

Postatrophic Hyperplasia of the Prostate Gland

Neoplastic Precursor or Innocent Bystander?

Rajal Shah,* Neil R. Mucci,[†] Anita Amin,*
Jill A. Macoska,[‡] and Mark A. Rubin*[‡]

From the Departments of Pathology,* Internal Medicine,[†] and
Surgery;[‡] Section of Urology, University of Michigan, Ann
Arbor, Michigan

Postatrophic hyperplasia (PAH) of the prostate gland often demonstrates overlapping histological features with prostatic adenocarcinoma (PCA). These features include small acinar growth and enlarged nuclei with prominent nucleoli. Recent work has demonstrated that PAH is a proliferative, noninvoluting lesion. PAH is also histologically distinct from simple atrophy (SA), which has intermediate- to large-sized glands, minimal cytoplasm, and inconspicuous nuclei. However, despite overlapping features between PAH and PCA, high-grade prostatic intraepithelial neoplasm (HGPIN) is still considered the only direct neoplastic precursor to PCA. HGPIN resembles PCA in its topographic distribution, cytological appearance, and molecular alterations including chromosome 8p loss and chromosome 8 centromeric gain. To examine the hypothesis that PAH is the earliest histologically distinct precursor to HGPIN or PCA, the frequency, distribution, proliferative state, and chromosome 8 gain of benign prostate, SA, PAH, HGPIN, and PCA were analyzed. Forty radical prostatectomy specimens from men with clinically localized PCA were systematically analyzed. Proliferation was determined by Ki-67 immunohistochemistry (MIB-1) on formalin-fixed, paraffin-embedded tissue and quantified by digital image analysis from a total of 5,510 sample areas with benign, SA, PAH, HGPIN, and PCA. A tissue microarray was constructed to evaluate 8c gain using interphase fluorescence *in situ* hybridization. SA foci ($n = 129$) and PAH foci ($n = 114$) were identified in the 40 cases of which 74% (95 of 129) and 88% (100 of 114) were seen in the peripheral zone, respectively ($P = 0.006$). PAH and SA were identified adjacent to PCA in 28% (32 of 114) and 14% (18 of 129) of foci examined, respectively ($P = 0.007$). The median number of proliferating nuclei increased significantly from benign (1.20%), SA (2.67%), PAH (3.62%), HGPIN (6.14%), to PCA (12.00%) ($P < 0.001$). The median percentage of nuclei with more than three centromeric probe signals (chromosome 8c gain) for SA, HGPIN, PAH, and PCA were 2.1, 2.8, 4.0, and 6.0%, respectively,

as compared to benign prostate with 1.3% ($P = 0.006$). In conclusion, the present study identified a strong topographic association between PAH and PCA. PAH is also seen often to be closely associated with chronic inflammation. Proliferation of PAH is significantly greater than benign prostatic epithelium and SA but less than HGPIN or PCA. Gain of 8c is significantly greater in PAH than benign prostate, SA, and even HGPIN. These findings demonstrate a strong association between PAH and PCA, supporting its role as a neoplastic precursor. (*Am J Pathol* 2001, 158:1767–1773)

Postatrophic hyperplasia (PAH) of the prostate gland consists of dilated ducts or acini with adjacent foci of small crowded glands with an atrophic appearance.¹ Nuclear enlargement and prominent nucleoli may often be observed (Figure 1A). PAH is best known to the surgical pathologist as a mimic of prostatic adenocarcinoma (PCA) because of its overlapping architectural and nuclear features, especially when these lesions are predominantly composed of small-crowded glands with nuclear enlargement and prominent nucleoli. Distinguishing PAH from PCA is particularly important on prostate needle biopsy because of the therapeutic implications.

In 1954, Franks² considered a specific subtype of PAH (“sclerotic atrophy with hyperplasia”) as a putative neoplastic precursor given its close association with PCA. Franks considered this atrophic lesion to be proliferative in nature. Liavag³ demonstrated a similar topographical association between PAH and PCA. Subsequently this hypothesis fell out of favor with the identification of high-grade prostatic intraepithelial neoplasia (HGPIN), which is now widely regarded as the most likely pre-invasive neoplastic precursor to PCA. The data supporting HGPIN as a precursor lesion includes its topographic association to PCA (peripheral zone), cytological resemblance to PCA, and similar molecular alterations, in particular chro-

Supported by the National Cancer Institute Special Program of Research Excellence (S.P.O.R.E.) in Prostate Cancer (grant CA69568). Presented in part at the United States and Canadian Academy of Pathology (New Orleans, LA., March 2000) and the American Urologic Association (Atlanta, GA., May 2000) meetings as poster presentations.

