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Review

Ovarian cancer and high-risk women—implications for prevention, screening, and early detection

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Abstract

Objectives. The aim of this study was to understand the strengths and limitations of current prevention, detection, and screening methods for ovarian cancer and to identify research areas to improve prevention, screening, and detection of the disease for all women as well as for women carrying a mutation in the *BRCA1/2* genes.

Methods. We convened an ovarian cancer symposium at the University of Pittsburgh in May 2002. Nineteen leading scientists representing disciplines such as epidemiology, molecular biology, pathology, genetics, bioinformatics, and psychology presented the latest data on ovarian cancer prevention, screening, and early detection.

Results. Ovarian cancer is the most common cause of death from a gynecologic malignancy in the United States. Because survival depends on stage of diagnosis, early detection is critical in improving clinical outcome. However, existing screening techniques (CA125, transvaginal ultrasound) have not been shown to reduce morbidity or mortality. Moreover, with the exception of oral contraceptives, there are no available chemopreventive agents. Bilateral salpingo-oophorectomy also has been shown to reduce incidence, but this procedure has several drawbacks in terms of a woman's reproductive, cardiovascular, skeletal, and mental health.

Conclusion. Better methods to prevent, detect, and screen for ovarian cancer in all women, but particularly in high-risk women carrying mutations in *BRCA1/2*, are urgently needed. This article reviews the current state of knowledge in the etiology, prevention, and early detection of ovarian cancer and suggests several areas for future clinical, epidemiologic, and laboratory-based research.

Introduction

Ovarian cancer is the most common cause of death from a gynecologic malignancy in the United States [1]. Currently, about 75% of women have advanced stage disease at the time of diagnosis [1]. Despite aggressive surgery and chemotherapy, the prognosis for these women is poor, with a 5-year survival rate of less than 30% [1]. This outcome is due, in large part, to the lack of effective prevention and early detection strategies; when diagnosed at an early stage, the survival rate is approximately 95% with current treatment modalities [1]. Thus, prevention and early detection of this disease would be of clear clinical benefit.

About 10% of ovarian cancers arise in patients who carry mutations in the cancer predisposing genes, BRCA1 and BRCA2 [2–5]. Compared to sporadic disease, *BRCA1/2*-associated ovarian cancer is often diagnosed at a later, less curable stage of disease [6,7]. Because mutation carriers are 10 times more likely to develop ovarian cancer [5,8,9],

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devising prevention and early detection strategies are particularly critical for this subset of women.

Unfortunately, the options for ovarian cancer prevention and early detection are limited. To date, only oral contraceptives have been shown to be effective as chemopreventive agents. Bilateral salpingo-oophorectomy also has been shown to reduce disease incidence [10,11], but this procedure has several drawbacks. It ends a woman's reproductive capabilities, it may increase her risk of cardiovascular disease and osteoporosis, and it has the potential to impact her quality of life. With respect to screening, only two techniques have emerged, measurements of CA125 levels and transvaginal ultrasound, but neither has been shown to reduce morbidity or mortality.

Thus, better methods to prevent, detect, and screen for ovarian cancer in all women, but particularly in high-risk women, are urgently needed. In the interim, we must understand the strengths and limitations of current prevention, detection, and screening methods as they apply to the general population and to women with a genetic predisposition.

To address these issues, we convened a meeting of 19 leading international scientists on May 6–7, 2002, in Pittsburgh, Pennsylvania. The program began with an in-depth discussion of the genetic and molecular epidemiology of ovarian cancer, focusing on recent data from studies in the United States, Canada, and Israel [5,12–15]. Preliminary results and impressions from screening and early detection trials such as the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial [16] and the Early Detection Research Network (EDRN) [17] also were presented. A detailed discussion of the biology and pathology of the disease was followed by presentations on the application of new technologies such as genomics and proteomics to further understand the pathogenesis of the disease. Finally, the program closed with presentations on chemoprevention and surgical prophylaxis, with a focus on the physical, social, and psychological effects of these clinical interventions on women and their families.

Here we report the highlights of the meeting, with an emphasis on new research directions that have emerged as a result of the high level of discussion among scientists from various disciplines, including epidemiology, genetics, molecular biology, cell biology, psychology, and bioinformatics. A video recording accompanied by the timed slide presentations from this symposium can be found at www.pitt.edu/~ovarian.

Epidemiology of ovarian cancer: genetic and environmental data

Malignant epithelial tumors are believed to arise from the surface epithelium of the ovary and account for about 90% of ovarian cancers [18]. They are divided histologically into five categories according to the type of cell into which they differentiate: serous, mucinous, endometrioid, clear-cell, and Brenner tumors. The remaining 10% of ovarian tumors are divided into two categories based on histogenesis and differentiation: sex cord–stromal tumors and germ cell tumors. In this review, we focus on epithelial ovarian tumors.

Ovarian cancer protective factors: oral contraceptives and parity

The most consistent protective factors for ovarian cancer are bearing children [19–38] and using oral contraceptives (OCs) [19–26,38–51]. Tubal ligation and breastfeeding also appear to be protective [38,52–54]. The most consistent risk factor for ovarian cancer is family history. Women with one affected first-degree relative have a 5% lifetime risk (i.e., 1 in 20 versus 1 in 100 for the general population). Those with two affected first-degree relatives have a 7% risk [55]. Three hereditary syndromes have been defined: the very rare Lynch syndrome II, which is associated with defects in DNA mismatch repair genes and hereditary nonpolyposis colorectal cancer; hereditary site-specific ovarian cancer; and hereditary breast/ovarian cancer, both of which are associated with *BRCA1/2* mutations.

