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TITLE: Identifying Molecular Targets for Chemoprevention in a Rat Model

PRINCIPAL INVESTIGATOR: Ralph W. deVere White, M.D.

CONTRACTING ORGANIZATION: University of California, Davis
Davis, CA 95616-8671

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Ralph W. deVere White, M.D.

E-Mail: rwdeverewhite@ucdavis.edu

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14. ABSTRACT
The purpose of this grant was to determine the molecular events that occur in the dorsal and ventral lobes of the rat prostate gland after 20 weeks of exposure to PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine). Our Final Report was submitted July 2007 and summarized our data from the completion of Task 1 and Task 2 (published in Neoplasia). Task 3 was still pending at that time. This Final Addendum Report includes data from Task 3. A manuscript is being prepared and will be submitted to the DOD upon completion.

15. SUBJECT TERMS
PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) Prostate Cancer, Rats, Chemoprevention

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(Previously Submitted with Final Report of July 2007)

Borowsky, A.D., Dingley, K., Ubick, E., Turteltaub, K.W, Cardiff, R.D, and DeVere-White, R**.** **"**Inflammation and Atrophy Precede Prostate Neoplasia in PhIP Induced Rat Model". **Neoplasia,** (8):708-715, 2006.

Title: Identifying Molecular Targets for Chemoprevention in a Rat Model.
Principal Investigator: Ralph W. deVere White, MD
PC040947 (Contract # W81XWH-05-1-0081)

Addendum Report (Task 3)
December 10, 2007

INTRODUCTION: The **subject** of this DOD grant was to develop a chemoprevention strategy for Prostate Cancer (CaP) because of its high incidence and long natural history. The **purpose** of this grant was to determine the molecular events that occur in the dorsal and ventral lobes of the rat prostate gland after 20 weeks of exposure to PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine). PhIP is a potent inducer of mutations in the rat prostate where we have shown that it forms bulky DNA adducts. In addition, it induces high levels of oxidative damage in the target tissues. The **scope of this research** included: Task 1) Generation of a rat model, Task 2) Analysis of the rats prostate after 20 weeks of PhIP, and Task 3) Gene chip microarray analysis. This Final Addendum Report focuses on Task 3 as Task 1 and 2 were previously reported in the July 2007 Final Report.

BODY:

Research accomplishments associated with:

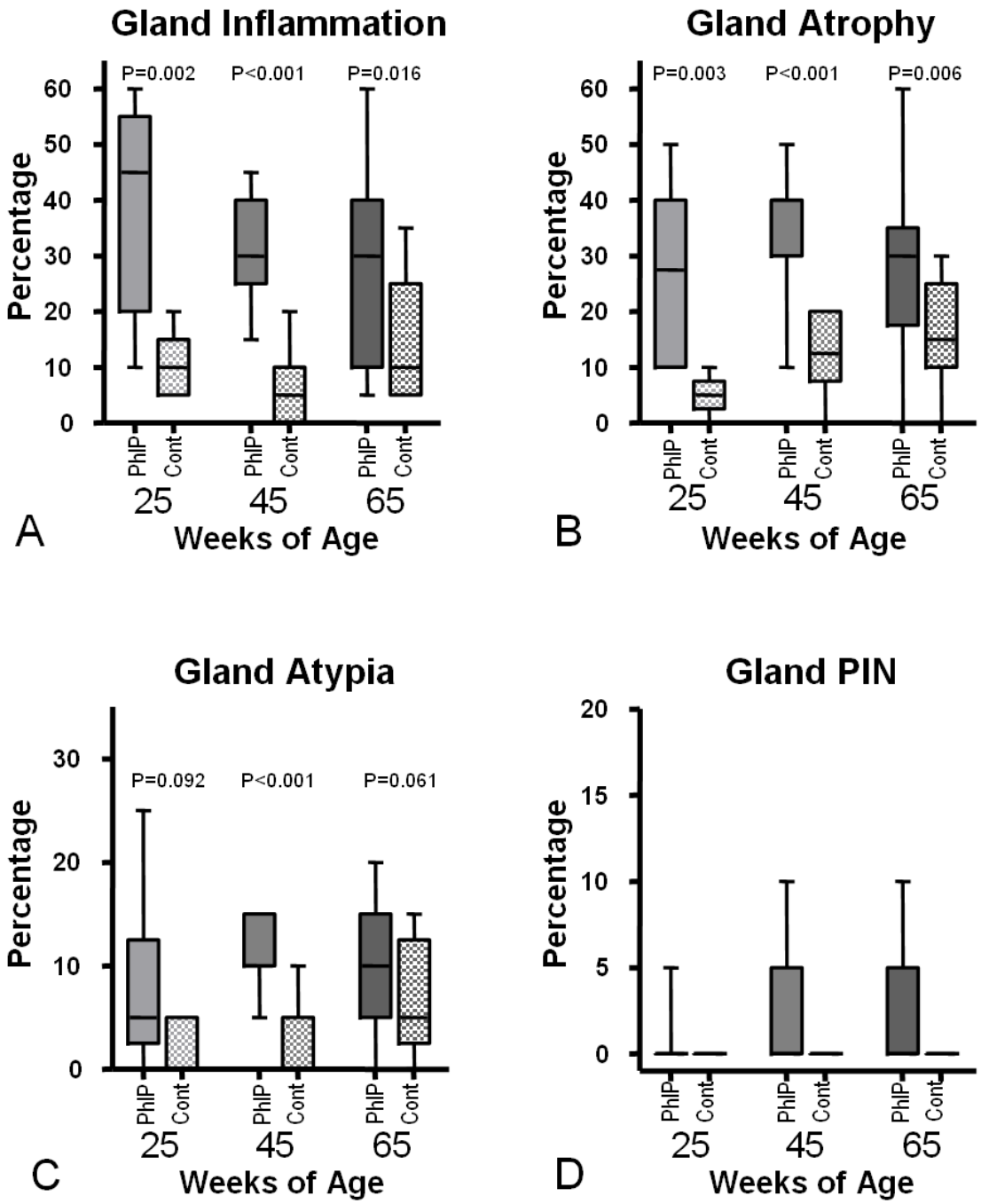
1) Generation of a rat model – (Completed) Male Fischer F344 Rats (Simonsen Laboratories, Gilroy,CA) were housed under the care of Lawrence Livermore National Laboratory Animal facility and treated per guidelines outlined in this DOD grant with approval by the LLNL Animal Care and Use Committee. Forty rats were fed PhIP in the diet for 20 weeks, beginning at 5 weeks of age. For the first 13 weeks, PhIP was fed to the animals at 400ppm in a certified basal diet. However, due to weight loss in the animals, the PhIP content was reduced to 200 ppm for the final 7 weeks. Forty controls received basal diet without the addition of PhIP. At 20 weeks, 10 PhIP-dosed and 10 control animals were euthanized.

