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# Molecular Approaches to Prevention and Detection of Epithelial Ovarian Cancer

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**Abstract** More than 90% of epithelial ovarian cancers arise from single cells. Malignant transformation can be associated with a number of molecular alterations including upregulation of tyrosine kinases and phosphatases, physiologic activation of *ras*, mutation of p53, amplification of *myc*, and increased activity of matrix metalloproteinases 2 and 9. Proliferation of transformed epithelial cells can be enhanced through the persistence of autocrine growth stimulation by TGF-α, loss of autocrine growth inhibition by TGF-β, as well as paracrine growth stimulation by macrophage derived cytokines and OCAF, a novel lyso-phospholipid. Ascites tumor cells retain responsiveness to growth inhibition by TGF-β which induces apoptosis in malignant ovarian epithelial cells, but not in normal ovarian surface epithelium.

Proliferation of surface epithelial cells following ovulation may contribute to the pathogenesis of ovarian cancer. Use of oral contraceptives that suppress ovulation has been associated with reduced risk of ovarian cancer in later life. Retinoids also deserve further evaluation for chemoprevention. Treatment with fenretinide was associated with decreased incidence of ovarian cancer. Additive or synergistic inhibition of ovarian tumor cell proliferation has been observed with TGF-β in combination with all-*trans*-retinoic acid.

Early detection of ovarian cancer could improve survival. Transvaginal sonography (TVS) and serum markers such as CA-125 have been evaluated in multiple clinical trials. The former lacks adequate specificity, whereas the latter is not sufficiently sensitive. Use of multiple serum markers can improve sensitivity. A combination of CA-125, M-CSF and OVX-1 has detected > 95% of Stage I ovarian cancers. If similar results are obtained with different data sets, multiple serum markers could be used to trigger the performance of TVS, providing a potentially cost effective screening strategy. Prospective trials will be required to demonstrate that screening for early stage ovarian actually impacts on survival.


**Key words:** Detection molecular markers, ovarian cancer, prevention

## MOLECULAR ALTERATIONS IN EPITHELIAL OVARIAN CANCER

Epithelial ovarian cancer is generally a clonal disease that arises from a single cell in more than 90% of cases [1,2]. Multiple genetic alterations must occur during the malignant transformation of a single ovarian surface epithelial cell. A var-

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iety of protooncogenes, tumor suppressor genes and growth factors have been studied in normal and malignant ovarian epithelial cells to identify alterations occurring in cancers from this particular site [3]. Molecular alterations associated with a fraction of ovarian cancers include persistence of autocrine growth stimulation by TGF-α through the epidermal growth factor receptor (EGFR), loss of autocrine growth inhibition by TGF-β, paracrine growth stimulation by macrophage derived cytokines, autocrine growth stimulation by macrophage colony stimulating factor (CSF-1) through the *fms* receptor, paracrine growth stimulation by the lyso-phospholipid OCAF, overexpression of HER-2/*neu*, overexpression of protein tyrosine phosphatase 1B (PTP1B), physiologic activation of *ras*, mutation of p53, amplification of *myc*, and increased activity of matrix metalloproteinases (MMP) 2 and 9.

Genetic alterations underlying these changes are most likely to occur and to be expressed in proliferating cells. The proliferation of ovarian surface epithelial cells is usually under tight control. Following ovulation, quiescent epithelial cells are stimulated to proliferate. A variety of growth stimulatory factors and cytokines have been detected in follicular fluid including TGF-α, tumor necrosis factor, and interleukin-1. Release of follicular fluid following ovulation may provide paracrine growth stimulation.

Paradoxically, quiescent surface epithelial cells express both the ligand TGF-α and its receptor EGFR [4,5]. Despite the potential for autocrine growth stimulation, surface epithelial cells have relatively low proliferative activity in the absence of ovulation. Autocrine and paracrine growth inhibition by TGF-β may be one of the factors that blocks proliferation of these cells.

When grown *ex vivo* normal ovarian surface epithelial cells express TGF-$\beta_1$ and TGF-$\beta_2$, activate TGF-β, and respond to the peptide factor with inhibiting proliferation [6]. Ovarian cancer cell lines have generally lost the ability to express, activate or respond to TGF-β [6]. In one ovarian cancer cell line that maintained each of these functions, addition of a monoclonal antibody that neutralized TGF-β increased proliferation, consistent with the persistence of an autocrine inhibitory loop. Ovarian cancer cells isolated directly from ascites fluid retained their responsiveness to TGF-β growth inhibition in more than 90% of cases, but lost expression of TGF-β in 40% of cases [7]. Consequently, ovarian cancer cells may have lost autocrine, but not paracrine growth inhibition by the peptide factor. TGF-β inhibits proliferation of both normal and malignant ovarian epithelium, but has induced apoptosis only in transformed cells [8]. This observation could have important implications for chemoprevention of ovarian cancer.

## PREVENTION OF OVARIAN CANCER

Epidemiologic studies point to the importance of ovulation in the pathogenesis of epithelial ovarian cancer. Early menarche, late menopause, nulliparity, and certain fertility-stimulating drugs increase the risk of ovarian cancer in later life; frequent pregnancies, prolonged lactation, and the use of oral contraceptive medication decrease the incidence of ovarian cancer. Use of oral contraceptives for as long as five years decreases the risk of ovarian cancer by 50% [9]. The impact of estrogens and progestins on autocrine and paracrine growth regulation of ovarian surface epithelium requires further study. Attempts to prevent breast cancer with the retinoic acid derivative (4-HPR) has been associated with a lower incidence of ovarian cancer in one recent study [10]. Retinoic acid derivatives have decreased proliferation in a minority of ovarian cancer cell lines and preparations of ovarian cancer ascites tumor cells [11]. Additive or synergistic inhibition of proliferation has been observed in cell culture when TGF-β was combined with all-*trans*-retinoic acid [11]. Clinical evaluation of chemopreventive strategies will depend upon identifying an adequate number of women with substantially increased risk of developing ovarian cancer.

