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# Differential Expression of Cytokeratin mRNA and Protein in Normal Prostate, Prostatic Intraepithelial Neoplasia, and Invasive Carcinoma

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***The expression of cytokeratin (CK) mRNA for CK5, -8, -14, -16, and -19 was investigated in normal prostate, prostatic intraepithelial neoplasia (PIN) lesions, and invasive carcinoma using* in situ *hybridization. Protein localization was carried out in adjacent sections using immunohistochemistry and correlated with mRNA expression. Snap-frozen human prostate samples including 22 examples of normal glands, 20 cases of PIN lesions, and 12 cases of invasive carcinoma were examined. CK5 and -14 mRNA and protein were prominently expressed only in the basal cells of normal glands and PIN lesions. CK14 mRNA was absent in the luminal cells of the most of the PIN lesions but was seen at a low level in some PIN lesions. CK14 protein was not detected in any PIN lesion, suggesting that, if the cell that makes up the PIN lesions is derived from a basal cell, CK14 translation is depressed although a low level of CK14 mRNA may persist. CK8 mRNA and protein were constitutively expressed in all epithelia of normal and abnormal prostate tissues. CK19 mRNA and protein were persistently expressed in both basal and luminal cells of the tubular portion of normal glands as well as PIN lesions, but were expressed heterogeneously in both basal and luminal cells of normal alveoli. CK16 mRNA was expressed in a similar pattern as CK19, but CK16 protein was not detected either in normal or in abnormal prostate tissues. In conclusion, the expression of CK19 in PIN lesions is similar to its tubular expression and would support an origin of PIN lesions from this structure rather than the alveolar portion of the glands. The similar cytokeratin expression between PIN lesions and invasive carcinoma further supports the concept that PIN is a precursor lesion of invasive carcinoma. (Am J Pathol 1997, 150:693–704)***

Prostatic intraepithelial neoplasia (PIN) is now generally accepted as a precursor of invasive carcinoma. There are numerous examples of phenotypical association between PIN lesions and invasive carcinoma[1,2]; however, little is known about the cellular origin of PIN lesions. The identification of the stem cells that give rise to PIN lesions is of critical importance to the understanding of prostate carcinogenesis.

Cytokeratins constitute the largest and most complex gene family of intermediate filaments. They have been widely used as molecular markers in the diagnosis of a variety of carcinomas as well as in the study of many nonmalignant lesions.[3] Using immunohistochemistry staining, we and others have previously reported that cytokeratins are differentially expressed in normal as well as abnormal prostate epithelia.[4–9] We have further reported that cytokeratin expression is altered in PIN lesions and invasive carcinoma compared with normal tissue.[6] We have extended our earlier immunohistochemistry studies by using more specific, well characterized antibodies and correlating protein expression with mRNA expression. A more exact knowledge of cytokeratin distribution in the prostate would provide preliminary information for the further understanding of cytoker-

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**Table 1.** *Antibodies Used*

| Antibodies | Specificity | Diutions | Sources |
|---|---|---|---|
| KA4 | CK14, -15, -16, -19 | 1:1000 | Dr. R. B. Nagle |
| KA1 | CK5, -14 | 1:1000 | Dr. R. B. Nagle |
| K4.62 | CK19 | 1:100 | ICN, ICN Biomedical Inc., Irvine, CA |
| LL025 | CK16 | 1:20 | Dr. E. B. Lane |
| M4740 | CK14 | 1:40 | American Qualex, La Mirada, CA |
| 17-2 | CK8 | 1:20 | Dr. M. Schmelz |

atin gene regulation as well as the role cytokeratin may play in progression of PIN lesions and invasive carcinoma.

In this study, expression of cytokeratin mRNA in normal prostate glands, PIN lesions, and invasive carcinoma was investigated by *in situ* hybridization using five different cytokeratin riboprobes. Protein localization was carried out in adjacent sections by immunohistochemistry staining using monoclonal antibodies with known specificities. Our data provide evidence of a ductal origin for the PIN lesion. The data also provide further support for the phenotypic association between PIN lesions and invasive carcinoma.

## Materials and Methods

### Tissues, Cell Lines, and Antibodies

Prostate tissues were immediately snap-frozen at the time of surgery or autopsy and were stored at −80°C. Frozen cryostat sections of prostate tissues were used for immunohistochemistry and *in situ* hybridization. Human skin samples fixed in 10% buffered formalin and embedded in paraffin were used as controls. DU145, a prostate carcinoma cell line, was obtained from the American Type Culture Collection (Rockville, MD). HaCat, a squamous carcinoma cell line, was a generous gift from Dr. Norbert Fusenig. Antibodies used in this study are shown in Table 1.

### Preparation of Cytokeratin Riboprobes

To facilitate preparation of riboprobes, the specific regions of CK5, -8, -14, and -19 cDNA were subcloned into appropriately digested pBluescript vectors (Stratagene, La Jolla, CA) containing T3 and T7 primers on both sides of the multiple cloning site (Table 2). CK16 cDNA was amplified by reverse transcriptase polymerase chain reaction from total RNA of normal frozen prostate tissue and then cloned into PGEM-T vector (Promega, Madison, WI). The primer pair used for reverse transcriptase polymerase chain reaction was 5′-CTCCTAGAACTGAGCTGCCTCTAC-3′ and 5′-ATCCTGTGTCCCACCTCCCACTTC-3′. See Table 2 for source and region of the cDNAs used in this study.

The resultant subclones were sequenced by the chain-termination method using the Sequenase version 2.0 kit (Amersham, Arlington Heights, IL) to ensure the correct orientation with respect to the primers for *in vitro* transcription. The specificity of the probes were further confirmed by Northern hybridization.

