Award Number: DAMD17-02-1-0045

TITLE: The Role of βII Isotype of Tubulin in Diagnosis and Development of Prostate Cancer

PRINCIPAL INVESTIGATOR: Richard F. Luduena, Ph.D.

CONTRACTING ORGANIZATION: University of Texas Health Science Center at San Antonio
San Antonio, Texas 78229-3900

REPORT DATE: January 2003

TYPE OF REPORT: Annual

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.
# The Role of βII Isotype of Tubulin in Diagnosis and Development of Prostate Cancer

**Author(s):**
Richard F. Luduena, Ph.D.

**Performing Organization Name(s) and Address(es):**
University of Texas Health Science Center at San Antonio
San Antonio, Texas 78229-3900
E-Mail: luduena@uthscsa.edu

**Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information):**
We have previously found that the βII isotype of tubulin occurs in the cell nuclei of a large number of prostate cancers. In this project, we have examined prostate cancers from two groups of prostatectomies, one set originating with patients whose cancer recurred and the other from a mixture of patients. We found that βII was expressed in a substantial fraction of prostate tumors in both sets of patients. We also observed that, for the subclass that expressed βII, the βII occurred more frequently in the nuclei and much less frequently in the cytoplasm alone in the set of patients whose cancer is likely to recur. We also examined the distribution of two other isotypes of tubulin, βI and βIII, in prostate and other cancers. We found that βI was common in all cancers, βIII was less common in prostate cancer and that nuclear occurrence of these isotypes was very infrequent in any cancer. Our results suggest that nuclear βI is a promising predictive biomarker for recurrence of prostate cancer while βI and βIII are not likely to become good biomarkers.
Table of Contents

Cover........................................................................................................................................... 1
SF 298........................................................................................................................................... 2
Table of Contents......................................................................................................................... 3
Introduction.................................................................................................................................... 4
Body.............................................................................................................................................. 5
Key Research Accomplishments.................................................................................................... 8
Reportable Outcomes..................................................................................................................... 9
Conclusions.................................................................................................................................... 10
References..................................................................................................................................... 11
Appendices..................................................................................................................................... 12
INTRODUCTION

The research reported here arose from two earlier observations: 1. that many cancer cells, specifically including prostate cancers, contain the $\beta_{II}$ isotype of tubulin in their nuclei (1-3); and 2. that otherwise normal cells adjacent to cancerous cells, also contain $\beta_{II}$ in their nuclei (3). The existence of nuclear $\beta_{II}$ is highly unusual, in that tubulin, the subunit protein of microtubules, is normally found exclusively in the cytoplasm (4). Our first observation, which has been reproduced in every type of cultured cancer cell we have examined and in 74% of the actual excisions, from many types of cancer, indicates that many cancer cells, in the process of becoming transformed, somehow incorporate $\beta_{II}$ into their nuclei. It also raises the possibility that nuclear $\beta_{II}$ could be a predictive biomarker for the diagnosis of prostate cancer. The second observation, that nuclear $\beta_{II}$ occurs in otherwise normal cells adjacent to the cancer, indicates that prostate cancer biopsies could become more accurate. In other words, even if the biopsy misses the actual cancer, the presence of cancer could be indicated by finding nuclear $\beta_{II}$ in adjacent cells. Accordingly, our two tasks in this research are 1) to determine if nuclear $\beta_{II}$ expression is a predictive biomarker for the diagnosis of prostate cancer; and 2) to test the hypothesis that nuclear $\beta_{II}$ is predictive of the diagnosis of prostate cancer in a subset of patients whose initial biopsy was negative. In accordance with the schedule in the original statement of work, we have not yet completed the first task nor have we begun the second task. We have analyzed two sets of prostate cancer patients. The aim was to get a background level for the correlation between the presence of nuclear $\beta_{II}$ and diagnosis of cancer. We have approached this by comparing prostatectomies from two sets of patients; one set consisted of a random selection of patients with no consideration of whether their cancer recurred. The second set consisted of those whose cancer recurred. We examined the expression and sub-cellular localization of $\beta_{II}$ in the cancers of these patients.
BODY

TASK 1: TO DETERMINE IF NUCLEAR \( \beta_{II} \) EXPRESSION IS A PREDICTIVE BIOMARKER FOR THE DIAGNOSIS OF PROSTATE CANCER

Rationale:

Since we had originally found a high fraction of prostate cancer excisions to contain nuclear \( \beta_{II} \), we analyzed prostatectomies from two sets of patients with prostate cancer. The rationale is to see how frequent is the expression of \( \beta_{II} \) (not normally expressed in prostate cells) in cancer cells and how frequent is the occurrence of nuclear \( \beta_{II} \) in these cells. We chose this set since it is a random mixture of prostate cancer patients, including some whose cancer was likely to recur and some whose cancer was not likely to recur.