BRCA1/2 mutations and ovarian cancer

In general, the lifetime risk of ovarian cancer is about 1.8%; however, the lifetime risk of ovarian cancer among *BRCA1/2* carriers is estimated to range between 16 and 30% [9,56,57]. Approximately 1 in 800 women carry a *BRCA1/2* mutation. Among Ashkenazi Jewish women, the prevalence is about 1 in 50 [58–60]. About 5–10% of malignant epithelial tumors contain germline *BRCA1/2* mutations [61–63] most of which are found in *BRCA1*. Among the Ashkenazim, approximately 45% of ovarian cancers arise from two *BRCA1* mutations (185delAGand 5382insC) and a single *BRCA2* mutation (6174delT) [64–67]. These three mutations are commonly found in other ethnic groups as well. The penetrance of *BRCA1* mutations for ovarian cancer is 36% by age 80 [5] and may depend on the mutation location [68,69]. The penetrance of *BRCA2* mutations in general is lower than that of *BRCA1* [5], and an ovarian cancer cluster region has been identified [70,71]. Elucidating factors that affect the penetrance of *BRCA1/2* mutations is an open area of research.

The *BRCA1* gene may be a tumor suppressor gene and is strongly expressed in the epithelial layer of the ovary, with expression reduced in malignant cells [72] and in sporadic ovarian tumors [73]. Exactly how *BRCA1/2* proteins suppress tumor formation and how defects in these genes contribute to the etiology of breast and/or ovarian cancer is the focus of current research. Recent evidence suggests a role for *BRCA1* in both transcriptional regulation and DNA repair [74].

Interestingly, almost all *BRCA1*-associated tumors are of the serous, endometrioid, or clear cell histologies and are invasive [5–7,65,66]. Such tumors are typically diagnosed about 7 years earlier than their sporadic counterparts. Five-year survival appears to be better for *BRCA1*-associated patients than for patients with sporadic disease [6,7,75]. In contrast, while *BRCA2*-associated tumors are also characteristically invasive and nonmucinous, the mean age of diagnosis is about the same as for sporadic tumors. Based on data from several large studies [5,65], factors predictive of a *BRCA1/2*-associated ovarian cancer include serous or endometrioid histology, high grade, two or more first- or second-degree relatives with breast and/or ovarian cancer, a family history of male breast cancer, Jewish ancestry, or early age at onset (*BRCA1* only). These factors may help identify women for whom genetic testing may be pursued.

Parity, oral contraceptives, BRCA1/2, and ovarian cancer risk

An open question is whether factors protective against ovarian cancer in general are also protective in *BRCA1/2* carriers. To date, only two studies have examined the question of OC use, with disparate findings. In a case–control study comparing 207 women with hereditary ovarian cancer to 161 of their unaffected sisters, OC use was less common among women with the disease (odds ratio (OR) for any past use versus never use: 0.5, 95% CI = 0.3–0.8) [14]. The risk decreased with increasing duration of use (*P* for trend, <0.001) and use of 6 or more years was associated with a 60% reduction in risk compared to never use [14]. This suggests that OC use may reduce the risk of ovarian cancer in women with a mutation in the *BRCA1* or *BRCA2* genes, just as it does in women without a *BRCA1/2* mutation. However, a recent study of 840 Israeli Jewish women with ovarian cancer and 2397 healthy controls found that the risk of ovarian cancer among carriers of a *BRCA1* or *BRCA2* mutation decreases with each birth (12% per birth, 95% CI = 2.3–21%) but not with increased duration of use of oral contraceptives [12].

While the levels of OC exposures differed between the two study populations, the distribution of an exposure does not affect risk estimates. Therefore, any differences in OC duration between the study populations cannot explain the contradictory findings. Other factors must play a role and further study is warranted. Thus, because OC use, especially at an early age or for more than 5 years, may increase the risk of breast cancer in *BRCA1* mutation carriers [76,77], it is premature to recommend OC use as a chemopreventive agent in these women. However, parity appears to be protective in both carriers and noncarriers [12,13]. More recently, tubal ligation has been shown to be protective as well (OR = 0.37, 95% CI = 0.21–0.63) [13].

Ovarian cancer etiology: existing and emerging hypotheses

There are two long-standing hypotheses to explain the etiology of epithelial ovarian tumors. The incessant ovulation hypothesis states that tumors result from recurrent minor trauma experienced by ovarian epithelial cells as a result of ovulation [78]. After each ovulation, the surface epithelium undergoes proliferation and repair. The greater the number of repairs, the greater the chance of an aberrant repair process that can lead to a malignant cell. According to this hypothesis, the risk of ovarian cancer is a function of the total number of ovulatory cycles in a woman's life. Any factor reducing that number would provide protection from the disease. In contrast, the pituitary gonadotropin hormone hypothesis states that high levels of circulating gonadotropins (follicle-stimulation hormone or luteinizing hormone) result in the production of estrogen or estrogen precursors, which stimulate ovarian surface epithelial entrapment in inclusion cysts. Under the influence of this environment, entrapped epithelial cells proliferate excessively, eventually leading to malignant transformation [79]. Accordingly, the risk of ovarian cancer is a function of the ovarys' lifetime exposure to gonadotropins. Any factor reducing the level of these hormones would be predicted to be protective against the disease.

