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Phenotypic Relationships of Prostatic Intraepithelial Neoplasia to Invasive Prostatic Carcinoma

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Thirty-one snap-frozen human prostate specimens containing examples of benign hyperplasia, prostatic intraepithelial neoplasia (PIN), and invasive carcinoma were analyzed using a panel of 24 antibodies and one lectin. Twenty-seven additional routinely processed radical prostatectomy specimens were studied using selected probes known to work on formalin-fixed paraffin-embedded material. Three probes, anticytokeratin KA4, anti-vimentin V9, and the lectin from* Ulex europaeus *(UEA-1), demonstrated phenotypic similarities between PIN and invasive carcinoma. Whereas the luminal cells of normal or hyperplastic prostatic epithelium are minimally reactive with KA4 (4%) or UEA-1 (0%) and strongly reactive with anti-vimentin (91%), both the PIN and invasive carcinoma are reactive with KA4 (89% and 93%, respectively) and UEA-1 (96% and 93%, respectively) and minimally reactive with anti-vimentin (15% and 0%, respectively). The increased KA4 staining was shown to be in part due to detection of cytokeratin 19, by using cytokeratin-19–specific antibodies, 4.62 and LP2K. The reasons for the increased expression of this cytokeratin and the decreased expression of vimentin are unclear but seem to indicate a phenotypic relationship between the PIN lesions and invasive carcinoma. (Am J Pathol 1991, 138:119–128)

Dysplastic epithelial alterations within prostatic ducts and acini believed to be premalignant changes first were mentioned by Oertel in 1925.[1] The first scientific description of prostatic dysplasia is that of Andrews.[2] Atypical lesions of prostate ducts and acini have subsequently been described under a confusing variety of terms.[3–23] Kovi et al[23] coined the term large acinar atypical hyperplasia to refer to lesions with atypical epithelial proliferation arising in the tubular glands of the prostate without new acini formation. On this basis they separated these lesions from atypical small acinar hyperplasia, in which there was formation of new acini characteristic of the previously described categories of atypical adenomatous hyperplasia,[9,10] atypical glandular proliferation,[11] adenosis of prostate,[17] and atypical hyperplasia, small acinar type.[12]

The dysplastic lesions of the large acinar type have cytologic abnormalities that, in their most severe forms, resemble carcinoma.[16,23] McNeal and Bostwick[16] referred to these lesions as intraductal dysplasia and have graded them into three categories. Bostwick and Brawer[21] have shown a progressive loss of basal cells accompanying increasing grades of prostatic intraepithelial neoplasia (PIN), with loss of more than one third of the basal cell layer in 52% of PIN3 compared with less than 2% in lower grades of PIN. The terminology 'prostatic intraepithelial neoplasia (PIN)' originally proposed by Bostwick and Brawer[21] was accepted as the preferred nomenclature for these lesions at the 1989 Workshop on Prostatic Dysplasia.[24]

The relationship of these cytologically abnormal lesions to invasive carcinoma remains unclear, although a number of studies using serially sectioned material have shown the incidence of dysplasia to be increased in association with carcinoma.[8,15,16,23,25,26] In addition to this finding, it is also known to be multifocal,[8,25] predominantly located in the peripheral prostate,[23] and more commonly seen associated with carcinoma when of high grade.[21,25] Thymidine labeling studies have shown a labeling index three times greater in these lesions compared with simple hyperplasia.[7] Recent immunohistochemical studies have shown a progressive loss of markers of secretory differentiation with increasing degrees of dysplasia, implying a

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progressive loss in regulatory control in these lesions similar to invasive carcinoma.[27-31]

In the present study, we have used immunohistochemistry to further examine the phenotypic relationship between PIN and invasive prostatic carcinoma. This analysis demonstrated similarities with respect to expression of cytoskeletal proteins and surface glycoprotein in the luminal cells of ducts and glands with PIN and invasive carcinoma.

Methods

Specimens

Thirty-one prostate specimens obtained at the time of surgery from needle biopsies (n = 8), transurethral resections (TURs; n = 18), or radical prostatectomy procedures (n = 5) were snap frozen in liquid nitrogen-cooled isopentane. In each specimen the PIN was graded 1 to 3, based on the highest degree of PIN identified, as shown in Figure 1, using the criteria of McNeal and Bostwick.[16] The carcinomas were graded using the Gleason grades 1 through 5. Fourteen cases contained invasive carcinoma (Gleason 2, n = 2; Gleason 3, n = 5; Gleason 4, n = 6; Gleason 5, n = 1), and 28 cases contained PIN (grade 1, n = 2; grade 2, n = 17; and grade 3, n = 9).

An additional 40 blocks from 27 routinely processed radical prostatectomy specimens were studied. They had been fixed in 10% phosphate-buffered formalin and embedded in paraffin. Routine hematoxylin and eosin (H&E)-stained sections were used to select areas containing examples of simple hyperplasia, dysplasia PIN, and carcinoma for immunohistochemical study. In total, there were 17 cases of carcinoma (Gleason 1, n = 1; Gleason 2, n = 6; Gleason 3, n = 7; and Gleason 4, n = 3). There were 22 cases with areas of PIN (grade 1, n = 2; grade 2, n = 12; and grade 3, n = 8).

