Distribution of cytokeratin polypeptides in epithelia of the adult human urinary tract

H.E. Schaalma1***, F.C.S. Ramaekers1, G.N.P. van Muijen1, E.C.M. Ooms2, and D.J. Ruiter1

1 Department of Pathology, University Hospital Nijmegen, Geert Grooteplein Zuid 24, 6525 GA Nijmegen, The Netherlands
2 Department of Pathology, Westeinde Hospital, Lijnbaan 32, 2501 CK The Hague, The Netherlands

Received April 20, 1988 / Accepted July 14, 1988

Summary. Cytokeratin expression was studied in the epithelia lining the normal human urine conducting system using immunohistochemistry on frozen sections employing a panel of 14 monoclonal antibodies. Eleven of these anticytokeratin antibodies reacted specifically with one of the 19 human cytokeratin polypeptides. Profound differences were found in the cytokeratin expression patterns between the different types of epithelia in the male and female urinary tract. In the areas showing morphological transitions of transitional epithelium to columnar epithelium and of nonkeratinizing squamous epithelium to keratinizing squamous epithelium gradual shifts of cytokeratin expression patterns were observed, often anticipating the morphological changes. However, also within one type of epithelium, i.e. the transitional epithelium, two different patterns of cytokeratin expression were found. Expression of cytokeratin 7 was homogeneous in the transitional epithelium of renal pelvis and ureter but heterogeneous in the transitional epithelium of the bladder. Furthermore, intraepithelial differences in cytokeratin expression could be shown to be differentiation related. Using a panel of chain-specific monoclonal antibodies to cytokeratins 8 and 18 conformational and/or biochemical changes in the organization of these intermediate filaments were demonstrated upon differentiation in columnar and transitional epithelium.

Introduction

Normal human epithelial cells and their tumors contain cytokeratins (CKs) as their intermediate filament constituents. It has been shown that CKs consist of a family of 19 different polypeptides (Moll et al. 1982) while subsets of 2 to 10 of these CK polypeptides are expressed in the different epithelia depending on their type of differentiation (Tseng et al. 1982; Quinlan et al. 1985). For many of these tissues data concerning CK distribution are available, as based on (two-dimensional) gel electrophoretic analyses of total epithelium or of isolated areas, as well as cell cultures (Moll 1982, 1983a, b; Achsttaeter et al. 1985; Rheinwald and O'Connell 1985). Monoclonal anti-CK antibodies, and especially chain-specific antibodies recognizing only one CK polypeptide, enable the study of expression of this type of intermediate filament proteins in more detail, even at the single cell level, using immunohistochemical methods (Ramaekers et al. 1987a; Cooper et al. 1985).

Achsttaeter et al. (1985) reported the presence of 11 different CK polypeptides in the different epithelia of the male urinary tract mainly using two-dimensional gel electrophoresis. Differences in CK patterns were related to known morphological differences in the different areas studied.

In the current study normal adult male and female epithelia, lining the urine conducting system were examined immunohistochemically using a large panel of monoclonal anti-intermediate filament antibodies, mainly chain-specific anti-CK antibodies. As a result, we were able to identify and localize separately 7 of 11 CKs reported by Achsttaeter et al. (1985) in these tissues. The advantages of this approach over gel electrophoretic analysis of tissues became clear by our observation that certain cytokeratins may be distributed heterogeneously throughout morphologically homogeneous epithelial layers.

Materials and methods

Tissues. The tissue specimens used in this study were obtained from the human urinary tract at autopsy, which was performed within less than 5 h of death. Tissues from six males and three females were snapfrozen and stored in liquid nitrogen. The age of the patients ranged from 61 to 85 years with an average of 74 years. In each case tissue samples were taken from at least 11 different sites, which included renal calyx, renal pelvis, ureteropelvic junction, ureter, bladder (dome, lateral wall, trigone), urethra (proximal, middle, distal) and external urethral orifice (see Fig. 1). Transitional epithelium of one male and urethral epithelium of two males appeared to be unsuitable for examination because of severe mechanical or autolytic damage. Of all nine cases at least six samples per site were examined. Examination of the tissues in H&E stained sections revealed no significant abnormalities.