Both the incessant ovulation and the pituitary gonadotropin hypotheses could potentially explain the associations of OC use and pregnancy with reduced ovarian cancer risk. OCs suppress ovulation and reduce gonadotropin levels. During pregnancy ovulation ceases and circulating gonadotropin are also reduced. However, over the past several years, much evidence to challenge these hypotheses has emerged. For example, if the gonadotropin hypothesis were correct, then hormone replacement therapy (HRT), which reduces circulating levels of gonadotropins, should protect against this disease. However, the data on HRT and ovarian cancer are contradictory; some studies show it to be protective [80–82], but most find no association or even an increased risk with HRT, especially with recent use [20,21,23,24,83–89]. In addition, both low- and high-dose oral contraceptives, which differentially affect gonadotropin levels, confer the same degree of protection against the disease [15]. Similarly, if the ovulatory hypothesis were correct, then women with ovulatory infertility would have a decreased risk. Existing data, however, suggest the contrary, that ovulatory infertility or menstrual infertility (which may be a surrogate marker for anovulation) has no effect on ovarian cancer risk [90] or may even elevate it [91,92]. Moreover, dizygotic births, which imply a higher number of ovulations, are also associated with a decreased risk for this disease [93]. Finally, after adjusting for "ovulatory life," OC use appears to reduce the risk of ovarian cancer by an additional 7%; that is, the effect of OC use on ovarian cancer risk exceeds the expected risk reduction from ovulatory inhibition alone [94]. Hence, new hypotheses that

more adequately account for these new epidemiologic data must be considered.

Two such new hypotheses have been made: the androgen and progesterone hypothesis [95] and the inflammation hypothesis [96]. Data exist to support both hypotheses; however, neither hypothesis fully accounts for all the existing epidemiologic data. An even more recent hypothesis involving stromal hyperactivity proposes yet another explanation for the disease origin [97], but it, too, does not account for all epidemiologic observations.

The androgen/progesterone hypothesis

Risch [95] has suggested that androgens and progestins may play a role in ovarian cancer etiology. Androgens are produced by ovarian theca cells, are present in follicular fluid, and are the principal sex steroid of growing follicles [98]. Postmenopause, a time when ovarian cancer rates sharply rise, the ovary is androgenic [99], and androgen receptors are found in normal ovaries [100], further supporting the activity of androgens within the organ. Epidemiologic evidence supports the androgen–ovarian cancer link. OCs suppress ovarian testosterone production by 35–70% [101–105]. A prospective study [106] found significantly higher levels of androstenedione in the serum of cases compared to controls. In the CASH study, cases were more likely to have a history of polycystic ovary syndrome (OR = 2.4; 95% CI = 1.0–5.9) [107], a condition that causes elevated androgen levels [108–110]. Finally, in a cohort study of 31,000 healthy women followed for more than 7 years, the risk of ovarian cancer increased with increasing waist-to-hip ratio ($P = 0.03$) [111], a marker of central obesity. Central obesity correlates with androgen levels in women [112–120].

There is also evidence for a protective role of progesterone in ovarian cancer. During pregnancy, the placenta causes a 10-fold increase in progesterone levels [121]. OCs also cause an increase in progesterone levels [122]. Progestin-only oral contraceptives, which do not totally suppress ovulation, are as protective against ovarian cancer as estrogen–progestin formulas [123]. Finally, the presence of progesterone receptors in normal ovarian epithelial cells [124] further supports the activity of the hormone in epithelial tissue.

The inflammation hypothesis

While the evidence for the androgen/progesterone hypothesis is compelling, it does not fully account for all the epidemiologic data. For example, it fails to address the associations of talc use, endometriosis, and pelvic inflammatory disease with ovarian cancer. In light of these observations, Ness and Cottreau [96] have suggested another possibility: that inflammation may play a role in the development of the disease. In support of this hypothesis, talc, a known inflammatory agent, has been repeatedly associated with ovarian cancer [125–131]. In addition, both tubal ligation and hysterectomy, which sever the upper genital tract from the lower genetic trac thereby potentially cutting off the pathway to the ovary, protect against the disease [132–141]. As well, medical conditions associated with inflammation, such as endometriosis [142] and pelvic inflammatory disease [143,144] have also been linked to ovarian cancer. Finally, the use of anti-inflammatory agents, such as aspirin and nonsteroidals (NSAIDS) [145–148] also appear to protect against the disease. Although these data support the inflammation/ovarian cancer link, the evidence is not always complete. For example, the effects of talc use are not specific and there is no clear association with duration and frequency of use.

The ovarian stromal hyperactivity hypothesis

While both the androgen/progesterone and the inflammation hypotheses provide potential explanations for the protective effect of OC use in disease initiation, there is still the question of why both OC use and parity, behaviors in which women are engaged in their 20's and 30's, provide protection against the development of a disease some 30–40 years later. It is possible that OCs have a residual effect on gonadotropin levels [97]. By reducing the amount of menstrual bleeding, OCs may also reduce retrograde menstrual flow, which is associated with endometriosis [149], believed to be a precursor to some ovarian tumors [142]. Most recently, Cramer et al. [97] hypothesized that OCs may reduce "stromal hyperactivity." In particular, during normal ovulation, granulosa and thecal cells proliferate to increase ovarian steroid production. Although it is believed that these cells undergo apoptosis, Cramer and colleagues hypothesize that some of the steroid-producing cells may in fact remain in the ovarian stroma. Hence, the greater the number of ovulatory cycles, the more follicles produced with granulosa–thecal cell compartments and the greater the number of residual (nonapoptosed) cells. These residual cells have been luteinized and may still retain steroid production capabilities. Hence, the more a woman ovulates, the greater the cumulative number of steroid-producing cells and potentially the greater cumulative lifetime exposure to ovarian steroids.

In support of this hypothesis, a longitudinal study of estradiol levels in 406 premenopausal women showed that a greater number of estimated ovulatory cycles was associated with higher estradiol levels ($P = 0.043$) [97]. It is possible that by reducing the cumulative number of lifetime ovulations, OCs reduce the cumulative number of residual steroid-producing cells that may have a proliferative effect on the ovarian epithelium. This new hypothesis together with the supportive preliminary data is exciting. Together with the primate data discussed below, these data suggest that OCs may exert their effects in various and potentially synergistic ways not only on the ovarian epithelial, but also on the ovarian stromal cells and/or via mediating intercellular communication between stromal and epithelial cells.

