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14. ABSTRACT

Prostate carcinogenesis is a multi-step process resulting in the transformation of prostatic epithelial cells into invasive carcinoma and metastasis. In recent years, mouse models have emerged that recapitulate salient features of prostate carcinogenesis found in human disease. These models illuminate the molecular events that result in transformation and disease progression. In addition, mouse models can be used to identify molecular targets and test chemotherapeutic agents that may alter the course of disease. We have generated a new mouse model to further delineate targets that may halt cancer progression and lead to regression of disease. Crossing the TRAMP mouse with the PSCA-TVA transgenic mouse has resulted in the TRAMP-TVA mouse that is destined to develop prostate cancer and expresses the avian viral receptor, TVA, on prostate cancer cells. This new transgenic mouse should enable specific gene transfer of imaging genes and small hairpin nuclear RNAs (shRNAs) resulting in knockdown of specific targets. TRAMP-TVA mice demonstrate PIN lesions at 8 weeks and develop adenocarcinoma at 6-15 months. We have been able to demonstrate PSCA-driven expression of the TVA viral receptor in these lesions. Intraperitoneal injection of virus containing the luciferase gene results in luminescence signal in the prostate. Further development of this model will enable the effect of target gene knockdown via RNA interference to be monitored non-invasively in mice. This approach will facilitate high throughput analysis of potential shRNA targets.

15. SUBJECT TERMS
Prostate Cancer, TRAMP, shRNA, TVA, viral gene transfer

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INTRODUCTION:

Prostate carcinogenesis is a multi-step process that results in the transformation of prostatic epithelial cells into invasive carcinoma with the ability to metastasize to distant sites. Along the continuum of prostatic cellular transformation, several phenotypes are observed, including benign hyperplasia, prostatic intraepithelial neoplasia (PIN), and invasive carcinoma. In recent years, numerous mouse models have been generated that recapitulate some of the salient features of prostate carcinogenesis that are found in human disease[1-3]. These models are critical to our understanding of the molecular events that result in transformation and disease progression. In addition, mouse models can be used to identify molecular targets and test chemotherapeutic agents that may alter the course of the disease. The overall goal of this proposal is to utilize an established, well characterized mouse model of prostate cancer to further delineate molecular targets that may halt cancer progression and/or lead to regression of metastatic disease. In order to rapidly evaluate a variety of select target genes in the long-term, we have created a new transgenic mouse, the TRAMP-TVA mouse, which allows for efficient and specific gene transfer of imaging genes into prostate epithelial cells that are destined to form cancer. The use of small hairpin nuclear RNAs (shRNAs) in this model may result in target gene knockdown and the ability to assess the effect of this action on prostate cancer development and progression in mice, non-invasively over time.

BODY:

The overall goal of this proposal is to utilize an established, well characterized mouse model of prostate cancer to further delineate molecular targets that may halt cancer progression and/or lead to regression of metastatic disease. In order to rapidly evaluate a variety of select target genes, we created a new transgenic mouse (TRAMP-TVA) to allow for efficient and specific gene transfer of imaging genes with small hairpin nuclear RNAs (shRNAs) that can result in target gene knockdown. The development of this mouse may enable prostate cancer development, progression, and the effect of shRNA introduction to be monitored non-invasively in mice over time.