Immunohistochemistry

A panel of 20 antibodies and one lectin was used to study the frozen material (See Table 1 for list of reagents, their specificity, and their source). All the anticytokeratin antibodies, as well as the antibodies to vimentin and desmin, were reacted using an indirect peroxidase technique.[32] The Ki-67 antibody was reacted using the peroxidase antiperoxidase (PAP) technique.[32] The lectin from *Ulex europaeus* (UEA-1) was localized by an avidin-biotin procedure.[33] All reagents listed in Table 1 were used to analyze the normal distribution of cytoskeletal proteins. From this large panel, 12 probes (shown by asterisk in Table 1) were applied to the 31 frozen specimens.

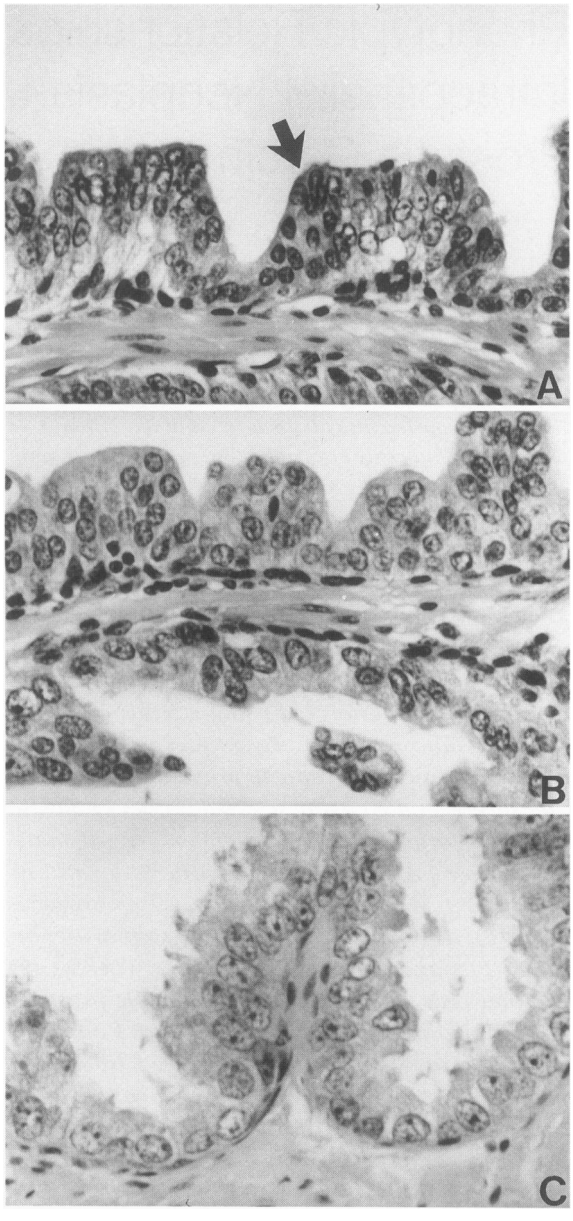

Figure 1. *Progressive cytologic atypia in grades of dysplasia (PIN).* **(A)** *Grades I (arrow) and II,* **(B)** *grade II,* **(C)** *grade III (×370).*

Four reagents (shown by double asterisk in Table 1) known to react with epitopes preserved in formalin-exposed material were applied to the formalin-exposed, paraffin-embedded material. Anti-cytokeratin antibodies KA4 and 10.11, as well as the anti-vimentin antibody V9, were detected by the PAP technique, and UEA-1 by the avidin-biotin technique.

Immunoreactivity was graded positive (+) or negative (−) in the respective areas of simple hyperplasia, dysplasia, or invasive carcinoma. All cytokeratins will be referred to by the numerical designations of Moll et al.[34]

Table 1. *Specific Antibodies Used*

Antibody	Source	Specificity
*KA1	1	Cytokeratins 4, 5, 6
†KA4	1	Cytokeratins 14, 15, 16, 19
*KA12	1	Cytokeratin 6
LP34	5	Cytokeratins 5, 6, 18
RCK102	3	Cytokeratins 5, 8
RCK103	3	Cytokeratin 5 (among others)
LP5K	5	Cytokeratin 7
LP3K	5	Cytokeratin 8
LP1K	5	Cytokeratin 8
†10.11	2	Cytokeratins 8, 18
RSKE60	3	Cytokeratin 10
*AE8	4	Cytokeratin 13
LL001	5	Cytokeratin 14
*RGE-53	3	Cytokeratin 18
*6.11	2	Cytokeratin 18
LE41, LE61, LE65	5	Cytokeratin 18
*4.62	ICN, Lisle, IL	Cytokeratin 19
*LP2K	5	Cytokeratin 19
†Vimentin	DAKO, Santa Barbara, CA	57-kd protein vimentin
†Ulex europaeus (UEA-1)	Vector, Burlingame, CA	Glycoproteins containing α-linked fucose residue
*Ki67	DAKO, Santa Barbara, CA	Proliferating cells

