVAR). The tumors were classified into differentiated, proliferative, immunoreactive, and mesenchymal type. Among these subtypes, the prognosis of patients with immunoreactive type is the best, and the prognosis is the worst in patients with mesenchymal type. In 2016 Murakami et al. [83] further associated the molecular typing with tumor morphology: the mesenchymal type often presents a significant desmoplastic reaction; the immunoreactive type usually manifests as plenty of lymphocyte infiltration in the tumor tissues; the proliferative type frequently has the solid growth pattern; and the differentiation type generally exhibits the papillary pattern. However, this molecular subtype needs be further confirmed by more studies before used clinically.

Common genetic and genomic alterations: The most prominent genetic features of high-grade serous carcinoma are genomic instability (the abnormalities of many DNA copy number and structure) and *TP53* mutations. According to genomic TCGA project, *TP53* mutation is found in almost all tumors tissues (96%) through a sequenced genomic analysis on 489 cases of high-grade serous carcinoma [28]. The *TP53* mutation rate is as high as 57% even in precancerous lesions, p53 signature [84]. *TP53* mutation is not only an initial event of high-grade serous carcinogenesis but also involved in tumor progression. In addition, nearly half of the high-grade serous carcinomas display *BRCA1* (17q21.31) and *BRCA2* (13q13.1) inactivating mutations (including germline mutation, somatic mutation, or promoter methylation). Further, common amplification of *CCNE1*, *NOTCH3*, *PIKCA3*, and *AKT*, and the inactivation of *RB* and *NF1* has also been observed in some tumors [28, 85]. Alterations in DNA copy number have been observed in early lesions like STICs [86]. High-grade serous carcinoma with *BRCA1/2* mutation is characterized by numerous alterations in DNA copy number but without *CCNE1* amplification, a very common event in primary and refractory tumors [81]. The minority of high-grade serous carcinomas with inherited mutations also has germline deletion mutations in *BARD1*, *BRIP1, MRE11*, *NBN*, *RAD51C*, *RAD51*, and *PALB2* genes involved in the signaling pathway of Fanconi anemia [87].

Although *TP53* mutation is a common event in high-grade serous carcinoma, the mutation alone in *TP53* is not sufficient to induce malignant transformation, suggesting other molecular genetic alterations also participate in the transformation process. Norquist et al. [88] found that the loss of *BRCA1/2* allele exists in STICs, but not in p53 signature, suggesting that both *TP53* mutation and *BRCA1/2* deletion are the key events in early carcinogenesis. Kim et al. [71] reported that ovarian/fallopian tubal high-grade serous carcinoma cannot be directly induced by *Tp53* mutation alone but by both *Tp53* and *Pten* mutation. Drapkin and Dinulescu et al. [70] also confirmed that the combination of *Brca1/2*, *Tp53*, and *Pten* mutations in tubal epithelial cells leads to STIC and high-grade serous carcinoma, whereas only *Tp53* mutation cannot induce tumor formation. The simultaneous loss of functions leads to a decrease in cell genome stability, a series of oncogene activation and/or tumor suppressor gene inactivation, and leads to multiple chromosomal breaks and deletions and the formation of aneuploidy or polyploidy, which is conducive to avoiding immune surveillance, over-proliferation, and the progression to high-grade serous carcinoma from STIC.

The prevalent view of carcinogenesis of high-grade serous carcinoma and its main molecular genetic alterations is shown in Fig. 4.3. Because of conflicting evidence and different views that is for or against the tubal theory, we ask readers of this chapter to take the views from different authors with great caution and make your own observation during the daily practice and decide which theory will best fit with what you observe, rather than blindly believe what are described by a particular author, journal, or academic group. We want to point out that health discussion and expression of different opinions should greatly help us to clarify controversial points and move field to next level. As Einstein puts it, "blind belief in authority is the greatest enemy of truth."

4.2.2 The Other Ovarian Epithelial Carcinomas

4.2.2.1 Endometrioid Carcinoma, Clear Cell Carcinoma, and Seromucinous Carcinoma

Ovarian endometrioid and clear cell carcinoma account for about 25% of all EOCs, which are the most common tumors after serous carcinoma. Seromucinous carcinoma (also known as endocervical-type mucinous or mixed epithelial carcinomas of Müllerian type) is very rare. It is composed predominately of serous and endocervical-type mucinous epithelium, foci of clear cells, and uncommon area of endometrioid and squamous differentiation. All of three tumors belong to type I EOCs and are believed that at least 1/3 of the cases originate in endometriosis [89, 90]. These tumors usually have endometriosis, endometrial benign, and/or borderline tumors in the background lesions.

