Cytokeratin Expression Patterns in Metastatic Transitional Cell Carcinoma of the Urinary Tract

An Immunohistochemical Study Comparing Local Tumor and Autologous Metastases

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The cytokeratin (CK) expression patterns of local, ie, primary or recurrent, high-grade-malignant transitional cell carcinomas (TCCs) of the human urinary tract and autologous lymphogenic and hematogenic metastases (n = 33) were compared. Special attention was paid to CK expression in the tumor invasion front and other areas where tumor-stroma interaction occurred to visualize cell populations with a metastatic phenotype. For this purpose, polypeptide-specific monoclonal antibodies to CKs 4, 7, 8, 10, 13, 14, 16, 17, 18, and 19 were used, employing the immunoperoxidase method. Results show that: 1) An increased expression of CK8 and CK18 is seen in the TCC tumor cells at the interface with peritumoral stroma in the tumor invasion front and with intra-tumoral stroma ('interface phenomenon'). Other than reflecting a quantitative change, this phenomenon might be explained by unmasking of CK8 and CK18 epitopes occurring in these regions. 2) Although in general the expression of CK13 in local TCC is decreased with increase of histopathologic parameters for progression, ie, grade and stage, an extensive proportion of CK13-positive tumor cells still can be found in some TCCs, even in metastases. 3) Morphologically recognizable types of aberrant differentiation in TCC, ie, pseudosarcomatous or squamous differentiation and marked loss of differentiation, show altered expression of many of the CKs studied. (Am J Pathol 1991, 139:1389–1400)

The cytokeratin (CK) protein family, which comprises the main type of intermediate filaments in normal and neoplastic epithelial cells, consists of at least 20 different polypeptides (numbered 1 to 201–5, not including the hair cytokeratins.6 Certain combinations of these CK polypeptides are synthesized in specific types of epithelium. Under certain circumstances, such as metaplasia,5 hyperproliferation,6 or neoplastic growth and progression, the CK expression pattern of a certain epithelium can change. Although initial studies showed that neoplasms retain the CK pattern of the normal epithelium from which they are derived,1,3 and CK typing could therefore be useful in determining the site of origin of a metastasis, deviating CK expression patterns in neoplastic lesions7 and cancers of different organ systems have recently been reported.8–11

Cytokeratins not expressed in the normal state of a certain epithelium can appear in cancers. Also a correlation between altered CK expression patterns and the degree of tumor cell anaplasia has been reported.8–12 Recently we and others10 showed that, during tumor progression of local transitional cell carcinomas (TCCs), a decrease of CK13 can be seen in the tumor cells,

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whereas in contrast CK14 became detectable using certain antibodies. Furthermore mainly the invasive TCC compartment showed an apparently altered CK cytoskeleton structure, as detected with monoclonal antibodies to different epitopes of CK 8 and CK 18. We interpreted these findings as an adaptation of the cytoskeleton to a more flexible and motile cell phenotype. This assumption was supported by our observations that umbrella cells in the urinary bladder, which have to be flexible during expansion of the organ, showed the same reaction pattern as compared with the invasive TCC cells with the different CK 8 and CK 18 antibodies.

In the present study, we examined the CK expression patterns in lymphogenic and hematogenic metastases of TCC of the urinary tract from 17 patients as the ultimate end point of TCC progression. In 10 of the 17 cases, the CK staining reaction of local tumor and metastasis could be compared, allowing us to recognize possible alterations in CK expression between these stages. We also considered the possibility that metastatic cells might be selected from a pool of primary tumor cells with a particular CK expression pattern; for example, those cells that exhibited specific CK 8 and CK 18 epitopes in the primary tumor, could be studied. Particular attention was paid to the specific location of the tumor cells, especially in the tumor invasion front and other areas where tumor-stroma interactions occur.