Antibodies. Fourteen monoclonal anti-CK antibodies, two monoclonal anti-vimentin antibodies and one monoclonal anti-desmin antibody were used in this study. The specificity of these antibodies has been summarized in Table 1, and has been described previously (for references see Table 1). Recent investigations have indicated that the M20 antibody does not recognize CK 18 but reacts exclusively with CK 8, which may show breakdown products in the CK 18 region (van Muijen et al. 1987a). Figure 2 shows the immunoblotting results of the M20 antibody on some carcinoma cell lines (T24, RT4 and A431), expressing among others CK 8. The results in lanes 1, 3
Fig. 1. Schematic representation of the urinary tract showing the sites from where the tissue samples were obtained. 1 renal calyx (in one sample also containing a renal papilla); 2 renal pelvis; 3 ureteroepithelial junction; 4 ureter; 5 near uretero-urethral junction; 6 bladder dome; 7 lateral bladder wall; 8 trigone; 9 proximal urethra; 10 mid urethra; 11 distal urethra and urethral orifice, in male containing distal urethra, fossa navicularis and glans penis. The main type of epithelium in 1–9 is transitional epithelium, while the main type of epithelium in 10–11 in female is nonkeratinizing squamous epithelium. In male these are (pseudo)stratified columnar epithelium in the urethra, nonkeratinizing squamous epithelium in the fossa navicularis and keratinizing squamous epithelium in the glans penis.

Table 1. Specificity of monoclonal intermediate filament antibodies used in this study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Protein(s) recognized</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>clone 80</td>
<td>most CKs</td>
<td>van Muijen et al. 1984</td>
</tr>
<tr>
<td>RCK102</td>
<td>CKs 5+8</td>
<td>Broers et al. 1986</td>
</tr>
<tr>
<td>RCK103</td>
<td></td>
<td>Ramaekers et al. 1987a</td>
</tr>
<tr>
<td>6B10</td>
<td>CK 4</td>
<td>van Muijen et al. 1986</td>
</tr>
<tr>
<td>RCK105</td>
<td>CK 7</td>
<td>Ramaekers et al. 1987a</td>
</tr>
<tr>
<td>LE41</td>
<td>CK 8</td>
<td>Lanc et al. 1982</td>
</tr>
<tr>
<td>M20</td>
<td>CK 8</td>
<td>van Muijen et al. 1987a</td>
</tr>
<tr>
<td>RKSE60</td>
<td>CK 10</td>
<td>Ramaekers et al. 1983, 1987b</td>
</tr>
<tr>
<td>1C7</td>
<td>CK 13</td>
<td>van Muijen et al. 1986</td>
</tr>
<tr>
<td>RG5E3</td>
<td>CK 18</td>
<td>Ramaekers et al. 1983</td>
</tr>
<tr>
<td>2C8</td>
<td>CK 18</td>
<td>unpublished</td>
</tr>
<tr>
<td>RCK106</td>
<td>CK 18</td>
<td>Ramaekers et al. 1987a</td>
</tr>
<tr>
<td>CK18-2</td>
<td>CK 18</td>
<td>Broers et al. 1986</td>
</tr>
<tr>
<td>LP2K</td>
<td>CK 19</td>
<td>Lanc et al. 1985, Broers et al. 1986</td>
</tr>
<tr>
<td>V9</td>
<td>vimentin</td>
<td>van Muijen et al. 1987a</td>
</tr>
<tr>
<td>RV202</td>
<td>vimentin</td>
<td>Ramaekers et al. 1987a</td>
</tr>
<tr>
<td>D33</td>
<td>desmin</td>
<td>van Muijen et al. 1987a</td>
</tr>
</tbody>
</table>

* Not yet fully characterized

Abbreviation: CK = cytokeratin

and 5 demonstrate that this antibody reacts with a protein band at 52 kDa, corresponding to CK 8. However, in the RT4 cell line preparation also some faint protein bands, representing CK 8 breakdown products are seen (lane 3). The position of the 52 kDa protein band is further confirmed by subsequent incubations with other monoclonal CK antibodies recognizing CK 7 and CK 18

Fig. 2. Immunoblotting study on cytoskeletal preparations from the two bladder carcinoma cell lines T24 (lanes 1 and 2) and RT4 (lanes 3 and 4), and the cell line A431, derived from an epidermoid carcinoma of the vulva (lanes 5 and 6). The nitrocellulose strips were incubated with antibody M20 (lanes 1, 3 and 5) and subsequently with antibodies RCK105, staining the CK 7 protein band, and RCK106, staining the CK 18 band (lanes 2, 4 and 6).

