AD_____

Award Number: DAMD17-01-1-0037

TITLE: Human Prostate Cancer in a Tissue Recombination Model

PRINCIPAL INVESTIGATOR: Karin Williams, Ph.D.
Simon Hayward, Ph.D.

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center
Atlanta, Georgia 31192-0303

REPORT DATE: December 2002

TYPE OF REPORT: Annual Summary

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	December 2002	Annual Summary (16 Apr 01 - 15 Nov 02)

4. TITLE AND SUBTITLE
Human Prostate Cancer in a Tissue Recombination Model

5. FUNDING NUMBER
DAMD17-01-1-0037

6. AUTHOR(S)
Karin Williams, Ph.D.
Simon Hayward, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Vanderbilt University Medical Center
Atlanta, Georgia 31192-0303

Email: simonh@itsa.ucsf.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

In vivo models based on human epithelial cells (hPrE) will provide powerful tools with which to investigate cancer initiation and progression. Tissue recombinations (TR) composed of hPrE and rat urogenital sinus mesenchyme (rUGM) grafted beneath the renal capsule of immunocompromised rat hosts recapitulate many key events in prostatic development and adult function. The stable integration of known or putative oncogenes into the hPrE component of such TRs is a powerful tool with which to study the effects of these genesin vivo. The SV40 large T antigen (a known oncogenic protein) and TSPY, a protein related to the NAP1 oncogene[1], have been introduced into hPrE. TSPY is not expressed in non-malignant prostate but is aberrantly present in low and high-grade human prostate cancer, suggesting a possible role in carcinogenesis.

The high efficiency LZRS/Phoenix retroviral system was used to insert the TSPY or SV40T gene into hPrE under the control of either the constitutively active CMV or the prostate-specific ARR2 probasin promoter. TR composed of infected hPrE and rUGM were made and grafted into athymic rats. Hosts were sacrificed after carrying the grafts for periods of 4-16 weeks.

Genes under the control of the CMV promoter are expressed at the time of grafting. We have visualized reporter gene expression at this time and confirmed transgene expression. Expression of GFP in grafted tissue has been detected by immunohistochemistry. The probasin promoter requires that the TR be fully differentiated, a state acquired at 12-18 weeks post grafting. Initiation of SV40T and TSPY expression occurs in this timeframe when the probasin system is used. The grafts are monitored histologically to determine the timing of key tumorigenetic events.

The present study demonstrates the feasibility of the TR approach to creating a 'transgenic' hPrE within an in-vivo hormonally manipulatable system. Locally growing and any metastatic cells that arise can be genetically and morphologically distinguished from host cells by their species of origin.

14. SUBJECT TERMS
prostate cancer, tissue recombinant, oncogene, retrovirus
tumor biology, prostatic carcinogenesis

15. NUMBER OF PAGES
12

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Statement of Work

The Investigation of Human Prostate cancer using a tissue recombination model

Task 1 To transfect human prostatic epithelium with cmv-ΔSV40, PB-ΔSV40, CMV-TSPY, PB-TSPY, PB-GFP and CMV-GFP and recombine with rUGM, followed by grafting into an athymic rat host. (Months 1-12)

a. Analysis of transfected cells in culture via EGFP (months 1-6)
b. Characterization of recombined transfected huPrE with rUGM and conformation of human prostatic cell architecture. (months 1-8)
c. Access the histological data and identify histologically altered cells (months 3-24)
d. Creation of cell lines from tissue removed from grafts at various time points and from any lesions observed (months 3-24)

Task 2 Steroid receptor localization and quantification in grafts consisting of untransfected huPrE, transfected huPrE both before and after castration (months 8-24)

a. Identification and analysis of ERß isoforms in the huPrE by IHC, western blotting and in situ hybridization (months 8-18)
b. Quantification of ERα immunoexpression and localization (months 12-24)
c. Analysis of AR expression, and AR mutation analysis by RT-PCR (months 12-24)
d. Investigation of PR expression in grafts both before and after castration (months 12-24)

Task 3 Analysis of apoptosis after hormonal depravation caused by castration (months 18-36)

a. Identification of hormone sensitive/insensitive cells within the recombinant grafts by use of the TUNEL assay (months 18-24).
b. Formation of cell lines derived from hormone insensitive cells (months 18-24)