**Materials and Methods**

**Tissues**

The 33 tumor samples (Table 1) were snap frozen and stored in liquid nitrogen immediately after surgery or during autopsy. No overlap between this series of tumor samples and those described previously exists. Approximately 5-μm thick cryostat sections were made and stained by the indirect immunoperoxidase procedure, essentially as described previously, but using 3-3′-diaminobenzidine tetrahydrochloride (DAB) as chromogen for the detection of peroxidase activity.

A more extensive panel of monoclonal CK antibodies was used than in our previous study, most of which are monospecific for individual CKs. To obtain a reference for the staining patterns of these additional antibodies, normal urothelium and a series of at least 15 local TCCs of different stage and grade were stained. These specimens were taken from the tissue bank described in two previous reports.

Metastatic and local high-grade malignant TCC specimens (n = 33) from the bladder or the upper urinary tract were examined. These included 23 TCC metastases from 17 different patients and 10 corresponding local TCCs, which were primaries or recurrences in the urinary tract. Samples from four patients were obtained at autopsy (see Table 1).

**Antibodies**

The panel of monoclonal CK antibodies used in this study includes most of the reagents described before and reacting specifically with CKs 4, 7, 8, 10, 13, 14, 18, or 19. Their reaction patterns in normal transitional epithelium are included in Table 2. In addition, the following antibodies were used:

- The mouse monoclonal antibody LL001 specific for CK14, reacts similar to antibody LL002 in normal urothelium and local TCC.
- The mouse monoclonal antibody RCK107, specific for CK14 reacts immunohistochemically more extensively than LL001 and LL002, by staining basal cells heterogeneously both in normal transitional epithelium and in G1 TCCs. In squamous metaplastic urothelium, all cells are positive except for basal and some parabasal cells.
- The mouse monoclonal antibody KA1, reacting with the CK 5/14 complex, stains virtually all basal and several suprabasal cells of normal transitional epithelium, whereas squamous metaplastic urothelium is homogeneously positive. In most G1 and G2 TCCs in a series of 55 tumors (predominantly the same series as described earlier), basal and several parabasal cells are positive, whereas a considerable part of the G3 TCCs is negative.
- The mouse monoclonal antibody KS8.12, described to be reactive with CKs 13 and 16 and as with CK5, CK14 was purchased from Sigma Chemical Co. through Bruinuwig Chemie, Amsterdam, the Netherlands. In normal transitional epithelium, all cells, except for the umbrella cells, are positive. In a series of 59 TCCs, this antibody reacted similarly to, although slightly more extensively than, our CK13 antibodies 1D7 and 2D7.
- The mouse monoclonal antibody LL025, specific for CK16, does not react with normal transitional epithelium and only weakly in squamous metaplastic urothelium, with negative reaction in basal and some parabasal layers. In a series (a part of the series described previously) of 15 TCCs (G1–3), sporadic positive tumor cells were observed only in three of nine G3 TCCs.
- The mouse monoclonal antibody E3, reacting with CK17, stains normal transitional epithelium heterogeneously, usually reacting with a minority of the basal cells, although in some samples all cell layers were reactive, with the most intense reaction on sporadic umbrella cells. In squamous metaplasia, extensive staining is observed in all but the basal and some parabasal cell layers. In 15 local TCCs (a part of the series described previously), basal cells were stained most intensely, whereas suprabasal cells were stained with variable intensity. In all
Table 1. Clinical Data and Morphologic Characteristics of TCCs Used in This Study (Local TCCs and Metastases)

