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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 2008

TYPE OF REPORT:
Annual

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

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REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188		
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1. REPORT DATE (DD-MM-YYYY) 30-06-2008		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 01 JUN 2007 - 31 MAY 2008	
4. TITLE AND SUBTITLE Dissecting Androgen-Dependent and Independent Signaling Pathways Using RNA Interference-Based Functional Genomics in Human Cells			5a. CONTRACT NUMBER W81XWH-07-1-0318		
			5b. GRANT NUMBER PC060290		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Isil Guney, Ph.D. Email: Isil_Guney@DFCI.HARVARD.EDU			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Dana-Farber Cancer Institute 44 Binney Street Boston, MA 02115			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. SPONSOR/MONITOR'S ACRONYM(S)		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION / AVAILABILITY STATEMENT Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
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15. SUBJECT TERMS Prostate cancer, RNAi, functional genomics, androgen signaling					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 7	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

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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 validating the candidate genes identified in this screen and investigating whether these genes contribute to prostate tumor progression. When completed, my studies will lead to the identification and characterization of androgen regulated genes involved in prostate cancer.

Subject Terms: Prostate cancer, RNAi, functional genomics, androgen signaling

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 to identify androgen responsive genes that are critical of androgen induced proliferation.

Body:

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

Over the past year, I have optimized the conditions for and 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). I am currently validating the set of genes that have scored in both the PSA production and the proliferation assays. The ability of each of these shRNAs to suppress their target genes is also being investigated to ensure correlation with functional phenotype (Figure 2).

Proliferation				PSA			
Ranking #	shRNA #	Z score		Ranking #	shRNA #	Z score	
1	238	-2.04358262	fli	1	238	-2.00120233	fli
2	15	-1.9927052	primase	2	15	-1.93077739	primase
3	41	-1.93425357		3	41	-1.90604766	
4	55	-1.72476613	c-maf	4	242	-1.66981732	
5	142	-1.72083589		5	14	-1.66377084	primase
6	14	-1.71598933	primase	6	55	-1.66104829	c-maf
7	242	-1.71401911		7	142	-1.65487237	NM_023078
8	144	-1.71340008	NM_023078	8	144	-1.62384325	NM_023078
9	57	-1.60752595	c-maf	9	233	-1.56680068	clecsf12
10	233	-1.5909379	clecsf12	10	264	-1.55129102	AR
11	237	-1.55619949	fli	11	237	-1.54537979	fli
12	245	-1.55613389		12	57	-1.5324615	c-maf
13	37	-1.54607488		13	102	-1.5215086	maguk
14	157	-1.54605651	acaca	14	37	-1.50553134	
15	264	-1.5437582	AR	15	245	-1.50449086	
16	83	-1.54045956		16	83	-1.49537023	
17	56	-1.53146117	c-maf	17	56	-1.47464551	c-maf
18	102	-1.52228734	maguk	18	222	-1.46231418	
19	166	-1.49715269	abcc4	19	157	-1.45724105	
20	78	-1.48321925		20	78	-1.4196147	
21	222	-1.48285431		21	285	-1.41369496	
22	285	-1.47643278		22	166	-1.40970603	abcc4
23	247	-1.40205587		23	247	-1.36839171	
24	178	-1.35147852	od21	24	165	-1.28352793	abcc4
25	125	-1.34326435		25	178	-1.27301629	
26	165	-1.34295582	abcc4	26	111	-1.25493973	NM_018081
27	111	-1.27647673	NM_018081	27	229	-1.24749359	clecsf12
28	30	-1.25221369		28	263	-1.24446441	AR
29	254	-1.25075943		29	125	-1.22879595	
30	112	-1.24126944	NM_018081	30	112	-1.223623	NM_018081
31	263	-1.23886258	AR	31	254	-1.20515475	
32	158	-1.23723709	acaca				
33	176	-1.20657276	od21				

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.

