10th Biennial Helene Harris Memorial Trust Meeting

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Summary

Improving the prognosis of ovarian cancer patients is a major challenge to scientists and clinicians. At a recent multidisciplinary meeting in Washington DC, advances in identification of precursor lesions, progress in disease biomarkers and animal models, the promise of nanotechnology, and strategies for manipulation of the innate and adaptive immune response offered prospects for real progress in this difficult-to-treat disease.

Recommendations for Future Research

- Further investigation of ovarian inclusion cysts as the precursor lesion of ovarian cancer.
- Establish international collaborations of sufficient sample size, with carefully selected controls, to study low-penetrance genes that influence susceptibility to sporadic ovarian cancer.
- Obtain a "standard" set of bloods, obtained months or years before diagnosis of ovarian cancer, for collaborative studies of biomarker panels that could be used for early detection.
- Set up prospective studies to determine the accuracy of symptom profiles in early detection of ovarian cancer.
- In high-risk women, determine the role of hysterectomy and hormone replacement therapy after risk-reducing surgery.
- Focus chemoprevention research on protecting the ovaries and endometrium without increasing the risk to breast tissue.
- Investigate the maternally imprinted tumor suppressor gene *ARHI*, Rab25, and DNA repair mechanisms in BRCA1/2 defective cells as new therapeutic targets.
- Develop therapeutic approaches that inhibit the immunosuppressive microenvironment of ovarian cancer and elicit tumor-specific immunity.
- Study developmental pathways in *Drosophila* to obtain insights into the development and growth of ovarian cancer.
- Develop mouse models that mimic the signaling pathways in human ovarian cancers.

The aim of the 10th Biennial Helene Harris Memorial Trust Meeting, held last April 2005, was to provide a forum in which all aspects of ovarian cancer research were discussed in the spirit of translating basic research to improved clinical outcomes. Participants at the meeting were selected as international experts in ovarian cancer research and treatment. In addition, six fellowships with oral presentations were awarded competitively from over 40 applications (see Appendix 1 for all speakers, fellows, and the topics they discussed).

Epithelial ovarian cancer is the most lethal gynecological cancer of the western world; the incidence and overall survival has changed little in the past 30 years. Although most forms are amenable to surgery and sensitive to chemotherapy, relapsed disease kills the majority of patients within 5 years. A major reason for the severity of this malignancy is that early-stage disease is often undetected. At presentation it has generally spread throughout, and even beyond, the peritoneum. Improved understanding of the molecular genetics and biology of ovarian cancer will drive new strategies for prevention, early detection, and therapy.

Early Detection of Ovarian Cancer

One major advance in developing early detection strategies would be identification of precursor lesions. Data presented at the meeting strengthen the theory that invagination of the surface epithelium to form inclusion cysts within the ovarian stroma may create a tumor-promoting microenvironment. Epithelial cells in inclusion cysts from women with BRCA mutations have a higher relative rate of p53 mutation, increased proliferative index, and greater incidence of aneuploidy than corresponding cells of the surface epithelium. These abnormalities are also found with less frequency in ovaries from women without BRCA mutations. Similar cysts are present in incessantly ovulating mice although this stimulus is not sufficient to induce malignant change.

Disease-Specific Biomarkers

Gene expression array analysis has identified a number of potential biomarkers for early detection or disease monitoring; β_8-integrin was one marker that was mentioned in some detail. We also learned that DNA methylation changes in candidate ovarian cancer genes can be detected in serum of ovarian cancer patients. These may not be sensitive enough to detect precursor disease but may be useful for disease monitoring. It is probable that a biomarker panel, with novel markers as well as the currently used CA125, will be needed to detect early lesions with sufficient sensitivity and specificity. Novel proteomics technologies may be useful in finding additional markers but the validity of these techniques for disease detection is still under discussion. It was clear from the meeting that nanotechnology and nanotools have potential to revolutionize biomarker discovery, pathway analysis, and early detection of disease, especially using a systems biology approach. Systems biology aims to study behavior of complex biological organizations and processes in terms of molecular constituents. Serial test reservoirs for simultaneous measurement of DNA and serum samples using nanochips are already under development.