The above cytokeratin cDNA subclones were linearized from one end of the insert with restriction enzymes. Approximately 1 $\mu$g of linearized template DNA was used for *in vitro* synthesis of cRNA probes using $^{35}$S-labeled CTP (Amersham) according to the manufacturer's protocol (Stratagene). Labeling activity of $2 \times 10^8$ to $4 \times 10^8$ cpm/mg was achieved.

**Table 2.** *Probes Used*

| cDNA clones | Specificity | Regions as probes (Genebank accession number) | Sources | Reference |
|---|---|---|---|---|
| pUR E20 | CK19 | 1074-1360 bp (Y00503) | Dr. E. Birgitte Lane, | 10 |
| | CK16 | 6-bp 3′ coding sequence plus 227-bp 3′ untranslated sequence | Authors (RT-PCR fragment) | 11 |
| pJK14.p | CK14 | 3′ sequence encoding for 383-467a amino acids | Dr. E. Fuchs | 12 |
| pK8-11 | CK8 | 1488-1724 bp (M34225) | Dr. R. G. Oshima | 13 |
| K5 intronless | CK5 | 1754-2276 bp (M21389) | Dr. E. Fuchs | 14 |

RT-PCR, reverse transcriptase polymerase chain reaction.

### In Situ *Hybridization*

*In situ* hybridizations were carried out as described[10] with the following modifications.

Frozen tissues of prostates were cut in a cryostat at 4 to 6 μm and placed onto silane-coated slides and stored at −80°C. Before use, the slides were thawed at room temperature for 10 minutes. The sections were fixed in 4% formaldehyde in phosphate-buffered saline for 10 to 30 minutes and then treated in TEA buffer (0.1 mol/L triethanolamine, pH 8.0, 0.25% acetic anhydride) for 10 minutes, dehydrated in increasing ethanol concentrations, and air dried .

Paraffin-embedded skin tissues were cut at 4 to 6 μm and placed onto silane-coated slides. The slides were baked at 65°C for 1 to 2 hours and stored in a desiccator at 4°C until use. Before hybridization, paraffin sections were dewaxed and rehydrated through xylenes and decreasing concentrations of ethanol. The sections were then incubated with 10 μg/ml proteinase K at 37°C for 15 minutes. TEA treatment was the same as for frozen sections.

The hybridization reaction was carried out at 42°C overnight in hybridization buffer (50% formamide, 0.3 mol/L NaCl, 10 mmol/L Tris/HCl, pH 8.0, 1 mmol/L EDTA, 1X Denhardt's, 500 μg/ml yeast tRNA, 500 μg/ml Poly A, 50 mmol/L dithiothreitol, 10% dextran sulfate). Approximately 40 to 80 μl of hybridization buffer containing $1 \times 10^6$ to $2 \times 10^6$ of $^{35}$S-CTP labeled cRNA probe were applied to each section. After hybridization, the slides were washed in 50% formamide, 2X standard saline citrate (SSC) at 50°C for 20 minutes to remove the coverslips and then washed for 1 hour in the same buffer. The sections were then treated with RNAse A (40 μg/ml, 500 μg/ml for each section) at 37°C for 30 minutes followed by a final wash in the above buffer at 50°C for 1 hour. The slides were dehydrated in increasing ethanol concentrations and air dried. The slides were then dipped in Kodak NTB-2 emulsion, air dried, and exposed in light-proof boxes at 4°C for 2 to 6 weeks. Finally, the slides were developed in Kodak D19 and counterstained with hematoxylin.

### *Northern Hybridization*

Total RNA was isolated from normal prostate frozen tissue and the prostate cell lines by using the Trizol RNA isolation kit (GIBCO BRL, Gaitherburg, MD). Approximately 10 to 20 μg of total RNA was loaded in each lane, separated on 1.2% agarose/formaldehyde gel, and transferred to nylon membrane (GIBCO BRL). The blots were probed both by [$^{32}$P]CTP- and [$^{35}$S]CTP-labeled cRNA probes specific for CK5, -8, -14, -16, and -19. The hybridization washes were performed at 42°C in 50% formamide, 2X SSC, 5X Denhardt's, 20 μg/ml salmon sperm DNA. Post-hybridization washing was performed in 0.1X SSC/0.1% sodium dodecyl sulfate at 65°C.

### *Immunohistochemistry*

Before immunostaining, frozen sections were removed from the −80°C freezer and fixed immediately in cold acetone for 5 minutes. The immunostaining was carried out using the standard indirect immunoperoxidase methods (See Table 1 for source and specificity of primary antibodies). Biotinylated secondary antibodies against mouse and rabbit were localized with streptavidin biotin conjugated to peroxidase (LSAB 2 kit, DAKO, Carpinteria, CA). The final color reaction was developed in diaminobenzidine substrate.

## *Results*

### *Specificity of Cytokeratin Probes and* in Situ *Hybridization*

The cytokeratin gene family is composed of more than 30 member genes that are highly homologous in the central α-helical domain, particularly among the members of the same subfamily.[3] Therefore, several steps were taken to ensure the specificity of both the probes and *in situ* hybridization conditions. By Northern hybridization, all five cytokeratin probes revealed a single transcript (Figure 1). The size of each transcript was in close agreement with those reported in the literature.[11–15] The specificity of the probes and hybridization conditions was further confirmed by *in situ* hybridization using as control tissues normal human skin and acanthotic skin in which the distribution of the cytokeratin mRNA is already known (Figure 2). The mRNAs for CK5 and -14 were restricted to the basal cells in normal skin (Figure 2, a and b). Both the protein and mRNA of CK16 were expressed in acanthotic epidermis as expected (Figure 2, c and d) but were not detected in normal epidermis.

