

# Comparison of Androgen-Independent Growth and Androgen-Dependent Growth in BPH and Cancer Tissue From the Same Radical Prostatectomies in Sponge-Gel Matrix Histoculture

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**BACKGROUND.** In order to determine androgen sensitivities of prostate cancer and benign prostatic hypertrophy (BPH) tissues from the same patient *in vitro*, we used a histoculture technique to measure androgen-independent and androgen-dependent growth and compared them in paired specimens of BPH and prostate cancer from 23 radical prostatectomies. Both androgen-independent growth and androgen-dependent growth are measures of important biological characteristics of benign and malignant prostate tissue.

**METHODS.** The effect of hydroxyflutamide and antiandrogens on dihydrotestosterone (DHT)-stimulated incorporation of <sup>3</sup>H-thymidine into both paired specimens of BPH and cancer was utilized to measure androgen-independent and androgen-dependent growth. The percentage decrease in <sup>3</sup>H-thymidine incorporation/ $\mu$ g protein in the flutamide-treated specimen compared to the DHT-treated specimen represented androgen-dependent growth. Residual <sup>3</sup>H-thymidine incorporation/ $\mu$ g protein during hydroxyflutamide administration represented androgen-independent growth.

**RESULTS.** Androgen-independent growth was significantly greater ( $P = 0.015$ ) in the BPH compared to the cancer paired tissue. Androgen-dependent growth was significantly higher in 23 paired specimens of cancer compared to BPH ( $P < 0.03$ ).

**CONCLUSIONS.** In paired specimens of BPH and prostate cancer from the same radical prostatectomy specimen, androgen-independent growth appeared greater in BPH compared to cancer specimens; androgen-dependent growth, however, was greater in prostate cancer than in BPH. There was no correlation of either growth parameter with Gleason tumor grade. Future clinical correlations will indicate whether either growth parameter represents an important prognostic factor for prostate cancer aggressiveness. stimulated <sup>3</sup>H-thymidine incorporation into DNA. *Prostate* 31:250-254, 1997. © 1997 Wiley-Liss, Inc.

**KEY WORDS:** dihydrotestosterone; BPH; prostate cancer; hydroxyflutamide; <sup>3</sup>H-thymidine

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## INTRODUCTION

Both benign and most malignant prostate tissues are known to have both androgen-dependent and androgen-independent growth. We undertook this study to compare these growth parameters in benign prostatic hyperplasia (BPH) and cancer from the same radical prostatectomy specimens, thus providing us with an ideal basis for comparison. These growth parameters represent defining biological characteristics of prostate cancer and BPH tissue.

There are prior reports of organ culture of prostate tissue in the literature by Schrodt and Foreman, [1] and Lasnitzki [2] from more than two decades ago. These studies were done using Trowell medium and tissue suspended on agar rafts supported on a Tantalum mesh. Although Lasnitzki [2] was able to show significant increases in  $^3\text{H}$ -uridine incorporation into RNA during incubation of prostatic organ cultures with dihydrotestosterone (DHT) compared to controls, no attempts at quantitation of biological growth parameters by using inhibitors of androgen-stimulated growth have been done until the present. In addition, significant advances in tissue-supporting sponge-gel-matrix and the use of defined media, as used in our studies, have been made since that time [3].

Our histoculture androgen sensitivity technique (HASA), as previously reported [3], provides a realistic reflection of *in vivo* growth, since stroma and epithelium are maintained in anatomical juxtaposition and the medium is carefully defined.  $^3\text{H}$ -thymidine incorporation into tissue per mcg of protein was the parameter measured under conditions of both androgen-stimulated and androgen-blocked incubation of the same tissue.

An additional long-term potential benefit of this study was to see if these growth parameters might predict prostate cancer aggressiveness. Therefore, all radical prostatectomy specimens were carefully staged and graded by a pathologist so that we could look in future studies for correlations in the defined growth parameters with long-term survival or time to prostate specific antigen (PSA) relapse in patients with the same pathological stage or disease.

