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PRINCIPAL INVESTIGATOR: Judd Moul, M.D.

CONTRACTING ORGANIZATION: Uniformed Services University of the Health Sciences
Bethesda, Maryland 20814-4799

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13. ABSTRACT *(Maximum 200 words)*

The congressionally mandated Center for Prostate Disease Research (CPDR) was established in 1992 as a program to study prostate cancer and prostatic disease in the U.S. military health care system. Initial funding through the Walter Reed Army Institute of Research (WRAIR) was used to establish a comprehensive laboratory for the molecular biologic study of genetic and cellular alterations that may contribute to the pathogenesis of prostate cancer. Through an MOU, the laboratory was established at the Uniformed Services University of the Health Sciences (USUHS) and contracted to the Henry M. Jackson Foundation for the Advancement of Military Medicine (HMJF). During the ensuing five years, HMJF scientists and technicians worked with the PI and other government and military members at USUHS to conduct numerous studies. The following report summarizes progress in the areas of genetic alterations and novel gene discovery, functional genetics, androgen mechanisms, prostate cell biology, and preclinical models of gene therapy. Through funding from USAMRMC and other private and public sources, the CPDR program continues to expand and is enhancing the understanding of the molecular mechanisms of prostate tumorigenesis.

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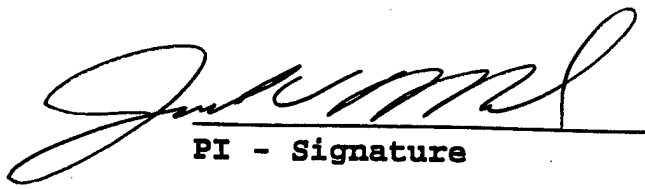

PI - Signature

10/25/98

Date

(4) Table of Contents

(5) Introduction

The Center for Prostate Disease Research (CPDR) at the Walter Reed Institute of Research (WRAIR) is an ongoing program since 1992 devoted to the study of prostate cancer and prostate disease in the U.S. military health care system. Funding from USAMRMC to WRAIR established a molecular biology laboratory program at the Uniformed Services University of the Health Sciences (USUHS) through a Memorandum of Understanding (MOU) between WRAIR and USUHS. The USUHS contracted the program to the Henry M. Jackson Foundation (HMJF) for the Advancement of Military Medicine for the hiring of personnel. The laboratory encompasses approximately 4000 square feet of space at the USUHS and scientists and support personnel are predominantly HMJF employees. In addition, military and civilian physicians, scientists and students affiliated with the USUHS, WRAMC, and WRAIR have been involved with research projects with the program over the last 5 years.

The CPDR program is a comprehensive multidisciplinary research endeavor involving basic scientists, urologists, pathologists and other researchers devoted to prostate molecular and cellular biology and translational research. The program involves five broad areas of study: Genetic Alterations/ Novel Gene Discovery, Functional Genetics, Androgen Mechanism, Prostate Cell Biology, and Preclinical Models of Gene Therapy. These studies are conducted on prostate cancer established cell lines, animal models of prostate tumorigenesis, and tissues and blood collected from consenting patients on Institutional-Review Board (IRB)-approved tissue and serum bank studies of prostate disease patients from Walter Reed AMC and other military health care facilities. When the program was initiated in 1992, little was known of the molecular and cellular mechanisms that contributed to prostate cancer. The CPDR program goal is to identify the genes and proteins that uniquely contribute to the development of prostate cancer and develop new detection, staging, and treatment methods to improve the care of men with this common disease.

(6) Body

CENTER FOR PROSTATE DISEASE RESEARCH
MOLECULAR BIOLOGY PROGRAM

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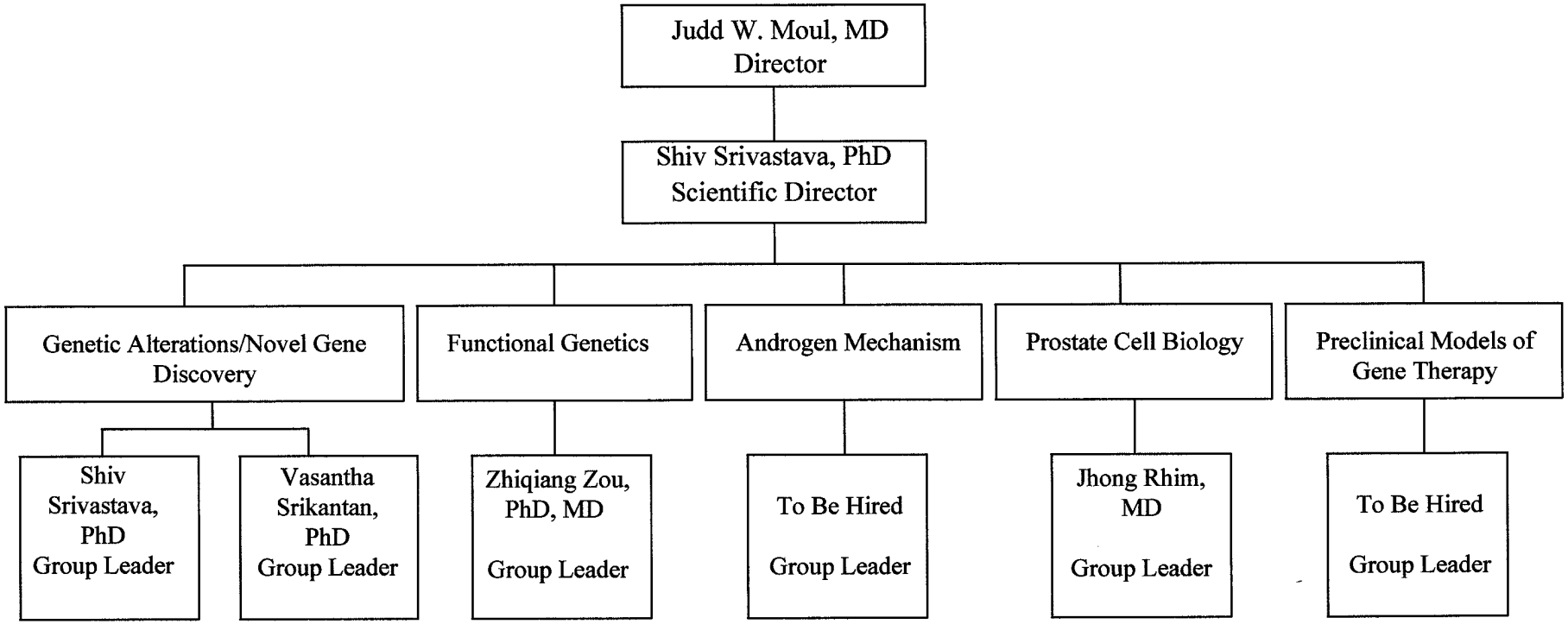
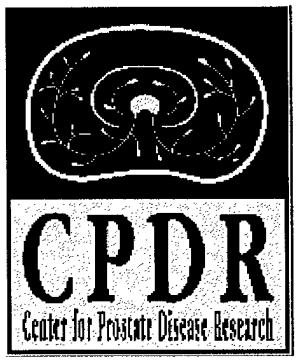
Center for Prostate Disease Research

Molecular Biology Program at USUHS





Center for Prostate Disease Research

Multi-disciplinary Molecular Biology Program

Walter Reed Army Medical Center Urology Service
•Tissue specimens
•Clinical information

⇩

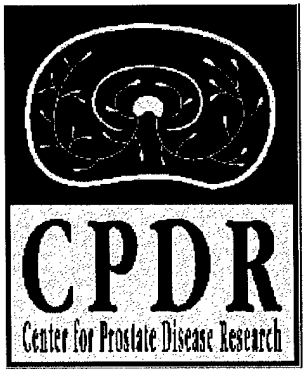
Armed Forces Institute of Pathology
Genito-urinary Pathology
•Pathologic characterization
•Tissue microdissections

⇩

Uniformed Services University of the Health Sciences
Department of Surgery, Center for Prostate Disease Research
•Molecular genetic alterations
•Mechanisms of prostate cancer progression