<table>
<thead>
<tr>
<th>Case—Site</th>
<th>Interval between the samples (months)</th>
<th>Therapy</th>
<th>Special morphologic aspects</th>
<th>Presence of Invasion front in the sample (Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Classical&quot; TCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Bladder Lung</td>
<td>2</td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>2-Bladder Liver</td>
<td>½</td>
<td>Bladder radiotherapy</td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>3-Bladder Liver</td>
<td>1½</td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>4-Bladder Lymphnode</td>
<td>—</td>
<td>Radiotherapy &amp; chemotherapy</td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>5-Bladder Lymphnode</td>
<td>1½</td>
<td>Chemotherapy</td>
<td>Necrosis (bladder) Severe polymorphism and tumor giant cells</td>
<td>N, Y</td>
</tr>
<tr>
<td>6-Renal pelvis Lymphnode</td>
<td>—</td>
<td>Nephrectomy</td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>7-Renal pelvis Lymphnode</td>
<td>—</td>
<td>Nephrectomy</td>
<td>Large eosinophilic cytoplasm</td>
<td>N</td>
</tr>
<tr>
<td>8-Lymphnode*</td>
<td>—</td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>9-Lymphnode*</td>
<td>—</td>
<td></td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>10-Lymphnode*</td>
<td>—</td>
<td>Chemotherapy</td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>11-Liver† Lung</td>
<td>—</td>
<td>Autopsy</td>
<td>Bassoid aspect of the cells</td>
<td>Y</td>
</tr>
<tr>
<td>2 Lymphnodes</td>
<td>—</td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>12-Lymphnode†</td>
<td>—</td>
<td></td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>Carcinomas “deviating” morphologically from classical TCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-Lymphnode*</td>
<td>—</td>
<td></td>
<td>Pseudosarcomatous carcinoma</td>
<td>N</td>
</tr>
<tr>
<td>14-Bladder 2 Lymphnodes</td>
<td>3</td>
<td>Bladder radiotherapy</td>
<td>Undifferentiated carcinoma with giant cells</td>
<td>N</td>
</tr>
<tr>
<td>Liver</td>
<td>—</td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>15-Bladder neck Liver pituitary gland</td>
<td>—</td>
<td>Autopsy</td>
<td>Oat cell-like, but neuro-endocrine marker N-CAM and electronmicroscopy are negative</td>
<td>Y, Y</td>
</tr>
<tr>
<td>16-Lung*</td>
<td>—</td>
<td>Autopsy</td>
<td>Chemotherapy</td>
<td>Extensive squamous metaplasia</td>
</tr>
<tr>
<td>17-Bladder Abdominal wall</td>
<td>26</td>
<td>Both after chemotherapy</td>
<td>Extensive squamous metaplasia</td>
<td>N</td>
</tr>
</tbody>
</table>

* Primary in ureter.  
† Primary in renal pelvis.  
‡ Primary in bladder.

grades, but especially in G3 TCC, homogeneously positive cases were found as well as less extensively stained tumors. One TCC of the “deviating” tumors, described in our previous report, was negative with E3.

The mouse monoclonal antibody K65, recognizing CKs 1, 9, and 10, does not stain normal transitional epithelium, whereas squamous metaplasia shows extensive staining except for basal and some parabasal cell layers. In a series of 55 TCCs (G1–3), most of the cancers are negative and only a few cases show sporadic positive cells.

The mouse monoclonal antibody CAM5.2, specific for CK8, reacts similarly to M20, another anti-CK8 antibody. With this antibody, both normal transitional epithelium and the 15 TCCs mentioned above were homogeneously positive. Squamous metaplastic urothelium is negative. With respect to CAM5.2, it should be kept in mind that this antibody reacted weakly with purified CK7 (unpublished results from CM Alexander, PC Stasiek, and EB Lane).

The mouse monoclonal antibody RV202 is directed against vimentin.

