Microenvironment and Pathogenesis of Epithelial Ovarian Cancer

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Abstract Multiple genetic alterations play a role in the pathogenesis of ovarian cancer. Although many key proteins and pathways involved in ovarian carcinogenesis and metastasis have been discovered, knowledge of the early steps leading to malignancy remains poorly understood. This poor understanding stems from lack of data from early-stage cancers and absence of a well-established premalignant state universal to all ovarian cancer subtypes. Existing evidence suggests that ovarian cancers develop either through a stepwise mutation process (low-grade

pathway), through genetic instability resulting in hastened metastasis (high-grade pathway), or more recently through what has been described as the “‘fimbrial-ovarian’ serous neoplasia theory.” In this latter model, ovarian serous cancers evolve from premalignant lesions in the distal fallopian tube called tubal intraepithelial carcinoma. In this manuscript, we review key genetic and molecular changes that occur in cancer cell progression and suggest a model of ovarian cancer pathogenesis involving both tumor cell mutations and microenvironmental factors.

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Introduction

Ovarian cancer is the most common cause of death from gynecologic malignancies and the second most common gynecologic cancer [1]. Epithelial neoplasms are the most common malignant ovarian neoplasm [2]. Most cases are diagnosed late, resulting in poor clinical outcome. Survival of epithelial ovarian cancer (EOC) patients remains <50% at 5 years in spite of recent advances in cytoreductive surgery and chemotherapeutic agents [1]. Despite the promise for better outcomes in recurrent EOC and advanced-stage ovarian cancer through biologic therapies, a better strategy could be achieved by detecting high-risk patients and offering risk-reducing surgery that has been shown to be successful in patients at high risk due to genetic mutations [3]. Conversely, EOCs are considerably heterogeneous. For instance, subtypes such as endometrioid, serous, mucinous, and clear cell all manifest differently and involve unique pathogenic molecular blueprints [4]. Among serous tumors, low- versus high-grade tumors also have different molecular pathogenesis. Consid-

erable progress has been made in comprehending the genetic makeup and physiological processes involved in ovarian cancer, but its underlying etiology is not well understood. In this review, we will focus on the existing science in characterizing the initial events in EOC.

Etiology of Sporadic Epithelial Ovarian Cancer

During embryogenesis, the coelomic layer develops into peritoneal mesothelium that surrounds the ovary and later differentiates through metaplasia to an epithelial layer [5]. Interestingly, contrary to other malignancies, as the ovarian epithelium acquires a malignant phenotype, it becomes more, rather than less, differentiated. This feature may explain its ability to transform into any cell type found in the Müllerian tract, such as those in the fallopian tube, uterus, cervix, and ovarian stroma [6]. Germline mutations are responsible for about 10% of ovarian malignancies and commonly involve BRCA1, BRCA2, or mismatch repair genes. Here, we will first focus on features of sporadic epithelial ovarian cancer.

For carcinogenesis to occur, the progenitor cell must surmount the physiologic checks and balances in order to become a clinically evident tumor [6]. Examples of such mechanisms include impairment of apoptosis, uncontrolled cell proliferation, angiogenesis, and metastasis. Several hypotheses have been proposed with regard to the possible origins of ovarian cancer (Table 1). The fact that there is a positive correlation between the number of ovulatory cycles with risk of ovarian cancer paved the way to the incessant ovulation hypothesis by Fathalla in 1971 [7]. According to this theory, with every ovulatory cycle, ovarian surface epithelial cells are injured, and subsequently, through repair mechanisms, the cells are predisposed to development of

mutations and later malignancies. Coherent with this hypothesis, multiparity [8–10], length of lactation [11], and oral contraceptive usage [8, 12] all decrease the risk of developing ovarian cancer. Moreover, confirmatory data from animal studies support the incessant ovulation hypothesis [13, 14]. But, this hypothesis is somewhat undermined by the fact that progesterone-only oral contraceptives are as effective as ovulation-inhibiting contraceptives despite not inhibiting ovulation [15]. In addition, although patients with polycystic ovarian syndrome (PCOS) are known to have decreased ovulatory or rather anovulatory cycles, these women are still at increased risk of EOC [16].

Pitfalls in the incessant ovulation theory and evidence of increased risk among women using fertility drugs in order to conceive led to the gonadotropin hypothesis. It states that ovarian epithelial cells are more predisposed for tumor transformation after exposure to follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Gonadotropin hormones (FSH, LH, and human chorionic gonadotropin) are known to activate EOCs to proliferate, and this process involves the mitogen-activated kinase pathway [17]. A recent study using human ovarian cancer cell lines (CaOV-3 and SKOV-3) showed that FSH/LH promote PGE2 production through cox-1 and cox-2 upregulation and increase cell invasion. In addition, treatment of these cells with cox inhibitors abrogated the stimulatory effects of gonadotropins on cell motility and cell invasion [18]. In addition, when the FSH receptor was overexpressed in non-tumorigenic SV40 Tag-immortalized ovarian surface epithelium (OSE)-derived cell lines, other proteins such as EGFR, HER-2, and c-myc were also upregulated [19]. The same was also shown when measuring other potential oncogenes in vitro such as cyclin G2, Meis-1, β -catenin, β -1 integrin, and IGF-1 [20, 21]. In a case-control study, Whittemore et al. [8] found that infertile women have a

