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TITLE: Comparative Analysis of Vitamin A (Retinol) Regulated Genes in African-American and Caucasian Prostate Cancer Patients

PRINCIPAL INVESTIGATOR: Sue Ellen K. Touma
Lorraine J. Gudas, Ph.D.
David M. Nanus, M.D.
Satish K. Tickoo, M.D.

CONTRACTING ORGANIZATION: Cornell University
New York, New York 10021

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**Title and Subtitle**

Comparative Analysis of Vitamin A (Retinol) Regulated Genes in African-American and Caucasian Prostate Cancer Patients

**Authors**

Sue Ellen K. Touma  
Lorraine J. Gudas, Ph.D.  
David M. Nanus, M.D.  
Satish K. Tickoo, M.D.

**Performing Organization**

Cornell University  
New York, New York 10021  
E-Mail: Set2002@med.cornell.edu

**Funding Numbers**

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**Abstract**

Vitamin A (retinol) and its related metabolites like retinoic acid (RA) have great potential in their roles as prostate cancer chemopreventive and chemotherapeutic agents by exerting regulation on cell growth and differentiation. Several studies have shown that there is a reduction in retinoid levels and retinoid receptors (e.g. RARα2) in prostate cancer. RA is being used to treat patients with prostate cancer and has been shown to inhibit tumor growth and reverse the events of carcinogenesis in animal models of prostate cancer. There is a disparity in prostate cancer among the African-American population and we hypothesize that more severe disruptions of retinoid signaling occur, contributing to this disparity. The purpose of this study is to examine the underlying causes for the clinical behavior of prostate cancer in African-Americans as compared to Caucasian patients. Preliminary results by immunohistochemical analysis have shown the expression of LRAT, an enzyme responsible for retinol esterification and storage as retinyl esters, to be reduced in tumor tissue specimens from prostate cancer patients as compared to adjacent nonmalignant tissue. Understanding the role of retinoid signaling in prostate carcinogenesis will lead to improved chemoprevention strategies and to the development of novel therapies for this disease.
INTRODUCTION

Prostate cancer is the most common cancer in men in the United States. A disparity exists in the African-American male population in the United States. Incidence rates of prostate cancer are about 70% higher than in white males, African-American patients present with more advanced and aggressive disease, and have poorer survival rates (1,2). A possible cause for this observed disparity is that there are more dramatic differences among known biological risk factors for African-Americans compared to other populations. Retinoids, retinol (vitamin A), and related metabolites like retinoic acid (RA) serve as cancer chemopreventive and chemotherapeutic agents by exerting regulation on cell growth and differentiation (3). Retinoid actions are mediated by binding two different families of nuclear RA receptors, RARs and RXRs, each with α, β, and γ subtypes (4,5). It has been shown that there is a reduction in the levels of retinoids and retinoid receptors (e.g. RARβ) in prostate cancer (6). Additionally, recent studies from our laboratory have shown that the levels of LRAT (lecithin:retinol acyltransferase), the primary enzyme responsible for the metabolism of retinol to retinyl esters, are reduced in many carcinomas, including oral cavity, skin, breast, bladder, renal and prostate (7-11).

The purpose of this study is to examine the underlying causes for the clinical behavior of prostate cancer in African-Americans as compared to Caucasian patients. I hypothesize that the molecular events, i.e. greater reductions in the expression of retinoid receptors or retinoid regulated genes, such as RARβ or LRAT, involved in the disruption of retinoid signaling in prostate carcinogenesis are more profound in the African-American population. Currently, the expression of selected retinoid receptors and target genes are being evaluated using immunohistochemical methods, utilizing paraffin-embedded sections obtained from African-American and Caucasian prostate cancer patients in a double-blinded fashion. Prostate tissue from African-American and Caucasian patients will also be used to examine protein and mRNA expression of retinoid-responsive genes. Additionally, the levels of retinol and its metabolites will be measured by reverse phase high pressure liquid chromatography in fresh prostate tissue samples from African-American and Caucasian prostate cancer patients.

BODY

The first aim of the proposed study is to examine the expression of selected retinoid receptors and retinoid regulated genes in formalin-fixed, paraffin-embedded tissue sections and fresh prostate tumor tissue samples from African-American and Caucasian prostate cancer patients. Tumors of the same Gleason grade as well as different Gleason grades will be obtained to determine if there are differences dependent on these scores.

Since statistical significance is a major consideration in this study, we have first performed a pilot study in which LRAT expression was evaluated in 19 paraffin-embedded tumor sections from patients of different races (Caucasian, African-American, Asian, and Hispanic) by immunohistochemical methods as described previously (Figure 1) (10). These prostate tissue specimens were obtained by the Department of Pathology and the Urological Oncology Division at New York Presbyterian Hospital- Weill Cornell Medical Center in a double-blinded fashion. Prior to immunohistochemical staining, hemotoxylin and eosin staining was used to confirm the malignant and benign phenotypes of the specimens. Tissue sections were deparaffinized followed by rehydration in a graded series of ethanol. Antigen retrieval was performed with an antigen unmasking solution in a pressure cooker. The expression of
LRAT was determined using an affinity purified polyclonal human LRAT antibody. Incubation with horseradish peroxidase conjugated secondary antibodies was followed by color development using diaminobenzidine (DAB) as substrate and counterstaining with hematoxylin. As a negative control, preimmune serum was used in place of primary antibody. LRAT staining was scored in a semiquantitative fashion by Satish Tickoo, our collaborating pathologist. Specimens were graded according to the intensity of staining within the tumor compared with the intensity of staining of the adjacent, benign tissue. Tumors were classified as 0 (no staining), 1+ (weak staining), 2+ (distinct staining, but weaker than the staining in benign tissue), and 3+ (staining equal to that in the benign tissue).

