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An immunohistologic characterization of human prostatic atypical hyperplasia*

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Summary. In an effort to better distinguish the morphologic relationship of atypical hyperplasia of the prostate to benign prostatic hypertrophy and prostatic cancer, 43 prostate specimens were analyzed with ten immunohistologic markers. Two cytokeratin antibodies appeared useful (Cyto M and Cyto P, with the latter slightly more discriminatory). In summary, it appears that atypical hyperplasia is immunohistopathologically related to both benign prostatic hypertrophy and prostatic cancer, having characteristics of both.

Key words: Benign prostatic hypertrophy – Prostatic atypical hyperplasia – Adenocarcinoma of the prostate – Immunohistopathology

In the human prostate there is a histologic variant that could be a morphologic transition between benign prostatic hypertrophy (BPH) and prostate cancer (CAP), that was first described as "atypical hyperplasia" (AH) by Moore [10] in 1943. There have been numerous reports by Mostofi [11], Kastendieck, [7], Tannenbaum [12], De Gaetani [3], Helpap [5], Baron [1], Miller [9], Harbitz [4], McNiel [8], Oyasu [13] and Bostwick [2] describing this entity since then, but its exact relationship to BPH and CAP still remains unclear. The definition of atypical hyperplasia (AH) (to simplify matters this report will employ Moore's original descriptive term) is related to changes in the histologic appearance of the prostate. Newer developments in immunohistologic staining allow better characterization of the various prostatic cell layers. This report is an attempt to morphologically relate AH to both BPH and CAP employing immunohistologic markers in eight BPH, nine AH and twenty-six CAP specimens.

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Materials and methods

Patients

Eight patients, with a histologic diagnosis of BPH and a mean age of 67.5 years, 9 patients with a histologic diagnosis of AH and a mean age of 66.3 years and 26 patients with a histologic diagnosis of CAP and mean age of 69.5 years were evaluated (Table 1). The CAP patients included six stage A patients; three stage B; five stage C; and twelve stage D when classified according to Whitmore [14].

Tissues

The tissues were obtained by either needle biopsies, transurethral resection or open (simple or radical) protatectomy. The tissue was fixed in 10% neutral buffered formalin, paraffin embedded and serially sectioned at 5 μm.

Antibodies

The antibodies employed were commercially purchased with reactivity against the markers cytokeratin (Cyto M and Cyto P; Biogenex), prostate specific antigen (PSA, Biogenex), prostatic acid phosphatase (PAP, Immulok), NK cells, (Leu-7, Becton-Dickenson), epithelial membrane antigen (EMA, Dako), leukocyte common antigen (LCA, Biogenex), desmin (Dako), vimentin (Biogenex) and fibronectin (Biogenex).

Immunohistochemistry

Blocks were sectioned in successive 5 μm cuts and floated onto glass slides which were previously coated with chrome gelatin to prevent

Table 1. Patients

Diagnosis	*N*	Age (mean)
BPH	8	67.5
AH	9	66.3
CAP	26	69.5

Table 2. Specimen reactivity

Marker	BPH ($n = 8$)	AH ($n = 9$)	CAP ($n = 26$)
Cyto P	8/8 100%	3/9 33%	2/26 8%
Cyto M	8/8 100%	9/9 100%	24/26 92%
PSA	8/8 100%	9/9 100%	26/26 100%
PAP	8/8 100%	9/9 100%	24/26 92%
NK	6/8 75%	9/9 100%	23/26 88%
EMA	6/8 75%	6/9 67%	19/26 73%
LCA	5/8 63%	8/9 89%	19/26 73%
DES	4/8 50%	6/9 67%	17/24 68%
VIM	5/8 63%	5/9 56%	18/26 72%
FN	0/8 0%	0/9 0%	0/26 0%

