National Cancer Institute Conference on Investigational Strategies for Detection and Intervention in Early Ovarian Cancer

Ovarian cancer is currently the leading cause of death from gynecological malignancy in the United States. In 1993, it is estimated that 21,000 new cases of ovarian cancer will be diagnosed in this country and that 13,000 women will die of the disease. Although the cure rate for stage I disease is nearly 90%, the majority of patients present with disease spread beyond the ovary. Despite aggressive surgical debulking and platinum-based chemotherapy, the 5-year survival rate for women with clinically advanced disease is only 15-20%. The magnitude of this problem is reflected in the recent SEER data which indicate that the 5-year survival of patients with ovarian cancer has increased only from 36% in 1975 to 39% in 1990. Clearly, advances in prevention and early detection are needed if mortality from this disease is to be significantly reduced in the near future.

Our current understanding of ovarian tumorigenesis is limited by the lack of a well-defined precursor lesion, our limited knowledge of the steps in tumor progression, and the relatively inaccessible location of the ovary within the peritoneal cavity. It has been hypothesized that ovarian cancer results from the entrapment of surface epithelium within the ovarian stroma following “incessant ovulation” and that malignant transformation then occurs under the influence of environmental and/or genetic factors. Although this theory seems plausible, a number of questions must be answered if we are to design more effective strategies for prevention and early detection of ovarian cancer.

Unlike colon and cervical cancer, a discrete precursor lesion has not been described for ovarian cancer. Benign cysts, borderline tumors, and low-grade malignancies may or may not represent transitional stages during transformation from benign epithelium to metastatic ovarian cancer. When multiple deposits of ovarian cancer are found throughout the peritoneal cavity, it has not been clear whether these represent metastases from an ovarian primary or multiple foci of independently transformed cells. Genes that contribute to familial ovarian cancer have not been well characterized. In sporadic disease, the profile of changes in growth factors, protooncogenes, and tumor suppressor genes has been only partially defined. Relatively little is known regarding interactions of ovarian epithelial cells with extracellular matrix and stromal cells. Whether existing serum markers and ultrasonographic techniques will permit earlier detection of ovarian cancer remains to be resolved. Optimal clinical management of early stage disease is still uncertain, and the mechanisms of drug resistance are poorly understood.

In an attempt to review our current knowledge of ovarian carcinogenesis and to develop future directions for research, the National Cancer Institute convened a Workshop on Investigational Strategies for Detection and Intervention in Early Ovarian Cancer April 12–15, 1992, in Annapolis, MD. This workshop, chaired by Drs. Holly Gallup and Robert Bast, brought together approximately 100 laboratory and clinical scientists together with 40 National Cancer Institute program staff and intramural scientists. The workshop was divided into six interrelated areas: (a) genetic events in ovarian carcinogenesis, (b) inheritance of risk, (c) early pathology and progression, (d) markers for detecting early ovarian cancer, (e) models for ovarian carcinogenesis, and (f) strategies for intervening in early ovarian cancer. The workshop format included brief state of the art presentations, followed by panel discussions and an open interchange among workshop participants.

Genetic Events in Ovarian Carcinogenesis

The first session of the workshop began with a review of the role of oncogenes and tumor suppressor genes in human malignancies (Berchuck, Duke University). Although >50 protooncogenes have been described to date, the role of only a few of these has been studied in ovarian cancer. Abnormal expression of the c-fms protooncogene and its ligand CSF-1 has been observed in the majority of ovarian cancers. Coexpression of the ligand and receptor may result in autocrine growth stimulation and increased invasiveness (Kacinski, Yale University). Approximately 30% of ovarian cancers exhibit amplification or overexpression of HER-2/neu that encodes a transmembrane tyrosine kinase which is closely related to the epidermal growth factor receptor. A similar fraction of ovarian cancers exhibit amplification of c-myc that encodes a nuclear transcription factor (Baker, University of Texas).

Expression of protooncogene products may be useful in estimating prognosis or in providing targets for novel therapy. Increasing serum values of CSF-1 can, for example, precede tumor recurrence, and elevated levels in ascites fluid predict poor survival (Chambers, Yale University). Antibodies to HER-2/neu inhibit proliferation of cells that overexpress HER-2/neu in vitro and inhibit growth of ovarian cancer heterotransplants in nude mice. A phase I clinical trial has been instituted using a murine monoclonal antibody reactive with the HER-2/neu oncogene to treat patients with refractory ovarian cancer (Karlan, UCLA). A single dose of antibody produced no apparent toxicity. The immunoglobulin could be detected at the site of recurrent tumor, suggesting that antibodies to oncogene products might have therapeutic value.