Immunohistochemistry. The indirect immunoperoxidase staining procedure was performed on frozen sections as described previously (van Muijen et al. 1986). Staining patterns were designated as homogeneously positive when all cells in a particular cell layer were positive, or heterogeneously positive when both negative and positive cells were observed in the same cell layer(s).

Results

In Fig. 3 the CK phenotype of the epithelial lining of the urinary tract at different sites, as recognized by the monoclonal anti-cytokeratin antibodies, is summarized per type of epithelium. All epithelial cells were stained by the antibodies clone 80 and RCK103 (not included in Fig. 3). RCK102 stained all epithelial cells with the exception of certain cell layers in squamous epithelium (see below). The staining patterns of the other anti-CK antibodies varied and will be described in detail. Vimentin positive epithelial cells were not observed. Throughout the different epithelia scattered vimentin positive cells were seen, most probably representing Langerhans or inflammatory cells. Desmin positivity was only observed in muscle cells (results not shown).

Renal calyx up to trigone

In one of the specimens of the renal calyx we observed the presence of pseudostratified columnar epithelium lining
a renal papilla (Fig. 3 A), adjacent to the transitional epithelium of the calyx. All cell layers of this columnar epithelium were positive for RCK105 (CK 7), M20 (CK 8), LP2K (CK 19) and only two of the CK 18 antibodies, i.e. RCK106 and CK18-2. A few basal cells were positive for 6B10 (CK 4; Fig. 4 A, upper part) and to an even lesser degree with 1C7 (CK 13; Fig. 4 B, upper part) and 2C8 and RGE53 (CK 18; Fig. 4 C, upper part). No reactivity was observed for LE41 (CK 8).

In transitional epithelium (Fig. 3 B and D) all cell layers were positive for M20 (CK 8, Fig. 4 D), RCK106 and CK18-2 (CK 18) and LP2K (CK 19). The other two CK 18 monoclonal antibodies (RGE53 and 2C8) stained only umbrella cells (Fig. 4 E) with cell extensions reaching down between intermediate cell layers or staining sporadically intermediate cells lying directly beneath the umbrella cells. All umbrella cells were also positive for LE41 (CK 8; Fig. 4 F), although cell extensions were not found to be stained. Only sporadically umbrella cells were positive for 6B10 (CK 4; Fig. 4 G), while most all basal and intermediate cells were heterogeneously positive for 1C7 (CK 13; Fig. 4 H), especially the lower intermediate cells. Only sporadically all layers except the umbrella cells were stained with this antibody (Fig. 4 I). Up to the bladder all epithelial layers of the transitional epithelium were positive for RCK105 (CK 7), but in the bladder areas of varying size were negative and alternated with CK 7 positive areas (Fig. 4 J).

**Trigone**

In the trigone the major part of the transitional epithelium reacted similar to bladder transitional epithelium. In the distal part of the trigone (Fig. 3 G), i.e. the bladder neck, transitions were observed in the staining patterns of antibodies 6B10 (CK 4), 1C7 (CK 13) and LE41 (CK 8). CK 4, which was detected only sporadically in umbrella cells of the tissues described above, was now found in an increasing number of cells in this umbrella cell layer (Fig. 5 A). More distally in the trigone also the intermediate and basal cell layers became increasingly positive, although they were stained weaker than the umbrella cell layer (Fig. 5 B). In the area were the umbrella cells became positive for 6B10 (CK 4), most cells, in all layers of the transitional epithelium, became positive for 1C7 (CK 13; Figs. 5 C and D). On the contrary, the expression of CK 8 in the umbrella cells, as monitored by LE41, diminished.

