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Potential Criteria for Cohort Selection in Chemoprevention Trials of Epithelial Ovarian Cancer

Vicki V. Baker, MD

Division of Gynecologic Oncology, University of Texas-Houston Health Science Center, Houston, TX 77030

Abstract Epithelial ovarian cancer is a heterogeneous disease. Epidemiologic studies have identified risk factors for this disease including advanced age, nulliparity, history of infertility, early age at menarche, late age at menopause, and perhaps ovulation induction. Cohort selection that includes women who have potential precursor lesions and alterations of select biomarkers may prove useful in the design of chemoprevention trials of epithelial ovarian cancer. Nuclear morphometry, specific genetic alterations, and markers of proliferation and differentiation may be useful biomarkers to monitor the efficacy of specific interventions.

Key words: Biomarkers, chemoprevention, ovarian cancer

Clinical trials to study the prevention of epithelial ovarian cancer will be problematic for three reasons: the disease is relatively rare, there are no effective screening strategies for this disease, and the ovary is a relatively inaccessible organ that cannot be easily monitored using currently available technology. For these reasons, cohort selection assumes particular importance in the design of a chemoprevention trial of epithelial ovarian cancer.

The goal of an ovarian cancer chemoprevention trial is to prevent the development of epithelial ovarian cancer in women who are at increased risk of disease development. The selection of this study cohort should be based upon epidemiologic risk factors and the presence of histopathologic precursor lesions. Epidemiologic risk factors for ovarian cancer include family history, advanced age, nulliparity, history of infertility, early age at menarche, and late age at menopause. These risk factors have in common the number of ovulatory events over time. Consistent with this observation, it has recently been suggested that ovulation induction is a particular risk factor for developing epithelial ovarian cancer.

PRECURSOR LESIONS

Potential precursor lesions for epithelial ovarian cancer include ovarian surface epithelial dysplasia [1,2], inclusion cysts [3,4], and perhaps adenomas and cystadenomas. An obvious problem in the prospective selection of study participants is to identify women who actually have one or more ovarian precursor lesions, since the ovary is relatively inaccessible for biopsy. Resta *et al.* [5] correlated the presence of hyperplastic lesions of the ovary with primary diseases of the female genital tract. Hyperplastic or metaplastic changes or inclusions cysts were found in 92% of women with a contralateral ovarian neoplasm, 76% of women with endometrial cancer, and 68% of women with polycystic ovarian disease. These

Send correspondence to Vicki V. Baker, Associate Professor and Director, University of Texas-Houston Health Science Center, 6431 Fannin, MSB 3.204, Houston, TX 77030.

observations suggest that polycystic ovarian disease might be an important criterion for entry into a study of ovarian cancer chemoprevention.

BIOMARKERS

Biomarkers to define cohort groups and assess the efficacy of interventions in chemoprevention trials of ovarian cancer are largely unknown. However, general genomic markers, specific genetic markers, proliferation markers, and differentiation markers have been described in normal and neoplastic ovarian tissue. Alterations in these markers may parallel the process of neoplastic transformation, its arrest, and perhaps its reversal.

General genomic markers include ploidy, karyotype, and nuclear morphometry. Studies of epithelial ovarian cancer that include precursor lesions are limited to the nuclear morphometry studies of Deligdisch *et al.* [6]. Using digital image analysis to quantitate nuclear perimeter, maximum nuclear diameter, and nuclear texture in addition to architectural changes, these investigators have refined the definition of ovarian dysplasia. Statistically significant differences were demonstrated between normal, dysplastic, and neoplastic ovarian epithelium.

Considerable information is available concerning numerous genetic alterations that occur in epithelial ovarian carcinoma [7]. However, information concerning genetic alterations in ovarian cancer precursor lesions is limited.

The c-*erb*B proto-oncogene encodes the 170 kD transmembrane receptor for epidermal growth factor (EGF). Normally the EGF receptor (EGFR) mediates cellular proliferation by binding EGF or transforming growth factor (TGF)-α. Henzen-Logman *et al.* [8] reported enhanced EGFR expression in 26 of 50 ovarian tumors. There was no association between EGFR expression and the histological type of tumor.

Wang *et al.* [9] evaluated the immunohistochemical expression of ERBB2 protein and EGFR by the ovarian surface epithelium, inclusion cysts, and adenocarcinoma specimens. Normal surface epithelium was weakly positive for ERBB2 protein and EGFR. Inclusion cysts exhibited stronger staining for ERBB2 protein and EGFR staining was absent.