Table 3. Mean Intensity of reactivity

Marker	BPH ($n = 8$)	AH ($n = 9$)	CAP ($n = 26$)
Cyto P	2.6	2.7	1.0
Cyto M	2.8	3.9	3.4
PSA	3.3	3.3	4.4
PAP	4.0	3.8	4.2
NK	3.2	4.0	4.0
EMA	1.8	2.2	2.4
LCA	1.7	2.0	2.2
DES	2.5	2.8	2.8
VIM	1.8	1.8	1.9
FN	0.0	0.0	0.0

tissue detachment during immunochemical staining. The method of Hsu et al. [6] was followed: Briefly, sections were deparaffinized in xylene, and dehydrated in absolute ethanol. Endogenous peroxidase activity was blocked by immersion in 10% methanol containing 3% H_2O_2. The slides were rehydrated with successive washes in 90 to 30% ethanol, until a final emersion in distilled water. Washes in phosphate buffered saline followed prior to trypsinization for 10–15 min using a 1% solution (Difco 1:250) in PBS containing 1% $CaCl_2$, pH 7.8. A final wash in PBS followed prior to treatment with non-specific serum (2%) of goat or horse origin. The primary marker antibody was added and left for 2 h at room temperature. Slides were poured off, washed with PBS and the link antibody (biotinylated antimouse/rabbit immunoglobulin) applied for 30 min, washed again, and the labeled avidin-biotin peroxidase complex added for 20 min. Following more washes in PBS the substrate 3-amino-9-ethylcarbazole (AEC) was added, allowed to incubate for 10 minutes and washed in distilled water, counterstained with hematoxylin and examined.

Analysis

Between three to ten slides (with a mean of approximately four) from each specimen were examined. The number of specimens that reacted with each antibody/the total examined (%) was recorded (Table 2). Degree (intensity) of reactivity (Table 3) was graded on a scale of 0 to 5 (most intense) for each slide according to the following scheme: Grade 0 reflects no cells reacting; Grade 1, less than 5% of the cells reacting; Grade 2, 5–25% of the cells reacting; Grade 3, 26–50% of the cells reacting; Grade 4, 51–75% of the cells reacting; and Grade 5 more than 75% of the cells reacting.

In order to determine the intra- and interobserver differences, slides were examined "in the blind" by each author which included two Board Certified Pathologists. Differences encountered between independent evaluations and observers were minimal.

Results

Table 2 lists the reactivity of BPH, AH and CAP specimens against marker antisera to cytokeratin (Cyto P and Cyto M), PSA, PAP, NK, EMA, LCA, desmin, vimentin and FN.

Cyto P and Cyto M marker reactivity was present in all BPH specimens. However Cyto P was reactive in only 3/9 (33%) and 2/26 (8%) of AH and CAP specimens respectively. Cyto M reactivity was present in all (9/9, 100%) AH and most (24/26, 92%) CAP specimens.

The degree of reactivity (Table 3) also varied between Cyto P and Cyto M. Cyto P had a similar degree of reactivity between BPH (2.6) and AH (2.7) but a lower degree of reactivity to CAP (1.0). Cyto M mean degree of reactivity was not significantly different between BPH (2.8), AH (3.9) and CAP (3.4).

The other markers: PSA, PAP, NK, EMA, LCA, desmin and vimentin were less helpful in that the AH staining pattern too closely resembled both BPH and CAP. FN was not taken up by AH, BPH or CAP.

Discussion

A consensus has arisen among urologists, pathologists and basic scientists that there is an intermediate pathologic variant between BPH and CAP. There have been a variety of names and corresponding definitions assigned to this disease process: atypical hyperplasia by Mostofi [11], Kastendieck [7], Tannenbaum [12], De Gaetani [3] and Helpap [5], atypical adenomatous hyperplasia by Baron [1], marked atypia by Miller [9], atypical glandular hyperplasia by Harbitz [4], intraductal dysplasia by McNiel [8], atypical prostatic hyperplasia by Oyasu [13] and intra-epithelial neoplasia by Bostwick [2]. We will employ the original term, atypical hyperplasia (AH). The significance of AH remains disputed, although in general it appears that these histologic changes are a transition entity between BPH and CAP.

