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TITLE: Pathogenesis of Ovarian Serous Carcinoma as the Basis
        for Immunologic Directed Diagnosis and Treatment

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13. ABSTRACT (Maximum 200 Words)

The etiology/pathogenesis of serous carcinoma, the most common type of ovarian cancer, is poorly understood. Until this is clarified the development of new diagnostic tests and therapy will remain empiric. The overall objective of our program is to identify biomarkers that define the molecular pathogenesis of serous carcinoma, and thereby develop an early detection test and antigen-specific immunotherapy for this deadly disease. During the first year of funding, significant progress has been made and the majority of specified tasks have been accomplished. This has led to five publications in high impact journals and the generation of important new research resources, including tissue and blood specimens, tissue microarrays, ovarian cancer cell lines, antibodies to novel ovarian cancer markers, and novel naked DNA vaccine constructs. In order to gain access to a wider variety of specimens including those that are not available in our tissue bank we have established a close collaboration with Dr Susanne Kruger Kjaer who is the principal investigator on the Danish population-based Malignant Ovary (MALOVA) study. This will improve the power of our studies . Continued support of this program will accelerate advances in diagnosis and treatment, leading an improved outcome for ovarian cancer patients.

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INTRODUCTION

Ovarian cancer is the most lethal gynecologic malignancy. Approximately, 23,000 women are diagnosed with ovarian cancer each year in the United States and 14,000 women die of this disease annually. Although new chemotherapeutic agents have prolonged survival, the overall mortality of ovarian cancer has remained unchanged. This is because conventional therapy is only curative for early stage disease, and there is currently no effective screen to detect serous carcinoma at this stage. Therefore the overall objective of our program is to identify biomarkers that define the molecular pathogenesis of serous carcinoma, and thereby develop an early detection test and antigen-specific immunotherapy for this deadly disease. We hypothesize that there are biomarker molecules uniquely expressed by serous carcinoma and linked to pathogenesis. Further we hypothesize that such molecules are immunogenic in patients with serous carcinoma and serve as important candidates for development of both an early detection blood test and for antigen-specific cancer immunotherapy. Progress towards a test and cure for ovarian cancer has been hampered by our lack of understanding of its causes. Understanding the basic biology of ovarian cancer will move research beyond trial and error to rational design of new diagnostic tests and therapies. Although ovarian cancer is often viewed as a single disease it is considerably more complex and represents a family of related but distinct tumors. However, serous carcinoma is responsible for the large majority of "ovarian cancer" related deaths and therefore is the focus of our program. At present, serous carcinoma is considered by most investigators to be a single entity. However we believe that 'serous carcinoma' comprises at least two distinctive types of tumors: the conventional type of serous carcinoma (CSC) grows rapidly and kills patients within 5 years despite aggressive treatment and the second type designated "micropapillary serous carcinoma (MPSC)"is low grade and indolent but fails to respond to conventional chemotherapy . Based on our files and a population based study, MPSC represent 35% of all serous carcinomas. Understanding the molecular basis that distinguishes CSC and MPSC is important to rational development of early diagnostic tests and effective, specific therapy.

In order to accomplish our objective we propose three complementary research projects, a Tissue and Blood Bank to provide specimens for study, and an Administration/Statistics Core to co-ordinate data analysis and promote synergy. In Project 1 Dr Shih will identify the specific genetic changes and genes that are expressed by the two different types of tumors using powerful molecular biologic techniques (digital PCR, SAGE and oligonucleotide-based microarray analysis) to provide biomarkers of disease pathways. The development of ovarian cancer is associated with the mutation and aberrant production of proteins that are recognized by the immune system as "foreign" and therefore result in the production of antibodies against their cancer. Molecules recognized by these antibodies are called tumor antigens and in Project 2,Dr Roden proposes to define their genetic sequence using a serologic analysis of recombinant tumor cDNA expression libraries (SEREX). Tumor-specific proteins relevant as biomarkers will be validated by immunohistochemistry in Projects 1 and 2. We have demonstrated that patients with serous carcinoma, but not healthy women make antibodies that are specific for particular tumor antigens and readily detectable using a simple blood test called enzyme-linked immunosorbant assay (ELISA). Having identified tumor antigens of early stage serous carcinoma, Dr Roden proposes exploration of the potential of the tumor antigen specific antibody for diagnosis of early stage serous carcinoma. A natural extension of this study, and Project 1 is to exploit advances in antigen presentation and immunotherapy to develop cancer specific vaccines that target tumor antigens and biomarkers uniquely expressed by cancer cells. In Project 3, Dr Wu proposes to develop specific vaccines first using a model ovarian cancer antigen, mesothelin, that is found in over 90% of serous carcinomas. Dr Wu has developed novel strategies to generate antigen-specific immune responses that are therapeutic in model tumor systems, and will be tested using the unique mesothelin-expressing mouse peritoneal/ovarian tumor model developed in our laboratory that resembles human ovarian serous carcinoma. In summary, this is an integrated multidisciplinary Project in which Projects 1 and 2 use molecular biologic and immunologic approaches to define biomarkers of serous carcinoma that are critical as probes for the etiology/pathogenesis of ovarian cancer, fundamental to the development of a blood test for early stage disease and represent targets for cancer vaccines generated using the novel strategies of Project 3.