Table 1 Hypotheses on physiologic susceptibilities to epithelial ovarian cancer

Hypothesis	Proposed mechanism	Evidence
Incessant ovulation	Ovulation leads to OSE damage and repair, making cells more predisposed to mutations	OCP use, breastfeeding, and pregnancy all decrease risk for EOC by diminishing the number of ovulatory cycles
Gonadotropin stimulation	FSH and LH activation leads to cell growth, cell mitosis, and genetic aberrations	Risk factors for EOC include PCOs and Infertility, whereas progestin-only OCPs are protective. In preclinical studies, FSH promotes oncogenes overexpression and cell proliferation
Hormonal stimulation	High levels of androgens in the tumor microenvironment are pro-oncogenic, where as progestins are protective	High androgenic conditions (PCOS, hirsutism, acne) increase risk, androgen is the predominant hormone in ovarian inclusion cysts; progestin use decreases risk of EOC, induces OSE cell death
Inflammation	Inflammation is involved in ovulatory damage of OSE, thus promoting reconstruction and mutation susceptibility	NSAIDs are believed to reduce risk; talc and asbestos have been associated with EOC; inflammatory mediators have been found in the microenvironment of tumor cells

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OSE ovarian surface epithelium, EOC epithelial ovarian cancer, FSH follicle stimulating hormone, LH luteinizing hormone, PCOS polycystic ovarian syndrome, OCPs oral contraceptive pills

higher chance of developing EOC (OR=2.8) and borderline tumors (OR=4.0) after taking infertility medications. However, other studies [22] have reported conflicting results, which prompted the idea that it is the infertility state, rather than gonadotropin medical therapy, that increases the risk of EOC [23]. Moreover, it has already been shown that FSH and LH receptors are frequently found on normal ovarian surface epithelial cells and 60% of malignant ovarian cancer cells [24]. Thus far, no evidence has been published showing that gonadotropins promote alterations of OSE to a malignant phenotype. However, animal studies have shown that gonadotropins play a key role in tumor growth [25], angiogenesis [25], vascular endothelial growth factor (VEGF) expression [26], and cell adhesion [27]. Therefore, current studies suggest a role for gonadotropins in ovarian cancer progression, but not necessarily in causality.

Progesterone has been shown to reduce the risk for ovarian cancer [15]. Similarly, progestin-only contraceptives are as valuable as combined OCPs in reducing the risk for ovarian cancer [15, 28]. The mechanism for this effect may be related to reduced ovarian testosterone levels [29]. Moreover, cell proliferation in OSE cells can occur through androgen receptors [30]. Developing follicles contain the highest androgen levels [31], providing an androgen-rich microenvironment around the epithelial cells. However, in vitro studies have not validated the effect of androgen derivatives on cancer cell growth [32]. Known hyperandrogenic diseases such as PCOS, hirsutism, and acne have been shown to be linked with increased EOC risk [16].

Various inflammatory processes are involved with each ovulation and are believed to be the causative process responsible for priming OECs to genetic damage and carcinogenesis. With every ovulatory cycle, inflammation occurs through the action of cytokines and chemotaxis of inflammatory cells and plays an important role in tissue repair [23]. The inflammation theory is supported with the observation that women taking anti-inflammatory medications (NSAIDS, ASA) have lower incidence of ovarian cancer [33]. The molecular pathway in this process involves intracellular effectors implicated in malignant transformation such as VEGF, NF- κ B, nitric oxide synthase, and cyclooxygenase-2 [33]. Other pro-inflammatory chemicals such as talc and asbestos have been shown to predispose patients to ovarian cancer [34]. However, no confirmation of the relationship between talc or asbestos exposure and ovarian carcinogenesis in an animal model has ever been established.

All of the aforementioned pathways may play a part in the overall process of ovarian carcinogenesis in certain groups of patients, but their direct relationship may be in doubt and suggests that other mechanisms may be involved. To enable detection of high-risk patients early, more research is required to identify key genetic and epigenetic factors.

Earliest Identifiable Processes in Tumor Progression

It is believed that EOCs originate from a single cell that has become abnormal. This belief is based on studies showing common characteristics between primary and metastatic lesions when analyzing loss of heterozygosity (LOH), X-chromosome inactivation, and DNA mutations [35]. Identification of early processes involved in tumor progression is complex since the tumors usually present late, making it difficult to clearly identify the earliest events.

