Cytokeratin Characterization of Human Prostatic Carcinoma and Its Derived Cell Lines

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ABSTRACT

Two murine monoclonal anti-cytokeratin antibodies with defined specificity were shown to distinguish between basal cells and luminal cells in human prostate tissue. Forty-one biopsies or transurethral resection specimens were characterized using these two antibodies. In cases of benign prostatic hyperplasia, focal loss of the basal cell layer was noted in areas of glandular proliferation. Ten cases of adenocarcinoma of the prostate, varying in Gleason’s histological grade from 2 to 4, were also studied. In each case the carcinoma was shown to represent the luminal cell phenotype with no evidence of involvement of the basal cell phenotype. An analysis of three established metastatic prostatic carcinoma cell lines (DU-145, PC-3, and LNCaP) using two-dimensional electrophoresis showed that the cytokeratin complement of each cell line was slightly different but retained the phenotype of the luminal cell. It was concluded that during both hyperplasia and neoplastic transformation of the prostate, the luminal cell phenotype is primarily involved and that the basal cell phenotype does not appear to contribute to either intraluminal proliferation or invasive cell populations.

INTRODUCTION

Although prostatic carcinoma remains one of the most common carcinomas affecting the male population, little is known about the basic biology of the cell types making up the prostatic acini. Previous observations have shown that the basal cell layer, sometimes referred to as the reserve cell layer, is lost during progression to the carcinoma (1). The function of these basal cells in the normal and neoplastic gland remains largely unknown.

The cytokeratin class of intermediate filaments has been shown to exist in all epithelia (2). There are now recognized 19 distinct cytokeratins expressed in human epithelia and each epithelial type has a distinct phenotype with regard to these proteins (3, 4). Early studies using polyclonal anticytokeratin made against human stratum corneum demonstrated that the basal cells of the prostate have cytokeratins that are immuno-reactively different from the luminal cells (5, 6). More recent studies using monoclonal anti-cytokeratin antibodies have differentiated the luminal and basal cell populations on the basis of their specific cytokeratin content (7). Using this approach Brawer et al. (7) examined the immunoreactivity of benign and neoplastic prostatic epithelium and was able to demonstrate focal loss of the basal cell layer accompanying proliferation of the luminal cells.

In the present study these findings were extended using well-characterized monoclonal antibodies with defined cytokeratin specificity which clearly differentiate luminal from basal cells in prostatic glands. Two-dimensional gel electrophoresis was used to establish the cytokeratin content of three established human prostatic cancer cell lines. These studies revealed that the cytokeratin content of each cell line was different but in each case was of the luminal phenotype. In addition the histochemical analysis of a variety of prostatic carcinoma biopsies revealed that the malignant cells expressed exclusively the cytokeratin phenotype of the normal luminal cells.

MATERIALS AND METHODS

Cultured Cells. Established human prostate cancer cell lines LNCaP (8) and DU-145 (9) were obtained from the original isolating investigators. The third cell line, PC-3 (10), was purchased from the American Type Culture Collection. RPMI 1640 (Gibco) supplemented with 10% fetal calf serum was used to maintain the PC-3 and the DU-145 cell lines that were grown as monolayer cultures in 75-cm² plastic flasks (Falcon Plastics). The LNCaP line was also maintained in RPMI media but supplemented with fetal calf serum was increased to 16%.

Biopsy Specimens. Surgical specimens of prostate tissue obtained by eitherneedle biopsy or transurethral resection were obtained from 41 patients. Ten cases of prostatic adenocarcinomas varying in Gleason’s grading from 2–4 were studied (11). Histological studies of the remaining 31 cases revealed one case of invasive transitional cell carcinoma and 30 cases of benign prostatic hyperplasia. Tissues were snap-frozen in isopentane supercooled in liquid nitrogen to −150°C, and stored at −80°C until processed for histochemistry.

Immunoreagents. Three murine monoclonal antibodies with different specificities were used for comparison. Individual cytokeratins were numbered using the method of Moll et al. (3). KA1, an antibody made against human cytokeratins derived from sole epidermis, has been shown by reacting with partially purified mixtures of basic cytokeratins dried onto nitrocellulose paper to react with a topographic epitope shared by cytokeratins 4, 5, and 6 (12). This antibody does not react with individual cytokeratins separated on polyacrylamide gel electrophoresis. KA4 is a monoclonal antibody also made against epidermal cytokeratins. This antibody has been shown by Western blotting against cytokeratins isolated from the epithelium of the glans penis and MCF-7 cells to react with cytokeratins 14, 15, 16, and 19 (12). RGE-53 (PAESEL GmbH and Co., Frankfurt am Main, Federal Republic of Germany) reacts specifically with cytokeratin 18 as shown by Western blotting against MCF-7 cells (13).

Immunohistochemical Procedures. Prostate cell lines were grown on coverslips using the media described above. The coverslips were washed in warm phosphate buffered saline and then fixed in cold methanol (−20°C) for 5 min. Just prior to staining, they were dipped five times in acetone for 3 s each and then stained for cytokeratins using an indirect peroxidase procedure with the reactivity localized by diaminobenzidine as described previously (14). Sections of prostate tissue, cut at 3–4 μm on a cryostat and fixed in acetone for 5 min, were reacted by the same method.

