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Award Number: W81XWH-06-1-0064

TITLE: The Role of HOX Proteins in Androgen-Independent Prostate Cancer

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REPORT DATE: November 2006

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. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-11-2006	Annual Summary	30 Oct 05 – 29 Oct 06

4. TITLE AND SUBTITLE
The Role of HOX Proteins in Androgen-Independent Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0064

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Colorado Health Sciences Center
Aurora CO 80045-0508

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: Our preliminary data demonstrated that HOXC8 and HOXC6 overexpression inhibits androgen receptor (AR)-mediated signaling in human prostate cancer (PCa) cells. Based on these findings, coupled together with previous reports demonstrating that homeodomain-containing proteins interact with and inhibit the histone-acetyltransferase (HAT) activity of the steroid receptor coactivators CBP and p3001, we hypothesized that HOXC8 inhibits AR-mediated signaling through inhibition of CBP/p300 HAT activity. In support of this hypothesis, we have recently shown that increased expression of CBP relieves HOXC8 induced inhibition of AR-mediated transcription in a dose dependent manner. Further, we have demonstrated by chromatin immunoprecipitation that hormone-induced histone acetylation at the androgen-responsive MMTV promoter in inhibited upon overexpression of HOXC8. We have created a series of PCa cell lines (LNCaP, DU-145, PC-3-AR and ALVA-31) stably overexpressing HOXC8. We wanted to demonstrate that HOXC8 inhibition of AR-mediated signaling is upheld in cells stably overexpressing HOXC8, not just in transient experiments. We have demonstrated that PSA induction is inhibited in LNCaP-HOXC8 when compared with LNCaP empty vector control cells. We have also performed various tumorigenicity assays in these HOXC8 overexpressing cells, such as cell proliferation, migration, invasion and anchorage independent growth. However thus far we have been unable to detect any significant difference between the HOXC8 overexpressing cell lines and control cell lines in these experiments. Because HOXC8 overexpression may be involved in early tumorigenesis, we believe that it will be important to perform similar tumorigenicity assays in cells lines derived from non-transformed "normal" prostate epithelial cells. We have therefore recently created cell lines stably overexpressing HOXC8 using RWPE-I and PWR-IE prostate epithelial derived cell lines. We are currently in the process of characterizing these cell lines..

15. SUBJECT TERMS
ANDROGENS, PROSTATE CANCER, HOMEOBOX PROTEINS, HOX, ANDROGEN INDEPENDENT PROSTATE CANCER

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	7	**19b. TELEPHONE NUMBER** *(include area code)*

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INTRODUCTION

In the androgen-responsive, normal prostate, essentially no expression of HOXC genes is seen. Our laboratory has previously shown that a subset of genes of the HOXC cluster are overexpressed in primary prostate tumors, metastases, and prostate cancer (PCa) cell lines[2]. Although the consequences of HOXC overexpression in the prostate are unknown, we have shown that HOXC6 or HOXC8 overexpression inhibits androgen receptor (AR)-mediated transcription in LNCaP cells. The goal of this work is to explore further the interplay between HOX expression and steroid receptor signaling, investigating both the underlying mechanisms and the consequences of HOXC overexpression on androgen action in prostate cells.

BODY

Two tasks were listed in the approved Statement of Work:

Task one: To characterize the consequences of HOXC expression on steroid signaling in human PCa cell lines.

Task two: To dissect the molecular mechanism of HOXC inhibition of androgen signaling.

Task One: To characterize the consequences of HOXC expression on steroid signaling in human PCa cell lines.

Our preliminary data demonstrated that HOXC8 and HOXC6 transient overexpression inhibits AR-mediated transcription of the androgen-responsive MMTV and probasin promoters in LNCaP PCa cells. We have since shown that transient overexpression of another homeobox containing protein, HOXB13, exerts a similar inhibition of AR-mediated transcription of the MMTV promoter (Fig. 1) and probasin promoter (data not shown) in LNCaP PCa cells. In order to demonstrate that HOXC8-induced inhibition of AR-mediated transcription is not somehow specific to LNCaP PCa cells, we have shown that HOXC8 overexpression results in similar inhibition of AR-mediated transcription of the MMTV promoter (Fig. 2) and probasin promoter (data not shown) in PC-3-AR PCa cells. The previous studies had been performed by transiently overexpressing HOX genes. In order to show that HOXC8 inhibits AR-mediated signaling when stably overexpressed in PCa cells, several PCa cell lines (LNCaP, DU-145, PC-3-AR, ALVA-31) were created in which HOXC8 was stably overexpressed by viral transduction. In reporter assays utilizing these LNCaP cell lines, AR-mediated transcription of the MMTV promoter (Fig. 3) and probasin promoter (data not shown) is inhibited in LNCaP-HOXC8 cells when compared with LNCaP control cells. In order to evaluate the impact of HOXC8 overexpression on androgen-mediated gene expression, hormone-induced PSA mRNA levels were analyzed by real-time PCR from LNCaP cells stably overexpressing HOXC8, compared with that of the control cell line. PSA induction is inhibited in LNCaP-HOXC8 cells when compared to LNCaP empty vector control cells (Fig. 4).