Comparison of Genetic Makeup of Early- Versus Late-Stage High-Grade Ovarian Cancers

Analyses of high-grade tumors have revealed overexpression of genes potentially responsible for cancer development and predicting clinical outcome (described later). While the earliest genetic events in ovarian cancer remain elusive, with the emergence of new technologies such as microarray expression profiling and comparative genome hybridization (CGH), many potential early genetic incidents have been discovered. At the expression level, early-stage tumors have profound changes in gene expression, which can be surprisingly similar to those found in late-stage tumors [36]. This goes against the hypothesis that advanced-stage tumors originate or progress from early-stage ones. But, when CGH analysis was used in the same study, differences were found at the genome levels that were more congruent with the evolution of cancer theory. Another study analyzing cancer specimens obtained from the ovary and omentum revealed 27 genetic changes that can be used to distinguish primary from metastatic cells [37]. The main pathway found to be altered was the P53 cascade; hence, this study revealed that this pathway may be critical for peritoneal seeding of ovarian cancer cells. Further studies are needed with larger power and microdissected specimens to elucidate differences between stromal and tumoral changes.

Genetic Disorders In general, the pattern of transmission for familial ovarian cancer is autosomal dominant with variable penetrance. Women with genetic predisposition for ovarian cancer are at high risk to develop the disease on average a decade earlier, with risk of clinical cancer increasing in the fifth decade [38]. BRCA1 and BRCA2 mutations constitute almost 90% of hereditary genetic disorders responsible for ovarian cancer. The lifetime risk of ovarian cancer in this population is estimated to be as high as 56% and 27%, respectively [39]. BRCA genes are the housekeepers of the cell's genome by mediating signals related to DNA injury, alerting and activating DNA repair mechanisms, monitoring the cell cycle through checks and balances, and promoting apoptosis [40–42]. BRCA has a close relationship with an array of important transcription

factors, including p53, STAT1, c-Myc, JunB, ATF-1, and others [43]. If the BRCA gene malfunctions, the cell will have an increased number of centrosomes, inadequate repair of damaged DNA, and will succumb to aneuploidy and will be predisposed to mutations [43]. Although cancers positive for these mutations will proliferate faster, better prognosis is observed [44]. Genetic evaluation revealed that BRCA1 and BRCA2 mutant tumors each have different gene expression profiles, while sporadic tumors had a profile similar to both BRCA1 and BRCA2 mutant tumors [45]. In the sporadic ovarian cancer population, mutations in the BRCA gene are uncommon [46]. Meanwhile, in the Jewish population, BRCA1 and BRCA2 mutations were only found in 4% of borderline tumors compared to early-stage ovarian tumors (24.2%), implicating a role for BRCA mutations in cancer initiation [47]. Factors affecting BRCA function include alternate splicing, epigenetic factors, and other genetic causes [48–50].

The remaining 10% of hereditary ovarian cancers consist of Lynch syndrome or hereditary nonpolyposis colorectal cancer (HNPCC). This syndrome is associated with alterations in DNA mismatch repair genes. It affects 5% of the population, and patients with this syndrome carry a risk of developing ovarian cancer of about 12% [51]. When the cell DNA repair apparatus malfunctions, DNA instability occurs and the cell becomes predisposed to unchecked mutations, leading to cell proliferation. Although ovarian carcinogenesis occurs in women with HNPCC, it has not been well studied. Other familial syndromes associated with stromal ovarian tumors include Peutz–Jeghers syndrome, which consists of hamartomatous polyposis (mutation in the STK11 gene, 21% lifetime risk), and Gorlin syndrome (mutation in PTCH, 20% lifetime risk), respectively.

Animal Models Several animal models have been designed in order to explore the pathogenesis of early ovarian carcinoma. To study the cell impact of various oncogenes, Orsulic et al. [52] used transgenic ovarian surface epithelial cells (p53-deficient) expressing the avian receptor TVA. After exposure to a variety of oncogenes, cells became tumorigenic when two out of three genes (c-myc, k-ras, Akt) were overexpressed. The transformed cells were then implanted into the bursal sac around the ovary of mice and later developed into peritoneal carcinomatosis similar to the metastatic pattern observed in human ovarian tumors. Connolly et al. [53] used genetically modified mice that express the transforming region of SV40 T-antigen controlled by the Müllerian inhibitory substance type II receptor gene promoter, resulting in de novo ovary-specific oncogenesis. In this model, the tumors were of high grade and were found in 50% of the mice along with carcinomatosis and ascites. Dinulesco et al. [54] established a model of endometrioid ovarian carcinogenesis. In this

