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Early Events in Ovarian Cancer Pathogenesis

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14. ABSTRACT A clear understanding of the molecular and biological events that occur early in the development of ovarian cancer would provide a basis for rational treatment and prevention. We have assembled a group of highly experienced investigators to systematically address these questions. During the funding period, we have developed the full Consortium grant according to the following three areas: 1) Molecular signatures of early tubal carcinogenesis. This project, led by Dr. Chris Crum, will validate the precursor sequence and identify markers of early malignancy based on expression or epigenetic profiles. 2) Identification of early molecular events in the pathogenesis of BRCA1 and BRCA2 associated ovarian cancer. This project, led by Dr. Karen Lu, will define the early molecular events in pathogenesis of BRCA1 and BRCA2 associated ovarian cancer and develop <i>in vivo</i> molecular imaging techniques for early detection. 3) Functional characterization of genes involved in fallopian tube cancer initiation, progression, and metastasis. This project, led by Dr. Sandra Orsulic, focuses on <i>in vitro</i> and <i>in vivo</i> functional characterization of genes that are suspected to play a role in tubal cancer. The proposed work described above is supported by the following Cores: 1) Administrative Core; 2) Pathology/Genomics Core; and 3) Biostatistics Core.				
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INTRODUCTION: This Consortium consists of a multi-disciplinary team of investigators from 12 major U.S. institutions, each with longstanding interest and existing infrastructure in ovarian cancer research. This synergistic team demonstrated their potential through the development phase to *address key questions related to the earliest molecular changes associated with* ovarian cancer precursor lesions, their transition to malignancy and the steps leading to early dissemination of ovarian cancer. The overarching goals of this multi-institutional consortium proposal are to: 1) reduce ovarian cancer deaths by identifying molecular markers and signatures for the precursor ovarian cancer pathway(s); 2) develop early detection methods by discovery, identification and validation of molecular markers; 3) develop marker-based non-invasive *in vivo* imaging methods, and; 4) build transgenic animal models to develop therapeutic solutions for ovarian cancer.

BODY: During the development grant, the Consortium has executed an exceptionally integrated effort to set up infrastructure (administrative, personnel, communications, data, technology and quality control) to distill innovative ideas into projects that impart the highest confidence to achieve the overarching goals. The Consortium has already gone beyond the developmental grant and generated preliminary scientific data that established strictest quality controls for the samples, validated next generation technologies, analytical methods, and generated epigenetic, gene, and protein expression and variation profiles; established cell line models; developed mouse models; and built the infrastructure for requisite databases and web-portals. To achieve the main goals of the Consortium, we solicited ideas for full projects from all Consortium members. From the 13 submissions received, the Executive Committee selected the 3 proposals that best addressed the challenges of early ovarian cancer to develop further. The critical questions included: ***What are the early precursor lesions for ovarian cancer? What is the role of fallopian tube lesions in development of ovarian carcinoma? What are the early molecular events in the development of ovarian cancer and what are the progressive events that lead to the spread of disease?***

The three highly synergistic projects proposed here are hypothesis-driven and are based on collaborations between Consortium members. Each of the Projects and Cores is led by acknowledged leaders in ovarian cancer research and involves investigators from multiple Consortium institutions that include prestigious cancer centers, hospitals, universities, and state and federal research centers across the U.S. One of the synergistic outcomes of the Consortium is the observation that *fallopian tube harbors a precursor pathway to high grade ovarian cancer*. To develop this observation further as the key question for the Consortium to follow, tissue banks from across the U.S. were queried and allowed us to identify relevant samples for the proposed project. In addition, our synergistic efforts have led to the validation of genomic and proteomic technologies, development of data sharing methodologies, and joint publications. This Consortium proposal focuses on *reducing the risk of death by ovarian cancer by identification of molecular markers and signatures for the precursor ovarian cancer pathway and development of early detection methods such as non-invasive imaging using molecular markers and transgenic mouse models*.

Administratively, the Consortium, under the leadership of Anil Sood (PI and the Consortium Director), and Michael J. Birrer (Co-PI and Core Leader) has hired a Grant Program Manager, administrative staff, coordinated and built communication web-portals, data repository and

sharing portals, identified regulatory protocols and procedures, developed standard operating procedures for all activities from administration to research, oversaw quality control measurements and reporting, established the Executive Committee, Advisory Committee, and developed an Administrative plan that encompasses tasks from coordination and execution of bi-weekly, and annual meetings, reporting of the data generated, conflict resolution, intellectual property protection, milestone evaluation, success measurement, contingency plans for resolving bottlenecks associated with data and any administrative issues. *With this level of preparation and fruitful development of the Consortium, we bring strong synergy to implement and execute the proposed objectives.*

KEY RESEARCH ACCOMPLISHMENTS:

The Consortium of research sites presented here was established based on the awarded DOD developmental grant. Over the period of the award, we have set up administrative, research, and communication channels and have scheduled regular bi-weekly conference calls to fully develop the Consortium, and solicit projects for full development. The milestones we have achieved are listed below (Fig. 1).