**Urethra**

(Pseudo)stratified columnar urethral epithelium (Fig. 3 F) was homogeneously positive for CK 4 (Fig. 5 E), CK 7, CK 8 (M20) and CK 13. Positive staining with anti-CK 18 antibodies was only observed with CK18-2 and RCK106, with a strong signal in the superficial cells and a variably weak to negative staining reaction in the other epithelial cell layers. Cells in the basal layer were found...
to be more intensely stained by RCK106 (Fig. 5F), than for CK18-2.

The female external urethral orifice and most of the urethra was lined by nonkeratinizing squamous epithelium with CK distributions similar to those observed in an area of nonkeratinizing squamous epithelium in one sample of a female trigone (Fig. 3C). Such squamous epithelium showed an abrupt change in the CK expression pattern as compared to the columnar or transitional epithelium. CKs 7, 8 (Fig. 5G), 10 and 18 (Fig. 5H and I) could not be detected in this type of squamous epithelium, while CK 4 (Fig. 5J) and CK 13 (not shown) were present in all except the basal cell layer. In a minority of these squamous areas LP2K (CK 19) reacted homogeneously positive (Fig. 5K). In most squamous areas this antibody showed a homogeneously positive reaction only in basal cell layers, while its reactivity was heterogeneous in the higher cell layers (Fig. 5L). RCK102 showed a distinct reaction in basal and lower suprabasal cell layers, while the other layers were predominantly negative (Fig. 5M). RCK103 showed a strong staining reaction in the basal cells, with a slightly less intense staining reaction in the other cell layers (Fig. 5N). Reactivity for RKSE60 (CK 10) was found only once in a few suprabasal cells (Fig. 5O).

**Fossa navicularis and glans penis**

Between urethral columnar epithelium and the nonkeratinizing squamous epithelium lining the fossa navicularis (Fig. 3E) an abrupt change in CK expression pattern was observed. Proximally in the fossa CK 19 was extensively but heterogeneously expressed (Fig. 6B). Up to the external urethral orifice reactivity for CK 19 decreased considerably...
Fig. 5A–O. Immunoperoxidase staining patterns of frozen sections from transitional epithelium in distal trigone for 6B10 A and B; B is further distally than A and for 1C7 C and D; urethral columnar epithelium for 6B10 E and RCK106 F; adjacent areas of nonkeratinizing squamous epithelium (at the left side of the figures) for M20 G, RGE53 H, RCK106 I, LP2K K, RCK102 M and RCK103 N; nonkeratinizing squamous urethral epithelium for 6B10 J, LP2K L and RKSE60 O. Note both homogenous K and heterogeneous L reactions for LP2K in this latter type of epithelium. A, C, G, H, I, K, M, N, ×80; J and L, ×100; O, ×150; B and D, ×200; E and F, ×280
Fig. 6A–L. Immunoperoxidase staining patterns of frozen sections from: nonkeratinizing squamous epithelium at proximal fossa navicularis (left a part of the urethra) for RCK102 A and LP2K B; distal fossa navicularis for LP2K C and RKSE60 D, the transition between fossa navicularis and glans penis lined by keratinizing squamous epithelium (right side) for RKSE60 E, 6B10 F and G and 1C7 H; the glans penis for RKSE60 I, 6B10 J, RCK102 K and 1C7 L. A–L. ×80

(Fig. 6C). Cells in the intermediate layers were heterogeneously positive for RKSE60 (CK10) with an increasing number of RKSE60 positive cells in the distal portion of the fossa (Figs. 6D–E). The positive reaction for RCK102 seen in the fossa decreased rather abruptly in the upper cell layers when going from the proximal to the distal part (Fig. 6A). The expression pattern of the other CKs was similar to that seen in nonkeratinizing epithelia in the ur-
etra as described above. However, with respect to the CK 4 expression pattern it should be noted that in most cases 6810 staining diminished before the transition into keratinizing squamous granular epithelium became morphologically evident. Initially only basal cells were unstained, while subsequently also the intermediate cell layers and finally the superficial layer became negative (Figs. 6F and G). CK 13 expression showed in most cases an abrupt change (Fig. 6H). The keratinizing granular epithelium (Fig. 3H) expressed CK 10 in all except the basal cell layer (Fig. 6I). CK 4 was found to be focally distributed in the stratum corneum (Fig. 6J). RCK 102 showed a positive reaction in the basal and lower suprabasal cells (Fig. 6K), while CK 13 was found occasionally in only a few cells (Fig. 6L).