study, ovarian bursal cavity was injected with adenoviral vectors expressing Cre recombinase, which resulted in the overexpression of k-ras and conditional PTEN deletion. Cells that overexpressed k-ras alone resulted in lesions histologically consistent with endometriosis, whereas cells that overexpressed k-ras along with PTEN knockout underwent rapid transformation to endometrioid carcinomas. Wu et al. [55] have introduced mouse models of ovarian endometrioid cancer containing deletion of APC and PTEN tumor suppressor genes resulting in a profile very similar to human cancer. A recent study used a syngeneic mouse model of human epithelial ovarian cancer model by orthotopically injecting murine ovarian carcinoma cells into transgenic mice [56]. This study allows research of the spread of disease to the peritoneum. Due to their ability to develop spontaneous ovarian tumors, the laying hen has also been a model animal used for ovarian cancer research. The CA125 ovarian cancer marker is also expressed in chicken ovarian cancer cells while absent in normal chicken ovarian cells [57]. Molecular studies of ovarian cancer have taken advantage of *Drosophila melanogaster* models. This model has given insight into genes involved in border cell migration and motility [58]. Recent studies in *Drosophila* have elucidated the role of transcriptional coactivator Yap as an ovarian cancer oncogene [59]. Wistar and Sprague–Dawley rats have also been found to develop spontaneous ovarian tumors and thus have served as models for cancer research [60]. Collectively, these models are aiding scientists to uncover the oncogenes responsible for early cancer development. Foreseen limitations to the application of these models include the difficulty in the delivery of genetically modified cells and the unclear role of SV40 T-antigen in human cancer.

Models of Ovarian Cancer

Clinical, translational, as well as genetic studies have elucidated two major categories of ovarian carcinogenesis based on the idea that tumors are heterogeneous: high-grade malignancies tend to be fast growing and chemosensitive, and the low-grade neoplasms typically grow slowly, but are less sensitive to chemotherapy [61]. Gershenson et al. presented a study comprising 112 low-grade ovarian serous carcinoma patients which identified the average age at diagnosis to be 43, much lower than the age for women with high-grade cancer. Women with low-grade ovarian cancer had prolonged median survival of 81 months compared to 57–65 months in those with high-grade epithelial ovarian cancer [62–64]. Pathologic findings show that 60% of low-grade ovarian serous carcinomas are

associated with a low malignant potential (LMP) neoplasm, whereas it was found in only 2% of high-grade ovarian carcinomas. Moreover, recurrent serous ovarian tumors of LMP are mostly low-grade lesions and have slow progression [65, 66]. Genetic and protein alterations in tumor cells also support the idea that the different types of ovarian cancers have unique pathogenesis (Table 2). Gene analyses have shown that b-raf, k-ras, and PTEN mutations occur more often in LMP tumors compared to high-grade tumors (30–50% versus 20%, respectively) [61, 67–69]. Conversely, HLA-G, HER2, and AKT levels are increased in high-grade tumors (61%, 20–66%, and 12–30%, respectively) as compared to LMP tumors [70, 71, 72]. While P53 is found to be mutated in >80% of high-grade tumors, it is rarely mutated in LMP tumors [73–75].

Assessment of whole genome profiles of ovarian cancers of various grades has shed light on the developmental association amongst the tumors. In such an approach, LMP tumors show closer similarity to normal ovarian epithelial cells rather than invasive cancers [4, 76]. Low-grade invasive cancers were also similar to borderline tumors. For example, wild-type P53 was found in LMP and low-grade tumors [4], suggesting that p53 dysfunction may not be required for these tumors. Moreover, developmental origin of these tumors was elucidated in loss of heterozygosity [77] and CGH [78] studies where LMP and benign adenomas share common genetic aberrations compared to high-grade tumors. Benign cystadenomas, LMP tumors, and mucinous adenocarcinomas have similar gene expression, which may be indicative of the sequential progression from adenoma to LMP and then to adenocarcinoma [79, 80].

In a recent study, Pothuri et al. [81] elucidated potential early aberrations in ovarian tumorigenesis. In this study, molecular, genetic, and morphologic analyses of early ovarian tumors and normal ovarian tissues revealed that EOCs originate from dysplastic precursor lesions within

ovarian inclusion cysts. In addition, laser microdissection and gene expression profiling of ovarian cystic epithelium revealed a quasi-neoplastic signature involving signal transduction, cell cycle control, and mitotic spindle formation. Moreover, these cells had a high proliferation index and aneuploidy. These findings suggest that at least some EOCs may arise in ovarian cystic inclusion sites, and these tumors may be preceded by identifiable dysplastic precursor lesions.

Compelling data offer evidence for a model where the distal fallopian tube is believed to be an early precursor for pelvic serous tumors. This theory emerged when pathology of specimens obtained from prophylactic bilateral salpingo-oophorectomy in BRCA-positive patients revealed the presence of premalignant lesions/early serous carcinomas in the distal fallopian tubes. Moreover, the same findings were found in more than 50% of patients with unknown BRCA status with pelvic serous carcinoma, establishing a potential link between the fallopian tube and pelvic serous cancer. Further analysis of tubal intraepithelial carcinoma (TIC) and their adjacent serous carcinoma showed shared P53 mutations [82], thus supporting an origin in the distal fallopian tube [83, 84]. Przybycin et al. [85] showed that TIC was found in 59% of high-grade serous carcinomas and 92% were found in the fimbriated end of the tube. When concomitant invasive serous carcinoma was present, TIC was found in 71% of cases. Additional work is needed to provide the mechanistic links between these potential precursor lesions and ovarian cancer.