Task 4 To identify genetic changes that associate with an altered phenotype within our huPrE/rUGS grafts (months 6-36)

a. Karyotyping of all cell lines derived from lesions observed in experimental animals and grafts (months 18-24)
b. CGH analysis of cell lines derived from metastatic tumors (months 6-18)
c. RT-PCR analysis combined with DNA sequencing of candidate oncogenes or tumor supressor genes (months 6-24)

Introduction

We are using a model in which **Human Prostatic Epithelial cells (huPrE)** are grown as a tissue recombinant with rat urogenital sinus mesenchyme (rUGM) and grafted back into the in vivo environment of an intact male athymic rat host. Manipulations of the **huPrE** allow us to examine the effects of retroviral transfection with SV40T or with putative human oncogenes (such as TSPY) within the **huPrE** and thus allow access to human tissue that has undergone the early events in cancer initiation, a stage at which tissue is very rarely recovered from human patients. It is highly desirable to study prostate cancer formation/progression in human prostatic epithelial cells, in an in-vivo setting, in order to minimize cell culture artifacts and more fully understand prostate cancer in vivo.

Key accomplishments

- Retroviral constructs have been constructed that allow transfer of TSPY or SV40ΔT into huPrE alongside the color marker EGFP. Cells can then be FACS to allow pure populations to be isolated for further study.
- The tissue culture conditions under which huPrE is infected with LZRS virus have been optimized and utilized in order to increase the retroviral gene transfer potential of the system.
- Immunocytochemistry has confirmed the expression of the 'retrovirally introduced transgene' (fig1) and expression of the color marker confirming the activity and efficiency of the Ires sequence.
- Western blotting has likewise been utilized to identify expression of the TSPY protein (fig2) and EGFP (fig2). Western blot analysis on the SV40ΔT expressing cells confirm the expression of only the large T antigen as expected (Small T antigen is deleted within our construct that was kindly contributed by Dr Matusik)
- Fluorescent microscopy has been utilized to detect both EGFP and DSRed2 (Fig3). FACS analysis for EGFP is standardized at the VA FACS core and we are about to begin optimization for DSRed 2.
- The components of the 3rd generation of LZRS constructs (fig 4) are fully assembled and we are at this point in time sequencing several potential LZRS clones
- Xenografts of huPrE and rUGM maintained in a rodent host for 45 weeks with testosterone and estradiol treatment are under histologic and IHC analysis (see fig 5 for preliminary data)
- Xenografts of [huPrE +TSPY-Ires-EGFP] and rUGM at 3 months (fig 6) and 6 months (not yet analyzed) have been harvested and are awaiting a full IHC analysis. To date no gross morphological changes have been observed however normal architecture has been obtained.
- Owing to the move from UCSF to Vanderbilt the steroid receptor localization and quantification study has been on hold while IHC conditions were evaluated using different equipment and new lot No of antibodies. We are now ready to proceed with this task.

