Colocalization of Basal and Luminal Cell-type Cytokeratins in Human Prostate Cancer

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ABSTRACT

In the epithelium of secretory acini of the prostate two different cell types can be discriminated on the basis of localization, morphology, and degree of differentiation, the luminal and basal cells. The possibility of a developmental relationship between basal and luminal cells has been a subject of interest in several studies. According to the stem cell model at least three cell types, i.e., stem, amplifying, and transit cells, can be discriminated in the epithelium of prostate secretory acini. We previously reported that in the process of degeneration and regeneration in normal rat prostate a population of cells could be identified as candidates for the amplifying cells. These cells showed a keratin expression profile intermediate between those of basal and luminal cells. We now show, by using keratin antibodies, that also in normal human prostate at least three subpopulations of cells can be identified, one of them putatively representing amplifying cells as defined in the stem cell model. Furthermore, these antibodies were used to obtain a better insight into the different cell types involved in the etiology and progression of prostatic carcinoma. Both primary and hormone-independent prostatic tumors were investigated. Our results indicated that the candidate stem cell population was absent in prostatic carcinoma. Unlike earlier reports on the unique presence of cells with luminal characteristics in prostatic carcinoma, we identified also a population of cells coexpressing basal and luminal cell-type cytokeratins in primary and hormone-independent prostatic carcinoma. Since amplifying cells are defined in the stem cell model as precursors of transit (luminal) cells in the hierarchical pathway of prostate epithelium differentiation, we postulate that on the basis of the keratin expression profile this subpopulation is most likely the target for neoplastic transformation.

INTRODUCTION

Prostate cancer, the most common malignancy in the Western male population, is characterized by its androgen responsiveness. Most prostatic cancers retain an androgen dependence for stimulation of their growth. From this finding, Huggins and Hodges (1) concluded that prostatic tumors could be treated by androgen ablation therapy. Indeed, prostatic cancers appear to retain an androgen responsiveness, yet unfortunately this response is of limited duration and the tumors show progression to an androgen-independent state. Various studies indicate that this unresponsive state is due to the presence of hormone-independent cells at the start of therapy (2, 3). Even though the presence of such a population is established, it has not been possible to elucidate the origin of these cells. Moreover, the molecular processes occurring during normal proliferation and differentiation of the prostate are poorly understood, and a better understanding of these mechanisms is an essential requirement for elucidation of the origin and progressive behavior of this malignancy. Recently the differentiation pathways in normal and malignant rat prostate have been immunophenotypically typed by using antibodies directed against keratins (4, 5). Keratins are major components of the cytoskeleton of epithelial cells and at least 20 different keratins can be distinguished in human epithelia. They are differentially expressed in specific epithelial cell types or in specific pathways of epithelial differentiation (6). In general, the epithelium of secretory acini of the prostate can be described as consisting of two different cell types, i.e., the luminal and basal cells, which differ in localization, morphology, and degree of differentiation. These two cell layers can be easily discriminated by antibodies directed against keratins (4, 5, 7–10). A more recent hypothesis postulates the existence of at least three different cell types, i.e., stem, amplifying, and transit cells (11). Cells that are morphologically intermediate, because they have structural features characteristic of both the basal and luminal cells, have been identified in the normal prostatic epithelium of monkeys (12), humans (13), and dogs (14); in addition, they are apparent in mice under conditions of androgen manipulation (15). Our study describing the process of degeneration and regeneration in normal rat prostate revealed a population of cells showing a keratin expression profile intermediate between those of basal and luminal cells (4). This observation was consistent with the concept, as described by Isaacs (3), that stem cells comprise a subpopulation of the basal cells that subserve a population of transient amplifying cells, which in turn give rise to the secretory tall columnar epithelial (transit) cells. The amplifying cells would then be phenotypically characterized by coexpression of basal and luminal cell-type cytokeratins. Antibodies directed against keratins can, therefore, serve as markers for the different cell types in the differentiation pathways and are used in this study to obtain more insight into the etiology and progression of prostate cancer.