Genetic and Protein Alterations in Ovarian Cancer

Most of the information about genetic and protein changes in ovarian cancer is based on advanced-stage tumor studies. However, it is important to examine low-stage cancers to understand some of the earliest genetic lesions. Therefore, based on the attributes that cancer cells need to obtain in order to become immortal [86], we will discuss these processes in the context of ovarian cancer (Table 3).

Independence from Growth Signals

Several oncogenes have been studied in ovarian pathology that enable cells to proliferate. One example is src, a tyrosine kinase that plays a functional role in cell proliferation, adhesion, angiogenesis, cell survival [87–89], and in chemotherapeutic drug resistance [90]. Src overexpression is found in 93% of late-stage ovarian cancers and in more than 80% of cell lines [91]. Inhibiting this proto-oncogene with antisense oligonucleotides or small molecule inhibitors results in the diminished growth of ovarian cancer in mouse models through inhibition of angiogenesis [87]. Another group of proteins that are

Table 2 Variability in biology in low- and high-grade tumors

Characteristic	LMP/low-grade	High-grade
p53 inactivity	Rare	50–80%
HLA-G	Rare	61%
HER2/neu amplification	Rare	20–66%
Akt amplification	Rare	12–30%
Apolipoprotein E expression	12%	66%
b-raf mutation	30–50%	Rare
k-ras mutation	30–50%	Rare
PTEN mutation	20% (endometrioid)	Rare
MSI	50% (endometrioid)	8–28%
ARID1A mutation	30% (endometrioid)	Rare

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Table 3 Select contributors to ovarian carcinogenesis

Protein	Function	Rate in EOC
Growth promotion		
EGFR(HER-1)	Membrane TK receptor, promotes growth	35–70%
HER-2	Membrane TK receptor, promotes growth	20–66% (HGS)
Src	TK, promotes growth, angiogenesis, survival	80–90%
CSF-1/fms	Ligand/receptor, inhibits anoikis	50–70%
ILGF/ILGFR	Peptide hormone/receptor, promotes growth	21–25%
k-ras	G-protein, promotes growth through MAP kinase pathway	30–50% (LGS)
b-raf	Promotes growth through MAP kinase pathway	30–50% (LGS)
Insensitivity to anti-growth signals		
TGF- β	Ligand, inhibits growth through Rb activation	Lost in 40%
myc	Transcription factor, cell cycle mediator	30%
Cyclin D/Cdk4/6	Advance from G1 to S phase	30–90%
Cyclin E/Cdk2	Advance from G1 to S phase	30–70%
Cyclin B/Cdk1	Advance cell cycle into M phase	80%
p16	Inhibits cyclin D/Cdk4/6	Lost in 30%
p27 (kip-1)	Inhibits cyclin E/Cdk2	Lost in 55%
p21 (WAF-1)	Inhibits cyclin B/Cdk1	Lost in 25–40%
NF κ B	Transcription factor, effector of many survival pathways	Unknown
NOEY(ARHI)	GTPase tumor suppressor, induces apoptosis through p21	40% LOH
Inhibition of apoptosis and immune surveillance		
PIP3/Akt	Akt (activated by PIP3) inhibits apoptosis	12–18% (HGS)
PTEN	Decrease Akt	20% (Endo)
p53	Promotes cell cycle arrest/apoptosis with DNA damage	50–90% (HGS)
BRCA1	Co-factor for transcription factors, “caretaker” of genome	6–82% ^a
BRCA2	Co-factor for transcription factors, “caretaker” of genome	1–3%
MLH1/MSH2	Mediates mismatch repair, promotes genetic stability	30% (Endo)
Fas ligand	Produced by tumor cells to induce apoptosis of T-cells	50–80%
HLA-G	Secreted by tumor cells to inhibit cytotoxic immune cells	61% (HGS)
Limitless replicative potential		
hTERT	Subunit of telomerase, maintains telomere length	80–85%
Enhanced angiogenesis		
VEGF/VEGFR	Ligand/receptor complex induces angiogenesis	40–100%
IL-8	Cytokine promoting angiogenesis	Unknown
EphA2	TK promotes angiogenesis and vasculogenic mimicry	76%
Promotion of invasion and metastasis		
MMPs	Matrix metalloproteinases degrade extracellular matrix	40–100%
α v β 3	Integrin, promotes survival and angiogenesis	95%
FAK	Co-factor TK promotes adhesion, proliferation, survival	70%
E-cadherin	Promotes adhesion	90–100%