⇩

Center for Prostate Cancer Disease Research
Patient Database
•Clinico-pathologic correlations of molecular findings



Center for Prostate Disease Research

Multi-disciplinary Molecular Biology Program

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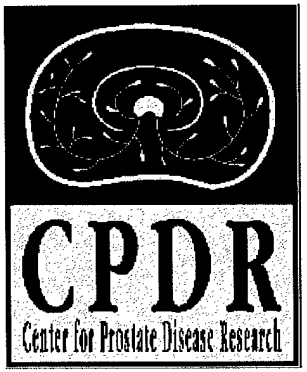

Center for Prostate Disease Research

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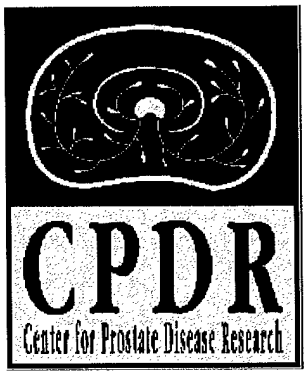

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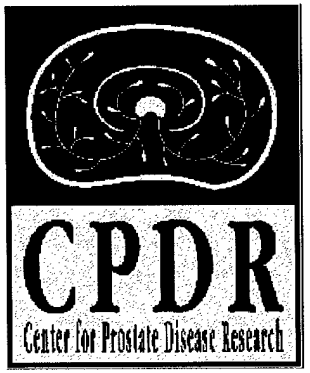

Center for Prostate Disease Research

Molecular Biology Laboratory

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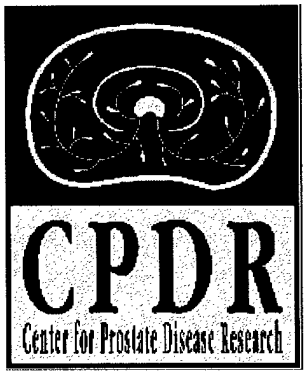
1994-1995	MAJ Howard B. Heidenberg, DO
1995-1996	MAJ John J. Bauer, MD
1996-1997	MAJ G. Bennett Stackhouse, MD
1997-1998	MAJ Robert C. Dean, MD
1998-1999	MAJ Greg L. Griewe, MD



Center for Prostate Disease Research

Molecular Biology Laboratory

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Prem Seth, PhD

NCI; Bethesda, MD

Meena Augustus, PhD

NHGRI, NIH; Bethesda, MD

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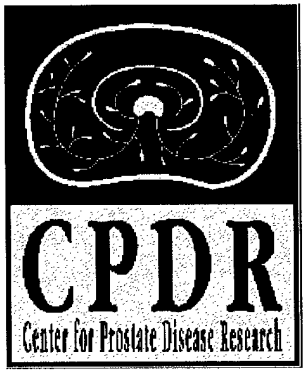
HGS; Rockville, MD

Jay Joshi, PhD

VA Medical Center; Washington, DC



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Anita Reynolds

I. Novel Gene Discovery

I.A. 6q-Tumor Suppressor Locus Project

Primary Investigators:

Vasantha Srikantan, D.V.M., Ph.D.
Zhiqiang Zou, M.D., Ph.D.
Robert Dean, M.D.
Shiv Srivastava, Ph.D.

Technical Help: Jeffery Livezey and Theresa Connell

Goal: To characterize 6q loci for involvement in prostate cancer progression and to identify the putative TSG on 6q locus.

Progress:

- Study was completed on 38 primary prostate cancer specimens by Dr. Srikantan and showed >3-fold increased 6q deletion with non-organ confined (pathologic stage C) disease as compared to organ confined disease.
- A follow-up study was continued with metastatic specimens. Poor quality of archival specimens have slowed the progress due to inconsistencies noted with the results. Mets showed high rate of 6q deletions. Study is being completed with LCM microdissected tissues.
- The study was presented by Dr. Srikantan at various meetings: AACR 1997, AUA 1997, and SBUR 1997.
- The manuscript is being submitted to *Int. J. Cancer.*
- Follow-up has been planned with consultant (Dr. Chandrasekharappa) from Human Genome Center at NIH

Current Plan:

- Dr. Dean performed a follow-up study on 6q archival specimens (in 80 patients with longer follow-up Bauer Group) using one 6q marker (D6S300), however he did not find clinical correlation. Paraffin embedded tissues did not amplify as well as frozen. These specimens were not analyzed from whole-mount prostate pathology. This locus most likely harbors a tumor suppressor gene which might play role in a subset of advanced prostate cancer as well as in other cancers. The research area on 6q is not as crowded as other lot loci in prostate cancer. We could do a much better job both with clinical correlations by increasing the number to 100 with frozen specimens as well as with mapping (new post-doc).

Need to talk to Dr. Chandrasekharappa, for a clear projection of personnel requirements.

- Dr. Srikantan is following up on strategies to refine the locus by additional markers. Need to develop as a clear focus/plan. A clear project plan needs to be written by Dr. Srivastava in consultation with Dr. Chandrasekharappa of the Genome Center, NIH. The meeting was convened and the consensus is to hire a dedicated Ph.D. or MD to work on this project.

- Dr. Zou has been developing alternative novel approaches to find candidate genes on frequently deleted locus on 6q. He has already identified a couple of promising candidates and is following up. A similar approach may be used for another not spot locus.

- C-kiss was shown to be regulated by the 6q14-22 locus. Expression analysis of c-kiss needs to be evaluated. This is an open project that needs further study.

- We need to hire a full-time post-doc to follow-up on positional cloning approaches supporting 6q follow-up who will work under Dr. Srivastava.

The studies from this project should yield reports for (2) meetings and (2) papers for publication.

1.B. Identification of a Novel Prostate Cancer Associated Gene by Differential Gene Expression Strategies.

Primary Investigators:

Vasantha Srikantan, Ph.D.
Zhiqiang Zou, M.D., Ph.D.
Meena Augustus, Ph.D.
Shiv Srivastava, Ph.D.

Technical Help: Jeffery Livezey
Tammy Lawrence (partial)

Goal: This long conceived project finally took off as a pilot project by Dr. Srikantan's initiative. The major goal of this project was and is to identify novel prostate cancer gene expression markers which may play a critical role in prostate cell biology and may serve as markers of prostate cancer progression. Our unique perspective was to utilize radical prostatectomy derived microdissected frozen specimens (instead of cell lines) and compare gene expression pattern in matched tumor normal tissues of the individual patients on whom comprehensive clinical information is available.

Progress:

We have made good progress on this project with respect to the identification of a novel prostate specific transcript: PCGEM1. Prostate tissue specificity has been established (very comprehensive). Androgen regulation is a very interesting aspect. The analysis of the protein coding sequence has been very intriguing as no clear open reading frame has been obtained. Drs. Zou and Srikantan have been performing a very comprehensive analysis and they have done a phenomenal job on this.

- Initial findings were presented at different meetings (AACR, AUA). Manuscript is in preparation.
- Patent application has been submitted to HMJ for processing.
- Two grants (VA/DoD and CaPCure) have been submitted on this research.

Follow-up:

- The main focus of this project is to quickly establish the nature/identity of PCGEM1 transcript and its functions and send the paper out (Dr. Srikantan). Regulation of PCGEM1 expression/promoter analysis and tissue specificity (Dr. Zou).

Dr. Srikantan has other candidates which also need to be characterized carefully.

General Comments:

We made great strides on this project.

- A good collaborative team effort between Drs. Srikantan and Zou has been in place for PCGEM1 characterization. The project has been moving at a fast pace. The major concern is the nature of PCGEM1, once clarified the project will move faster. Specific tasks have been distributed among primary investigators. PCGEM1 currently represents one of the major focus of the lab. Great focus for DoD or CaPCURE grants if we know what PCGEM1 is.

- Differential Gene Expression approach needs to be better developed (DD or DNA array) to identify markers for cancer recurrence. My recommendation is that one investigator (Dr. Segawa, the Japanese post-doctoral fellow) need to devote 100% time of this project.

