Focus on epithelial ovarian cancer

Robert F. Ozols,* Michael A. Bookman, Denise C. Connolly, Mary B. Daly, Andrew K. Godwin, Russell J. Schilder, Xiangxi Xu, and Thomas C. Hamilton

Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111
*Correspondence: robert.ozols@fccc.edu

Introduction
Epithelial ovarian cancer (EOC) will be diagnosed in 24,400 women in the United States in 2004, with an estimated 14,300 deaths (Jemal et al., 2003). Neoplasms from the surface epithelium of the ovary exhibit a variety of Müllerian-type cells, including serous, mucinous, endometrioid, and clear cell, reflecting a common pathway in embryological development. In the western world, EOC is the most lethal gynecologic cancer, accounting for more deaths than endometrial and cervical cancer combined. Spread of the disease via the lymphatics and by peritoneal implantation is not associated with any specific signs or symptoms, and the vast majority of women are diagnosed with disseminated intraperitoneal carcinomatosis (FIGO Stage III). Also contributing to the high mortality is the advanced age at diagnosis (median 63 years), with an increase after menopause. While ultrasound and computerized tomograms are useful in definition of sites of bulk disease, surgical evaluation is necessary for accurate staging and to remove large metastases (cytoreduction). Due to the propensity for diffuse small-volume disease, surgery is rarely able to render patients disease free, and postoperative chemotherapy is usually required. While overall mortality rates have remained relatively constant for the past two to three decades, five-year survival rates have increased from 30% in the 1960s to over 50% in the past several years.

Etiology and cellular mechanism of epithelial ovarian cancer
Consistent epidemiological data indicate that the risk of EOC increases with the number of ovulatory events. For the last few decades, two major theories, the incessant ovulation (Fathalla, 1971) and the gonadotropin hypotheses (Cramer and Welch, 1983), have been proposed to explain the same epidemiological data (Riman et al., 1998). The incessant ovulation hypothesis postulates that the repetitive wounding and recurring cell proliferation in postovulatory repair of the ovarian surface epithelium result in mutations accumulating in the epithelial cells and ultimately tumor formation. This straightforward and conceptually obvious explanation easily gained acceptance. Support for this concept was also provided by laboratory experiments demonstrating spontaneous transformation of rat ovarian surface epithelial cells following prolonged subculturing (Godwin et al., 1992; Testa et al., 1994). The passing of the cells in culture mimics repeated injury and proliferative repair of ovarian surface epithelium imiled by incessant ovulation. Recent experimental evidence supports the idea that higher ovulatory activity is associated with more inclusion cysts and other changes in the ovarian surface, such as invaginations. It has been suggested that these inclusion cysts are a fertile environment for ovarian cancer development (Feeley and Wells, 2001). In support of this hypothesis, many (but not all) studies of the ovaries of ovarian cancer-prone individuals, i.e., women with a family history of ovarian cancer and/or a deleterious BRCA1 or BRCA2 mutation, have reported more changes in their surface epithelium than control ovaries (Schlosshauer et al., 2003).

The gonadotropin theory postulates that the surges of pituitary gonadotropins that initiate each ovulation and persist in high levels for years following menopause also stimulate the ovarian surface epithelial cells and induce cell transformation. Abundant epidemiological data and animal models exist to support this idea (Cramer and Welch, 1983). Gonadotropins have unremarkable effects on ovarian surface epithelial cells in culture. Thus, inflammation of the ovarian epithelium was suggested as a mechanism by which gonadotropin stimulation and ovulation contribute to ovarian cancer risk (Ness and Cottreau, 1999, 2000), since inflammation is a well-known factor contributing to cancer (Ames et al., 1995). Ovulation is an inflammatory-like process involving multiple cytokines and proteolytic enzymes, and their actions ultimately lead to tissue rupture (Espey, 1994). Inflammation can also provide an explanation for the increased risk associated with talc and asbestos exposure, endometriosis and pelvic inflammatory disease, and mumps viral infection. The ovarian epithelial inflammation caused by ovulation or other factors may contribute to cancer risk by increasing mutations in epithelial cells, as suggested (Ness and Cottreau, 1999, 2000). However, gonadotropins may also stimulate an ovulation-like loss of the ovarian surface epithelial basement membrane (Roland et al., 2003). The loss of basement membrane may dramatically alter the biology of the epithelial cells in tissue organization and cell contact signaling. For example, the epithelial cells may upregulate their survival mechanisms in the absence of an interacting extracellular basement membrane, or the lack of basement membrane may favor the selection of apoptosis-resistant cells. Thus, it can be postulated that the frequent placement of the surface epithelium in such a basement membrane-less state by repetitive gonadotropin stimulation may lead to the selection of preneoplastic cells and increase the chance for a subpopulation of the epithelial cells, the cancer-prone cells with accumulated genetic mutations, to transform (Zeimet and Marth 2003). Even after cessation of ovulation due to the depletion of follicles, the high levels of gonadotropins immediately following menopause may still stimulate the ovulation-like process involving the expression of cytokines and proteolytic enzymes in the surface epithelial compartment (follicles are depleted) of the ovaries. The inflammatory stimulation may lead to the loss of basement membrane of the surface and inclusion cysts of ovarian epithelium, perhaps contributing neoplastic transformation. Since the inflammation-like ovulation is COX-2-dependent, this mechanism may also provide support for the chemopreventive function of COX-2 inhibitors in ovarian cancer.