Sources
1. Nagle RB, Böcker W, Davis J, et al: Characterization of breast carcinoma by two monoclonal antibodies distinguishing myoepithelial from luminal epithelial cells. J Histochem Cytochem 1986, 34:869–881.
2. Gift from Dr. Robert Cardiff, University of California, Davis, CA.
3. Gift from Dr. Frans Ramaekers, University Hospital, Nijmegen, The Netherlands.
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5. Gift from Dr. E. Birgit Lane, Imperial Cancer Research Fund, Herts, England.

* Reagents that were applied to all frozen specimens.
† Reagents that were applied to all fixed specimens.

Statistical Analysis

Statistical analysis of the data set used Fisher's exact test[35] for analysis of simple associations, and logistic models[36] for identifying more complex interactions. The *P*-values reported in the following section were determined using a two-tailed Fisher's exact test.[35]

Results

Frozen Tissue Immunohistochemistry Results on Normal and Hyperplastic Ducts and Glands

The normal anatomic divisions of the epithelium were defined using the various anti-intermediate filament antibodies as described below. The most distal alveoli were composed of two cell types, with the luminal cells expressing cytokeratins 8 and 18 (Figure 2A), and the basal cells consistently expressing cytokeratins 5, 6, 8, 10, 13, 14, and 18 (Figure 2B). The tubular portions of the tubuloalveolar glands were slightly different in that the luminal cells expressed cytokeratins 8, 18, and 19. The basal cells of the tubular portions express cytokeratins 5, 6, 8, 10, 13, 14, 18, and 19 (Figure 2C). Antibodies against cytokeratin 19 showed variable expression in the most distal alveolar basal cells, with most areas staining but with foci in which staining is absent (Figure 2D).

The more proximal larger ducts appear pseudostratified and resemble transitional epithelium. The luminal cells express cytokeratins 5, 7, and 13 (Figure 3A) in addition to 8, 18, and 19, which are seen in the more distal tubuloalveolar glands. The basal cells here are similar to the more distal tubuloalveolar glands and express cytokeratins 5, 6, 8, 10, 13, 14, 18, and 19. Focal areas of squamous metaplasia were reactive with antibodies against cytokeratin 6 (Figure 3B). Focal areas of basal cell hyperplasia were demonstrated with antibodies to cytokeratins 5 and 14 (Figure 3C).

Vimentin was present in luminal cells of the tubuloalveolar glands in a primarily subnuclear pattern (Figure 3D). It was absent from the basilar cells and totally absent from the pseudostratified ductular luminal cells. Vimentin was present also in the stromal fibroblasts, stromal smooth muscle cells, and endothelial and smooth muscle cells of vessels (Figure 3D). Desmin was confined to the smooth muscle cells of the stroma and vessels.

The lectin UEA-1 was bound to both basal and luminal cells of the major ducts and the basal cells of the tubuloalveolar glands, highlighting focal areas of basal cell hyperplasia. The basal lamina surrounding periacinar cap-