Protein Electrophoresis. Cells from each cancer cell line grown in two 75-cm² plastic flasks (approximately 6–10 × 10⁶ cells) were extracted with cold, high-salt detergent buffer (1.5 M KCl-0.5% Triton X-100-5 mM EDTA-0.4 mM phenylmethylsulfonyl fluoride-10 mM Tris-HCl, pH 7.4). Following 30 min of incubation in this buffer, the insoluble material was sedimented (5 min at 8000 rpm). The pellet material was washed in low-salt detergent buffer (10 mM Tris-HCl, pH 7.4).
7.4-5 mM EDTA) and analyzed by two-dimensional nonequilibrium pH gradient electrophoresis using 12.5% acrylamide gels for separation in the second dimension. Gel electrophoresis was always performed using internal standards including phosphoglycerol kinase (M, 43,000, pl 7.4), bovine serum albumin (M, 68,000, pl 6.34), and α-actin (M, 42,000, pl 5.4) (Sigma Chemical Co., St. Louis, MO). Gels were stained with Coomassie blue or ammoniacal silver (12).

RESULTS

The cytokeratin profiles of the three established human prostatic cancer cell lines are shown in Fig. 1. PC-3 contained cytokeratins 7 (M, 54,000), 8 (M, 52,500), 18 (M, 45,000), and 19 (M, 40,000); LNCaP contained cytokeratins 8 and 18; and DU-145 contained cytokeratins 7, 8, and 18 as well as vimentin. These findings were corroborated by the reaction pattern of monoclonal antibodies KA1, KA4, and RGE-53 on cells obtained at the same cultured passage and grown on coverslips. RGE-53 (anti-18) reacted diffusely with all three cell lines as predicted (Fig. 2, a, c, and e). In contrast, KA4 (anti-14, -15, -16, and -19), reacted diffusely only with PC-3 (Fig. 2b). PC-3 was seen to contain cytokeratin 19 on gel electrophoresis explaining its reactivity (Fig. 1a). In contrast, approximately 1% of the cells in DU-145 cell line reacted with the monoclonal antibody KA4 (Fig. 2d) but none of the cytokeratins detected by this antibody (cytokeratin 14, 15, 16, or 19) were present in sufficient amount to be detected by gel electrophoresis. Antibody KA1 (anti-4, -5, and -6) did not react with any cell line.

Reactivity of the three antibodies with normal or hyperplastic glands is shown in Fig. 3. KA1 stained only the basal cells (Fig. 3a), KA4 stained both basal and luminal cells (Fig. 3b), and RGE-53 stained only the luminal cells (Fig. 3c). In areas of glandular hyperplasia containing proliferating luminal cells with some atypia, the proliferating cells reacted only with RGE-53 (Fig. 4b) and KA4 (Fig. 4c). In these areas of proliferation there was a disappearance of the basal cell layer as demonstrated by the lack of reaction with antibody KA1 (Fig. 4a).

The reactivity of the 3 antibodies with the 10 cases of prostatic cancer and 1 case of transitional cell carcinoma is shown in Table 1. All of the adenocarcinomas of the prostate reacted diffusely with RGE-53 (Fig. 5c) and either diffusely or in a mosaic pattern with KA4 (Fig. 5b). Conversely, none of them reacted with KA1 (Fig. 5a). The single case of transitional cell carcinoma invading prostate tissue showed a different pattern, with diffuse reactivity of KA1 and KA4 but no reactivity with RGE-53.

In summary, the gel electrophoresis and immunohistochemistry of the prostatic cell cancer lines and the biopsied carcinomas showed a phenotype containing cytokeratins 8 and 18 with variable expression of cytokeratins 7 and 19. Also, some of the cases of adenocarcinoma appeared to stain in a mosaic pattern with KA4, comparable to the focal staining of cell line DU-145.

DISCUSSION

Murine monoclonal anticytokeratin antibodies can be used to distinguish two populations of cells in the normal prostatic glands and ducts. One population, designated the basal cell, reacts with most polyclonal antibodies (5, 6) and with the monoclonal antibody KA1 (anti-4, -5, and -6). The luminal cells react with the monoclonal antibodies RGE-53 (anti-18) and KA4 (anti-14, -15, -16, and -19). Although the cytokeratin phenotype of prostates has been chemically determined (15), the separate profiles of the basal and luminal cells are not...
Fig. 2. Prostatic cancer cell lines reacted with anti-cytokeratin and anti-vimentin antibodies. Cell lines, PC-3 (a, b) DU-145 (c, d, and f), and LNCaP (e). a, c, and e were reacted with antibody RGE-S3 specific for cytokeratin 18. b and d were reacted with anti-cytokeratin monoclonal antibody KA4 specific for with cytokeratins 14, 15, 16, and 19. f was reacted with polyclonal antivimentin. a, b, c, d, and e were reacted using a secondary antibody conjugated to peroxidase; f was reacted with a secondary antibody which was conjugated with fluorescein isothiocyanate. Bar, 50 μm.
Fig. 3. Immunohistochemistry of anti-cytokeratin antibodies reacted with hyperplastic prostate tissue. Sections of human prostate from a case of benign prostatic hypertrophy reacted with monoclonal anti-cytokeratin antibody K VI (a), KA4 (*), and RGE-53 (c). KA1 reacts only with the basal cell layers (a), KA4 reacts with both the basal cells (more intensely) and luminal cells (less intensely) (*), RGE-53 reacts with luminal cells only (*). Bar, 50 μm.