Epidemiology: summary and future directions

In summary, although we are currently left with no unifying hypothesis to guide research into the epidemiology of ovarian cancer, new concepts and ideas will no doubt shape the future of this important area of investigation. In particular, a deeper understanding of the biology of the ovary and the pathogenesis of ovarian cancer, in conjunction with epidemiologic studies, will be needed in order to establish such a unifying hypothesis. Such an understanding also will facilitate the identification of chemopreventive agents and other preventive modalities. To date, only one chemopreventive agent has been identified (OCs); however, we do not fully understand yet how OCs exert their protective effects and we are left with no clinical recommendations for the use of OCs as chemopreventive agents in high-risk women. Research into chemopreventive agents for women in general, and especially for high-risk women, is urgently needed.

Chemoprevention

There are several factors that have hindered progress in identifying chemopreventive agents for ovarian cancer. First, while the disease is quite virulent, it is not very common: approximately 23,000 women in the United States will be diagnosed with the disease in 2003 [1]. Therefore, prospective studies of chemopreventive agents would require extremely large cohorts of women in order to have enough power to demonstrate reduced incidence. Second, while the disease is believed to originate from the epithelial layer of the ovary, the exact cell of origin is unknown. This makes development of chemopreventive agents difficult, since the target cell is unknown. Third, a precancerous lesion has not been identified. Thus, the molecular changes that could potentially serve as screening and/or chemopreventive targets are unknown. Fourth, there is a lack of understanding of the link among current epidemiologic, biologic, and pathologic data for ovarian cancer. For example, while OCs and parity have long been established epidemiologically as being protective against the disease, the exact biologic mechanism remains unknown. Finally, as discussed below, there is a lack of an animal model that can be used to rapidly screen prospective agents, and there is no established surrogate endpoint biomarker to evaluate in an animal model.

Despite these limitations, there are several agents that are actively being investigated in both human and animal models, including progestins, retinoids, and vitamin D.

Progestins

Rodriguez et al. [150] randomized 75 female cynomolgus macaques (*Macaca fascicularis*) to receive no hormones ($n = 20$), ethinyl estradiol plus levonorgestrel ($n = 17$), ethinyl estradiol alone ($n = 20$), or levonorgestrel ($n = 18$) for a period of 35 months. The endpoint under evaluation was the number of apoptotic cells. The cynomolgus macaque is a plausible animal model for evaluating endpoints relevant to ovarian cancer because it is a nonhuman primate with a 28-day menstrual cycle similar to that of humans [151–153]. However, the animal does not develop epithelial ovarian tumors. Nonetheless, the macaque ovary enables the study of potential intermediate biomarkers of biologic effects on surface epithelial, and these data could potentially apply to humans.

In the Rodriguez study, monkeys randomized to the estrogen + progestin treatment and to the progestin-only group had a significant four- to six-fold increase in the proportion of apoptotic cells ($P > = 0.01$), with the maximum sixfold effect seen in the progestin-only group. These data suggest that progestins induce apoptosis. They also support the observation that the protection against ovarian cancer afforded by OCs extends beyond that of ovulation suppression [94] and that high-dose progestin OC formulations may be more protective than low-dose formulations [154]. Finally, the primate data provide a mechanism for the observed reduction in risk: the protective effects of OCs may be caused, in part, by progestin-mediated apoptosis of epithelial cells.

More recent data [155] from this animal cohort showed TGF-β expression to be differentially regulated in the epithelium of primates that received progestin, either alone or in combination with estrogen. TGF-β, a family of growth-inhibitory factors, has been implicated as a mediator of the biologic effects of chemopreventive agents, including tamoxifen in breast tissue [156] and retinoids in prostate tissue [157]. In the monkey study, progestin treatment was associated with a significant decrease in TGF-β1 expression ($P < 0.001$), together with a significant increase in TGF-β2/3 expression ($P < 0.001$). In addition, the change in TGF-β isoform expression was highly correlated with the number of apoptotic cells ($r = -0.998$, $P = 0.002$ for TGF-β1; $r = 0.973$, $P = 0.03$ for TGF-β2/3). Exactly how progestins regulate TGF-β is unknown.

These results may have important implications for ovarian cancer prevention. First, they help explain the risk reduction observed among (premenopausal) OC users in general. They further explain the observation that progestin-only formulations, which do not suppress ovulation, are as protective as combined estrogen and progestin formulations [123]. In addition, these data support the recent observation that OC formulations with higher progestin doses afford greater protection than low-dose formulations [154]. More importantly, these animal data further suggest that progestin-based interventions may be useful postmenopausally, a time when ovarian cancer rates dramatically increase [158]. Indeed, progestin-containing hormone replacement therapy formulations *used in a continuous regimen* have recently been shown not to be associated with an increase in ovarian cancer risk, whereas both estrogen-only formulations and

formulations in which the progestin component is used sequentially were both associated with an increased risk in that same study [159]. In particular, the animal data together with the data from this recent study implicate unopposed estrogens as risk factors for ovarian cancer.

Clinically, progestin-containing HRT is prescribed only for women with an intact uterus in order to reduce the risk of endometrial cancer associated with unopposed estrogens. However, the primate data, in conjunction with emerging epidemiologic data, suggest that further study is needed to evaluate whether all postmenopausal women, regardless of their uterine status, should consider a progestin-containing HRT formulation used in a continuous regimen. This recommendation should be taken cautiously, because use of combined HRT formulations for extended periods of time has been associated with an increase in breast cancer risk [160]. Indeed, these conflicting data from the ovarian and breast cancer literature highlight the need for further investigation of specific progestin (and estrogen) formulations and their potential tissue-specific effects on the various hormone-sensitive tissues.