## MATERIALS AND METHODS

### Tissue Preparation

Radical prostatectomy specimens from patients with early prostate cancer were placed in Eagle's minimal essential medium (MEM) solution on ice in the operating room and brought to the Department of Pathology (Mercy Hospital and Medical Center, San Diego, CA). The pathologist weighed and measured

the specimens and marked the prostate surface with green ink to aid in the pathological staging of the tumor. The tissue was cut serially in its anatomical position so that the 50% of the gland above and below a midline axial cut was labeled as anterior and posterior, respectively. A sagittal cut running from distal to proximal divided the prostate into right and left sections; approximately 0.5-cm cuts were then made beginning at the distal urethral end of the prostate. Each section was identified with a letter label. The pathologist selected representative specimens of cancer and BPH grossly for our study; anatomical mirror-image sections of the specimens for histoculture were kept by the pathologist for determination of microscopic anatomy. The tissue was then sent to our laboratory in ice-cold MEM solution.

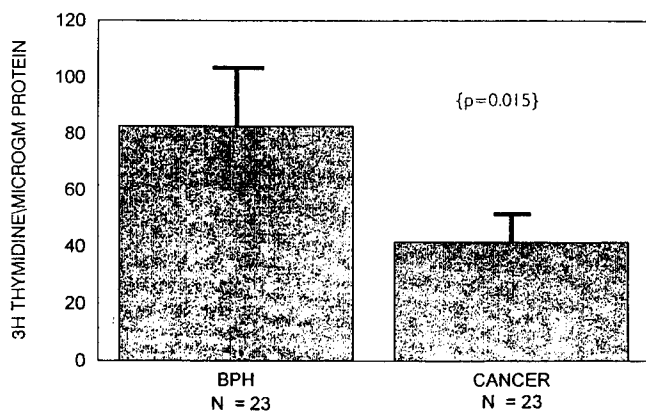
### Histoculture

Paired BPH and cancer specimens from the same prostate were separately and finely cut into 1-cubic-mm minces and separately planted on incubation plates with wells containing collagen-gel matrices and with media containing MEM and appropriate concentrations of DHT and hydroxyflutamide (HF1). We arbitrarily decided that if the microscopic mirror-image sections of gross specimens of BPH and cancer provided to us contained >10% cancer in the BPH specimen, or <70% cancer in the cancer specimen, the tissue was to be discarded. A total of 23 patient specimens meeting these criteria was available for analysis. Histoculture, as previously described [3] was performed for 5 days. DHT ( $2 \times 10^{-8}$  M) was added daily on days 2-5 of histoculture to one plate of both BPH and cancer; HF1 ( $2 \times 10^{-5}$  M) was added to a parallel plate of BPH and cancer tissue on a similar schedule.  $^3\text{H}$ -thymidine (8  $\mu\text{c}$ ) was added on day 5 to all plates, and the incubations were stopped 24 h later. The tissues were kept frozen following incubation until analysis.

### Measurement of $^3\text{H}$ -Thymidine Incorporation

Processing of tissues for measurement of  $^3\text{H}$ -thymidine incorporation per mcg of protein was done by first washing tissues to remove nonspecific  $^3\text{H}$ -thymidine. Tissues were then homogenized in sucrose buffer and centrifuged. The pellet obtained was digested with benzomethonium hydroxide for  $^3\text{H}$ -thymidine counts per minute, and the supernate was analyzed for protein concentration. Results were expressed as  $^3\text{H}$ -thymidine incorporation per mcg protein for both BPH and cancer tissues histocultured with both HF1 and DHT.

## ANDROGEN-INDEPENDENT GROWTH



**Fig. 1.** Left bar represents androgen-independent growth measured as  $^3\text{H}$ -thymidine incorporation per mcg protein in BPH specimens in histoculture with hydroxyflutamide  $2 \times 10^{-5}$  M. Right bar represents the same study in a paired sample of tissues from the cancer-containing part of the prostate. Twenty-three pairs were studied. There is a significantly higher level of androgen-independent growth in the BPH compared to the cancer specimens ( $P = 0.015$ ).

