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TITLE: Genetic Alterations in Epithelial and Stromal Compartments of Prostate Adenocarcinomas

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Fort Detrick, Maryland 21702-5012

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Genetic Alterations in Epithelial and Stromal Compartments of Prostate Adenocarcinomas

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Fort Detrick, Maryland 21702-5012

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Genetic analyses on prostate cancer has been occurring for over a decade. However, such studies were uniformly performed on the entire tumor without regard to its components despite the fact that a few groups were quite aware of both epithelial and stromal components of tumors, and the cell biology of the tumor "microenvironment" has been described for the last 20 years. Thus, until now, when a genetic alteration, be it intragenic mutation, regional amplification, or deletion manifested by loss of heterozygosity of markers (LOH) is attributed to a prostate cancer, it is unclear if the alteration is actually occurring in the epithelial compartment, the surrounding stromal compartment or both. Our own preliminary data on breast carcinomas demonstrate that LOH and even somatic mutations can occur in surrounding stromal fibroblasts. Therefore, this proposal proposes to search for genetic alterations in the stroma of prostate cancers and to determine if such alterations can influence clinical outcome. In the almost 2 years, we suffered technical difficulties mainly with the quality of the blocks. In year 3, the PI has accrued 90 non-M1 adenocarcinomas of the prostate. Of these 90, 70 have been subjected to LCM and total genome LOH scanning data available on 50 with the other 20 in various stages of processing and analysis.
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Year 3 Annual Report [No Cost Extension Requested]

Proposal Title: Genetic alterations in epithelial and stromal compartments of prostate adenocarcinomas
PI: Charis Eng, MD, PhD

INTRODUCTION
Prostate cancer is common in the West and is uniformly lethal once metastasized. Thus, there is growing interest in examining the genetic alterations in prostate cancer. Until recently, however, solid tumors such as prostate carcinoma were treated as a single amorphous entity. Genetic studies were uniformly performed on the entire tumor without regard to its components despite the fact that a few groups were quite aware of both epithelial and stromal components of tumors, and the cell biology of the tumor “microenvironment” has been described for the last 20 years. Thus, until now, when a genetic alteration, be it intragenic mutation, regional amplification, or deletion manifested by loss of heterozygosity of markers (LOH) is attributed to a prostate cancer, it is unclear if the alteration is actually occurring in the epithelial compartment, the surrounding stromal compartment or both. Recently, Moinfar and colleagues, using a limited subset of samples and markers, demonstrated that LOH of markers representing three chromosomal loci can occur in the stromal compartment of a small pilot series of invasive breast adenocarcinomas (1). Further, the PI has demonstrated LOH of a limited set of markers in the stroma of invasive breast adenocarcinomas (2). More importantly, somatic intragenic mutations of TP53 and PTEN have been found in the stroma, but are mutually exclusive within any single compartment (3). This has never been examined in prostate cancers. Nonetheless, the mechanisms, especially the genetic mechanisms, by which the different cells in the micro-environment interact with the epithelial component to initiate and/or promote tumor growth is not well understood. Thus, the overall hypothesis of the submitted proposal was that genetic changes in the stromal and epithelial compartment of prostate adenocarcinomas differentially contribute to tumor growth, such that they affect clinical outcomes differently. The hypothesis is to be addressed by two Objectives:

1. To determine the relative frequency of genetic alterations within the stromal versus epithelial compartment of human prostate adenocarcinomas, and to build a genetic model for multistage stepwise carcinogenesis involving the epithelium and stroma in prostate cancer;

2. To determine the clinical consequences of genetic alterations within the stromal versus epithelial compartment of adenocarcinomas of the prostate.

BODY

Objective 1: To determine the relative frequency of genetic alterations within the stromal versus epithelial compartment of human prostate adenocarcinomas, and to build a genetic model for multistage stepwise carcinogenesis involving the epithelium and stroma in prostate cancer
This objective can be viewed as a two-stage task. The first step is the accrual of prostate cancer specimens for the analysis. The second step is laser capture microdissection (LCM) of neoplastic epithelium, surrounding stroma, and corresponding non-neoplastic germline tissue, followed by total genome LOH scanning and final analyses. At the end of Year 1, the PI reported procuring epithelial and stromal cells by LCM from 55 non-M1 prostate adenocarcinomas and that a total genome scan was commencing. Unfortunately, after almost 9 months of attempting a genome scan with the Research Genetics set of 400 markers, the PI and team have ascertained that the DNA from all but 5 samples were so degraded (or the formalin may not have been buffered) that PCR was impossible. This is not a systematic technical issue in the PI’s lab because in parallel, the PI has just successfully completed a 389-microsatellite marker total genome LOH scan of DNA from LCM-processed cells in the epithelial and stromal compartments of 135 sporadic invasive adenocarcinomas of the breast (4) and completed a 389-marker total genome LOH scan as well as TP53 mutation analysis of DNA from epithelial and stromal compartments LCM-processed from 12 invasive adenocarcinomas of the breast originating from individuals with germline BRCA1/2 individuals (funded by DOD BCRP). Thus, in the latter 3 months of Year 2, the PI has changed sources (dates — obtaining newer blocks which is a trade off for longer follow up) for obtaining prostate adenocarcinoma archived blocks. At the end of year 2, genomic DNA from stroma and epithelium from 10 prostate adenocarcinomas with Gleason score 2+2 have been obtained and a 389-marker (Research Genetics) total genome LOH scan successfully completed. In the last year (Year 3), the PI has accrued 90 non-M1 adenocarcinomas of the prostate with Gleason scores 2+2, 2+3 (or 3+2), 3+3 and 3+4 (or 4+3). Of these 90, 70 have been subjected to LCM and total genome LOH scanning data available on 50' with the other 20 in various stages of processing and analysis.