The *ras* gene family includes three structurally and functionally related genes: c-Ha-*ras*, Ki-*ras* and N-*ras*. These genes encode proteins, collectively designated p21, that exhibit guanosine triphosphate (GTP) binding/GTPase activity. Yaginuma *et al.* [10] studied 39 epithelial ovarian neoplasms. Immunohistochemical evidence of *ras* gene expression was not detected in normal ovarian epithelium, but a progressive increase in expression was demonstrated among benign versus invasive ovarian neoplasms of both serous and mucinous cell types. Scambia *et al.* [11] investigated *ras* p21 expression in normal, benign, and malignant ovarian neoplasms using Western blot analysis. Normal ovarian tissue and benign tumors exhibited similar levels of p21 expression based upon densitometric analysis, whereas the expression of p21 was significantly elevated in ovarian carcinoma tissue.

The metastasis-related genes *nm23*-H1 and *nm23*-H2 have also been studied in epithelial ovarian cancer. Mandai *et al.* [12] have reported that ovarian neoplasms expressed higher mRNA *nm23* transcripts than normal or benign ovarian tumor tissue, although the level of expression was not related to histological subtype or stage of disease.

Henriksen *et al.* [13] studied the expression of platelet-derived growth factor (PDGF), the α receptor for PDGF, and the β receptor for PDGF in ovarian cancer specimens. These investigators found that the α receptor was present in 16 of 45 malignant tumors, but absent in 16 benign ovarian tumors and 10 normal ovaries. The β receptor was not detected in normal ovarian tissue or benign or malignant ovarian tumors. PDGF was detected in 33 of 45 malignant ovarian tumors, but in none of 20 benign ovarian tumors or 11 normal ovaries.

Proliferation markers described for precursor lesions are limited to nucleolar organizer regions (AgNORs), which reflect cellular and nuclear activity. AgNORs reliably differentiate benign and malignant epithelial ovarian neoplasms [14–16]. Kinsey *et al.* [15] reported that benign tumors exhibited a mean AgNOR count of 1.13, whereas malignant tumors exhibited a mean AgNOR count of 4.89. Mauri *et al.* [14] have reported similar results.

Markers of differentiation and function have been studied to a limited extent in precursor lesions of ovarian cancer. Blaustein *et al.* [17] described the presence of placental lactogen in a significant proportion of inclusion cysts, serous

papillary cystadenomas, and serous adenocarcinomas. The β subunit of human chorionic gonadotropin, α-2-glycoprotein, and β-1-glycoprotein were present in a smaller proportion of these lesions.

van Niekerk *et al.* [18] have reported a marker profile of tumor-associated antigens, keratins, and glycoproteins in ovarian cystomas, cystadenomas, and cystadenocarcinomas. These investigators reported that the antigens OA3, CA-125 and Mov18 were strongly expressed in ovarian carcinoma specimens. The majority of cystadenomas were also positive, but the intensity of expression was much less. The CEA antigen was expressed in most of the specimens at comparable levels of intensity and did not discriminate among these lesions.

Hanigan *et al.* [19] investigated the immunohistochemical patterns of γ-glutamyl transpeptidase (GGT) staining in normal and carcinomatous ovarian tissue. These investigators reported that GGT was absent in normal ovarian surface epithelial cells, expressed in some epithelial inclusion glands, and commonly found in epithelial ovarian neoplasms.

CONCLUSION

The relative rarity of epithelial ovarian cancer in conjunction with limited information regarding the initiation and progression of precursor lesions will make the design of chemoprevention trials problematic. Identification of individual women most likely to benefit from preventive interventions is difficult. Positive family history, a history of infertility and polycystic ovarian disease, a negative history of oral contraceptive use, and perhaps ovulation induction are clinical criteria that should be considered in selecting cohorts for a chemoprevention trial of epithelial ovarian cancer. Further definition of the cohort study group should be based on the known or suspected presence of a precursor lesion. With the increasing use of office laparoscopy and development of fluorescence spectroscopy to direct ovarian biopsies, it will be feasible to monitor alterations of biomarkers to assess the efficacy of chemoprevention. Potential biomarkers include nuclear morphometry and the expression of PDGF receptor, EGFR, the *ras* gene, and OA3, CA-125, Mov18, and GGT.

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