Award Number: DAMD17-02-1-0118

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

PRINCIPAL INVESTIGATOR: Charis Eng, M.D., Ph.D.

CONTRACTING ORGANIZATION: The Ohio State University Columbus, OH 43210-1063

REPORT DATE: January 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
Genetic analyses on prostate cancer have 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 second year,
# Table of Contents

Cover..............................................................................................................

SF 298.............................................................................................................1

Table of Contents.............................................................................................2

Introduction.......................................................................................................3

Body..................................................................................................................3

Key Research Accomplishments........................................................................4

Reportable Outcomes........................................................................................4

Conclusions........................................................................................................5

References.........................................................................................................5

Appendices.........................................................................................................6
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 was 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 or other 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 the PI has just successfully completed a 389-microsatellite marker total genome LOH scan of DNA from LCM-procured cells in the epithelial and stromal compartments of 125 sporadic invasive adenocarcinomas of the breast (Eng, unpublished) and completed a 389-marker total genome LOH scan as well as TP53 mutation analysis of DNA from epithelial and stromal compartments LCM-procured 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. In the last 3 months, the PI has obtained 20 blocks, non-M1 and >90% Gleason’s score 2+2 and 2+3 (or 3+2), as a pilot. 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. Inspection demonstrates LOH of markers in the epithelial and/or stromal compartments. The PI will therefore continue to accrue non-M1 prostate adenocarcinoma samples from similar sources as the initial batch of 20.

**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 3). While we are trading off shorter follow-up for technical success, the initial new batch of 20 all have clinico-pathologic information linked to the samples.

**KEY RESEARCH ACCOMPLISHMENTS**

The first 20 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 10 with differential LOH of markers in epithelial and/or stromal compartments observed.

**REPORTABLE OUTCOMES**

Elected Fellow, AAAS, Sept., 2003

Chair, Scientific Program Committee, 53rd Annual Meeting of the American Society of Human Genetics, Los Angeles, CA, Nov. 4-8, 2003
CONCLUSIONS
The first nine months of the second year of this project was mainly dedicated to technically achieving a total genome scan on epithelium and stroma LCM-procured from the first 55 samples from Year 1. Unfortunately either because of massive degradation of nucleic acids and/or non-buffered formalin of these samples, PCR was impossible despite massive efforts. This was not due to systematic issues in the PI’s lab as the PI has completed total genome LOH scans from LCM-procured epithelium and stroma from >100 breast adenocarcinoma samples. Nonetheless, a separate set of 20 non-M1 prostate adenocarcinomas (more recent samples) were accrued in the latter 3 months, 10 of which have had epithelial and stromal cells procured by LCM, and subjected to a total genome LOH scan. Inspection reveals LOH of certain markers in the epithelium and/or stroma. We are not able to rapidly accrue the remaining samples and subject them to total genome LOH scanning, without further issues.

REFERENCES CITED

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/Principal Investigator

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 (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Chicago, Chicago, IL</td>
<td>B.A.</td>
<td>1978-82</td>
<td>Biological Sci</td>
</tr>
<tr>
<td>University of Chicago, Chicago, IL</td>
<td>Ph.D.</td>
<td>1982-86</td>
<td>Development. Bio</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>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
1994-1995 Instructor in Medicine, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA
1995-1998 Assistant Professor of Medicine, Dana-Farber Cancer Institute and Harvard Medical School, Boston
1992-1995 Active Staff Physician, Adult Oncology, Dana-Farber Cancer Institute, Boston, MA
1995-1998 Associate Physician, Brigham and Women’s Hospital, 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, OH
2001-present 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, OH
2002-present Dorothy Klotz Chair of Cancer Research, The Ohio State University, Columbus, OH
2002-present Director, Division of Human Genetics, Department of Internal Medicine, The Ohio State University

Honors and Awards
1982 Phi Beta Kappa
1982 Sigma Xi Associate Membership and Sigma Xi Prize
1987 Sigma Xi Promotion to Full Membership
1988 Alpha Omega Alpha
1995 First Lawrence & Susan Marx Investigator in Human Cancer Genetics, Dana-Farber Cancer Institute
1999 American College of Physicians, Promotion to Fellowship
2001 Elected Member, American Society for Clinical Investigation
2002 Harry de Lozier Memorial Lectureship
2002 Stephanie Spielman Breast Cancer Research Award, The Ohio State University, Columbus
2002 Doris Duke Distinguished Clinical Scientist Award (2002-2007)
2003 Elected Fellow, AAAS

Selected Recent Additional Professional Activities
1998-present North American Editor and Cancer Genetics Editor, Journal of Medical Genetics
1998-present NCCN Genetics/High Risk Screening Panel
2001-2003 American Soc. of Clinical Oncology Subcommittee to Revise Guidelines for Cancer Genetic Testing
2001-present American Cancer Society Molecular Biology and Oncogenes Study Section

B. Selected Publications (selected from a total of 203 peer reviewed original publications)


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.

09/01/02-08/31/04
3) NIH/NCI 1R21CA030722-01
Title: Genetic etiologies of esophageal Barrett's and cancer
PI: Charis Eng, M.D., Ph.D.
This is a proposal to accrue families with Barrett esophagus and adenocarcinoma of the esophagus to begin whole genome scans to search for the predisposing gene(s).

08/01/00-07/31/03
4) Army Med R&D Command DAMD17-00-1-0390
Title: A Novel Phosphatase Gene on 10q23, MINPP, in Hereditary and Sporadic Breast Cancer
PI: Charis Eng, M.D., Ph.D.
The goals of the study are to explore whether another phosphatase which genetically localizes in proximity to PTEN and which has overlapping activity with PTEN could play a role in inherited and sporadic breast tumorigenesis as well.

03/25/02-04/24/05
5) 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 BRCA1-related breast adenocarcinomas.

08/01/00-07/31/03
6) 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

03/01/01-02/28/04
7) V Foundation, Jimmy V Golf Classic Research Award
Title: Genetic Analysis of the Role of the Microenvironment in Epithelial Tumor Progression
PI's: Charis Eng, M.D., Ph.D., Gustavo Leone, Ph.D. and Michael C. Ostrowski, Ph.D.
The goal of this award is to provide seed moneys to gather preliminary data and make reagents related to tumor-microenvironmental interactions so that a group grant, e.g. PPG, may result from such work, as well as novel targets for therapy and prevention.

09/15/01-10/14/04
8) Army Med. R&D Command DAMD17-01-1-0357
Title: Rosiglitazone Therapy in Breast Cancer
PI: Lisa Yee, M.D.
Co-I: Charis Eng, M.D., Ph.D.
The goal of this study is to examine the anti-cancer effects of rosiglitazone, a synthetic ligand for PPARgamma, in early stage breast cancer as it relates to several genetic and molecular markers.
12/15/02-12/14/07
9) Doris Duke Distinguished Clinical Scientist Award
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.