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Differential Expression of Keratins in the Basal and Luminal Compartments of Rat Prostatic Epithelium During Degeneration and Regeneration

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The role of the different epithelial compartments during degeneration and regeneration of the rat prostate is examined on basis of intermediate filament protein (IFP) expression pattern. With the monoclonal antibodies RCK 103 and RGE 53, directed against specific keratins, it was possible to differentiate between the basal (RCK 103+) and luminal (RGE 53+) cells of the prostatic epithelium. After testosterone deprivation, by orchietomy, an extensive and rapid cell loss was observed which appeared to affect mainly the luminal cells. In the process of prostate regeneration, induced by testosterone administration, using silastic implants, the luminal compartment rapidly regained its normal thickness. A heterogeneous population of morphologically luminal cells was observed showing keratin expression patterns intermediate between basal and luminal cells. These findings support the idea of a relationship between basal and luminal cells as being members of the same lineage of differentiation.

Key words: prostatic epithelium, keratins, differentiation

INTRODUCTION

More information about the basic biology of the different cell types of the secretory prostatic epithelium may give us better insight into the development of prostatic carcinoma, one of the most common malignancies affecting the male population.

The prostatic epithelium has generally been described as consisting of two different cell types [1–3]. The luminal or glandular cells are highly differentiated and are often referred to as the functionally active prostatic secretory cells. On the contrary, the basal cells are less differentiated and are located at the base of the epithelium. Their function remains largely unknown, although a function as reserve cell has frequently been suggested [4].

The two cell types may exist independently of each other, although the
possibility of a developmental relation is suggested. In several studies a simple relationship between the two cell types has been described. The basal cells are often indicated as the stem cell population, which can proliferate and subsequently differentiate into the luminal cells [5,6]. Aumüller et al. [7] indicate—although not in prostatic epithelium, but in the epithelium of the seminal vesicle—that the basal cells only proliferate in the absence of androgen or when affected otherwise. Moreover, this suggests that the new cells do not differentiate into glandular cells. Evans and Chandler [8], however, recently reported that they could not find evidence for the theory that the basal cells alone represent the proliferative compartment or that these cells are responsible for the replacement of luminal cells during normal turnover. They suggested that the basal and luminal cells are self-replicating cell types. Furthermore, it has been suggested by Deane and Wurzelmann [9] that the basal cells may represent a degenerated form of luminal cells. Alternatively, a more complex stem cell model has recently been proposed [10]. In this model the existence of three different cell types is suggested, i.e., stem cells and amplifying cells, both androgen-independent, as well as transit cells, which are androgen-dependent.

In this study the role of basal and luminal cells during prostate degeneration and regeneration are studied, using antibodies to keratins as specific markers for the two epithelial compartments. The keratins, a class of intermediate filament proteins, have been shown to exist in virtually all epithelial cells. So far, 19 different human keratins have been characterized and their expression pattern has been shown to be specific for the different epithelial compartments [11–14]. With these markers the relationship between the basal and luminal cells of the rat prostate has been investigated.

MATERIALS AND METHODS

Tissue Specimens

Twenty-seven 6-month-old male Wistar rats were castrated and divided into nine groups of three rats each. After 3, 6, 8, 10, and 14 days, respectively, three rats were sacrificed, their prostates removed, weighed, and immediately stored in liquid N₂ until use. In the remaining 12 rats a 2.5-cm-long testosterone-filled silastic capsule was implanted subcutaneously in the flank, which restored the serum testosterone to intact control values (approx. 3 ng/ml) [10]. After 3, 7, 9, and 14 days postimplantation, three rats were sacrificed, their prostates removed, and the tissues processed as described above.

From three Wistar rats, a control group, prostates were collected and immediately stored in liquid N₂ after dissection.

Indirect Immunofluorescence Technique

Frozen sections of the prostatic tissue (6 μm) were cut on a cryostat and air-dried before fixation in methanol (−20°C, 5 min) and acetone (3 times, 5 sec). After being washed in phosphate-buffered saline (PBS) for 10 min, the tissue sections were incubated with 10% normal goat serum (NGS) in PBS at room temperature (30 min), and subsequently with the primary antiserum for 45 min. After repeated washing in PBS (3 times, 10 min) the fluorescein (FITC)-conjugated goat antimouse IgG (1:25 in 10% NGS, Nordic, Tilburg, The Netherlands) was applied. After incubation for 30 min and extensive washing in PBS (3 times, 10 min) the tissue was
mounted in Gelvatol (Monsanto, St. Louis, MO) containing 100 mg/ml 1.4-
diazobicyclo-(2.2.2)-octane (DABCO, Janssen Pharmaceutica, Beerse, Belgium).