Although molecular mechanistic studies provide further support for the incessant ovulation, gonadotropin stimulation,
inflammation, and basement membrane loss hypotheses, all the theories concerning the etiology of ovulation-associated ovarian cancer are not mutually exclusive. Additional hypotheses concerning steroid hormones and the retrograde transport of carcinogens through fallopian tubes (Riman et al., 1998) may also contribute to ovarian cancer risk in certain circumstances and magnitude. The mechanism of gonadotropin-stimulated basement membrane loss may not be mutagenic, but rather promote the transformation of predisposed cells that have acquired mutations from other events, such as ovulatory proliferation and DNA modification by mutagens.

Genetics of ovarian cancer
Much remains to be discovered regarding the molecular events that underlie ovarian cancer development. Both hereditary and nonhereditary forms of EOC develop in a multistep process that involves alterations in many specific genes. The normal ovarian surface epithelial cell has multiple mechanisms that regulate its growth and differentiation, and several separate events are required to override these control mechanisms. The fundamental mechanisms underlying the genetic basis of cancer are continually being defined and redefined and involve alterations in a number of genes, including protooncogenes, tumor suppressor genes, and DNA repair genes. Ovarian cancers display defects in many genes, including AKT, EGFR, ERBB2, RAS, PIK3CA, MYC, DOC-2/DAB2, γ-synuclein (SNCG), and TP53, as well as a myriad of cytogenetic abnormalities (Prowse et al., 2003).

These defects result from both genetic and epigenetic changes and can occur at varying frequencies in different pathologic subtypes, both early and late in the transformation process. Genetic factors that are unique to ovarian cancer have been somewhat difficult to identify. Two of the most well known genes associated with susceptibility to breast and ovarian cancer, BRCA1 and BRCA2, discovered almost a decade ago, were expected to illuminate not only the rare forms of these inherited cancers, but the more common forms (i.e., sporadic) as well. Their roles in hereditary forms of ovarian cancer are now well documented and described below. However, initial enthusiasm for a significant role of these genes in the pathogenesis of the approximately 95% of ovarian cancers without a family history has waned somewhat. Somatic mutations of BRCA1 and BRCA2 genes have proven rare, yet epigenetic changes in the form of promoter methylation resulting in transcriptional silencing of the BRCA1 gene have been demonstrated in about 5% to 10% of nonfamilial ovarian cancer cases. In contrast, BRCA2 is generally hypomethylated and overexpressed in ovarian cancers. The puzzle that endures is why breast tissue, and ovarian tissue to a lesser extent, is so susceptible to mutated BRCA1 and BRCA2. Hormonal factors, such as estrogen, have been central to this hypothesis and are under active investigation. A recent study has found that BRCA1 interacts specifically with, and is in part concentrated on, the inactive X chromosome (Xi) in female somatic cells (Ganesan et al., 2002). There, it interacts with the large, noncoding, Xi-associated RNA, Xist, and promotes the proper localization of Xist on Xi. In cells devoid of/depleted in BRCA1, Xi lacks both Xist and the specialized histone, MacroH2A (MH2A), and shows signs of incomplete silencing. This may explain why women, as opposed to men, so often develop cancer when they have inherited a mutant gene. Dissecting BRCA1 and BRCA2 gene function has raised further excitement with regard to uncovering additional cancer-associated genes. Recent studies have found that BRCA2 and FANC01, a gene associated with Fanconi anemia, were one and the same (Howlett et al., 2002) and that disruption of FA-BRCA pathway, which occurs in ovarian cancers, alters sensitivity to cisplatin (Taniguchi et al., 2003). Novel BRCA1 and BRCA2 protein complexes are also being...
discovered, and components of these complexes have been shown to be abnormally expressed in cancer (Dong et al., 2003). Like so many other BRCA discoveries regarding the BRCA proteins, it is still not clear how these will all fit into the bigger picture. However, proteins within this network are likely to have implications in many cancers, including ovarian.