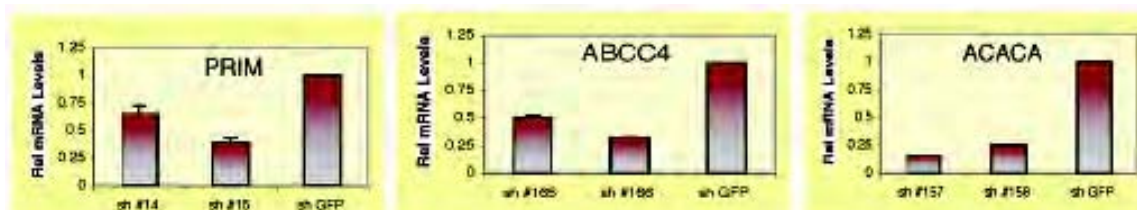


FIGURE 2: Ability of shRNAs to suppress target gene expression. The efficiency of gene silencing by each of the shRNAs that scored in the proliferation and PSA screens is being assayed by real-time PCR in triplicate to monitor correlation with functional phenotype. Relative mRNA levels are shown where expression was normalized to control GFP hairpin. Data from three representative assays are shown.

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

With the validation phase for Task 1 being near complete, I have begun to conduct preliminary experiments to undertake Task 2. 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 am conducting *in vitro* proliferation and soft agar assays to determine whether the genes that were identified in this screen play a role in prostate cancer progression. For example, c-Maf scored in the screen, and I have assessed the consequences of suppressing c-Maf in the androgen dependent cell line LNCaP (Figure 3 and data not shown). Additionally, I am assaying for the proliferation of the cells in androgen depleted medium to determine whether the genes function in androgen independence. I will soon be initiating *in vivo* xenograft experiments to corroborate the *in vitro* data.

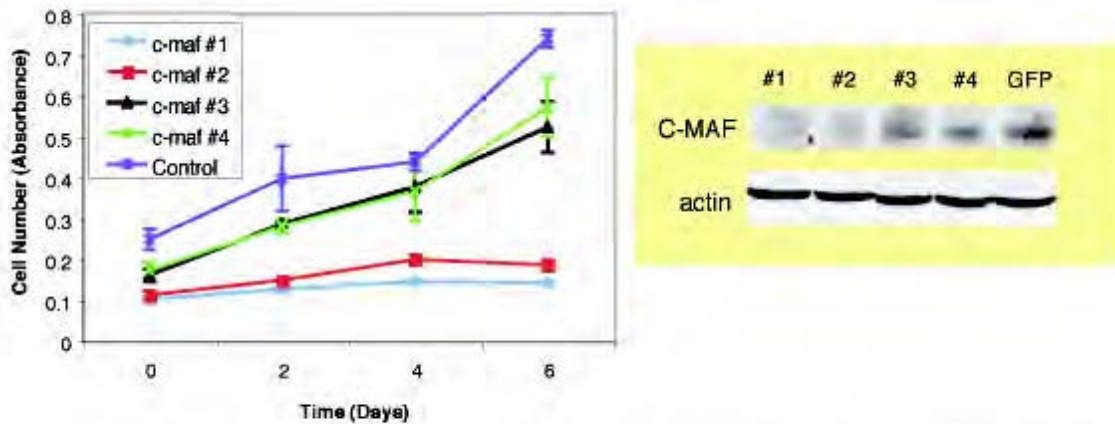


FIGURE 3: In vitro proliferation following C-MAF RNAi. LNCaP cells were infected with shRNAs targeting C-MAF and proliferation was monitored over six days using the luminescence based Cell Titer Glo assay. Immunoblotting was performed to monitor efficiency of gene silencing.

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- Acquired significant expertise in manipulating high throughput genomic tools.

Reportable Outcomes:

Once the aforementioned studies are completed, I will write a manuscript describing the results for publication.

Conclusions:

My studies have identified a set of androgen regulated genes that are critical for androgen mediated proliferation. I will continue my investigations as scheduled and 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.

References:

- 1- Berger et al. (2004) "Androgen-induced differentiation and tumorigenicity of human prostate epithelial cells." *Cancer Res* 64(24):8867-75.