

AD_____

Award Number: W81XWH-07-1-0147

TITLE: Role of Hsp90 in Androgen-Refractory Prostate Cancer

PRINCIPAL INVESTIGATOR: Zhou Wang, Ph.D.

CONTRACTING ORGANIZATION:
University of Pittsburgh

Pittsburgh, PA 15260

REPORT DATE: March 2009

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT:

Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 01-03-2009		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 1 March 2008-28 Feb. 2009	
4. TITLE AND SUBTITLE Role of Hsp90 in androgen-refractory prostate cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-07-1-0147	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Zhou Wang, Ph.D.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Pittsburgh Pittsburgh, PA 15260				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 Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT A major challenge in prostate cancer research is to develop novel therapies that can delay or prevent the progression of androgen-sensitive prostate cancer to androgen-independence. Androgen receptor (AR) is often overexpressed and plays an essential role in androgen-refractory prostate tumors. Our preliminary studies suggest that heat shock protein 90 (Hsp90) is required for androgen-independent AR nuclear localization in androgen-refractory prostate cancer cells. This project will test our research hypothesis that Hsp90 plays a critical role in ligand-independent AR nuclear localization, an essential step leading to androgen-refractory prostate cancer. In the last year of the funding period, we showed that Hsp90 deacetylation by HDAC6 is a critical step involved in castration resistance of prostate cancer cells.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 13	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4-11
Key Research Accomplishments.....	12
Reportable Outcomes.....	12
Conclusion.....	12
References.....	12-13
Appendices.....	13

Introduction

Prostate cancer (CaP) is the most common cancer and the second most common cause of cancer death among men in the United States (1). The androgen deprivation therapy (ADT) is the standard treatment for metastatic CaP. However, patients invariably recur with hormone-refractory or androgen-independent (A-I) CaPs. Effective treatment for A-I CaP is desperately needed for this lethal disease. Recent studies showed that, although the A-I tumors are androgen depletion independent, their growth is androgen receptor (AR)-dependent (2, 3). Novel strategies to inhibit ligand-independent AR activity may be effective against A-I prostate tumors. Androgen receptor (AR), a member of the steroid receptor superfamily, is a ligand-dependent transcription factor that controls the expression of androgen-responsive genes (4). Intracellular trafficking is an important mechanism in the regulation of many transcription factors, including AR. In order to access its target genes, a transcription factor requires localization to the nucleus. Likewise, retention of a transcription factor in the cytoplasm prevents its activity. Thus, a key regulatory step in the action of AR is its nuclear translocation. In androgen-sensitive cells, AR is localized to the cytoplasm in the absence of ligand. Upon addition of androgens, AR translocates to the nucleus and transactivates target genes. However, in A-I prostate cancer cells, AR remains in the nucleus even in the absence of androgen and transactivates androgen-responsive genes, leading to A-I growth of prostate tumors (2, 3). Our preliminary studies demonstrate that the Hsp90 inhibitor, 17-AAG, prevents A-I nuclear localization and activation of AR in the androgen-refractory C4-2 cells (5). This study will further determine the role of Hsp90 in ligand-independent AR nuclear localization and prostate cancer progression to A-I using the C4-2 androgen-refractory prostate cancer cell line as a model.

Body

Task 1: Determining the effect of HSP90 level on AR nuclear localization in the absence of androgen (months 1-18).

- A. Develop lenti-viral expression vector for HSP90 protein and siRNA specific for HSP90 (months 1-10)
- B. Infect LNCaP cells with HSP90 expression lenti-virus and assay for the effect of HSP90 overexpression on AR intracellular localization (months 10-18)
- C. Infect C4-2 cells with HSP90 siRNA lenti-virus to assay the effect of HSP90 downregulation on the intracellular localization of AR (months 10-18)

Lentiviral expression vectors are chosen to express Hsp90 protein and siRNA in this project because they offer high infection efficiency. We have purchased lentiviral vectors pCDH cDNA cloning vector (Cat# CD511A-1) and pSIH-H1 shRNA cloning vector (Cat# SI500A-1) from System Biosciences (SBI) (**Fig. 1**). Both vectors express green fluorescence protein (GFP), which allows us to monitor the infection efficiency conveniently. We have generated one lenti-viral vector that expresses Hsp90 protein and another one that expresses siRNA specific for Hsp90. Unfortunately, the lenti-viral Hsp90 protein expression vector had a stop codon in the Hsp90 open reading frame, as indicated in our previous progress report. We have recently corrected the mutation in the expression vector. We are in the process of generating and characterizing pseudoviral particles for Hsp90 overexpression.