**Experimental models of ovarian cancer**
The lack of ovarian cancer-prone mammals has hampered our ability to experimentally determine whether and how changes in the surface epithelium occur and if they are an aspect of ovarian oncogenesis. Recently, significant inroads have been made in genetic modeling of EOC in immunocompetent mice (reviewed by Nikitin et al., 2004). In one model, whole ovaries from mice genetically engineered to express the avian retroviral receptor, TVA, were isolated, cultured, and transduced with several oncogene-bearing avian retroviruses individually and in various combinations. Reintroduction of ovarian cells exhibiting loss of p53 in combination with a minimum of two oncogenic lesions, including c-Myc, K-Ras, or Akt, to the ovarian bursa results in the development of ovarian carcinoma in mice. Other investigators have shown that conditional inactivation of both the p53 and Rb tumor suppressor genes in the ovarian surface epithelium of mice leads to the development of ovarian carcinomas in 97% of cases. Additionally, a transgenic mouse model of EOC that recapitulates human disease by expression of the simian virus 40 large and small TAg genes under control of the 5′ upstream regulatory sequences of the Müllerian inhibitory substance type II (MISII) receptor gene promoter has recently been developed. Female TgMISII-TAg transgenic mice develop bilateral ovarian adenocarcinomas (Figure 1) accompanied by ascites and peritoneal implants. Although female TgMISII-TAg transgenic mice are nearly always infertile, a stable transgenic line was derived from a male founder. Hopefully, these models will lead to a better understanding of the biological basis for ovarian cancer initiation, and aid in finding better ways to prevent, treat, and diagnose this frequently fatal disease.

**Screening and prevention**
Epidemiologic studies have identified several factors that increase a woman’s risk for EOC, including increasing age, a family history of ovarian and/or breast cancer, and nulliparity. Exposure to long-term (greater than 10 years) estrogen replacement therapy confers a 2- to 3-fold increase in risk (Lacey et al., 2002). The highest level of risk for ovarian cancer is associated with germline mutations in BRCA1, BRCA2, and the HNPCC genes (Narod and Boyd, 2002; Brown et al., 2001). Estimates of the lifetime risk for ovarian cancer range from 16% to 60% for BRCA1/2 mutation carriers. Among Ashkenazi Jewish women, in whom one of the three founder BRCA mutations has been found, lifetime ovarian cancer risks were 54% for BRCA1 and 23% for BRCA2 mutations (King et al., 2003).