BODY

There are no substantial changes or modifications of the original statements. The accomplishments associated with each task outlined in the approved statement of work are detailed below, point by point. Note that the Statement of work for the overall program was contained in Core A.

ADMINISTRATION/STATISTICS CORE (CORE A)

Task 1 Leading each of the projects and Core B in order to coordinate their activities and to keep the focus of the overall project on track, Months 1-36

The research progress for each project is fully described in each individual progress report. In brief,

Project 1: The purpose of project 1 is to elucidate the pathogenesis of serous carcinoma by identifying the molecular genetic changes and preferentially expressed genes of different histological types of serous neoplasms. We hypothesize that the development of serous carcinoma proceeds along two main pathways: one is rapid progression from ovarian surface epithelium to high-grade serous carcinoma without well-established morphological precursors ("de novo" pathway) and the other is a gradual development from borderline tumors, to non-invasive micropapillary serous carcinomas then to low-grade carcinomas (stepwise pathway). The first pathway results in a high-grade neoplasm (conventional serous carcinoma) and the second leads to the development of a low-grade indolent tumor. Both types of carcinomas and the putative precursor lesions of invasive MPSC are characterized by distinctive molecular genetic alterations and specific gene expression. We found that mutations in *KRAS* and *BRAF* genes characterize the development of low-grade serous carcinomas. Expression of HLA-G and high levels of chromosomal instability are confined to high-grade serous carcinomas. This project, designed to test our proposed model of diverse pathways in the pathogenesis of ovarian serous carcinoma, provides the basis for the other two projects.

Project 2: The goal of this project is to develop an early detection screening test for serous carcinoma using cDNAs of autologous tumor antigens recognized by sera of patients with early stage serous carcinoma, but not controls. While our ovarian cancer cDNA library is construction for SEREX analysis, we have developed an alternative, complementary methodology. We employed patient sera to immunoprecipitate proteins from autologous cancer cells and identified these antigens by mass spectrometry. One antigen, SMAP-1, has homology to genes that facilitate cell division. Another goal of this project is to identify autologous tumor antigens expressed in serous carcinoma but absent from, or a low level in normal tissue. We have expressed recombinant SMAP-1 protein and generated specific antisera. The SMAP-1 mRNA is ubiquitous in all tissues tested thus far, and immunohistochemical studies are currently underway. We have already identified several other SEREX antigens, including the homeobox HOXB7 transcription factor and have generated peptide antiserum to HOXB7. Using this antibody we have over-expression of HOXB7 in cancer cell lines by confirmed HOXB7 over expression in cancer cell lines. Interestingly, we also note a shift from a nuclear localization in normal tissue to the cytoplasm in most carcinomas. The significance of this phenomenon is under investigation.

Project 3: The purpose of this study is to test whether the greater extent of intracellular spreading of encoded antigen will generate a higher degree of antigen-specific immunity and anti-tumor effects in vaccinated mice. We previously showed that a DNA vaccine, HVP22 linked to a model antigen, E7 generates strong CD8 specific T cell response and anti-tumor effects. We generated DNA vaccines encoding BVP22 and MDV-1 VP22 linked with E7, respectively and demonstrated that compared with mice vaccinated with DNA encoding wild-type E7, mice vaccinated with BVP22/E7 and MDV-1 VP22/E7 DNA exhibited a significant increase in number of E7-specific $CD8^+$-T-cell precursors as well as stronger anti-tumor effects. Furthermore, our data indicated that the anti-tumor effect was CD8 dependent. These results suggest that the development of vaccines encoding VP22 fused to a target antigen might be a promising strategy for

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improving DNA vaccine potency. In addition, we have generated the pcDNA3-HVP22, pcDNA3-BVP22, pcDNA3-MVP22, pcDNA3-mesothelin, pcDNA3-HVP22/mesothelin, pcDNA3-BVP22/mesothelin and pcDNA3-MVP22/mesothelin for the control of ovarian cancer in task 1 of statement of work from months 0 to 9.