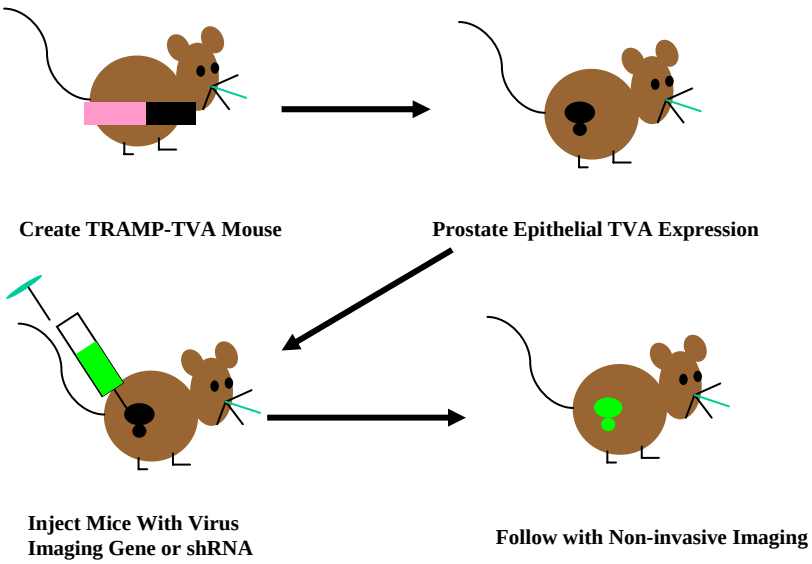

Figure 1: Viral-mediated gene transfer of TRAMP-TVA transgenic mouse. The TRAMP-TVA mice express the TVA avian viral receptor in prostate tissue under the direction of the PSCA promoter. Genetically engineered avian viruses containing the luciferase imaging gene and/or shRNA directed at specific target genes are intraperitoneally or orthotopically injected. Mice are imaged for reporter gene expression with change in tumor development/progression over time.

The concept of the TRAMP-TVA mouse model is shown in Figure 1. We have previously generated a PSCA-TVA mouse that utilizes the PSCA promoter to drive expression of the avian viral receptor, TVA, in prostate epithelial cells (see original proposal). This receptor is required for efficient gene transfer of avian viruses that are genetically engineered to express imaging genes, oncogenes, or shRNAs. The advantages of the TVA system include stability of gene transfer (viral genes integrate into host genome) and the ability to introduce multiple genetic changes into a single cell via multiple rounds of infection. The TRAMP mouse is a well-established mouse model that utilizes the prostate-specific probasin promoter to drive expression of the SV40 large T antigen, resulting in PIN, adenocarcinoma, and eventually, metastatic disease in male mice[1]. Previous work has demonstrated increased PSCA expression in prostate cancer cells from TRAMP mice[4]. Therefore, we predicted that prostate cancer cells from TRAMP-TVA mice would express high levels of the TVA receptor. The creation of TRAMP-TVA mice should enable transfer of genetic information via avian viruses in order to study the effect of shRNA on disease initiation and progression. Combined transfer of imaging genes (luciferase and/or GFP) will allow this process to be followed non-invasively over time. The specific aims of this project are listed below:

AIM 1: Establish a TRAMP-TVA transgenic line with characterization of prostate tumorigenesis as well as TVA expression within prostate cells

AIM 2: Non-invasive imaging of tumor development and progression in TRAMP-TVA mice

AIM 3: Demonstration of viral mediated shRNA delivery to TRAMP-TVA mice cancer cells

Tasks Involved in Accomplishing Aim 1:

1) Cross-breeding of TRAMP and PSCA-TVA mice

2) Confirmation of TVA expression and prostate tumor development in mice generated from the TRAMP-TVA cross

Materials/ Methods/Outcomes (Aim 1): In our first year of funding, we examined males generated from TRAMP-TVA crossbred mice for development of PIN and progression to prostate adenocarcinoma (Figure 2). Mice were genotyped by PCR for the presence of the TVA gene and SV40 large T antigen (data not shown). TRAMP-TVA mice were sacrificed at various timepoints to determine cancer initiation and progression. Immunohistochemistry with anti-TVA antibodies confirmed the presence of the TVA receptor expression in PIN lesions and well-differentiated adenocarcinoma of TRAMP-TVA mice (Figure 2A-B). Ki-67 activity was markedly increased in the TRAMP-TVA mice with PIN (Figure 2F). Proliferative activity is an important parameter in determining viral infection efficiency, since the avian retroviruses require cellular proliferation for infection.