In order to further characterize the consequences of HOXC expression in the context of prostate cancer, we have utilized various tumorigenicity assays in LNCaP cells stably overexpressing HOXC8. These assays include cell proliferation (Hoechst staining), invasion and migration (Boyden chamber), and anchorage-independent growth (soft agar colony formation). Thus far we have been unable to detect any significant difference between the HOXC8 overexpressing cell lines and control cell lines in these assays (data not shown). Because HOXC8 overexpression may be involved in early tumorigenesis, we reasoned that it would be important to perform these tumorigenicity assays in cells lines derived from non-transformed

"normal" prostate epithelial cells. We have therefore recently created cell lines overexpressing HOXC8 using RWPE-I and PWR-IE prostate epithelial derived cell lines. We are currently in the process of characterizing these cell lines.

Task two: To dissect the molecular mechanism of HOXC inhibition of androgen signaling.

Our initial findings that HOXC8 and HOXC6 overexpression inhibit AR-mediated transcription in PCa cells, coupled with reported data demonstrating that homeodomain-containing proteins interact with and inhibit the histone-acetyltransferase (HAT) activity of the steroid receptor coactivators CBP and p300[1], lead us to hypothesize that HOXC proteins inhibit AR-mediated signaling through inhibition of CBP/p300 HAT activity. In support of this hypothesis, we have recently demonstrated that increased expression of CBP relieves HOXC8 induced inhibition of AR-mediated transcription of the androgen-responsive MMTV promoter in a dose dependent manner in LNCaP PCa cells (Fig. 5). Further, we have demonstrated by chromatin immunoprecipitation (ChIP) that hormone-induced histone acetylation (histone H4) at the androgen-responsive MMTV promoter is inhibited upon overexpression of HOXC8 in LNCaP PCa cells (Fig. 6, "H4" panel). Further, preliminary data suggests that hormone-induced AR loading at the PSA promoter is inhibited in the presence of HOXC8 (Fig. 6, "AR" panel).

KEY RESEARCH ACCOMPLISHMENTS

- Development and characterization of several HOXC8 overexpressing PCa and non-transformed prostate epithelial cell lines by viral transduction
- Successful implementation of ChIP analysis using transiently transfected target DNA
- Successful siRNA knockdown of HOXC8 protein levels in LNCaP PCa cells

REPORTABLE OUTCOMES

Abstract: HOXC Gene Expression Modulates Androgen- and Vitamin D- Mediated Actions in Human Prostate Cancer Cells; IMPaCT meeting, September 5-8, 2007.

CONCLUSIONS

We have extended upon our initial observations demonstrating that HOXC6 and HOXC8 inhibit AR-mediated transcription in LNCaP PCa cells in transient reporter assays to include congruent data from an additional HOX family member (HOXB13), an addition PCa cell line (PC-3-AR), and cell lines stably overexpressing HOXC8, all further demonstrating HOX inhibition of AR-mediated transcription in PCa cells.

We hypothesize that HOX proteins inhibit AR-mediated signaling through inhibition of HAT activity of the steroid receptor coactivators CBP/p300. In support of this hypothesis, we have demonstrated that increased expression of CBP relieves HOXC8-induced inhibition of AR-mediated transcription in a dose dependent manner. Further, we have demonstrated by ChIP analysis that hormone-induced histone acetylation at the androgen-responsive MMTV promoter in inhibited upon overexpression of HOXC8.

We have also performed various tumorigenicity assays in HOXC8 overexpressing PCa cell lines, such as cell proliferation, migration, invasion and anchorage-independent growth. Although we have been unable to detect any significant difference between the HOXC8 overexpressing cell lines and control cell lines in these experiments, we believe that HOXC8 overexpression may be involved in an early step in tumorigenesis, and therefore it will be critical to perform these assays in non-transformed prostate epithelial cell lines. We have therefore recently created cell lines stably overexpressing HOXC8 using RWPE-I and PWR-IE non-transformed prostate epithelial derived cell lines. We believe characterization of these cell lines will prove extremely informative in elucidating the role of HOXC8 in androgen receptor-mediated signaling and prostate tumorigenesis.

REFERENCES

(1) Shen WF, Krishnan K, Lawrence HJ, Largman C. The HOX homeodomain proteins block CBP histone acetyltransferase activity. Mol Cell Biol. 2001 Nov;21(21):7509-22.

(2) Miller GJ, Miller HL, van Bokhoven A, Lambert JR, Werahera PN, Schirripa O, Lucia MS, Nordeen SK. Aberrant HOXC expression accompanies the malignant phenotype in human prostate. Cancer Res. 2003 Sep 15;63(18):5879-88.