We evaluated eight patients with BPH. Nine patients with AH, and twenty-six patients with CAP employing ten markers for both percent of specimens reacting to the marker antibody and degree of reactivity. PSA and PAP

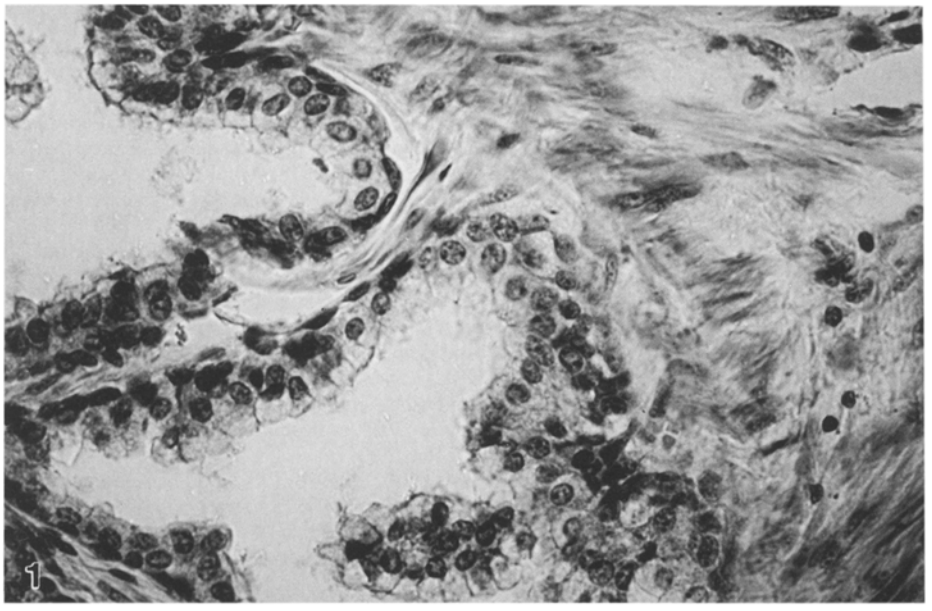

Fig. 1. Immunohistochemical staining for keratins in benign prostatic hypertrophy ×40 utilizing Cyto P. Note the staining pattern in the basal cell layer. Grade 3+

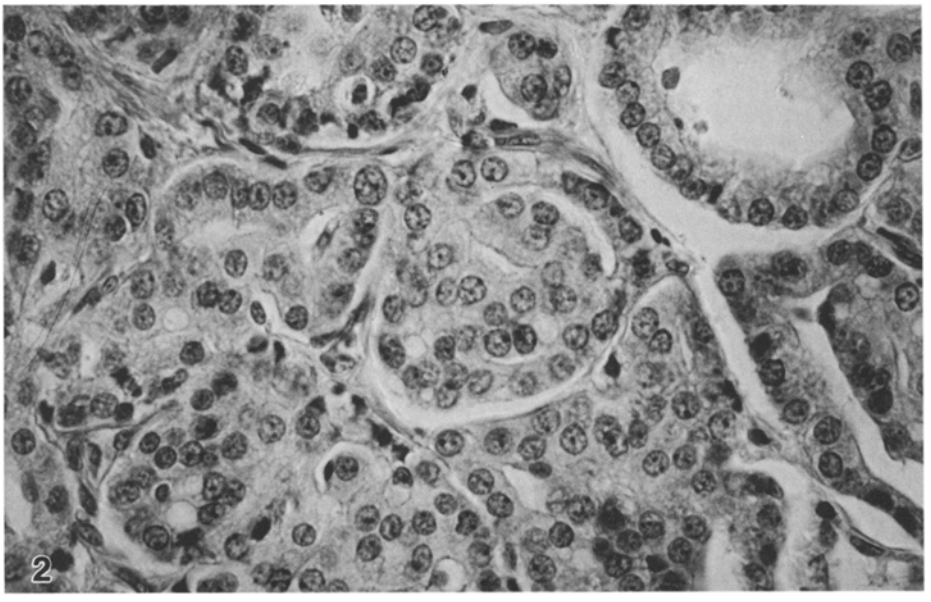

Fig. 2. Immunohistochemical staining for keratins in carcinoma of the prostate ×100 utilizing Cyto P. Note the lack of reactivity. Grade 0