Anti-inflammatory agents, vitamin D, and retinoids

Several other chemopreventive agents are under study. Data from both epidemiologic [145–148] and cell culture studies [161] suggest that NSAIDs hold promise for chemoprevention. In particular, COX-2 inhibitors appear to decrease cell proliferation and mitotic activity, while at the same time increase apoptosis in ovarian cancer cell lines [161]. Data from international studies indicate that ovarian cancer incidence varies according to latitude, with the greatest incidence found in Nordic countries and the lowest found in African countries [162]. In the United States, ovarian cancer incidence follows a similar trend, with declining incidence found as one moves from north to south [163]. These data suggest that vitamin D may play a preventive role, just as it may in prostate cancer [164–166], where the disease incidence follows a geographic trend similar to that of ovarian cancer [163]. Preliminary studies of the effects of vitamin D on ovarian cancer development in aging hens, the only other species in which ovarian tumors arise in the surface epithelium [167], are currently under way (Rodriguez, unpublished). Moreover, the Women's Health Initiative is conducting a controlled clinical trial of vitamin D and its effect on hip fracture, other fractures, and colorectal cancer [168]. Approximately 45,000 women will be randomized to the vitamin D or placebo arm, with an average 9 years of follow-up expected. This prospective cohort will prove valuable in evaluating the effects of vitamin D on ovarian cancer incidence.

Data from a study of *N*-(4-hydroxyphenyl) retinamide (4-HPR or fenretinide) as an adjuvant treatment for breast cancer suggest that 4-HPR may reduce the incidence of ovarian cancer [169]. In the original breast cancer study, women were randomized to receive either 200 mg of 4-HPR or placebo daily for 5 years [170]. Notably, during the 5-year intervention period 4-HPR reduced ovarian carcinoma occurrence (zero cases in the 4-HPR arm versus six cases in the control arm, $P = 0.0327$). However, the effect was no longer evident after the 5-year intervention period (six new cases in the 4-HPR arm versus four new cases in the controls, $P = 0.7563$). This suggests that the protective effects of 4-HPR extend only during the active intervention period and cease once the drug is discontinued. The use of 4-HPR as an ovarian cancer chemopreventive agent is plausible because 4-HPR is known to have anti-proliferative, apoptotic, and differentiating effects on ovarian surface epithelium [171] and ovarian cancer cell lines [172–176], and retinoid receptors as well as high levels of retinoid-binding protein have been found in the ovary [171]. A small trial of the effects of 4-HPR on the histology and cell biology of prophylactically removed ovaries from high-risk women is currently under way (GOG 0190).

Animal models for the study of chemopreventive agents

One of the major hindrances to advances in developing chemopreventive agents is the lack of a suitable animal model of ovarian carcinogenesis. In order to develop a high-throughput system to study chemopreventive agents, we must develop animal models that quickly and spontaneously develop ovarian epithelial carcinomas.

Although several rodent models [177–181] have been developed, they are not sufficient for chemopreventive studies for several reasons; these animals differ from humans reproductively, they tend to develop stromal and germ cell rather than epithelial ovarian carcinoma [182–185], and they do not *spontaneously* develop ovarian tumors at a rate sufficient enough to support chemopreventive studies [177–179,186]. Several researchers have attempted to address these limitations by trying to induce ovarian tumors in rodents through a variety of mechanisms, such as intraperitoneal injection of transformed ovarian cancer cells [187] and induction of genetic lesions in ovarian surface epithelium using a retroviral gene delivery technique [188]. However, even these models are limited because the induced tumors may differ biologically and histologically from spontaneous tumors. Moreover, these rodent models often require the use of immunocompromised animals. Because the immune system may play an important role in ovarian carcinogenesis, recent efforts have focused on developing rodent models with intact immune systems [189,190]. These models may prove more suitable for studying chemopreventive agents than those lacking full immune function.

In contrast to the rodent models, nonhuman primates more closely resemble humans in reproductive function and anatomy, as well as in hormonal and menstrual patterns [191–193]. In particular, histochemical analyses and hormone activity [192] of the nonhuman primate ovary indicate substantial similarities. Moreover, the microanatomy is almost identical among primates [191]. These factors support

nonhuman primates as an excellent model to study ovarian chemopreventive agents. However, this model is also limited the animals are costly to obtain and maintain, their development time is long, and their reproductive rates are low compared to rodents. Moreover, they do not develop epithelial ovarian tumors.

Perhaps one of the most promising animal models is the laying hen (*Gallus domesticus*), which has high rates of spontaneous development of ovarian adenocarcinoma [194]. In a study of 466 hens 2–7 years old, 19% had histologically confirmed spontaneous ovarian adenocarcinoma. Similar to humans, a trend toward increasing incidence was observed with increasing age: 12% at mean age 3.9 years versus 50% at mean age 6.1 years. These findings have been confirmed by other studies [195,196], suggesting overall a 30–40% rate of spontaneous ovarian adenocarcinoma in 4-year-old hens [194–196]. These avian ovarian and oviduct tumors are histologically similar to human ovarian adenocarcinomas and express antibodies that cross-react with antigens expressed in human ovarian cancer tissue [195]. Moreover, similar to humans, the proposed etiology of the avian tumors is incessant ovulation (laying hens ovulate every 28 h). This hypothesis is supported by a very recent study in which administration of medroxyprogesterone acetate reduced ovulation as well as the frequency of adenocarcinoma in the avian model [197]. Thus, the relatively fast and frequent rate of spontaneous ovarian tumor development in *G. domesticus* and the relative ease of procuring and caring for these animals make them a promising model for the study of chemopreventive agents.