Accepted for publication February 5, 2001.

Address reprint requests to Mark A. Rubin, M.D., Assistant Professor, Pathology and Surgery-Urology Section, University of Michigan, 1500 E. Medical Center Dr., Room 2G 332/Box 0054, Ann Arbor, MI 48109-0054. E-mail: marubin@umich.edu.

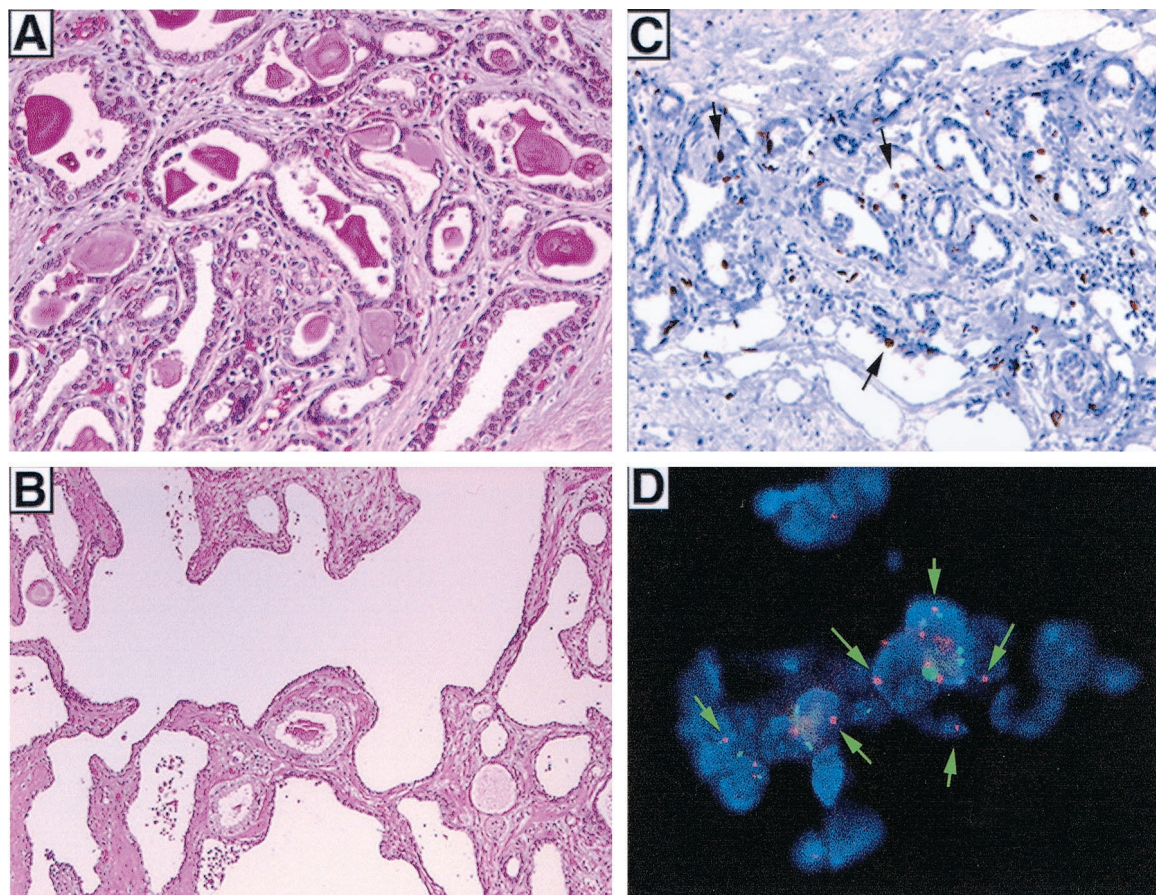


Figure 1. **A:** Postatrophic hyperplasia (H&E). **B:** Simple atrophy (H&E). **C:** Elevated proliferation of postatrophic hyperplasia determined by Ki-67 (arrows). **D:** Gain of 8c in postatrophic hyperplasia determined by FISH (red probe for 8c (arrows) and green probe for chromosome 12c control).

mosome 8p22 loss and 8c gain.⁴⁻¹² The major distinction between HGPIN and PCA is the presence of a basal cell layer in HGPIN, which can be demonstrated using the basal cell-specific antibody 34 β E12.¹³

