AD_____

Award Number: DAMD17-02-1-0045

TITLE: The Role of β_{II} 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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	January 2003	Annual (1 Jan 02 - 31 Dec 02)

4. TITLE AND SUBTITLE
The Role of β_{II} Isotype of Tubulin in Diagnosis and Development of Prostate Cancer

5. FUNDING NUMBERS
DAMD17-02-1-0045

6. AUTHOR(S) :
Richard F. Luduena, Ph.D.

7. 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

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. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates: All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** ***(abstract should contain no proprietary or confidential information)***

We have previously found that the β_{II} 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 β_{II} was expressed in a substantial fraction of prostate tumors in both sets of patients. We also observed that, for the subclass that expressed β_{II}, the β_{II} 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, β_{I} and β_{III}, in prostate and other cancers. We found that β_{I} was common in all cancers, β_{III} was less common in prostate cancer and that nuclear occurrence of these isotypes was very infrequent in any cancer. Our results suggest that nuclear β_{II} is a promising predictive biomarker for recurrence of prostate cancer while β_{I} and β_{III} are not likely to become good biomarkers.

14. SUBJECT TERMS:	15. NUMBER OF PAGES
prostate cancer, tubulin, cell nucleus, diagnosis, biomarker	13
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

The research reported here arose from two earlier observations: 1. that many cancer cells, specifically including prostate cancers, contain the β_{II} isotype of tubulin in their nuclei (1-3); and 2. that otherwise normal cells adjacent to cancerous cells, also contain β_{II} in their nuclei (3). The existence of nuclear β_{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 β_{II} into their nuclei. It also raises the possibility that nuclear β_{II} could be a predictive biomarker for the diagnosis of prostate cancer. The second observation, that nuclear β_{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 β_{II} in adjacent cells. Accordingly, our two tasks in this research are 1) to determine if nuclear β_{II} expression is a predictive biomarker for the diagnosis of prostate cancer; and 2) to test the hypothesis that nuclear β_{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 β_{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 β_{II} in the cancers of these patients.

BODY

TASK 1: TO DETERMINE IF NUCLEAR β_{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 β_{II}, we analyzed prostatectomies from two sets of patients with prostate cancer. The rationale is to see how frequent is the expression of β_{II} (not normally expressed in prostate cells) in cancer cells and how frequent is the occurrence of nuclear β_{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 β_I and β_{III} isotypes of tubulin in prostatectomies as well as in other cancers. The rationale is to see if the β_{II} isotype is a more promising biomarker for prostate cancer than are the other isotypes.

Experimental Results:

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

Distribution of β_{II} Expression and Nuclear β_{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 β_{II} and what percentage had β_{II} in the nuclei. The results are as follows:

1. 68% of the patients expressed β_{II}.
2. 47% had nuclear β_{II}.
3. 39% had β_{II} only in the nuclei and not in the cytoplasm.
4. 21% had β_{II} only in the cytoplasm and not in the nuclei.
5. 17% had β_{II} in both nuclei and cytoplasm.
6. 32% of the patients expressed no β_{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 β_{II}.
2. 40% had nuclear β_{II} I.
3. 20% had β_{II} only in the nuclei and not in the cytoplasm.
4. 5% had β_{II} only in the cytoplasm and not in the nuclei.
5. 20% had β_{II} in both nuclei and cytoplasm.
6. 55% of the patients expressed no β_{II}.

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

As can be seen, some tumors had β_{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 β_{II}. However, if one analyzes the various outcomes as a percentage of the β_{II}-positive patients, an interesting difference becomes apparent.

β_{II} distribution	Random mixture of patients	Patients whose cancer recurred
Nuclear β_{II}	69%	89%
β_{II} in nuclei only	57%	44%
β_{II} in cytoplasm only	31%	11%
β_{II} in both nucleus and cytoplasm	25%	44%

It appears therefore that a substantial fraction of prostate tumors do not express β_{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 β_{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 β_{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 β_{II} (Figure 2), some had β_{II} only in the nuclei (Figure 5) and some had β_{II} only in the cytoplasm (Figure 3).

We are illustrating different types of β_{II} distribution in the photographs attached to this report.

Distribution of the β_I and β_{III} isotypes in prostate and other tumors

In order to verify that β_{II} is indeed, among the tubulin isotypes, the best potential biomarker for prostate cancer, we examined the distribution of the β_I and β_{III} isotypes in prostate, lung, melanoma, breast, renal and colon cancers. We found that β_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 β_I in the nuclei.