Chemoprevention: summary and future directions

In conclusion, several potential chemopreventive agents have been identified through epidemiologic and basic biologic studies, as well as through secondary endpoints in other clinical trials. These agents warrant further investigation in cell cultures as well as in animal models. In particular, a greater understanding of how these agents work at the cellular and molecular level is needed before intervention studies at the population level are warranted. Even when cell culture and animal studies are supportive, population-based studies will be difficult because of the large sample size needed to see a significant decrease in disease incidence. For example, assuming an annual incidence rate of 30 per 100,000, a two-arm study would require 42,000 women to achieve 80% power to detect a 35% reduction in disease incidence over a 15-year follow-up period. This is comparable to undertaking a study the size of the Women's Health Initiative [168].

Finally, little research has focused on chemoprevention in high-risk women. Given the contradictory data on OCs and ovarian cancer prevention in *BRCA1/2* carriers, it is unclear whether chemopreventive data specific for women in the general population can be applied to high-risk women without additional study.

Screening

Given the paucity of chemopreventive agents available for ovarian cancer, an important step in reducing morbidity and mortality is to employ screening efforts to identify women as early in the disease process as possible. The rationale is that earlier detection will lead to reduced mortality.

Currently, there are two screening approaches: the serum-based CA125 marker and transvaginal ultrasonography (TVU). However, these techniques can fail to detect ovarian cancer at an earlier, more curable stage [198–201]. Moreover, neither technique has been shown to reduce morbidity and mortality. Two ongoing trials, one in the United States and one in the United Kingdom, are assessing the effect of variations of these screening techniques on ovarian cancer mortality in general.

The PLCO screening trial and the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS)

The PLCO Cancer Screening Trial is a large, multicenter, randomized clinical study of the effects of screening on site-specific mortality [16]. From 1993 through 2000, 10 screening sites throughout the United States enrolled 154,958 participants age 55–74 (over 74,000 women), who were randomly assigned to either the intervention arm or the observational arm. Participants will be followed for a minimum of 13 years. Intervention for ovarian cancer screening includes baseline measurements of CA125 levels and TVU, followed by annual CA125 readings for 5 years and TVUs for 3 years. The UKCTOCS is a randomized trial with three arms: no screening (control group), a multimodal group (annual screening with serum CA125 as the primary test and ultrasound as the secondary test), and an ultrasound group (annual screening with ultrasound as the primary test and repeat ultrasound in 6–8 weeks as the secondary test). Ovarian cancer mortality will be assessed 7 years after randomization from 2001 to 2010; an estimated 200,000 women are expected to enroll.

Together, PLCO and UKCTOCS have ample power to assess the impact of screening on ovarian cancer mortality. However, while PLCO has no specific exclusion criteria for women at high risk for ovarian cancer, UKCTOCS explicitly excludes high-risk women, defined as two affected first- or second-degree relatives with confirmed epithelial ovarian cancer [202]. Thus, the evaluation of screening on high-risk women is limited to the PLCO trial, with greatly reduced power.

Risk of ovarian cancer algorithm

Data from a screening trial in which 21,935 women were screened for an average of 5 years indicate that CA125 has a specificity of 99.9% but a sensitivity of only 71% [203].

Notably, the behavior of CA125 levels over time differed between ovarian cancer cases and noncases, suggesting that using information on longitudinal CA125 levels can lead to substantial improvement of screening programs. This observation led to the development of the Risk of Ovarian Cancer Algorithm (ROCA) [204], in which the risk of ovarian cancer for an individual is calculated using a computerized algorithm. The algorithm, which is based on Bayes theorem, compares each individual's serial CA125 levels to the pattern in known cases of ovarian cancer and controls. The closer the CA125 profile to the CA125 behavior of known cases of ovarian cancer, the greater the risk of ovarian cancer. The final result is presented as the individual's estimated risk of having ovarian cancer during the year following the test.

The advantage of ROCA is that it enables systematic calculation of risk for all participants. Moreover, there is substantial efficiency gained by dynamically allocating screening resources to higher risk participants. The costs include a small increase in the number of CA125 measurements, as well as the logistics of tracking individual CA125 levels over time. Simulations indicate that the ROCA sensitivity for preclinical early stage disease is approximately 60%, compared to 25% in clinical practice and 40% with a screening cutoff of CA125 >30 U/ml [205]. Currently, ROCA is being evaluated in the UKCTOCS as well as in a small pilot study of 2400 high-risk women in th United States [206].

Screening: summary and future directions

In summary, no single effective ovarian cancer screening strategy has been developed. One of the major challenges for ovarian cancer screening is that, unlike cervical cancer in which the Pap smear is used to identify precancerous cellular changes, there are no known premalignant lesions or cellular changes that make ovarian cancer amenable to screening using this morphologic approach. Thus, a better understanding of the biology and pathogenesis of ovarian cancer is critical not only for developing effective chemopreventive strategies, but also for developing effective screening modalities.

Biology, pathology, and emerging technologies

One of the major difficulties in studying epithelial ovarian carcinomas is that they are among the most complex of all solid tumors [207]. The cell type from which they arise is believed to be the ovarian surface epithelium (OSE), a monolayered squamous-to-cuboidal epithelium which is separated from an underlying stroma by a basement membrane [208]. The OSE is derived from the celomic epithelium, which is the mesodermally derived epithelial lining of the intraembryonic celom [208]. The OSE directly transports materials to and from the peritoneal cavity. During a woman's reproductive life, the OSE also assists in the breakdown of the ovarian cortex during ovulation [209] as well as in repair of the ovarian cortex after ovulation [210–212].

The origin of ovarian cancer: surface epithelium and inclusion cysts

As the ovary ages, the OSE forms clefts and inclusion cysts, possibly from normal epithelial cells trapped in ruptured follicles after ovulation [213,214]. Cells within these lesions tend to undergo metaplastic changes, including producing markers such as CA125 not typically expressed in normal epithelial cells but found to be expressed in ovarian neoplasms [208] as well as in müllerian duct-derived neoplasms [215]. Moreover, OSE cells within cysts are also common sites for neoplasia [216,217]. An open question is why epithelial cells within inclusion cysts (rather than cells on the ovarian surface) tend to be the site of neoplastic transformation. Another unexplained observation is that if inclusion cysts arise from the site of ruptured follicles, why are they more common in multiparous women [216] compared to nulliparous women, who would ovulate more than their parous counterparts and have a greater risk of ovarian cancer? Similarly, why are inclusion cysts found more often in women with polycystic ovary disease [107], a condition characterized by anovulation and increased ovarian cancer risk?