Several recent studies have re-examined the role of PAH as a potential neoplastic precursor.^{1,14-16} Two studies examining the topographic location of PAH with respect to PCA concluded that PAH is extremely common in the periphery of the prostate gland.^{1,14} Immunohistochemical analysis has identified some surprising features regarding PAH. First, Ruska and colleagues¹⁶ demonstrated that prostatic atrophy including PAH is not undergoing involution but instead is proliferative in nature as determined by high MIB-1 expression by immunohistochemistry (Ki-67). This confirmed previous observations by Feneley and colleagues¹⁷ and Liavag.³ Second, De Marzo and colleagues¹⁵ identified expression of the carcinogen-detoxifying enzyme, π -class glutathione S-transferase (GSTP1) in the basal cell layer of benign nonatrophic prostatic epithelium, which they believe protects cells from DNA damage. Prostatic atrophy including PAH for the most part overexpresses GSTP1 except for focal areas, which have low or absent GSTP1 expression. They concluded that the majority of prostatic atrophy is protected from incurring DNA damage because of the protective role of GSTP1. However, some of the secretory cells are potential targets for genetic alterations and po-

tentially neoplastic transformation. Finally, work from Macoska and colleagues¹⁸ recently identified chromosome 8p22 loss and 8 centromere (8c) gain in a small number ($n = 7$) of atrophic lesions using interphase fluorescence *in situ* hybridization (FISH).

This study examines PAH as a neoplastic precursor lesion by comparing topography, proliferation, and 8c gain between PAH, HGPIN, and PCA in a series of radical prostatectomies from men with clinically localized PCA.

Materials and Methods

Case Selection

Between the years 1996 and 1998, 632 patients underwent radical retropubic prostatectomy as a monotherapy (ie, no hormonal or radiation therapy) at the University of Michigan Hospital for clinically localized PCA. Clinical and pathology data for all patients was acquired with approval from the Institutional Review Board. From these cases, 40 formalin-fixed and paraffin-embedded whole-mount radical prostatectomies were randomly identified for the purposes of this study. The preoperative and pathology data for all cases is summarized in Table 1. These cases are representative of the larger population of cases; no statistically significant differences between

Table 1. Preoperative and Prostatectomy Data on Study Cases ($n = 40$)

		Range
Preoperative demographics		
Age	60.5 years median	39–75 years
Serum PSA	5.1 ng/ml median	0.9–14.2 ng/ml
Clinical stage		
T1a	70% (28/40)	
T2a–c	30% (12/40)	
Prostatectomy results		
Weight	51.8 g median	21.2–102 g
Maximum tumor dimension	1.2 cm median	0.2–3.2 cm
Gleason Score		
5	2.5% (1/40)	
6	47.5% (19/40)	
7	50% (20/40)	
Extraprostatic extension	20% (8/40)	
Seminal vesicle invasion	2.5% (1/40)	
Positive surgical margins	12% (5/40)	

the 40 cases in the present study and the 632 cases were seen (data not shown). Hematoxylin and eosin-stained sections were evaluated for foci of simple atrophy (SA), PAH, HGPIN, and PCA. SA was defined as large dilated glands with normal architecture composed of lining epithelial cells with scant cytoplasm and absence of nuclear enlargement or prominent nucleoli. PAH was defined using previously described criteria.¹ In brief, PAH included dilated acini with adjacent small, crowded glands. Architecturally these foci have a lobular, well-circumscribed appearance at low magnification. High magnification demonstrated scant to moderate amounts of cytoplasm, regular chromatin pattern, and occasional nucleoli. Focal nuclear enlargement was also observed within the microacinar foci of PAH. Foci demonstrating these features were marked and analyzed for several parameters: 1) number of foci with SA and PAH; 2) location within the prostate gland as peripheral or transition zone; 3) association of SA and PAH with HGPIN and PCA as adjacent, near, or distant (adjacent was defined as glands merging with each other, near was defined as being within one high-power field from each other and distant was defined as separated by more than one high-power field); and 4) number of SA and PAH foci associated with acute and chronic inflammation.

Proliferation Study

Immunohistochemistry

Indirect immunohistochemistry was performed to evaluate proliferation using the monoclonal mouse IgG Mib-1 antibody for Ki-67 (1:150 dilution; Coulter-Immunotech, Miami, FL). Microwave pretreatment (30 minutes at 100°C in Tris-ethylenediaminetetraacetic acid buffer) for antigen retrieval was performed using 3,3'-diaminobenzidine tetrahydrochloride as a chromogen. Negative controls were derived by omission of the primary antibody. Four slides per case ($n = 168$ slides total) were evaluated for proliferation index in areas of benign, SA, HGPIN, and PCA using a digital image analysis system (CAS200; Bacus

Labs, Lombard, IL). Measurements were recorded as the percentage of total nuclear area that was positively stained. All positive nuclear staining, regardless of the intensity, was measured. Sites for analysis were selected to minimize the presence of nonsecretory epithelium (eg, stromal and basal cells). Each site contained ~50 to 100 epithelial nuclei. Proliferation was measured from 5,510 sites.