**Objective 2: To determine the clinical consequences of genetic alterations within the stromal versus epithelial compartment of adenocarcinomas of the prostate**

This objective is entirely dependent on completion of the total genome LOH scan and analysis as proposed in Objective 1 (which is envisioned to complete in the final quarter of Year 4).

**KEY RESEARCH ACCOMPLISHMENTS**

The first 90 prostate adenocarcinoma samples with full clinical and pathologic information have been accrued, and subjected to LCM and DNA extraction. Total genome scanning has successfully been completed on 70 (with data on 50 to date, with the rest in process) with differential LOH of markers in epithelial and/or stromal compartments observed.

**REPORTABLE OUTCOMES**


Elected Member, Association of American Physicians (AAP), April, 2004

Appointed Member, American Association for Cancer Research (AACR) Publications Committee, April, 2004 —
CONCLUSIONS
After taking almost 2 years to overcome technical issues (poor tissue specimens), we have been able to obtain 90 (and continuing) adenocarcinomas of the prostate (from a different source). Seventy have been subjected to compartment-specific LCM and 50 have completed total genome LOH scanning for both compartments. Inspection reveals LOH of certain markers in the epithelium and/or stroma. Although we proposed to obtain 175 total samples, our work with breast carcinomas has shown that statistical power would be adequate with a sample size between 120 and 150 (135 for the breast study (4)). We are confident we can accrue and analyze the remaining samples within the no-cost-extension period.

REFERENCES CITED
APPENDIX
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2. Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME
Eng, Charis, M.D., Ph.D.

POSITION TITLE
Professor of Medicine and Human Cancer Genetics

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
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<tr>
<td>University of Chicago, Chicago, IL</td>
<td>B.A.</td>
<td>1978-82</td>
<td>Biological Sciences</td>
</tr>
<tr>
<td>University of Chicago, Chicago, IL</td>
<td>Ph.D.</td>
<td>1982-86</td>
<td>Development Biology</td>
</tr>
<tr>
<td>University of Chicago, Chicago, IL</td>
<td>M.D.</td>
<td>1982-88</td>
<td>Medicine</td>
</tr>
<tr>
<td>University of Cambridge, Cambridge, UK</td>
<td>(Post-Doc)</td>
<td>1992-95</td>
<td>Human Cancer Genetics</td>
</tr>
</tbody>
</table>

A. Positions and Honors

Academic Appointments
1988-1991 Residency in Internal Medicine, Beth Israel Hospital, Boston, MA
1991-1994 Clinical Fellowship, Medical Oncology, Dana-Farber Cancer Institute, Boston, MA
1992-1995 CRC Dana-Farber Fellowship in Human Cancer Genetics, University of Cambridge, UK
1992-1995 Senior Registrar in Clinical Cancer Genetics, University of Cambridge Addenbrooke's Hospital, Cambridge, UK and Royal Marsden Hospital, London, UK
1995-1998 Assistant Professor of Medicine, Dana-Farber Cancer Institute and Harvard Medical School, Boston
1995-1998 Active Staff Physician, Adult Oncology, Dana-Farber Cancer Institute, Boston, MA
1999-2002 Associate Professor (with tenure) of Medicine, The Ohio State University, Columbus, OH
1999-present Director, Clinical Cancer Genetics Program, James Cancer Hospital and Solove Research Institute, Comprehensive Cancer Center, Ohio State University, Columbus
2001-02 William C. and Joan E. Davis Professor of Cancer Research, The Ohio State University
2002-present Professor (with Tenure) of Medicine, The Ohio State University, Columbus
2002-present Dorothy E. Klotz Chair of Cancer Research, The Ohio State University, Columbus
2002-present Director, Division of Human Genetics, Department of Internal Medicine, The Ohio State University, Columbus

Honors and Awards
1982 Phi Beta Kappa; Sigma Xi Associate Membership
1987 Sigma Xi Promotion to Full Membership
1988 Alpha Omega Alpha
1999 American College of Physicians, Promotion to Fellow
2001 Elected Member, American Society for Clinical Investigation (ASCI)
2002 Doris Duke Distinguished Clinical Scientist Award (2002-2007)
2003 Elected Fellow, American Association for the Advancement of Science (AAAS)
2004 Elected Member, Association of American Physicians (AAP)