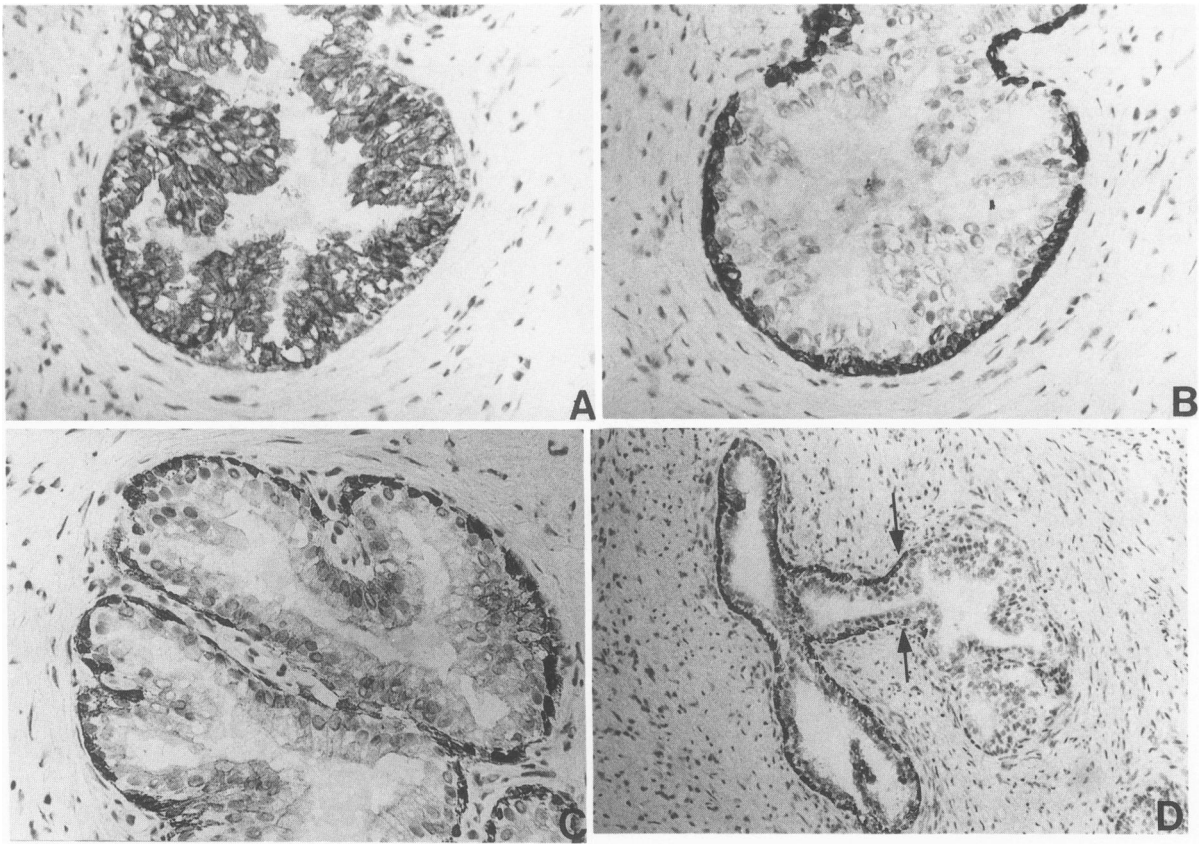

Figure 2. *Immunohistochemical demonstration of cytokeratins in normal human prostate.* **A:** *Reactivity of antibody RGE-53 directed against cytokeratin 18. Note staining of luminal as well as basal cells (×300).* **B:** *Reactivity of antibody KA1 directed against cytokeratin 5. Note staining in basal cells and absence of staining in luminal cells (×300).* **C and D:** *Reactivity of antibody 4.62 directed against cytokeratin 19. Note intense staining of basal cells but absence of staining of luminal cells in* **C** *(×300). Note in* **D** *the heterogeneous staining of basal cells in the proximal portions of the tubuloalveolar glands but absence in the more distal portions (×120).*

illaries was intensely reactive, but neither the basal nor luminal cells of the acini were reactive.

The antibody Ki-67 showed reactivity focally with the cytoplasm of basal cells within ducts and tubuloalveolar glands, but did not show the characteristic diffuse nucleolar staining pattern that we routinely see in breast and lymphoproliferative lesions. This is probably due to the low rate of cellular renewal in the prostatic epithelium.

Frozen Tissue Immunohistochemical Findings in PIN

Areas of PIN showed piling up of epithelial cells, which usually revealed the spectrum of cytologic abnormality (grades 1 to 3) described by McNeal and Bostwick.[16] In some of the frozen material, the nuclear detail was less well preserved and often lacked sufficient cytologic detail to allow definitive classification by routine H&E. Immunohistochemical examination of areas with the unmistakable cytology of PIN were used to define the phenotype of PIN lesions. Using these results, PIN in the less well-preserved material could be easily separated from normal structures having more than two cell layers, such as larger ducts or glands having basal cell hyperplasia. For example, ducts could be distinguished by staining with antibody AE8 reactive with cytokeratin 13. Basal cell hyperplasia, which is reactive with antibody KA1 specific for cytokeratin 5, could also be distinguished from PIN, which was unreactive with this antibody.

Three consistent immunohistochemical changes were therefore seen in the PIN that deviated from the pattern described above for normal and simple hyperplasia of the tubuloalveolar glands (Figure 4). The results obtained on the frozen specimens are shown in Table 2. First there was a significant increase ($P < 0.001$) in reactivity of the dysplastic luminal cells (23/27 cases) as compared with normal or hyperplastic luminal cells (2/23 cases) with the antibody KA4, which is specific for cytokeratins 14, 15, 16, and 19 (Figure 4A, B). Reactivity with the antibodies specific for cytokeratin 19 (4.62 and LP2K) seemed to

