AWARD NUMBER: W81XWH-07-1-0318

TITLE: Dissecting Androgen-Dependent and Independent Signaling Pathways Using RNA Interference-Based Functional Genomics in Human Cells

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REPORT DATE: June 2009

TYPE OF REPORT: Final

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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**4. TITLE AND SUBTITLE**
Dissecting Androgen-Dependent and Independent Signaling Pathways Using RNA Interference-Based Functional Genomics in Human Cells

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**14. ABSTRACT**
We had previously identified androgen responsive genes in LNCaP prostate cancer cells using microarray technology. I have performed a high throughput loss of function screen using RNA interference (RNAi) in order to identify androgen responsive genes that are critical for androgen induced proliferation. I am currently investigating whether the genes identified in my screen function in prostate tumor progression. At the same time, in collaboration with scientists from the Broad Institute of Harvard and MIT, we screened by RNAi LNCaP cells among a panel of human cancer cell lines to identify synthetic lethal partners of oncogenic KRAS, and identified TBK1 as a gene that is essential in cells with mutant KRAS. The results from this study are currently in review for publication.

**15. SUBJECT TERMS**
Prostate cancer, RNAi, functional genomics, androgen signaling
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Introduction:

Prostate cells depend on a crucial level of androgenic stimulation for proliferation and survival. Although hormone therapy is the mainstream treatment for prostate cancer, the targets of androgen receptor signaling crucial for prostate cell growth have not been elucidated. The identification of androgen responsive genes and pathways that are critical for proliferation will shed light into the mechanism of androgen action, and yield insight into how cancer cells subvert regulation of controlled proliferation and become refractory to hormone manipulation. Using microarray technology, we had previously identified 58 androgen responsive genes in LNCaP human prostate cancer cells. In this project, I have conducted a loss of function screen in order to identify androgen responsive genes that are critical for androgen induced proliferation and also function in prostate cancer progression, with the objective of discovering novel therapeutic targets for the treatment of prostate cancer.

Given that many known oncogenes have proven to be challenging therapeutic targets, an alternative approach to direct targeting of known cancer alleles is to identify genes whose suppression selectively impair the viability of cells harboring an oncogenic allele. In collaboration with scientists from the Broad Institute, we screened by RNAi LNCaP prostate cancer cells among a panel of human cancer cell lines in order to identify synthetic lethal partners of oncogenic KRAS,

Body:

Task 1: Perform a loss of function screen to identify genes critical for androgen receptor stimulated cell proliferation

I have conducted a high throughput RNA interference screen in LNCaP cells to ablate the expression of androgen regulated genes. 300 short hairpin RNAs were used in the screen and were delivered into cells by lentiviral infection. PSA production and proliferation were monitored to identify androgen target genes critical for proliferation (Figure 1). Some of the genes that have scored in both the PSA production and the proliferation assays have been validated (Figure 2).
FIGURE 1: Androgen target genes critical for proliferation. Short hairpin RNAs (shRNA) targeting the expression of androgen regulated genes were delivered into LNCaP cells by lentiviral infection. Cell proliferation and PSA production were monitored using luminescence based Cell Titer Glo assay and ELISA assay, respectively, to identify androgen target genes critical for proliferation. shRNAs that reduced proliferation and PSA production to at least 1 standard deviation below the mean are shown. The Z score represents the number of standard deviations the observed phenotype is relative to the mean. Genes that scored in both of the assays are also indicated.

FIGURE 2: Ability of shRNAs to suppress target gene expression. The efficiency of gene silencing by each of the shRNAs that scored in the proliferation and PSA screens is being assayed by real-time PCR in triplicate to monitor correlation with functional phenotype. Relative mRNA levels are shown where expression was normalized to control GFP hairpin. Data from three representative assays are shown.
I also conducted a loss of function RNAi screen LNCaP cells as a part of a larger effort to screen 17 human cancer cell lines in order to identify genes whose suppression selectively affects the viability of cancer cells harboring oncogenic KRAS. Using a lentiviral library consisting of 5002 shRNAs targeting 957 genes encoding kinases and cancer related genes, we found that the suppression of the kinase TBK1 consistently induced apoptosis in cell lines with mutant KRAS (data not shown).

**Task 2:** Study the relevance of the validated genes in tumor progression and its possible role in the development of androgen independent prostate cancer

I have overexpressed the genes identified in Task 1 in immortalized and transformed prostate epithelial cells that my lab had previously generated (1). I have also overexpressed the genes in androgen dependent LNCaP and LAPC4 cells. I have conducted in vitro proliferation and soft agar assays to determine whether the genes play a role in prostate cancer progression (Figure 3, data not shown). I will be conducting in vivo xenograft experiments to corroborate the in vitro data.

![Cell proliferation graph](image1)

**FIGURE 3:** In vitro proliferation following C-MAF RNAi. LNCaP cells were infected with shRNAs targeting C-MAF and proliferation was monitored over six days using the luminesence based Cell Titer Glo assay. Immunoblotting was performed to monitor efficiency of gene silencing.

**Key Research and Training Accomplishments:**
1- Optimized conditions for conducting a high throughput screen in LNCaP cells.
2- Conducted an RNAi screen in LNCaP cells.
3- Identified a synthetic lethal partner of oncogenic KRAS.
4- Acquired significant expertise in manipulating high throughput genomic tools.

**Reportable Outcomes:**
Once I identify androgen induced genes that function in prostate cancer progression, I will write a manuscript describing the results for publication. The manuscript describing...
TBK1 as a synthetic lethal partner of oncogenic KRAS has been submitted for publication.

**Conclusions:**
My studies have identified a set of androgen regulated genes that are critical for androgen mediated proliferation. I am continuing to investigate whether these genes function in prostate tumor progression. I expect that I will achieve my objective of identifying genes that mediate androgen induced proliferation that also play a critical role in prostate cancer. In collaboration with scientists from the Broad Institute, my studies have also identified TBK1 as a therapeutic target in KRAS mutant cancers.

**References:**