Most endometrial carcinomas are low-grade (FIGO grade 1), whose clinical manifestations are consistent with the features of type I EOCs. Only a few endometrial carcinomas

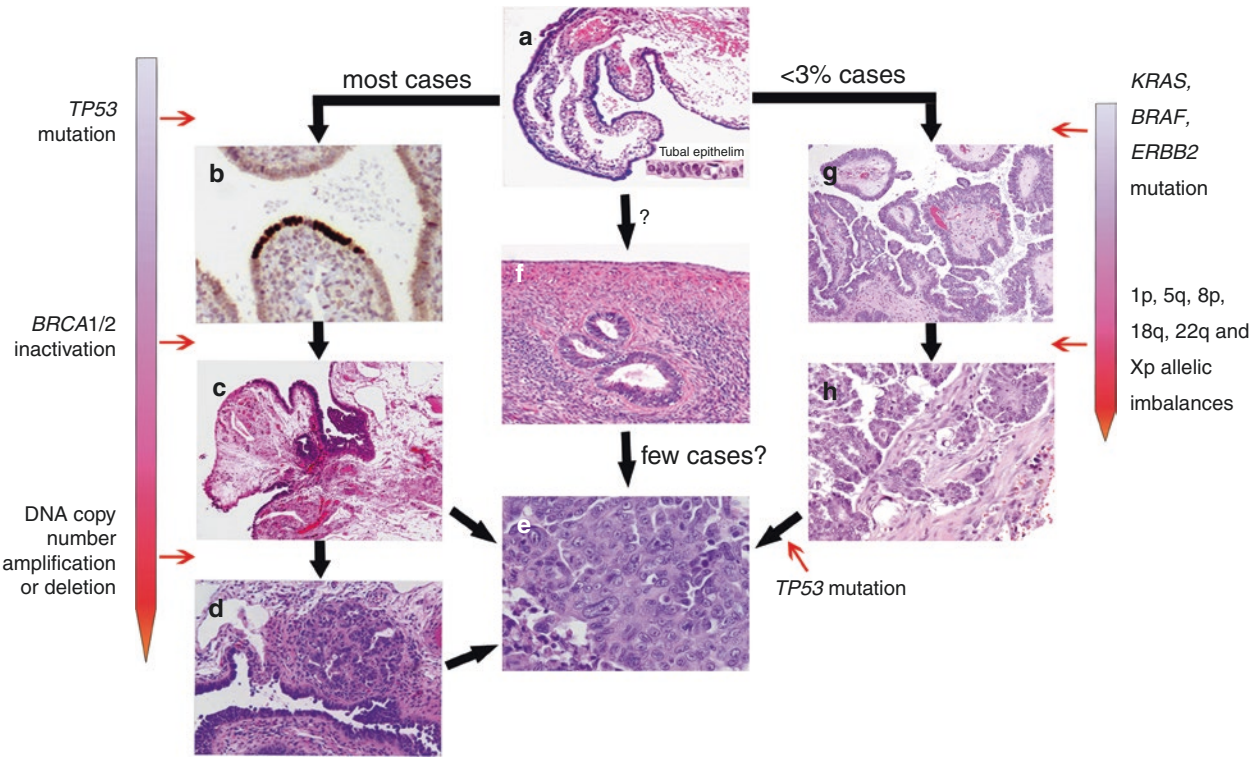

Fig. 4.3 The progression of high-serous serous carcinoma and associated molecular genetic alterations. The normal mucosa epithelium of fallopian tubal fimbria (**a**) undergoes p53 signature (**b**), serous tubal intraepithelial carcinoma (**c**), serous tubal invasive carcinoma (**d**), and ovarian high-grade serous carcinoma via the shedding and implantation in ovary (**e**). The normal tubal epithelium may seed on the ovarian surface during ovulation process and form the tubal OEIs (**f**). Few ovarian high-grade serous carcinomas may be derived from the OEIs. The *TP53* and *BRCA1/2* mutations and numerous amplifications and deletions of DNA copy number involve in above two pathways. Less than 3% of high-grade serous carcinomas are originate in direct progression of low-grade serous carcinoma (**h**) or the recurrence of borderline serous tumor (**g**). This process may also be related to *TP53* mutations

belong to intermediate-grade or high-grade (FIGO grade 2 or 3). In these tumors, it is frequently found the component of low-grade carcinoma or the presence of some molecular genetic alterations similar to concurrent low-grade carcinoma, which suggests that these intermediate-grade or high-grade components may be transformed via dedifferentiation of low-grade carcinoma [52]. Few high-grade endometrioid carcinomas have the same clinical features and genetic alterations (such as *TP53* mutation) as those of high-grade serous carcinomas. Recent evidence supports the notion that these tumors are more suitable for being classified to a variant of high-grade serous carcinomas [80, 90, 91].