Selected Recent Additional Professional Activities
1998-present North American Editor and Cancer Genetics Editor, Journal of Medical Genetics
1998-present NCCN Genetics/High Risk Guidelines Panel
2001-2003 Am Soc Clinical Oncology Subcommittee to Update Policy for Cancer Genetic Testing
2001-present American Cancer Society Molecular Biology and Oncogenes Study Section
2004- Senior Editor (Molecular Biology, Pathobiology and Genetics), Cancer Research

B. Selected Publications (selected from a total of 220 peer reviewed original publications)


Chung JH, Ginn-Pease ME, Eng C. PTEN has NLS-like sequences for nuclear import mediated by MVP. (submitted)
C. Research Support

Ongoing Research Support

07/01/01-06/30/05 1) NIH/NICHD 1 R01 HD39058-01A1
Title: RET complex polymorphisms in Hirschsprung disease
PI: Charis Eng, M.D., Ph.D.

The goal of this project is to identify and characterize common low penetrance alleles within RET and the genes which encode its ligands and co-ligands in “sporadic” medullary thyroid carcinoma as well as sporadic Hirschsprung disease.

07/01/02-06/30/06 2) American Cancer Society RSG-02-151-01-CCE
Title: Genetics of PTEN in Cowden and Related Syndromes and Familial Cancer
PI: Charis Eng, M.D., Ph.D.

The goals of the project are to determine the individual-as-unit PTEN genotype-organ-specific phenotype risk of cancer in individuals with PTEN mutations, as to determine the risk and age of onset of each type of cancer.

12/15/02-12/14/07 3) Doris Duke Charitable Trust Distinguished Clinical Scientist Award
Title: Title: Genetics of PTEN and molecular-based patient care
PI: Charis Eng, MD, PhD

This is an award for translational research and mentorship activities on the platform of the comprehensive analysis of PTEN in cancer as a paradigm for clinical cancer genetics translational research.

03/25/02-04/24/05 4) Army Med R&D Command DAMD17-02-1-0528
Title: Genetics of Epithelial-Stromal Interactions in Hereditary Breast Cancer
PI: Charis Eng, M.D., Ph.D.

To determine the frequency of genetic alterations at various chromosomal loci across the genome in the epithelial and stromal compartments of BRCA1 invasive adenocarcinomas of the breast, to determine the clinical consequences of genetic alterations at various chromosomal loci across the genome in the epithelial and stromal compartments of BRCA1 invasive adenocarcinomas of the breast; and to determine the dependency of genetic alterations to one another in the epithelial and stromal compartments of BRAC1-related breast adenocarcinomas.

12/01/01-01/15/05 5) Army Med R&D Command DAMD17-02-1-0118
Title: Genetic Alterations in the Epithelial and Stromal Compartment of Prostate Adenocarcinomas
PI: Charis Eng, M.D., Ph.D.

The goal of this project is to examine, from a genetic point of view, the contribution of the epithelial and stromal compartments to human prostate carcinogenesis.

05/01/03-04/30/07 6) BRTT Ohio
Title: Gastrointestinal Cancer Genetics
PI: Joseph Nadeau, PhD

OSU PI’s: Albert de la Chapelle, MD, PhD & Charis Eng, MD, PhD

This is an award from the State of Ohio for an Ohio-wide multi-institutional effort to discover and validate new genes related to gastrointestinal cancer pathogenesis in human and mouse models.

05/01/03-04/30/07 7) BRTT Ohio
Title: Bioinformatics Platform
PI: Joel Saltz, MD, PhD

Co-I: Charis Eng, MD, PhD

This award funds the development and implementation of a bioinformatics infrastructural platform for multidisciplinary biomedical investigation, prominently of which are genomic and epigenomic analyses.

9/15/04-9/14/09 8) National Cancer Institute 1P01CA97189-01A2
Program Title: Genetic analysis of the role of the tumor microenvironment in breast cancer progression
Project 1 Title: Genetic alterations in the epithelial and stromal compartments of breast adenocarcinomas
PI: Charis Eng, MD, PhD (Project) / Michael Ostrowski, PhD (Program)
The goal of Project 1 within the PPG is to examine and characterize total genome genetic alterations in the epithelium of human sporadic invasive adenocarcinomas of the breast and the surrounding stroma and how they impact clinical outcome. Further, we begin to functionally validate our stromal data by proposing to selectively knock-out Pten in the epithelium and separately in the stroma of mouse mammary glands and functionally assess the effects thereof.

9/15/04-9/14/09
Program Title: Interrogating Epigenetic changes in cancer genomes
Project 2 Title: Integrating genomic and epigenomic alterations in cancer and its microenvironment
PI: Charis Eng, MD, PhD (Project) / Tim Huang, PhD (Program)
The goal of this project is to integrate genomic and epigenomic alterations in stroma during breast cancer progression using experimental and computational biology strategies.