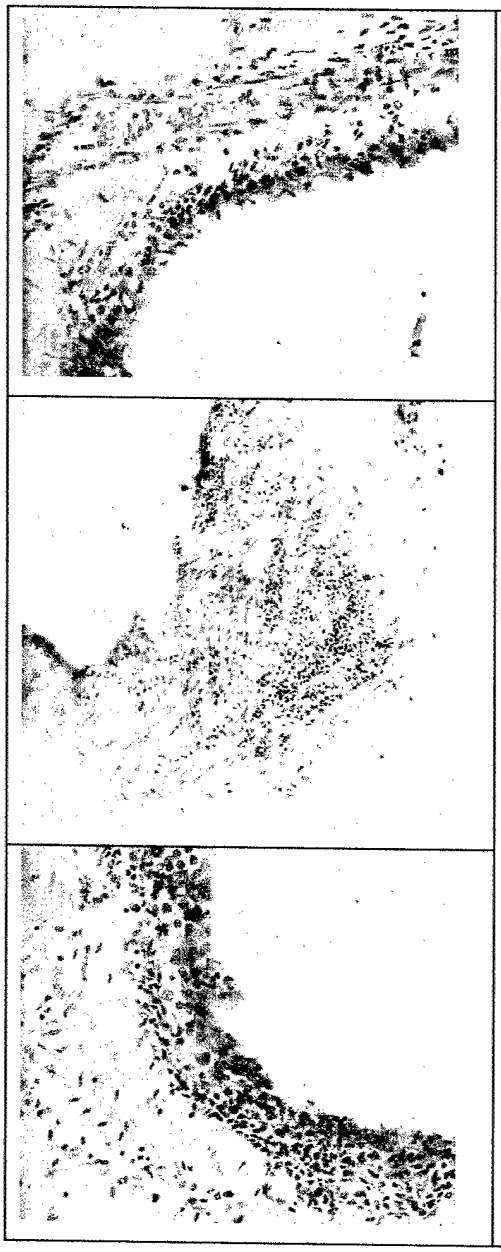


Fig 5

Xenograft of human Prostatic Epithelium recombined with rat urogenital mesenchyme and grafted into a hormone treated (testosterone & estradiol) nude rat. The grafts were harvested after 45 weeks and examined by Dr Scott Schappell a urogenital pathologist with extensive expertise in xenograft and mouse models of prostate cancer. Top panel shows the presence of a major blood vessel that has maintained the graft. The epithelium displays a nuclear atypia with prominent nucleoli. The position of the nucleus is atypical and the cellular organization is atypical. Middle panel displays shows the lymphocytic infiltrate which is often associated with malignant transformation within the xenograft model. lymphocytic infiltrate is not normally present in benign xenografts. Lower panel shows the squamous differentiation that is induced as a result of hormonal treatment. Again the nuclear atypica is present in the epithelium.

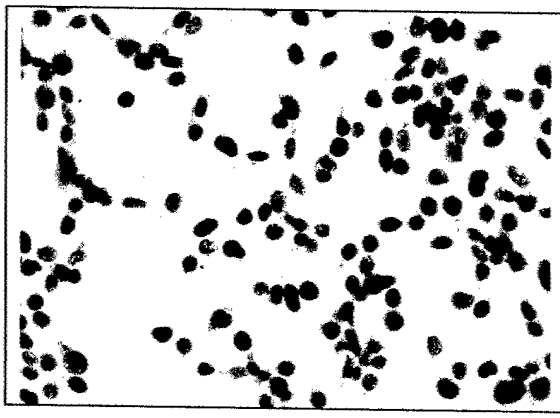


Fig1

Immunocytochemistry demonstrating the expression of SV40T in huPrE cells infected with a retrovirus carrying the SV40ΔT Ires EGFP construct. Immuno positive cells displayed EGFP expression visible on a fluorescent microscope (data not shown).

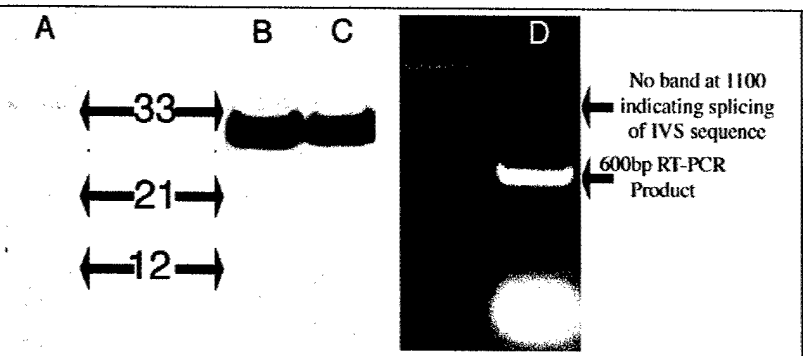


Fig2
Western blot analysis for TSPY (lane A) and GFP (lane B & C), demonstrating specific localization of a 33kDa protein (TSPY) or 27.5kDa (GFP). MW marker values are shown in kDa. Lane A and B contain samples infected with a retrovirus carrying the cmv-TSPY-Ires-GFP construct. Lane C contains a sample derived from cells infected with a virus carrying the GFP construct alone driven from the LTR demonstrating the powerful constitutive promoter present in the 3'LTR (lane D) RT-PCR OF TSPY mRNA transcript. The absence of a band at 1100bp indicates that the amplification is of mRNA origin and not 'viral RNA' or integrated DNA. RT-PCR on non-infected cells was negative (data not shown)