Endometriosis is an estrogen-dependent inflammatory disease. Ovary is the most common organ involved by endometriosis. Thus far its origin is still in dispute. Its major theory is the reflux menstruation and the coelomic metaplasia [92]. In 2005, Zheng et al. [93] found the earliest morphological changes of endometriosis and named it "initial endometriosis." Its histological feature is a transition from minimal to mature endometriosis in normal ovarian tissue. This change presents both in the ovarian epithelium and in the cortical OEI, and the latter is most common. That is, some ovarian OEI cells exhibit the endometrioid morphological changes. In combination of the recent views that some OEIs are derive from the seeding of tubal epithelial cells [54, 90], it is possible that fallopian tube may contribute the histogenesis of ovarian endometriosis. Lately, Yuan et al. [94] found that 18 cases (56%) showed a high level of FMO3 and a low level of DMBT1 expression in 32 ovarian endometriosis cases by a gene differential array analysis, which is similar to fallopian tubal profile. This result suggests that approximately 60% of the ovarian endometriosis may be derived from the fallopian tube, whereas about 40% of the cases may be of endometrial origin. Using a fallopian tube-specific promoter *OVGP1* gene, Wu et al. [95] developed an animal model with specifically biallelic inactivation of *Apc* and *Pten* in oviductal or ovarian epithelium using *Ovgp1-iCreER*^{T2} mice and found that there was the formation of endometrioid carcinoma-like malignant epithelial tumors in the fallopian tubes or ovaries, respectively. On the basis of the morphology and global genetic profiles, the oviduct-derived tumors more closely resemble human ovarian endometrioid carcinomas than do OSE-derived poorly differentiated ovarian carcinoma. The abovementioned results provide strongly experimental evi-

dence that tubal epithelial cells may be one of the potential origins of ovarian endometrioid carcinoma.

Recent data suggest that clear cell carcinoma may arise from ciliated cells in the endometrium (both eutopic endometrium and endometriosis) and fallopian tubes, whereas endometrioid carcinoma may arise from secretory cells rather than histotype-specific mutations. The cystathionine gamma-lyase (CTH) and methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) can serve as specific markers for ciliated and secretory cells, respectively [96].

Common genetic and genomic alterations: At the genetic level, several unique genes have been found in the endometrioid carcinoma. The most frequently mutated gene is *ARID1A* tumor suppressor gene. Its encoded protein BAF250a can bind to the AT-rich DNA sequence and is a key component of the switch/sucrose non-fermentable (SWI/SNF) chromosome remodeling complex. The function of this complex is to mobilize nucleosomes, regulate gene expression and chromosome kinetics, and participate in the regulation of a variety of cell processes (e.g., development, differentiation, proliferation, DNA repair, and tumor suppression). Mutations in *ARID1A* have been detected in 46–57% of cases of clear cell carcinoma, 30% in cases of endometrioid carcinoma, and 33% in cases of seromucinous carcinoma; on other hand, there are no detected *ARID1A* mutations or deletions in serous and mucinous carcinoma and malignant Brenner tumor, suggesting that these mutations are histotype-specific. Interestingly, and unexpectedly, while it is widely believed that endometriosis is a precursor lesion of endometrioid, clear cell, and seromucinous carcinoma. These mutations are also found in endometriosis tissues adjacent to cancer tissues [97], suggesting that these commonly believed cancer-causing mutations are actually not the drivers of malignant transformation. *ARID1A* may be an early event of endometriosis progression to cancer [98–100]. In addition to *ARID1A* gene, endometrioid, clear cell, and seromucinous carcinomas have other special genetic alterations. It is now clear that sole *ARID1A* inactivation is not sufficient to induce tumorigenesis. The deletion of both *Arid1a* and *Pten* leads to endometrioid and undifferentiated carcinoma *in vivo* studies [101]. And the combinations of deletions of *ARID1A* and *PIK3CA* can cause clear cell-like tumors via the activating inflammation signaling pathways [102, 103].

The genetic alterations in the endometrioid carcinoma appear to be different from that of clear cell carcinoma. Mutations in *PI3K/PTEN* and Wnt/β-catenin signaling pathways are frequently detected, including *CTNNB1* (15–40%), *PIK3CA* (20%) and *PTEN* (14–21%). *CTNNB1* mutation is associated with squamous differentiation, low-grade tumors, and good prognosis. *PIK3CA* and *PTEN* mutations can occur simultaneously. 13–20% cases of endometrial carcinoma possess microsatellite instability (MSI), which usually manifest as the deletion expression of hMLH1 (human mutL homolog 1) or hMSH2 (human mutS homolog 2) protein. *KRAS* and *BRAF* mutations are rare, with an incidence of less than 7% [9, 52].

Compared with endometrioid carcinomas, clear cell carcinomas rarely have abnormal Wnt/β-catenin signaling pathway. The most common genetic alteration is *ARID1A* inactivation mutation. It is documented that the expression status of the SWI/SNF complex serves as an independent prognostic factor. The loss of one or multiple SWI/SNF complex subunits demonstrates aggressive behaviors and poor prognosis [83]. Clear cell carcinoma also has a high *PIK3CA*-activated mutation (40%) and *PTEN* deletion mutation (10%). More than 70% *PIK3CA* mutation occurs simultaneously with *ARID1A* mutation [52]. Clear cell carcinoma shows similar frequency of MSI [104, 105] as endometrioid adenocarcinoma except that MSI is mainly manifested as *hMSH2* germline mutation. Furthermore, clear cell carcinoma has a unique epigenetic alteration, which involves in the methylation of multiple gene promoters in α-estrogen receptor alpha (ERα) and hepatocyte nuclear factor 1 (HNF-1) signal pathways [106]. The changes in cell metabolism induced by HNF-1β expression are conducive to tumorigenesis and cell survival [107].