### Calculation of Androgen-Independent and Androgen-Dependent Growth

Androgen-independent growth was measured as  $^3\text{H}$ -thymidine incorporated per mcg protein when androgen was blocked by  $2 \times 10^{-5}$  M HF1 in histoculture. Androgen-dependent growth was calculated as the percent decrease in  $^3\text{H}$ -thymidine incorporation in HF1 histoculture compared to the same tissue in histoculture with  $2 \times 10^{-8}$  M DHT.

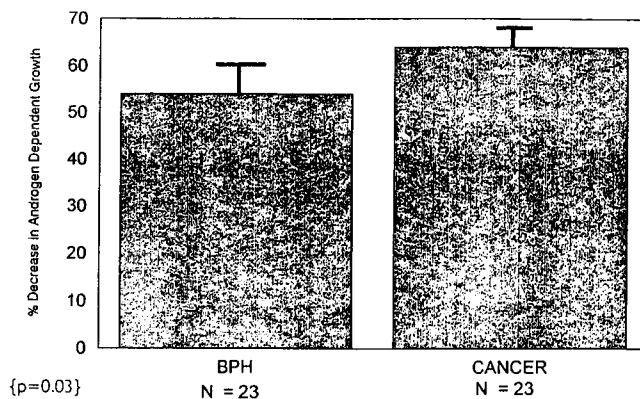
Gleason grade was assessed by the pathologist for each cancer. Correlation of Gleason grade with both androgen-independent and androgen-dependent growth was done.

## RESULTS

### Measurement of Androgen-Independent Growth

Androgen-independent growth was significantly higher in 23 BPH as compared to paired cancer tissues (see Fig. 1). Following incubation with HF1, three quarters of the BPH specimens had higher values of thymidine incorporation than the cancer paired samples; mean androgen-independent growth expressed as  $^3\text{H}$ -thymidine incorporation per mcg protein averaged  $82.7 \pm 95$  SD (range, 3.9–415) in BPH and  $41.8 \pm 40.3$  SD (range, 2.6–132) in prostate cancer, a statistically significant difference ( $P \leq 0.015$ ), as measured by Student's *t*-test using paired analysis.

## ANDROGEN DEPENDENT GROWTH



**Fig. 2.** Androgen-dependent growth in BPH and cancer-paired tissues from 23 radical prostatectomies were measured. Androgen-dependent growth represents the percentage decrease in  $^3\text{H}$ -thymidine incorporation per mcg protein in the hydroxyflutamide-treated histoculture compared to the DHT-treated histoculture. Note that the right bar, which represents mean cancer androgen-dependent growth, is significantly higher than the left bar representing BPH ( $P = 0.03$ ).

### Androgen-Dependent Growth Measurement

$^3\text{H}$ -thymidine incorporation was higher in 12/23 BPH compared to cancer histocultures incubated with DHT alone. Androgen-dependent growth, as defined, was greater in 70% of prostate cancer specimens compared to BPH from the same prostate. The value for prostate cancer was  $64 \pm 17\%$  SD (range, 34–88%), while that for BPH was  $54 \pm 28\%$  SD (range, 5–88%), a significant difference (see Fig. 2) ( $n = 23$ ,  $P \leq 0.03$ ), as measured by paired *t*-test analysis.

### Correlation of Gleason Grade With Androgen-Independent and -Dependent Growth

The  $R^2$  value for androgen-independent growth and Gleason grade was 0.0587; a *t*-test indicated no correlation between the two parameters.

$R^2$  value for androgen-dependent growth was 0.1187; a *t*-test indicated  $P > 0.1$ , again denoting an absence of any significant correlation with Gleason grade.

## DISCUSSION

The measurement of  $^3\text{H}$ -thymidine incorporation under the conditions of optimal stimulation with DHT and optimal blockade with HF1 is critical to the inter-

pretation of androgen-independent and androgen-dependent growth as defined.

Kennealey and Furr [4] showed that  $10^{-5}$  M HF1 maximally inhibits  $^3\text{H}$ HR1881 binding to the rat prostate androgen receptor. Based on these studies, we decided upon the use of  $2 \times 10^{-5}$  M HF1 in our histocultures for androgen inhibition. That this concentration was not toxic to human prostate tissue was confirmed in our laboratory (data not shown) using fluorescence of cells treated with the dye 2',7'-bis-(2-carboxyethyl)-5-(and -6) carboxy-flourescein acetoxy-methylester as the criterion for cell viability [5].