Double-label indirect immunofluorescence studies were done by using the
monoclonal antibody RGE 53 directly labeled with biotin in combination with the
unlabeled monoclonal antibody RCK 103. The procedure was the same as described
above except that after incubation with the primary unlabeled monoclonal antibody,
the tissue sections were incubated with Texas-Red conjugated sheep antihorse whole
Ig (1:50 in 10% NGS, Amersham, England). To saturate additional binding sites on
this second antibody, it was incubated with 10% normal mouse serum (NMS) in PBS
(30 min). After extensive washing with PBS (3 times, 10 min) the second primary
monoclonal antibody RGE 53, directly labeled with biotin, was applied to the tissue
sections (45 min). After washing with PBS (3 times, 10 min) FITC-conjugated
streptavidine (1:100 in 10% NGS, 30 min, Amersham, England) was applied. After
the final washing steps with PBS the tissue was mounted as described above. In
control experiments 10% NGS was used instead of the primary antiserum.

Gel Electrophoresis and Immunoblotting

Frozen sections (20 μm) of prostatic tissue were collected and extracted with cold,
high-salt detergent buffer (0.5% Triton X-100, 1.5 M KCl, 10 mM Tris-HCl
[pH = 7.4], 5 mM EDTA, and 0.5 mM phenylmethylsulfonyl chloride [PMSC,
Merck]). After incubation (20 min, 0 °C) the insoluble material was sedimented (5
min, 800g); washed in low-salt detergent buffer containing 10 mM Tris-HCl
[pH = 7.4], 5 mM EDTA, and 0.5 mM PMSC; dissolved in sodium dodecyl sulfate
(SDS)-sample buffer (2% SDS-10% glycerol, 5% β-mercaptoethanol, and 0.1 M
Tris-HCl, pH = 6.8); and heated for 2 min at 100 °C.

Proteins were separated according to their molecular weight on 13% SDS
polyacrylamide gels as described by Laemmli [15]. Transferring of the proteins to
nitrocellulose was performed in 192 mM glycine, 25 mM Tris, pH = 8.3, and 20%
methanol at 50 V/250 mA (overnight). For detection of antigens specific binding
sites on the nitrocellulose blot were first blocked by incubation of the blot with
pre-incubation buffer (3% bovine serum albumin [BSA], 350 mM NaCl, 10 mM
Tris-HCl [pH = 7.4], and 0.5 mM PMSC) for 1 hr and then with the first antibody for
2 hr. After extensive washing with buffer 1 (0.3% BSA, 150 mM NaCl, 10 mM
Tris-HCl, 0.5% sodium deoxycholate [DOC; BDH Chemicals Ltd, Poole, England],
and 0.1% SDS) (3 times, 10 min) and buffer 2 (0.5% Triton-X 100 in PBS) (10 min),
the blot was incubated for 1 hr with horseradish-peroxidase-labeled rabbit-antihorse
IgG [heavy and light chain specific; Nordic] diluted 1:500 in PBS containing 0.5%
Triton-X 100 and 0.5% BSA. After the final washing steps in buffer 2 (3 times, 10
min), the peroxidase activity was visualized by incubation of the blot in 0.5 mg/ml
4-chloro-1-naphtol and 0.012% H2O2 in PBS for approximately 10–15 min.

Intermediate Filament Protein Antibodies

The following antibodies directed against intermediate filament proteins (IFPs)
were used in this study (see Table 1):