Discussion

The distribution of cytokeratin (CK) polypeptides was studied immunohistochemically in the epithelial lining of the normal adult human urinary tract, using a panel of monoclonal antibodies. This technique allows light microscopic interpretation at the single cell level.

The panel of monoclonal antibodies used in this study recognized separately seven of the 11 CKs reported by Achstatter et al. (1985) to occur in the male urinary tract epithelium.

A limitation of the immunohistochemical approach is that the antigenic epitope recognized by a certain monoclonal antibody can vary in protein structure as a result of biological activity and malignant transformation. This may result in an alteration of the detectability or accessibility of the component under investigation, i.e. the antigenic epitope is masked or unmasked. This phenomenon is well illustrated by the fact that four different CK 18 antibodies showed two completely different expression patterns in the urinary tract epithelium. RGE53 and 2C8 mainly stained the superficial umbrella cells, while all cell layers were positive with RCK106 and CK18-2 (compare Ramaekers et al. 1985; Nadakavukaren et al. 1984; Achstatter et al. 1985; Feitz et al. 1986). Also in the (pseudo)stratified columnar epithelium of urethra and renal papilla these two subgroups of CK 18 antibodies could be distinguished on basis of their reaction patterns. A similar observation was made for the anti-CK 8 antibodies LE41 and M20. These results may possibly be explained by a phenomenon recently described by Franke and coworkers (1987), who showed that a CK 18 dependent antibody Ks18.18 interacted only with its antigen when this was present in heterotypic coiled-coil complexes, notably with CK 8. It can also not be excluded that (de)phosphorylation may play a role in the phenomenon (Sterner and Sterner 1983).

Apparently the epitopes recognized in CK 18 by RGE53 and 2C8, but also by the monoclonal antibody CK1, described by Achstatter et al. (1985), and in CK 8 by LE41, are structurally or biochemically different in the basal and intermediate cell layers as compared to the umbrella cell layer.

In transitional epithelium other CKs studied showed differences in expression depending on the site. This indicates that differences in CK distribution exist between morphologically identical transitional epithelium. It was striking to note that CK 7 was found heterogeneously distributed in the epithelium of the bladder, including the trigone, whereas it was homogeneously expressed in the renal pelvis and ureter. This observation may be the result of epitope masking as described above for CKs 8 and 18. It remains to be examined whether or not these differences in CK 7 structure or content are related to functional differences between the bladder on one hand and the higher urinary tract on the other hand. Epithelia of other hollow organs with a reservoir function, such as the digestive tract, seem to lack CK 7 completely, while ductal structures (for example bile ducts) frequently show the presence of CK 7 (Osborn et al. 1986; Ramaekers et al. 1987a).

CK 4 expression in transitional epithelium was most pronounced in the distal trigone and was sporadically found in the more proximal transitional epithelium. Gel-electrophoretic data published by Moell et al. (1982) and Achstatter et al. (1985) showed that CK 4 was found occasionally only at low levels in transitional epithelium. In the same distal trigonal area all cell layers including umbrella cells were CK 13 positive, while more proximally only basal and intermediate cells were stained heterogeneously. Also in this area CK 8 expression in umbrella cells, as detected by LE41, diminished. We considered that here the CK expression in transitional epithelium partially anticipates the morphological transition into the columnar urethral epithelium. Our observations for CK 4 and CK 13 differed from those reported previously (van Muizen et al. 1986) in that these authors found CK 4 and CK 13 in suprabasal cells. Most probably squamous metaplasia has been examined for this study. Our observations of the CK 13 expression are partly in accordance with those of Huszar et al. (1986), who reported that their antibody Ks8.12, recognizing both CK 13 and CK 16, in some sections did not react well with certain individual luminal cells, most probably due to the fact that the epitope recognized by Ks8.12 was less accessible in these cells. We prefer to consider the umbrella cells as a separate cell type with a CK expression pattern only partly related to that of the underlying cells.