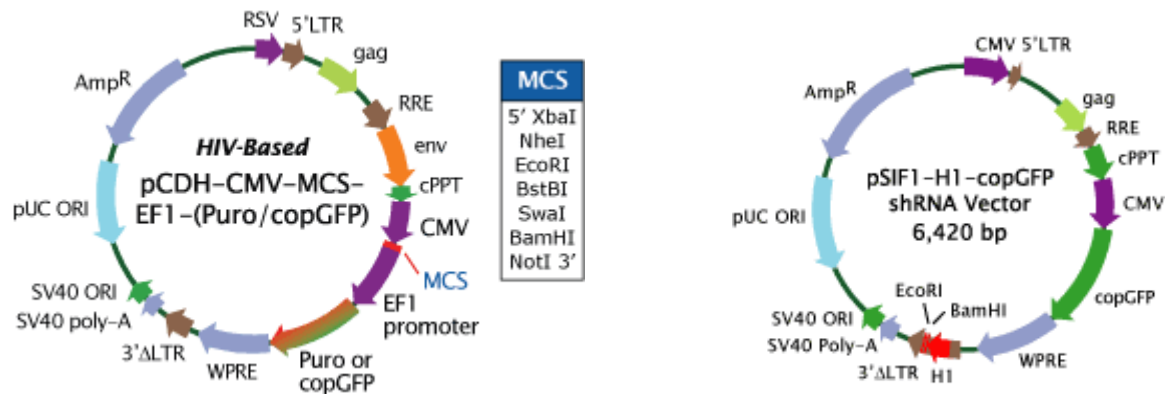


Fig. 1. Lenti-viral vectors. Left diagram shows the pCDH cDNA cloning vector (Cat# CD511A-1) for Hsp90 overexpression. This vector includes a CMV promoter before the multiple cloning sites and an EF1 promoter just before a green fluorescence protein (GFP) as a reporter for transduced cells. Right diagram shows the knockdown lentivector, pSIF1-H1 shRNA cloning vector (Cat# SI500A-1). This vector includes a CMV promoter for constitutive expression of GFP and an RNA polymerase III promoter to drive the expression of the siRNA sequence. Both vectors were purchased from System Biosciences (SBI) Mountain View California.

We have designed siRNA sequence using Integrated DNA Technologies RNAi online software tool (IDT, Coralville, IA). The sequence of siRNA specific for Hsp90 is 5'GGACCAGGTAGCTAACTCA3'.

To generate Hsp90 expression vector, the human Hsp90 cDNA sequence was PCR amplified using forward (For) and reverse (Rev) primers with indicated sequences:

For: 5' CAGGCTAGCACCATGCCTGAGGAAACCCAGACCCA 3'

Rev: 5' TCAGGCGGCCGCTTAGTCTACTTCTTCCATGCG 3'.

The amplified cDNA was restriction digested and cloned into the multiple cloning sites (MCS).

Lentiviral vectors were next packaged into packaging cell line 293TN (SBI, California). An RSV-5LTR or CMV-5LTR is included in each of the vectors for expression of constructs into the producer cell line (293TN). The pPACK H1 plasmids: pPACKH1-GAG, pPACKH1-REV, and PVSV-G provide all the necessary elements to produce VSV-G pseudoviral particles. These particles provide the most efficient method for high titer transduction into target cells.

The siHsp90 construct was sequence verified and packaged. The pseudoviral particles were transduced into C4-2 prostate cancer cells. Based on GFP expression, about 60% of the cultured C4-2 cells were transduced by siHsp90 lenti-viral particles in our experiments. We have isolated siHsp90 vector transduced C4-2 cells by fluorescence activated cell sorting (FACS) analysis. **Fig. 2** shows that FACS sorted C4-2 cells are positive with GFP expression. These GFP-positive cells were used to determine if siHsp90 lenti-viral infection is effective in down-regulating Hsp90 expression. The data thus far are very promising.

The down-regulation of Hsp90 mRNA by siRNA specific for Hsp90 was revealed by real-time PCR (**Fig. 3**). The primer pair used for Real-time PCR are 5' TCTGGAAGATCCCCAGACAC 3' (Forward) and 5' AGTCATCCCTCAGCCAGAGA 3' (Reverse).