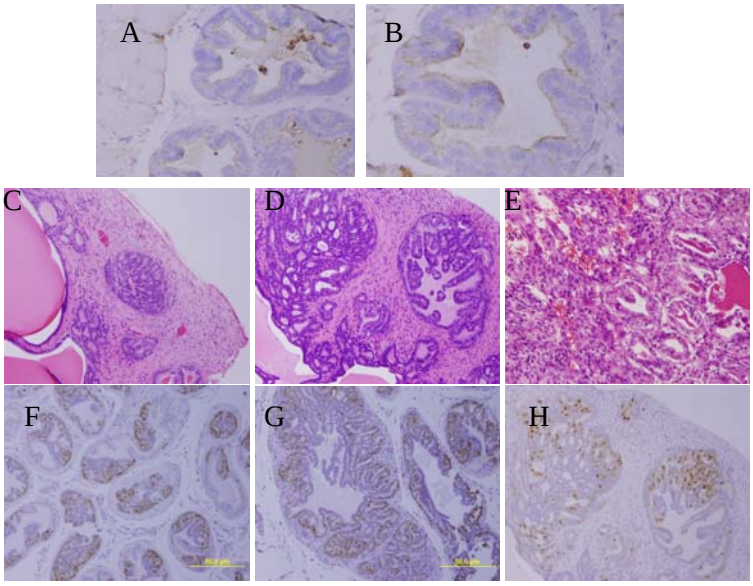

Figure 2: Prostate Epithelial Cell TVA Expression and Tumor Development and Progression in TRAMP-TVA Mice. TRAMP-TVA crossbred mice were confirmed by tissue genotyping and males were sacrificed at 8 weeks to determine TVA expression. The TVA viral receptor was demonstrated by immunohistochemistry on the apical surface of TRAMP-TVA mice (A&B). TRAMP-TVA mice develop PIN lesions by 8 weeks, as shown on H&E sections and with increased Ki-67 staining (C, F). These lesions progress to well-differentiated (D,G, H) and poorly differentiated adenocarcinoma (E) at 4 months and 12-15 months, respectively.

Issues encountered in 2007-2008 Year: We had some problems arise over the course of the past funding year. Our primary technician relocated, which resulted in breeding delays, while a new technician was hired and trained. We also endured breeding problems with the TRAMP mouse strain that persisted for approximately 3 months. We began observing mauled TRAMP pups by the birth mother. This problem was attributed to excess noise from a generator in the mouse room. With the assistance with the staff veterinarian in our mouse core facility, the problem was identified and solved by moving breeding cages to an alternate location. More recently, we began experiencing difficulty genotyping the TVA mice by PCR. We eventually determined that our primer set was unusually sensitive to degradation. Additionally, a contaminating band on generated by a faulty PCR reaction coincided with our expected product, resulting in genotyping errors, until the problem was recognized and solved by creating new primer sets. It has taken some time to evaluate and correct all of these issues, but currently we are back on track with breeding and genotyping TRAMP-TVA animals.

Tasks Involved in Accomplishing Aim 2:

1) Infection of TRAMP-TVA prostate epithelial cells with viral vectors containing luciferase at various timepoints in development
2) Imaging of infected TRAMP-TVA mice with charged coupled device (CCD) camera over time

Materials/Methods/Outcomes (Aim 2):

Previously we have shown that intraperitoneal and orthotopic injection of avian virus containing luciferase is capable in infecting the prostate of PSCA-TVA mice (see original proposal). In our first funding year, evaluated imaging gene transfer into prostate tissue of TRAMP-TVA mice. IP injections were performed in

TVA mice at 2-days, 1 month, and 3 months of age. These IP injections were performed daily for 7 days. Mice were imaged with the CCD camera following the last day of injection and showed photon emission from the pelvis that correlated with prostatic infection (data not shown). CCD imaging 4-months after the initial injection demonstrated durable prostatic luciferase expression (Figure 3A). Pelvic exploration and prostate exposure confirmed all signal was being emitted from infected prostatic tissue (Figure 3B). When IP injections were performed in mice over the age of one month, we were not able to observe luciferase expression, despite escalation in viral titer and increased number of injections (data not shown). The reason for the inability to demonstrate efficient gene transfer in older mice could be due to the mature immune system resulting in neutralization of virus when given IP.

