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TITLE: Role of Estrogen Metabolism in the Initiation of Prostate  
Cancer: Biomarkers of Susceptibility and Early Detection

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## Introduction

The purpose of this research is to investigate the hypothesis that estradiol ( $E_2$ ) initiates prostate carcinogenesis and testosterone promotes the process. This is being explored in male Noble rats, which develop prostate tumors when treated with  $E_2$  and testosterone [1]. We think that estrogens are involved in the initiation of prostate cancer by a mechanism that involves oxidation of endogenous 4-catechol estrogen (CE) metabolites to CE-3,4-quinones (CE-3,4-Q). Reaction of CE-3,4-Q with DNA results in tumor initiation as the first step in the events leading to prostate cancer. Formation of depurinating DNA adducts by CE-3,4-Q, which generate apurinic sites in DNA, would be the critical event leading mutations that initiate the cancer [2]. To study the role of CE-Q in the initiation of prostate cancer, we are (1) treating male Noble rats with E by i.p. injection at various doses and for various times, analyzing the estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts and comparing their levels in the various regions of the prostate [3]; (2) investigating the conversion of testosterone into  $E_2$  in the prostate by analyzing the same compounds in prostate tissues from rats treated with testosterone or testosterone plus the aromatase inhibitor letrozole; and (3) determining the expression of four enzymes involved in the activation and deactivation of estrogens, cytochrome P450 (CYP) 19 (aromatase), CYP1B1, catechol-*O*-methyltransferase (COMT) and quinone oxidoreductase (QOR). The results of these studies will provide information on the relationship between estrogen activation and deactivation in relation to tumor initiation in the prostate.

## Body

In the first year of this research project, significant progress has been made on the projected tasks, as detailed in the Statement of Work. Because the analyses are in progress as this report is being written, we do not yet have results to report. We can report, however, that data are being successfully acquired.

**Task 1:** Conduct the  $E_2$  dose-response study of CE metabolites, GSH conjugates and DNA adducts.

The animals were treated with 0, 16, 32 or 48 mg/kg of  $E_2$  by i.p. injection, and after 3 h the prostate tissues were collected and sent to UNMC for analysis. The HPLC analyses with electrochemical and mass spectrometric detection are currently being conducted.

In addition, animals were treated with testosterone by implantation for 2 wk or by i.p. injection of 0 or 52 mg/kg for 6 h. The prostate tissues were collected and sent to UNMC for analysis. The HPLC analyses with electrochemical and mass spectrometric detection are currently being conducted. The dose-response and time course experiments with testosterone will be conducted based on the results of this study.

**Task 2:** Conduct the  $E_2$  time course study of CE metabolites, GSH conjugates and DNA adducts.

Initiation of this task awaits the results of the  $E_2$  dose-response study, and it will be conducted in the next few months.

Task 3: Synthesize primers for analyses of mRNAs.

This has been accomplished and the primers are being used to analyze expression of the enzymes.

Task 4: Analyze the expression of estrogen-metabolizing enzymes in control animals.

Analysis of the four enzymes, CYP19, CYP1B1, COMT and QOR, in control rats is currently being conducted.

Task 5: Begin analysis of the expression of estrogen-metabolizing enzymes in E<sub>2</sub>-treated animals.

Analysis of the four enzymes, CYP19, CYP1B1, COMT and QOR, in rats treated with testosterone (as described in Task 1) is currently being conducted. This will be followed by analysis of the enzymes in E<sub>2</sub>-treated animals.

### **Key Research Accomplishments**

1. Groups of rats were treated with estradiol (3 different doses injected for 3 h) or testosterone (implanted for 2 wk or injected for 6 h), the prostates were excised and dissected into the dorsolateral prostate, ventral prostate, seminal vesicle, coagulating gland and urethra, and the tissues were shipped to UNMC for analysis.
2. Tissues from the testosterone experiment are being analyzed for expression of the estrogen-metabolizing enzymes cytochrome P450 (CYP) 19 (aromatase), CYP1B1, catechol-*O*-methyltransferase and quinone oxidoreductase at the mRNA level.
3. Tissues from the testosterone experiment are being analyzed for the levels of estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts by HPLC with electrochemical and mass spectrometric detection.

### **Reportable Research Accomplishments**

None thus far.

### **Conclusions**

In this first year, we have established the minimum amounts of tissue needed to extract RNA successfully and to analyze estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts. We have also established the protocol necessary to extract tissues for analysis of estrogen derivatives by HPLC. In addition, we have found a compound to serve as an internal standard, so that we can validate the level of recovery of estrogen derivatives from tissue samples. These parameters are all necessary so that the results of these studies are as precise and meaningful as possible.

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