### *Cytokeratin Expression in Normal Prostate Tissues*

Fifteen samples of normal prostate glands obtained from uninvolved areas in prostatectomy specimens and an additional seven samples obtained from

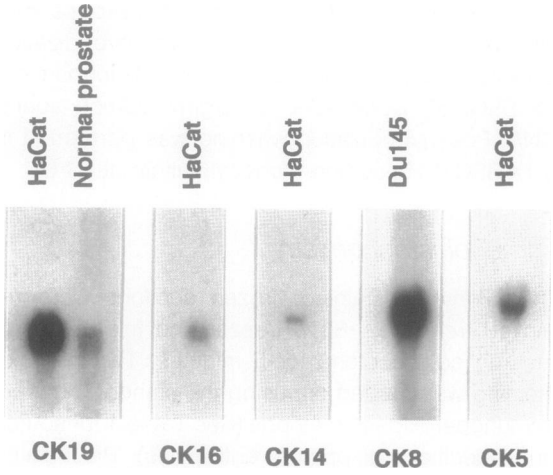


**Figure 1.** *Northern hybridization revealing a single transcript with each cytokeratin probe. Approximately 10 μg of total RNA was loaded in each lane. The samples were separated in 1.2% formaldehyde agarose gel and then transferred onto nylon membrane. The blots were hybridized with [a-$^{32}$P]CTP-labeled specific cytokeratin riboprobes.*

healthy young men dying from trauma were examined by *in situ* hybridization and immunohistochemistry. Whereas protein and mRNA of CK8 were expressed in all epithelia (Figure 3, a and b), the protein and mRNA of CK14 were exclusively expressed only in the basal cells of normal alveoli and ducts (Figure 3, c and d). Both protein and mRNA of CK5 exhibited a similar basal-specific expression pattern as that of CK14 (see Table 3).

Both the protein and mRNA of CK19 were persistently expressed in normal ducts at high levels (Figure 4, a and b), although their expression in the alveoli of normal prostate epithelia was heterogeneous (Figure 5). Expression of CK16 mRNA exhibited a similar pattern as that of CK19, but CK16 protein was not detected in normal prostate epithelia (see Table 3).

## *Cytokeratin Expression in High-Grade PIN Lesions*

All PIN lesions in this study were identified as high grade by hematoxylin and eosin (H&E)-stained frozen sections using the criteria of McNeal and Bostwick.[16] Using *in situ* hybridization and immunohistochemical staining, 20 cases of frozen prostate tissues containing high-grade PIN lesions were examined for the expression of cytokeratins. Both the protein and mRNA of CK19 were expressed in PIN lesions in all cases (Figure 6, a and b). The mRNA of CK16 was also expressed in PIN lesions; however, CK16 protein was not detected in any case (Figure

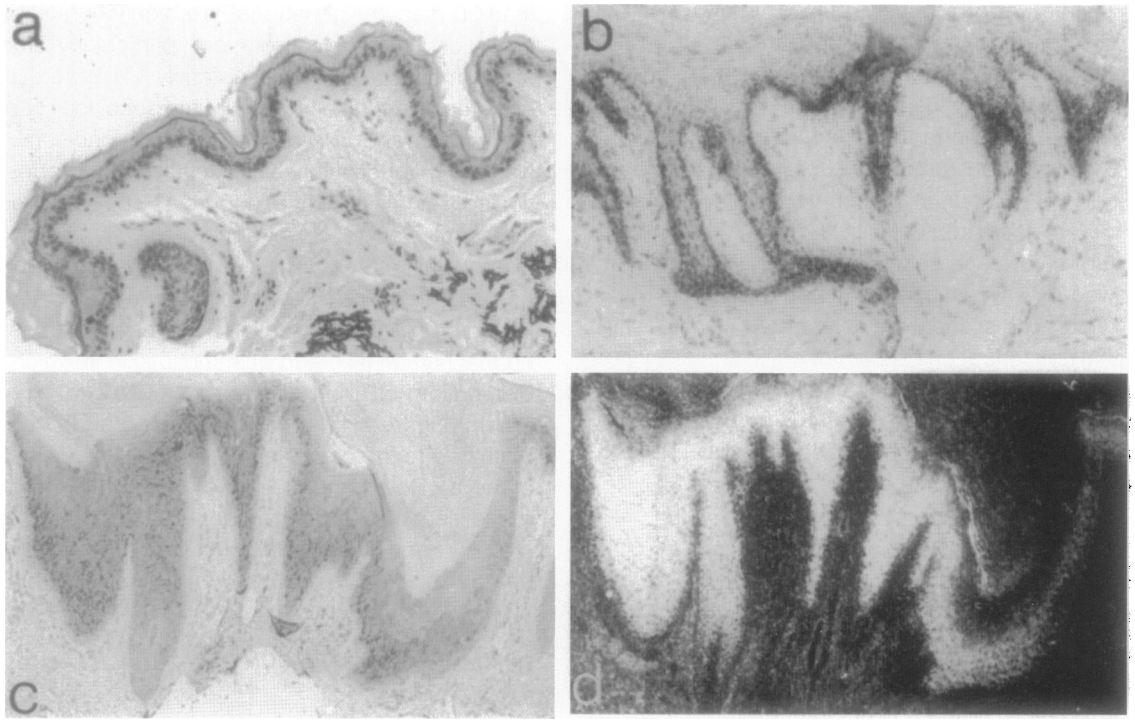


**Figure 2.** *Specific localization of cytokeratins in human skin sample controls confirmed the specificity of* in situ *hybridization. Paraffin* (a, *CK5*) *and frozen* (b, *CK14*) *skin sections were probed with* $^{35}$*S-labeled antisense riboprobes as a tissue control. The specificity of basal cell hybridization indicated that the probes* (*CK5 and CK14*) *were specific and the* in situ *hybridization conditions were stringent. Both protein* (c) *and mRNA* (d, *dark-field*) *of CK16 were expressed correspondingly in a sample of acanthotic epidermis.*