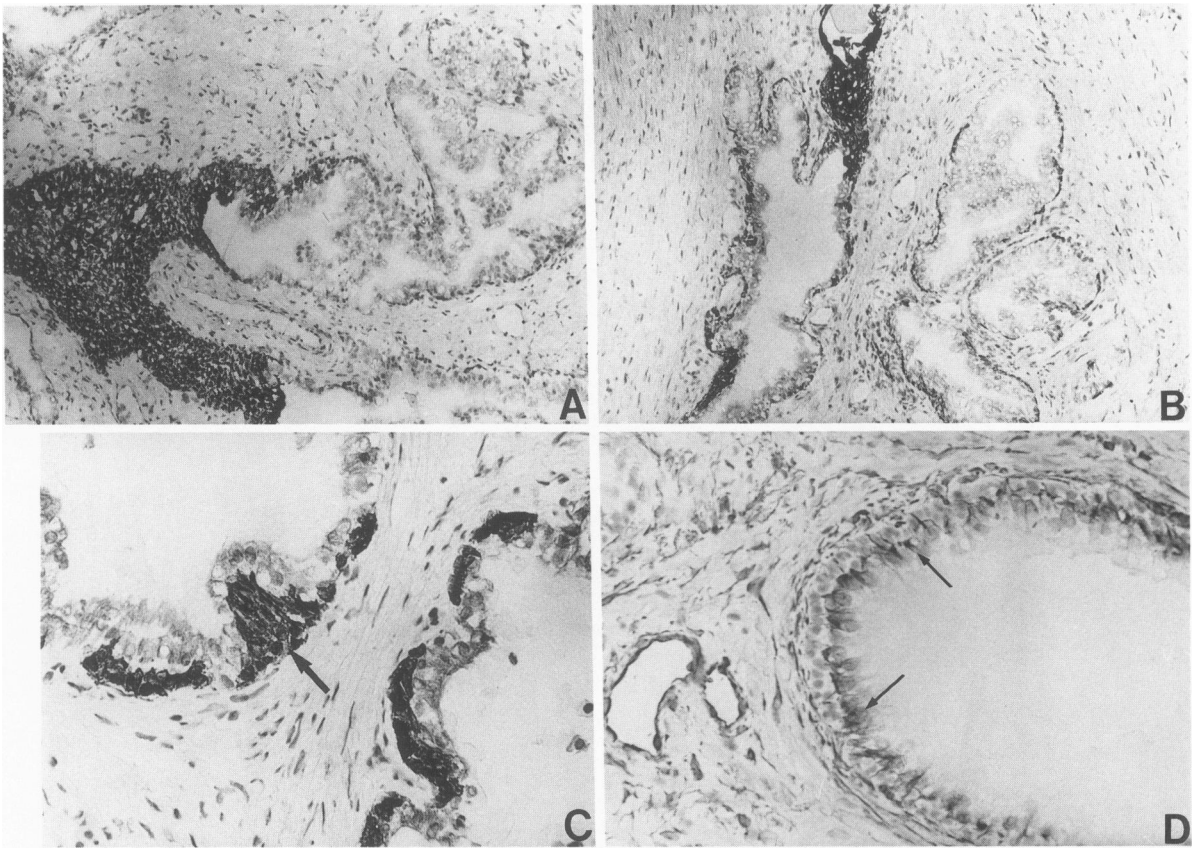

Figure 3. *Immunohistochemical distribution of cytokeratins in normal human prostate.* **A:** *Reactivity with anticytokeratin antibody AE8 directed against cytokeratin 13. Note full-thickness staining of urothelium of a proximal duct and basal cell staining of the distal tubuloalveolar glands (×120).* **B:** *Reactivity of antibody KA12 directed against cytokeratin 6. Note full-thickness staining of an area of squamous metaplasia in upper center and basal cell staining of the remainder of the tubuloalveolar glands (×120).* **C:** *Reactivity with antibody KA4 directed against cytokeratins 14, 15, 16, and 19. Note focal areas of basal cell hyperplasia (×300).* **D:** *Reactivity with anti-vimentin. Note staining of stromal cells, endothelial cells, and subnuclear regions of the luminal cells, but lack of basal cell staining (×300).*

indicate that the staining with KA4 was in fact due to increased expression of cytokeratin 19.

Second there was a significant ($P < 0.001$) loss of the normal luminal cell expression of vimentin, with only 4 of 26 cases of PIN being positive, compared with 20 of 22 cases of simple hyperplasia (Figure 4D, E).

Third there was significant ($P < 0.001$) increased binding of the lectin UEA-1 in PIN (26/27 cases) as compared with simple hyperplasia (0/23 cases) (Figure 4G, H). These findings are summarized in Table 3. There were no dramatic differences in these patterns related to the three grades of PIN (Table 2).

Frozen Tissue Immunohistochemical Findings in Invasive Carcinoma

The immunohistochemical findings did not vary with Gleason grade of carcinoma. All carcinomas stained diffusely positive with the 10.11 antibody directed toward epitopes of cytokeratins 8 and 18, and 14 of 15 cases reacted at least focally with anticytokeratin KA4 ($P < 0.001$) (Figure 4C). Vimentin antibodies failed to react with the invasive carcinoma cells (0/14 cases) (Figure 4F) or PIN (4/26 cases) (Figure 4E), but reacted with benign or hyperplastic glands (21/23 cases, $P < 0.001$) (Figure 4D).

The lectin UEA-1 stained all but one case of carcinoma diffusely (14/15 cases) (Figure 4I). These findings are summarized in Table 3.

Fixed Tissue Immunohistochemical Findings

When these studies were repeated on fixed material, the distinctions seen with KA4 and vimentin antibodies in PIN and carcinoma were lost (P values $\gg 0.05$), while UEA-1 staining remained consistent in its pattern ($P < 0.001$) (Table 4).