Tissue Microarray Construction

To evaluate all lesions of interest on a single glass slide, a tissue microarray was assembled using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD). This procedure has previously been described.^{19,20} The instrument consists of thin-walled stainless steel needles with an inner diameter of ~600 μm and stylet used to empty and transfer the needle contents. The assembly is held in an X-Y position guide that is manually adjusted by digital micrometers. In this technique, small biopsies were retrieved from selected regions of donor tissue and transferred to a new paraffin block. Tissue cores are 0.6 mm in diameter and range in length from 1.0 mm to 3.0 mm depending on the depth of tissue in the donor block. Cores are inserted into a 45 \times 20 \times 12-mm recipient block (standard size) and spaced at a distance of 0.8 mm apart. After construction, 3- μm sections of the resulting microarray block were cut and a hematoxylin and eosin-stained slide was reviewed to confirm that the targeted tissue was actually transferred into the tissue array. Because of the small size of PAH and HGPIN lesions, only a total of 17 cases (43% of 40 cases) were represented on the final tissue microarray. The identity of each 0.6-mm tissue sample was tracked using their coordinate (X-Y) position and linked to clinical and pathology data on a relational database (Microsoft Access; Microsoft, Redmond, WA).

FISH

FISH analysis for 8c gain was performed as previously described^{18,21–23} using the rhodamine-labeled chromosome 8 pericentromeric probe DNA as the 8c probe (CEP8 Spectrum Orange DNA Probe; Vysis Inc., Downer's Grove, IL) on formalin-fixed, paraffin-embedded tissue cut at a thickness of 5 μm . Standard slides and tissue microarray slides were used to optimize this protocol. As an initial control of nuclear ploidy, a pericentromeric DNA probe for chromosome 12 was used (CEP 12 spectrum green DNA probe, Vysis Inc.). Slides were incubated in 4 mg/ml pepsin (Sigma P-7012 in 0.9% NaCl, pH 1.5, Sigma Chemical Co., St. Louis, MO) at room temperature for 5 minutes. Slides were hybridized for 16 hours in a humidified chamber and then counterstained with DAPI II and visualized using appropriate fluorescent light filters (Zeiss Axio-plan, Germany). Tissue microarray samples (0.6-mm diameter) were analyzed at $\times 1,000$ final magnification by counting the number of fluorescent signals in at least 100 nuclei per tissue microarray sample. Morphology was con-

Table 2. Comparison between Simple Atrophy (SA) and Postatrophic Hyperplasia (PAH) from 40 Radical Prostatectomies

	SA	PAH	P value
Location			
Peripheral zone	95 (74%)	100 (88%)	0.006
Transitional zone	34 (26%)	14 (12%)	0.005
Adjacent to			
PCA	18 (14%)	32 (28%)	0.007
High-grade PIN	12 (10%)	16 (14%)	0.3
Associated with inflammation			
Acute	18 (14%)	21 (18%)	0.3
Chronic	37 (29%)	49 (43%)	0.02

firmed by bright-field microscopy before evaluating the next tissue sample.

Statistical Analysis

Using commercial software two nonparametric tests were applied. The Kruskal-Wallis (for more than two categories) and Wilcoxon ranked sum tests (for two categories) were used to determine differences between histological tissue types and test results (8c gain and Ki-67 expression).

Results

Phenotypic and Topographic Association of PAH and PCA

SA and PAH were both found frequently and topographically located near or adjacent to PCA in the peripheral zone of the prostate gland. Examination of 40 consecutive whole-mount radical prostatectomy specimens revealed SA in 39 of 40 (98%) cases and PAH in 31 of 40 (78%) cases. SA foci ($n = 129$, mean 3.2/case) and PAH foci ($n = 114$, mean 2.9/case) were identified. Peripheral zone location, the most common site of PCA and HGPIN was common for both foci of SA and PAH, yet PAH tended to be more common. Seventy-four percent (95 of 129) foci of SA and 88% (100 of 114) of PAH were present in peripheral zone, respectively (chi-square test, $P = 0.006$). There was a significant difference between the number of SA and PAH foci located directly adjacent and near to PCA, with 14% (18 of 129) and 28% (32 of 114) foci of SA and PAH, respectively (chi-square test, $P = 0.007$). No such difference was noted for the number of SA and PAH foci located directly adjacent to and near to HGPIN with 10% (13 of 129) and 14% (16 of 114), respectively (chi-square test, $P = 0.3$). Foci of PAH were more often associated with chronic inflammation than SA, 43% (49 of 114) versus 29% (37 of 129), respectively

(chi-square test, $P = 0.02$). These results are summarized in Table 2.