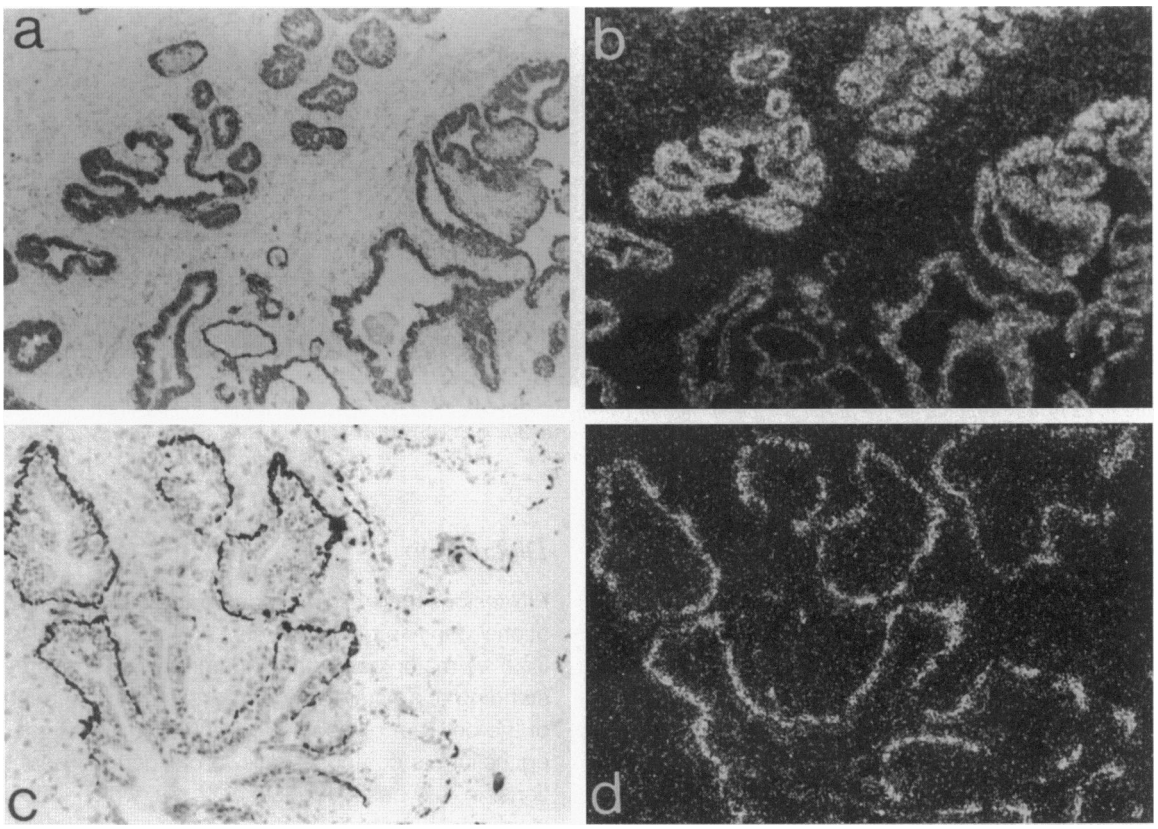


**Figure 3.** *Protein and mRNA distribution of cytokeratins in normal prostate tissues. Immunohistochemical staining and* in situ *hybridization (dark-field) were carried out in adjacent sections of normal frozen prostate tissues. Protein* (a) *and mRNA* (b) *of CK8 were expressed in both basal and luminal cells, whereas protein* (c) *and mRNA* (d) *of CK14 were expressed only in basal cells of normal prostate epithelia.*

6. c and d). CK14 protein was expressed in the basal cells but not in the luminal cells of PIN lesions. In contrast, a low level of CK14 mRNA expression was detected in luminal cells of PIN lesions in only 6 of 20 cases. Expression of CK14 mRNA was seen in basal cells of PIN lesions as expected and corresponded to the CK14 protein expression (Figure 6, e and f). Although both protein and mRNA of CK8 were expressed constantly in PIN lesions, the protein and mRNA of CK5 were expressed only in basal cells but not in luminal cells of PIN lesions (data not shown).

Of the 20 cases with PIN lesions, 3 showed PIN lesions involving ducts. The expression of the cytokeratins in these PIN lesions exhibited a pattern similar to the normal ducts. The data for CK19 and 16 are shown in Figure 7.

**Table 3.** *A Summary of Cytokeratin Expression in Normal Prostate and PIN Lesions*

| | | Protein | | | | mRNA | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ducts | | Alveoli | | Ducts | | Alveoli | |
| | Cytokeratin | B | L | B | L | B | L | B | L |
| Normal prostates (22 samples) | CK19 | +++ | ++ | +/− | +/− | +++ | ++ | +/− | +/− |
| | CK16 | − | − | − | − | +++ | ++ | +/− | +/− |
| | CK14 | + | − | + | − | + | − | + | − |
| | CK8 | + | + | + | + | + | + | + | + |
| | CK5 | + | − | + | − | + | − | + | − |
| PIN lesions (20 samples) | CK19 | +++ | ++ | ++ | ++ | ++ | + | + | + |
| | CK16 | − | − | − | − | ++ | + | + | + |
| | CK14 | + | − | + | − | + | −/+ | + | −/+ |
| | CK8 | + | + | + | + | + | + | + | + |
| | CK5 | + | − | + | − | + | − | + | − |

B, basal cells; L, luminal cells.