Seromucinous tumors are very rare. Morphologically, seromucinous tumors in addition to serous and endocervical-type mucinous epithelium contain endometrioid, undifferentiated, and squamous-type epithelium with frequent expression of ER and PR, lack of expression of CK20 and CDX2, and infrequent expression of WT1. This tumor is also frequently associated with endometriosis-like endometrioid or clear cell carcinoma. Although seromucinous carcinomas were introduced as a new entity in 2014 WHO classification, it has been argued that its name may have a serious flaws that obscure that nature of neoplasms [90]. Due to its rarity, there are few reports about its molecular genetics except *ARID1A* inactivation mutation. In 2017, through the study of 32 tumors diagnosed as seromucinous carcinomas from 2 medical centers, the authors found that these tumors had *KRAS* (70%), *PIK3CA* (37%), *PTEN* (19%), and *ARID1A* (16%) mutations. 30% of cases harbored concurrent *KRAS* and *PIK3CA* mutation [108]. The immunophenotype and molecular genetics of seromucinous carcinoma overlapped predominantly with endometrioid and low-grade serous carcinoma. After integrating morphology, immunophenotype, and genotyping, 32 cases of seromucinous carcinomas were reclassify to endometrioid (23 cases), low-grade serous (8 cases), and mucinous carcinoma (1 case). It has been proposed to reclassify this group of tumors as "mixed Müllerian tumors" which can be subcategorized as "mixed Müllerian cystadenomas," "mixed Müllerian atypical proliferative (borderline) tumors," and "mixed Müllerian carcinomas." Therefore, seromucinous carcinoma may be due to the heterogeneity and lineage infidelity of different histotypes and may not be a real distinct subtype of EOCs [108].

4.2.2.2 Mucinous Carcinoma and Malignant Brenner Tumor

According to the 2014 WHO classification, ovarian mucinous carcinoma refers to the gastrointestinal-type. With further studies on clinical pathology and molecular genetics, it is clear that the vast majority (more than 90%) of ovarian mucinous carcinoma (especially advanced tumors) are secondary, mostly from the gastrointestinal tract (especially colorectum and appendix). The other common metastatic sites include pancreas, bile duct, gallbladder, endocervix, bladder, etc. The real primary mucinous carcinomas are less than 5% of EOCs [7, 109].

The size of primary ovarian mucinous carcinoma is often large (more than 10 cm). Histologically, there are significant heterogeneity changes in tumor tissue, such as the presence of components of benign mucinous cystadenoma/adenofibroma, borderline tumor, and carcinoma. The vast majority of tumors are low-grade, which fully reflects the tumorigenesis of type I EOCs. Clinically, the patients with stage I ovarian mucinous tumors (borderline with/without intraepithelial carcinoma or microinvasion, and invasive carcinoma) have excellent prognosis, whose 5-year survival rate is more than 90%. Advanced ovarian mucinous carcinomas are uncommon and have a poor prognosis. The overall survival rate of these patients is the same as that of metastatic adenocarcinoma [110].

Compared with metastatic mucinous carcinoma, most primary tumors are confined to unilateral ovaries, suggesting that its precursor lesions should be located in the ovaries. Accumulating molecular genetic evidence suggests that at least a subset of mucinous tumors (including mucinous cystadenomas, borderline tumors, and mucinous carcinomas) may arise from mucous epithelium within mature cystic teratomas (germ cell origin) and Brenner tumor (non-germ cell origin).

The cell origin of teratomas is believed to be postmeiotic ovarian germ cells [111]. There are mucinous tumors in about 2–11% of ovarian mature cystic teratoma. Around 3–8% mucinous tumors are associated with teratomas. Using short tandem repeat analysis, genotypical concordance between the teratomas and coexisting mucinous tumors are found, which provide the evidence that the cell origin of some mucinous tumors is germ cells [111, 112].

Mucinous tumors are often associated with Brenner tumors. Seidman et al. [113] found that 20% of mucinous tumors have Brenner tumor components and 16% of Brenner tumors contain concurrent mucinous tumors. In the molecular genetics, there is 12q14-21 amplification both in ovarian mucinous carcinoma and coexistence of Brenner tumors using an analysis of comparative genomic hybridization [114]. It is also reported that all two components in most mixed Brenner and mucinous tumors have a concordant X-chromosome inactivation pattern via a human androgen receptor gene assay [115]. These results confirm that the components of Brenner and mucinous tumors share a same clonal relationship. Some mucinous tumors originate from the Brenner tumor and the pure mucinous tumor may develop from a Brenner tumor in which the component of Brenner tumor is compressed and obliterated by an expanding mucinous neoplasm. The transitional cell nests in Brenner tumors frequently contain mucinous cells, prompting that the overgrowth of these mucinous epithelial cells eventually lead to the occurrence of mucinous tumors.