We also examined the distribution of the \( \beta_{I} \) and \( \beta_{III} \) isotypes of tubulin in prostatectomies as well as in other cancers. The rationale is to see if the \( \beta_{II} \) isotype is a more promising biomarker for prostate cancer than are the other isotypes.

Experimental Results:

Antibodies to the \( \beta_{I} \), \( \beta_{II} \), and \( \beta_{III} \) isotypes of mammalian tubulin were prepared as previously described (5-8).

Distribution of \( \beta_{II} \) Expression and Nuclear \( \beta_{II} \) Occurrence in Patients with Prostate Cancer

We examined prostatectomies from 47 patients with prostate cancer. This includes patients whose subsequent prognosis is unknown. In other words, some of them may have had prostate cancer that did not recur and others had prostate cancer that did recur. The aim was to see how what percentage of the patients expressed \( \beta_{II} \) and what percentage had \( \beta_{II} \) in the nuclei. The results are as follows:

1. 68% of the patients expressed \( \beta_{II} \).
2. 47% had nuclear \( \beta_{II} \).
3. 39% had \( \beta_{II} \) only in the nuclei and not in the cytoplasm.
4. 21% had \( \beta_{II} \) only in the cytoplasm and not in the nuclei.
5. 17% had \( \beta_{II} \) in both nuclei and cytoplasm.
6. 32% of the patients expressed no \( \beta_{II} \).

We then examined prostatectomies from 31 patients whose PSA began to increase within five years after the operation and are presumed to be patients whose cancer is
recurring. Of these patients, the sampling missed the cancer in 11. Of the remaining 20, these broke down as follows:

1. 45% expressed $\beta_{II}$.
2. 40% had nuclear $\beta_{II}$ I.
3. 20% had $\beta_{II}$ only in the nuclei and not in the cytoplasm.
4. 5% had $\beta_{II}$ only in the cytoplasm and not in the nuclei.
5. 20% had $\beta_{II}$ in both nuclei and cytoplasm.
6. 55% of the patients expressed no $\beta_{II}$.

In all cases with nuclear $\beta_{II}$, staining is most intense in small intra-nuclear areas that are likely to correspond with nucleoli.

As can be seen, some tumors had $\beta_{II}$ mainly in the nuclei (Figure 1), some in the mainly in the cytoplasm (Figure 4), and some in both.

At first glance, there do not seem to be interesting differences between the two sets of data. In fact, it appears that patients with a likely recurrence of prostate cancer are less likely to express $\beta_{II}$. However, if one analyzes the various outcomes as a percentage of the $\beta_{II}$-positive patients, an interesting difference becomes apparent.

<table>
<thead>
<tr>
<th>$\beta_{II}$ distribution</th>
<th>Random mixture of patients</th>
<th>Patients whose cancer recurred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear $\beta_{II}$</td>
<td>69%</td>
<td>89%</td>
</tr>
<tr>
<td>$\beta_{II}$ in nuclei only</td>
<td>57%</td>
<td>44%</td>
</tr>
<tr>
<td>$\beta_{II}$ in cytoplasm only</td>
<td>31%</td>
<td>11%</td>
</tr>
<tr>
<td>$\beta_{II}$ in both nucleus and cytoplasm</td>
<td>25%</td>
<td>44%</td>
</tr>
</tbody>
</table>

It appears therefore that a substantial fraction of prostate tumors do not express $\beta_{II}$ at all. However, among the ones that do, a much higher fraction of the patients with prostate cancers that are likely to recur have significantly higher levels of nuclear $\beta_{II}$ than a random mixture of those with and without recurrence of prostate cancer. Also, the cancers that are likely to recur contain significantly fewer cases of $\beta_{II}$ in the cytoplasm only.