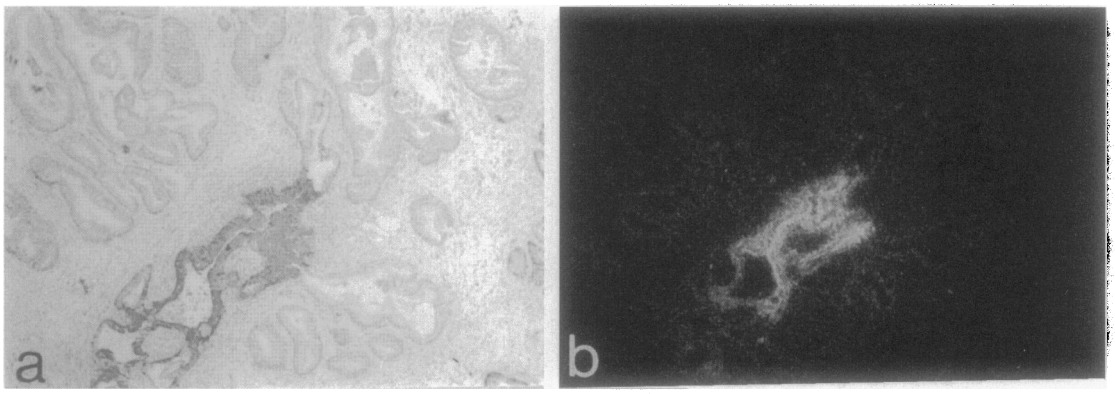


Figure 4. *CK19 was expressed in the ductal portion but not in the alveoli of normal prostate epithelium. Immunohistochemistry and* in situ *hybridization (dark-field) were carried out in adjacent sections. Both protein* (a) *and mRNA* (b) *of CK19 were expressed correspondingly at high levels in the ductal portion but not in the alveoli that are continuous with the duct.*

## *Cytokeratin Expression in Invasive Carcinoma*

Twelve frozen prostate samples containing invasive carcinoma of Gleason patterns III to V were examined. Both the CK8 (Figure 8a) and CK19 (Figure 8b) proteins were consistently expressed in carcinoma. Neither CK14 (Figure 8c) nor CK16 (Figure 8d) were detected. The mRNA expression of each cytokeratin corresponded to that of the protein expression except for CK16, which persistently expressed mRNA at low levels despite the absence of detectable protein expression (Table 4).

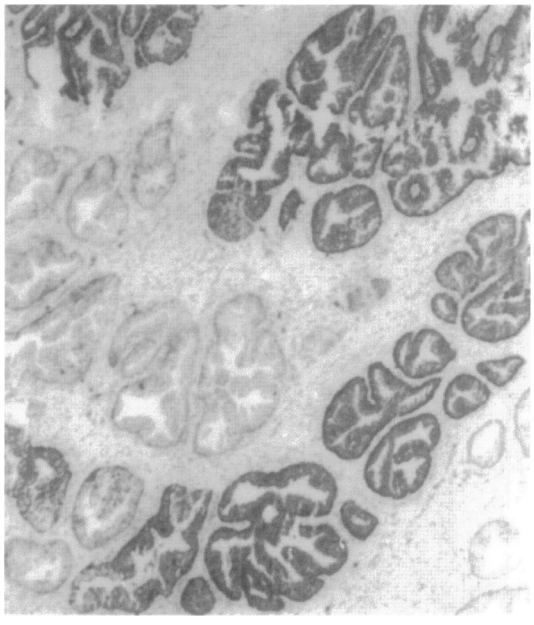

Figure 5. *Heterogeneity of CK19 protein expression in normal prostate tissue. Immunohistochemical staining of CK19 monoclonal antibody revealed coexistence of both CK19-positive and CK19-negative alveoli.*

## *Discussion*

PIN lesions have been described as cellular proliferations within prostatic ducts, ductules, and alveoli.[1,16] A major question fundamental to our understanding of prostate tumor progression is the identity of the cell that gives rise to the PIN lesion. Prostate epithelium is composed of two distinct cell populations, ie, the basal and luminal cells, which differ in localization, morphology, and degree of differentiation. In the normal prostate, the basal cells and luminal cells are easily separated on the basis of cytokeratin expression.[3,4,8,9] Although there are reports suggesting that both basal cells and secretory luminal cells retain the ability to divide,[17,18] a number of studies have demonstrated that basal cells represent the proliferative compartment of the prostatic epithelium in normal and hyperplastic conditions.[9,19–23] Therefore, it has been postulated that PIN lesions may be derived from a stem cell subpopulation of the basal cells.[24]

Our previous immunohistochemistry studies indicated that cytokeratin expression is altered in the PIN lesion as well as in carcinoma.[5,6] This study has clarified that it is CK19, not CK14, that was responsible for the earlier reported KA4 antibody staining in PIN lesions and carcinoma.[6] CK14, which is a specific marker of normal basal cells in ducts as well as alveoli, was not expressed in the luminal cells of PIN lesions. In contrast, CK19 mRNA and protein were persistently expressed in PIN lesions. CK19 mRNA and protein were also persistently expressed in both basal and luminal cells of normal ducts but variably expressed in both basal and luminal cells of the alveoli in the prostate of normal young men. This finding would support the hypothesis that PIN lesions are primarily derived from ductular epithelium[25] but

