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Perspective

The Monkey, the Hen, and the Mouse: Models to Advance Ovarian Cancer Chemoprevention

Perspective on Romero et al., p. 792

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Abstract This perspective on Romero et al. (beginning on p. 792 in this issue of the journal) discusses the available animal models of ovarian cancer, including the laying hen, non-human primate, and transgenic rodent models, and their relevance to ovarian cancer chemoprevention studies.

Epithelial ovarian cancer is highly lethal. In 2009, there will be an estimated 21,550 new cases of and 14,660 deaths from ovarian cancer in the United States (1). The vast majority of women with ovarian cancer present with advanced-stage disease with little chance for a long-term cure. Despite a sustained research effort to discover new markers, there currently are no effective early-detection strategies for this disease.

Although a strong research effort continues in novel therapeutics for ovarian cancer, ovarian cancer chemoprevention remains an understudied area. Contrasting with growth of the overall field of cancer chemoprevention, ovarian cancer chemoprevention is hindered by significant challenges, many of which are related to the biology of ovarian cancer. First, epithelial ovarian cancer is a heterogeneous disease composed of different histologic types, each with a unique molecular signature. Second, ovarian cancer has no clear premalignant lesion such as those associated with endometrial or colon cancer. Third, there is no clear understanding of the early molecular events in epithelial ovarian cancer pathogenesis. Fourth, there are few relevant and easily manipulated animal models of ovarian carcinogenesis for chemoprevention-related studies. Last, the ovaries are not easily accessed for tissue sampling.

Multiple epidemiologic factors, including one or more full-term pregnancies, use of oral contraceptive pills (OCP), breast-feeding a child, and a tubal ligation or hysterectomy, are associated with a lower risk of ovarian cancer (2). OCPs are by far the most effective factor in preventing ovarian cancer. Epidemiologic studies have consistently found that OCP use is associated with an ~30% to 60% decreased ovarian cancer risk. Pooled data from 45 large epidemiologic studies involving 25,257 cases and 87,303 controls found that the overall relative risk of ovarian cancer for OCP ever users versus never users was 0.73 (95% confidence interval, 0.70-0.76). The protective effect correlated with duration of use. Furthermore, the risk reduction lasted for decades after OCP use had stopped (3). The theories to explain how combined estrogen and progestin OCPs decrease ovarian cancer risk have centered on two mechanisms. First, the risk reduction may be related to decreasing the repetitive cycle of epithelial damage and subsequent proliferative repair of the ovarian surface epithelia due to ovulation. Second, OCPs may reduce ovarian cancer risk by decreasing the levels of the gonadotropins (follicle-stimulating hormone and luteinizing hormone) elevated levels of which directly stimulate the ovarian epithelium. Both theories are plausible, but the OCP-related molecular alterations that contribute to ovarian cancer prevention have not been well-defined.

Given the well-established effectiveness of OCPs in reducing ovarian cancer risk, a reasonable starting point for ovarian cancer chemoprevention studies is to evaluate the effects of OCPs on the ovarian surface epithelium. This analysis has the potential to provide insight into possible molecular mechanisms of protection as well as into molecular alterations contributing to ovarian cancer pathogenesis. Indeed, this approach has been used in monkeys, chickens, and now, as discussed below, in a mouse model of ovarian cancer. In monkeys (cynomolgus macaques), combined estrogen and progestin oral contraceptives increased apoptosis rates in the ovarian surface epithelium (4). Progestin alone had an even higher rate of apoptosis, suggesting that the progestin component of OCPs may be the dominant chemopreventive agent. Similar studies in rhesus monkeys have further validated the utility of primate models for ovarian cancer chemoprevention studies (5). With similarities to humans in anatomy and menstrual cycles, monkey models may be very useful for more-detailed chemopreventive studies. For example, this similarity allows scientists the opportunity to test the current hypothesis that a subset of ovarian cancers may derive from the fimbria of the fallopian tube rather than from the ovarian surface epithelium, an important site-of-origin question. However, monkey models for ovarian cancer prevention have several biological limitations, including a lack of ovarian tumor development and an absence of validated surrogate biomarkers, as well as

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practical challenges, including a high cost and need of specialized skills.

As discussed recently in this journal (6), the laying hen represents a unique model for studying ovarian cancer chemoprevention. It develops spontaneous ovarian tumors believed to result from a high frequency of ovulation. In a chemoprevention study by Barnes et al. (7), ovarian tumor formation was significantly decreased in chickens treated with progestin (depot-medroxyprogesterone) versus untreated controls. More recently, Hakim et al. confirmed that genetic alterations of ovarian tumors in the laying hen model, including altered p53, Ras, and HER-2/neu, are similar to those in women (8). With spontaneously developing ovarian tumors, this model may be particularly useful for ovarian cancer chemoprevention studies, although the hen's anatomic dissimilarity to humans may limit its utility.