- We have started testing the commercially available gene chip arrays for differential gene expression. As a test we have used androgen regulated and p53 regulated genes and they are being evaluated. If successful, a similar strategy will be followed for other projects in the pipeline.

1.C. Analysis of Novel CaP Associated Alterations by Spectral Karyotyping and CGH

Primary Investigators:

Meena Augustus, Ph.D.
Evelyn Schröck, MD
Thomas Ried, MD
Isabell A. Sesterhenn, MD
Judd W. Moul, MD
Shiv Srivastava, Ph.D.

Goal: To identify novel prostate cancer associated alterations by this new technology.

- This project was initiated by Dr. Augustus. Following is the summary from her write up.

Progress:

With the advent of a revolutionary technique like spectral karyotyping (SKY) which uses a multi-color fluorescence-in-situ (FISH) strategy to recognize every chromosome in man and in mouse by a unique color, it has convincingly been shown that there are about twice the number of involved gene loci in cancers than has been previously reported. Even well characterized leukemia cases showed hidden abnormalities when repeated using SKY. It is therefore, more than likely that hitherto uncovered chromosomal loci of great import would see the light of day in solid tumors as well. In a well-planned and systematically orchestrated analysis of the complicated genetics of prostate cancer, SKY could lay bare the spectrum of clinically implicated chromosome alterations.

A glimpse into complex prostate genomes - combining the strengths of CGH and SKY for the analysis of both primary and metastatic prostate cancer - was possible in a recently reported study from our group (NIH meeting abstract Augustus, et al, 1998). Apart from being able to precisely characterize every marker chromosome that has defied definition by conventional G-banded analysis in the metastatic prostate cell lines LNCaP, DU145 and PC3, the biology of a primary, immortalized prostate cancer cell line, CPDR-1 was also elucidated using these tools. The tumor, pT3 (2) c, was obtained from a radical prostatectomy, and immortalized after three passages with E6/E7 genes in a retroviral construct (Davis et al, 1998 meeting abst.). The tumor showed evidence of multifocal PIN (nuclear grad N1-II). While CGH reflected only gross coy number changes with a gain of chromosome 18 and 20, SKY unraveled some additional cytogenetic aberration characteristic of advanced stage cancer, like clonal gains of #5 and #8 and loss of 8p21-pter and del (17) (p13). Additionally, a cryptic der (y;11) t(q10;p10) which is possible only by SKY. Tissue in-situ hybridization using centromeric chromosome enumeration probe on the tumor tissue in whole prostate from which CPDR-1 was derived in culture, very elegantly showed evidence for three copies of chromosomes 18 and 20.

SKY analyses of the three most commonly studied models for prostate tumorigenesis, the metastatic cell-lines LNCaP, DU145 and PC3, suggested very strongly that the karyotypic complexities are barely interpretable by CGH, and a lot of very valuable, critical data could possibly have been over-looked (Fig.2). LNCaP shows very stable and characteristic reciprocal balanced translocations (Fig.3 - copy of the figure and legend in the review chapter), and one of these break points at t(6;16) has also been recently characterized and cloned (Croce et al...). Translocations, like these that are so commonly seen in the hematological malignancies and some solid tumors have largely contributed to our understanding of cancer genes at particular chromosomal loci.

Follow-up:

- Various types of primary, immortalized and established prostate cancer cells are being analyzed by SKY in the on-going prostate cancer research endeavor at the CPDR and by Dr. Rhim, NCI (who is planning to join our program) together wit the NHGRI. It is now possible to identify genetic events occurring very early in the onset on CaP that would help us to identify cancers with a higher risk of metastatic potential and treatment refractory profiles. Manuscript by Dr. Augustus is in preparation.

II. Androgen Regulation of Prostate Growth (Molecular Mechanisms)

IIA. Identification of androgen regulated genes by SAGE

Primary Investigators:

Linda Xu, M.D.
Ping Su, M.D.

Goal: Growth and differentiation of prostate is dependent upon physiological levels of androgen. Depletion of physiological levels of androgen by hormonal therapy is the mainstay for the treatment of advanced prostate cancer. It is hypothesized that androgen regulated genes play critical role in growth/maintenance of prostate cancer. The disregulated expression of such genes may play a role in uncontrolled prostate growth (BPH or cancer) Identification of the full repertoire of androgen regulated genes will be central to the understanding of prostate cell growth. Up until now, androgen regulated genes have been discovered as incidental findings. More intense and directed approaches are needed to unravel the full breadth of such genes. Using global gene expression technologies (SAGE and DNA arrays) and normal and cancer epithelial cells, we propose to develop the data base of the androgen regulated genes. Using SAGE which provides both qualitative and quantitative information simultaneously, we also have a good chance of identifying novel androgen regulated genes which may serve as surrogate marker of AR activation. Such AR activation markers could be analyzed in the context of decreased CAG repeat or increased IGF1 levels.

Progress:

Dr. Xu had made a remarkable accomplishment in getting SAGE protocol working. Our laboratory represents a handful of labs worldwide with this technology. If we succeed in utilizing this novel technology, we are going to be in a unique position in the field of prostate cancer. All the logistics are in place to analyze the ditags as soon as possible. By the end of year we will analyze 30,000 tags from androgen treated/untreated LNCaP cells which might already represent 50%-75% of all the expressed genes. To prove the potential of this technology, we have already shown identification of PSA tags as the most inducible gene by androgen in early analysis of 12,000 tags. In the first phase we have analyzed 12,000 tags (6,000 each from control and androgen treated LNCaP cells) and the abstract has been submitted for AUA.

Follow-up:

- Write first comprehensive paper on 30 to 50K tags for publication by 6/99 with characterization of one or two novel genes.
- Follow-up and stream-line this technology for utilization in other situations, e.g., normal versus cancer as well as androgen regulated genes in normal prostatic epithelial.

- Will need to employ a couple of people if this project takes off.
- Write DoD Prostate Grant (this cycle) on the basis

General Comments:

This is a novel project in the prostate cancer field. We should push this as one of the key projects of the laboratory. There is risk involved, however, I believe new information will come out of this project. It may be possible to identify a new class of genes regulated by AR using this approach.

II.B. Novel Prostate Tissue Specific/Androgen Genes (Human Genome Sciences - Collaborations)

Primary Investigators:

Linda Xu, MD
Bennett Stackhouse, MD
Meena Augustus, Ph.D.
Ken Carter, Ph.D.
Shiv Srivastava, Ph.D.

Goal: To identify prostate specific genes which may play a central role in prostate cell biology and may have utility as additional prostate cancer markers like PSA.

II.C. NKX3.1 Gene

Progress:

The prostate specific androgen regulated homeobox gene NKX3.1 provoked a lot of interest due to its chromosomal localization at 8p21, a region most frequently deleted in prostate cancer. Dr. Xu has completed a comprehensive study of NKX3.1 expression and its regulation by androgen in human prostate cancer tissues. The new unexpected finding is that NKX3.1 expression is increased in one-third of prostate cancer patients. The paper on these results has been submitted for publication.

General Comments:

NKX3.1 is tightly regulated by androgen and may play an important role in prostate cell biology. Need to review the knock-out mice experiment of Dr. Abate.

Follow-up:

- Role of NKX3.1 overexpression in prostate cell biology, cell proliferation: (a) Is NKX3.1 expression cell cycle regulated? (b) Develop prokaryotic/eucaryotic expression vectors (c) Cell type specificity?
- Since NKX3.1 is so tightly regulated by androgen it will be interesting to see what downstream targets of NKX3.1 are regulated using DNA arrays.
- If transfection experiments/cell cycle experiments yield good results, it is a good focus for and NIH grant.
- Characterization of NKX3.1 promoter to show that AR directly regulated NKX3.1 expression.

- Antibody reagents to analyzed NKX3.1 proteins. We have made antibodies (need to characterize).

- Firm up NKX3.1 expression in peripheral blood (one way or another) and if it holds good, carry out the study of circulating prostate cells using this marker. Also reconfirm NKX3.1 expression tissue specificity using multiple tissue dot blot.