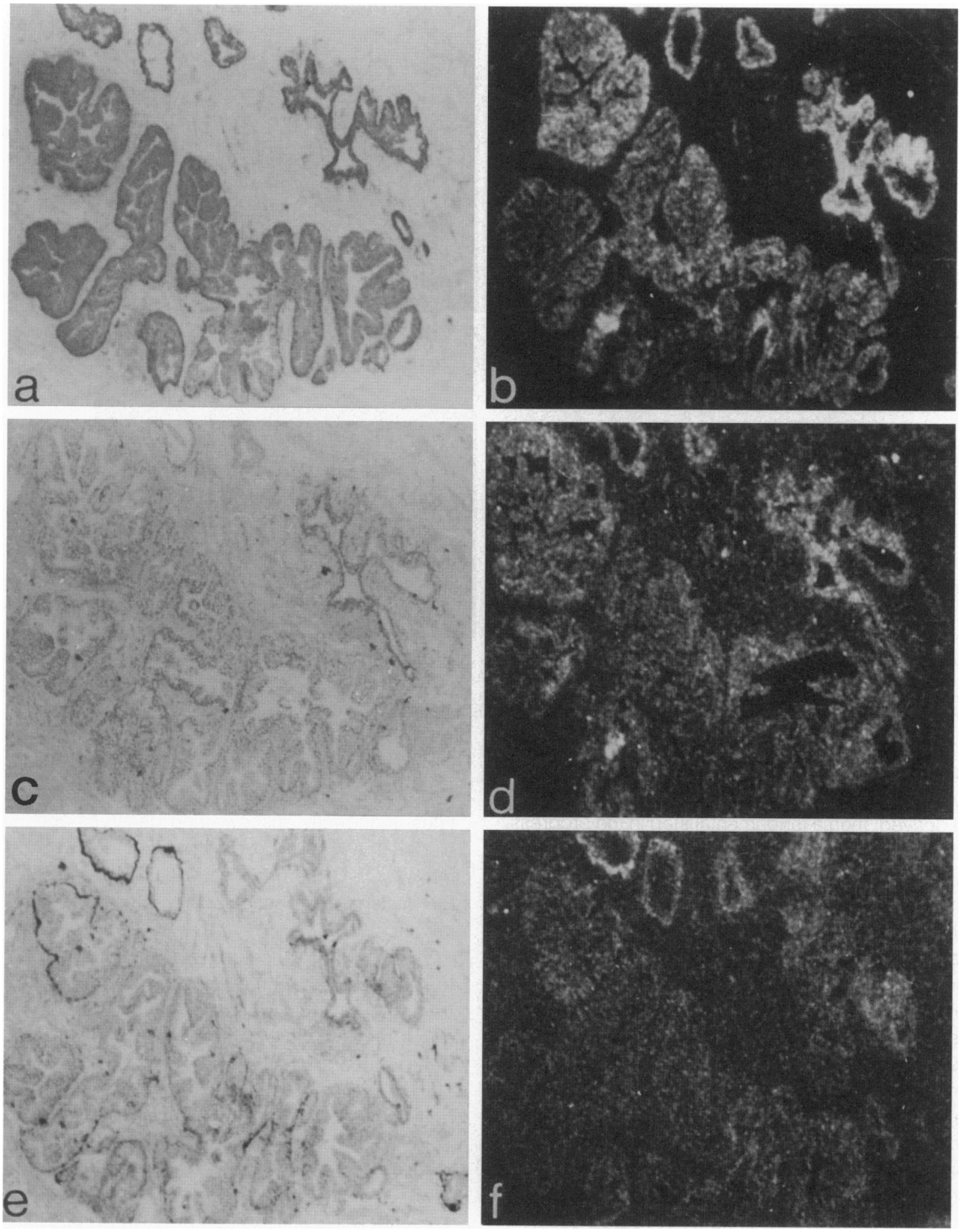


**Figure 6.** *Both protein and mRNA of CK19 were persistently expressed in PIN lesions. Immunohistochemistry and* in situ *hybridization (dark-field) were carried out in serial sections of frozen prostate tissue containing PIN lesions. Both protein* (a) *and mRNA* (b) *of CK19 were highly expressed in PIN lesions. Although CK16 mRNA* (d) *was expressed in PIN lesions, the CK16 protein* (c) *was not detected. CK14 protein* (e) *was discontinuously expressed in basal cells but not in the luminal cells of the PIN lesions, and the mRNA* (f) *appeared to be expressed at a very low level in both the basal and luminal cells of the PIN lesions.*