In this issue of the journal, Romero et al. extend the study of oral contraceptives to a transgenic mouse model of ovarian cancer (9). They inject an adenoviral vector expressing Cre recombinase (AdCre) under the ovarian surface (intrabursal injection) to induce a conditional knockout of *PTEN* and activation of oncogenic *KRAS*. This process rapidly induced endometrioid-type ovarian tumors, and intraperitoneal metastases developed in the majority of mice. Mice treated with combined estrogen and progestin or progestin alone prior to the AdCre injection had a trend toward decreased mean tumor weight. However, the authors did not show a delayed time to tumor development, which would be a more direct measure for cancer chemoprevention. Although the study by Romero et al. begins to address the effect of OCPs on ovarian tumor development, future studies in genetically engineered mouse models of ovarian carcinogenesis must carefully consider whether the end point addresses cancer prevention or treatment effects on tumor growth or metastases.

Several other mouse models of ovarian cancer have been developed for studies of the antitumor effects of therapeutic agents. These models include the inactivation of *PTEN* and *APC* through intrabursal injection of AdCre to produce tumors resembling endometrioid ovarian carcinoma (10); a mouse model of ovarian serous carcinoma that Fleskens-Nikitin et al. (11) developed by injecting AdCre to inactivate p53 and Rb in the ovary; and a model relying on *ex vivo* infection of p53-null ovarian surface epithelial cells with vectors carrying oncogenes (*c-MYC, KRAS,* and/or *AKT*), followed by surgical implantation of these cells under the ovarian bursa. Adding at least two oncogenes produced tumors in the last model (12). Despite generating ovarian tumors, these mouse ovarian tumor models may have limited usefulness for chemoprevention because of their dependence on strong oncogenic driving forces that may not reflect the pathogenic process of genetic changes leading to ovarian cancer in humans. Furthermore, even though these mouse models produce tumors that phenotypically resemble human histologic subtypes of ovarian cancer, the genetic signatures of these tumors may be different from those in humans. Also, other factors such as the ovarian microenvironment, which is essential for ovarian cancer initiation, are not replicated in existing ovarian cancer models.

Transgenic animal models of human ovarian disease associated with germ line mutations of the tumor suppressors *BRCA1* and *BRCA2* may have an important role in chemoprevention studies. Because homozygous deletion of *BRCA1* is lethal in mice, models of conditional inactivation of *BRCA1* using Cre recombinase in the ovary have been developed. For example, an AdCre injection to inactivate *BRCA1* in mouse ovarian surface epithelial cells induced histologic changes (13). Using follicle-stimulating hormone receptor Cre to inactivate *BRCA1* in ovarian granulosa cells resulted in ovarian cystadenoma formation (14). Also, conditional inactivation of *BRCA1* and *p53*, combined with the expression of oncogenic *MYC*, has been shown to produce *BRCA1*-associated serous ovarian carcinoma in mice (15).

The high lifetime risk of ovarian cancer in women with *BRCA* mutations makes it especially important to develop animal models to test novel chemoprevention strategies for them. Women with *BRCA1* mutations carry a 39% lifetime risk for developing ovarian cancer, often at younger ages compared with their sporadic counterparts (16). Women with *BRCA2* mutations have a lower, although substantial 22% lifetime risk of developing ovarian cancer (16). Prevention options for *BRCA* mutation carriers have been thus far primarily surgical. Prophylactic removal of the ovaries and fallopian tubes reduces the risk of ovarian cancer by 85% to 90% (17). This benefit comes at a great price, however. Early menopause and loss of fertility are difficult consequences for women in their late 30s and early 40s—ages at which prophylactic surgery is currently recommended. Although the interest in chemoprevention for these women is strong, only preliminary studies of this approach have been completed to date. A small pilot study showed that it is feasible to conduct a clinical trial of a cyclooxygenase-2 inhibitor (celecoxib) in this population (18). A Gynecologic Oncology Group trial of fenretinide for ovarian cancer chemoprevention in women at high-risk had to be closed because of slow accrual; a Gynecologic Oncology Group study of a progestin (levonorgestrel) in women at high-risk is ongoing (19). Epidemiologic studies indicate that, similar to women in the general population, women with *BRCA1* and *BRCA2* mutations are protected against ovarian cancer by oral contraceptives (20). Therefore, examining the ovaries of women at high-risk after treatment with a progestin is likely to yield valuable information. However, in part because of the possibility of an increased risk of breast cancer associated with oral contraceptives (21), developing novel and effective chemoprevention for this population is still urgently needed.

It is important to remember that no animal model is likely to perfectly reflect the biology of human ovarian cancer. These models are tools that must be matched appropriately to our scientific questions and understood to have certain strengths and weaknesses. Newly developed high-throughput screening technologies also will enable us to better define the fundamental molecular and genetic changes associated with ovarian cancer pathogenesis in humans. As our understanding of human ovarian cancer advances, rodent models will join the hen and the monkey as important investigational tools in advancing the field of ovarian cancer chemoprevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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