Loss of critical tumor suppressor genes may also play an integral role in early ovarian oncogenesis. Functional loss of tumor suppressor gene activity frequently occurs via loss or deletion of one allele associated with mutational inactivation of the remaining allele. In the case of the Rb and p53 genes, deletions have sometimes been large enough to be seen on karyotypic analysis. Although structural abnormalities involving chromosomes 1, 3, 5, 7, and 11 occur frequently in sporadic ovarian cancers, no specific breakpoints or deletions have been identified (Gallion, University of Kentucky). Loss of heterozygosity in marker genes that detect submicroscopic loss of chromosomal material has, however, revealed ovarian tumor-specific allelic deletions, both on the short arm of chromosome 17 and on the long arm of chromosome 17 distal to the BrCa1 region, suggesting the presence of closely linked tumor suppressor gene(s) on this chromosome (Gallion, University of Kentucky; Jacobs, Duke University; Duebau, University of Southern California). Losses on 6q and 17p were observed in borderline and grade 1 tumors, whereas 11p, 13q, and 17q losses were most commonly seen in high-grade, advanced stage tumors. This pattern of allelic loss suggests that chromosomes 6q and 17p contain tumor suppressor genes important in early ovarian tumorigenesis and that losses on 17q and other chromosomes may be later events. Alternatively, losses on 17q and elsewhere may lead inevitably to the formation of high-grade tumors without transition...
through borderline and low-grade intermediates. The finding of mutation and overexpression of the p53 tumor suppressor gene in 50% of advanced ovarian cancers compared to only 16% of stage IA tumors suggests that the p53 gene may be important in the development of metastatic potential (Berchuck, Duke University). To establish whether ovarian cancer is a clonal disease, allelic loss, p53 mutation, and X chromosome inactivation have been compared in ovarian primaries and in putative tumor metastases. In 15 of 17 cases, epithelial ovarian cancer proved to be a monoclonal rather than a multifocal disease (Jacobs, Duke University).

Inheritance of Risk

Hereditary ovarian cancer occurs in at least two identifiable syndromes: hereditary breast-ovarian cancer syndrome, which is linked to a 17q chromosomal locus, and the Lynch syndrome II, which is characterized primarily by the development at a young age of colorectal, endometrial, and ovarian carcinomas (Lynch, Creighton University). Careful analysis of families thought to develop only site-specific ovarian cancer has often detected cancers that arise from other primary sites. The average annual risk for ovarian cancer in the general population is approximately 10 per 100,000 at age 40 years and 60 per 100,000 at age 60 years, compared to 100 per 100,000 in Lynch syndrome II families and nearly 1,000 per 100,000 in hereditary breast-ovarian cancer families (Watson, Creighton University).

Between 1981 and 1991, 658 families were accessioned into the Gilda Radner Familial Ovarian Cancer Registry, including 1568 familial ovarian cancer cases (Piver, RPMI). The most common clusters observed were between mothers and daughters (49.5%), followed by clusters between sisters (38.5%). Analysis of these families indicated that the mean and median age at the time of diagnosis for mothers was significantly greater than that for their daughters. Interestingly, oral contraceptive use was more prevalent in index cases without ovarian cancer than in index cases with ovarian cancer. In the United Kingdom, a nationwide study of women with two or more close relatives with ovarian cancer has been initiated and guidelines drafted for screening and management of women at risk (Ponder, Cambridge University). Blood and other tissues have been collected for research. Genetic linkage studies to date have indicated that most families with multiple cases of breast and ovarian cancer were associated with a predisposing gene on the long arm of chromosome 17, referred to as BrCa1 (Ponder, Cambridge University). In the near future, DNA-based analysis of individual risk will be possible in these families.

Early Pathology and Progression

At least three models have been proposed to explain the epidemiology of ovarian cancer, including (a) incessant ovulation, (b) foreign body carcinomatosis, and (c) gonadotrophin stimulation with premature depletion of germ cells (Cramer, Harvard University). The first would increase production of inclusion cysts within the ovary, the second would lead to cortical granulomas, a potential source of growth factors, and the third could induce stromal hyperplasia that contributes to the differentiation and proliferation of cells which line inclusion cysts. Theoretically, ovarian cancer could arise either de novo or from precursor lesions such as endometriosis or benign neoplasms. A histological review of >90 ovarian tumors revealed areas of benign epithelium in 100% of low malignant potential “borderline” tumors and a zone of transition from benign to atypical epithelium in 79% (Powell, University of Kentucky). Moreover, areas of benign epithelium were found in the majority of invasive carcinomas, suggesting that in some instances malignant epithelial ovarian tumors may arise from preexisting benign neoplasms. Analysis of microscopically detectable early ovarian cancers, including three which were detected retrospectively only after tumor recurrence, suggests that extraovarian spread may occur from extremely small tumors resulting in advanced disease from inception (Scully, Harvard University).