Core A has been responsible for overall direction of this program and for facilitating interactions between the investigators. In order to supplement frozen tissue and serum samples from our tissue and blood bank that are available for each project, we have initiated a collaboration (and sub-contract) with Dr Susanne Krüger Kjær of the Danish Cancer Society, Copenhagen. This will improve the power of our studies and provide access to a wider variety of specimens. Dr Kjaer is a principle investigator on the MALOVA study, for which enrollment is complete. In 1994 Dr Susanne Krüger Kjær initiated this multidisciplinary study which covers epidemiology (life style factors), biochemistry (tetranectin, CA-125, OVX-1, gonadotropins, sex-steroids, inhibin), immunohistochemistry and molecular biology (*p53, ras*, LOH). Women (35-79 years) who are diagnosed with ovarian tumors over a 3 year period were included as potential cases from most gynecological departments in Denmark. About 700 patients with ovarian cancer or borderline tumors as well as 200 patients with benign ovarian tumors were enrolled in the study. The control group consists of a random sample of 1500 age-matched women drawn from the central population registry. A personal interview to collect information on suspected risk factors for ovarian cancer, and blood samples were obtained from all participants. In addition, fresh frozen ovarian cancer tissue was collected on each case. This study was approved by Danish Scientific Ethical authorities and informed consent was obtained from each patient. The collaborative study was also approved by our local IRB.

Task 2 Organize regular meetings of investigators to promote cross fertilization of ideas between projects and an annual review process with the Advisory Panel, Months 1-36
We have a weekly meeting to discuss issues relating to Gynecologic Pathology, and monthly presentations relating to ovarian and cervical cancer. (See Task 1 above.)

Task 3 Over see the budgets of all the projects, Months 1-36
Spending for this fiscal year has followed our original proposal except for the inclusion of a new, DoD-approved subcontract.

Task 4 Prepare required progress reports, Months 1-36
This document represents the first annual progress report for this award.

Task 5 Provide administrative support for all the projects and Core B, Months 1-36
Donna Sevigny has supported this research program by assisting with meeting organization, filing, copying and preparing the progress report.

Task 6 Provide statistical support for the three research projects, Months 1-36
This core has provided statistical support for the study design and analysis as detailed in the attached publications.

Task 7 Maintain a liaison with our patient advocate involved with our ovarian cancer web site by providing regular updates on research findings, Months 1-36
We have continued to build up the information available on our website, including a recent feature on radiology for ovarian cancer patients. We are conducting an awareness campaign together with a patient advocate, Sean Patrick, and have published several enewsletters describing our research findings. Information on this is available at http://ovariancancer.jhmi.edu

CORE B: TISSUE AND BLOOD BANK

Task 1: To assemble a comprehensive collection of tumor and normal tissues together with matching blood samples and clinical histories from healthy patients and those with ovarian tumors.
(months 1-36) Specimens from approximately 500 patients.

The tissue bank has enrolled 80 patients with gynecological malignancies in the tissue acquisition study J9883. Fifty ml of blood was obtained from 65 of these patients and tissue specimens were obtained on all 80. We also obtained tissue only from an additional 81 patients.

Task 2: To assemble a collection of viable specimens, including primary cell cultures and PBMC.
(months 1-36) Viable PBMC from approximately 500 patients, and 50 primary ovarian cancer cultures.

The tissue bank has banked viable freezes from 65 patient blood specimens. Primary cultures of gynecologic specimens were generated and viable samples frozen for 18 ovarian carcinomas.

Task 3: To integrate the data generated by the research projects with clinical histories of the patients and available specimens into the Filemaker Pro relational database.
(months 1-36) Clinical and research data from approximately 500 patients.

Pathology reports have been obtained for all 161 patients and input into the database of specimens.

KEY RESEARCH ACCOMPLISHMENTS

- Addition of MALOVA study samples to our studies to improve their power and gain access to a wider variety of ovarian tumors.
- Banking of specimens from 181 gynecologic oncology patients.
- Co-ordination and management of this research program resulting in 5 high quality publications, and one more that has been submitted for publication.