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There was much discussion on issues of study power, quality control, proper designation of "normal" controls, and use of the most carefully collected and annotated samples. "Preclinical" samples obtained months to years before diagnosis are needed and collaboration is required to ensure that the best candidates are evaluated together in a standard set of bloods.

Identification of Individuals at Risk of Ovarian Cancer

Identification of high-risk individuals is central to screening and prevention of ovarian cancer. In addition to the highly penetrant effect of BRCA mutations, other genetic variants may have an impact. Single nucleotide polymorphism population analysis may provide genetic clues to understanding low-penetrance genes that influence susceptibility to sporadic cancer. However, to avoid false-positives, DNA from a least 1,000 cases, with carefully matched controls, is required. Success may be enhanced by enriching the sample set with families in which there are two or more cases of "sporadic" cancer by haplotype/single nucleotide polymorphism tagging and by improving candidate gene selection. Large-scale international collaborative studies of single nucleotide polymorphisms in candidate genes are now under way using DNA from ovarian cancer patients and controls.

Timely Identification of the Signs and Symptoms of Ovarian Cancer

Symptoms of ovarian cancer may occur before clinical diagnosis in early as well as advanced disease. There is considerable overlap in symptoms between women with ovarian cancer and healthy women or women with benign conditions. A recent study showed that symptoms are more frequent and severe in women who have ovarian cancer. However, even the most discriminative combination of symptoms is absent in >50% patients and present in 8% of healthy women. Further prospective studies are needed to establish the accuracy of symptom profiles in diagnosis of ovarian cancer.

Screening and Surgery of Women at High Risk of Ovarian Cancer

Screening with CA125 and ultrasound is of uncertain efficacy and may fail to detect high-grade, poor prognostic types of epithelial ovarian cancer at an early stage. The effect of general screening will be determined in two large prospective randomized trials but this is not feasible/acceptable in high-risk populations. In the absence of clear evidence that screening can reduce mortality from ovarian cancer, the uncertainties about screening should be explained to women in the high-risk group and they should be offered the option of risk-reducing surgery. There is now convincing evidence that salpingo-oophorectomy in high-risk women who have completed their families greatly reduces or eliminates the risk of ovarian cancer and breast cancer. However, the incidence of primary peritoneal cancer is unaffected. A degree of caution is still required in view of the short duration of follow-up in these studies. The optimal timing for oophorectomy, the role of hysterectomy, and the place of hormone replacement therapy after risk-reducing surgery still need to be determined.

Preventing Ovarian Cancer

Childbirth, oral contraceptives, and menopause all decrease the relative risk of developing ovarian cancer. Chemoprevention strategies may be devised from understanding how these different stimuli affect ovarian function. During pregnancy, the ovaries are inactive and the ovarian surface is not undergoing the tearing and repairing of ovulation. Oral contraceptives mimic this by preventing follicle development and ovulation. However, the contraceptive pill is associated with a higher risk of breast cancer and does not decrease ovarian cancer risk as much as pregnancy. The goal should be to achieve as effective chemoprotection to the ovaries and endometrium as a pregnancy without increasing the risk to breast tissue. This could be accomplished, for instance, by using gonadotrophin-releasing hormone to stop ovulation and by protecting the endometrium by delivery of progestin via an intrauterine device. Another method could be to increase the levels of progestin in the pill to those found during pregnancy.