In normal cells, peptide growth factors regulate proliferation or differentiation by binding to specific cell membrane receptors (Berek, UCLA). As in other epithelial malignancies, both stimulatory and inhibitory factors have been observed in ovarian cancers. A poor prognosis is associated with continued expression of the EGF receptor, overexpression of HER-2/neu (c-erbB-2), and the novel expression of fms. Concomitant expression of these growth factor receptors and their respective ligands may provide autocrine growth stimulation.

Most ovarian carcinoma cell lines appear to be relatively resistant to EGF in culture, whereas monolayer cultures of normal human epithelial cells are consistently stimulated by EGF (Berchuck, Duke University). Loss of responsiveness to EGF in ovarian carcinoma cells does not appear to result from differences in EGF receptor number or affinity. Moreover, simultaneous and concurrent expression in ovarian carcinomas of mRNAs encoding ligands and receptors of the EGF superfamly, including EGF, TGF-α, amphiregulin, EGF receptor, and c-erbB-2, supports the possible role of EGF-related genes in autocrine growth control (Stromberg, FDA).

In many epithelial systems, transforming growth factor-β inhibits proliferation. Normal ovarian epithelial cells express, activate, and respond to TGF-β with autocrine growth inhibition. Loss of autocrine growth inhibition has been observed in different epithelial ovarian carcinoma cell lines that failed to express, activate, or respond to TGF-β. In an ovarian cancer cell line in which the autocrine inhibitory loop appeared to be intact, neutralization of TGF-β with specific antibodies stimulated proliferation (Berchuck, Duke University). A TGF-β-like factor may be responsible for the immunosuppressive effect of malignant ascites (Hirte, Hamilton).

Growth factor receptors may provide novel targets for prevention and treatment. Murine monoclonal antibodies that bound to the HER-2/neu gene product (p185) also inhibited anchorage-dependent and -independent growth of ovarian cancer cell lines that overexpressed p185 (Bast, Duke University). This activity depended on the kinase activity of the receptor. Immunotoxins were also cytotoxic to HER-2/neu positive cells. However, inactive HER-2/neu (c-erbB-2), and the novel expres

1 The abbreviations used are: EGF, epidermal growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor; TVS, transvaginal sonography.
formation of metastases after i.p. injection in nude mice, suggesting that TNF may contribute to the i.p. spread of human ovarian cancer (Malik, Ontario). In the mouse, intermediate cells which represent a transition between benign and malignant cells are frequently TNF sensitive, whereas cancer cells are TNF resistant (Mutch, Washington University). Most human cancer cell lines are also resistant to TNF. The mechanism of resistance to TNF does not appear related to the overexpression of superoxide dismutase. Cytokines like TNF-α may play important roles in ovarian tumor growth, not only by acting as growth factors for tumor cells but also by affecting the host immune response. The observation that resistance to doxorubicin or cisplatin can be reversed by TNF in both multiple drug resistance positive and multiple drug resistance negative ovarian cancer cell lines suggests that combinations of immune and nonimmune modalities may be clinically useful in overcoming drug resistance (Bonavida, UCLA).

Markers for Detecting Early Ovarian Cancer

A panel of serum markers, including CA 125, MKS-15, M-CSF, and OVX1, has improved sensitivity for detecting ovarian cancer when compared to CA 125 alone (Bast, Duke University). Serum levels of M-CSF have also been shown to correlate with disease progression in ovarian cancer patients (Kacinski, Yale University). In addition, urinary gonadotrophin fragment, a secreted antigen, derived from the β chain of human chorionic gonadotropin, reflected tumor regression more accurately than did CA 125 in at least one clinical trial (Cole, Yale University). Preliminary reports of in vivo diagnostic imaging of ovarian tumors using technetium-99m-labeled monoclonal antibodies (HMFG1, SME) against polymorphic epithelial mucin also appeared promising (Epenetos, ICRF).