Proliferation of PAH, HGPIN, and PCA

The median Ki-67 labeling rate (ie, percentage of total nuclear area staining positive for Mib-1) analyzed from >5,510 sites revealed a progressive increase from benign to PCA (Table 3). As a base line, 1.20% (95% confidence intervals 0.82 to 1.74%) of benign, nonatrophic prostate glands demonstrated nuclear proliferation as determined by Mib-1 staining for Ki-67. A significant increase in nuclear proliferation from normal prostate was seen along a continuum: SA (2.67%) < PAH (3.62%) < HGPIN (6.14%) < PCA (12.00%) ($P < 0.001$).

Chromosome 8 Gain for PAH, HGPIN, and PCA

FISH analysis was successfully performed on the tissue microarray slide constructed with multiple replicate samples of benign prostate, SA, PAH, HGPIN, and PCA. Fifty-one samples could be adequately evaluated for 8c gain (Table 4). Chromosome 8 gain was divided into three groups ranging from zero to two fluorescent signals, three signals (tri-ploidy), and greater than three signals. A gain of more than three signals for 8c was observed in 1.3% of the benign nuclei. A gradual increase in the percentage of nuclei with more than three signals for 8c was found for SA (2.1%), HGPIN (2.8%), PAH (4.0%), and PCA (6.0%) ($P = 0.006$). Therefore significant 8c gain was observed more often in PAH than in either SA or HGPIN.

Discussion

The topographic association between PAH and PCA was the focus of a recent study by Anton and colleagues.¹ In their systematic evaluation of 272 radical prostatectomies, they found PAH in 32% of cases (86 of 272) with 91% occurring in the peripheral zone. PAH was identified within 2 mm of PCA in a third of the PAH foci (33%). However, when they examined the mirror image area on the opposite side of the prostate without PAH, they found PCA in 40% of cases. They concluded that PAH was common in the peripheral zone but was not specifically associated with PCA. The present study identified PAH in 31 of 40 (78%) prostatectomy specimens examined, with 88% occurring in the peripheral zone of the prostate. PAH was seen adjacent to PCA in 28% of the foci identified. Taken together these studies suggest that PCA is often but not exclusively associated PAH. Anton and colleagues¹ suggest that because PAH was seen as often in

Table 3. Proliferation of Postatrophic Hyperplasia Compared to Benign, High-grade PIN, and PCA

	Benign	SA	PAH	HGPIN	PCA
Measurements	2,560	910	750	540	750
Proliferation*	1.20 (0.82–1.74)	2.67 (2.07–3.44)	3.62 (2.9–4.5)	6.10 (4.6–8.2)	12.10 (9–15.9)

* Ki-67 index (95% confidence intervals). $P < 0.001$.

Table 4. Percentage Nuclei Exhibiting Chromosome 8 Gain in Atrophy Compared to Benign, HG PIN, and PCA

Tissue Type	n	Percentage nuclei with chromosome 8 signal		
		0-2 (SD)	3 (SD)	>3 (SD)
Benign	7	93.1% (5.0)	6.3% (4.5)	1.3% (0.6)
SA	10	85.5% (6.3)	12.6% (6.0)	2.1% (0.8)
PAH	15	86.4% (9.8)	11.2% (7.6)	4.0% (2.9)
High-grade PIN	8	86.8% (8.1)	11.1% (6.1)	2.8% (2.1)
Prostate cancer	11	81.7% (13.5)	14.5% (9.4)	6.0% (4.4)
		P = 0.20	P = 0.24	P = 0.006

SD, Standard deviation.

the absence of PCA, PAH is not etiologically associated with PCA. In fact, it is precisely the common occurrence in the peripheral zone that makes PAH an interesting putative precursor lesion. One might expect to see numerous precursor lesions give rise to a smaller number of tumors. This model would then predict that there is a high association between PAH and PCA but that PAH would often be seen in isolation.