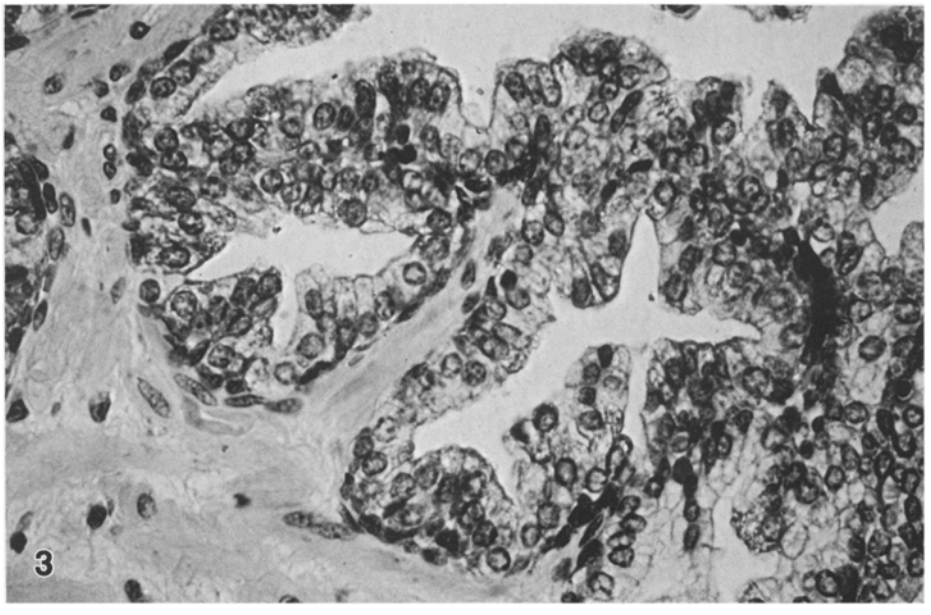

Fig. 3. Immunohistochemical staining for keratins in atypical hyperplasia ×100 utilizing Cyto P. Note that both the luminal cells and the discontinuous layer of basal cells are positive. Grade 4+

were strongly reactive in virtually all specimens. NK reacted intensely but fewer specimens reacted. EMA, LCA, desmin and vimentin reacted more weakly and fewer specimens were reactive. Fibronectin was uniformly absent from the specimens. None of these markers were able to reliably distinguish between the various tissues.

The only marker that could more clearly distinguish between BPH, AH and CAP was cytokeratin (Cyto P and Cyto M). Cyto P (100% reactive in BPH, 33% reactive in AH and 8% reactive in CAP) was more discriminating than Cyto M (100% reactive in BPH, 100% reactive in AH and 92% reactive in CAP). The difference in mean degree of reactivity also suggests that Cyto P (2.6 in BPH, 2.7 in AH, and 1.0 in CAP) was more discriminatory than Cyto M (2.8 in BPH, 3.9 in AH, and 3.4 in CAP).

An evaluation of our material suggests that Cyto P reacts preferrentially with basal cells (Fig 1) and Cyto M stains for both basal and secretory cells. An examination of our CAP specimens suggests that there is a significant loss of basal cells and therefore an absence in Cyto P staining (Fig. 2). The loss of basal cells is one of the characteristics of CAP [12]. The AH specimens also had a relative loss of basal cells and therefore a corresponding decrease in Cyto P staining (Fig. 3). Since the relative secretory cell content was similar in both BPH and CAP, as well as AH, the Cyto M staining was strong in all three.

The results of this investigation suggest that in AH there is a loss of the basal cell layer of the prostate acini and that this loss can be best detected by the marker Cyto P. This also suggests that AH may be a transition phase in the development of CAP. The results of this study, while it cannot be concluded with certainty, suggest that AH may be an incipient form of CAP and that BPH may be related to this process.

The relevance of this work for clinical medicine is the fact that there is a marker (cytokeratin) that can detect AH and that the presence of AH might suggest the presence of CAP elsewhere in the prostate. We conclude that cytokeratin (Cyto P and Cyto M) is potentially a useful marker in the understanding of disease processes of the human prostate.

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