Adapted from *JCO* 2008;995–1005 (Feb 20) with permission
TK tyrosine kinase, *LOH* loss of heterozygosity, *HGS* high grade serous, *LGS* low-grade serous, *Endo* endometrioid

^a Inherited mutation in 6–7% of all cancers may play a role in up to 82% of sporadic cancers

altered in tumorigenesis include the type I tyrosine kinase receptor family HER (Erb). It comprises four monomers: epidermal growth factor receptor (aka EGFR/Erb1/HER-1), HER-2 (proto-oncogene neu), HER-3, and HER-4. EGFR is the most common of the four and is overexpressed in 35–70% of epithelial ovarian cancers [92]. HER-2 lacks an extracellular ligand-binding domain, yet it dimerizes with other type I receptors. HER-2 is overexpressed in 20–30% of ovarian cancer cases [93].

Another well-known oncoprotein is RAS. It is a G-protein involved in cell proliferation. Activated RAS turns on a series of serine/threonine and tyrosine non-receptor kinases, leading to phosphorylation of Erk1 and Erk2 transcription factors, which function in cell growth and proliferation. K-ras mutations have been detected in 61% of borderline tumors, 68% of low-grade tumors, 50% of mucinous adenocarcinomas, and only in 5% of high-grade serous carcinomas [69, 94]. However, Wong et al. [95]

analyzed 91 human ovarian tumor samples and found that among the low-grade serous carcinomas, only 19% expressed a KRAS mutation.

Non-responsiveness to Anti-growth Signals

For early malignant transformation to occur, anti-growth pathways must be surpassed. Although not much is known about the genetic mechanisms or the sequence of events, anomalies in cell cycle proteins have been noted. Examples of cell cycle mediators include cyclins, cyclin-dependent kinases (CDK), CDK inhibitors (block the binding of cyclins to CDK), and transcription factors such as pRb, TP53, and E2F. The G₁ phase checkpoint (the restriction point), after which the cells are committed to enter S phase, is controlled by cyclins D and E's phosphorylation of Rb and release of E2F. Cyclin E is associated with poor patient outcome and is expressed in borderline and malignant tumors (40% and 70%, respectively), whereas it is expressed in only 9% of benign tumors [96]. Cdk2, another protein involved in G1-S phase transition with cyclin E, is also found to be elevated in high-grade malignant ovarian tumors as compared to low-grade or even benign tumors [96]. Cyclin D1 is abundantly found in ovarian cancer cell cytoplasm (89%) and nucleus (30%) and scarcely found in normal epithelial ovarian cells [97]. Entry to the M phase is regulated by CDK1/cyclin B complex, which is highly expressed in 80% of malignant cells, but largely undetectable in normal ovarian epithelial cells [98].

Another pathway important in cell cycle control is the RAS-Raf pathway, which involves the myc oncogene/transcription factor. Myc is overexpressed in 30% of ovarian cancers. Moreover, AHRI (aka NOEY2, a GTPase tumor suppressor gene) [99] is a protein found in normal epithelial ovarian cells, but is completely absent in cancer cells. Yu et al. [100] suppressed the clonogenic growth of cancer cells after re-expression of NOEY2 by transfection. Therefore, we can conclude that various genetic anomalies are involved in the disruption of the cell cycle and may give ovarian cancer cells the advantage needed to expand and grow without host supervision.

Surviving Apoptosis

Many proponents of this mechanism strongly believe that avoiding cell death is the most critical step in order to achieve carcinogenesis rather than cell proliferation alone. Various genes and proteins play a part in the mechanism of cell death evasion. One example is the p53 gene and its protein TP53. In normal physiology, it functions by detecting damaged DNA, arresting the cell cycle, initiating mechanisms of repair, and directing the cell cycle into the path of apoptosis [101, 102]. When endogenous p53 is

knocked down with small interfering RNA, serous ovarian borderline cancer cells show increased cell invasion and cell survival [102]. Most p53 aberrations are missense mutations [103]. P53 null mutations tend to be associated with a more aggressive disease pattern in ovarian cancer patients and may play a role in the metastatic process [104]. When p53 mutations are absent, its function can also be altered through degradation by ubiquitination, or through translation of TP53 binding protein. Moreover, pathology of ovaries obtained from prophylactic surgery in BRCA1 patients revealed that p53 mutations are detected in inclusion cysts adjacent to cystadenocarcinomas and in microscopic ovarian malignant cells [105]. Collective data suggest that p53 mutations and dysfunction may be one of the critical early steps toward tumor transformation and progression.

Another pathway involved in apoptosis evasion is the PI3-kinase/Akt pathway. It is present in 30% of ovarian pathology [70]. It is also involved in neoangiogenesis, cell invasion, and resistance to chemotherapy [106]. PTEN is the major regulator in this pathway, which acts as a tumor suppressor gene by dephosphorylating PIP3 back to PIP2 and leading the cell to apoptosis. Animal models have shown that PTEN mutations may be an important early event in the pathogenesis of the endometrioid subtype of ovarian cancer [54].