A. B.

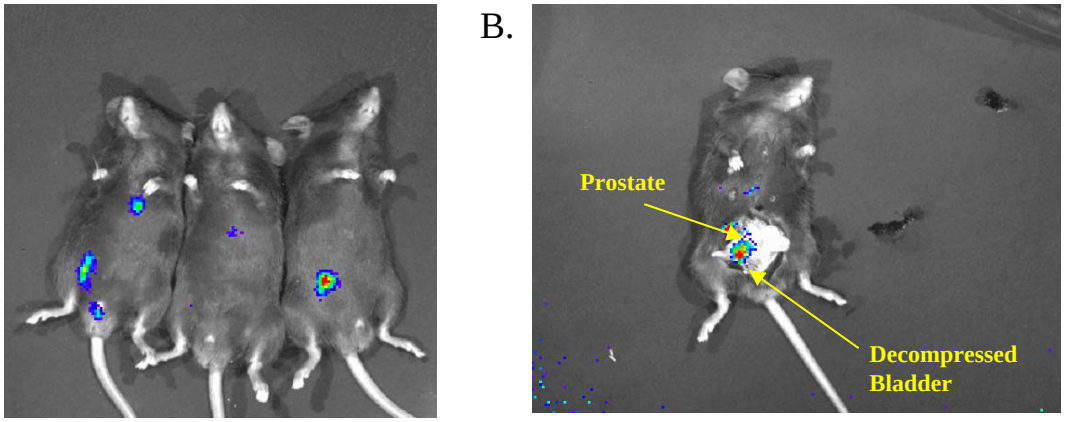

Figure 3: TRAMP-TVA mice intraperitoneally injected with avian virus containing the luciferase gene at 2 days of age were imaged with the CCD camera four months later for stable luciferase activity in the prostate (A). Two/three mice demonstrated signal in the area of the prostate. One of the mice was opened to expose the prostate and confirm the sight of the signal. The bright-red signal was indeed emitted from the prostate and can be compared to the lack of signaling seen from the bladder. TVA is also expressed in the stomach of TRAMP-TVA mice, so some luciferase activity is noted this region as well.

Trouble-Shooting Approach in Year 2007-20008: In order to address the issue of inhibition of viral infection in immunocompetent mice, we decided to breed the TVA mice into a SCID background. Creating an immunodeficient TVA strain will likely enable the following; 1) efficient IP infection of TVA+ tissues in older mice; 2) multiple rounds of infection within a single mouse. We have begun to crossbreed SCID and TVA and have analyzed the first generation mice after IP injection at 1-month of age. For the first time, we are able to visualize a luciferase signal emanating from the prostate in the male mice (Figure 4). This result is very encouraging, and we predict that subsequent generations of SCID-TVA crossbred mice will result in our ability to establish the PSCA-TVA model that will enable multiple rounds of infection at various time-points in development with increased efficiency and intensity.

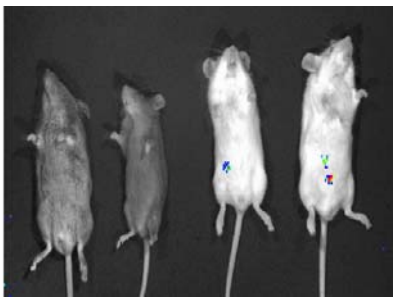

Figure 4: TVA mice were crossbred with SCID mice in order to generate a SCID-TVA cross that would demonstrate decreased immune response to intraperitoneal injection of TVA virus. Approximately 25% of first generation SCID-TVA mice injected with luciferase virus demonstrated pelvic luminescence that correlated with prostate tissue gene transfer.

Tasks Involved in Accomplishing Aim 3:

1) Creation of RCAS-shRNA vectors targeted against luciferase and SV40 large T antigen
2) In vitro and in vivo analysis of target gene knockdown by RCAS-shRNA viruses

Materials/Methods/Outcomes (Aim 3):

The goal of this aim is to create oligonucleotide sequences will produce shRNAs capable of target gene knockdown and clone them into the avian viral system for in vivo gene transfer into TRAMP-TVA mice. In our first funding year, we acquired a DNA vector that contains shRNAs against luciferase as well as a GFP reporter. Transient transfection of our viral producer cell lines has demonstrated a >80% knockdown in luciferase signal (Figure 5). We are currently in the process of cloning the DNA fragment containing the luciferase shRNA and GFP into our RCAS viral vector. This RCAS-RNAT vector should enable targeting of prostate epithelial cells in TRAMP-TVA mice that have been previously infected with luciferase. We anticipate that with sufficient infection efficiency, we will be able to visualize the emergence of GFP signal and the loss of luciferase. Once the cloning of this vector is complete, we will first confirm infection efficiency and luciferase knockdown capability in vitro in viral producer cells. Following this, we will perform in vivo infections in TRAMP-TVA mice previously infected with luciferase with prominent, stable activity in the prostate. Depending on the success of the luciferase knockdown experiments, we will go on to produce other shRNAs directed at specific molecular targets, such as SV40 large T antigen, and investigate the in vivo effect on prostate cancer initiation and progression using luciferase or GFP as a co-infected imaging gene.