Synergy and strength of the Consortium. The Consortium includes 12 research sites spanning the entirety of the United States and brings together experts in the field of ovarian cancer research with an emphasis on innovative methods for ovarian cancer risk prediction, early diagnosis and therapy. The multiple strengths of our Consortium include: the reputation and infrastructure available at each of the institutions to provide access to sample banks reduces potential for biases inherent at a single site; technological expertise of next generation technologies and biostatistics to support such a Consortium; a multi-pronged and multifaceted approach to understanding ovarian cancer through a targeted approach of focusing on a novel precursor pathway in fallopian tubes, overall expertise of each of the researchers, and the collaborative history of the PI with the Consortium members.

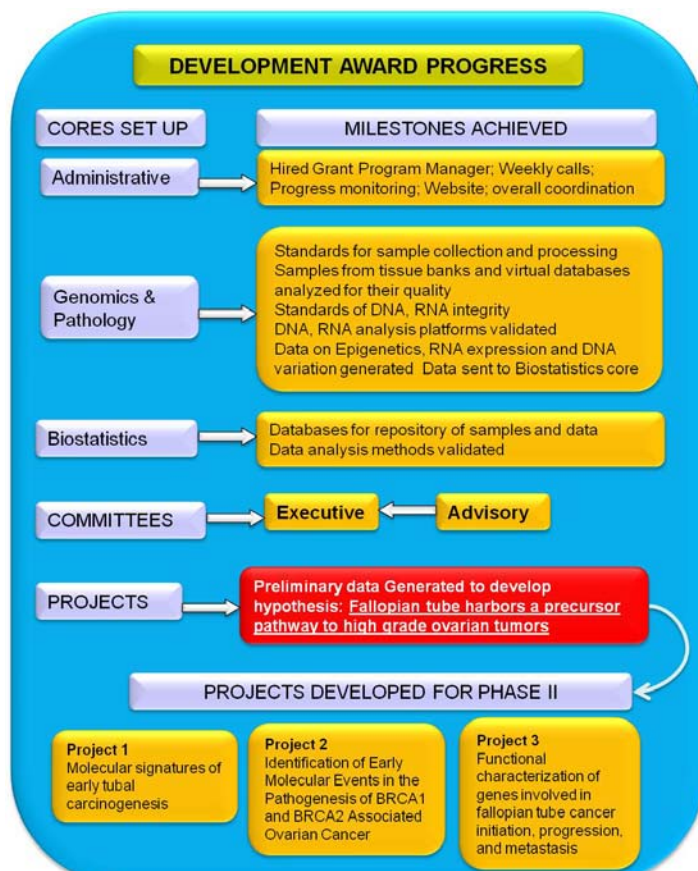


Fig. 1. Development grant progress.

MILESTONES

•**Administrative Core.** The Administrative Core has been established and has been used during the developmental project period effectively for setting up a web-based communication portal to oversee all aspects of the Consortium. The infrastructure to communicate, exchange data, and develop the phase II proposal has been accomplished. Program personnel including a Grant Program Manager has been hired to run the Administrative Core. Currently, through the Administrative core, we conduct the bi-weekly meetings, annual meetings, and exchange data to integrate all of the research sites.

This Core also worked with investigators to generate the final proposals for the Consortium, and oversee administrative duties including regulatory procedures to ensure compliance with the individual research site review boards for animal and human subjects protection, and other regulatory requirements for all sites.

- Pathology/Genomics Core:** A Pathology/Genomics Core has been established and is fully operational. Currently, we are using this Core to check the uniformity in case review across the members; for applying an integrated biomarker and histopathologic classification system for discriminating normal and precursor lesions in the fallopian tube; for establishing a collaborative FFPE tissue bank from the pathology review of Project 1 (Aim 1), from which a sufficient number of validated tissue targets can be obtained for Projects 1 and 2. This Core will provide de-identified and consented frozen tissues for validation studies in Project 1 (Aim 3). We have generated preliminary data, critical to execute proposed work, for gene expression, DNA methylation analysis of signatures of clinicopathologic importance from microdissected serous ovarian cancers, matched fallopian tube lesions and normal tubal epithelium using whole genome expression profiling; and characterizing the molecular origin of ovarian cancer cells as possibly arising from fallopian tube lesions. The flow of data from Pathology/Genomics Core to Biostatistics Core has been established to test the analytical methods. Embedded within this process is a quality assurance evaluation to ensure the highest quality pathology specimens and nucleic acids derived from them. All molecular platforms have been tested and preliminary data obtained to attest to the robust nature of the technologies.
- Biostatistics Core:** The Biostatistics Core has tested statistical methods for various platforms (e.g., Infinium Illumina Human Methylation27 BeadChip data in Project 1) for quality control. Unsupervised analytical methods have been developed and tested using singular value decomposition (SVD), adapted to the methylation data, to determine the number of significant components of variation and their association with phenotypes. This Core will provide comprehensive support that integrates the biostatistical activities and data management for the preclinical and clinical studies conducted at the various Consortium sites.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