Discussion

Studies of carcinogenesis in the prostate have been hampered by a number of factors, including the relative in-

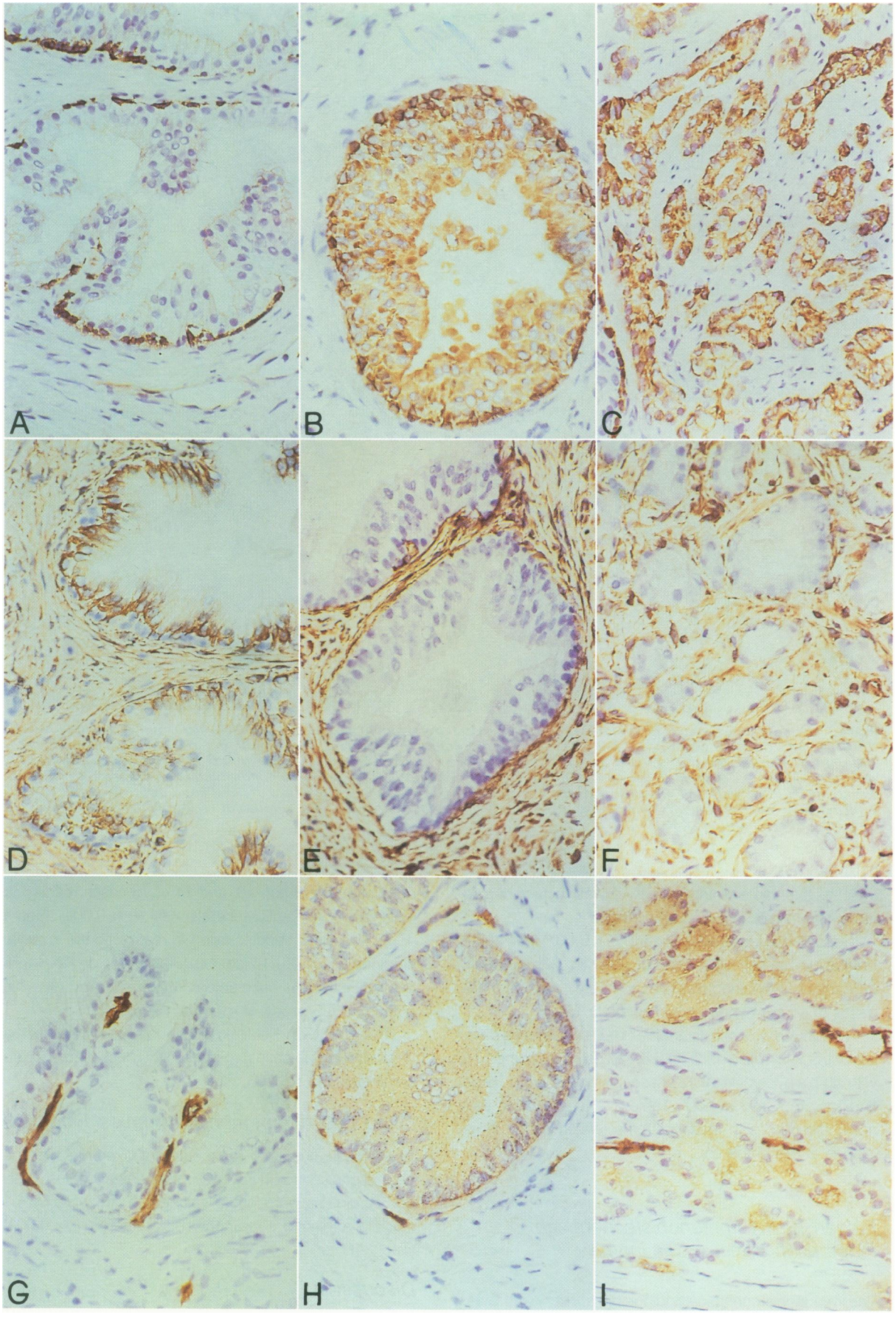
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Figure 4. *Immunohistochemical changes in dysplasia and invasive carcinoma compared with normal prostatic glands.* **A, D, and G:** *Normal glandular epithelium.* **B, E, and H:** *dysplastic epithelium.* **C, F, and I:** *Invasive carcinoma.* **A, B, and C:** *Reacted with antibody KA-4 directed against cytokeratins 14, 15, 16, and 19.* **A:** *Normal gland, basal cells are stained only.* **B:** *Increased staining of the dysplastic luminal cells.* **C:** *Invasive carcinoma cells positively stained.* **D, E, and F:** *Reacted with anti-vimentin.* **D:** *Normal epithelium with reactivity seen in stromal cells and luminal cells only.* **E:** *Staining of the luminal cells in the dysplastic epithelium is lost.* **F:** *Invasive carcinoma shows lack of staining of the epithelium, however, the stroma is positive.* **G, H, and I:** *Reacted with UEA-1.* **G:** *Normal glands showing vascular basement membranes and lack of staining of epithelium.* **H:** *Dysplastic epithelium shows positive staining of cytoplasm.* **I:** *Invasive carcinoma stains positively (×300).*