MATERIALS AND METHODS

Human Prostatic Tumor Tissue. Surgical specimens were categorized into three groups on the basis of the GS2 (16). Primary prostatic tumors were obtained by transurethral resection or radical prostatectomy from 49 patients (GS 2–3, 22; GS 4, 17; GS 5, 10). Surgical specimens from 15 (GS 2–3, 5; GS 4, 5; GS 5, 5) patients with recurrent prostatic cancer and a history of androgen ablation therapy (either orchietomy or hormonal therapy) were obtained by transurethral resection and are referred to as hormone-independent tumors. These patients had complaints caused by locally recurrent tumors. Apart from the clinical progression, high Ki67 labeling indices indicated that the tumor cells indeed were proliferating, despite the androgen withdrawal (17). The tissues were kept frozen at −200°C until use.

Indirect Immunofluorescence and Immunoperoxidase Technique. Frozen sections of the prostatic tissue (6 μm) were cut on a cryostat and air-dried before fixation in acetone for 10 min. After washing in PBS for 10 min, the tissue sections were incubated with 10% normal goat serum in PBS at room temperature for 30 min and then incubated with the primary antiserum for 1 h. After repeated washing in PBS (3 times, 2 The abbreviations used are: GS, Gleason score; PBS, phosphate-buffered saline; BSA, bovine serum albumin; PMSC, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate.
Tilburg, the Netherlands) was applied. After incubation for 30 min and extensive washing in PBS (3 times, 10 min) the sections were mounted in Gelvatol (Monsanto, St. Louis, MO) containing 100 mg/ml 1,4-diazobicyclo[2.2.2]octane (Janssen Pharmaceutica, Beerse, Belgium). For the immunoperoxidase method horseradish peroxidase-labeled rabbit anti-mouse IgG (heavy and light chain specific; Nordic) diluted 1:25 in 10% normal goat serum was applied. After incubation for 30 min and extensive washing in PBS (3 times, 30 min) the peroxidase activity was detected with 3,3′-diaminobenzidine (0.5 g/liter Sigma, St. Louis, MO) containing 0.6% imidazole (Sigma) and 0.04% H2O2. After washing with water the reaction was intensified with CuSO4 (0.5% in 0.9% NaCl). After washing with water the sections were counterstained with hematoxylin. Double-label direct immunofluorescence studies used the monoclonal antibody RGE 53 directly labeled with biotin in combination with the unlabeled monoclonal antibody RCK 103, following the procedure previously described (4). For each section five areas of similar grade were analyzed semiquantitatively, by two independent investigators, for the fraction of cells staining.

**Electrophoresis and Immunoblotting.** Crystall sections (20 μm) of prostatic tissue of homogeneous degree of differentiation were extracted with cold, high-salt, detergent buffer containing 0.5% Triton X-100, 1.5 M KCl, 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, and 0.5 mM PMSC (Merck). After extraction (20 min, 0°C) the insoluble material was sedimented (5 min, 800 × g), washed in low-salt detergent buffer containing 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, and 0.5 mM PMSC, dissolved in SDS sample buffer (2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.1 M Tris-HCl, pH 6.8), and finally heated for 2 min at 100°C. Proteins were separated according to their molecular weight in 13% SDS polyacrylamide gels, as described by Laemmli (18). Transfer of the proteins to nitrocellulose was performed in 192 mm glycine, 25 mM Tris, pH 8.3, 20% methanol, at 50 V/250 mA (overnight). Non-specific binding sites on the nitrocellulose blot were blocked by incubation of the blot for 1 hr with preincubation buffer containing 3% BSA, 350 mM NaCl, 10 mM Tris-HCl, pH 7.4, and 0.5 mM PMSC. Then the appropriate dilution of the primary antibody in buffer 1, containing 0.3% BSA, 500 mM NaCl, 10 mM Tris-HCl, 0.5% sodium deoxycholate (BDH Chemicals Ltd, Poole, England), and 0.1% SDS was applied for 2 hr. After extensive washing in buffer 1 (3 times, 10 min) and PBS containing 0.5% Triton X-100 (10 min), the blot was incubated for 1 hr with horseradish peroxidase-labeled rabbit anti-mouse IgG (heavy and light chain specific; Nordic) diluted 1:500 in PBS containing 0.5% Triton X-100 and 0.5% BSA. Finally, after washing 3 times for 10 min in 0.5% Triton X-100 in PBS the peroxidase activity was visualized by incubation of the blot for approximately 10–15 min with 0.5 mg/ml 4-chloro-1-naphthol and 0.012% H2O2 in PBS.