BAF250A, the protein encoded by ARID1A [the AR-rich interactive domain 1A (SWI-Like) gene], is one of the accessory subunits of the SWI-SNF complex that is believed to be responsible for providing specificity in gene expression regulation. The SWI-SNF complex is ubiquitous in eukaryotic cells and is involved in cellular development, differentiation, proliferation, DNA repair, and tumor suppression [107]. It consists of ATP-dependent motion of nucleosomes, therefore controlling the accessibility of transcription genes to promoters. RNA sequencing revealed that ARID1A mutations were found in 46% of ovarian clear cell carcinomas and in 30% of endometrial cancers [108]. These findings correlated with BAF250A loss by immunohistochemistry and were absent in high-grade serous ovarian serous ovarian carcinomas. Hence, ARID1A is a new potential tumor suppressor gene that may play a part in early events leading to ovarian carcinogenesis. NF-KappaB (NFκB), a pleiotropic transcription factor, inhibits cell apoptosis and promotes cancer cell survival. Deregowski et al. [109] used RT-PCR and DNA hybridization microarrays to show that when NFκB is upregulated in transfected cells, BCL-2 family members are also upregulated along with apoptotic inhibitors and other genes important for cell survival. Another study also showed that when NFκB was blocked in mice, the levels of interleukin-8 (IL-8) and VEGF were also decreased, resulting in diminished malignant potential in these ovarian cancer cell lines [110].

Cell Immortality

Cell senescence followed by apoptosis is achieved after the normal cell divides a number of times. The ends of the chromosomes contain DNA/protein complexes that protect the DNA from degradation. If the telomeres are lost, the chromosomes are exposed to defects, which allow p53 and other proteins to initiate apoptosis. Using telomere-specific fluorescence in situ hybridization, 82% of serous tubal intraepithelial carcinomas (STIC), a putative precursor of ovarian high-grade serous carcinoma (HGSC), were found to have short telomeres compared to normal tubal epithelium. In contrast, HGSC had longer telomeres than STIC. These findings suggest that telomerase activity is an important occurrence in the initiation of ovarian tumorigenesis [111]. Most ovarian cancer cells (81–86%) overcome the apoptosis pathway by producing telomerase, which is a reverse transcriptase made of RNA (hTR) and protein (hTERT) [112]. The major subunit linked to tumorigenesis is the overexpression of hTERT [113]. Expression of hTERT and p53 knockdown can transform ovarian surface epithelial cells [114]. Inhibition of telomerase activity is achieved via a functional BRCA [115]. These findings suggest that telomerase activation is an important occurrence in the initiation of ovarian tumorigenesis.

Early Events in the Tumor Microenvironment: Angiogenesis, Invasion, and Metastasis

The tumor microenvironment consists of tumor cells, matrix components, inflammatory cells, and stromal cells. The communication between these various components allows for angiogenesis, invasion into stroma, and metastatic growth in distant organs. The concept of metastasis usually is synonymous with advanced stage of disease, but it has been shown in breast cancer that early tumors may also have subclinical metastasis [116]. In ovarian cancer, changes in the stroma and peritoneum may allow the tumor to spread [117]. Oxygen and nutrients are essential for all cells, including malignant, benign, or normal cells. For cells to receive this basic need, they must reside within 100 μm of a capillary [118]. For a malignant cell to increase in size beyond 1 mm^3 , genesis of new vessels around its premises must occur. Angiogenesis consists of a complex balance between pro- and anti-angiogenic factors in the cellular microenvironment. VEGF-A is the primary regulator of angiogenesis [119, 120]. It augments vascular permeability, acts as a positive effector for endothelial cell proliferation and migration, modifies endothelial cell gene expression, and allows the cell to evade apoptosis [121, 122]. Studies have shown that VEGF expression induces ovarian cancer cell lines to metastasize and produce ascites and carcino-

matosis [123]. The clinical outcomes of ovarian cancer patients are correlated with VEGF levels [124, 125].

Mediators of angiogenesis can either originate from the host or from the tumor cells. Among these, IL-8 is elevated in ovarian cancer patients [126] and is believed to be a key factor for cancer growth and new vessel formation [127]. Integrin subunits also play a key role in invasion and angiogenesis. Davidson et al. [128] studied the expression of α_v and β_3 integrin subunits in cancer cells and endothelial cells. The $\alpha_v\beta_3$ integrin was primarily located on newly developing vascular endothelial cells and on ovarian cancer cells. Another group concluded that EphA2, a tyrosine kinase receptor involved in oncogenesis, is overexpressed in 75% of ovarian cancers [129]. When EphA2 is inhibited, cancer growth was also slowed down by anti-angiogenic mechanisms [130, 131]. The patient-specific tumor microenvironment is an important feature that will regulate tumor angiogenesis and modulate the outcome of anti-angiogenic therapy [132].