- If tissue specificity data is solid then detailed analysis of promoter is warranted for tissue specific/gene therapy approaches.

II.C.2. HPRAJ Gene: (Human Genome Science Collaboration)

This is an HGS characterized gene which Dr. Bennett Stackhouse, MAJ, MC, USA had discovered through the electronic Northern blotting. We confirmed the data mining experiment with Northern blot. The expression is prostate specific, however we did not detect in epithelial cells. Very preliminary results did suggest overexpression in tumor tissues. Dr. Stackhouse can write the paper as is by describing protein characteristics of the gene and tissue specificity. Dr. Ping Su has taken over the project.

Follow-up:

- Dr. Stackhouse needs to complete his paper.

- In situ hybridization/in situ PCR to firm up cell type specificity.

- Northern blot on cell lines (Dr. Srikantan)

II.C.3. Novel Gene Encoding Secretory Protein (HGS Collaboration)

This is a new gene, we obtained from HGS. We have not pursued anything on this. Dr. Zou has the material from HGS.

III. Cell Cycle Check-point and Prostate Cancer (p53, p21, and p27)

III. p53 Alterations in Prostate Cancer

Primary Investigators:

Howard Heidenburg, MD, MAJ, MC, USA
John Bauer, MD, MAJ, MC, USA
Bennett Stackhouse, MD, MAJ, MC, USA
Robert Dean, MD, MAJ, MC, USA
Greg Griewe, MD, MAJ, MC, USA
Naga Shanmugam, MS
Kekule Asgari, MD
Prem Seth, Ph.D.
Isabell Sesterhenn, MD
Judd W. Moul, MD, LTC, MC, USA
Shiv Srivastava, Ph.D.

Goal: We have performed a number of studies of p53 alterations in prostate cancer as well as preclinical models of p53 gene therapy. Most of these studies are published and have resulted in over 10 papers and couple of review articles. Despite these studies the heterogeneous pattern of the p53 immunostaining remains intriguing and causes confusion over the true role of p53 alterations in prostate cancer. p53 alterations in prostate cancer must be reconciled with the multifocal nature of disease.

Current Projects/Follow-up:

III.A. p53 Immunostaining in Biopsy Specimens/Clinical Correlations

II Unlike the study of p53 alterations in radical prostatectomy specimens, the prostate biopsy study did not provide a significant correlation of p53 staining with pathologic stage or cancer recurrence. This result however was anticipated due to known heterogenous nature of p53 alterations in primary CaP.

II Dr. Stackhouse completed the study and paper is being submitted to *J. Urol.* (pending review).

III.B. p53 Immunostaining Guided LCM and p53 Mutational Analysis

- Due to the reasons discussed in the Goals of this section, it is critical to carefully establish the rate of p53 alterations in wholemounted prostate cancer.

- The goal of this project is to analyze p53 mutations in the context of multifocal prostate cancer as well as to understand the meaning of heterogeneous p53 staining at the levels of p53 mutations. Utilizing wholemounted sections from 25 patients and p53 staining guided region are microdissected by laser capture microdissection and are analyzed by SSCP/DNA sequencing for mutations. These

studies will be followed with the analysis of p53 mutation in LCM of tumor regions by H&E staining of the multifocal prostate cancers from 25 patients. The results of p53 stained vs. H&E stained groups will clarify the prevailing confusion over the true rate of p53 alterations in prostate cancer.

- This project was started by Dr. Dean and is followed up by Dr. Griewe and Mr. Shanmugam.
- One AUA abstract and potentially very relevant paper is expected from this further effort.
- This project really needs to be done as there is no comprehensive study to date on this aspect. Now with LCM capability, we can stream line the issues. Also this study may serve as a model for molecular analysis using LCM/wholemounts and DNA technology.
- Revised NIH grant on this subject has been submitted. Focus on the multifocal prostate cancer/p53 mutational analysis.

III.C. Novel p53 Related Projects

Primary Investigators:

Chun Ling Gao, MD
Zhiqiang Zou, MD, Ph.D.
Leland Davis, BS

Novel p53 Regulated Genes (HSP27, MASPIN):

We have started this project three years ago. However, this project was always done as a side project with low priority addressing basic molecular mechanisms of p53 action. The goal of this project is to identify a novel p53 induced gene (especially apoptosis) in context of prostatic epithelial cells as p53 mediated apoptosis has been shown to be cell context specific.

Progress:

- Using Differential Display, Dr. Gao identified several candidates which unfortunately did not show confirmatory results using gene specific RT-PCR assays. These cDNAs did not detect specific transcripts on Northern blots probably due to low level expression. Dr. Gao tried RNAse protection assays but was not successful. Alternative approaches are being pursued.
- Simultaneously Dr. Gao employed DNA arrays from Clonetech and has identified several genes regulated by p53 that have not been described before. We have selected one of the most induced ones, HSP27, for a detailed analysis. A series of

biochemical experiments has been performed which suggest that induction of HSP27 is p53 specific and not a secondary effect of cell death or cell growth arrest. This represents a novel observation. Additional mechanistic studies are being pursued. Regulation of HSP27 by p53 is very interesting because in animal models of prostate cancers HSP27 expression is elevated in regressing prostate in response to androgen ablation. Manuscript should be ready for publication in a couple of months time.

- Dr. Zou, in collaboration with Dr. Gao, has recently obtained very interesting and novel information with respect to regulation of a tumor suppressor gene, MASPIN (this gene was discovered by Dr. Zou at Harvard Medical School) by p53 and he is expediently pushing the analysis with the hope of writing a manuscript as soon as possible.

Follow-up:

- At least two to three publications are expected from this project.
- We have prepared RNA from early time points of p53 infected prostate cancer cells with the hope of identifying additional interesting targets which may provide a better system of direct p53 targets. We will use a gene chip based array for this.
- In animal models of prostate cancer, HSP27 induction by p53 correlated with androgen ablation induced apoptosis. Regulation of HSP27 expression in prostate cancer may be of some significance. Needs to be done. We need to do a translational project on HSP27 expression and prostate cancer prognosis.

III.D. Novel p53 Related Genes (Search for Another One)

Recently three new members of the p53 gene family have been described (p73, p40, p51). In fact, p40 showed relatively specific expression in GU organs: prostate and bladder. Leland Davis as part of his Ph.D. thesis has undertaken a project which aims to identify novel p53 related genes and study the regulation of p73/p40 expression in prostate cancer. Using degenerate RT-PCR and low stringency cDNA library screening, Leland is attempting to identify novel p53 related genes. Using prostate tumor lines derived RNA and laser microdissected, Leland is initiating expression/mutation analyses of p40 and p73 in prostate cancer cells/specimens.

III.E. Analysis of p27 Expression in Prostate Cancer Specimens:

- p27 alterations have been shown to be a prognostic factor in breast tumor progression. This translational project was assigned to Dr. Dean. A status report is still due from Dr. Dean.

III.F. Adenovirus p53, p16, p21, and p27 Expression Vectors:

Our collaboration with Dr. Prem Seth has been very successful and productive and resulted in several publications (7+) over the last five years. We have all the vectors available to us for preclinical models as well as any basic science project. Our support to him of a technician has been extremely fruitful. He does not have our technical support anymore, however, we still continue the collaboration. Possible technical support may be necessary if we want him to develop specific adenovirus expressing vectors for our projects.

IV. Preclinical Models of Prostate Cancer Gene Therapy

IV.A. p53 Gene Therapy

Our studies of p53 gene therapy in a preclinical cellular/animal model has been completed with AdWTp53 vector. Several reports have been published. One more paper is pending for publication. We could not translate nude mouse findings to immune competent Dunning model of CaP because the rat cells could not be infected with AdWTp53 vectors. We have now succeeded in introducing AdWTp53 in Lobund Wistar Rat model of prostate cancer. Following studies are proposed:

IV.B. Lobund-Wistar Rat Prostate Cancer Model for *in vivo/in vitro* Gene Therapy: Effects of the AdWTp53

- All the key experiment using PAIII cells have been completed and we have obtained convincing data on the cytotoxic effects of AdWTp53 on a Lobund-Wistar rat prostate cancer derived PAIII cell line which now can be used in experiments of animal model. Dr. Pollard is conducting animal studies with our p53 vectors and has already obtained very interesting preliminary observations. This model system may also serve as a resource for collaboration with Dr. Esther Chang (IV-C-3).