APPENDICES- none

SUPPORTING DATA

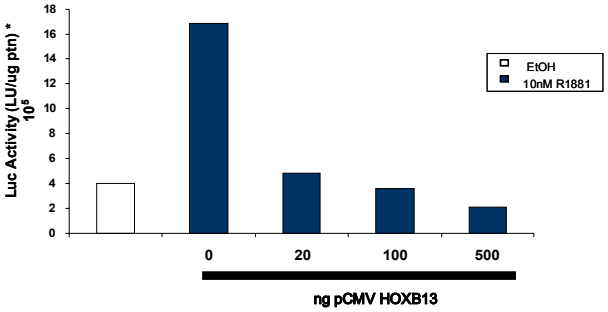

Fig. 1. Increased HOXB13 overexpression inhibits androgen induction of the MMTV promoter in LNCaP PCa cells.

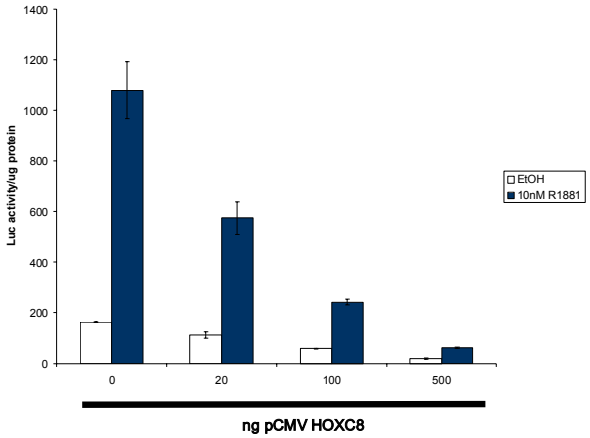

Fig. 2. Increased HOXC8 overexpression inhibits androgen induction of the MMTV promoter in PC-3-AR PCa cells.

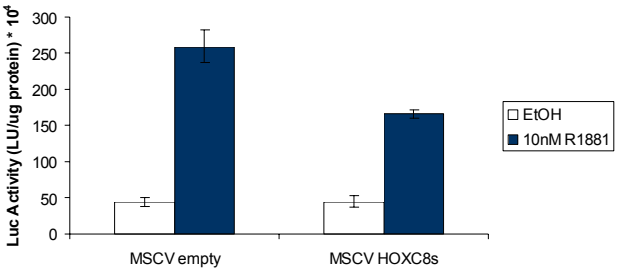

Fig. 3. AR-mediated transcription of the MMTV promoter is inhibited in LNCaP cells stably overexpressing HOXC8.

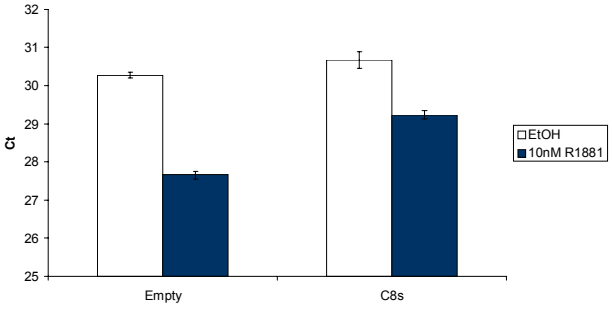

Fig. 4. PSA induction is inhibited in LNCaP-HOXC8 cells by real-time PCR analysis.

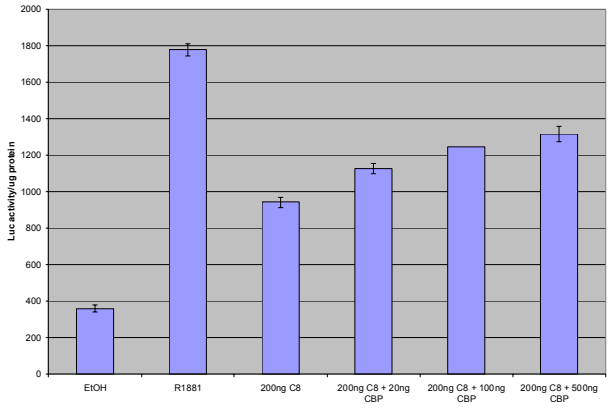

Fig. 5. CBP overexpression relieves HOXC8-induced inhibition of AR-mediated transcription of the MMTV promoter in LNCaP PCa cells.

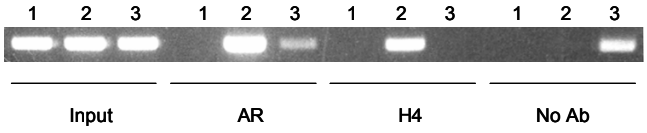

Fig. 6. HOXC8 overexpression inhibits histone H4 acetylation and AR loading at the MMTV promoter by ChIP analysis. Treatment conditions: (1) MMTV + EtOH; (2) MMTV+ 10nM R1881; (3) MMTV + 10nM R1881 + 200ng/ml HOXC8. Total transfected DNA was balanced in each treatment with empty vector DNA. Cells were hormone or vehicle treated for one hour.