Down-regulation of Hsp90 protein by siRNA in C4-2 cells was reproducibly observed in Western blot analyses using anti-Hsp90 antibody (**Fig. 4**). This result indicates that our siRNA lenti-viral vector is effective in down-regulating Hsp90 protein expression. Also, the expression of PSA was down-regulated in C4-2 cells with shHSP90 expression, supporting our research hypothesis that HSP90 enhances AR activity. However, the HSP90 levels rebounded after a few more passages (data not shown). We are trying to overcome this technical problem by investigating the timing and possible reasons of HSP90 rebound in C4-2 expressing shHSP90.

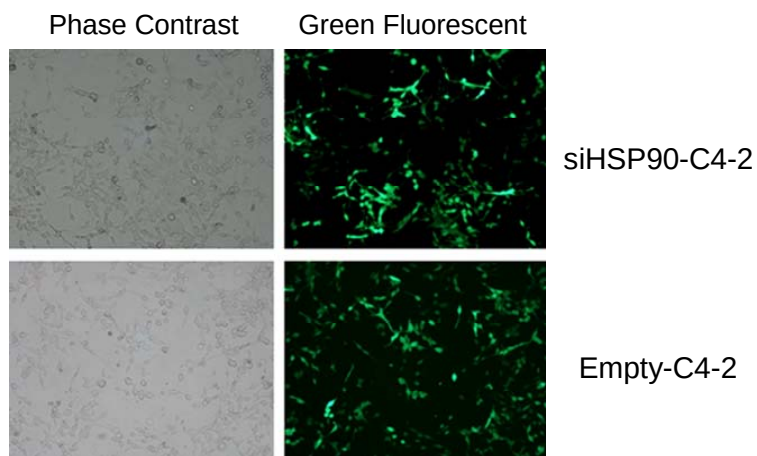


Fig. 2. C4-2 Cells Expressing GFP and siHsp90 Lentiviral Vector. Lentiviral Expression vector was packaged into 293TN cells. The viral particles were then used to infect parental C42 cells. Lentiviral vector was transduced and integrated into the genomic material of the parental cells. Cells expressing GFP include transduced lentivirus containing the sequence of interest. Since infections did not reaching 100% efficiency, the transduced cells were FACS sorted for GFP-positive cells.

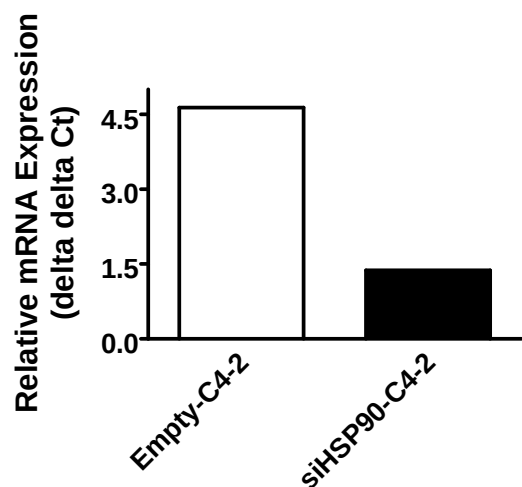


Fig. 3. Real-Time RT-PCR Analysis of Hsp90 mRNA expression in C4-2 Cells Transduced by Lentiviral siHsp90 Vector. Cells were harvested for at 90% confluency from a 6-well plate. RNA was isolated with Trizol® Reagent. cDNA synthesis and qPCR by Sybr Green were performed. The resultant graph is generated from one experiment done in triplicate. Expression of Hsp90 mRNA in siHSP90-C42 stable cells is knocked down by 3.4 fold relative to the empty vector infected control cells.

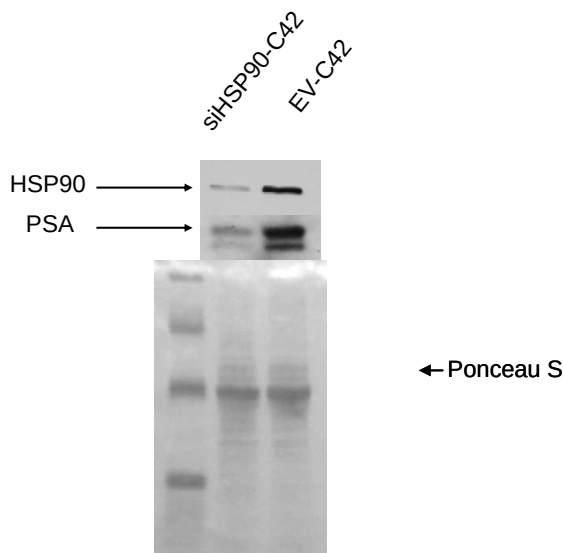


Fig. 4. Western Blot Analysis of Hsp90 Expression in C4-2 Cells Transduced by siHsp90 Lentiviral Vector. Cells were harvested in RIPA buffer containing protease inhibitors at ~90% confluency. Lysates were run on an SDS gel and transferred to membrane. Hsp90 was probed using anti-HSP90 $\alpha\beta$ (N-17). Empty vector infected C4-2 cells were used as a control in parallel.