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

It thus appears that neither β_I nor β_{III} would be superior to β_{II} as a biomarker for prostate cancer.

TASK 2: TO TEST THE HYPOTHESIS THAT NUCLEAR β_{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 β_{II} than do those that are not likely to recur.

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

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

- β_{II} is a much better biomarker for prostate cancers than either the β - or β_{III}-tubulin isotypes.

- β_{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 β_{II}-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 β_{II} 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 β_{II} than do those of patients whose cancer is not likely to recur. Thus, for patients whose cancer does not express β_{II}, our approach would not be likely to have predictive value. However, for patients whose cancer does express β_{II}, those whose cancer is likely to recur are much more likely to have β_{II} in the nucleus and much less likely to have β_{II} in the cytoplasm only. The distribution of β_I and β_{III} in prostate and other tumors suggest that these are not promising biomarkers for prostate cancers.

REFERENCES

1. Walss-Bass C, Xu K, David S, Fellous A & Ludueña RF (2002) Occurrence of nuclear β_{II}-tubulin in cultured cells. *Cell and Tissue Research* **308**, 215-223.
2. Xu K & Ludueña RF (2002) Characterization of nuclear β_{II}-tubulin in tumor cells: a possible novel target for taxol. *Cell Motility and the Cytoskeleton* **53**, 39-52.
3. Yeh IT & Ludueña RF, unpublished results.
4. Walss C, Kreisberg JI & Ludueña RF (1999) Presence of the β_{II}-isotype of tubulin in the nuclei of cultured rat kidney mesangial cells. *Cell Motility and the Cytoskeleton* **42**, 274-284.
5. Banerjee A, Roach MC, Wall KA, Lopata MA, Cleveland DW & Ludueña RF (1988) A monoclonal antibody against the type II isotype of β-tubulin. Preparation of isotypically altered tubulin. *J. Biol. Chem.* **263**, 3029-3034.
6. Banerjee A, Roach MC, Trcka P & Ludueña RF (1990) Increased microtubule assembly in bovine brain tubulin lacking the type III isotype of β-tubulin. *J. Biol. Chem.* 265, 1794-1799.
7. Banerjee A, Roach MC, Trcka P & Ludueña RF (1992) Preparation of a monoclonal antibody specific for the class IV isotype of β-tubulin. Purification and assembly of $\alpha\beta_{II}$, $\alpha\beta_{III}$, and $\alpha\beta_{IV}$ tubulin dimers from bovine brain. *J. Biol. Chem.* 267, 5625-5630.
8. Roach MC, Boucher VL, Walss C, Ravdin PM & Ludueña RF (1998) Preparation of a monoclonal antibody specific for the class I isotype of β-tubulin: the β isotypes of tubulin differ in their cellular distributions within human tissues. *Cell Motility and the Cytoskeleton* **39**, 273-285.

APPENDIX

Figure 1: A low grade cancer showing nuclear staining for β_{II}-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 β_{II}.

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

Figure 5: An example of PIN showing nuclear staining.