1. The monoclonal antibody RCK 102, directed against keratins 5 and 8
TABLE I. Specificity of Antibodies Used in This Study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Ig subclass</th>
<th>Antigen recognized in rat prostate</th>
<th>Tissue specificity in rat prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCK 102</td>
<td>IgG1</td>
<td>Keratins, 45–58 kD</td>
<td>All epithelial cells</td>
</tr>
<tr>
<td>RGE 53</td>
<td>IgG1</td>
<td>Keratin, 45 kD</td>
<td>Luminal epithelial cells</td>
</tr>
<tr>
<td>CK 18-2</td>
<td>IgG1</td>
<td>Keratin, 45 kD</td>
<td>Luminal epithelial cells</td>
</tr>
<tr>
<td>RCK 103</td>
<td>IgG1</td>
<td></td>
<td>Basal epithelial cells</td>
</tr>
<tr>
<td>RV 203</td>
<td>IgG1</td>
<td>Vimentin</td>
<td>Mesenchymal tissue</td>
</tr>
<tr>
<td>RD 301</td>
<td>IgG2b</td>
<td>Desmin</td>
<td>Smooth muscle cells</td>
</tr>
</tbody>
</table>

(nomenclature according to Moll et al.[12]), stains virtually all epithelial tissue types [16].

2. The monoclonal antibody RGE 53, which is specifically directed against keratin 18, recognizes glandular epithelial cells from digestive, respiratory, and urogenital tracts; endocrine and exocrine tissues; and mesothelial cells. In general no reaction is found in squamous epithelial or nonepithelial tissues.[17]

3. The monoclonal antibody CK 18-2, also directed against cytokeratin 18, is comparable with RGE 53 [16].

4. The monoclonal keratin antibody RCK 103 stains the basal cell compartment in human prostate [18].

5. The monoclonal antibody RV 203 is specific for vimentin.

6. The monoclonal antibody RD 301 is specific for desmin [19,20].

RESULTS

Characterization of the Antigens in Rat Prostate Recognized by the IFP Antibodies

The IFP antibodies used in this study were characterized by immunoblotting for their antigen specificity in rat prostatic tissues. From the immunoblot pattern depicted in Figure 1, it can be noted that the antibody RCK 102 (lane 1) showed reactivity with major protein bands in the 45–58-kD molecular weight region, probably representing rat counterparts of the human keratins 5, 8, and 18. The antibody RCK 103 (lane 2) did not show any significant reactivity in immunoblotting. The antibodies RGE 53 (lane 3) and CK 18-2 (lane 4) both recognize a protein band at the level of 45 kD and additionally a protein band with a slightly lower weight, which most probably represent a proteolytic degradation product. The antibody CK 18-2, however, showed a much stronger reaction in immunoblotting as compared to RGE 53. Antibodies RV 203 and RD 301 showed a strong reaction at the level of vimentin, 57 kD (lane 5), and desmin, 53 kD (lane 6), respectively.

Intermediate Filament Expression Pattern in Normal Rat Prostate

The antibodies directed against intermediate filament proteins showed different reaction patterns in the epithelial tissues of the prostate of normal rats. No reaction was found in nonepithelial cells with the keratin antibodies. The broadly cross-reacting monoclonal antibody RCK102 strongly stains all epithelial cells of the prostate (Fig. 2a). The monoclonal antibody RGE 53, to keratin 18, reacts specifically with the luminal compartment of the prostatic epithelium (Fig. 2b). Because this antibody gave a much stronger reaction than CK 18-2 in the immuno-
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\[ M_r \times 10^3 \]

58  45

1 2 3 4 5 6

Fig. 1. Characterization of the antigens in rat prostate detected by the antibodies directed against intermediate filament proteins in immunoblotting. The individual blot strips, originating from the same blot, were incubated as follows: Lane 1: RCK 102, which recognizes keratin bands in the 45–58 kD region. Lane 2: RCK 103, showing no significant reaction in the immunoblotting assay. Lane 3: RGE 53, which very weakly recognizes the 45 kD keratin and some of its degradation products. Lane 4: CK 18-2, shows the same reaction pattern as RGE 53 but stains much stronger. Lane 5: RV 203, directed against vimentin, shows a band at 57 kD and a degradation product at 40 kD. Lane 6: RD 301, directed against desmin, reacts with a single protein at 53 kD.

fluorescence assay, RGE 53 was normally used for this purpose. When parallel sections of the normal prostate were incubated with the antibody RCK 103 specifically, the basal cell compartment and not the luminal epithelial compartment was stained (Fig. 2c). Double-label studies of RCK 103 in combination with RGE 53 directly labeled with biotin indicate that, although difficult to interpret in the immunofluorescence assay, both staining reactions are mutually exclusive (Fig.
Fig. 2. Indirect immunofluorescence staining pattern of rat prostatic tissue with different monoclonal antibodies directed against intermediate filament proteins. a: RCK 102, shows staining with the total prostatic epithelial cell layer. b: RGE 53, specifically recognizes the luminal cells of the epithelium. c: RCK 103, specifically recognizes the basal cells of the epithelial layer. d: RD 301, directed against desmin recognizes smooth muscle cells, which surround each prostatic acinus. e: RV 203, directed against vimentin stains the connective tissue. f,g: Double-label immunofluorescence staining pattern of RGE 53 (F) and RCK 103 (G). ×300.