- We will need the support of a post-doc to fully realize the utility of this model system.

IV.C. Prostate Cancer Vaccine Approaches

IV.C.1. PSMA Vaccine - Rat Model:

In collaboration with Dr. Al Boynton (Pacific Northwest Cancer Institute, Seattle, WA) we proposed to develop prostate cancer animal models for dendritic cell based vaccine approaches. Dr. Asgari (a former CPDR employee) started the project to develop rat prostate cancer model for PSMA dendritic cell treatments. Dr. Asgari learned how to grow dendritic cells and did the first experiments with AT6.1 - whole cell lysate treated dendritic cells. This experiment did not show any effect. However, in this experiment PSMA transfected cells grew considerably slower. We need to test this observation in nude mice (Shanmugam). A follow-up experiment on dendritic is warranted. Not sure who will do it. One possibility is to hire a post-doc to do this project.

IV.C.2. PSA-DNA Vaccine Project:

Based on Dr. Srikantan's prior experience on DNA vaccines, we started a project to develop animal model in Dunning rats to test the efficacy of PSA DNA vaccine. Although we were successful in creating PSA expression vector in pcDNA, we could clearly establish the presence of exogenous PSA mRNA or protein by Northern and Western blots. We unequivocally showed the expression

of PSA in transfectants by RT-PCR and immunohistochemistry. We are not sure about the discrepancy noted above. Animal experiments were also very confusing. At this point this project is shelved until a clear plan is developed.

IV.C.3. Collaborative Projects on Vaccine Related Approaches

Using NKX3.1, PCGEM1 (once we know the protein) or PCSA tumor vaccine is being planned with Dr. George Peoples, MAJ, MC, USA from WRAMC.

CPDR interest will be to push dendritic cells - AdWTp53 or MTp53 treatments for immune therapy of MTp53 containing cancer. I would like to pursue this idea. However, lack of tumor immunology expertise in the group prevents us from testing these ideas.

Follow-up:

We need a tumor immunologist (post-doc) for this project. Since no one in our group has tumor immunology expertise, it is prudent to let MAJ George Peoples pursue this area as he is well trained in these aspects. We can support MAJ George Peoples with a technician/post-doctoral fellow.

IV.D. p53 Gene Therapy Collaborations with Dr. Esther Chang

Dr. Esther Chang, a noted molecular biologist at Georgetown University, has developed efficient lyposome based systemic delivery of p53 in tumor targeted fashion. Moreover, combining p53 and current chemotherapy/radiation therapy regimens, she has shown amazing results in nude mouse animal models of different types of cancer including prostate cancer. At the lab scale we have provided her with all our results and experience we have in prostate cancer cell and animal models. Similarly, Dr. Moul has contributed greatly toward the phase I potential of this approach. We hope to continue this collaboration to develop into Phase I/II study at WRAMC under Dr. Moul's direction. We may need to hire one post-doctoral fellow (MD) to support clinical study.

V. Prostate Cancer - Animal Model

Lobund-Wistar Prostate Cancer Model:

Molecular alteration in tumors of Lobund Wistar rats-Pollard model needs to be characterized for p53 mutations and AR status in tumors. Pollard model is very interesting as these animals are genetically susceptible to spontaneous prostate cancer as well as hormone/chemically induced cancers. We need to validate this model as virtually no molecular characterization has been done. We have full cooperation of Dr. Morris Pollard from Notre Dame University in terms of animal model. Need good ideas to productively use this opportunity. In the short term we are establishing a base line for the following information:

- Androgen/IGF levels in young animals to compare with nonsusceptible Wistar Rats and progenitors.
- p53 mutations and AR expression in rat tumors.
- Rat equivalent of human 8p alterations in Lobund model (need Dr. Augustus' help).
- Comparison of metastatic/primary tumor tissue to identify CaP metastasis related genes.
- Currently one technician is working on this project and we will expand once we have some more information.

VI. Novel Molecular Diagnostics: Strategies for the Detection of Prostate Cancer Progression

VI.A. Evaluation of the Prognostic Value of Circulating Prostate Cells in Blood and Bone Marrow of Prostate Cancer Patients

Primary Investigators:

Chun Ling Gao, MD
Robert Dean, MD
Shiv Srivastava, Ph.D.
Judd W. Moul, MD

Progress:

Initial goal of analyzing 100 plus patients for the presence of circulating prostate cells in blood and bone marrow by RT-PCR PSA assay has been accomplished.

- Abstracts have been published.
- Both the manuscripts are accepted for publication in *J. Urol.* and *Urol.*
- The exciting preliminary data showed a statistically significant correlation of bone marrow RT-PCR-PSA positivity and cancer recurrence.

Follow-up:

- To improve the reproducibility of RT-PCR assay we are enriching prostate cells from circulation by Dyna-beads conjugated with anti-epithelial cell specific antibody. The isolated cells are being analyzed for PSA and cytokeratin expression.
- Since PSA/cytokeratin based detection of prostate cells does not tell about the cancer phenotype of the cells, we propose to analyze the magnetic beads enriched prostate cells for CaP associated DNA alterations such as microsatellite analysis for chromosome deletions 8p21, 18q, 10q.
- Not sure if it is feasible but explore the possibility of doing spectral karyotyping on enriched cells (maybe using the cell culture dishes coated with anticytokeratin antibody).

VII. Development of Prostate Cell Culture Bank for Molecular Analysis

Characterization of Primary and Immortalized Prostate Cancer Cells from WRAMC Patients:

Primary Investigators:

Leland Davis, BS
Ping Su, MD
Meena Augustus, Ph.D.
Tammy Lawrence, MS
Amjad Choudhary, DVM, MPh
Isabell Sesterhenn, MD
Judd W. Moul, MD
Shiv Srivastava, Ph.D.

This CPDR initiative has been developing enormous resources for several types of molecular and cell biology experiments. Leland Davis established the immortalization protocols which are now routinely used in the laboratory. Leland was able to immortalize four primary prostate cancer derived cell cultures. That really provided a firm footing for following up this avenue of research.

Dr. Ping Su has done a remarkable job in organizing the primary cell culture as well as immortalized cell derived RNAs and DNAs for molecular analysis. She herself has immortalized clonetics normal prostate epithelial cells as well as three additional primary cancer cells cultures. She together with Leland has developed a great resource of primary culture and immortalized cell derived DNA and RNAs (7 immortalized cancer lines plus an additional four upcoming; 1 immortalized normal culture; about 35 primary cultures). These cells are being analyzed for p53 mutational status, androgen receptor, PSA, cytokeratin, NKX3.1 expression as well as chromosomal alterations frequently noted in CaP at DNA level by LOH assays.

Dr. Augustus along with Dr. Su and a technician has been spearheading the generation of primary culture derived cells suitable for SKY analysis. Dr. Augustus has been very successful. Recently she has over a dozen primary cultures in the pipeline for SKY analysis. Dr. Chaudhary has successfully performed the *in vivo* tumorigenicity assays of established prostate cancer cell lines as well as the new developed immortalized cells. Dr. Chaudhary has analyzed these cells in subcutaneous and orthotopic models. Only CPDR1 exhibited a rare mouse tumor which is currently being analyzed in the laboratory.

This initiative is proposed to be expanded by Dr. Rhim's (a pioneer in prostate cell primary culture) joining our program. He is proposing to develop a National Prostate Cell Center under the CPDR umbrella.