Since KA1, which selectively reacts with the cytokeratins in basal cells, recognizes an epitope seen in mixtures of cytokeratins 4, 5, and 6 exclusively, there must be minor amounts of at least one or more of these cytokeratins in the reserve cells which are either masked or absent in the luminal cell. The luminal cell reactivity with KA4 most probably represents cytokeratin 19, which is commonly seen combined with cytokeratins 7, 8, and 18 in simple types of epithelium (3).

Histochemically the glandular and ductular elements of the prostate are similar to eccrine sweat glands and breast where antibody KA1 identifies the myoepithelial cells lining the ducts and lobules and antibody KA4 reacts with the luminal cells along the entire length of the duct-lobular unit (12). Neoplastic transformation of the breast and prostate is also similar in that all of the carcinomas appear to react with KA4 suggesting a luminal cell origin in both malignancies. There is a major difference, however, in the two organs in that the myoepithelial cells in the breast (KA1 positive) persist as an intact cell layer becoming attenuated by the growing intraluminal carcinoma and are finally separated as the carcinoma invades the stroma (12). In the prostate it would appear that when proliferation of the luminal cell occurs there is, in contrast, an early loss of the basal cell layer (7). The relationship of the breast myoepithelial cell layer to the basal cell layer in the prostate is uncertain, but at least in their response to luminal proliferation they appear to be quite different.

Our finding of a focal loss of antibody KA1-reactive basal cells, external to luminal epithelial proliferation, is in agreement with the recent publication of Brawer et al. (7). All the prostatic adenocarcinomas thus far studied appear to represent the luminal cell phenotype and not the phenotype of the basal cell (Table 1). This fails to support previous concepts which have alleged that carcinomas arise from the proliferation of basal cells.

It is clear from the current study that the two cell populations of the prostate, like other lobular ductal epithelial systems, consist of two distinct cell populations with regard to their cytokeratin content. Why the basal cells should have different cytokeratins than the luminal cell is not known. It is also clear from the present study that the cell type, as defined by cytokeratins, which is confined in the normal gland to the basal layer is lost focally during hyperplasia. The cell type which becomes invasive shares the cytokeratins which are normally seen in the luminal cells. Whether there is a maturational process with respect to the cytokeratin in the transformation of the basal cell to the luminal cell is unknown. This phenotypic change could occur by posttranslational modifications of the epitopes such that the antibodies detecting basal cells would no longer detect the same cytokeratins in a luminal cell. This possibility could only be tested by chemical analysis of separated basal cells and luminal cells. This could be achieved only if sufficient cells could be separated and then separately chemically ana-

### Table 1 Immunohistochemical reactivity of prostatic carcinomas

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* Focal staining only.

known since these two cells have not thus far been separated and studied. The major cytokeratins making up the phenotype of the whole prostate as reported by Achstätter reveals cytokeratins 7, 8, 18, and 19 (15).
The use of the two antibodies described in the present paper should be capable of separating the cell types by flow cytometry if techniques can be devised to isolate a sufficient number of cells from fresh tissue.

Our chemical determination of the cytokeratin phenotypes of three established human prostatic carcinoma cell lines also suggests that all three cell lines synthesize cytokeratin 18, which is a normal component of the luminal cell. Cytokeratin 19 expression is variable in these cell lines with PC-3 cells producing it in all cells but only relatively few of the DU-145 cells producing it \textit{in vitro}. This correlates with the immunohistochemical data on the clinical cases presented which indicate that thus far all cases studied react diffusely with RGE-53 (anti-18) but less actively and sometimes only focally (e.g., Table 1, Case 5) with KA4 (anti-19).

It is clear from the study of the cultured metastatic prostate cell lines that although there is some variability, all three cell lines appeared to share the same cytokeratins which are demonstrated immunohistochemically in the luminal cells. There is no evidence of persistence of cytokeratins 4, 5, and 6 which are
detected in the basal cells histochemically. Additional studies using genetic probes and cell modulation are planned to determine the true capacity of these two cell types with regard to cytokeratin synthesis in both benign and neoplastic glands.

REFERENCES


Fig. 5. Reactivity of prostatic adenocarcinoma (Case 4) with anti-cytokeratin antibodies KAI (a), KA4 (b), and RGE-53 (c). Note the residual normal glands which are identified by their basal cell reactivity with KAI (a). The adenocarcinoma, infiltrating the adjacent tissue, does not react with this antibody but is reactive with both antibody KA4 and RGE-53. Bar: 50 μm.
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