Another important observation made by Anton and colleagues¹ was the high degree of association between chronic inflammation and PAH. Chronic inflammation was present at 88% of the PAH foci. This was considerably greater than that of the 43% of PAH lesions with chronic inflammation seen in the present study and the 32% association identified by Ruska and colleagues.¹⁶

Ruska and colleagues¹⁶ renewed interest in the significance of prostatic atrophy by demonstrating that prostatic atrophy is not undergoing involution but is instead proliferative in nature as determined by the proliferation marker Ki-67 (Mib-1). This observation was confirmed by De Marzo and colleagues¹⁵ using prostatectomy specimens. The present study performed a global analysis of proliferation with >5,000 measurements. The results demonstrated a proliferative gradation going from benign prostate (1.20%), SA (2.67%), PAH (3.62%), HGPIN (6.14%), to PCA (12.00%). The present study found a significant difference in proliferation between each category, including SA and PAH. SA had a proliferation index of 2.67%, which was greater than benign prostate (1.20%), but significantly less than PAH (3.62%). The difference in proliferation between SA and PAH is compatible with their distinct histological appearances.

De Marzo and colleagues¹⁵ have attempted to link the strong association with proliferative atrophic lesions and chronic inflammation, noting that longstanding chronic inflammation seems to predispose one to the development of carcinoma. In their study, they combined focal SA and PAH, coining the term proliferative inflammatory atrophy. They observed expression of the carcinogen-detoxifying enzyme, π -class glutathione S-transferase (GSTP1) in the basal cell layer of benign nonatrophic prostatic epithelium; the induction of GSTP1 is believed to protect cells from DNA damage. In contrast, the secretory-type epithelial cells in proliferative inflammatory atrophy (PAH and SA) overexpress GSTP1 except for focal areas, which have low or absent GSTP1 expression. Therefore, according to their study, the majority of proliferative inflammatory atrophy is protected from incurring DNA damage because of the protective role of GSTP1;

some of the secretory cells, however, are potential targets for genetic alterations and potentially neoplastic transformation.

We recently reported 8c gain in 7 atrophic lesions.¹⁸ The present study expanded on that observation by evaluating SA and PAH lesions using FISH for 8c gain. Interestingly, PAH demonstrated an increase in three fluorescent signals for 8c that was greater than SA or even HGPIN. One novel approach taken in this project was the development and utilization of a tissue microarray composed of targeted lesions. Therefore, we were able to assemble PCA, PAH, and benign tissue from the same case on the same slide, allowing better control of experimental variables, such as antibody binding and *in situ* hybridization efficiencies. Another potential benefit is that histologically benign epithelium adjacent to PCA may not be genetically normal. Perhaps more importantly, HGPIN adjacent to PCA may in instances represent intraductal spread of tumor and not discrete dysplastic lesions. Therefore our sampling method, taking lesions away from tumor, may avoid a field effect bias. This approach is in contrast to most studies looking at molecular alterations in HGPIN using FISH. These studies have confined their analysis to a single slide per case.^{18,24,25} The tissue microarray, as recently demonstrated by Bubendorf and colleagues,²⁶ is also an efficient way to profile a large number of tumors.

8c gain has been reported to be a common molecular alteration found in HGPIN.²⁵ 8c gain in prostate tumors is believed to be a marker for poor prognosis.^{18,21,24,27} For example, Sato and colleagues²⁴ reported that 34.7% (50 of 144) of stage III (T3N0M0) prostate tumors had 8c gain. Macoska and colleagues,¹⁸ recently reported a significant association between poor outcome and 8c gain. The mechanism for 8c gain was recently demonstrated to be because of isochromosome 8 formation with 8p loss.^{7,28} Putative proto-oncogenes on 8q²⁹ and tumor suppressor genes on 8p^{8,11} have also been reported.

The present study found that PAH demonstrated a significant increase in the percentage of nuclei with greater than three signals for 8c as compared to HGPIN, SA, and benign prostate. This percentage was only less than the 6.0% found in PCA. This observation along with the proliferative data suggests that SA and PAH are distinct from one another. However, they may represent a continuum in the process of tumorigenesis. We believe that the absolute percentage for chromosome 8c gain may be underestimated because of inefficient hybridization. For each sample, as described in the Materials and

Methods section, at least 100 nuclei were counted. There was a significant proportion (~70%) that demonstrated either one or no signals. This would suggest that improvement in our ability to perform hybridization with the 8c probe would demonstrate an even higher percentage of prostate tissue with chromosome 8 gain. The present study also identified an increase in the percentage of nuclei with trisomy for 8c in the atrophic lesions (SA and PAH), HGPIN, and PCA with respect to benign prostate. This difference was, however, not statistically significant ($P = 0.24$). This lack of significance may be because of an insufficient number of cases evaluated.