2) Analysis of the prostate after 20 week of PhIP - (Completed) Inflammation in the PhIP treated rat prostate was the earliest, and most obvious change as compared to the control group (Figure 1a). Inflammation was found in all prostatic glands, but was least prominent in the anterior or coagulating gland. Inflammation persisted after discontinuing the PhIP treatment, and epithelial layers at all of the scheduled time points were intact, even in areas of luminal microabcess. Many of the areas of inflammation were accompanied by a reactive stromal proliferation resulting in a distinct thickening of the thin muscular layer surrounding individual glands. These proliferations did not appear to over grow the reactive process or to become neoplastic, as has been reported in some mouse models of prostate cancer [1].

Large areas of glandular atrophy, particularly affecting the ventral prostate were seen in all treated animals. Non-treated animals also appeared to be prone to glandular atrophy, particularly in the ventral prostate, but with less area of the prostate involved (Figure 1b).

No invasive carcinoma was seen in any of the animals. There were areas of inflammation induced stromal proliferation and high levels of inflammatory atypia. Not all of the animals were examined, but of the 20 animals examined at least with segmental histology of the intestine, only one had a full fledged polypoid adenoma and only two others had areas of early adenomatous changes.

Figure 1.



3) Gene chip microarray analysis – (Task 3 Completed-Manuscript Pending) Initially we planned to dissect subgross lesions in the prostate identified by surgical microscopy. Unfortunately the PIN lesions we eventually identified by histologic processing and transmitted light microscopy were too small to be seen in the surgical microscope. Furthermore, the

amount of tissue (and thus RNA) present in these foci would require amplification of the nucleic acid to obtain enough for analysis by the Affymetrix arrays. This confounded the results so we modified Taks 3 to evaluate the intermediate conditions of inflammation and atrophy in the prostate by gene expression analysis.

A second batch of PhIP-containing diet was prepared to match the original dosing protocol. We prepared PhIP-containing diets and challenged rats daily with PhIP in this diet for 0, 5 weeks, and 10 weeks. Tissues were collected and stored for microarray analysis. RNA was isolated from the tissues using the Qiagen's RNeasy micro RNA isolation kit according to the manufacturer's instructions. RNA quality was evaluated spectroscopically using OD. 260/280 and 28s/18s ratios All RNA had 260/280 ratios equal to or greater than 2.0 and 28s/18s ratios equal to or greater than 1.5.

Microarray analysis was then carried out using Affymetrix Rat 34A and Mouse 430 version 2.0 ChIP. Each rat sample was independently analyzed 3 times. Of the 40,000 genes on the chip, there were approx 200 with significant changes in expression during the 20 weeks of exposure (P> 0.05 and expression changes greater than 2- fold relative to unexposed control animals). Of this 200, 37 were affected at all three time points although the trends were not linear (see Figure 2 for examples of genes significantly upregulated at week 10). Of the 37 that were most up or down regulated at week 20, the biggest changes were in genes that have functions in stromal remodeling, cell fate (apoptosis or senescence), cell adhesion or motility. Of particular note, Clusterin was up-regulate over 20-fold by week 20 of the study. A publication is in preparation and will be submitted to the DOD upon completion.