accessibility of the gland, heterogeneity of the glandular components,[37,38] biologic variability in the rapidity of growth and rate of spread, and lack of a good animal model.[39] Little is known concerning the factors involved in neoplastic initiation, although epidemiologic studies report higher incidence in blacks, especially in industrial countries, suggesting that both genetic and environmental factors may be important. The data presented in this report suggest that there may be a progression from PIN to invasive cancer. Cytogenetic confirmation has thus far not been reported to support this concept. Androgen stimulation appears to be one of the factors involved in tumor promotion, but it is now clear that most prostate cancer is testosterone sensitive but not dependent, and, although the majority of patients will show an initial response to androgen removal or inhibition, all will eventually remit and appear androgen independent.[39] Perhaps the most vexing problem in the management of men afflicted with this malignancy is the fact that although tumor grade, size, and stage are useful predictors of survival, in any given patient it is currently impossible to predict whether the tumor will be indolent or rapidly fatal.

As discussed above in the introduction, lesions with cytologic abnormalities approaching carcinoma but confined to the ductal glandular components have long been recognized and thought to be precursor lesions of invasive carcinoma.[8,26] McNeal and Bostwick recently established a system of grading of these lesions and criteria for their recognition.[16] Modern authors have shown that these intraepithelial lesions are associated more frequently with

Table 2. *Luminal Cell Immunohistochemistry Results in Frozen Specimens*

Case	CA Grade	PIN Grade	KA4			ULEX			Vimentin		
			CA	PIN	BPH	CA	PIN	BPH	CA	PIN	BPH
1	3	3	+	+	−	+	+	−	ND	ND	ND
2	3	2	+	+	−	f+	f+	−	−	−	−
3	A	2	A	+	A	A	+	A	A	−	A
4	A	3	A	+	−	A	f+	−	A	f+	+
5	4	3	f+	+	−	+	+	−	−	−	+
6	A	3	A	+	A	A	+	A	A	−	A
7	A	2	A	+	−	A	A	−	A	A	+
8	3	3	+	+	−	+	f+	−	−	−	+
9	A	2	A	−	−	A	f+	−	A	f+	+
10	A	2	A	+	−	A	+	−	A	−	+
11	4	3	f+	+	−	+	f+	−	−	−	+
12	A	2	A	+	−	A	f+	−	A	−	+
13	5	A	+	A	A	−	A	A	−	A	A
14	2	3	+	+	−	+	+	−	−	f+	+
15	4	3	f+	f+	−	+	+	−	−	−	+
16	4	A	f+	A	A	+	A	A	−	A	A
17	A	2	A	−	−	A	+	−	A	f+	+
18	A	2	A	f+	−	A	+	−	A	−	+
19	3	1	f+	+	−	+	−	−	−	−	+
20	4	2	−	f+	A	+	+	A	−	−	A
21	A	2	A	+	A	A	+	A	A	−	A
22	A	1	A	+	A	A	+	A	A	−	A
23	2	2	+	f+	A	+	f+	A	−	−	A
24	A	A	A	A	−	A	A	−	A	A	+
25	4	2	+	+	−	+	+	−	−	−	+
26	A	2	A	f+	f+	A	+	−	A	−	+
27	A	2	A	f+	−	A	+	−	A	−	+
28	3	3	+	+	−	f+	+	−	−	−	+
29	A	2	A	−	−	A	f+	−	A	−	+
30	A	2	A	+	−	A	f+	−	A	−	−
31	A	2	A	f+	A	A	f+	−	A	−	+

f, focal; A, absent; +, positive; −, negative; ND, not done.

Table 3. *Summary of Luminal Cell Immunohistochemistry Results in Table 2.*

Probe	Benign hyperplasia	Dysplasia	Carcinoma
KA4	1/22 (4%)	25/28 (89%)	13/14 (93%)
Vimentin	20/22 (91%)	4/26 (15%)	0/13 (0%)
Ulex europaeus	0/23 (0%)	26/27 (96%)	13/14 (93%)

Number of positive cases/Total number of cases.

carcinoma than benign hyperplasia,[16] are multifocal and more commonly found in the peripheral zone,[23,29] have a peak occurrence that antedates the occurrence of carcinoma,[23] and show an increased proliferative capacity as measured by thymidine uptake.[7] Occasional reports have demonstrated microinvasive lesions arising from these lesions.[21] In this report, we establish that there are at least three biochemical phenotypic changes shared by intraepithelial neoplasia and invasive carcinoma that deviate from normal or hyperplastic epithelium.