REPORTABLE OUTCOMES

Articles published:
Singer G, Oldt 3rd R, Cohen Y, Wang B, Sidransky D, Kurman RJ, Shih IM. Mutations in BRAF and KRAS Ras characterize the development of low-grade ovarian serous carcinoma. J Natl Can Inst, 95:484-486, 2003.

Singer G, Kurman RJ, Chang H-W, Cho SKR, Shih IM. Diverse tumorigenic pathways in ovarian serous carcinoma. Am J Pathol, 160:1223-1228, 2002.

Singer G, Shih IM, Truskinovsky A, Umudum H, Kurman RJ. Mutational analysis of K-ras segregates ovarian serous carcinomas into two types: Invasive MPSC (a low-grade tumor) and conventional serous carcinoma (a high-grade tumor). Int J Gynecol Pathol, 22:37-41, 2003.

Singer G, Rebmann V, Chen Y-C, Cheng C-C, Liu H-T, Ali SZ, Reinsberg J, McMaster MT, Pfeiffer K, Chan DW, Wardelmann E, Grosse-Wilde H, Kurman RJ, Shih I-M. HLA-G is a potential tumor marker in malignant effusion. Clin Cancer Res, Oct 1 issue, 2003.

Hung CF, He L, Juang J, Lin TJ, Ling M, Wu TC. Improving DNA vaccine potency by linking Marek's disease virus type 1 VP22 to an antigen. J Virol. 2002 Mar;76(6):2676-82.

Article submitted for review:
Shih I-M, Kurman RJ. Ovarian tumorigenesis- a proposed model based on morphologic and molecular genetic analysis. Submitted.

Research resource:
Specimens from 181 gynecologic oncology patients in the tissue and blood bank.

CONCLUSIONS

Ovarian epithelial tumors are the most common type of ovarian cancer and are the most lethal gynecologic malignancy. Based on clinicopathological and molecular observations, we have provided evidence supporting a new model for their development. In this model, ovarian serous tumors are divided into two types designated low-grade and high-grade carcinomas which correspond to two main pathways of tumorigenesis. Low-grade neoplasms arise in a stepwise fashion from borderline tumors whereas high-grade tumors arise from ovarian surface epithelium or inclusion cysts for which morphologically recognizable precursor lesions have not been identified, so-called "de novo" development.

In project 1, we show that low-grade tumors are associated with distinct molecular changes that are rarely found in high-grade tumors, specifically *BRAF* and *KRAS* mutations. There are very limited data on the molecular alterations associated with the high-grade tumors and we continue to explore the molecular alterations that contribute to the development of high-grade tumors.
This model of carcinogenesis in ovarian serous tumors reconciles the relationship of borderline tumors to invasive carcinoma and provides a morphologic and molecular framework for studies aimed at elucidating the pathogenesis of ovarian cancer. Identification and characterization of the panoply of molecular changes associated with ovarian carcinogenesis will facilitate development of diagnostic tests for early detection of ovarian cancer and for the development of novel therapies aimed at blocking key growth-signaling pathways.

In project 2, we have developed a methodology for the identification of ovarian cancer-associated antigens that is complementary to SEREX. While we are generating the cDNA expression library for SEREX screening, we have used this alternate immunoprecipitation and mass spectrometry-based approach to identify two new ovarian cancer associated antigens. Almost nothing is known about the function of either antigen. For one of these new antigens, SMAP-1, we have performed a survey of transcript expression and have developed a specific antibody reagent. Furthermore, we have built upon our previous work in which HOXB7 was identified by SEREX as an ovarian cancer antigen. We have demonstrated over expression of the HOXB7 protein in ovarian cancer, as compared to normal surface epithelium. This immunohistochemical data supports our previous analysis of HOXB7 transcript expression. We also observed a mislocalization of HOXB7 in ovarian cancer. The significance of this exciting finding is under investigation.

In project 3, we have demonstrated that intercellular molecules of HVP22, BVP22, and MDV-1 VP22 linked to a model antigen E7 are able to induce strong immune response and anti-tumor effects. These findings suggest that the intercellular spreading strategy is a powerful tool to manipulate the immune system and generate strong anti-tumor effects. This strategy is potentially used for other antigens such as ovarian cancer antigen, mesothelin.

REFERENCES
None

APPENDICES
None