Clinical genetic testing and counseling are now available for women suspected to have a hereditary susceptibility to ovarian cancer to better define their risk profiles and to develop appropriate preventive strategies. Recommendations fall into four general categories: increased surveillance, surgical prophylaxis, pharmacologic interventions (chemoprevention), and lifestyle changes. Screening recommendations are problematic for ovarian cancer, for which no test or series of tests have been found to be sufficiently sensitive and specific. The identification of a precursor lesion to EOC would also facilitate earlier diagnosis. Despite their limitations, however, many practitioners have begun screening with the combination of pelvic exam, transvaginal ultrasound, and CA-125, either annually or semiannually, particularly in women with a strong family history of ovarian cancer. In order to improve the positive predictive value of screening strategies, current efforts are being directed toward identifying novel molecular markers, including genomic and proteomic markers (Petricoin et al., 2002), or panels of markers which may be combined for use in conjunction with ultrasonography to improve the predictive value of the screening process (Barnes et al., 2002). Prophylactic oophorectomy is often considered by women with a family history of ovarian cancer, particularly those who are BRCA1/2 mutation carriers, due to the uncertain nature of screening and the high case-fatality rate of advanced stage cancer. Recent studies have demonstrated a greater than 90% reduction in ovarian cancer and a 50% reduction in breast cancer among women undergoing oophorectomy for prophylaxis (Rebbeck et al., 2002). Prophylactic surgery does not, however, eliminate the risk for primary peritoneal cancer and may result in long-term adverse physical and psychological sequelae. To date, there have been no phase III randomized chemoprevention trials for ovarian cancer. However, because of the strong epidemiologic association between oral contraceptive (OC) use and a reduction in ovarian cancer rates, many gynecologists are recommending their use in women with an increased risk, either due to family history or nulliparity. While data from some studies of women with BRCA1/2 mutations suggest that they enjoy the same degree of protection (approximately 40%) from OCs as do women in the general population, others have not found a protective effect, and there is some concern about an increased risk for breast cancer in this population. Small pilot studies are now underway to determine the chemopreventive role of other agents, including members of the retinoid family, progestational agents, and COX-2 inhibitors. There is intense interest on the part of high-risk individuals about opportunities to reduce their ovarian cancer risk by changes in diet, exercise, or other lifestyle modifications. There is concern, for example, about exposure to fertility drugs and their effect on ovulation. Unfortunately, most of the factors linked to ovarian cancer risk are reproductive in nature (e.g., nulliparity) and their manipulation is, therefore, confounded by social concerns. The exact role of diet, micronutrient supplementation, and exercise remains elusive for ovarian cancer, and any recommendations given must be on the basis of general health and wellbeing.

**Current treatment of epithelial ovarian cancer**
EOC is considered to be a chemosensitive neoplasm, with initial overall response rates to systemic therapy exceeding 80% when integrated with cytoreductive surgery. However, among women with advanced-stage disease at diagnosis, long-term survival remains poor due to eventual tumor recurrence and emergence of drug-resistant disease. While effective cytoreductive surgery at the time of diagnosis has been correlated with improved survival, the optimal integration of surgery and chemotherapy remains to be determined. Primary postoperative chemotherapy has evolved from single alkylating agents to cisplatin and cisplatin-based combinations, followed by incorporation of paclitaxel and substitution of carboplatin for cisplatin, but not without a degree of controversy. In particular, although mature results from GOG111 (McGuire et al., 1996) established the superiority of cisplatin-paclitaxel compared to cisplatin-
In view of the central role of platinum, there has been particular interest in the incorporation of other active cytotoxic agents that may accentuate the platinum response, including taxanes (paclitaxel and docetaxel), gemcitabine, topotecan, polyethylene glycosylated (PEG)-liposomal doxorubicin, and prolonged oral etoposide. In addition to their clinical activity in patients with platinum-resistant disease, preclinical models have suggested that sequential therapy with single-agent platinum followed by paclitaxel can achieve equivalent outcomes. In spite of improved median survival with carboplatin and paclitaxel, long-term survival and disease mortality have remained largely unchanged (Figure 2).

Recent phase III trials have evaluated a number of potential strategies, including increased dose intensity of platinum or paclitaxel, increased regional drug exposure through intraperitoneal delivery, and extended maintenance therapy. While each of these approaches has been associated with increased toxicity, none has yet achieved a meaningful improvement in quality-adjusted survival or replaced the current standard of care.

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In developing the current phase III Gynecologic Cancer Intergroup (GCIG) trial (GOG182-ICON5), it was elected to include four experimental arms to evaluate the addition of three new drugs (topotecan, gemcitabine, and PEG-liposomal doxorubicin) using two different strategies for drug administration (sequential doublets and triplet combinations) (Bookman, 2003). Based on the current rate of accrual and events, it is anticipated that an interim analysis will be performed in May 2004 and GOG enrollment will be completed by October 2004, with extension of enrollment at international sites. Results from GOG182-ICON5 will be maturing in conjunction with data from other international randomized trials evaluating new combinations, including cisplatin-topotecan, epirubicin, and gemcitabine.

