Retinoids for Ovarian Cancer Prevention: Laboratory Data Set the Stage for Thoughtful Clinical Trials

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Ovarian cancer is a highly lethal disease and represents one of the leading causes of cancer death among women in Western countries. Since effective methods of screening and early diagnosis are lacking, prevention is being sought as one of the most promising ways to reduce mortality. While prophylactic oophorectomy is increasingly used for high-risk women, its short- and long-term morbidity is unknown. More important, most ovarian cancers are sporadic, and the widespread use of prophylactic oophorectomy in average-risk women is unimaginable. An epidemiologic study (1) has shown that oral contraceptive use is associated with reduced ovarian cancer risk among the general population as well as in BRCA1 heterozygotes. In women with a family history of breast cancer, however, oral contraceptive use is associated with additional risk of that disease (2). Thus, other agents are being studied that might replace or be combined with oral contraceptives for prevention of ovarian cancer. Among these agents, retinoids have been the subject of the most intensive investigation of their potential to prevent cancer (3).

The first evidence of an in vivo effect by retinoids on ovarian cancer came from the study by Formelli and Cleris (4) using the retinoic acid analogue fenretinide (N-4-hydroxyphenylretinamide). The potential of retinoids for the prevention of ovarian cancer was subsequently confirmed in the phase III trial of fenretinide for the reduction in the incidence of second breast cancer (5). During the 5-year intervention period, a statistically significant lower incidence of ovarian cancer was noted in the fenretinide group compared with the control group (no cases versus six cases, respectively) (6). However, because of the unexpected results and the limited number of events, the observation should be viewed with extreme caution. In addition, the apparent benefit was partially lost during the subsequent follow-up period, suggesting that the potential preventive effect of fenretinide was not lasting and that treatment would have to be continued indefinitely for the benefit to continue (5). However, attention to secondary clinical trial endpoints often yields new hypotheses for exploration. Thus, the finding prompted different laboratories to provide further insight into this clinical finding.

Since that initial clinical observation (6), several in vivo and in vitro studies (4,7–9) have consistently shown that fenretinide, as well as other natural and synthetic analogues of vitamin A, can effectively inhibit ovarian cancer growth and induce apoptosis at clinically relevant concentrations. More important, ovarian cancer is a steroid hormone-dependent cancer that tends to express high concentrations of estrogen receptors (ERs) in several situations, particularly in borderline tumors and in well-differentiated invasive cancers, where measurable responses to tamoxifen may be observed (10,11). Data (11) also indicate that ovarian cancer tissue expresses substantial amounts of retinoic acid receptor (RAR)-α and that these levels are highly associated with ER expression and degree of differentiation. Most retinoids, including fenretinide, are known to exert a greater antitumor activity in ER-positive breast cancer cell lines. Fenretinide is known to inhibit carcinogenesis through several pathways, one of which involves binding to RARs, presumably by inhibition of retinoic acid catabolism (12).

Since RAR-α is under ER regulation and is positively stimulated by estradiol, one explanation for the benefit of fenretinide in second breast and ovarian cancers in premenopausal women may, therefore, be related to the growth inhibition through binding to RAR-α. In line with this notion, we have shown fewer (although not statistically significant) ER-positive and progesterone receptor-positive second breast cancers in a subgroup of premenopausal women treated with fenretinide compared with a control arm in our randomized trial (13). Another mechanism of fenretinide activity might be interference with the carcinogenesis pathway associated with mutation of BRCA1 or BRCA2. Notwithstanding the mechanisms involved, these findings suggest a common pathway for growth and differentiation of the breast and ovaries under the regulation of estrogen and retinoid signaling.

In this issue of the Journal, Guruswamy et al. (14) offer further support for the use of retinoids for the prevention of ovarian cancer. They use an innovative approach to determine the effects of different classes of retinoids (receptor-dependent versus receptor-independent compounds) on proliferation, glandular differentiation, and cell death of organotypic ovarian cultures of primary and established cell lines. Several approaches are of particular interest: 1) the use of clinically relevant concentrations (≤1 μM), which correspond to the mean plasma level attained with 200 mg of fenretinide, a daily dose that, unlike higher doses (15,16), can be administered long-term without bothersome adverse events (5); 2) the systematic comparison between apoptogenic and antiproliferative (differentiating) retinoids; 3) the use of organotypic cultures mimicking the glandular structures and thus resembling the in vivo histologic architecture; and 4) the study of the association between growth inhibition or apoptosis and mucin or MUC1 expression.