We have tried to determine the endogenous AR localization in C4-2 cells infected with empty vector or with shHSP90 lenti-virus. However, immunohistochemical analysis revealed variable endogenous AR localization. Our results were not reproducible. Thus, we are doing troubleshooting now. Variability of our observations in AR localization could be due to the re-expression of HSP90 in the C4-2 cells infected with shHSP90. In fact, we showed that HSP90 expression was increased after several passages in C4-2 cells infected with shHSP90. Also, HSP90 expression can respond to stress, which could cause variability in our studies. While we are trying to resolve the technical difficulties, we explored alternative approaches to inhibit HSP90 activity. One mechanism of regulating HSP90 is its acetylation/deacetylation by HDAC6 (6-9). Thus, we decided to test the effect of HDAC6 on HSP90 acetylation and AR intracellular localization in C4-2 cells. One advantage is that HDAC6 knockdown did not affect cell viability (data not shown), which is consistent with the fact that HDAC6 knockout mice develop normally (10).

HDAC6 knockdown inhibits AR transcriptional activity.

To define the role of HDAC6 in the regulation of AR function, we established C4-2 cells stably expressing shRNA specific to HDAC6 using a lentiviral shRNA expression system. As shown in Figure 5A, HDAC6 protein level was knocked down approximately 50% as compared to the uninfected or control shRNA infected C4-2 cells. Moreover, protein level of HDAC10, which is most closely related to HDAC6 was not affected, suggesting that the shRNA effect was HDAC6 specific. We also observed that the protein levels of Hsp90 and AR were not affected by HDAC6 knockdown. HDAC6 knockdown inhibited both ligand-independent and androgen-induced PSA expression in C4-2 cells (Fig. 5A). Interestingly, DHT elevated PSA expression above basal levels in HDAC6 knockdown cells (Fig. 5A). This suggests that HDAC6 knockdown inhibits ligand-independent activation of AR while retaining androgen-responsiveness. Consistent with the results shown in Figure 5A, Real-time RT-PCR verified that HDAC6 knockdown inhibited PSA mRNA in C4-2 cells while retaining androgen-responsiveness (Fig. 5B). As expected, HDAC6 knockdown resulted in hyperacetylation of Hsp90 in C4-2 cells (Fig. 5C). However,

DHT treatment did not affect the level of acetylated Hsp90 (Fig. 5C). Expression of codon-switched HDAC6 expression vector, which is resistant to shHDAC6 treatment (Fig. 5D), restored PSA mRNA in HDAC6 knockdown C4-2 cells (Fig. 5E). This confirms that the shRNA effect is mediated through loss of HDAC6 and not by off target effects.