2f,g). The monoclonal antibody to desmin specifically stains the layer of smooth muscle cells surrounding each prostatic acinus (Fig. 2d), while the monoclonal antibody to vimentin stains the connective tissue, which surrounds the epithelium-lined acini (Fig. 2e).

Intermediate Filament Protein Expression in the Degenerating Prostate

When rats were orchiectomized an extensive cell loss of the prostate rapidly occurred. During the first 8 days after orchiectomy the prostate showed a tremendous decrease in volume and weight (see Fig. 3) and reached a stable state after which no further degeneration seemed to occur.

In frozen sections of the prostate during degeneration a decline in thickness of the epithelial layer of the acini was seen. This effect was nicely detectable with the
monoclonal antibody RCK 102 (Fig. 4, No. 1 in A–C). The reduction of the epithelial layer seemed to be caused mainly by a reduction of the luminal cell compartment, as can be concluded from the reaction patterns specific of the monoclonal antibody RGE 53 (Fig. 4, No. 2 in A–C). Fourteen days after orchietomy, the time of maximal involution, a very thin luminal cell layer was left (Fig. 4C2). On the contrary, the basal cell compartment seemed to be mostly unaffected, as can be seen from the staining patterns with the monoclonal antibody RCK 103 (Fig. 4, No. 3 in A–C). The stromal component surrounding the acini was specifically stained for vimentin, and the layer of smooth muscle cells reacted positively for desmin. At all time intervals desmin and vimentin reaction patterns were comparable to those shown in Figure 2d,e.

**Intermediate Filament Protein Expression Pattern in the Regenerating Prostate**

Testosterone administration to the 14-day castrates induced a rapid regeneration of the prostate (Fig. 3). The prostate almost regained its normal weight within approximately 14 days of exogenous testosterone treatment.

From our IFP expression studies on frozen sections of the regenerating prostate, it was obvious that the prostatic epithelium very rapidly regained its normal thickness.
Fig. 4. Indirect immunofluorescence staining pattern of rat prostate 3 days (A), 6 days (B), and 14 days (C) after orchectomy and incubated with RCK 102 (1), RGE 53 (2) and RCK 103 (3). × 300.
Fig. 5. Indirect immunofluorescence staining pattern of rat prostate 3 days (A), 7 days (B), and 14 days (C) after administration of testosterone and incubated with RCK 102 (1), RGE 53 (2) and RCK 103 (3). × 300.
Fig. 6. Double-label indirect immunofluorescence staining pattern of rat prostate 7 days after administration of testosterone showing the different types of morphologically luminal cells. A: representation of cell types a (RCK 103+/RGE 53+) and b (RCK 103+/RGE 53-). B: representation of cell types c (RCK 103+/RGE 53+) and d (RCK 103-/RGE 53-).
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Three days after testosterone administration the normal morphology of the luminal cell layer seemed to be almost completely restored, as shown by staining with RCK 102 (Fig. 5A1) and RGE 53 (Fig. 5A2). A typical phenomenon observed in the regenerating prostatic epithelium was the presence of morphologically luminal cells which were now partly positive with the monoclonal antibody RCK 103. Three days after testosterone administration only a few luminal cells were positive for this basal compartment antibody (Fig. 5A3). However, 7 and 9 days after testosterone administration the number of luminal cells positive with RCK 103 had increased (Fig. 5B3). After 14 days of testosterone administration the number of RCK 103 positive luminal cells was diminished again (Fig. 5C3). The staining patterns of the regenerating prostate with RGE 53 (Fig. 5, No. 2 in A–C) showed a heterogeneous character, especially when examined 14 days after testosterone administration. Not all luminal cells were positive for RGE 53, while the positive cells differed in intensity (Fig. 5C2). Double-label immunofluorescence studies showed that on basis of their keratin expression pattern the following morphologically luminal cell types can be distinguished in the regenerating prostatic epithelium:

a: morphologically luminal cells, negative with RCK 103 and positive with RGE 53 (Fig. 6A)
b: morphologically luminal cells, positive with RCK 103 and negative with RGE 53 (Fig. 6A)
c: morphologically luminal cells, positive with RCK 103 and negative with RGE 53 (Fig. 6B)
d: morphologically luminal cells, negative with RCK 103 and negative with RGE 53 (Fig. 6B)

In the regenerating prostate, also as in the degenerating prostate, desmin and vimentin gave positive reaction patterns comparable to those in Figure 2d,e.