For optimal stimulation of prostate growth, a DHT concentration of  $2 \times 10^{-8}$  M was chosen, since it is close to the physiologic levels of DHT found in the human prostate. Shao et al. [6] showed this to be an optimal concentration for stimulation of growth of the rat prostate.

In 12 out of 23 paired samples,  $^3\text{H}$ -thymidine incorporation was higher in the BPH than in the cancer specimens in histoculture with added DHT alone. Androgen-independent growth, however, as defined, was higher in the majority (18 out of 23) of BPH specimens and significantly greater ( $P = 0.015$ ) than cancer specimens (see Fig. 1) because of the greater percentage decrease in  $^3\text{H}$ -thymidine incorporation in HF1-treated cancer compared to BPH. By contrast, however, androgen-dependent growth was greater in 70% of the cancer as compared to the paired BPH tissue specimens. These differences were statistically significant ( $P \leq 0.03$ ) (see Fig. 2). We interpret this as strong evidence for an increased androgen-dependence in prostate cancer as compared to BPH tissue from the same specimen.

Prior evidence for the increased sensitivity of prostate cancer to androgen was noted by Levine et al. [7], who noted that  $10^{-14}$ – $10^{-18}$  M DHT stimulated LNCAP cell line growth in culture.

Similar findings were noted by Labrie et al. [8] in clones of the androgen-sensitive Shionogi carcinoma, which is not a prostate tissue.

Currently, Gleason grade of tumor tissue is the most frequently used and best-established of indices for predicting aggressiveness of prostate cancer, although there are many exceptions noted. We did look for correlations between Gleason grade of our 23 prostate cancers and the androgen-independent as well as androgen-dependent growth parameters. We did not find a significant correlation of either parameter with Gleason grade. This suggests that the tissue growth parameters represent different biological characteristics of prostate cancer that may or may not provide a better measure of tumor aggressiveness. This can only be determined by future clinical outcomes in our patients.

It is possible that an increased epithelial cell mass, theoretically represented by prostate cancers, might give higher  $^3\text{H}$ -thymidine incorporation and be more androgen-responsive compared to the mixture of epithelial and stromal tissue which one finds in BPH, and therefore might create a bias in favor of a greater response to androgen blockade in cancer specimens. There are studies in the literature showing that epithelial cell structures are slightly more responsive to androgen deprivation than stromal cells by Laroque et al. in dogs [9] and by Peters and Walsh in humans [10]. However,  $^3\text{H}$ -thymidine incorporation per mcg protein in our DHT-treated histocultures of BPH and cancer was higher in 12 of the 23 paired BPH compared to cancer specimens, strongly suggesting that there was no bias in favor of increased  $^3\text{H}$ -thymidine incorporation by the cancers. It is of course also well-known that the stroma as well as epithelium has androgen receptors and therefore is responsive to both androgen administration as well as androgen withdrawal.

Whether the androgen-dependent and/or androgen-independent growth characteristics of prostate cancer provide any insight into tumor aggressiveness will depend upon future retrospective analyses of correlations of these parameters with clinical outcomes, such as time to PSA relapse in these patients. A broad range of values was noted for androgen-independent growth as well as androgen-dependent growth. If a correlation does exist, this HASA assay may well have an important practical use in identifying the aggressiveness of prostate cancer and therefore in defining appropriate therapy.

## CONCLUSION

Comparison of growth parameters in BPH and cancer appears valid, using our histoculture technique. Androgen-independent growth, measured as  $^3\text{H}$ -thymidine incorporation in histoculture under conditions of androgen blockade with HFL, was significantly increased in BPH compared to paired cancer specimens. However, androgen-dependent growth, measured as percentage decrease in DHT-stimulated  $^3\text{H}$ -thymidine incorporation by HF1, was significantly greater in cancer than in BPH. No correlation of either growth parameter with Gleason grade was noted. A wide range of values for androgen-independent and androgen-dependent growth was noted for both BPH and cancer, and we plan to follow patients retrospectively to search for a correlation of one or both of these markers with time to PSA relapse.

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