For metastasis to occur, the basement membrane must be invaded. This involves interactions between the invading cell and the surrounding stroma. To allow endothelial cell migration during angiogenesis, remodeling of the extracellular matrix is necessary and key proteins in this process include the matrix metalloproteinases (MMPs). MMPs are zinc-dependent endopeptidases that degrade collagen and other ECM constituents. They also have the capability to promote angiogenesis through VEGF [133]. Ovarian cancers have been shown to express high levels of MMP2 and MMP9 [18, 134] and at the same time are associated with clinical stage [135] and patient outcome [131]. Huang et al. [136] showed that host-derived MMP-9 expression appears to play a major role in angiogenesis and progression of human ovarian tumors compared to MMPs from tumor cells. Another theory that may potentiate invasion is the psychoneuroimmunomodulation hypothesis where catecholamines are released due to chronic stress. Abundant preclinical evidence supports the role of chronic stress in activating the cholinergic/sympathetic pathways, which can lead to increased invasion and metastasis [137, 138]. Moreover, epidemiological studies show that patients with poor social support status and chronic stress may be at greater risk for worse cancer outcome [138].

The tumor microenvironment consists of inflammatory cells that have the ability to recognize foreign components of the tumor cell and promote tumor cell apoptosis. In an effort to evade recognition by the immune system, which would eventually lead to their destruction, tumor cells have acquired the ability to produce Fas ligand that induces apoptosis in lymphocytes [139] as well as secretion of HLA-G that can inhibit NK cell activity [72, 140]. Cytokine production by mesenchymal cells as well as by the tumor cells helps the tumors to grow and prevent

Fig. 1 Proposed model of ovarian carcinogenesis (adapted from JCO 2008:995–1005 (Feb 20) with permission). Normal ovarian epithelium is exposed to physiologic processes that may predispose to malignant transformation, such as prolonged androgen exposure. A number of characteristics must be obtained, primarily through mutations or other genetic changes, to be transformed to a malignant state. These include unregulated growth, resistance to anti-growth signals, inhibition of apoptosis, evasion of recognition by the immune system, achieving limitless replicative potential, induction of angiogenesis, and invasion of the basement membrane. Examples of specific proteins known to play a role in each of these processes in ovarian cancer are listed in *italics*. The order in which these mutations may occur is not well understood, but the timing and specific protein affected may be

significant in producing different histological subtypes and grades of ovarian cancer. For example, if mutations favoring growth and resistance to apoptosis occurred early, prior to achieving the potential for invasion and metastasis, an intermediate pathologic subtype would be noted more often, such as *k-ras* mutations in LMP tumors. A mutation leading to genetic instability, such as *p53*, that occurred early would predispose cells to other mutations and rapid progression to a metastatic phenotype, as seen in high-grade malignancies. Permissive or contributing factors of the microenvironment, such as production of MMPs by fibroblasts (pictured in red), infiltration of inflammatory cells (pictured in blue), and proliferation of endothelial cells for angiogenesis, may be just as important as mutations in the tumor cells

apoptosis [141, 142]. Immunohistochemistry in 186 specimens showed that increased numbers of T cells correlate with an improvement in survival [143]. The role of specific immune cell populations in controlling versus promoting tumor growth remains to be fully defined [144].

An advanced stage of tumor usually is associated with metastasis, which may occur earlier than once thought [116]. Although cells escape from the primary tumor into the vasculature, this does not necessarily mean that they will anchor and grow in different distant organs [145]. Only 30% of stage I cancers showed positive peritoneal cytology [146]. Due to the shedding capability of ovarian cancer, an early role may be played by cell survival promoters such as focal adhesion kinase (FAK) and E-cadherin [147–150]. Moreover, E-cadherin is expressed only in tumor cells (LMP, benign, or low and high grade) and notably in ovarian inclusion cysts, but absent in normal ovarian epithelial cells [151].

Proposed Model of Ovarian Carcinogenesis and Concluding Remarks

The understanding and identification of key players in the development of ovarian cancer is important in order to provide better targeted therapies. In the broad dual-pathway model of ovarian carcinogenesis, we propose that not only are the early genetic events important but also that the stroma plays a significant role in tumorigenesis (Fig. 1). The sequence of events is quite variable, but important genetic alterations may lead to specific tumor types. For example, KRAS mutations can lead to a LMP tumor, whereas a p53 or BRCA mutation can lead to cancer development through the high-grade pathway. Both pathways include certain characteristics such as evasion from the immune system and invasion into the stroma and peritoneal cavity while continuing to grow and to vascularize. Despite similarities in both pathways, clinically and histologically, each cancer is distinct and a large spectrum of unknown genetic transformations or pathways may play a role in the early steps in carcinogenesis. Therefore, strife exists to identify these early factors responsible for the initiation of oncogenesis in ovarian pathology, enabling clinicians to detect cancer early and to target specific factors to improve clinical therapy outcomes.

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