## DETECTION OF EARLY STAGE OVARIAN CANCER

Ovarian cancer is detected in Stage I in less than 20% of patients using conventional pelvic examination. Transvaginal sonography (TVS) has been evaluated for its ability to detect ovarian cancer at an early stage in a larger fraction of patients. In collected series, TVS was used to screen 11,283 women who underwent 486 laparotomies to detect 13 Stage I cancers, only 5 of which were invasive [12]. Consequently, 37 operations were performed for each cancer detected

and a majority of the lesions were of borderline histology. Although TVS is quite sensitive, it is not adequately specific. In addition, the expense of screening every woman over the age of 40 every year by this modality would be substantial.

Detection of molecular markers in serum has been evaluated as an alternative strategy to identify a larger fraction of patients with early stage disease. CA-125 is elevated (> 35 U/mL) in only 50% of Stage I ovarian cancers [13]. Elevated CA-125 has been detected several years prior to diagnosis of ovarian cancer, both in anecdotal reports [14,15] and in a larger study performed with the Janus serum bank [16]. CA-125 can, however, be elevated by a number of benign gynecologic conditions, by hepatic disease, and by inflammation of serosal surfaces. Specificity can be improved by combining CA-125 and ultrasound or by monitoring CA-125 over time. One study in the United Kingdom used elevated CA-125 (> 30 U/mL) to trigger transabdominal sonography [17]. When the sonogram was abnormal, a laparotomy was performed. Among 22,000 women screened, CA-125 was elevated in 340, sonograms were abnormal in 40, and cancer was detected in 11. Not all cancers were of early stage and ovarian cancers were subsequently detected in 18 women who had had normal CA-125 levels. Another trial conducted in Stockholm screened 5,550 apparently healthy women [18]. Two percent of women > 50 years of age had elevated CA-125 (> 30 U/mL), suggesting that CA-125 could provide a sufficiently specific trigger for ultrasound to avoid a prohibitive number of sonograms if a larger trial were conducted to determine the impact of screening on survival. Six patients with ovarian cancer were detected, with four in Stage I or II. Three patients were subsequently found to have ovarian cancer despite normal CA-125 levels. Consequently, CA-125 is adequately specific to permit large scale screening trials in postmenopausal patients, but is not optimally sensitive.

Based upon a review of these studies, an NCI Consensus Panel felt that neither TVS nor CA-125 should be used to screen for ovarian cancer. A more effective strategy might utilize multiple serum markers to prompt TVS, taking advantage of the strengths of each approach. A combination of serum markers would, however, have to exhibit sensitivity approximating that of TVS. A number of molecular markers have been tested that might complement CA-125 to detect patients who would be missed with the single assay. A partial list includes carcinoembryonic antigen, CA19-9, CA15-3, TAG72, HMFG-2, placental alkaline phosphatase, tissue peptide antigen, lipid associated sialic acid, NB/70K, and urinary gonadotropin fragment.

In a retrospective study, one of three markers (CA-125, OVX-1, and M-CSF) was elevated in 98% of 46 patients with Stage I ovarian cancers [19]. The specificity of this panel was 89% in a postmenopausal population. At least in theory, if the serum markers were used to trigger TVS, nearly 90% of sonograms could be avoided without missing an ovarian cancer. In a case report, 11 months lead time has been observed in a patient with a Stage Ic tumor [20]. When stored sera were analyzed, one of the three markers was also elevated in 40% of the 18 patients who had been missed in the earlier United Kingdom study. Future studies will utilize CA-125 and OVX-1 to trigger TVS in a population over the age of 50 to determine whether screening impacts on survival. In the meantime, use of these modalities is not recommended for early detection of ovarian cancer in the general population.

## DISTINGUISHING BENIGN FROM MALIGNANT PELVIC MASSES

For a postmenopausal population, a strongly elevated CA-125 (> 95 U/mL) distinguishes benign from malignant disease with a positive predictive value of > 95%. When both premenopausal and postmenopausal patients are considered, more modest elevations of CA-125 (> 35 U/mL) distinguish malignant from benign pelvic masses with a sensitivity and specificity of 78% and 77%, respectively [21]. Greater specificity might be attained by studying the coincident elevation of additional markers. In past studies that have utilized multiple markers, increases in specificity have generally produced a coincident decrease in sensitivity. When eight serum markers were evaluated for their ability to distinguish 192 malignant from 273 benign pelvic masses, classification and regression tree (CART) analysis produced a sensitivity of 92% and specificity of 93% [21]. While prospective evaluation of additional data sets will be needed to confirm the utility of this approach, it appears that the use of

multiple markers and CART analysis might permit an increase in specificity without a concomitant decrease in sensitivity. If sensitivity and specificity in excess of 90% can be attained, values for multiple markers could aid in the cost-effective management of both premenopausal and postmenopausal patients.

Novel approaches using molecular techniques to identify new markers. Using 2-dimensional polyacrylamide gel electrophoresis, a 30 Kd protein with a pI of 6.1 has been detected in culture supernatants from ovarian cancer cells lines, but not from normal ovarian surface epithelium [22]. Several groups are using subtraction cloning of cDNA libraries and differential expression of genes from normal and malignant ovarian surface epithelium to isolate novel genes preferentially expressed by ovarian cancers.

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