In addition to examining the above cancers, we also examined a few cases of prostatic intra-epithelial neoplasia (PIN) and found that some expressed no $\beta_{II}$ (Figure 2), some had $\beta_{II}$ only in the nuclei (Figure 5) and some had $\beta_{II}$ only in the cytoplasm (Figure 3).

We are illustrating different types of $\beta_{II}$ distribution in the photographs attached to this report.
Distribution of the $\beta_I$ and $\beta_{III}$ isotypes in prostate and other tumors

In order to verify that $\beta_{II}$ is indeed, among the tubulin isotypes, the best potential biomarker for prostate cancer, we examined the distribution of the $\beta_I$ and $\beta_{III}$ isotypes in prostate, lung, melanoma, breast, renal and colon cancers. We found that $\beta_I$ occurs in both benign and malignant prostate tissue as well as in the other tumors. However, it is almost exclusively cytoplasmic. Only one prostate tumor showed $\beta_I$ in the nuclei.

The $\beta_{III}$ isotype also occurs in both benign and malignant prostate tissue and in the other tumors as well. In fact, $\beta_{III}$ is expressed with greater frequency in the non-prostate tumors. However, with one exception, $\beta_{III}$ is found entirely in the cytoplasm. The one exception was renal clear cell carcinoma where $\beta_{III}$ occurs in the nucleus.

It thus appears that neither $\beta_I$ nor $\beta_{III}$ would be superior to $\beta_{II}$ as a biomarker for prostate cancer.

**TASK 2: TO TEST THE HYPOTHESIS THAT NUCLEAR $\beta_{II}$ IS PREDICTIVE OF THE DIAGNOSIS OF THE DIAGNOSIS OF PROSTATE CANCER IN A SUBSET OF PATIENTS WHOSE INITIAL BIOPSY WAS NEGATIVE**

Not yet commenced (see initial Statement of Work)
KEY RESEARCH ACCOMPLISHMENTS

- Prostate cancers that are likely to recur express less $\beta_{II}$ than do those that are not likely to recur.

- In the fraction of patients whose cancers express $\beta_{II}$, those whose cancer is likely to recur have a much greater likelihood of $\beta_{II}$ occurring in the nucleus and a much lower probability of occurring only in the cytoplasm.

- $\beta_{II}$ subcellular localization may be a good predictive factor for recurrence of prostate cancer in patients who have had a prostatectomy.

- $\beta_{II}$ is a much better biomarker for prostate cancers than either the $\beta$ - or $\beta_{III}$-tubulin isotypes.

- $\beta_{III}$ is more commonly expressed in non-prostate cancers.
REPORTABLE OUTCOMES

1. Manuscripts, Abstracts, Presentations:

   None

2. Funding Applied for Based on Work Supported by this Award:

   We submitted a grant to the EDRN (Early Diagnosis Research Network) program of the National Cancer Institute titled "Blood βII-Tubulin as a Biomarker for Prostate Cancer". The grant has been funded. It will explore the possibility of developing a blood test for prostate cancer by looking for nuclear βII in prostate cancer cells in the serum.
CONCLUSIONS

We have examined a large number of prostate cancers. We have found that cancers from patients whose cancer is likely to recur express less $\beta_{II}$ than do those of patients whose cancer is not likely to recur. Thus, for patients whose cancer does not express $\beta_{II}$, our approach would not be likely to have predictive value. However, for patients whose cancer does express $\beta_{II}$, those whose cancer is likely to recur are much more likely to have $\beta_{II}$ in the nucleus and much less likely to have $\beta_{II}$ in the cytoplasm only. The distribution of $\beta_{I}$ and $\beta_{III}$ in prostate and other tumors suggest that these are not promising biomarkers for prostate cancers.
REFERENCES


3. Yeh IT & Ludueña RF, unpublished results.


APPENDIX

**Figure 1:** A low grade cancer showing nuclear staining for βII-tubulin. Note intense nuclear staining in most of the cancer cells.

**Figure 2:** An example of prostatic intra-epithelial neoplasia (PIN) showing no staining.

**Figure 3:** An example of PIN showing cytoplasmic staining for βII.

**Figure 4:** A grade 4+3 = 7/10 Gleason's score tumor showing cytoplasmic staining.

**Figure 5:** An example of PIN showing nuclear staining.