Mucinous carcinomas commonly possess the activation of RAS/MEK signaling pathways. More than 90% of cases present *KRAS*, *BRAF*, and/or *ERRB2* mutation. Among them the most common is *KRAS*-activated somatic cell mutation (64.5%) [116]. The amplification of *ERRB2* is about 15% to 20%. *KRAS* mutation is thought to be an early event of mucinous carcinogenesis because *KRAS* mutation is found in mucinous cystadenomas and borderline tumors adjacent to cancer tissues [52]. Moreover, *TP53* mutation rates in mucinous carcinomas and borderline tumors are 56.8% and 11.5%, respectively [116]. About 21% cases of mucinous carcinomas had an inactivated mutation of *RNF3* tumor suppressor gene, similar to pancreatic mucinous tumors [117].

Brenner tumors are composed of urethral/transitional epithelium nested around with fibromatous stroma. The vast majority of tumors are benign. The malignant tumors are very rare, and around 80% of tumors are confined to the ovary (FIGO stage I), with a typical progression process of type I EOCs, i.e., from benign to borderline to malignant Brenner tumors.

Its origin is still unknown because the morphology of Brenner tumors is totally different from Müllerian epithelium and lacks the expression of Müllerian immunohistochemical markers (PAX-8 and PAX-2). Some scholars believe that its origin is independent of ovarian OEI [8]. Many tissue or cell types, including transitional cell metaplasia from OSE, mesonephric remnant, the rete ovarii, mucinous tumor, fallopian tube, and teratoma, have been presumed to be the origin of Brenner tumor [113].

Walthard cell nests consist of transitional epithelium and usually exist at/near the tuboperitoneal junction and frequently coexist with Brenner tumors. It has long been considered the origin of Brenner tumors because they display epithelia with the same morphology and immunophenotype (e.g., GATA3 and p63 positive, germline marker SALL4 negative) as Brenner tumors [8, 118]. Roma et al. [118] found that 43% of patients with Brenner tumors had Walthard cell nests in the soft tissue surround fallopian tubes/ovaries. Through a morphologic and immunohistochemical analysis in Brenner tumors, Kuhn et al. [119] found that tubal secretory cells, transitional metaplasia, Walthard cell nests, and the epithelial component of Brenner tumors shared a similar immunophenotype, consistently expressing AKR1C3 (an enzyme involved in androgen biosynthesis) and androgen receptor, but not calretinin. The stromal cells that closely surrounded the epithelial nests showed strong expression of calretinin, inhibin, and steroidogenic factor 1 (markers of steroidogenic cells) in the majority of tumors. There were

small groups of cilia in transitional metaplasia and Walthard cell nests, multifocal stretches of cilia and/or ciliated vacuoles in benign tumors, and well-developed cilia in atypical proliferative tumors. These findings suggest a tubal origin of Brenner tumors via transitional metaplasia and Walthard cell nests under the effect of androgenic stimulation.

Malignant Brenner tumors are low-grade EOCs with the activation of PI3K/AKT signaling pathway. *PIK3CA* mutation has been reported in an individual case [120]. In benign Brenner tumors, the positive rate of p16 immunohistochemistry is 92%, while completely negative expression of p16 is found in the borderline/atypical proliferative Brenner tumor. The data from fluorescence in situ hybridization confirm that there is loss of heterozygosity of *CDKN2A* gene (encoding p16 protein), suggesting that *CDKN2A* deletion may play a role in progression from benign to borderline Brenner tumor [9].

4.2.2.3 Undifferentiated Carcinoma and Malignant Mixed Müllerian Tumor

Both of these histotypes are rare high-grade and aggressive malignant tumors. Undifferentiated carcinomas lack the features of high-grade serous or endometrioid carcinoma. Nearly half of the cases have deficiencies of mismatch repair proteins. It is not clear whether the tumor is an independent histological subtype or a poorly differentiated variant of high-grade serous or endometrioid carcinoma. MMMTs have significant biphasic differentiation of high-grade carcinoma and sarcoma elements. It is believe that these tumors belong to high-grade carcinoma with sarcomatoid differentiation due to the same clonality of these two elements. Its most common molecular genetics alterations are *TP53* mutation and *CDKN2A* amplification [9].

In summary, many of EOCs come from extraovarian tissues, and the ovaries could be secondary involvement; a significant number of ovarian cancer may also arise from multifocal origin of different type of Müllerian epithelial cells. Recent studies have provided increasing evidence that a number of EOCs may arise directly or indirectly from tubal epithelial cells except that a part of mucinous carcinoma and endometriosis-associated EOCs (endometrioid, clear cell, and seromucinous carcinoma) derive from ovarian germ cells and glandular epithelial cells of endometriosis, respectively (Fig. 4.4). However, readers should consider these models only as hypotheses. As more evidence accumulates in the future, these models will be revisited and updated.