A second possible transition zone is observed in the epithelium of the renal papilla, consisting of two or three layers of columnar cells without umbrella cells. This epithelium lies between the epithelium of the renal collecting tubules (negative for CK 4 and CK 13, unpublished data) and the transitional epithelium of the renal calices (heterogeneously positive for CK 4 and CK 13). The CK distribution in the epithelium of the renal papilla is different from both adjacent types of epithelium in the expression of CK 4 and CK 18 (as recognized by RGE53 and 2C8) in several basal cells, contrasting with the expression of these two CKs only in umbrella cells for regular transitional epithelium.

In the columnar epithelium of the urethra, which is histologically classified as a pseudostratified or stratified type of epithelium (Ham 1965; Bloom and Fawcett 1975), we observed staining of CK 18 only with RCK106 and CK18-2 and of CK 8 only with M20. These results are in contrast to the gel electrophoretic data of Achstatter et al. (1985), who could not find CKs 8 and 18 in the urethra, and show the additional value of cytokeratin immunohistochemistry. The presence of CK 4 and CK 13 in this type of epithelium supports its stratified rather than pseudostratified nature (see Sun et al. 1985). The female urethra is mainly lined by nonkeratinizing squamous epithel-
lium, while in male only foci of squamous epithelium can be found (Bloom and Fawcett 1975). Neither could we demonstrate CK 7, CK 8 or CK18 in this type of epithelium, nor significant levels of CK 10 expression in urothelial squamous epithelium, with the exception of one sample in which a few RKS620 positive cells were detected. CK 4 and CK 13 were expressed in all suprabasal cells as reported previously (van Muijen et al. 1986). The heterogeneous CK 19 pattern seen in the former nonkeratinizing squamous epithelium was also found in the nonkeratinizing squamous epithelium of the proximal fossa navicularis. In the distal fossa an increase of CK 10 positive cells was observed and a decrease of CK19 and CK 4 reactive cells. Normally the glans penis is lined by a keratinizing squamous epithelium (Ham 1965) as observed also in all six male patients examined by us. The CK distribution in this type of epithelium was, however, not identical to that in epidermis (Huszar 1986, van Muijen 1985, 1987b; Moll et al. 1982). For example, CKs 4 and 13 are normally not found in adult epidermis, while they are detected in the keratinizing glanular epithelium. Achtsaeter et al. (1985) reported that apparently nonkeratinizing squamous glanular epithelium contained CK 13 and CK 19, but also CK 1, which is a marker for keratinization. When comparing the staining patterns of the CK 8 antibodies LE41 and M20 with those of RCK102, recognizing CKs 5 and 8, one may conclude that the positive reaction of RCK102 in stratified squamous epithelia represents the distribution of CK 5 in these tissues.

In summary, we can conclude that monoclonal antibodies to individual cytokeratin polypeptides are valuable markers for the detection and characterization of the different morphological types of epithelia occurring in the human male and female urinary tract. Furthermore, within one type of epithelium cytokeratin patterns can be related to stage of differentiation. Future studies with these antibodies in neoplasms of these epithelia will have to reveal whether cytokeratin expression is related to morphology, site of origin or degree of tumor progression.

Acknowledgements. We would like to thank Corrie Vellema and Klaas de Groote for excellent technical assistance and Anita Huijsmans and Gert Schaart for help with the preparation and immunoblotting of monoclonal antibodies. Ton van Eupen is acknowledged for his assistance in the preparation of the illustrations. We are grateful to Dr. E.B. Lane (ICRF, Clare Hall Labs, Hertfordshire) for providing us the antibodies LE41 and LP2K.

References


Ramakers FCS, Huijsmans A, Schaart G, Moesker O, Vooijs