Results

The CK expression patterns seen in the series of TCC metastases from 17 patients, including 10 cases with cor-
<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>6B10</th>
<th>2D7</th>
<th>Ks8.12</th>
<th>LL025</th>
<th>E3</th>
<th>KA1</th>
<th>LL001</th>
<th>RCK107</th>
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<tbody>
<tr>
<td>Cytokeratin subtype</td>
<td>4</td>
<td>13</td>
<td>13, 15, 16</td>
<td>16</td>
<td>1, 5, 10</td>
<td>17</td>
<td>5, 14</td>
<td>14</td>
</tr>
<tr>
<td>Normal urothelium(^{13})</td>
<td>S(^+)</td>
<td>B(^{-})</td>
<td>B(^{-})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>invasive G3 TCC(^{8})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&quot;Classical&quot; TCC</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1-Local</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Meta</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>S/(^{B})</td>
<td>B/(^{+})</td>
<td>B/(^{-})</td>
</tr>
<tr>
<td>2-Local</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Meta</td>
<td>S</td>
<td>-</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>F/(^{-})</td>
<td>F/(^{+})</td>
<td>F/(^{-})</td>
</tr>
<tr>
<td>3-Local</td>
<td>S</td>
<td>-</td>
<td>B/(^{+})</td>
<td>-</td>
<td>-</td>
<td>B/(^{-})</td>
<td>B/(^{-})</td>
<td>B/(^{+})</td>
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<td>4-Local</td>
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<tr>
<td>Meta</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>-</td>
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<tr>
<td>5-Local</td>
<td>S</td>
<td>S</td>
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<tr>
<td>Meta</td>
<td>S</td>
<td>-</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>B/(^{+})</td>
<td>B/(^{-})</td>
<td>B/(^{-})</td>
</tr>
<tr>
<td>6-Local</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Meta</td>
<td>S</td>
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<td>S</td>
<td>S</td>
<td>S</td>
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<td>-</td>
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<tr>
<td>7-Local</td>
<td>S</td>
<td>S</td>
<td>B/(^{-})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Meta</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Meta</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10-Meta</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11-Meta</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12-Meta</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| Carcinomas, morphologically "deviating" from classical TCC | - | - | - | - | - | - | - | - |
| 13-Meta | - | - | - | - | - | - | - | - |
| 14-Local | - | - | - | - | - | - | - | - |
| Meta | - | - | - | - | - | - | - | - |
| 15-Local | F | +/\(^{-}\) | S | - | - | - | - | - |
| Meta | S | S | S | S | S | - | - | - |
| 16-Meta | F | +/\(^{-}\) | S | - | - | - | - | - |
| 17-Meta | F | +/\(^{-}\) | S | - | - | - | - | - |

**Immunohistochemical staining results of cytokeratin markers for stratified squamous and transitional epithelium and basal cell components in TCC metastases and several autologous local high-grade malignant TCCs, showing heterogeneous relations between the results with the different cytokeratins, between antibodies recognizing the same cytokeratins, and sometimes between samples from one case.**

1. \(\times\): two staining patterns are observed: dominating pattern of the tumor (>50%)/minor pattern (<50%); S = sporadically positive cells (<5%); F = focally dispersed positive cells (5% to 30%); \(\times\) = homogeneously strongly positive; \(\times\) = weakly positive; \(\times\) = = no staining observed; and S = basal cells positive.
2. Only positive in superficial (umbrella) cells.
3. (Several) cells, which are adjacent to the stroma, i.e., "basal" cells, are more intensely stained.
4. Also staining in several parabasal cell layers; in normal transitional epithelium are all cells except the umbrella cells positive with Ks8.12.
5. In small area also staining of all epithelial cells.
6. Small totally negative areas are present.
7. The most intense staining reaction is situated at the periphery of the whole tumor lesion.
8. In several cells the most intense staining is present at the stromal side of basal cells.
9. Basal cells are negative.
10. Negative with antibody KA5.
11. Tumor localization in the abdominal wall, 26 months after cystectomy.
responding local tumors, are summarized in Table 2 and depicted in Figures 1 to 4. Morphologically the TCCs could be subdivided into two groups, one with features of classical TCCs and the other group with a morphology deviating from classical TCC.

**CK Expression in "Classical" TCCs and Their Metastases**

In 12 cases (with 22 specimens), the lesions were morphologically typical transitional cell carcinomas.