The findings of the present study build on previous work from Ruska and colleagues¹⁶ and De Marzo and colleagues.¹⁵ Prostatic atrophy is a common, histologically distinct entity found in the peripheral zone of the prostate. PAH is in a state of proliferation and not involution. PAH is often associated with chronic inflammation and PCA. PAH also demonstrates 8c gain, a molecular alteration often seen in HGPIN and PCA. These results support the earlier hypothesis by Franks² that PAH or a subtype of prostatic atrophy is a neoplastic precursor. It is important to recognize that the evidence for HGPIN as the direct precursor to PCA is identical to findings of PAH and to a lesser degree SA. There is a spectrum of changes seen from SA to PAH and various studies have used slightly different definitions for PAH. For example, the present study has a broader definition of PAH that is more consistent with Anton and colleagues.¹ Ruska and colleagues¹⁶ confined their study to include a narrow range of lesions and avoided lesions with overlapping features.

In conclusion, the present study identifies a strong topographic association between PAH and PCA. PAH is also seen often to be closely associated with chronic inflammation. Proliferation of PAH is significantly greater than benign prostatic epithelium and SA but less than HGPIN or PCA. Gain of 8c is significantly greater in PAH than benign prostate, SA, and even HGPIN. These findings demonstrate a strong association between PAH and PCA, supporting its role as a neoplastic precursor.

However, a number of questions still need to be answered. Does PAH give rise to HGPIN or directly to PCA? What is the role of inflammation in this process? Do random molecular events in PAH occur that cause a chronic inflammatory response? Or conversely does chronic inflammation produce these molecular alterations? Further studies using laser capture microdissection and expression array technology should help to more completely elucidate the relationship between PAH and PCA.

Acknowledgment

We thank Sally Pilon for her secretarial assistance.

References

1. Anton RC, Kattan MW, Chakraborty S, Wheeler TM: Postatrophic hyperplasia of the prostate: lack of association with prostate cancer. *Am J Surg Pathol* 1999, 23:932-936

2. Franks L: Atrophy and hyperplasia in the prostate proper. *J Pathol Bacteriol* 1954, 68:617-621
3. Liavag I: Atrophy and regeneration in the pathogenesis of prostatic carcinoma. *Acta Pathol Microbiol Scand* 1968, 73:338-350
4. Qian J, Jenkins RB, Bostwick DG: Genetic and chromosomal alterations in prostatic intraepithelial neoplasia and carcinoma detected by fluorescence in situ hybridization. *Eur Urol* 1999, 35:479-483
5. Qian J, Jenkins RB, Bostwick DG: Determination of gene and chromosome dosage in prostatic intraepithelial neoplasia and carcinoma. *Anal Quant Cytol Histol* 1998, 20:373-380
6. Cher ML, MacGrogan D, Bookstein R, Brown JA, Jenkins RB, Jensen RH: Comparative genomic hybridization, allelic imbalance, and fluorescence in situ hybridization on chromosome 8 in prostate cancer. *Genes Chromosom Cancer* 1994, 11:153-162
7. Virgin JB, Hurley PM, Nahhas FA, Bechuk KG, Mohamed AN, Sakr WA, Bright RK, Cher ML: Isochromosome 8q formation is associated with 8p loss of heterozygosity in a prostate cancer cell line. *Prostate* 1999, 41:49-57
8. Lutchnan M, Pack S, Kim AC, Azim A, Emmert-Buck M, van Huffel C, Zhuang Z, Chishti AH: Loss of heterozygosity on 8p in prostate cancer implicates a role for dematin in tumor progression. *Cancer Genet Cytogenet* 1999, 115:65-69
9. Matsuyama H, Pan Y, Skoog L, Tribukait B, Naito K, Ekman P, Lichter P, Bergerheim US: Deletion mapping of chromosome 8p in prostate cancer by fluorescence in situ hybridization. *Oncogene* 1994, 9:3071-3076
10. Massenkeil G, Oberhuber H, Hailemariam S, Sulser T, Diener PA, Bannwart F, Schafer R, Schwarte-Waldhoff I: P53 mutations and loss of heterozygosity on chromosomes 8p, 16q, 17p, and 18q are confined to advanced prostate cancer. *Anticancer Res* 1994, 14:2785-2790
11. Macoska JA, Trybus TM, Benson PD, Sakr WA, Grignon DJ, Wojno KD, Pietruk T, Powell IJ: Evidence for three tumor suppressor gene loci on chromosome 8p in human prostate cancer. *Cancer Res* 1995, 55:5390-5395
12. Prasad MA, Trybus TM, Wojno KJ, Macoska JA: Homozygous and frequent deletion of proximal 8p sequences in human prostate cancers: identification of a potential tumor suppressor gene site. *Genes Chromosom Cancer* 1998, 23:255-262
13. Wojno KJ, Epstein JI: The utility of basal cell-specific anti-cytokeratin antibody (34 beta E12) in the diagnosis of prostate cancer. A review of 228 cases. *Am J Surg Pathol* 1995, 19:251-260
14. Billis A: Prostatic atrophy: an autopsy study of a histologic mimic of adenocarcinoma. *Mod Pathol* 1998, 11:47-54
15. De Marzo AM, Marchi VL, Epstein JI, Nelson WG: Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am J Pathol* 1999, 155:1985-1992
16. Ruska KM, Sauvageot J, Epstein JI: Histology and cellular kinetics of prostatic atrophy. *Am J Surg Pathol* 1998, 22:1073-1077
17. Feneley MR, Young MP, Chinyama C, Kirby RS, Parkinson MC: Ki-67 expression in early prostate cancer and associated pathological lesions. *J Clin Pathol* 1996, 49:741-748
18. Macoska JA, Trybus TM, Wojno KJ: 8p22 loss concurrent with 8c gain is associated with poor outcome in prostate cancer. *Urology* 2000, 55:776-782
19. Mucci NR, Akdas G, Manely S, Rubin MA: Neuroendocrine expression in metastatic prostate cancer: evaluation of high throughput tissue microarrays to detect heterogeneous protein expression. *Hum Pathol* 2000, 31:406-414
20. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP: Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 1998, 4:844-847
21. Takahashi S, Alcaraz A, Brown JA, Borell TJ, Herath JF, Bergstrahl EJ, Lieber MM, Jenkins RB: Aneuploidies of chromosomes 8 and Y detected by fluorescence in situ hybridization are prognostic markers for pathological stage C (pt3N0M0) prostate carcinoma. *Clin Cancer Res* 1996, 2:137-145
22. Qian J, Bostwick DG, Takahashi S, Borell TJ, Brown JA, Lieber MM, Jenkins RB: Comparison of fluorescence in situ hybridization analysis of isolated nuclei and routine histological sections from paraffin-embedded prostatic adenocarcinoma specimens. *Am J Pathol* 1996, 149:1193-1199
23. Persons DL, Takai K, Gibney DJ, Katzmann JA, Lieber MM, Jenkins RB: Comparison of fluorescence in situ hybridization with flow cytometry