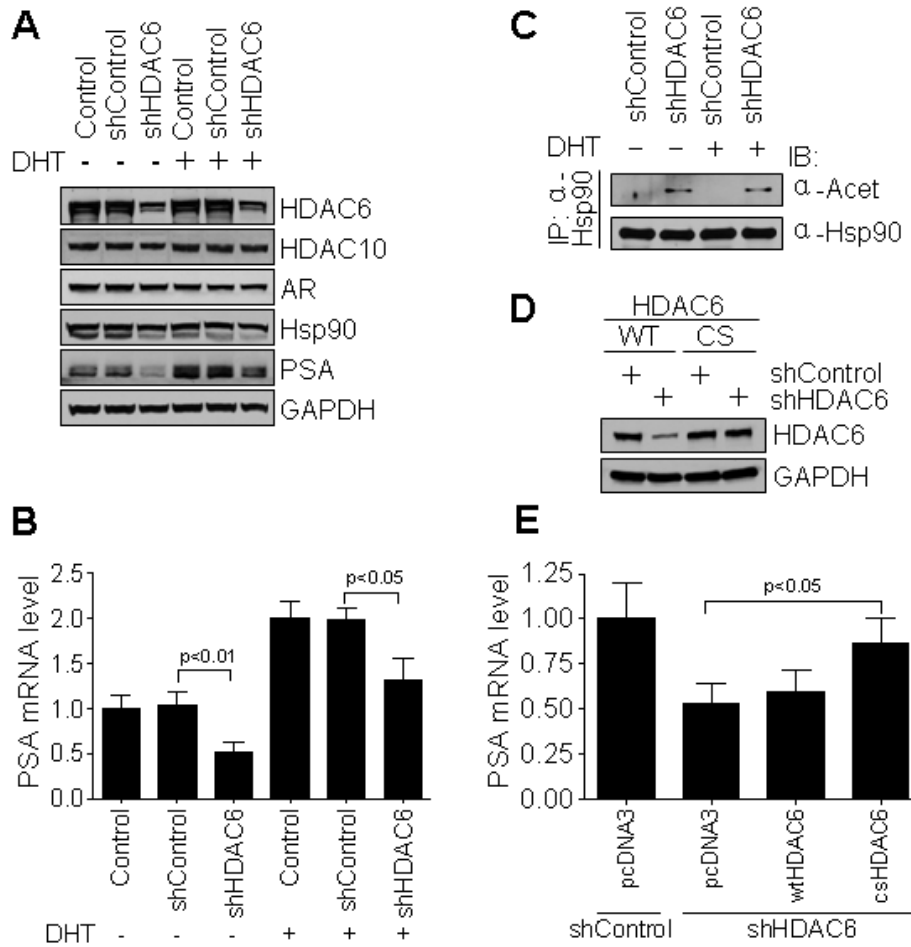


Fig.5. Effect of HDAC6 knockdown on endogenous AR expression and its transcriptional activity in prostate cancer cells. (A) C4-2 cells were infected with the lenti-virus encoding for shRNA to HDAC6 or Luciferase and stable cells were cultured in ligand-free conditions for 24 hours prior to treatment with (+) or without (-) 1nM DHT for additional 16 hours. Then the cell lysates were immunoblotted with anti-HDAC6, HDAC10, AR, Hsp90, PSA, or GAPDH antibody. (B) Total RNA isolated from C4-2 cells identically treated as Fig. 5A were used for real-time RT-PCR analysis to determine PSA mRNA level. (C) C4-2 cells with HDAC6 knockdown or shRNA control were cultured in conditions with or without DHT for 16 hours, and then Hsp90 was immunoprecipitated (IP) from the cell lysates and immunoblotted (IB) with either anti-Hsp90 or anti-acetylated lysine antibody. GAPDH served as loading control or internal control. (D) C4-2 cells were cotransfected with shHDAC6 or shControl vector and wild type (WT) or codon-switched (CS) HDAC6 expression vector and Flag-tagged HDAC6 expression was detected by anti-Flag antibody. (E) GFP positive cells were sorted and endogenous PSA mRNA was determined using CellsDirect One-Step qRT-PCR. Error bars represent $\pm$ SD. A P-value <0.05 or <0.01 was generated using an unpaired *t*-test in GraphPad Prism (GraphPad Software, Inc.).