No consistent differences in the expression patterns of the different CKs were found when the local TCC and hematogenic or lymphogenic metastasis were compared. The vimentin antibody did not stain tumor cells, and the broad-spectrum epithelial marker RCK102 stained all cases extensively, as did LP2K (to CK19). The reactivity patterns of the other antibodies reacting with individual CKs can be summarized as follows:

**Cytokeratin 7**

Homogeneous positive reaction was observed in the 22 specimens of both local and metastatic TCC, indicating a complete conservation of CK7 during malignant progression.

**Cytokeratins 8 and 18**

The different CK8 and CK18 antibodies showed different staining patterns. LE41 stained fewer tumor cells than the other two CK8 antibodies, CAM5.2 and M20. The CK18 antibody 2C8 stained fewer tumor cells than RGE53, which in its turn stained less extensively than the other CK18 antibodies CK18-2 and RCK106. As a result of the different degree of staining (both qualitatively and quantitatively), particular staining patterns emerged, which can be described as follows (Figures 1 and 2a–h):

1) Peripherally located carcinoma cell clusters within a tumor lesion were more intensely and extensively stained than the more centrally located cell clusters (Figures 2a–c). This staining pattern was most prominent with LE41 and 2C8 in cases 3 (liver), 4 (lymph node), 5 (lymph node), 6 (renal pelvis), 8, 9, 14 (lymph node), and 15 (pituitary).

2) Peripheral individual tumor cells within a given tumor cell cluster ("basal" cells) reacted more frequently and more strongly than those located more centrally within the cluster, depending on which antibody was used (Figures 2d, e) and on the localization of the cluster in the whole tumor lesion (see above). This staining pattern was most prominent with LE41 and 2C8 and in a lesser degree with RGE53 and was observed only a few times with the other, more extensive staining antibodies (cases 3 [bladder], 9, and 11 [lung and lymph node]). In cell clusters with minimal CK8 and CK18 expression, staining was often observed to be polarized within the cell, located at the stromal side of individual basal cells (Figure 2f, g).

3) In small tumor clusters, relatively more cells seemed to be positive than in larger tumor cell groups (Figure 2h).

These distribution patterns were observed consistently, and they hold true for the local TCC as well as the metastases. In some cases the phenomenon described above was less prominent when a diffuse strong staining reaction was observed even with the generally "less extensive" staining antibodies (LE41, 2C8, and RGE53). in

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Figure 1. Schematic representation of the "interface phenomenon" in TCC based on CK8 and/or CK18 staining. It is characterized by preferential staining of carcinoma cells located at the interface with stroma, especially in the invasion front. This results in a gradient in expression from the invasion front to the center of the tumor. The degree of staining (A, local expression; B, moderate extensive expression; C, extensive expression) depends on the antibody used as well as tumor qualities.
Figure 2. Immunoperoxidase staining patterns of frozen sections of local transitional cell carcinomas or metastases, showing typical staining patterns for the different CKs and CK13 antibodies, illustrated for 2D7 (A, case 4—lymph node metastases), CK19-2 (B, some case as in A), RCK105 (C, case 9), RCK53 (D, case 3—bladder), LE41 (E, case 6—lymph node), RCK53 (F, case 3—bladder), 2D7 (G, case 3—bladder) and RCK105 (H, case 9). Magnifications, A–C, H, ×50; D–F, ×200; G, ×300.

Figure 3. Immunoperoxidase staining patterns of frozen sections of local transitional cell carcinomas or metastases, including 2D7 (A, B, C, respectively, case 4—lymph node, case 7—lymph node, case 11—liver), GB19 (D, case 11—liver), LL025 (E, case 6—kidney), E3 (F, G, H, case 7—lymph node, case 6—kidney and case 10, respectively). Magnifications, B–D, F, H, ×50; A, G, ×75; E, ×100.
fact, the expression of CK8 and CK18 appeared to be conserved throughout the final steps of malignant progression.