New Therapeutic Targets

Several promising new targets were presented at the meeting. A maternally imprinted tumor suppressor gene, *ARHI*, which encodes a small GTPase of the ras superfamily, is down-regulated in 60% ovarian cancers. Reexpression of ARHI reversibly decreases tumor cell growth *in vitro* and *in vivo*, inducing autophagy. *ARHI* gene expression can be induced by combinatorial inhibition of methylation providing a potential therapeutic approach to treating tumors with silenced ARHI. Two other targets that were discussed were RAB25 at chromosome 1q22 and protein kinase Cι at 3q26.2. These are prognostically significant in ovarian and breast cancer and exhibit enzymatic activity amenable to therapeutic intervention. Rab25 regulates vesicle recycling, altering sensitivity to apoptosis, and protein kinase Cι seems to be involved in the regulation of cell polarity and cell proliferation. Over the past 5 years, there has been considerable interest in PIK3CA as a therapeutic target. At the meeting, we learned that somatic activating point mutations of PIK3CA are found in ~7% of ovarian carcinomas, with a predominance of mutations occurring in tumors of endometrioid and clear cell histologies. In contrast, PIK3CA amplification is common in all histologic types and is mutually exclusive with point mutation.

A New Approach to Treatment

In the absence of reliable biomarkers for screening and early detection of the disease, a major emphasis must be on improved treatment. BRCA1 and BRCA2 mutations predispose to ovarian cancer and have significant roles in maintenance of genome integrity via homologous recombination. Rad51 foci are concentrations of the Rad51 protein that appear after certain kinds of DNA damage. They are presumptive sites of the repair of DNA breaks by homologous recombination. The formation of Rad51 foci on DNA damage provides a useful functional assay of BRCA repair pathways. Loss of these foci accompanies disruption of BRCA repair pathways. Targeting redundancy in DNA repair mechanisms may enhance the defect in homologous recombination in BRCA dysfunction. Poly(ADP-ribose) polymerase is an important enzyme involved in repair of DNA lesions by base excision repair. Recent experiments reported at the meeting have shown that poly(ADP-ribose) polymerase inhibitors are selectively and profoundly lethal to BRCA1/2 mutant cells independent of their p53 status. This promising strategy will now be tested in women with breast or ovarian cancer who carry BRCA1/2 mutations. Widespread clinical application will be dependent on the extent of BRCA pathway disruption in sporadic ovarian cancer.

The Innate and Adaptive Immune System in Ovarian Cancer

There has been significant progress in defining the roles of innate and specific immune system in ovarian cancer and a range of clinical trials are under way to test hypotheses. The consensus of the meeting was that combination approaches focused on inhibiting the immunosuppressive microenvironment and eliciting tumor-specific immunity are key to successful translation of laboratory studies.

The number of tumor infiltrating lymphocytes correlates with both overall survival and progression-free survival in ovarian cancer patients, and these monoclonal T cells have significant tumor-specific immunity. However, the inflammatory infiltrate of ovarian cancer also has immunosuppressive components. For instance, immunosuppressive plasmacytoid dendritic cells are attracted to, and functionally modified by, the chemokine CXCL12 in the ovarian cancer tumor microenvironment. CXCL12 is also involved in ovarian tumor cell spread and survival, with the chemokine receptor CXCR4 being expressed by ovarian cancer cells *in vivo* and *in vitro.* Another immunosuppressive cell population in ovarian cancer are the T regulatory cells that can inhibit the function of tumor specific effector cells. Patients with low levels of circulating T regulatory cells have improved overall survival. Strategies to deplete T regulatory cells are now in clinical trial and with some evidence of clinical efficacy.

Some therapeutic interventions are being developed to elicit immunity and circumvent the immunosuppressive microenvironment. These include an adenoviral vector expressing IFN-β and lentiviral transduction of CTL with scFv for the ovarian cancer biomarker mesothelin and T-cell receptor-ζ and 4-1BB signaling modules. In addition, some therapeutic strategies result in productive and tumor inhibitory inflammation. Depleting T regulatory cells, for instance, will modulate the immune suppressive environment to support immune enhancement. Eliciting antigen-specific $CD4^+$ T cells that home to tumors can result in "epitope spreading" or a broadening of the immune response. After such vaccination, immunity can persist for >5 years. However, selective immunologic pressure targeting a single antigen may result in the development of antigen negative variants. Because of high-throughput techniques for antigen discovery, it is now possible to design multiple antigen vaccines.