Women with early stage disease (FIGO I and II) have a significantly better prognosis than patients with advanced-stage disease. Stage I patients with favorable prognostic factors have more than a 90% cure rate with surgery alone. Early-stage patients with unfavorable prognostic features (such as poorly differentiated tumors or evidence of extracapsular spread [FIGO Ic]) have a 70% to 80% cure rate. Recent clinical trials have demonstrated that five-year survival can be improved from 74% to 82% with chemotherapy administered immediately after diagnostic surgery compared to observation with chemotherapy administered at relapse (Trimbos et al., 2003).

**Targeted therapies for ovarian cancer**

Future phase III trials will be driven by the rapid development of novel compounds, including antiangiogenic reagents, humanized monoclonal antibodies, selective hormonal agents, and small molecules that target key components in signal transduction pathways associated with cell growth, tumor vascularity, and invasive potential. With rapid emergence of new agents, it becomes important to efficiently and decisively evaluate as many agents as possible. GOG182-ICON5 has demonstrated that large multiarmed phase III trials appear feasible with international collaboration. However, it is also important to consider other innovative strategies, such as dual (or bifactorial) design and randomized phase II trials, coupled with a robust phase I–II developmental therapeutics program to identify and evaluate promising new agents and develop feasible combinations for future phase III initiatives.

Thus far, most studies of targeted therapy in EOC have focused on the epidermal growth factor receptor (EGFR), which is expressed in 30% to 70% of EOC. Many of the cellular processes associated with the malignant phenotype are initiated by activation of EGFR (Baselga, 2002). Receptor function can be inhibited by blocking the binding of ligand by the receptor, e.g., with monoclonal antibody (cetuximab, ABX-EGF) or inhibition of the enzyme activity of the receptor tyrosine kinase with small molecules, such as gefitinib or erlotinib.

These drugs have been evaluated in clinical trials. Erlotinib was found to have a response rate of 8.8% as a single agent in heavily pretreated patients (median: three prior regimens) with ovarian cancer (Finkler et al., 2001). Gefitinib induced a response and long-term stable disease in three additional patients in 27 patients with similar characteristics (Schilder et al., 2003). The two most common toxicities for these two agents were mild to moderate acneiform rash and diarrhea. Similar trials are planned with cetuximab and include correlative laboratory studies, such as the levels of EGFR and phospho-EGFR expression, measuring downstream effects, such as increase in p27 in tumor biopsy specimens, and microarray analyses on tumor tissue specimens before and after treatment with these EGFR antagonists. It remains to be determined how these agents will be best incorporated into combination chemothera-
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Several caveats already are evident with these new forms of therapy. It is an oversimplification to attempt to categorize these agents based purely on the anticipated molecular target. For example, EGFR inhibitors have been demonstrated to also potentiate antiangiogenic activity. In addition, in spite of encouraging preclinical models, early results from large, randomized trials in other disease sites have not shown a benefit of adding a molecularly targeted agent to a conventional chemotherapy regimen as demonstrated by the recent INTACT trials in nonsmall cell lung cancer (carboplatin and paclitaxel with or without gefitinib). However, more recently, bevacizumab improved the overall survival of patients with colon cancer when added to 5-fluorouracil, leucovorin, and irinotecan. The presence of a target on a tumor by itself may not be sufficient for these drugs to have activity. It is likely that these compounds will only have activity in the subset of patients where the target has a critical biological role in the growth of the cancer, such as is seen with gain-of-function mutations. New tools that will allow highly specific tumor profiling, such as genomic microarray and proteomic analyses, will facilitate more accurate patient selection for treatment with these agents (Sawiris et al., 2002).

Future studies
While EOC has become more of a chronic disease, to significantly impact on mortality will require advances in screening, treatment, and prevention. Proteomic screening may lead to earlier diagnosis, but large-scale validation of this technology is still necessary. The current international randomized trial of different chemotherapy combinations will establish the regimen of choice for the next decade, but it is unlikely that a major improvement in survival will result from any combination of current cytotoxic agents. To be effective, targeted therapy will require molecular profiling to match biochemical abnormalities in a patient’s tumor with a specific therapy. A better understanding of the biology of ovarian cancer and the identification and molecular characterization of a precursor lesion to invasive EOC is essential to translational studies of prevention and early diagnosis.

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References
Dong, Y., Hakimi, M.A., Chen, X., Kumaraswamy, E., Cooch, N.S., Godwin,