Recent Abstracts Submitted for Meetings
October 1998

p53 MUTATION ANALYSIS IN LASER CAPTURE MICRODISSECTIONS OF p53 POSITIVE FOCAL REGIONS OF WHOLE-MOUNT PROSTATES IN MEN WITH CLINICALLY ORGAN-CONFINED PROSTATE CANCER

Greg L. Griewe, Robert C. Dean, Isabell A. Sesterhenn, Naga Shanmugam, Wei Zhang, David G. McLeod, Judd W. Moul and Shiv Srivastava, Washington, D.C. (Presented by Dr. Griewe).

INTRODUCTION AND OBJECTIVES: p53 tumor suppressor gene, the most frequently altered gene in human cancers, is involved in cell cycle regulation and apoptosis. A wide range of p53 mutations (5-65%), detected by various methods has been reported in primary prostate cancer. DNA based mutation detection assays generally find a low rate of p53 alterations (5-10%), while immuno-staining infers a much higher frequency of p53 mutations in primary prostate cancer. In part because of these discrepancies, the significance of p53 in prostate cancer remains uncertain. Immunostaining of radical prostatectomy specimens shows marked heterogeneity of focally distributed p53 positive cells from tumor to tumor as well as within single tumors. Our goal is to better define the role of p53 mutation in prostate cancer.

METHODS: Whole-mount prostates from men with clinically organ-confined prostate cancer were immunostained for p53. Laser capture microdissection (LCM) was used to excise foci of p53 positive cells from areas of tumor and prostatic intraepithelial neoplasia. DNA from microdissected cells were amplified for p53 exons 5-8 by polymerase chain reaction and analyzed for mutations by single strand conformation polymorphism and DNA sequencing.

RESULTS: Mutation analysis of exons 5-8 of heterogeneous p53 positive focal regions (1+ to 4+) of whole-mount prostate sections from 22 specimens representing tumors in 17 patients has been performed. Our ongoing studies have revealed p53 mutations in at least one specimen from 6 of 17 patients (35%). Suspected alterations by SSCP in an additional 5 of the 17 patients are pending sequence.

CONCLUSIONS: Prostate tumors with focally positive immunostaining for p53 have been confirmed to contain mutations by DNA analysis. P53 immunostaining guided LCM combined with DNA based analyses has potential to define the role of p53 in primary prostate cancer.

SOURCE OF FUNDING: United States Army Medical Research and Materiel Command, Henry Jackson Foundation, Center for Prostate Disease Research

EXPRESSION PROFILE OF AN ANDROGEN REGULATED PROSTATE SPECIFIC HOMEOBOX GENE *NKX3.1* IN PRIMARY PROSTATE CANCERS

Linda L. Xu, Vasantha Srikantan, Meena Augustus, and Shiv Srivastava, Bethesda, MD; Kenneth C. Carter, Rockville, MD; Isabell A. Sesterhenn, Robert Dean, David G. McLeod, and Judd W. Moul, Washington, D.C. (Presented by Dr. Xu)

INTRODUCTION AND OBJECTIVES: *NKX3.1*, a new member of the homeobox gene family, exhibits prostate tissue specific expression and may play a critical role in prostate development. Furthermore, chromosomal localization of *NKX3.1* to 8p21, a region frequently deleted in prostate cancer (CaP) provided the impetus to evaluate the role of *NKX3.1* in human prostate tumorigenesis by studying expression patterns in primary CaP specimens, established CaP cell lines and a variety of normal tissues.

METHODS: *NKX3.1* expression was analyzed in matched, microdissected normal and tumor tissues from radical prostatectomies from 53 patients by semiquantitative RT-PCR and *in situ* hybridization and correlated with androgen receptor regulation. *NKX3.1* expression was also analyzed in variety of normal tissues by RT-PCR.

RESULTS: *NKX3.1* expression in matched normal and tumor tissues revealed overexpression in 32% of tumors , decreased expression in 21% and no change in 47%. When these patterns were stratified by organ confined (T2) and non-organ confined (T3) disease status, a higher percentage of patients exhibited *NKX3.1* overexpression in T3 (40%) vs. T2 (22%) tumors. *In situ* hybridization analysis of representative tissues showed prostatic epithelial cell specific expression of *NKX3.1* and confirmed RT-PCR observations of overexpression in tumors. Androgen ablation therapy responsive tumor cells from primary CaP exhibited lack of *NKX3.1* expression. *NKX3.1* expression correlated with androgen receptor expression ($P<0.01$) *in vivo* in human normal and primary CaP.

CONCLUSIONS: Increased *NKX3.1* transcripts associated with a higher fraction of non-organ confined disease is suggestive of its role in aggressive phenotypes of CaP. The androgen regulation of *NKX3.1* in the physiologic context of prostate development, differentiation and proliferation appears vital enough to merit further investigation.

SOURCE OF FUNDING: CPDR, USAMRMC, HM Jackson Foundation

IDENTIFICATION OF THE ANDROGEN REGULATED GENES (ARGs) BY SERIAL ANALYSIS OF GENE EXPRESSION (SAGE)

Linda L. Xu, Yongping Su, Ron LaBiche, Judd W. Moul, and Shiv Srivastava, Bethesda, MD; David G. McLeod, Washington, D.C (Presented by Dr. Xu)

INTRODUCTION AND OBJECTIVES: The biologic effects of androgen on target cells are mediated by transcriptional regulations of ARGs by the androgen receptor. ARGs most likely play critical role in androgen dependent proliferation and differentiation of prostate as well as in androgen ablation induced apoptosis in prostate gland. Therefore, a comprehensive characterization of ARGs may lead to identification of promising candidates, which play important role in prostate tumorigenesis. SAGE allows for a high throughout, quantitative analysis of gene expression (Velculescu et al; Science, 270: 484, 1995) and has potential to identify novel ARGs. Here we report a preliminary analysis of ARGs differentially expressed in LNCaP cells with/without androgen treatment.

METHODS: Control LNCaP cells or synthetic androgen R1881 treated LNCaP cells grown in charcoal stripped serum containing media were used to prepare polyA RNA. SAGE libraries were generated by using 5 μg polyA RNA from control and treated group. SAGE tags representing transcripts were analyzed with SAGE software kindly provided by Dr. K. Kinzler.

RESULTS: A total of 12,000 SAGE tags derived from transcripts of control and androgen treated LNCaP cells (approximately 6000 tags from each group) have been isolated, sequenced and analyzed for this preliminary report. These SAGE tags represented a total of approximately 7000 genes and their relative abundance varied between 0.016% and 5.83%. Comparison of the transcripts between control and treated groups revealed 19 genes upregulated and 14 genes down regulated (>4 fold) in response to androgen. Among the SAGE tags analyzed for upregulation prostate specific antigen (PSA)-SAGE tags showed the highest level induction (10 fold) in response to androgen.

CONCLUSIONS: Our preliminary evaluation of SAGE analysis of ARGs revealed several ARGs not described before. Our results showing the SAGE tags of PSA as the most induced transcript in response to androgen validates the utility of this approach in finding physiologically relevant ARGs. Analysis of additional SAGE tags from our ongoing studies should provide a comprehensive global profile of ARGs in prostatic epithelial cells.

SOURCE OF FUNDING: CPDR, USAMRMC, HM Jackson Foundation

MOLECULAR CYOGENETIC ALTERATIONS IN PROSTATE CANCER DETECTED BY SPECTRAL KARYOTYPING: A SCHEMA OF EVENTS FOR PROSTATE TUMORIGENESIS.

Meena Augustus, Isabell Sesterhenn, Tammy Lawrence, Yongping Su, Wei Zhang, Evelin Schrock, F.K. Mostofi, David G. McCleod, Jhong Rhim, Thomas Ried, Judd Moul, Shiv Srivastava, Bethesda, MD., and Washington D.C.(Presented by Dr. Meena Augustus)

INTRODUCTION AND OBJECTIVES: Prostate cancer (CaP) remains the second leading cause of death in American men. Translational research from the laboratory to the clinic is a critical link in the chain from cancer to cure. Molecular cytogenetic analysis by spectral karyotyping (SKY) was used as one such vital link to elucidate and track chromosome markers for prostate cancer genesis/progression.