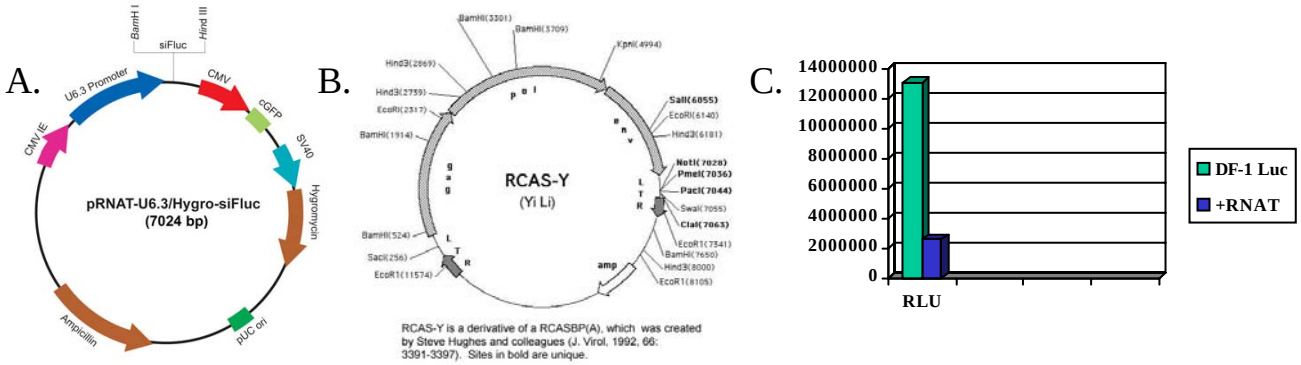

Figure 5: Construction of RCAS vector containing siRNA against Luciferase. A. RNAT vector (Genscript corporation) contains RNAi against luciferase driven by the U6.3 promoter and enhanced with the CMV enhancer. An approximately 2250 bp fragment was amplified from the RNAT backbone and inserted into the Cla and Pac sites (B). Transient transfection of luciferase-infected DF-1 viral producer cells were transiently transfected with RNAT and luciferase activity was measured after 72 hours (C). Greater than 80% reduction in luciferase activity was seen in cells containing RNAT than in controls.

The timeline initially outlined in the statement of work for the proposed experiments is shown below:

Year 1: Perform mouse breeding to generate cross, analyze mice for timeframe of tumor development and metastasis, confirm TVA expression within prostate cells and tumors, infect mice with luciferase and follow with imaging

Year 2: Continue with imaging experiments; optimize imaging protocol for orthotopic and IP injections, Begin shRNA design and cloning

Year 3: Complete cloning of shRNAs, test in vitro and in vivo, and follow mice with optical imaging

KEY RESEARCH ACCOMPLISHMENTS:

Generation of TRAMP-TVA Mice
Generation of SCID-TVA Mice
Analysis of Prostate Cancer Initiation in TRAMP-TVA mice

Confirmation of TVA Expression in Prostate Epithelial Cells of TRAMP-TVA mice
Prostate Viral-Mediated Luciferase Gene transfer of TRAMP-TVA mice
Prostate gene transfer into adult SCID-TVA mice
Cloning of Anti-Luciferase shRNA virus

REPORTABLE OUTCOMES: We have not yet submitted our results for publication, however, we are well underway to developing this model as a reproducible and efficient method for viral-mediated gene transfer of imaging genes and shRNAs into prostate epithelial cells. We presented a poster at the DOD Prostate Cancer Meeting in 2007 summarizing the results we have achieved thus far.

CONCLUSIONS:

Since initiation of funding, we have accomplished many of the goals outlined in the original statement of work, and despite several drawbacks encountered this past year, we remain on target with the proposed timeline. We have established a new mouse line that is capable of multiple rounds of viral-mediated gene transfer of imaging and other target genes. We hope that we will be able to apply this model of prostate cancer development and progression for the evaluation shRNA knockdown of a variety of genes.

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APPENDICES: N/A

SUPPORTING DATA: N/A