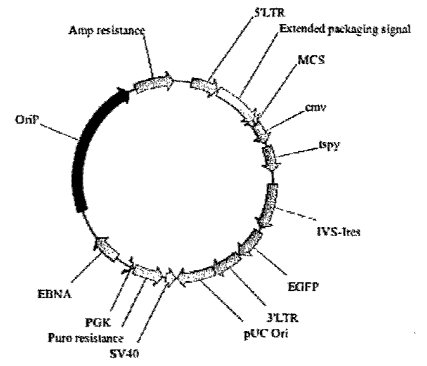
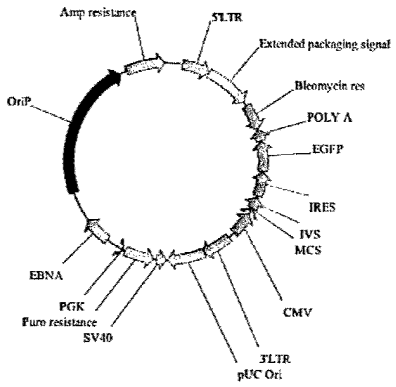


Fig4
Left 1st generation of plasmid that utilizes a color marker for selection by FACS. Right 3rd generation plasmid that has both a color marker and biochemical selection marker. The CMV may be replaced with a regulatable or tissue specific promoter



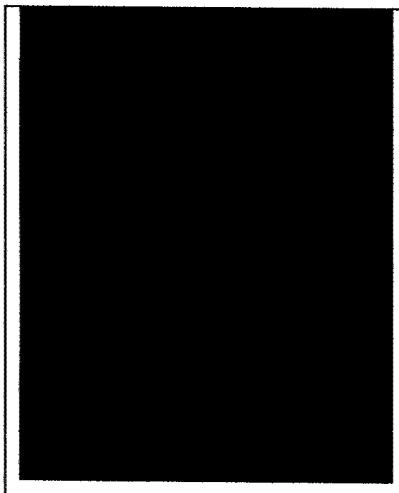


Fig3
Fluorescent microscopy of cells infected with either LZRS-DSRed2 or EGFP. We have incorporated several different color markers into the LZRS plasmid. This permits selection of cells into which more than one oncogene has been retrovirally integrated into the genome.

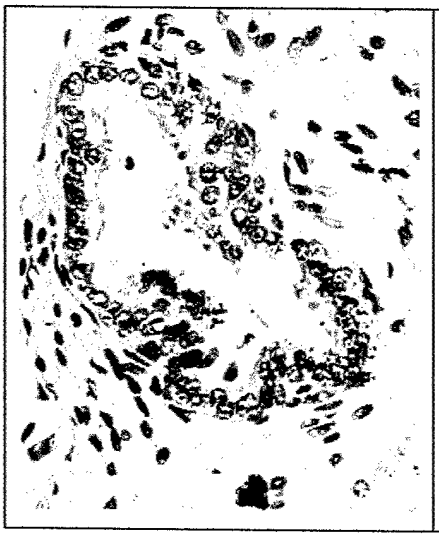
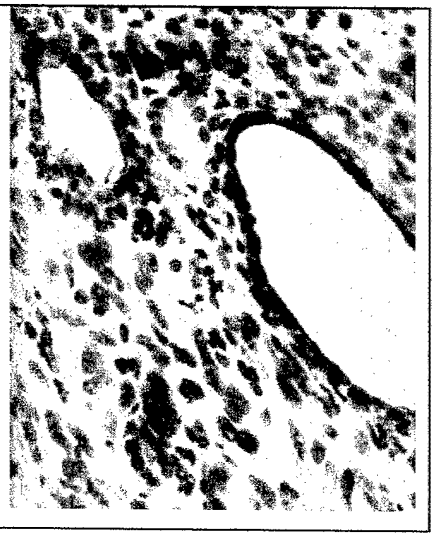


Fig 6
Left. H+E of [huPrE + TSPY-Ires-EGFP] and rUGM at 3 months post grafting.
Right IHC of a retrovirally infected cell line demonstrating our ability to detect EGFP expression within a xenograft model



Training

I have attended and participated in the 2001 SBUR/ESUR Fall Meeting /4th World Congress in and the 2002 SBUR fall meeting both Tucson, AZ USA. I presented posters at both meetings and was the recipient of a travel award at the 2002 meeting.

In July 2002 I was accepted to the AACR Pathobiology of Cancer 'Cancer Camp' in Keystone Colorado. This was a laboratory-based workshop at which I presented a poster and attended several round table discussions, while receiving training in the histopathologic and gross characterization of human cancer.

I was also a participant at the Joint Host-Tumor Interactions Program of the Vanderbilt Ingram Comprehensive Cancer Center & the Department of Cancer Biology Retreat, Lake Barkley, Kentucky. In 2001 I gave an oral presentation and in 2002 I presented a poster.