**Cytokeratin 4 and 13**

Expression of CK13, as recognized by the antibodies 1C7 and 2D7, was low in 10 of the 12 cases, and only sporadically positive cells were found in most of these local and metastatic tumors (Figure 3a). In the remaining two cases, numbers 7 and 11, all six specimens, including one local TCC, were extensively positive for both CK13 antibodies (Figure 3b, c). Expression of CK4 (Table 2) was also very low in most cases and was found to be associated with the degree of CK13 expression. The two cases that were extensively positive for CK13 showed a slightly higher expression level for CK4 as compared with the other 10 cases (Figure 3d).

**Cytokeratin 16**

CK16 (Table 2), as recognized by LL025, was mostly found to be focally expressed in a few dispersed cells in a minority of the local and metastatic tumors (Figure 3e). The antibody KS8.12, which recognizes CK13, CK16, and CK15, stained the TCCs more extensively than the monospecific CK13 and CK16 antibodies taken together, suggesting the presence of some CK15. In three cases that were all negative with the two specific CK13 antibodies, and of which only one showed some positive reaction with the monospecific CK16 antibody LL025, there was positive staining with KS8.12.

**Cytokeratin 17**

The expression of CK17 (Table 2) was variable between the samples, even within individual cases (Figure 3f–h). Extensively positive cases were found next to cases that showed sporadic positivity in the high-grade malignant local TCC as well as in metastases.

**Cytokeratins 1, 9, and 10**

Only sporadic staining was observed in some tumor cells with antibodies RK5G60 and KAS5, used to recognize cytokeratins expressed in keratinizing differentiation. Their expression appeared unrelated to expression of other CKs (Table 2).

**Cytokeratin 14**

The three monoclonal antibodies that specifically react with CK14 (Table 2) all gave similar staining patterns when the local and metastatic TCCs were compared.
They preferentially stained the basal and parabasal cells (i.e., cells at the interface with the stroma), with occasional staining in virtually all tumor cells. Antibodies LL001 and LL002 reacted similarly. The antibody RCK107 stained more cases than did the former two, and its reaction pattern was more extensive, in that it stained more cells. Antibody KA1, reacting with both CK14 and CK5, reacted in a pattern that was neither related to the CK14-specific antibodies nor to any of the other CK antibodies.

**Discussion**

Several rules for the distribution of the various cytokeratins and their combinations have been described for adult human epithelial tissues, both in the normal tissues and benign as well as malignant tumors. For example, CKs 4 and 13 are related to epithelial stratification, whereas CKs 7, 8, 18, and 19 are described to be merely associated with glandular differentiation. After examination of extensive series of normal and malignant epithelial tissues, however, several exceptions to these general rules were reported. For example, our immunohistochemical studies on urothelium and cancers derived thereof showed alterations in cytokeratin expression related to tumor progression. Another complicating factor is introduced by a diversity in reaction patterns of individual monoclonal antibodies all reactive with the same CK protein. Furthermore, different tumor compartments showed differences in their cytokeratin expression patterns, whereas the morphologic phenotype also influences the intermediate filament composition of cells. In the present study, we compared the cytokeratin expression patterns in local high-grade malignant TCCs, i.e., a primary tumor or a local recurrence, with those in TCC metastases, with special emphasis on aberrations, as described previously.