**Antibodies Directed against Keratins.** The following monoclonal antibodies were used in this study. (a) RGE 53 (IgG1), indicated in this study as Kbasal, stains the basal cell compartment in human prostate (4, 5, 20). (b) RCK 103 (IgG1), indicated in this study as K18, is monospecific for keratin 18 in immunoblotting (19). (c) RCK 101 (IgG1), indicated in this study as Kbasal, stains the basal cell compartment in human prostate (4, 5, 20). (d) LL002 (IgG3), indicated in this study as K14, is monospecific for keratin 14 (21). (d) RCK 107 is also directed against keratin 14. Because RCK 107 gave results similar to those obtained with LL002, it is also indicated as K14. The results with K14 shown in this study are those with LL002. (e) LP2K, indicated in this study as K19, recognizes only keratin 19 in the immunoblotting assay (22).

**RESULTS**

**Cytokeratin Immunophenotypes in Nonmalignant Human Prostatic Secretory Acini.** In the nonmalignant human prostatic secretory acini, keratin expression patterns were examined using the monoclonal antibodies Kbasal, K14, K18, and K19. The keratins recognized by these reagents are all expressed in the glandular cells (Fig. 1). Kbasal and K14 specifically stain the basal cell population, while K18 and K19 react with the luminal cell population, as summarized in Table 1. Occasionally K19 reactivity was also detected in the basal cells. Besides the two predominant immunophenotypes, K14+/Kbasal+/K18- and K14-/Kbasal+/K18+, a third cell type was detected that showed a keratin expression pattern intermediate between those of basal and luminal cells. These cells can be characterized as K14-/Kbasal+/K18-. In the morphologically defined basal cell population the combination of K14 and Kbasal enables discrimination of two cell types. Cells positive for K14 were always positive for Kbasal, as shown in Fig. 1. However, a subpopulation of cells was detected that was positive for Kbasal but negative for K14, as shown in Fig. 1A and confirmed by double-immunofluorescence studies (data not shown). This indicates that sometimes K18- cells were found that contained the marker Kbasal but lacked K14. These are indicated as K14-/ Kbasal+/K18- (see also Table 1). Morphologically defined luminal cells are characterized by the absence of staining for K14 and Kbasal. The luminal cells were positive for K18 and K19, although not all luminal cells were positive for K19 (data not shown). In the morphologically defined luminal cells of the normal human prostate, occasional cells were found that were positive for Kbasal but negative for K14. These cells were also positive for K18 and are indicated as K14-/Kbasal+/K18+, as shown in Fig. 1B (see also Table 1).

**Keratin Expression Patterns in Primary Prostatic Tumors.** Tissue sections of 49 cases of human primary prostatic tumor were examined for the expression of keratins in the tumor cells. In all cases K14 did not react with any of the tumors, as shown in Figs. 2 and 3 and summarized in Table 2. Kbasal reacted with some of the tumor cells. Examples of Kbasal+ tumors are shown in Fig. 2, while the Kbasal− tumors are illustrated in Fig. 3. Of all the well differentiated tumors investigated, an average of 35% of the cells were positive (see Table 2). The different tumors showed a great variability in reactivity of Kbasal (0–90%). With malignant progression and decrease of degree of differentiation the number of Kbasal− cells also decreased. In poorly differentiated tumors fewer cells positive for Kbasal were found (an average of 25%, with a range of 0–45% of cells staining). In anaplastic tumors no Kbasal+ cells were found, although a few positive cells were present. The tumor cells were always positive for K18, while K19 stained 50–75% of the tumor cells (see Table 2).

**Keratin Expression Patterns in Hormone-independent Prostatic Tumors.** Tissue sections of 15 human hormone-independent prostatic tumors were also examined for expression of the various keratins. It appeared that in all the cases examined the tumor cells were negative for K14, as summarized in Table 3. Kbasal expression was heterogeneous and similar to the patterns described for the primary tumors. In the well differentiated and poorly differentiated tumors approximately 10–15% of the cells were positive for Kbasal (Table 3). Kbasal− cells were also positive for K18. Anaplastic tumors were totally negative for Kbasal, although sometimes a few positive cells were found. K18 was expressed in all tumor cells. K19 again showed a heterogeneous expression pattern but definitely decreased from 90% positive for the well differentiated tumors to 50% positive for the poorly differentiated tumors and 25% positive for the anaplastic tumors (Table 3).