Despite these questions, molecular evidence supports the hypothesis that transformed OSE within inclusion cysts are the cell and site of origin of ovarian carcinoma. Boyd et al. (unpublished) combined molecular genetic analyses with morphological analyses of the normal ovarian epithelium from *BRCA1/2* mutation carriers diagnosed with stage I ovarian cancer. In each of five cases analyzed, inclusion cysts contained regions of neoplastic cells separated from normal OSE by a region of dysplastic cells. Moreover, in each case, loss of heterozygosity at the *BRCA1* locus and the same p53 mutations were found in both the tumor and the dysplastic regions. In two cases, these same genetic alterations were found in the histologically normal OSE as well. These data support the hypothesis that the normal epithelial tissue is giving rise to the dysplastic cells which in turn are giving rise to the neoplastic lesion. Similar data from inclusion cysts identified in the OSE of stage I sporadic ovarian tumors further support the theory (Boyd, unpublished).

Although this molecular evidence is compelling, it still does not explain a key factor necessary for the malignant transformation: because epithelial ovarian tumors are morphologically indistinguishable from müllerian-duct derived neoplasms [218], a necessary event in ovarian carcinogenesis is the transformation of the less differentiated OSE to a more well-differentiated müllerian cell type. Indeed, the fact that ovarian carcinomas are müllerian-like instead of mesothelioma-like (as would be expected given the origin of OSE) raises the perennial question of whether OSE cells are

the cell of origin for epithelial ovarian tumors [218]. In this regard, components of the secondary (extrauterine) müllerian system also have been suggested to play a role in ovarian tumorigenesis [218].

Determining the cell of origin and the sequence of transforming events leading to ovarian neoplasia are important factors in devising prevention (and possibly screening) strategies. If OSE must first undergo metaplastic changes to become müllerian-like in the pathway to carcinoma, then identifying agents that prevent this event might be useful in preventing malignant progression. On the other hand, if ovarian carcinomas arise from the secondary müllerian system, then preventive strategies will need to focus on the changes in müllerian cells that accompany malignant transformation.

Genes implicated in the etiology of sporadic epithelial ovarian cancer

Both sporadic and hereditary ovarian cancer require the accumulation of genetic changes, and both are characterized by a high degree of genetic alterations. It is not known, however, whether these alterations are needed to initiate and/or promote tumorigenesis or whether they result from the genomic instability inherent in the resulting tumor.

Both tumor suppressor genes and oncogenes have been implicated in the development of ovarian cancer (reviewed recently in Wenham et al. [219] and Liu and Ganesan [220]). Mutations in the *p53* tumor suppressor gene are the most frequently reported genetic alterations [221–223]; more than 50% of ovarian tumors lack a functional *p53* gene product. Other tumor suppressor genes, including *PTEN* [224] and *p16* [225], appear to be inactivated in ovarian tumors. The mechanisms of tumor suppressor gene inactivation range from point mutation to whole gene deletion. More recently, promoter region hypermethylation has been implicated as another way to inactivate tumor suppressor genes [226].

Proto-oncogenes are often mutated or amplified in ovarian cancer. However, the frequency of these mutations is less than that of tumor suppressor genes [219]. Among the oncogenes implicated in ovarian carcinogenesis are c-*myc* [227–229], K-*ras* [230–235], *HER2/neu* (*erbB2*) [236], and *Akt* [237–241], each of which plays a role in cell growth, proliferation, and/or death.

Notably, the patterns of genetic alterations differ according to histologic subtype (reviewed in Feeley and Wells [242] and Aunoble et al. [243]). For example, the *p53* tumor suppressor gene is often mutated in serous tumors, whereas K-*ras* mutations are seen predominantly in mucinous tumors. This supports the hypothesis that although the various subtypes of epithelial ovarian cancer presumably all arise from the same cell of origin, they represent histopathologically, genetically, and biologically distinct diseases. Understanding the molecular basis and the underlying biology of ovarian cancer could, therefore, lead to the development of targeted chemopreventive agents. Until recently, most of the molecular evidence has been based on immunohistological examination of ovarian tumors. The advent of advanced technologies, however, is rapidly changing our knowledge base.

Genomics and proteomics: identifying chemoprevention and screening targets

Identifying the cell of origin and the nature of premalignant changes also will support early detection efforts. The NCI's EDRN is a national consortium focused on developing, evaluating, and validating biomarkers of early cancer detection and risk assessment [244]. Several strategies are being employed in ovarian cancer, including serial analysis of gene expression [245], molecular probing, and proteomics [246]. These strategies combine recent advances in molecular biology, including the mapping of the human genome, with new technologies, such as laser capture microdissection and advances in existing technologies, such as mass spectrometry. These combinations hold promise for identifying premalignant changes that may serve as targets for early detection and possibly chemoprevention efforts.

For example, Petricoin et al. [246] used a surface enhanced laser desorption ionizing time-of-flight mass spectrometry approach to identify protein expression patterns in the serum of 50 women with ovarian cancer and 50 healthy controls. They then employed computer-assisted pattern recognition to identify a protein expression pattern that differentiated the cases from the noncases. The computer-generated algorithm was then used to classify the case/control status of an independent set of 116 blinded samples (50 cases, 66 controls). The technique correctly classified all 50 cases, including 18 stage I tumors (100% sensitivity, 95% CI 93–100%) and 63/66 controls (95% specificity, 95% CI 87–99%). The positive predictive value (PPV) was 94% (95% CI 84–99%) compared with only 35% for CA125 for the same samples. Notably, all the cases and most of the controls were high-risk women who had been followed for at least 5 years with annual CA125 measurements and three-dimensional color Doppler flow ultrasound examination [247]. Thus, the high positive predictive value is acceptable for potential clinical application in this high-risk group, but is not sufficient for general population-based screening, where the relatively low disease incidence requires a PPV of almost 100% to avoid generating too many false-positive tests.