**Increased Expression of Cytokeratins 8 and 18 in Areas of Tumor–Stroma Interaction**

From the results presented here, the increased detectability of CK8 and CK18, reported earlier by us to occur during malignant progression of TCC, can be placed in a new perspective. By the use of our panel of specific CK8 and CK18 antibodies, which show different reaction patterns in normal and malignant urothelium, given tumor areas were positive for a set of CK8 and CK18 antibodies, whereas on the same area another set of such antibodies gave negative results. The use of this panel of antibodies showing different degrees of reactivity with CK8 and CK18 allows a crude histochemical titration of antigen accessibility, if not protein quantity. Quantitation at the cell level is generally not possible with the immunoperoxidase detection because the method depends for its sensitivity on a saturation amplification reaction. Furthermore, we observed regional differences in the staining intensity of TCCs with the antibodies. We often noticed the most intense reaction in tumor cells in close proximity to stroma and in the periphery of the tumor nodule, i.e., the invasion front. At one extreme, in several cases antibodies LE41 and 2C8 showed exclusive reactivity with cells in these peripheral areas. Similar observations were made in the invasion front of otherwise
Cytokeratin 13 Can Be Expressed Extensively in TCC Metastases

Our earlier observations\(^8\) and those of Moll et al\(^9\) showed that, with progression of TCC (G1pTa to G3pT2), CK13 expression decreased in the tumor cells. Therefore we assumed we would find low CK13 expression levels in TCC metastases. In an extended series of metastatic TCCs, this hypothesis was only partly confirmed by the present study. The 23 metastases examined showed CK13 expression similar to that described for high-grade malignant local TCCs.\(^6\) Of the 10 cases in which the reaction patterns could be directly compared with those in the matching local cancer, nine cases showed a similar staining pattern in both tumor stages. For example, in three cases (cases 7, 11, and 17), where the local TCC showed an extensive CK13 expression, this was also found in the metastases. In other cases (except one), where the local tumor showed low CK13 levels, the metastasis also showed a very limited reaction with the CK13 antibodies. Case 15 showed extensive CK13 reactivity in the local tumor but loss of this reactivity in the metastases. Only in one case (case 17) could squamous metaplasia explain the extensive CK13 expression in this otherwise typical high-grade malignant TCC. Moll et al\(^10\) described absence of CK13 in their series of five TCC metastases except for one case, which also showed squamous metaplasia. In our series, the extensive CK13 expression of two cases (cases 7 and 11) remained unexplained, although in one case the cytoplasm of the tumor cells was relatively large and eosinophilic as seen by hematoxylin and eosin (H&E) staining, which might indicate early stages of squamous differentiation. It should be noted, however, that carcinomas of the squamous phenotype do not necessarily contain CK13. For example, Broers et al\(^11\) reported a decrease in CK13 expression on dedifferentiation of squamous cell carcinomas of the lung, resulting in 50% CK13 negative cases in poorly differentiated squamous carcinomas. Kuruc et al\(^12\) showed that CK13 is not expressed in squamous cell carcinomas of the skin, although this had been expected on the basis of the results of Nischet et al\(^13\) and because of its transient expression in certain stages of fetal skin development.\(^14\) Our preliminary studies in head and neck mucosal squamous cell carcinomas are in line with these observations, in that CK13 is not a major component in these tumors (to be published).

Cytokeratin 16 as an Indicator of Squamous Differentiation

CK 16 has been reported to be related to hyperproliferation in stratified epithelial cells.\(^6,16,17,18\) It also has been correlated with a basal cell phenotype in certain malignancies.\(^19\) As expected, we did not find a significant reaction in normal transitional epithelium for CK16. In squamous metaplasia in the bladder, however, CK16 expression was found in most suprabasal cells. In both local bladder cancers as well as in the metastases, CK16 (as
recognized by LL025) was only found sporadically in
scattered cells, except for the two cases with squamous
metaplasia, which displayed more extensive positive re-
activity. Our immunohistochemical observations con-
cerning CK16 are generally in line with gel electrophore-
tic data.10,34 The results suggest a relation of CK16 with
squamous differentiation, rather than with proliferative
state, at least in the bladder. This assumption may be
supported by the observation that the metastasis of case
7 also showed a relatively high CK16 level. In this case,
CK16 expression might be related to the expression of
CK13, a marker for stratified, squamous epithelium that is
not keratinizing. In this particular case, however, morpho-
logic characteristics for such a type of differentiation were
not found, although a suggestion was present in the ex-
tensive eosinophilic cytoplasm of some cells.