Laboratory meeting discussions and journal club presentations are integral to the running of the department as is interaction with a branching morphogenesis journal club run by the Department of Nephrology. I attend and have presented within this forum.

Lunchtime and evening seminars and mini symposiums as well as the Vanderbilt DOD Prostate Cancer Center Retreat also contribute to an overall training strategy in place within the department.

Reportable outcomes

Talks

November 16-17, 2001 Joint Host-Tumor Interactions Program & Department of Cancer Biology Retreat, Lake Barkley, Kentucky. Human prostate carcinogenesis initiated by Retroviral gene transfer.

Poster Abstracts

Karin Williams., Suzanne Fernandez, Kenichiro Ishii, Simon W Hayward. (2002) Retroviral gene transfer into human prostatic epithelium permits investigation of putative oncogenes in a tissue recombination model. SBUR Fall Meeting, Tucson, AZ USA Dec 5-Dec 8, 2002

Karin Williams., Fernandez S, Ishii K, Love H, Hayward S. November 15-16, 2002 2nd.Joint Host-Tumor Interactions Program & department of Cancer Biology Retreat, Lake Barkley, Kentucky. Retroviral gene transfer: a tool for the modern molecular biologist

Karin Williams., Chris Lau, Simon W Hayward.(2002) Human Prostate Cancer in a tissue Recombination model. Pathobiology of Cancer Keystone CO,USA July 14-21

Williams, K., Lau C, Hayward, S.W (2001) Human Prostatic Carcinogenesis Initiated by Retroviral Gene Transfer SBUR/ESUR 2001 Fall Meeting and the 4th World Congress in Urologic Research Tucson, AZ USA Nov 29-Dec 2, 2001

Book Chapters

Williams, K., and Hayward, S.W. Stem cells in glandular organs. In: Stem Cell Handbook (Ed Sell, S.) Humana Press, Totowa, N.J. (in press)

Reagents Generated

A number of modified LZRS constructs have been generated within this project they are in use within our laboratory for a number of projects and by Cancer researchers within Vanderbilt University including the laboratory of Professor Moses. The Ires DSRed2 construct is also of considerable interest to several other laboratories as the commercially available plasmid is unsuitable to shuttle into LZRS.

Appendices

Registration Form

Host-Tumor Interactions Program & Department of Cancer Biology

2nd Annual Joint Retreat

Please fill out completely. Return completed forms to michael.a.davis@vanderbilt.edu.

Name:	Karin Williams
Lab:	AA1302
Telephone number:	2-5883
e-mail address:	karin.williams@vanderbilt.edu
Campus Address (including 4 digit zip):	AA1309 Dept. Urologic Surgery, MCN 37232-2765

Check one in each group.

1) Male: Female:x

2) Staff: Student: Post-Doc:x Faculty:

All rooms are doubles. PI's will be given a room to themselves. All others must share a room.

Indicate if you will need a room for Friday, November 15th. Yes: x No:

Please list someone with whom you would like to share a room. Suzanne Fernandez

Your abstract, including the title and authors, must fit in the following box. The font must be 12pt "Times New Roman."

RETROVIRAL GENE TRANSFER: A TOOL FOR THE MODERN MOLECULAR BIOLOGIST

Karin Williams, Suzanne Fernandez, Kenichiro Ishii, Harold Love, Simon Hayward.

Retroviruses are capable of stably introducing foreign DNA into the genome of eukaryotic cells. We have modified the LZRS/Phoenix system that was developed at Stanford by Gary Nolan to permit the use of tissue specific promoters, color visualization and biochemical selection of infected cells. Rapidly dividing cell lines give exceedingly high integration rates although in our hands some lines e.g. LnCaP are reasonably resistant to infection. Primary cell cultures maintained for short periods of time have shown infection rates that appear to exceed 50%.

We have developed a great many LZRS-based retroviral vectors primarily to investigate the response of human/rodent prostate cells to genes that are under investigation for their potential role in prostate cancer initiation and progression. We have inserted SV40T, TSPY & the TGFβ RII dominant negative genes into primary cultures of human prostatic epithelium. Due to the limitations of tissue culture in providing a physiological environment for cells we have utilized the cell recombinant xenograft model to assess the in vivo effects of these genes. In this model we utilize the kidney capsule of an immunologically compromised host (athymic or SCID) as a well-vascularized physiological incubator. We can alter the hormonal environment of the host; introduce chemotherapeutic agents or carcinogens to the animal and thus to the graft that it harbors. The recombinant cells continue to express the 'transgene' in the graft and the grafts respond to the alteration in hormonal environment for example by androgen ablation or testosterone/estrogen supplementation.