This research finding is exciting not only because it holds promise as a cost-effective, high-throughput screening modality especially for high-risk women, but also because the complex serum proteomic patterns might reflect the underlying pathological state of the ovary. Currently, the origin and identity of the discriminating proteins detected using these methods are unknown but are being investigated [248]. These serum proteins, once identified, may serve as

both early detection markers and guides for the development of more effective chemopreventive agents.

Surgical prophylaxis

With the lack of chemopreventive agents and effective screening modalities available to high-risk women, the only recommended method of preventing ovarian cancer occurrence is bilateral prophylactic oophorectomy. Until recently, the data on the effectiveness of this approach have been limited and based largely on expert opinion [249]. However, two recent prospective studies provide supportive data on the efficacy of the procedure in reducing ovarian cancer risk [10,11]. In one study of 259 women who underwent prophylactic oophorectomy and 292 matched controls who had not undergone the procedure followed for an average of 8.8 years, surgery significantly reduced the risk of coelomic epithelial cancer (hazard ratio, 0.04; 95% CI 0.01–0.16) [11]. In the other study, 170 women chose to undergo surveillance or bilateral salpingo-oophorectomy. During a mean follow-up of 24.2 months, only 1 of 98 women undergoing surgery developed peritoneal cancer compared to 4 ovarian cancers and 1 peritoneal cancer in the surveillance group [10].

While these studies support the role of bilateral oophorectomy in primary prevention of ovarian cancer, this surgical procedure is not without certain risk. First, because *BRCA1/2* carriers are also at risk for fallopian tube cancer, removal of the ovaries alone is insufficient. Therefore, the procedure also should include removal of the fallopian tubes, and possibly the entire uterus, to minimize the risk of cancer developing in the small amount of fallopian tube tissue remaining after a salpingo-oophorectomy [250,251]. Even with the removal of the entire uterus, the chance of primary peritoneal carcinomatosis remains [252]. Hence, in order to clearly communicate that the procedure does not completely remove the risk of cancer in high-risk women, a more appropriate term may be "risk-reducing salpingo-oophorectomy" RRSO. As well, because prophylactically removed ovaries and surrounding tissue may contain microscopic disease foci [10,11], careful examination of the tissue is necessary to reduce the likelihood of a missed early stage cancer.

In addition, the procedure renders women infertile and forces premature menopause. Surgical menopause is not without substantial morbidity, including potential negative effects on the cardiovascular system [253,254], the skeletal system [255], and quality of life, such as alterations in mood, sleep disturbances, and adverse psychosexual effects [256–261]. There is also the concern about using hormone replacement therapy in women with an increased risk of breast cancer due to *BRCA1/2* carriage [76,160]. Finally, there has been no long-term follow-up study of the physical and psychological effects of the procedure on women and their families. Plans for such a study are currently underway (Gynecologic Oncology Group protocol 0199, M.H. Greene, PI).

In summary, while RRSO has been demonstrated to reduce the incidence of ovarian cancer, no study has established the efficacy of the approach in reducing overall mortality in high-risk women. The potential morbidity associated with this procedure is a concern. This should be discussed with women during the decision process. Once a woman has chosen the procedure, close clinical monitoring is needed to minimize the potential adverse effects of surgical menopause.

Conclusions

Traditional large-scale epidemiologic studies have provided us with information on lifestyle and environmental factors associated with ovarian cancer risk. Yet these data have not successfully informed the design of prevention and screening strategies, especially for high-risk women. The only current recommendation for these women is surgical removal of the ovaries, which can be associated with significant morbidity. Conversely, recent progress has been made in understanding the molecular and genetic basis of the carcinogenic process. Yet these data have yet to be applied to epidemiologic studies in a way that has served to identify modifiable lifestyle factors associated with ovarian cancer risk.

Hence, several research challenges remain, including identifying the cell of origin in ovarian cancer, determining the early cellular processes that lead to the malignant phenotype, identifying premalignant lesions, developing animal models that exhibit spontaneous carcinogenesis, identifying surrogate endpoints useful for prevention and screening trials in humans and animals, devising more precise tumor classification schemes, characterizing sporadic and hereditary tumors at the molecular level, and translating these data into effective screening and prevention modalities. Progress in these areas will only be made through the marriage of several disciplines, including epidemiology, molecular biology, pathology, and biochemistry, along with advances in technologies such as proteomics, genomics, transcriptionomics, and bioinformatics, which will provide more accurate and large-scale data on cellular processes in general and on the genetic and molecular basis of cancer in particular.

In the interim, several clinically applicable avenues merit further investigation, including the timing of prevention interventions in average- and high-risk women; continuous OC use to reduce breakthrough bleeding and retrograde menstrual flow, as well as potentially reducing the cumulative number of steroid-producing cells in premenopausal women; the use of progestational agents in postmenopausal women with and without an intact uterus; the investigation of other chemoprevention agents such as retinoids and vitamin D; and the effects of RRSO on the physical and psychological well-being of high-risk women and their fam-

ilies. The multidisciplinary symposium held in Pittsburgh, Pennsylvania, in May 2002 represents a first step in bringing together experts from such diverse disciplines, along with consumer advocates and clinicians, in the hope that such an exchange of information will stimulate future studies and better inform the research community about emerging topics that warrant further study.

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