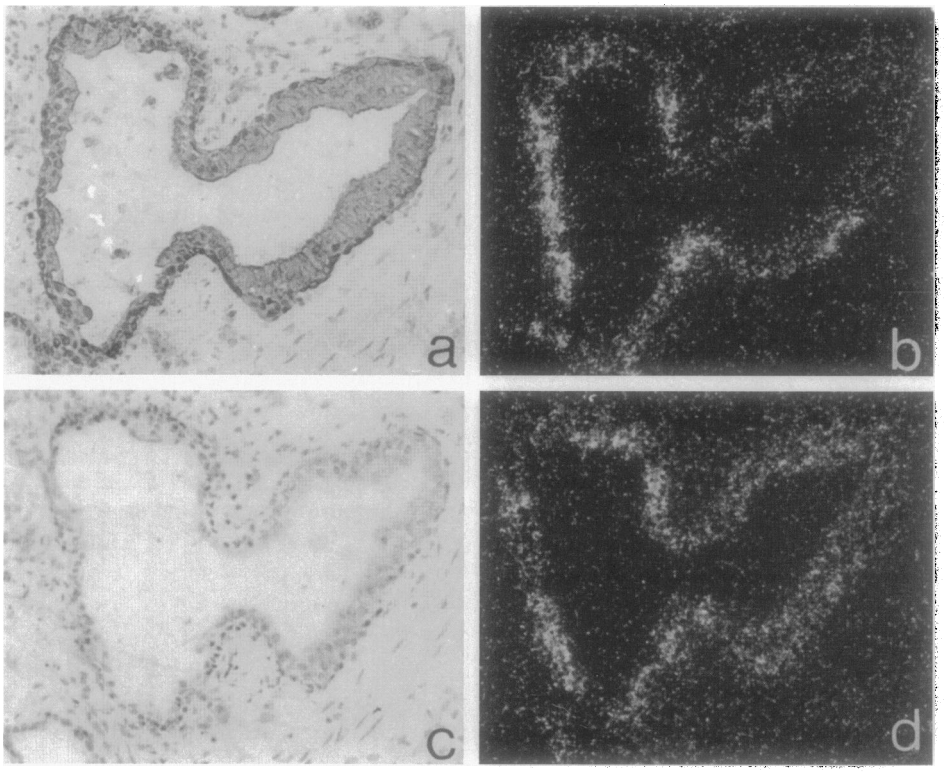


Figure 7. *Expression profile of cytokeratins in a PIN lesion derived from a duct. Immunohistochemistry and* in situ *hybridization (dark-field) were carried out in serial sections of frozen prostate tissue containing a PIN lesion arising from a duct. Both protein* (a) *and mRNA* (b) *of CK19 were expressed at relatively higher levels in the ductal portion and were also expressed substantially in the PIN lesion. Although CK16 mRNA* (d) *was expressed in a similar pattern as CK19, its protein* (c) *was absent in the normal duct and the PIN lesion.*

does not resolve whether the PIN cells are the progeny of basal cells or luminal cells. If they are basal cell derived, then a fundamental step in the progress of the PIN lesion would have to be the suppression of CK5 and -14 expression. Recently, the transcription factor AP-2 has been shown to regulate the expression of the CK14 gene.[26–28] The CK5 gene 5′-upstream sequence also contains AP-2 binding motifs.[26] Both CK14 and CK5 also have SP-1 binding motifs.[29] The Oct-6 transcription factor has also been shown to be expressed in basal and suprabasal cells in a variety of stratified epithelia and has been shown to be capable of specifically repressing the expression of CK5 and CK14.[30] These transcription factors and their influence on cytokeratin expression in the prostate have not as yet been investigated.

Table 4. *Cytokeratin Expression in Invasive Carcinoma*

| Cytokeratin | Protein | mRNA |
|---|---|---|
| CK19 | + | + |
| CK16 | − | + |
| CK14 | − | − |
| CK8 | + | + |
| CK5 | − | − |

The reason for the dramatic variability of CK19 expression in prostatic alveoli even in normal prostates is currently unknown and deserves additional study. The similar expression patterns of CK16 mRNA with CK19 provides additional evidence that PIN lesions are phenotypically similar to the duct tubules.

High-grade PIN lesions are cytologically similar to invasive carcinoma. They occur with greater frequency in men whose prostates harbor carcinoma and are now generally considered to be a precursor of invasive carcinoma.[1,2,25] A striking similarity between high-grade PIN and invasive carcinoma has been demonstrated with respect to cytological comparison and chromosomal abnormalities as well as numerous molecular markers.[31–39] In our study, the similar cytokeratin expression patterns between high-grade PIN lesions and invasive carcinoma provides additional evidence of their phenotypic association. Although there is little doubt that high-grade PIN lesions are highly associated with invasive carcinoma, low-grade PIN lesions are more controversial but may represent an early stage of tumorigenesis process.[32]