**Characterization of Keratin Expression by Immunoblotting.** The presence and identity of the various keratins were also characterized by immunoblot analysis. In Fig. 4 the immunoblot pattern of normal human prostate is shown, in which all four antibodies recognized keratin bands with the expected molecular weights (K14, Mr 50,000; K18, Mr 45,000; K19, Mr 35,000). In the immunoblot assay (22) of the prostatic tumor some of the tumor cells. Examples of Kbasal+ tumors are shown in Fig. 2, while the Kbasal− tumors are illustrated in Fig. 3. Of all the well differentiated tumors investigated, an average of 35% of the cells were positive (see Table 2). The different tumors showed a great variability in reactivity of Kbasal (0–90%). With malignant progression and decrease of degree of differentiation the number of Kbasal− cells also decreased. In poorly differentiated tumors fewer cells positive for Kbasal were found (an average of 25%, with a range of 0–45% of cells staining). In anaplastic tumors no Kbasal+ cells were found, although a few positive cells were present. The tumor cells were always positive for K18, while K19 stained 50–75% of the tumor cells (see Table 2).

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BASAL CELL-TYPE CYTOKERATINS IN PROSTATIC CARCINOMA

Fig. 1. Expression pattern of cytokeratins in nonmalignant secretory acini of the prostate. A, illustration of the predominant staining pattern in the acini. Left, staining of the basal cells, with K\textsubscript{basal} staining all basal cells. Middle, immunohistochemical staining with K14, detecting a subpopulation of basal cells. Right, staining of all luminal cells with K18. B, illustration of a staining pattern occasionally observed in the acini. K\textsubscript{basal} also stains a significant portion of the luminal cells.

Table 1 General keratin expression pattern in the different cell types in normal prostate

<table>
<thead>
<tr>
<th>Keratin expression</th>
<th>Stem cells (basal cells)</th>
<th>Amplifying cells</th>
<th>Transit cells (luminal cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K14</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K\textsubscript{basal}</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K18</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>K19</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

40,000; for K\textsubscript{basal} a band with an apparent molecular weight of 58,000 was observed, which may possibly represent keratin 5). Fig. 4B shows the immunoblot pattern of a lesion of a poorly differentiated primary prostatic tumor in which only K\textsubscript{basal}, K18, and K19 showed a positive reaction.

DISCUSSION

Relatively little is known about the fundamental cell biology of prostatic epithelium. On the basis of localization and differentiation the prostatic epithelium has been described as consisting of basal and luminal cells. The relationship between basal and luminal cells is still a matter of discussion, although recent observations (23) strongly suggest that cells from the basal compartment proliferate and differentiate into luminal cells during prostatic development. According to the stem cell model (11) the prostatic epithelium consists of at least three different cell types, i.e., stem, amplifying, and transit cells. In this hypothesis the three components are arranged in an expanding hierarchy. Stem cells have a high potential for cell proliferation but are normally slow-cycling. On the basis of
Fig. 2. Human prostate cancers expressing Kbasal. From left to right, staining with Kbasal (RCK 103), K14 (LL002), K18 (RGE 53), and K19 (LP2K). A-D, expression pattern in four different tumor samples of varying GS (GS 2–4).
BASAL CELL-TYPE CYTOKERATINS IN PROSTATIC CARCINOMA

Table 2 Percentage of the tumor cells of all investigated primary prostatic tumors that are positive for the various keratins

<table>
<thead>
<tr>
<th>Keratin-positive tumor cells (%)</th>
<th>GS1-3</th>
<th>GS4</th>
<th>GS5</th>
</tr>
</thead>
<tbody>
<tr>
<td>K14</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kbasal</td>
<td>35</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>K18</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>K19</td>
<td>75</td>
<td>50</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 3 Percentage of the tumor cells of all investigated recurrent prostatic tumors that are positive for the various keratins

<table>
<thead>
<tr>
<th>Keratin-positive tumor cells (%)</th>
<th>GS1-3</th>
<th>GS4</th>
<th>GS5</th>
</tr>
</thead>
<tbody>
<tr>
<td>K14</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kbasal</td>
<td>15</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>K18</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>K19</td>
<td>90</td>
<td>50</td>
<td>25</td>
</tr>
</tbody>
</table>

Fig. 3. Human prostate cancers negative for Kbasal. From left to right, staining with Kbasal (RCK 103), K14 (LL002), K18 (RGE 53), and K19 (LP2K). A, example of a Kbasal well differentiated tumor. B, example of a Kbasal poorly differentiated tumor.