- etry and static image analysis in ploidy analysis of paraffin-embedded prostate adenocarcinoma. *Hum Pathol* 1994, 25:678–683
24. Sato K, Qian J, Slezak JM, Lieber MM, Bostwick DG, Bergstralh EJ, Jenkins RB: Clinical significance of alterations of chromosome 8 in high-grade, advanced, nonmetastatic prostate carcinoma. *J Natl Cancer Inst* 1999, 91:1574–1580
 25. Qian J, Bostwick DG, Takahashi S, Borell TJ, Herath JF, Lieber MM, Jenkins RB: Chromosomal anomalies in prostatic intraepithelial neoplasia and carcinoma detected by fluorescence in situ hybridization. *Cancer Res* 1995, 55:5408–5414
 26. Bubendorf L, Kononen J, Koivisto P, Schraml P, Moch H, Gasser TC, Willi N, Mihatsch MJ, Sauter G, Kallioniemi OP: Survey of gene amplifications during prostate cancer progression by high-throughout fluorescence in situ hybridization on tissue microarrays. *Cancer Res* 1999, 59:803–806
 27. Takahashi S, Qian J, Brown JA, Alcaraz A, Bostwick DG, Lieber MM, Jenkins RB: Potential markers of prostate cancer aggressiveness detected by fluorescence in situ hybridization in needle biopsies. *Cancer Res* 1994, 54:3574–3579
 28. Macoska JA, Beheshti B, Rhim JS, Hukku B, Lehr J, Pienta KJ, Squire JA: Genetic characterization of immortalized human prostate epithelial cell cultures. Evidence for structural rearrangements of chromosome 8 and i(8q) chromosome formation in primary tumor-derived cells. *Cancer Genet Cytogenet* 2000, 120:50–57
 29. Chang GT, Tapsi N, Steenbeek M, Blok LJ, van Weerden WM, van Alewijk DC, Eussen BH, van Steenbrugge GJ, Brinkmann AO: Identification of a gene on human chromosome 8q11 that is differentially expressed during prostate-cancer progression. *Int J Cancer* 1999, 83:506–511