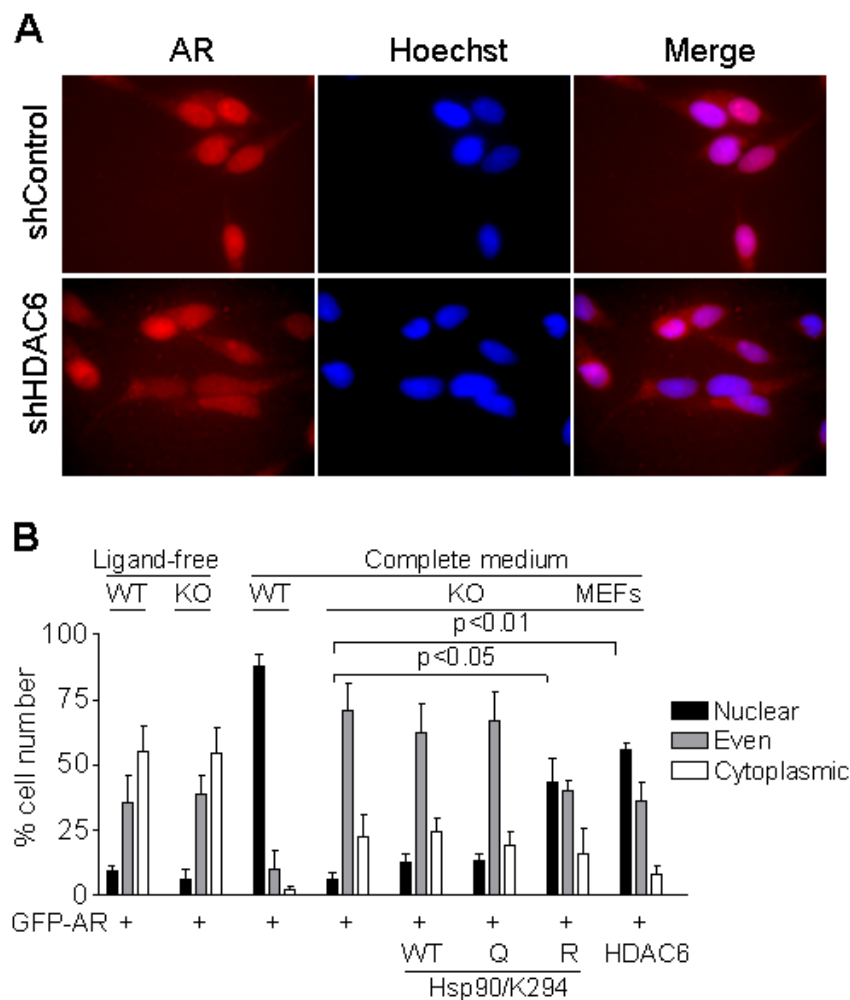


Fig. 6. Effects of HDAC6 knockdown or knockout on AR intracellular localization. (A) Control and HDAC6 knockdown C4-2 cells were cultured in ligand-free conditions for 24 hours and then the localization of AR was determined by immunostaining with an anti-AR antibody. Nuclei were stained with 1 μ g/ml Hoechst 33342. (B) Wild type and HDAC6 knockout MEFs were transfected with GFP-AR or HDAC6 knockout MEFs were co-transfected with GFP-AR and Hsp90 mutants or HDAC6 expression vector. Four hours after transfection, the cells were cultured in ligand-free or complete medium. Localization of GFP-AR was assessed by fluorescence microscopy after additional 16 hours culture. Results are the average of five experiments, where >100 cells were analyzed for each experiment. Error bars represent $\pm$ SD. A P-value <0.05 or <0.01 was generated using an unpaired *t*-test in GraphPad Prism (GraphPad Software, Inc.).

HDAC6 regulates AR nuclear localization by deacetylation of Hsp90.

We determined the effect of HDAC6 knockdown on the intracellular localization of AR in C4-2 cells. HDAC6 stable knockdown and shRNA control C4-2 cells were cultured in ligand-free conditions for 24 hours and then cultured in ligand-free medium or medium containing 1 nM DHT for additional 16 hours. Localization of endogenous AR was determined by immunofluorescence experiments. As expected, in ligand-free conditions, endogenous AR is predominantly present in the nucleus of C4-2 cells expressing shRNA control (Fig. 6A). However, in HDAC6 knockdown C4-2 cells, AR is evenly distributed between cytoplasm and nucleus. Treatment with 1 nM DHT for 16 hours was sufficient to prevent HDAC6 knockdown-induced cytoplasmic localization of AR (Data not shown). Thus, the loss of HDAC6 prevents ligand-independent nuclear localization of endogenous AR in C4-2 cells. To better define the role of HDAC6 and explore its mechanism in regulating AR trafficking, we utilized wild type (WT) and HDAC6 knockout (KO) MEF cells to examine the GFP-AR intracellular localization in the absence or presence of ligand. As shown in Figure 6B, no difference in GFP-AR distribution was observed between WT and HDAC6 KO MEFs when the cells were cultured in ligand-free conditions. In both types of MEF transfected cells, ~35% of the cells showed even localization of GFP-AR and ~55% of the transfected cells showed cytoplasmic GFP-AR. In complete medium, which contains ligand, ~86% of transfected WT MEFs exhibited nuclear GFP-AR, whereas ~18% and ~70% of the transfected HDAC6 KO MEFs displayed nuclear and even GFP-AR localization, respectively. As a control, co-transfection of HDAC6 and GFP-AR plasmids in HDAC6 KO MEF cells increased the percentage of transfected cells with GFP-AR nuclear localization from ~18% to ~50% in complete medium. These results indicate that depletion of HDAC6 impaired AR nuclear localization in complete medium. We next investigated the mechanism by which HDAC6 inhibition suppresses nuclear localization of AR. Since HDAC6 deficiency caused hyperacetylation of Hsp90 in C4-2 cells (Fig. 5C) and MEF cells (10), and the acetylation state of Hsp90 K294 is critical for its chaperone function (11), we used Hsp90 K294 mutants to determine whether acetylation/deacetylation of Hsp90 is responsible for HDAC6 mediated nuclear localization of AR. Expression of a wild-type or an acetylation mimic Hsp90 (Hsp90K294Q) slightly increased the percentage of nuclear GFP-AR, while a deacetylation mimic Hsp90 mutant (Hsp90K294R) markedly restored nuclear localization of GFP-AR, to the extent similar to the restoration by HDAC6 re-expression. These observations argue that defect of GFP-AR nuclear translocation caused by HDAC6 deficiency is mediated mainly through acetylation of Hsp90.