Previous findings had shown that retinoids, acting through multiple pathways, such as fenretinide and CD437, can inhibit growth and induce differentiation at low concentrations and induce apoptosis at higher concentrations via receptor-independent mechanisms (12). Guruswamy et al. (14) strengthen this notion by demonstrating that classic apoptotic retinoids, such as fenretinide and CD437, as well as new compounds defined as het-

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eroarotinoids, administered at low concentrations, inhibit proliferation and induce differentiation in a similar manner to conventional, receptor-mediated retinoids. However, these retinoids also maintain their ability to induce apoptosis at clinically achievable concentrations. Consequently, the authors suggest that apoptotic retinoids are preferable, since they may act through different pathways to inhibit carcinogenesis. They bring about decreased proliferation in cells that have retained their ability to undergo differentiation under appropriate stimuli, and when the differentiation potential is lost, they induce cell death. While this flexible functional ability of apoptotic retinoids needs to be assessed in future studies, the loss of benefit on ovarian cancer might be associated with progression of carcinogenesis. There (7) suggests that, in humans, the effect of fenretinide is antiproliferative rather than apoptogenic at a concentration of 1 μM.

Another important finding by Guruswamy et al. (14) is the strong association between mucin expression, MUC1 overexpression and induction of glandular differentiation, decreased proliferation, and apoptosis by the retinoids. Human epithelial mucins constitute a family of high-molecular-weight glycoproteins with a large, variable number of O-glycosylated tandem repeat domains. Considerable attention has been focused on these complex molecules, given their ability to react with many antibodies developed against malignant epithelial cells, thus providing a potential target for selective cancer immunotherapy (17). While during differentiation of the glandular structures, these molecules are overexpressed on the apical side of secretory epithelial cells, where they contribute to important functions of tissue morphogenesis, such as organization and maintenance of lumen of glandular and endothelial structures, progression of carcinogenesis is associated with the loss of polarization in their local overexpression, distribution over the entire cell surface and in the cytosol, and subsequent release into the blood stream (18).

Moreover, the expression of these MUC1 molecules, the prototypes of which are CA 15–3 and mucin-like carcinoma-associated antigen, may not merely be a passive signal of disease extent but, rather, may alter the biologic characteristics of adenocarcinomas by reducing cell-adhesion properties and by altering the function of immune-effector cells, two characteristics that may confer increased invasive and metastatic potential (19).

As the authors correctly point out, the complex regulation and function of MUC1 suggest caution when discussing the therapeutic implications of the retinoids’ effects on MUC1 expression. While during early phases of carcinogenesis, retinoids may reverse metastatic potential by increasing MUC1 polarization and glandular differentiation, one cannot exclude the hypothesis that induction of MUC1 expression during advanced disease might be associated with progression of carcinogenesis. There are several examples in which retinoids either reverse or promote carcinogenesis, depending on the carcinogenesis phase and the context (20,21). These examples strengthen the contention that retinoids should be studied with caution, especially in the therapeutic setting of advanced disease and also in secondary prevention trials, since their spectrum of pleiotropic effects might even be associated with increased cell invasiveness and metastatic potential.

Altogether, the potential opposing effects of MUC1 on carcinogenesis underline the importance of carefully conceived mechanistic clinical studies to assess the effect of retinoids on ovarian carcinogenesis. One ideal setting is a presurgical study in the increasingly available cohort of high-risk family members who opt for prophylactic oophorectomy for ovarian and breast cancer risk reduction. Induction of MUC1 expression and glandular differentiation, increased ER and RAR expression, decreased proliferation as measured by Ki-67, and an increase in apoptosis as assessed by TUNEL (i.e., terminal deoxynucleotidyl transferase-mediated biotin-deoxyuridine triphosphate nick-end labeling) may well be used as potential surrogate endpoint biomarkers to assess the activity of different retinoids. Likewise, subtle alterations possibly reflecting premalignant lesions of the ovary could be assessed in high-risk women (22,23). Since most ovarian cancers are sporadic, however, perimenopausal women undergoing hysterectomy for benign disorders, such as large myoma or uncontrollable bleeding, may also be an ideal cohort to screen for the activity of these agents in short-term, presurgical trials.

In conclusion, new in vitro data support the notion that ovarian cancer is extremely sensitive to the inhibitory effect of retinoids at clinically achievable concentrations, particularly to the class of apoptogenic compounds that include fenretinide and novel retinoid compounds. These data, together with previous clinical observations, provide sound rationale for the implementation of thoughtfully designed clinical studies to assess the activity of selected retinoids for the prevention of ovarian cancer. Given the complexity of the clinical model and the pleiotropic, context-dependent activity of retinoids, short-term, dose–response trials using surrogate biomarkers in women undergoing ovarian surgery are recommended. Hopefully, these studies will provide important clues for the implementation of large, randomized trials aimed at reducing the incidence of this deadly cancer.

REFERENCES


NOTE

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