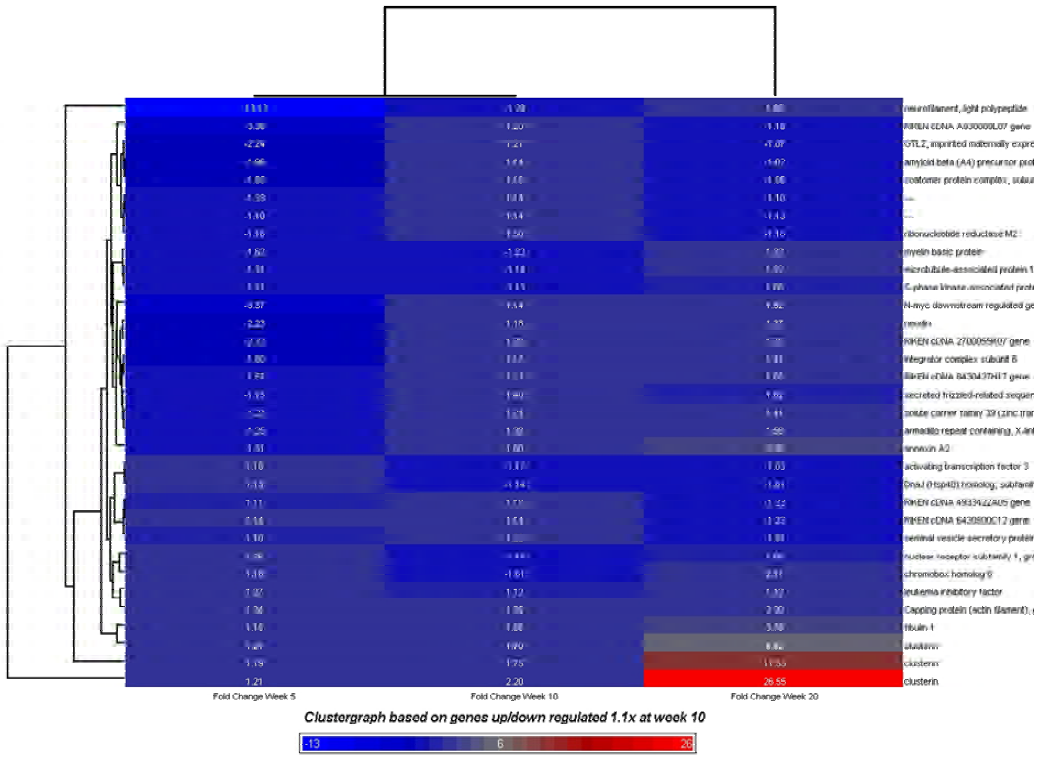


Figure 2. Cluster analysis of genes with at least a 2-fold change in expression at week 10 relative to untreated control animals.

KEY RESEARCH ACCOMPLISHMENTS:

- **Histopathologic analysis of PhIP induced prostate pathology**
- **Generation of an inflammatory/atrophy/proliferation model**
- **Data generated which refutes a previous (more simplistic) model of neoplastic progression.**
- **Data generated which calls into question some previously reported findings.**
- **Samples of prostate available for molecular analysis by microarray**

REPORTABLE OUTCOMES:

Borowsky, A.D., Dingley, K., Ubick, E., Turteltaub, K.W, Cardiff, R.D, and DeVere-White, R**.** (2006) Inflammation and Atrophy Precede Prostate Neoplasia in PhIP Induced Rat Model. Neoplasia 8, 708-715.

CONCLUSIONS:

The importance of this research is that it has shown that the PhIP treated rat can be a useful model of human disease suitable for testing prevention strategies targeting either DNA adduct formation or prostate inflammation as potential initiators and promoters of prostate cancer progression. This is a model that can be highly controlled in the laboratory so that quantitative and objective studies can be done to test and evaluate various chemopreventive strategies to prevent or delay development of clinical prostate cancer. In particular it offers the ability to study the role of inflammation in this cancer which is a unique opportunity. Overall, this research provides a model to evaluate efficacy of chemoprevention strategies and also to provide supportive and validating data on the molecular pathways affected. Thus a variety of chemoprevention agents and dosing regimens can be evaluated in a controlled setting so that the most effective strategies can be moved into clinical trials faster. We believe this work ultimately has the potential to spare patients from dealing with the effects of this disease.

REFERENCES:

1. Shappell SB, Thomas GV, Roberts RL, Herbert R, Ittmann MM, Rubin MA, et al. Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the Bar Harbor meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. Cancer Res 2004;64:2270-305.

APPENDICES: (Previously Submitted with July 2007 Final Report)

Borowsky, A.D., Dingley K., Ubick E., Turteltaub K.W, Cardiff R.D, and DeVere-White R. (2006) Inflammation and Atrophy Precede Prostate Neoplasia in PhIP Induced Rat Model. Neoplasia 8, 708-715.