SCIENTIFIC MILESTONES ACHIEVED THAT LED TO PROJECT DEVELOPMENT:

Selection of full projects: To achieve the main goal of the development of the Consortium, we solicited ideas for full projects from all Consortium members. From the 13 submissions received, the Executive Committee selected the 3 proposals that best addressed the challenges of early ovarian cancer to develop further. The selection process for the projects involved several levels:
a) The critical research questions concerning early development and spread of ovarian cancer were identified based on the following preliminary data generated:

The preliminary data generated by the Consortium members helped us address two important and fundamental issues: 1) identification of genes involved in the early pathogenesis of tubal precursor development, and 2) the cell types involved in this process. Using microarray technologies, we have identified gene signatures of clinicopathologic importance from microdissected serous ovarian cancers, matched fallopian tubal lesions and normal tubal epithelium using whole genome expression profiling. This goes well beyond what we initially proposed in the development plan, but paves a path to explore the precursor pathway for ovarian

cancer. Other preliminary data obtained here include DNA methylation data and the characterization of the molecular origin of ovarian cancer cells as possibly arising from fallopian tube lesions. For the animal studies, several lines of data have been established and a parabiosis model using either nude or immunocompetent mice has been established. *Ex vivo* models of cancer cell attachment to omentum and mesothelial cells have been established. Endothelial and ovarian cancer cells from human ovarian cancer and omental samples have been isolated and profiled to identify candidate genes involved in the early steps in the metastasis process. We have generated triple transgenic mice with Cre-mediated conditional inactivation of p53 and BRCA1 in the female reproductive tract (p53lox/lox; Brca1lox/lox; Amhr2-Cre mice). Cell line models were established to define key genes that can cooperate with loss of p53 and BRCA genes to develop high grade serous carcinoma from normal fallopian tubal epithelial cells. We have also established the important scientific modes of analysis involving expression profiling, copy number variation (CNV), and methylation.

b) The proposed hypotheses were then juxtaposed with available specimen resources, potential technology platforms and important clinical paradigms.

c) Scientific leaders with expertise in these technologies and/or experience in these scientific areas were identified and approached for possible participation in the Consortium.

The critical questions involved in the early events in ovarian cancer included:

- ***What are the early precursor lesions for ovarian cancer?***
- ***What is the role of fallopian tube lesions in development of ovarian carcinoma?***
- ***What are the early molecular events in the development of ovarian cancer and what are the progressive events that lead to the spread of disease?***

Plan for sharing authorship of published data: The authorship of major projects will reflect major contributions including development of the hypothesis, supplying critical specimens, genomic and biological assessment, statistical analyses, and writing the manuscript. All manuscripts will be reviewed by all Consortium members. The data and resources generated will be shared as follows:

1. The Project data will be made available encrypted *via* a secure, password protected, web site to investigators in the Consortium.
2. Findings will be reported at scientific meetings and published in peer-reviewed journals.
3. Data will not be disseminated outside the Consortium (except at scientific meetings) until the data are published or in press.
4. Model organisms and cell lines will be made available through postings on the Consortium web site after they are reported in the scientific literature.
5. If a study involves intellectual property issues, the data or resources will not be shared outside the Consortium institution until the issues are resolved (e.g., a patent has been filed).

The intellectual property plan is given below.

Plan for assessing performance of each research site and synergy: The Executive Committee in conjunction with the Advisory Committee will evaluate the productivity, efficiency, and relevance of each research site on a continued basis with an annual in-person meeting. Decisions regarding continuation, modification, or termination of a research site will be made based upon the input from these committees. Bi-weekly conference calls and exchange of the information will be done to ensure continued synergy among research sites and projects and cores to accomplish the goals of the project. To assure that there is a rigorous administrative structure in place, the co-investigators, will have overall responsibility for the progress, budget and direction

of the grant, and will participate in the general oversight for research design and conduct of projects, data collection and quality control, data analysis, and preparation/review of publications.

CONCLUSION:

This Consortium proposal brings together eminent cancer researchers from 12 premier institutions across the US, and focuses on reducing the risk of death by ovarian cancer by identification of molecular markers and signatures for the precursor ovarian cancer pathway and the development of early detection methods such as non-invasive imaging using molecular markers and transgenic mouse models. The three highly synergistic projects proposed here are hypothesis-driven and are based on collaborations between Consortium members. Investigation into the fallopian tube harboring a precursor pathway to a high grade ovarian cancer is a synergistic outcome of the Consortium. To address this question further, tissue banks from across the US were queried and allowed us to identify high grade samples for the proposed project. In addition, the synergistic efforts have led to validation of genomic and proteomic technologies; development of data sharing methodologies; and resulted in joint publications.