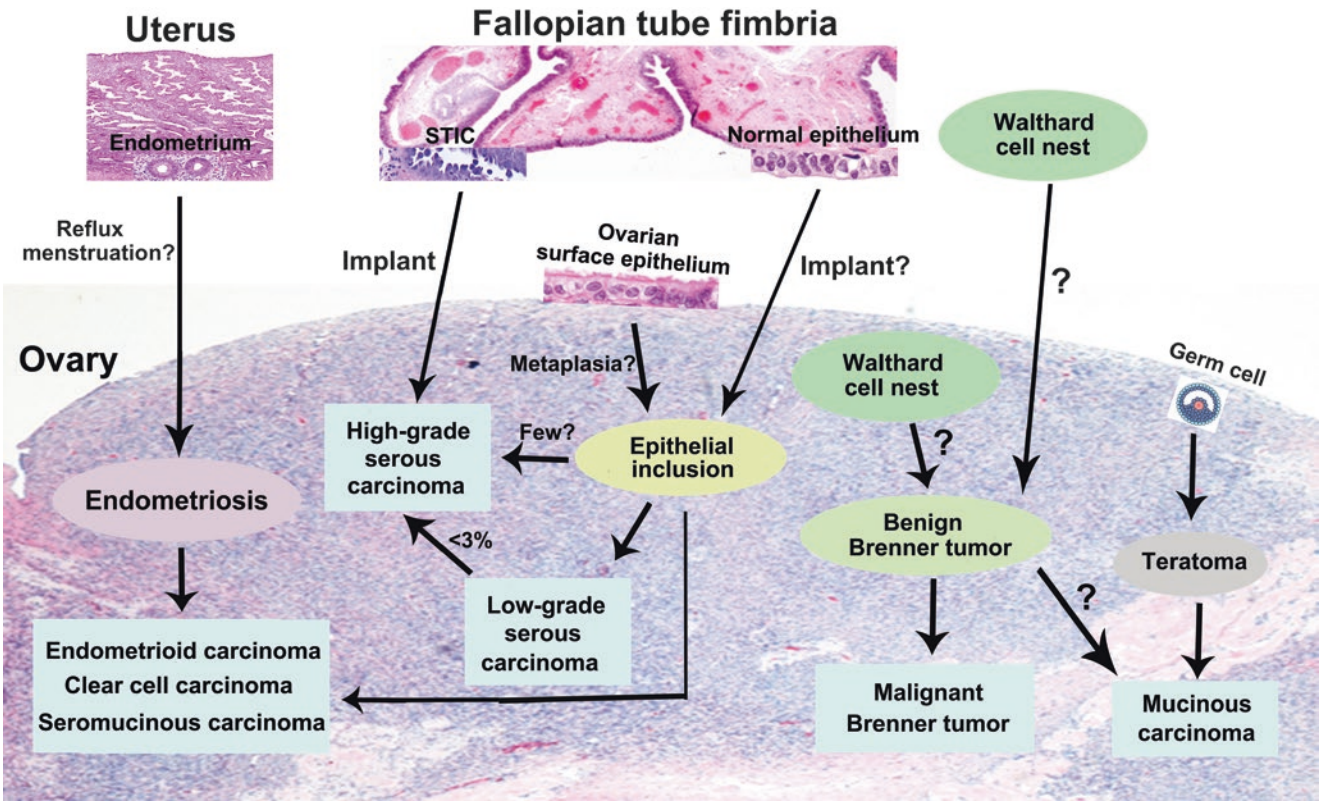

Fig. 4.4 The proposed models of ovarian epithelial carcinogenesis. Normal mucosa epithelial cells of fallopian tubal fimbria shed and implant on the ovarian surface. The OEIs are formed via the epithelial invagination during ovulation process and then undergo metaplasia. Endometrioid, clear cell, and seromucinous carcinoma originate in endometriotic glands and OEIs. Low-grade serous carcinoma arises from OEIs. High-grade serous carcinoma mostly comes from the seeding of serous tubal epithelial carcinoma (STIC). A very small number of them are derived from OEIs. And less than 3% of cases are from the progression of low-grade serous carcinomas or the recurrence of borderline serous tumors. Malignant Brenner tumors progress from benign Brenner tumors that may derive from the Walthard cell nests, although the cell origin of Walthard cell nests is unclear. Mucinous carcinomas originate in mucosal epithelium within the mature cystic teratomas and benign Brenner tumors

4.3 Newly Clinical Applications Based on Ovarian Epithelial Carcinogenesis Models

These above new models on carcinogenesis and the mechanisms of molecular genetics suggest that diagnosis, prevention, and treatment of the patients with EOCs should be determined by new strategy.

4.3.1 The Adjustments in Early Screening

Traditional strategy in early screening of EOC is "the triple methods" worldwide, which includes gynecological examination, transvaginal ultrasound, and serum CA125 detection. This screening strategy is based on the traditional concept that EOC form mass in the early stage. In fact, only a small number of patients with type I EOCs are diagnosed at the early stage due to the mass formation. The majority of type II EOCs, the most common type of EOCs, is often missed because there may not form mass at the early stage.