The ovarian cancer tumor microenvironment is rich in tumor-promoting inflammatory cytokines and chemokines. Targeting such mediators may have direct effects on tumor growth and progression and may also help restore specific immune responses to the tumor. Antagonists of the inflammatory cytokine tumor necrosis factor-α (TNF-α) have been used with success to combat inflammatory diseases such as rheumatoid arthritis and Crohn's disease and may disrupt the tumor-promoting cytokine cross-talk in ovarian cancer patients. In one particular trial, use of low-density cDNA arrays showed down-regulation of proinflammatory chemokine and cytokine mRNA in ascites cells from ovarian cancer patients after infusion of an anti-TNF-α antibody.

Animal Models of Ovarian Cancer

Improved animal models will aid in the development of new treatments for ovarian cancer. One promising new tool is a conditional and tissue-specific K-*ras* transgenic mouse that develops ovarian and peritoneal implants histologically indistinguishable from human endometriosis. A substantial proportion of ovarian carcinomas of endometrioid and clear cell histologies are believed to arise within endometriotic lesions. When the K-*ras* transgenic mice were crossed with mice harboring a conditional, tissue-specific *Pten* knockout, ovarian carcinomas of endometrioid histology developed. These models should prove invaluable in studying the early natural history of these gynecologic conditions and experimental therapeutics. However, the relative infrequency of K-*ras* mutations in human endometrioid ovarian cancer and the lack of information on the frequency of concurrent mutation of K-*ras* and *Pten* suggest that results from these models will require extensive confirmation in human disease.

Insights into human ovarian cancer may come from developmental pathways in *Drosophila*. Loss of expression of homeobox (*HOX*) genes in a segment or the inappropriate expression of another *HOX* gene in that location causes the segment to assume a different morphologic identity. Mammalian *HOX* genes *HOX-A9, HOX-A10*, and *HOX-A11* are differentially expressed in fallopian tube, uterus, and vagina of the mouse. Expression of different *HOX* genes in a poorly differentiated human ovarian cancer cell line produced serous, endometrioid, and mucinous differentiation. This observation could potentially resolve one of the long-standing questions in how a relatively undifferentiated ovarian surface epithelium can give rise to several differentiated tumor histotypes. Border cell migration in the *Drosophila* ovary is a normal developmental process that resembles metastasis in higher animals. Myosin VI is required for ovarian border cell migration and is also associated with high-grade epithelial ovarian cancer.

Another transgenic murine model for epithelial ovarian cancer has been developed by expressing the transforming region of SV40 under transcriptional control of the 5′ upstream regulatory sequences of the Mullerian inhibitory substance type II receptor (*MISIIR*) gene. Approximately 50% of transgene positive Tg*MISIIR-TAg* founder mice developed bilateral poorly differentiated ovarian cancers with occasional cysts and papillary structures at the ovarian surface. These tumors disseminated i.p., invaded omentum, and were frequently accompanied by bloody ascites. The cancers expressed cytokeratins 8 and 19, but not α-inhibin, consistent with an epithelial origin.

There is still debate about the relevance of animal models to clinical trials in patients with epithelial ovarian cancer. Even primary cancers in transgenic mice may not reflect the heterogeneity that is characteristic of human ovarian cancer and, in particular, may not mimic the complex interaction of multiple genetic events. It would be interesting to induce incessant ovulation in mice manipulated to express the common genetic lesions of human ovarian cancer. Mouse models that mimic the signaling pathways in human ovarian cancers will also be particularly useful. Many inhibitors of signal transduction may require use in combination with other drugs. Testing all combinations in clinical trials may not be feasible with current trial designs and a limited number of trial participants. Human ovarian cancer cell lines, xenografts, or relevant transgenic murine models might all be used to identify synergistic interactions between drugs.

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