Task 2: Test the hypothesis that HSP90 modulates the sensitivity of AR-positive xenograft tumors to androgen ablation (months 10-30)

We were not able to test the effect of HSP90 knockdown on xenograft tumor growth because of phenotype changes of C4-2 cells expressing shHSP90. Specifically, HSP90 levels rebounded after a few passages in C4-2 sublines with shHSP90 expression. One alternative is to conduct the xenograft tumor studies using C4-2 cells with HDAC6 knockdown instead of with HSP90 knockdown. This is not surprising because HSP90 is essential for cell survival. Since HDAC6 regulates AR activity via HSP90 acetylation/deacetylation, we decided to test the effect of inhibiting HSP90 via blocking its deacetylation on C4-2 xenograft tumor growth.

HDAC6 knockdown inhibits C4-2 xenograft tumor development and growth accompanied by decreased PSA levels in xenograft tissues. To determine whether HDAC6 silencing could affect AR transcriptional activity and inhibit prostate cancer growth *in vivo*, we established human prostate xenograft tumors in nude mice (Fig. 7A). Both C4-2/shControl and C4-2/shHDAC6 cells generated tumors in six out of six (100%) intact mice (Fig. 7C). HDAC6 knockdown inhibited tumor growth in intact mice 6 weeks after injection compared with control (Fig. 7B). The means of the tumor volumes of C4-2/shControl ($2.12 \pm 0.33\text{cm}^3$) and C4-2/shHDAC6 ($0.96 \pm 0.26\text{cm}^3$) were significantly different ($p < 0.05$), suggesting that HDAC6 gene function is important to the growth of androgen-independent prostate xenograft tumors. The inhibition of xenograft tumor growth corresponded with a marked decrease in PSA expression levels in tumor tissues without affecting AR expression levels (Fig. 7D). This indicated that HDAC6 knockdown inhibited AR transcriptional activity even in intact male mice. No tumor was formed in castrated nude mice injected with C4-2/shHDAC6 cells while C4-2/shControl cells formed tumors in two out of six (33%) castrated mice (Fig. 7C). These findings argue that HDAC6 knockdown inhibited the establishment of castration-resistant prostate cancer *in vivo*.

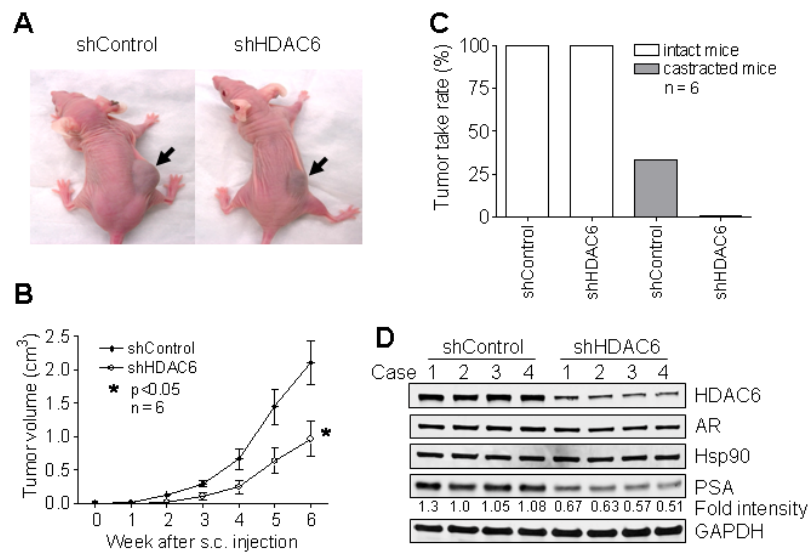


Fig. 7. HDAC6 knockdown inhibits C4-2 xenograft tumor growth. (A) Xenograft tumor formation in intact mice, indicated by an arrow. (B) Tumor growth is shown as a function of tumor volume at indicated days post s.c. injection. Error bars represent $\pm$ SEM. * A P-value < 0.05 was generated using an unpaired *t*-test in GraphPad Prism (GraphPad Software, Inc.). (C) Tumor take rate was calculated 12 weeks after s.c. injection. (D) Tissue lysates of xenografts collected from intact mice were immunoblotted with anti-HDAC6, anti-Hsp90, anti-AR and anti-PSA antibodies. GAPDH served as loading control.