DISCUSSION

To obtain more insight into the relationship between the different cell types of the prostate we studied the intermediate filament protein expression patterns in the different compartments of the prostate.

As in the human prostate [1,2], in the secretory acini of the rat prostate two different epithelial cell compartments, the basal and luminal cell layer, could also be distinguished on the basis of their keratin expression patterns.

In earlier studies it was already noticed that after orchietomy a rapid involution of the prostate occurs. A decline in total prostatic cell number of 75–88% was noticed [21–24]. Based on our keratin expression studies, we can conclude that the luminal compartment especially is diminished, while the basal compartment seems to be mostly unaffected.

Although orchietomy induces extensive cell loss in the prostate, cells which retain the capacity to respond to exogenous testosterone administration remain present. After administration of testosterone the prostate seemed to return to its original volume. The luminal cells regained their original cylindrical morphology, as was visualized by the keratin 45-kD antibody. This is in agreement with the observations of other investigators, such as English et al. [22], who reported that after testosterone administration the glandular cell number in orchietomized rat increased toward values similar to those of the intact prostate.
A surprising observation in our study was the fact that during the process of regeneration of the rat prostate several types of morphologically luminal cells could be distinguished on the basis of their keratin expression patterns. While the basal cell compartment seemed unaffected, luminal cells expressing luminal-type keratins as well as luminal cells expressing basal-type keratins were found. Furthermore, some of the morphologically luminal cells appeared to express both types of keratins or none at all.

These observations suggest the existence of certain pathways of differentiation
in the prostatic epithelium, which have been influenced by testosterone deprivation. Apparently, in the normal prostate, both compartments can be distinguished on the basis of their keratin expression. However, after orchiectomy, morphologically luminal cells are present that have a keratin expression with characteristics of both compartments. A hallmark of the basal type cells is now found in luminal cells, indicating that basal and luminal cells probably are related in a hierarchical pathway of differentiation. The fact that this is not found in all luminal cells suggests that there are more steps in the differentiation pathway of secretory prostatic epithelium. A schematic representation of the keratin expression pattern in a hypothetical model of differentiation pathways in prostatic epithelium as based on the stem cell model of Isaacs [10] is illustrated in Figure 7. The stem cell model for the organization of the prostate consists of three components arranged in an expanding hierarchy. The first component comprises the stem cells, a small fraction of epithelial cells. These cells have an extensive proliferative capacity and are androgen independent. The second component, a population of amplifying cells, arises from the stem cells. These cells have a limited proliferative activity and also are androgen independent. The amplifying cells give rise to the third component, a population of transit cells. These cells only have a very limited proliferative activity and are androgen dependent. On basis of this stem cell model, the transit cells will disappear after orchiectomy, since these cells are androgen dependent. In our study the highly differentiated luminal cells seemed to be especially affected after orchiectomy and, therefore these cells are indicated as the population of transit cells (Fig. 7). So, after testosterone deprivation, the prostatic epithelium only consists of two cell populations, i.e., stem cells and amplifying cells. As indicated by English et al. [22], virtually all basal cells and the cuboidal luminal cells are amplifying cells. Since the luminal cells seem to have a limited proliferative capacity [8,22], the presence of morphologically luminal cells with a luminal keratin expression pattern during the testosterone induced restoration can be explained. This pathway of luminal proliferation is illustrated in Figure 7. The pathway of basal proliferative activity can explain the presence of morphologically basal cells with the normal basal type keratin expression pattern. The model of Figure 7 also illustrates some hypothetical differentiation pathways comprising the heterogeneous population of morphologically luminal cells on basis of their keratin expression pattern.

So the pathway of differentiation in the prostatic epithelium is likely to be a more complex process than was at first assumed.

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