Figure 1: LRAT expression in human prostate tumor tissue specimens. Paraffin-embedded tissue sections from radical prostatectomy specimens (n=19) containing prostate cancer were stained with affinity-purified LRAT antibodies and counterstained with hematoxylin. Negative controls were incubated with preimmune serum instead of primary antibody (not shown). LRAT protein expression is visualized by dark brown staining and is present in basal epithelial cells in areas of normal epithelium. LRAT immunostaining was graded as follows: 1+ (weak staining), 2+ (distinct staining, but weaker than the staining in benign tissue), and 3+ (staining equal to or greater than that in the benign tissue).

A) Representative images for each grade are shown; A, 1+, B, 2+, and C, 3+.

B) Race, Gleason, and staining scores for each patient. A, Asian, B, Black/African-American, C, Caucasian, and H, Hispanic.

Preliminary results have shown the expression of LRAT to be reduced in tumor tissue specimens from prostate cancer patients as compared to adjacent nonmalignant tissue (Figure 5).
However, there were no detectable trends correlating staining with race or Gleason grade (Figure 1B). This could be due to the fact that the sample size was very small. A biostatistician, Quanhong Ni, from the Clinical Research Methodology core facility at Weill Medical College of Cornell University was consulted regarding sample size requirements and statistical significance of the preliminary data. The results of this analysis are presented here using statistical tests to determine an appropriate sample number in each group (African-American vs. other races (including Caucasian, Asian, and Hispanic patients)) that will be statistically significant in future experiments.

**A)** Outcome variable (Staining) as a continuous variable. Mean and standard deviation for two groups are listed as follows:

<table>
<thead>
<tr>
<th>Race</th>
<th>Staining(Staining)</th>
<th>Mean</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-American</td>
<td>2.1667</td>
<td>0.7528</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>1.6154</td>
<td>0.7679</td>
<td></td>
</tr>
</tbody>
</table>

A sample size of 35 in each group will have 80% power to detect a difference in means of 0.552 (the difference between African-American Group (mean, $\mu_A$, of 2.167) and the Others group (mean $\mu_O$, of 1.615)) assuming that the common standard deviation is 0.763 using a two group t-test with a 0.050 two-sided significance level. This is also verified by Wilcoxon rank test.

**B)** Outcome variable (Staining) as ordered categories. Frequency and proportions for each category are listed in the following table:

<table>
<thead>
<tr>
<th>Table of Race by Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race</td>
</tr>
<tr>
<td>Frequency Row Pet</td>
</tr>
<tr>
<td>African-American</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

When the sample size in each of the two groups is 35, a 0.050 level Chi-square test will have 80% power to distinguish between the groups when the proportions in the 3 categories are as above table. This is also verified by Wilcoxon rank test.

At this time, we have requested paraffin-embedded prostate tumor samples from the National Cancer Institute's Cooperative Prostate Cancer Tissue Resource. A letter of intent was submitted in January 2005 but the reviewing committee responded back requesting more information regarding the study design and additional statistical analysis. A revised letter of
intent will be submitted in March 2005. Once samples can be obtained, a more thorough immunohistochemical analysis of LRAT expression will be performed.

KEY RESEARCH ACCOMPLISHMENTS

- I learned how to stain paraffin-embedded tumor tissue sections using immunohistochemical methods and optimized this procedure for the human LRAT antibody.
- I completed a pilot study looking at LRAT staining in prostate cancer patients of different races and obtained the necessary statistical analysis to move ahead with this project.
- I have designed a larger scale experiment examining LRAT staining in prostate cancer patients using immunohistochemical methods.

REPORTABLE OUTCOMES

Poster Presentation
American Association for Cancer Research Third Annual International Conference on Frontiers in Cancer Prevention Research, Seattle, WA, October 16-20, 2004
Title: Analysis of Retinoid Signaling and Metabolism in Prostate Cancer.
Authors: Sue Ellen Touma, Satish K. Tickoo, David M. Nanus, Dean Bok, and Lorraine J. Gudas

CONCLUSIONS

This proposal addresses fundamental aspects of retinoid signaling and prostate cancer biology. Although preliminary, we have shown that there is a decrease in LRAT expression in tumor tissue as compared to the adjacent benign tissue in prostate tumor samples. We must increase our sample size to determine if we can further distinguish differences in LRAT staining based on race and/or Gleason grade. Differences in retinoid regulated gene expression may account for the observed disparity and serve as markers for enhanced detection of this disease.
REFERENCES