TVS provides an alternative approach for detecting early stage ovarian tumors. The transvaginal approach is particularly well suited for screening because it is safe, time efficient, and well tolerated by the patient. Among 1300 asymptomatic postmenopausal women screened with TVS (van Nagell, Kentucky), 27 ovarian tumors were detected, including two primary ovarian cancers. Both women with ovarian cancer had normal pelvic examination findings, a CA 125 of <35 units/ml, and stage I disease. With >2000 patients now screened for ovarian cancer in this series, the specificity of TVS was 98.1%, and the sensitivity was apparently 100%. Potential methods to increase the specificity of TVS for ovarian malignancies have included immunoblotting, morphology, and Doppler flow studies. Low pulsatility and resistance indices as measured by Doppler flow sonography reflected the presence of neovascularity that is characteristic of neoplastic growth. This new technology may increase the specificity of transvaginal sonography in the detection of ovarian malignancies (Karlan, UCLA). In another large screening trial, a series of 22,000 asymptomatic women older than 44 years of age were screened initially with CA 125 followed by transabdominal sonography if CA 125 values were elevated (Jacobs, Duke University). Of the 340 women with elevated serum CA 125, 41 had abnormal ultrasound and 11 cases of ovarian cancer were detected, 8 of which had spread beyond the ovary. The sensitivity of this approach was 78%, and the specificity was 99.9%. Eight ovarian cancers developed in 21,660 women with normal CA 125 levels. Because of the low prevalence of ovarian cancer in the general population, efforts should be made to define high-risk populations most likely to benefit from screening. In addition to women older than 50 years of age, women with a family history of the disease are at high risk for ovarian cancer and are an ideal population for screening studies (Schwartz, Yale University). A national trial to determine the efficacy of ovarian cancer screening should be undertaken.

Experimental Models for Ovarian Carcinogenesis

Ovarian cancer research has suffered from a lack of relevant animal models because most species develop ovarian cancer only rarely, and these malignancies differ from human tumors in their histology and probable site of origin (Auersperg, University of British Columbia). Domestic fowl may provide an ideal model system in that aged hens have a high incidence of ovarian epithelial adenocarcinomas. These tumors metastasize widely throughout the peritoneal cavity resulting in bowel obstruction and inaniation similar to that observed in human ovarian cancer patients (Fredrickson, National Cancer Institute). If, however, this model is to be widely used, methods need to be developed to induce these carcinomas in a shorter period of time, the cell of origin needs to be identified, and the role of sex hormones in their induction needs to be clarified. The rabbit ovarian surface mesothelium, which undergoes cyclic changes and forms papillae histologically similar to borderline tumors of the human ovary, provides another potential animal model for epithelial ovarian cancer (Nicosia, University of South Florida). In addition to animal model systems, methods to culture human fetal ovarian epithelial cells which appear to be derived from the rete ovarii have been described. These culture methods constitute an excellent model to address the relationship between ovarian granulosa cell tumors and epithelial ovarian cancers (Dubeau, University of Southern California).

Currently, cultured ovarian cancer cells are being studied to enhance our knowledge regarding ovarian tumorigenesis and differentiation. For instance, ovarian cancer cells in culture have been shown to secrete and respond to ovarian steroids and gonadotropins, suggesting the existence of a steroid-mediated autocrine loop in ovarian tumorigenesis (Wimalasena, University of Nebraska). Moreover, subpopulations of ovarian cancer cells can be induced to a more differentiated, less aggressive phenotype (Grunt, University of Vienna). The ability to induce differentiation has obvious therapeutic potential.

Our understanding of ovarian tumorigenesis, invasion, and metastasis has been further enhanced by analyzing the interrelationships between ovarian surface epithelium and the underlying ovarian stroma and extracellular matrix. Adjacent stroma may play an active role in the remodeling of the normal epithelial layer and in the formation of preneoplastic lesions (Auersperg, University of British Columbia). A tissue culture model in which benign and malignant ovarian neoplasms are grown on extracellular matrix proteins has been used to demonstrate the capacity of these neoplasms to modify their microenvironment. Borderline tumors exhibit distinctive abnormalities both in karyotype and in growth characteristics on extracellular matrix that may provide useful diagnostic and prognostic criteria (Crickard, SUNY Buffalo). Moreover, substances which interfere with the complex processes of invasion and metastasis may be clinically useful, and a phase I trial of CAI, a factor known to inhibit invasion, has already been instituted (Kohn, National Cancer Institute).

Intervention in Early Stage Disease

Patients with early stage ovarian cancer have an excellent 5-year survival. Less than 30% of women with cancer of the ovary, however, have disease confined to the ovaries or pelvis (Ozols, Fox Chase). Because of the low incidence of early stage disease as well as a failure to enter patients on clinical trials, information concerning adjuvant therapy from randomized trials is limited (Thigpen, University of Mississippi). Patients can be divided into those at low risk for recurrence (grade 1 or 2 disease, no tumor on the surface of the ovary, no ascites, negative peritoneal cytology, and no extravarian disease) and those at high risk (grade 3 disease or tumor on the surface of the ovary or ascites or positive peritoneal cytology or extravarian disease).
Patients at low risk for recurrence have an excellent 5-year disease-free survival with surgical resection alone and no adjuvant therapy. By contrast, high-risk patients have a relatively high (≥30%) likelihood of relapse and should receive adjuvant treatment. Current adjuvant therapy for patients with high-risk limited disease should consist of platinum-based chemotherapy.