METHODS: 12 prostatic cell lines (normal prostatic epithelium (PrEC-Clonetics), E6/E7 immortalized PrEC, SV-40 transformed prostatic epithelium (267B1), HPV-18 immortalized prostatic epithelium (CRL-11609), Nitroso-Methyl Urea (NMU) transformed/tumorigenic cell line (Nu5002), spontaneously immortalized primary CaP (ND1), 3 E6/E7 immortalized primary CaP tumors (CPDR 1,2 and 3), 3 metastatic prostate cell lines (LN CaP, PC3 and DU145) and 8 primary prostate tumors were characterized using SKY – a multi-color, fluorescence–*in-situ*–hybridization tool.

RESULTS : Genetic alterations involving chromosomes 2,8p,18 and 20q as early events for cancer initiation to late events involving chromosomes 6q,8q,10q,11,12,16, 17p,18q and other occult, cryptic rearrangements were accurately defined. Evidence for the same chromosome markers *in-vivo* was obtained by tissue *in-situ* hybridization using centromere specific chromosome enumeration probes (CEP) on the whole mounts of prostates from which the tumors were derived and maintained in culture. These markers were useful in distinguishing low grade tumors from those indicative of inherent invasive/metastatic potential, as was seen in CPDR1, derived from a moderately differentiated tumor with prostatic intraepithelial neoplasia (PIN). Primary prostatic tumors though essentially diploid, carried unbalanced translocations leading to critical gains and losses of specific chromosomes and regions.

CONCLUSION : Recurring sites of chromosomal aberrations reflect underlying molecular events that participate in cancer progression. SKY- while providing a precise genome wide look at such events- dispels the 'gray zones' of conventional cytogenetics that confound issues of prostate tumorigenesis.

SOURCE OF FUNDING: Henry M. Jackson Foundation for the Advancement of Military Medicine, Rockville, MD; USAMRMC; CPDR; and the Office of International Affairs, NCI (for M.A.)

NKX3.1 GENE A MOLECULAR MARKER FOR DEFINING PROSTATIC ORIGIN OF CELL CULTURES DERIVED FROM PRIMARY PROSTATE CANCER SPECIMENS

Yong Ping Su, Leland Davis, Linda Xu, Meena Augustus, Judd W. Moul and Shiv Srivastava, Bethesda, MD; Isabell A. Sesterhenn, Washington, DC
(Presented by Dr. Su)

INTRODUCTION AND OBJECTIVES: Well characterized primary prostate cancer derived cells are critically needed to evaluate molecular genetic alterations contributing to prostate tumorigenesis. Further molecular analysis of androgen regulation of prostate cells is dependent on availability of androgen receptor (AR) expressing prostatic epithelial cells. Here, we report characterization of a variety of primary prostate cancer derived cell cultures and immortalized cell lines for the expression of AR and markers of prostatic epithelial cells.

METHODS: Radical prostatectomy derived short term cultures (~2 months) were prepared from 28 prostate cancer patients. Seven prostate cancer derived cells and one normal prostate epithelium derived cell culture (Clonetics) were immortalized by infection of E6/E7 gene retroviral expression vector. Using RT-PCR based assays, expression of housekeeping gene: glyceraldehyde phosphate dehydrogenase (GAPDH), epithelial cell marker: cytokeratin 19, prostatic epithelial cell markers: prostate specific antigen (PSA); NKX3.1, a recently discovered prostate specific androgen regulated gene and androgen receptor (AR) were analyzed in these cells.

RESULTS: All the primary as well as immortalized cell cultures expressed GAPDH and cytokeratin 19. A majority of short term cultures (>75%) expressed AR as well as the NKX3.1 gene. However, most of the primary cultures as well as immortalized cell lines lacked PSA expression. One prostate cancer derived immortalized cell line was obtained that expressed PSA, NKX3.1 and AR. Immortalization resulted in loss of AR and NKX3.1 expression in few cell lines.

CONCLUSIONS: A significant association of NKX3.1 and AR was observed in short term as well as several prostate cancer derived immortalized cell cultures. Loss of PSA expression in short term as well as immortalized cultures is intriguing and needs to be further evaluated. Presence of AR and NKX3.1 served as molecular markers to define the prostate origin of cells studied here.

SOURCE OF FUNDING: CPDR, USAMRMC, HJF

CYTOTOXIC EFFECTS OF THE ADENOVIRUS P53 EXPRESSION VECTOR IN RAT PROSTATE CANCER MODELS.

Naga Shanmugam, Judd W. Moul, Shiv Srivastava, and Prem Seth, Bethesda, MD; Morris Pollard, South Bend, IN. (Presented by Mr. Shanmugam)

OBJECTIVES: Our previous studies established antitumorigenic effects on human prostate cancer cells grown in nude mice. We also reported long-term antitumorigenic effects of AdWT p53 following single intratumor injections suggesting for the role of bystander effects. In order to extend these observations to immune competent animal models, we have evaluated the feasibility of using PAIII rat prostate cancer cells in the Lobund Wistar (L-W) rat prostate cancer model and AT 6.1 rat prostate cancer cells in the Dunning rat prostate cancer model. Here we report the differential cytotoxic effects of AdWT p53 on PAIII and AT 6.1 rat prostate cancer cells.

METHODS: Effects of AdWT p53 were studied on two rat cancer cell lines AT 6.1, and PAIII. DU145 human CaP cells were used as a control for cytotoxic effects of AdWT p53. PAIII is a rat prostate adenocarcinoma-III which was derived from a spontaneous tumor in an aged L-W rat. AT 6.1 cells are a highly metastatic derivative from Dunning rat prostate cancer cells. These cell lines were infected with different dosages of AdWTp53 or dl312 control adenovirus. The efficiency of adenoviral infection was evaluated by infecting these cell lines with Ad β-Galactosidase(Ad-β gal) virus. Further, we studied p53 protein expression by Western blot.

RESULTS: AdWT p53 vector exhibits potent growth inhibitory effects in PAIII cells. However, AT 6.1 cells were refractory to the effects of AdWT p53. Ad β-gal infection of PAIII and AT 6.1 cells revealed that AT 6.1 cells were significantly resistant to the infection by the adenovirus expression vectors as compared to the PAIII cells. These observations are also supported by abundant p53 protein detected in AdWT p53 PAIII cells. Mutational analysis of p53 in rat prostate cancer cells is underway.

CONCLUSIONS: Our studies revealed that PAIII rat prostate cancer cells serve as efficient target cells for evaluating gene therapeutic potential of adenovirus based vectors including AdWT p53. Further evaluations of AdWT p53 in L-W rats is in progress.

SOURCE OF FUNDING: CPDR, USAMRMC, HJF

STRUCTURE AND EXPRESSION OF A NOVEL PROSTATE SPECIFIC GENE: PCGEM1

Zhiqiang Zou, Vasantha Srikantan, Leland D. Davis, Jeffery Livezey, Isabell Sesterhenn, Linda Xu, Kash F. Mostofi, David G McLeod, Judd W. Moul, and Shiv Srivastava, Bethesda, MD (Presented by Dr. Zou)

OBJECTIVE: Prostate cancer (CaP) is the most common malignancy in men in the United States. Traditional prognostic markers such as Gleason score, clinical stage and pre-treatment PSA are of limited value in following up the disease progression in individual patients. In order to identify consistent gene expression alterations in CaP which may define specific stages of tumor progression, we have employed differential display (DD) technology on histologically well characterized normal and tumor tissues from CaP. Characterization of one of the promising candidates, PCGEM1 cDNA is reported here.

METHODS: The full length cDNA of PCGEM1 was obtained by 5' and 3' racing and by screening the prostate cDNA library using the originally identified 530bp fragment as probe. The genomic DNA was cloned from BAC library. The DNA sequencing was performed by ABI 310 DNA sequence machine. The PCGEM1 expression in variety of normal tissues and prostate cancer cell lines and tissues has been analyzed by a combination of Northern blot and RT-PCR base assays.