In addition, the prognosis of patients with EOCs depends not only on tumor staging but also on the biological behaviors of specific histological subtypes of tumors. In general, type II EOCs show more rapid growth, more aggressive clinical behaviors, and higher lethality rate compared with type I EOCs. Therefore, it is unlikely to achieve early diagnosis only by conventional screening method in type II EOCs. A published literature from the large-scale clinical trial result has exhibited the ineffectiveness of this screening method and provides further support for this view [121].

In view that there is no obvious mass formed in some type II EOCs in the ovary and/or pelvic cavity at early stage [122], the concept of early diagnosis for EOC has changed to a new idea by trying to find a small and/or tiny ovarian tumors in pelvic cavity. Based on the theory that the fallopian tube fimbria is also an important source of EOCs, not only ovaries but also fallopian tubes should be checked in order to find STIC, a precursor of high-grade serous carcinoma, which will also affect FIGO staging in high-grade serous carcinomas. The standardized sampling, Sectional and Extensively Examining the FIMbria (SEE-FIM) proposed by Crum et al. [123], has been widely adopted in pathological examination of fallopian tube and greatly increase the detection rate of STIC. However, it must be emphasized that approximately 50% of pelvic serous carcinomas have no defined STIC; the pathologic examination of the fallopian tube along is unlikely to find all of precursor lesions for all pelvic serous carcinoma.

In patients with EOCs with familial genetic defect (such as *BRCA1/2* mutation and Lynch syndrome-related MSI), it is particularly important for these high-risk groups to be taken into consideration of close follow-up and early screening. Although diagnostic value for high-risk patients with asymptomatic tumor in early stage by ultrasound examination is very limited [124], other imaging techniques, such as multispectral fluorescence imaging and magnetic resonance imaging (MRI) diffusion-weighted imaging, could help to improve the detection rate of small-volume tumors [125, 126].

In conclusion, the decreases in mortality of EOCs should focus on the detecting small volume of type II EOCs and identify high-sensitivity molecular markers or genetic alterations in this type of tumors. The morphology, molecular genetics, and biological behavior in STICs are similar to high-grade serous carcinoma, which are different from the precancerous lesion of type I EOCs because most of them existed as benign and borderline tumors. Even though the lesions are confined to the epithelium of tubal mucosa, the cells in STICs have markedly decreased adhesion and easily shedded and implanted into pelvic cavity. Tumor mutation-specific DNAs can be detected in ovarian cystic fluid [127], given their biological characteristics; it may be possible to detect these lesions using detection of cytology in combination with genetic probes or a highly sensitive molecular imaging technology in the fallopian tube, although it is unlikely to detect all of high-grade serous carcinoma due to alternative multicentric origins for these tumors.

4.3.2 The Changes in Prevention Strategy

As the significant number of EOCs is secondary to extra-ovarian tissue, especially the significant number of high-grade serous carcinoma arising from the mucosa of fallopian tube fimbria, it is not surprising that prophylactic bilateral salpingo-oophorectomy can effectively reduce the EOC risk in women with *BRCA* gene mutation or the family history of EOCs. In addition, bilateral salpingo-oophorectomy has been gradually replaced by bilateral salpingectomy so that the female fertility and endocrine function can be maintained as well as decreased risk in cardiovascular diseases caused associated with removal of ovary [128]. Toward this end, the Gynecologic Oncology Group (GOG) underwent the clinical trials that bilateral salpingo-oophorectomy was replaced by bilateral salpingectomy to prevent hereditary EOCs. In 2015, Falconer et al. [72] analyzed data from the Swedish Cancer Registry on women with previous surgery on benign indication (sterilization, salpingectomy, hysterectomy, and bilateral salpingo-oophorectomy; n = 251,465) compared with the unexposed population (n = 5,449,119) between 1973 and 2009 using Cox regression models. They found that there was a statistically significantly lower risk for ovarian cancer

among women with previous salpingectomy (HR = 0.65, 95% CI = 0.52 to 0.81) when compared with the unexposed population. Bilateral salpingectomy was associated with a 50% decrease in risk of EOC compared with the unilateral procedure (HR = 0.35, 95% CI = 0.17 to 0.73, and 0.71, 95% CI = 0.56 to 0.91, respectively). These data support that salpingectomy by itself, or in combination with other benign surgery, is an effective method to reduce EOC risk in the general population.

The most direct and effective preventive method is that the patients with benign diseases undergo prophylactic removal of bilateral fallopian tubes (so-called opportunistic salpingectomy) and keep their ovaries when they need hysterectomy surgery, because there is no physiological significance for the fallopian tubes after hysterectomy. In addition, for women who are willing to have sterilization surgery, the application that traditional tubal ligation is replaced by bilateral salpingectomy may have a good preventive effect on EOCs that originate in fallopian tubes and endometrium. However, as mentioned above, in our opinions, the evidence that majority of pelvic serous carcinoma is still a hypothesis and likely to be multicentric origins. Great caution should be excised in clinical practice as not all of normal physiologic functions are known in the ovarian and other organs in women.