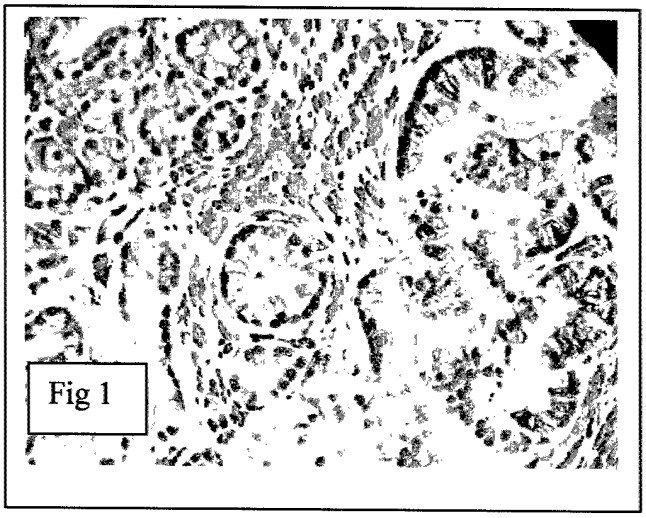

Fig 1

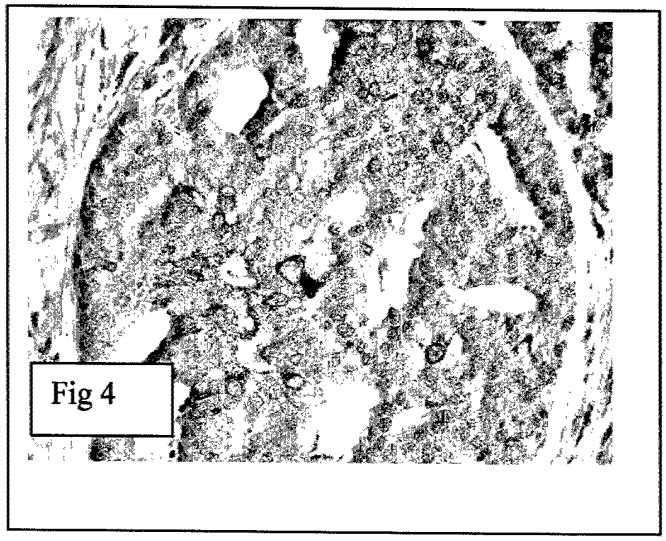

Fig 4

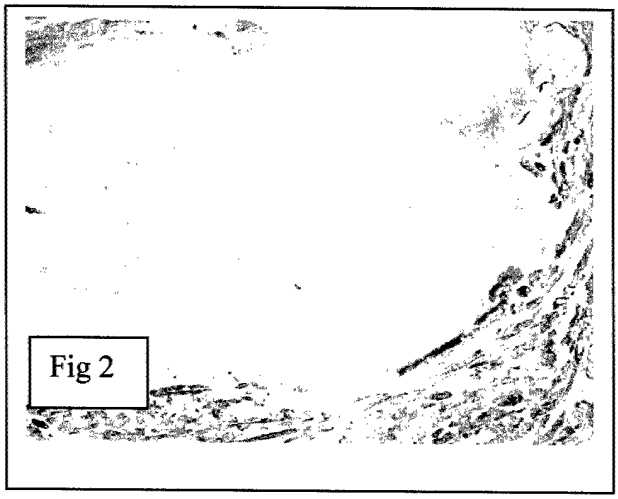

Fig 2

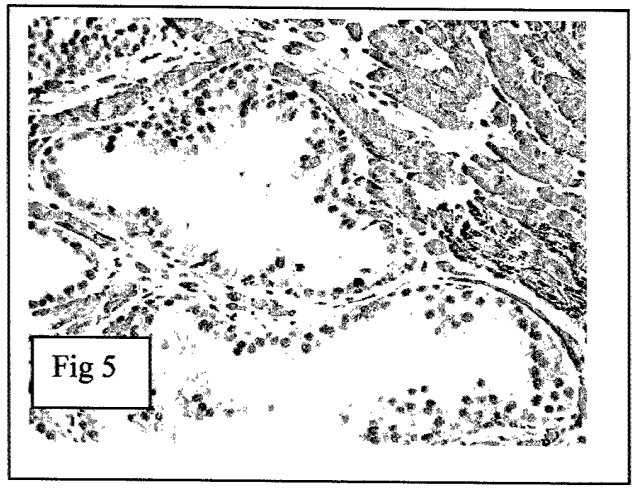

Fig 5

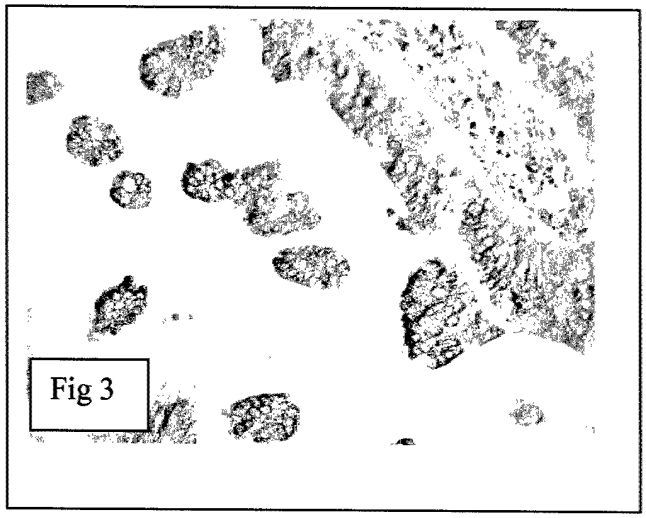

Fig 3