Table 2 Percentage of the tumor cells of all investigated primary prostatic tumors that are positive for the various keratins

Fig. 4. Western blot analysis of the expression of cytokeratins in nonmalignant (A) and malignant (B) prostate tissue. Blots were incubated with antibodies against Kbasal (RCK 103) (lane 1), K14 (LL002) (lane 2), K19 (LP2K) (lane 3), and K18 (RGE 53) (lane 4). Note that a weak but definitely visible expression of Kbasal is found in the tumor, whereas K14 is absent in the cancer specimen (B, lane 2).

morphology and location the stem cells are most likely part of the basal cell population, although no clear evidence has been presented to support this idea unequivocally (24–26). Stem cells give rise to more rapidly dividing amplifying cells, which have a limited capacity to proliferate. Characteristic features of the amplifying cells have not yet been described, although in monkeys, humans, dogs, and mice (12–15) morphologically intermediate types were identified on the basis of structural features characteristic of both the basal and the luminal cells. The transit cells are highly differentiated and are referred to as the functionally active prostatic secretory cells; they are localized luminally. Further identification of the various cell types is, however, still hampered by the lack of suitable markers. The
cell kinetics associated with normal cell growth and differentiation have yet to be elucidated and will be of crucial importance in the understanding of the etiology of prostatic cancer. We, therefore, first focused on normal human prostatic epithelium, to identify specific cell populations. It appeared that, as in rat (4), at least three staining patterns could be discriminated on the basis of keratin expression. We demonstrated that antibodies K14 and K_{basal} are specific markers for basal cells in normal prostate, suggesting that the K_{basal}/K14+ cell population is a candidate for the stem cells. Morphologically defined luminal cells with the characteristics K14+/K_{basal}/K18+ colocalize with secretory cells and are, therefore, most likely the transit cells. The K_{basal}/K14+ cells may in fact be candidates for the hypothetical amplifying cell population, because they show a keratin expression pattern intermediate between those of the basal and the luminal cells (see Table 1). It is obvious that the cell type from which a neoplastic lesion may have arisen is an important factor in the etiology of prostatic cancer development. Therefore, in this study prostatic tumors were analyzed with the goal of obtaining more insight into the different cell types of which they are composed. Cells that were positive for both K14 and K_{basal} could not be detected in human prostatic tumors, indicating that the immunophenotype that in the stem cell model was postulated to be associated with the stem cells is not present in prostatic carcinoma. Cells with an expression pattern characteristic of transit cells were definitely present in prostatic carcinoma, a phenomenon also found in other studies (7, 8, 27, 28).

Interestingly, a population positive for K18 and K_{basal} was also detected in prostatic carcinoma. Cells characterized by a K14+/K_{basal}/K18+ keratin profile were also found in the differentiation pathway induced by androgen administration after orchiectomy (4) and were considered candidates for the hypothetical amplifying cells. The presence of cells with this keratin phenotype in primary and hormone-independent prostatic tumors suggests the existence in human prostatic carcinoma of a subpopulation of cells that we now associate with amplifying cells. No difference between primary and hormone-independent tumors could be found in this respect. The fact that we have identified in nonmalignant and malignant prostatic glandular epithelial cells a population of cells with a keratin immunophenotype intermediate between those of basal and luminal cells is in agreement with the stem cell model for prostatic epithelial differentiation (3), which predicts the existence of such a cell type (‘amplifying cells’) in prostatic epithelial differentiation. Moreover, this phenotype was also found in prostatic carcinoma, whereas the keratin expression profile of a subpopulation of basal cells that putatively serve as stem cells was not. Since in the hierarchical pathway of prostatic epithelial differentiation the amplifying cells are precursors of the transit cells we postulate that this subpopulation is most likely the target for neoplastic transformation.

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