Intraperitoneal chemotherapy appears to be most effective against microscopic disease and may have its greatest utility in the management of early stage ovarian cancer (Markman, Memorial Sloan-Kettering). Taxol should have a significant pharmacological advantage when administered i.p. Randomized trials comparing Taxol and cisplatin to cyclophosphamide and cisplatin have already been initiated in patients with advanced stage ovarian cancer. Treatment of patients with early stage ovarian cancer with an intraperitoneal Taxol-containing regimen should also be considered. Future trials of chemotherapy for early stage disease should consider prospective randomized comparisons of i.p. and systemic therapy.

In addition to chemotherapy, radiation therapy has been used in the management of early stage ovarian cancer. Recent randomized trials from Europe have demonstrated an advantage for chemotherapy (single-agent cisplatin) over the standard approach of i.p. radiotherapy with $^{32}$P chromatophosphate. An ongoing GOG trial compares i.p. $^{32}$P to a relatively aggressive combination of cisplatin plus cyclophosphamide. Whole abdominal radiation therapy can be delivered with acceptable toxicity to patients with ovarian cancer (Thomas, Toronto-Bayview). Reportedly, whole abdominal radiation therapy constitutes curative therapy in >70% of patients with “intermediate risk” disease, (FIGO, stage II). Radiation therapy can, however, be used only in patients with <2 cm of residual disease in the pelvis and no macroscopic disease in the upper abdomen.

Radiolabeled antibodies have been evaluated for external imaging and intraoperative radioimmunodetection of ovarian cancer (Rubin, Memorial Sloan-Kettering). Monoclonal antibodies coupled to isotopes, biological toxins, or drugs are being evaluated in the treatment of ovarian cancer and have important potential for (a) improving the diagnosis particularly of early stage disease, (b) monitoring response to therapy, and (c) treatment of ovarian cancer. Radiouclide-antibody conjugates have also shown promise in the management of microscopic residual or recurrent disease (Epenetos, ICRF).

Photodynamic therapy offers yet another novel approach to the management of minimal residual disease. In patients with platinum-refractory intraabdominal disease, cytoreductive surgery has been followed by photodynamic therapy using laser light to excite intracellular photoporphyrins. Disease-free intervals of >1 year have been observed in some patients (Reed, National Cancer Institute).

Drug resistance appears to be the primary factor that prevents cure of all stage I and II ovarian cancers with surgery and chemotherapy. Several factors have been associated with drug resistance, including (a) decreased accumulation of platinum compounds, (b) increased inactivation of alkylating agents by glutathione-S transferase or by direct interaction with glutathione, and (c) increased DNA repair of both intra- and interstrand lesions of DNA (Ozols, Fox Chase). In preclinical ovarian cancer models, lowering glutathione levels by exposure of tumor cells to buthionine sulfoximine as well as inhibition of the DNA repair process by DNA polymerase inhibitors leads to increased sensitivity of drug-resistant cells to alkylating agents and platinum compounds. Clinical trials of buthionine sulfoximine with melphalan are in progress, and studies combining aphidicolin with carboplatin will soon be initiated. In tumor specimens from advanced ovarian cancers, overexpression of TGF-α, EGF-R, myc, jun, metallothionein, and VEGF mRNA expression correlated with clinical outcome (Bauknecht, University of Freiburg). Tumors that responded to chemotherapy were usually those with an activated transcription unit. Strategies aimed at reversing drug resistance should benefit patients both with drug-refractory early stage ovarian and advanced ovarian cancer.

Conclusions

From the summary discussions, a number of research directions should be explored in ovarian cancer: (a) preclinical research is needed to define events in the initiation and early progression of ovarian cancer; (b) relevant model systems should be developed; (c) cost-effective approaches are required for the diagnosis of premalignant and early ovarian cancer, including the detection of women genetically predisposed to ovarian cancer, the development of prognostic factors to identify women at high risk for recurrence, and the improvement of screening strategies that include serum tumor markers, TVS, and radiographic techniques; and (d) prospective, randomized trials should be carried out to resolve clinical issues regarding the merits of standard chemotherapy, i.p. $^{32}$P, whole abdominal radiation therapy, and experimental alternatives in women with early disease who are at high risk for recurrence.

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