Antibody Ks.12 showed a more extensive staining
pattern than we would expect from the combined results
of antibodies 1C7, 2D7, and LL025. Recent reports now
suggest that this antibody reacts with CK15 16,18 as well
as the earlier described CK13 and CK16 reactivity. Data
presented here therefore point to the presence of CK15,
particularly in TCCs with 'deviating' morphology, but not
or as a much lesser extent in the typical TCCs. This also
might explain why expression of CK15 in TCC was not
reported earlier.10,34

Cytokeratin 14

In this study, the panel of CK14 antibodies was exten-
sed from our earlier study. Two different sets of results
were obtained. LL001 and LL002 were both negative in
normal transitional epithelium; conversely, RCK107 was
reactive with several basal cells in normal urothelium. This
difference also was present in the cancers, frequently
with more staining by the latter antibody. In general,
LL001 and LL002 reacted similarly. In our previous
study,8 an increase of CK14 expression was noted in
parts of the tumors during tumor progression. We re-
ported that this increase was not consistently present in
tumors infiltrating muscle. From the results of the present
study, we could even state that there is no relation of
CK14 expression to the metastatic potential of TCC. Ex-
tensive CK14 expression was found only in one case of
the two TCCs with squamous metaplasia. No constant
relation between increase of CK14 and CK13 expression
was detected.

The antibody KA1, described as recognizing com-
plexes of CK5 and CK14, reacted extensively in basal
cells of normal transitional epithelium and in the majority
of G1 and G2 TCCs studied in our series. These results
are in accord with data of Moll et al.10 No consistent pat-
tern of CK expression was noted with increasing degree
of malignancy for this antibody.

Independent of morphologic characteristics, the ex-
pression patterns of individual CKs observed in this se-
ries of TCCs showed interrelationships only for CK8 and
CK18 and to a minor degree between CK4 and CK13;
some crude quantitative relation appeared to exist be-
tween CK17 and CK14 if CK14 expression was deter-
mined with antibody RCK107.

Cytokeratin 7 as Marker for Transitional Cell
Differentiation

In normal transitional epithelium, CK7 is expressed ho-
mogeneously in the upper urinary tract but heteroge-
neously in the lower part.13 This is observed with two
independent monoclonal antibodies to CK7.12 In TCCs,
also those in the bladder, the expression of CK7 is in
general homogeneous. In the differential diagnosis of
carcinomas, CK7 can be used as an indicator for urothe-
llial differentiation,12 especially in the male, where it can
distinguish urothelial malignancies from, for example,
prostate cancer, renal cell carcinomas, etc. We must
keep in mind, however, that the expression of CK7 can
be found to be decreased, particularly when squamous
differentiation is present.8

Cytokeratin 17

Using our immunohistochemical procedure, the ex-
pression of CK17 appears to be less extensive in normal
urothelium than in local TCC and metastases. No relation
was observed between advanced stage and increased
CK17 expression, because a number of advanced can-
cers with only limited expression of CK17 also were
found. No morphologic explanation or relation to expres-
sion patterns of other CKs could be found in these TCCs
with limited CK17 expression. It was striking that, in the
two TCC cases with squamous metaplasia, no increase
of CK17 expression could be seen, although extensive
CK17 expression was found in normal squamous meta-
plastic urothelium. Our immunohistochemical results ap-
ppeared to be in line with the gel electrophoretic data of
Moll et al.10

Conclusions

We conclude from the present and previous studies that
for human urothelium, CK expression is not fully conser-
vaive during neoplastic progression. Expression pat-
terns in TCC depend on morphologic characteristics and
type of differentiation, the degree of anaplasia, and on
interactions with the microenvironment.
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