A second class of retroviral constructs is presently under construction in which siRNA technology is being introduced to silence genes in cells. The U6 promoter makes active RNA molecules which can be targeted towards specific mRNAs resulting in destruction of the RNA and resultant loss of protein expression. This technology therefore allows us to down regulate genes of interest in both tissue culture cells and cells in tissue recombinants. One important power of this technique is the ability to generate specific gene deletions, which might be lethal in whole animals.

Society for Basic Urologic Research Fall 2002 Meeting
Loews Ventana Canyon Resort Tucson, AZ
December 5 - 8, 2002

Abstract Deadline: October 15, 2002

RETROVIRAL GENE TRANSFER INTO HUMAN PROSTATIC EPITHELIUM PERMITS INVESTIGATION OF PUTATIVE ONCOGENES IN A TISSUE RECOMBINATION MODEL.

Karin Williams, Suzanne Fernandez, Kenichiro Ishii, Simon W Hayward. Department of Urologic Surgery, MCN, Vanderbilt University Medical Center, Nashville TN USA

In vivo models based on human prostatic epithelial cells (hPrE) will provide powerful tools with which to investigate cancer initiation and progression. Tissue recombinations (TR) composed of hPrE and rat urogenital sinus mesenchyme (rUGM) grafted beneath the renal capsule of immunocompromized rat hosts recapitulate many key events in prostatic development and adult function. The stable integration of known or putative oncogenes into the hPrE component of such TRs is a powerful tool with which to study the effects of these genes within the hPrE. This has been accomplished using a retrovirus capable of infecting hPrE in vitro. The SV40 large T antigen (a known oncogenic protein) and TSPY, a protein related to the NAP1 oncogene, have been introduced into hPrE. TSPY is not expressed in non-malignant prostate but is aberrantly present in low and high-grade human prostate cancer, suggesting a possible role in carcinogenesis.

The high efficiency LZRS/Phoenix retroviral system was used to insert the TSPY or SV40T gene under the control of either the constitutively active cmv or the prostate-specific ARR2 probasin promoter into hPrE. TR composed of infected hPrE and rUGM were made and grafted into athymic rats. Hosts were sacrificed after carrying the grafts for periods of 4-16 weeks.

Genes under the control of the cmv promoter are expressed at the time of grafting. We have visualized reporter gene expression at this time and confirmed transgene expression. Expression of GFP in grafted tissue has been detected by innunohistochemistry. The probasin promoter requires that the TR be fully differentiated, a state acquired at 12-18 weeks post grafting. Initiation of SV40T and TSPY expression occurs in this timeframe. The grafts are being monitored histologically to determine the timing of key tumorigenetic events.

The present study demonstrates the feasibility of the TR approach to creating a 'transgenic' hPrE within an in-vivo hormonally manipulatable system. Locally growing and any metastatic cells that arise can be genetically and morphologically distinguished from host cells by their species of origin (human). In addition each clonally derived cell cluster can be examined according to its site of gene integration. We have developed a new technology that bridges animal models and human tissue culture and allows us to examine human prostate cancer initiation in a manipulatable in-vivo system.

Person to whom correspondence should be directed:

Name: KARIN WILLIAMS E-mail: karin.williams@vanderbilt.edu

Full address: Department of Urologic Surgery, Vanderbilt University Medical Center, AA1309 , Medical Center North 1161 21st Ave. S Nashville TN 37232-2765 USA

Phone: ——— Fax: ———

Name of person presenting abstract: KARIN WILLIAMS

Would you like your abstract to be considered for a travel award? [x] Yes [ ] No

Instructions:

(1) Please type abstract single-spaced and within border.
(2) Title should be typed in uppercase letters; do not list academic degrees. Type authors names, institution, city, and country in upper and lower case letters. Leave no margins.
(3) Send original abstract and 3 copies to: SBUR Fall 2002 Meeting, Department of Urology, 2193 MEBRF, University of Iowa, 375 Newton Road, Iowa City, IA 52242. (4) Deadline for submission is **October 15, 2002.** For more information, please contact Linda Buckner at (319) 356-4275 or by fax at (319) 335-6971.