Task 3: Determine the efficacy of HSP90 inhibitor in the inhibition of the prostate cancer progression to androgen-independence in LNCaP xenograft tumor model (months 20-36):

We will start Task 3 in the third year funding period.

Key Research Accomplishments

1. HSP90 acetylation is required for ligand-independent AR nuclear localization in C4-2 castration-resistant prostate cancer cells.
2. HDAC6 regulation of ligand-independent AR nuclear localization in C4-2 cells is mediated through HSP90 acetylation/deacetylation.
3. HDAC6 knockdown by shRNA inhibited establishment of C4-2 xenograft tumors in castrated nude mice.

Reportable Outcomes

1. Junkui Ai, Yajuan Wang, Javid A. Dar, Lingqi Liu, Joel B. Nelson, Zhou Wang: HDAC6 silencing inhibits androgen receptor activity and tumor growth in a xenograft model of human prostate cancer, Abstract accepted by American Urological Association 2009 annual meeting.

Conclusion

HSP90 acetylation/deacetylation, which is modulated by HDAC6, is a critical step involved in ligand-independent nuclear localization of AR and the development of castration resistance of prostate cancer cells.

References

1. Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., and Thun, M. J. Cancer statistics, 2008. *CA Cancer J Clin*, 58: 71-96, 2008.
2. Chen, C. D., Welsbie, D. S., Tran, C., Baek, S. H., Chen, R., Vessella, R., Rosenfeld, M. G., and Sawyers, C. L. Molecular determinants of resistance to antiandrogen therapy. *Nat Med*, 10: 33-39, 2004.
3. Zegarra-Moro, O. L., Schmidt, L. J., Huang, H., and Tindall, D. J. Disruption of androgen receptor function inhibits proliferation of androgen-refractory prostate cancer cells. *Cancer Res*, 62: 1008-1013, 2002.
4. Zhou, Z., Wong, C., Sar, M., and Wilson, E. The androgen receptor: an overview. [Review]. *Recent Progress in Hormone Research*, 49: 249-274, 1994.
5. Saporita, A. J., Ai, J., and Wang, Z. The Hsp90 inhibitor, 17-AAG, prevents the ligand-independent nuclear localization of androgen receptor in refractory prostate cancer cells. *Prostate*, 67: 509-520, 2007.
6. Kovacs, J. J., Murphy, P. J., Gaillard, S., Zhao, X., Wu, J. T., Nicchitta, C. V., Yoshida, M., Toft, D. O., Pratt, W. B., and Yao, T. P. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. *Mol Cell*, 18: 601-607, 2005.

7. Murphy, P. J., Morishima, Y., Kovacs, J. J., Yao, T. P., and Pratt, W. B. Regulation of the dynamics of hsp90 action on the glucocorticoid receptor by acetylation/deacetylation of the chaperone. *J Biol Chem*, 280: 33792-33799, 2005.
8. Bali, P., Pranpat, M., Bradner, J., Balasis, M., Fiskus, W., Guo, F., Rocha, K., Kumaraswamy, S., Boyapalle, S., Atadja, P., Seto, E., and Bhalla, K. Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis for antileukemia activity of histone deacetylase inhibitors. *J Biol Chem*, 280: 26729-26734, 2005.
9. Aoyagi, S. and Archer, T. K. Modulating molecular chaperone Hsp90 functions through reversible acetylation. *Trends Cell Biol*, 15: 565-567, 2005.
10. Zhang, Y., Kwon, S., Yamaguchi, T., Cubizolles, F., Rousseaux, S., Kneissel, M., Cao, C., Li, N., Cheng, H. L., Chua, K., Lombard, D., Mizeracki, A., Matthias, G., Alt, F. W., Khochbin, S., and Matthias, P. Mice lacking histone deacetylase 6 have hyperacetylated tubulin but are viable and develop normally. *Mol Cell Biol*, 28: 1688-1701, 2008.
11. Scroggins, B. T., Robzyk, K., Wang, D., Marcu, M. G., Tsutsumi, S., Beebe, K., Cotter, R. J., Felts, S., Toft, D., Karnitz, L., Rosen, N., and Neckers, L. An acetylation site in the middle domain of Hsp90 regulates chaperone function. *Mol Cell*, 25: 151-159, 2007.

Appendices

None.