Mounting evidence has shown a close correlation among EOCs, ovulation, inflammation caused by the process of ovulation, and the release of sex hormones. The decrease of ovulation (such as oral contraceptives, multiple pregnancies, breastfeeding, delayed menarche, etc.) can effectively reduce the incidence of EOCs [129, 130]. It is reported that oral contraceptives can reduce the incidence of ovarian cancer by 50%. The longer duration women have used oral contraceptives, the greater reduction is in the EOC risk. However, the incidence of mucinous tumors seems little affected by oral contraceptives [131]. Unfortunately, long-term use of oral contraceptives has multiple potential side effects. Because the rupture of ovarian follicle releasing the ovum and follicular fluid is a prostaglandin-mediated inflammatory process that can be stopped by nonsteroidal anti-inflammatory drugs (e.g., aspirin) and lead to pharmacologic production of a luteinized unruptured follicle, which mimics a normal nonconception cycle with unaltered steroid patterns/levels and cycle length, it is suggested that non-hormonal periodic interruption of incessant ovulation could be recommended for women who are at high EOC risk. Recent data clearly reveal that the usage of low doses of aspirin can lessen the risk of ovarian cancer [132]. Considerable further research is required to validate the potential of this approach. In short, as knowledge accumulation on the ovarian epithelial carcinogenesis, more and more drugs with low side effects are being developed for the prevention of EOCs.

4.3.3 The Improvements in Therapeutic Methods

Today, ovarian cancer remains one of the most challenging diseases on treatment. Since EOCs are comprised of a heterogeneous group of diseases, the different histological subtypes have different pathogenesis patterns, cell origin, and molecular genetic alterations. Therefore, it is necessary for patients to receive individualized therapy according to the specific histologic and molecular features of tumors.

The majority of type I EOCs is confined to the ovary (FIGO stage IA). All of them are classified as low-grade carcinomas, with the exception of clear cell carcinoma. For FIGO IA tumor, the main clinical therapeutic strategy is surgical resection, including tumor and ipsilateral ovary with/without pelvic lymphadenectomy. For advanced tumors with extraovarian dissemination, the patients must undergo adjuvant chemotherapy. However, due to an indolent biological behavior, tumor cells are often not sensitive to chemotherapy and easy to develop drug resistance. In consideration of constitutive activation of MAPK signaling pathway induced by *KRAS*, *BRAF*, or *ERBB2* mutation, it is possible for MAPK inhibitor-based therapy to become an effective adjuvant therapeutic approach. Indeed, this sort of treatment has showed efficacy for advanced and/or recurrent type I EOCs in the clinical trials [133, 134]. In addition, in view of the correlation between some type I EOCs (e.g., endometrioid and clear cell carcinoma) and the deficiency of mismatch repair proteins and a large number of tumor-infiltrating lymphocytes within the tumor stroma, the patients may benefit from the current anti-PD-1 (programmed death 1) inhibitors in immune checkpoint therapy. For those type I EOCs with *ARID1A* mutation (such as endometrioid, clear cell, and seromucinous carcinoma), it is interesting to note that tumor cells exhibit sensitivities to EZH2 inhibitors [135, 136] and poly (ADP-ribose) polymerase (PARP) inhibitors [137] *in vitro*. Additional work is needed to determine if these inhibitors can be explored for clinical application in the future.

The vast majority of type II EOCs is advanced stage at presentation. The main strategy of clinical treatment is reductive surgery in combination with chemotherapy. The tumor cells are prone to have secondary drug resistance, although they are sensitive to drugs in the initial phase of chemotherapy [81]. In patients with high-grade serous carcinoma, nearly 50% of cases have the deficiency of homologous recombination proteins and about 20% of patients having *BRCA1* or *BRCA2* mutations (16% of cases are germline mutations; 4% of cases are somatic mutations). Since the European Medicines Agency and the US Food and Drug Administration approved the development of PARP inhibitors (olaparib) for cisplatin-sensitive, recurrent, or advanced

high-grade serous carcinoma with *BRCA* mutation in 2014, the application of olaparib has been used in patients with BRCA mutations. In addition, several the other PARP inhibitors are also at the various stages of clinical trials [138]. The therapeutic strategies of platinum-based medicines in combination with PARP inhibitors may prolong disease-free survival of the patients with *BRCA* mutation-related EOCs [139]. On the other hand, current immunotherapies have been largely ineffective in advanced and current ovarian cancer patients [140]. Therefore, great challenges lie ahead why the ovarian cancers are largely unresponsive for commonly used check point inhibitor for immunotherapy. Finally, if our hypothesis of somatic blastomere origin is correct, treatment should be focused on preventing formation of PGCCs and redifferentiating the PGCCs toward benign lineages, which will offer a totally new paradigm for cancer prevention and therapy. It is expected that more and more efficient therapeutic strategies will be developed to clinical treatment as we gain additional insight into the mechanism of ovarian carcinogenesis.

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