The first two of these chemical changes involve intermediate filaments of the cytoskeleton. Vimentin is a member of the multigene family of intermediate filament proteins that forms homopolymeric 10-nm filaments that act as major components of the cytoskeleton.[40] Its expression is primarily confined to mesenchymal cells, but it is also coexpressed in certain epithelia, ie, mesothelium, thyroid, endometrial, and prostatic glands. In a number of epithelial cell types, this protein is not seen in the native epithelium, but is expressed when these cells are grown in tissue culture.[41] The function of vimentin in epithelium and the controlling factors for its regulation are currently unknown. In this study, we have shown that its expression in the prostate is heterogenous. As expected, the protein is seen in the smooth muscle, periglandular fibroblasts, as well as the endothelial cells lining the vascular spaces. It is not expressed in the normal ducts, but is prominently demonstrated in the subnuclear aspects of the glandular cells of the acini but not in the basal cells. The functional significance of this distribution is currently not understood. We were quite surprised to discover that the epitope that the V9 monoclonal anti-vimentin antibody detects was lost in both invasive carcinoma and PIN. This could be due to either repression of the gene and resultant decreased expression of the protein or a post-translational modification of the protein in the antigenic site resulting in 'masking' of the protein. Studies with two-dimensional electrophoresis and additional anti-vimentin antibodies are underway to investigate this question.

An interesting finding is the peculiar effect of formalin fixation on the ability to demonstrate vimentin with the V9 antibody in human prostate tissue. In fixed material, the epitope is demonstrated in the stromal cells, but is completely lost in the luminal epithelial cells, where it is present in fresh frozen material. This suggests that the epitope is differentially presented in the epithelium and stroma and is blocked in the epithelium through the single or combined effects of fixation, alcohol dehydration, and paraffin embedding.

Preliminary studies describing the distribution of cytokeratins in normal and pathologic prostate have been previously reported.[42–46] Our observation of increased reactivity of the cells to monoclonal anti-cytokeratin antibody KA4 in the PIN and invasive carcinoma lesions, when compared with the hyperplastic lesions, could have been due to an unmasking or increased synthesis of cytokeratins 14, 15, 16, or 19. The use of the specific monoclonal antibodies to cytokeratin 19 (LP2K, 4.62) showed increased staining of the PIN and carcinoma lesions, indicating that at least cytokeratin 19 is one of the keratins increased in these lesions. Interestingly, this cytokeratin has been shown to be variably expressed in the three commonly studied prostatic cell lines (Du-145, PC3, and LnCap), with PC3 being the only line expressing the protein in sufficient quantity to be detectable on two-dimensional protein electrophoresis.[47] It might be expected that the cytokeratins 6 and 16 normally expressed in hyperproliferative epithelium would be also expressed and, although KA4 would detect cytokeratin 16, we could not confirm the presence of cytokeratin 6 using antibody KA12 specific for cytokeratin 6. Microdissection studies and gel electrophoresis will be required to completely resolve this question.

The increased binding of the lectin UEA-1 indicates the presence of an alpha-linked fucose unit that is present in both the PIN and invasive cancer, but is not seen in normal or hyperplastic glands. McNeal et al[28] described absence of UEA-1 staining in normal peripheral zone, but up to 75% of peripheral zone prostate containing dysplastic lesions showed equivocal to intense staining. McNeal et al described moderate staining in less than 25% of dysplastic foci. Perlman and Epstein[30] have recently reported results similar to ours with 15 of 16 cases of dysplasia showing increased UEA-1 staining similar to the adjacent carcinoma.

Table 4. *Comparison of Frozen and Fixed Tissue Immunohistochemical Results*

Probe	Benign hyperplasia	Dysplasia	Carcinoma
KA4			
Frozen	1/22 (4%)	25/28 (89%)	13/14 (93%)
Fixed	4/30 (13%)	11/35 (31%)	11/24 (45%)
Vimentin			
Frozen	20/22 (91%)	4/26 (15%)	0/13 (0%)
Fixed	3/27 (11%)	1/36 (3%)	1/25 (4%)
Ulex			
Frozen	0/23 (0%)	26/27 (96%)	13/14 (93%)
Fixed	3/27 (11%)	29/35 (82%)	20/24 (83%)

Number of positive cases/Total number of cases.

The study by McNeal et al[28] demonstrated reduction of immunoreactivity to prostate-specific antigen (PSA), prostate acid phosphatase (PAP), and Leu-7 antigen in dysplastic glands.[29] They interpreted these findings as indicative of reduced differentiation in the early stages of prostate carcinogenesis.

It appears clear from these findings and those presented in this paper that there are changes in cytoskeletal proteins, secreted proteins, and states of glycosylation that are similar in PIN and invasive prostatic carcinoma. It is probable, but thus far unproved, that changes in cytoskeletal protein can effect transport of cell products and therefore might explain the differences in secretory protein distribution demonstrated in PIN by McNeal et al.[29] These changes in distribution patterns might also be related to changes in glycosylation states, but thus far a chemical analysis of these proteins has not been performed. It seems highly probable that these changes indicate that high-grade PIN and invasive carcinoma represent different stages of the same process. The events that initiate the cellular changes that lead to these observed differences in protein expression are still unknown and will require further understanding of the genetic changes that occur in this series of lesions of the glandular epithelium of the prostate.

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