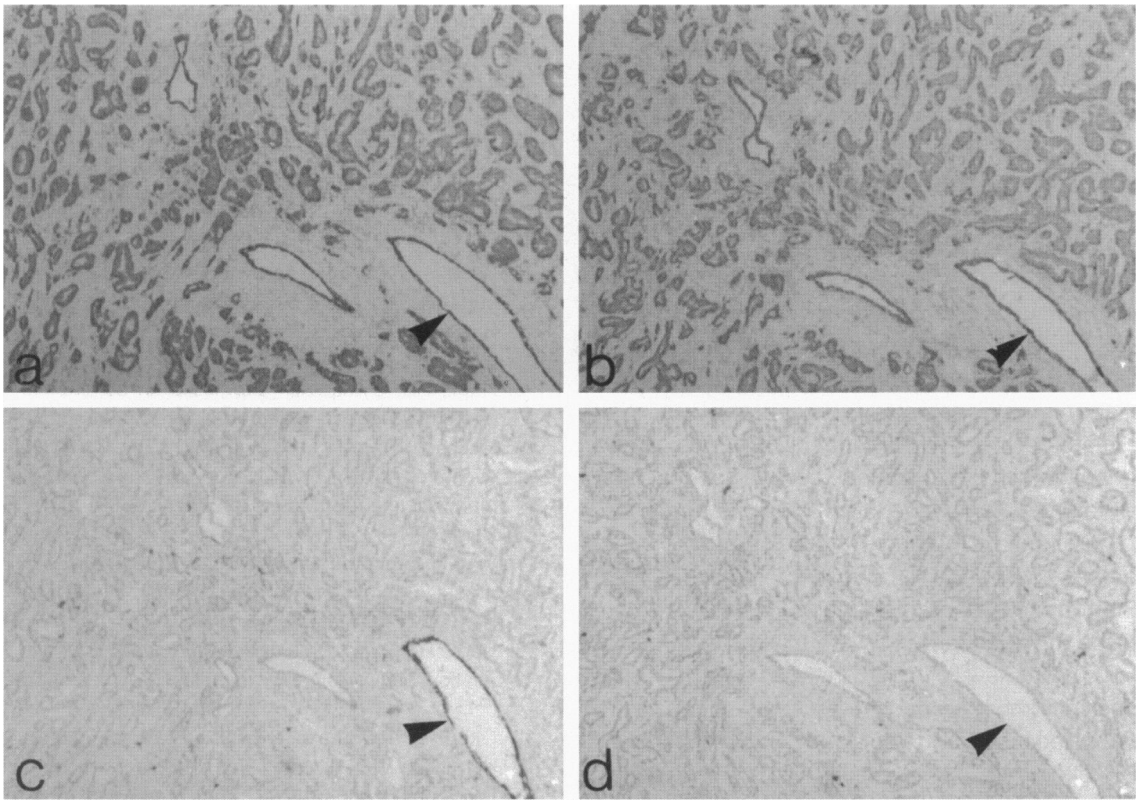


**Figure 8.** *Differential expression of cytokeratins in invasive carcinoma. Immunohistochemical staining revealed expression of CK8* (a) *and CK19* (b) *in invasive carcinoma. Neither CK14* (c) *nor CK16* (d) *was expressed. The* arrow *denotes a normal ductal structure.*

In this study, the expression of cytokeratin mRNA was correlated with protein expression in terms of the localization and level of the expression. These data strongly indicated that cytokeratin gene expression is primarily regulated at the transcriptional level. The exception to this rule was the failure to demonstrate CK16 protein even though its mRNA was detected in a pattern similar to CK19. This remains unexplained, but both protein and mRNA were demonstrated in control acanthotic skin samples, suggesting that the antibody to CK16 was specific. Masking of the epitope by a protein specific to prostate is one possibility and should be further explored.

Cytokeratins are characteristically expressed in pairs,[40] that is, one type I cytokeratin is usually co-expressed with a member of the type II cytokeratins. Transfection studies in fibroblasts and mouse L cells demonstrated that expression of CK8 or CK18 alone resulted in proteolytic degradation, and co-expression of CK8/CK18 was essential for the formation and stabilization of cytokeratin filaments.[13] The CK16/CK6 pair is expressed constitutively in a number of stratified squamous epithelia as well as in the hyperproliferative stage of epidermis.[41–43] The discrepancy between expression of CK16 mRNA and protein in prostate tissues might be explained by the proteolytic degradation of inappropriately paired CK16 protein; eg, CK6 protein was not appropriately co-expressed.[13] As CK16 expression is associated with hyperproliferative states, it is of particular interest in the studying of the cytokeratin gene regulation. Alternatively, the fact that CK16 was not seen in PIN lesions could be related to a relatively low rate of proliferation for PIN lesions.[36]

The CK19 gene is positively regulated by retinoic acids at the transcriptional level in HeLa cells.[44] It has been recently reported that expression of the CK19 gene was activated by a 3′ enhancer containing an AP1 site.[45] Studies from mutant p53-transfected keratinocytes indicated that CK19 expression was suppressed by the tumor suppressor gene, p53. The strict correlation between CK19 expression and TP53 mutation was also observed in a set of human skin tumors.[44–46] As p53 gene mutation has been detected in 2 of 19 prostate carcinomas,[47] it suggests that CK19 expression may be activated in PIN lesions and prostate carcinoma through additional mechanisms. CK19 was persistently expressed in normal ducts as well as PIN lesions and invasive carcinoma but was heterogeneously expressed in

alveoli of normal prostate epithelium. Analysis of CK19-positive and CK19-negative alveoli of normal prostate through microdissection promises to provide clues for the factors that may be involved in the activation of CK19 gene expression. The strong correlation between the protein and mRNA expression of CK19 makes the possibility of significant post-transcriptional defects unlikely.

CK8 and CK18 were expressed in various prostate tumor cells.[3] In our study, both protein and mRNA of CK8 were expressed constitutively in both basal cells and luminal cells of prostate epithelia. This pair seems to represent the essential intermediate filament component of normal prostate epithelium. The luminal cells also constitutively express vimentin.[6] In PIN lesions and invasive carcinoma, CK8 and -18 persist, but vimentin expression is lost.[6]

Both protein and mRNA of CK14/5 were expressed in basal cells but were totally lost in carcinoma. The low level of mRNA that was seen in 6 of 20 PIN lesions most probably represents untranslated mRNA. Because the detected level was just above the background, the possibility of cross-hybridization with another type II mRNA cannot be entirely excluded. These two cytokeratins provide important molecular markers that distinguish benign from malignant glands.[48]

In summary, both protein and mRNA of CK8 were constitutively expressed in all epithelial prostate cells. CK19 protein and mRNA were persistently expressed in both basal and luminal cells of ducts at high levels. CK19 protein and mRNA were expressed variably in both basal and luminal cells of normal alveoli. PIN lesions resemble ducts in that they consistently express relatively high levels of both CK19 protein and mRNA. CK16 mRNA was expressed in similar patterns as CK19, whereas CK16 protein was not detected. Both protein and mRNA of CK14 and CK5 were specifically expressed in the basal cells of normal prostate epithelium as well as in basal cells of PIN lesions. CK14 mRNA was variably expressed at very low levels in PIN lesions, but CK14 protein was never detected. These findings are consistent with a ductular origin of PIN lesions. The findings do not confirm CK14 or CK5 expression by PIN lesions and therefore do not necessarily support a basal cell origin for those PIN cells. The similar cytokeratin mRNA and protein expression patterns further supports the phenotypic association between PIN lesions and invasive carcinoma.

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