RESULTS: PCGEM1 cDNA spanning about 1.7kb sequence did not reveal any significant homology to DNA sequences in NCBI gene database. A 1.8kb messenger RNA was specifically detected using PCGEM1 cDNA probe, and was exclusively expressed in prostate tissue. PCGEM1 expression was detected only in LNCaP cells and absent in DU145 and PC3 cells. When the LNCaP cells were treated with androgen, expression of PCGEM1 was greatly increased. RT-PCR analysis of prostate tumors found that over 45% of the tumor cells overexpress PCGEM1 gene.

CONCLUSION: The PCGEM1 was expressed only in androgen-dependent tumor cell LNCaP cells and was up-regulated by androgen. Prostate tissue specificity and overexpression of PCGEM1 in prostate tumor cells suggests that it plays an important role in prostate cell growth and tumor development. The remarkable tissue specificity of PCGEM1 could provide a valuable diagnostic marker for prostate cancer.

SOURCE OF FUNDING: CPDR, USAMRMC, HJF

CYCLIN-DEPENDENT KINASE INHIBITOR p27/kip1 PROTEIN EXPRESSION IN PROSTATE CANCER

Robert C. Dean, David G. McLeod, F. Kash Mostofi, Isabell A. Sesterhenn, Shiv Srivastava, and Judd W. Moul. Washington, DC (Presented by Dr. Dean)

INTRODUCTION AND OBJECTIVES: Loss of expression of p27 has recently been implicated as a marker for a tumor's aggressive potential in diverse cancers. The expression of p27 is recognized as a negative regulator of the cell cycle and the overexpression triggers apoptosis. In recent reports, decreased p27 correlated with prostate cancer (PC) progression. In this study, p27 protein expression in PC specimens detected by immunohistochemical methods was correlated with Gleason score, glandular differentiation, and disease progression following radical prostatectomy (RP).

METHODS: Expression of p27 protein was evaluated using a polyclonal antibody (Santa Cruz Biotechnology, CA) against p27 in archival paraffin-embedded RP specimens followed from 2 to 12 years (median=7 years). The percentage of nuclei expressing p27 antigen was measured in normal and tumor cells then assigned a p27 score (none to rare=<2%, +1=up to 25%, +2=26-50%, +3=51-75%, +4=>75%). The relationship of p27 score and Gleason score, glandular differentiation, and serological recurrence postoperatively were analyzed using Kaplan-Meier survival analysis.

RESULTS: Of 128 patients analyzed, 120 tumors (94%) had some p27 expression. All samples had +4 nuclear staining of the normal prostate epithelium. The vast majority (96%) of PC specimens demonstrated decrease p27 expression. In Kaplan-Meier analysis, Gleason score ($p<0.01$), glandular differentiation ($p<0.001$), pathological stage ($p<0.01$) were statistically significant predictors of disease-free survival; however, the p27 score did not predict disease progression ($p=0.40$).

CONCLUSION: Although 96% of PC specimens demonstrated a decrease in p27 expression indicating a loss of a cell cycle regulator, there is no correlation of p27 expression and recurrence of PC following radical prostatectomy. These findings suggest p27 protein expression is altered in prostate carcinoma cells, perhaps occurring at different stages in tumor progression. The expression of p27 cyclin-dependent kinase protein appears not to be a useful prognostic marker in prostate cancer.

Source of funding: USAMRMC, HJ Foundation, CPDR

MUTATIONAL ANALYSIS OF MASPIN GENE IN PROSTATE CANCER CELLS

Zhiqiang Zou, Theresa Connell, Leland D. Davis, Judd W. Moul and Shiv Srivastava, Bethesda, MD. (Presented by Dr. Zou)

OBJECTIVE: Maspin, a tumor suppressing serpin, is expressed in normal breast and prostate epithelial cells, but its expression is lost during tumor progression. In vitro and in vivo studies have shown that expression of maspin inhibited tumor cell invasion and metastasis of breast tumor. Maspin was located on chromosome 18q21.3 where frequent loss of heterozygosity (LOH) has been found in variety types of tumors including prostate, colon and breast. LOH of 18q21 was associated with aggressive behavior of prostate tumor. This study was undertaken to identify mutation of maspin in prostate tumor cell lines and primary tumor cell culture.

METHODS: The entire coding sequence of maspin (1167 nucleotides) was amplified from total RNA of primary cell culture of prostate tumor and tumor cell lines. DNA sequencing was performed on ABI 310 DNA sequence machine.

RESULTS: Regions of maspin from 10 to 1233 nucleotide spanning the entire coding sequence was analyzed from 13 tumor cell line including PC3, DU145, LNCaP, Dupro and 9 primary cell culture from prostate tumor. Normal prostate mRNA purchased from Clontech (26 pooled normal individual) was also sequenced for control. Two primary cell cultures of tumor specimens had a change at codon 176 from proline to serine (CCT to TCT). The result also indicated a polymorphism change at codon 319, either an isoleucine (ATC) or valine (GTC) residue present at about equal frequency. The normal RNA samples indicated mixture of A and G on the chromatogram.

CONCLUSION: The complete coding sequence of maspin was analyzed in prostate tumor samples. A possible mutation at codon 176 was found in two tumor cell samples. A polymorphic change occurred at codon 319. The results indicated that mutation of maspin could be present in a subset of prostate tumor cells. Mutation of maspin may be involved in tumor invasion and metastasis of the prostate tumor.

SOURCE OF FUNDING: CPDR, USAMRMC, HJF

EVALUATION OF REVERSE TRANSCRIPTASE (RT)-POLYMERASE CHAIN REACTION (PCR)-PROSTATE SPECIFIC ANTIGEN (PSA) ASSAY IN EPITHELIAL CELLS ISOLATED FROM PERIPHERAL BLOOD OF PROSTATE CANCER (CaP) PATIENTS

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OBJECTIVES: The detection of circulating prostate cancers cells in the blood and bone marrow of CaP patients may improve the staging and follow-up of the disease. Several laboratories including ours are focusing on this important issue. However, presently available RT-PCR based assays to detect circulating prostatic epithelial cells face a technological challenge with respect to inter-laboratory as well as intra-laboratory variations resulting from the sensitivity limits of these assays. To improve the sensitivity/reproducibility of these assays, we have now assessed RT-PCR-PSA assays in peripheral blood derived epithelial cells of CaP patients.

METHODS: The circulating epithelial cells were isolated from the peripheral blood using antiepithelial cells antibody Ber-EP4 coated magnetic beads (Dynal Corporation). Total RNA prepared from cells fractionated on the beads were analyzed for the expression of glyceraldehyde-3-phosphated dehydrogenase (GAPDH) and PSA using nested RT-PCR assays. Controls included a (1) no RT-PCR reaction on RNA specimens to rule out for genomic DNA and pseudogene interference, (2) no template PCR reaction as a negative control and (3) a positive control RNA to validate the assay.

RESULTS: Sensitivity of RT-PCR-PSA assays were defined as the detection limit of one LNCaP cell spiked into 10^7 white blood cells derived from a healthy female. Peripheral blood from 24 of 51 (47%) CaP patients analyzed here were positive in RT-PCR-PSA assay. Reproducibility of RT-PCR positive and negative specimens was 70% and 100% respectively in two RT-PCR assays.

CONCLUSIONS: Peripheral blood of about half of the CaP patients yielded epithelial cells that were positive for PSA. Isolated epithelial cells should provide a good source for the analysis of CaP specific molecular alterations.

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(7) Conclusions

The CPDR research program at the WRAIR has developed into a comprehensive molecular biology program devoted to the study of prostate cancer. Under an MOU and contracting, the Uniformed Services University of the Health Sciences (USUHS) has provided outstanding laboratory infrastructure and the Henry M. Jackson Foundation (HMJF) for the Advancement of Military Medicine has provided exemplary scientists and technicians. These civilian contract personnel working closely with military and government civilian scientists, physicians and support personnel have created a multifaceted dynamic group pursuing prostate pathogenesis. This USAMRMC funding has allowed the creation of an ongoing program that is making novel genetic and cellular discoveries that hopefully will translate into better diagnostic, staging and treatment approaches for men with prostate cancer.

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