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# Immunohistochemical Demonstration of Tumor-Associated Antigens with the Aid of Monoclonal and Polyclonal Antisera in Carcinoma of the Bladder

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**Summary.** Keratin was identified with the aid of polyclonal antisera in the cytoplasm in over 90% of the transitional cell carcinomas investigated. The intensity of staining increased with the degree of dedifferentiation. Detection of cytokeratin with monoclonal antibodies was successful in over 80% of samples. All squamous cell carcinomas of the bladder were strongly positive for keratin and cytokeratin. CEA was found in 20% of the G1 and 40% of the G2 and G3 carcinomas of the bladder. Both the prostatic epithelium markers PSA and PAP and the monoclonal antibody Ca1 were negative in all cases.

**Key words:** Immunohistochemistry, Bladder carcinoma, Keratin, Cytokeratin, Carcinoembryonic antigen, Ca1, Prostate-specific antigen, Prostate acid phosphatase.

## Introduction

The development of new tumor markers has opened up new fields in oncological diagnosis and treatment and has stimulated the search for new, sensitive tumor markers for biochemical diagnosis.

No specific tissue markers have as yet been found for urothelial carcinoma of the bladder, which has been the most intensively studied of all tumors falling within the sphere of the urologist [1–3, 7].

Since it has been possible to detect carcinoembryonic antigen (CEA) in serum and urine [16–19, 23, 26, 28, 31, 38] and also by immunocytochemical methods [21, 23, 35, 40, 41] this oncofetal antigen has been considered to be a good indicator of the growth of bladder carcinoma rather than as a specific tumor marker.

Keratin has also been detected in transitional cell tumors by means of immunohistochemical light microscopy [33] and electron microscopy [43].

In the present study we investigated the incidence and localization of these tumor markers in 50 transitional cell carcinomas exhibiting various grades of differentiation and 7 squamous cell carcinomas of the bladder using the peroxidase-anti-peroxidase method.

The monoclonal antibody Ca1, discovered in 1982, and which is reportedly only detectable in malignant tumors of varying origin, was also included in the investigation [5, 25].

The prostate-specific epithelium markers prostate-specific antigen (PSA) and prostate acid phosphatase (PAP) were also incubated, in order to exclude the possiblity of prostatic carcinoma.

## Material and Methods

Three investigators reclassified all the bladder carcinomas according to the WHO grading as follows:

*50 Transitional Cell Carcinomas of the Bladder*

- 15 well differentiated carcinomas (G1)
- 15 moderately differentiated carcinomas (G2)
- 20 dedifferentiated carcinomas (G3)

*7 Squamous Cell Carcinomas of the Bladder*

4-$\mu$ thick sections were prepared from representative material embedded in paraffin blocks. We investigated the following markers using the peroxidase-antiperoxidase method described by Sternberger [37]:

- keratin
- cytokeratin
- carcinoembryonic antigen (CEA)
- Ca1
- prostate acid phosphatase (PAP)
- prostate-specific antigen (PSA)

*Monoclonal Antibodies*

The *monoclonal antibodies against cytokeratin* were supplied by Camon (Wiesbaden, FRG) (1 : 100 dilution).

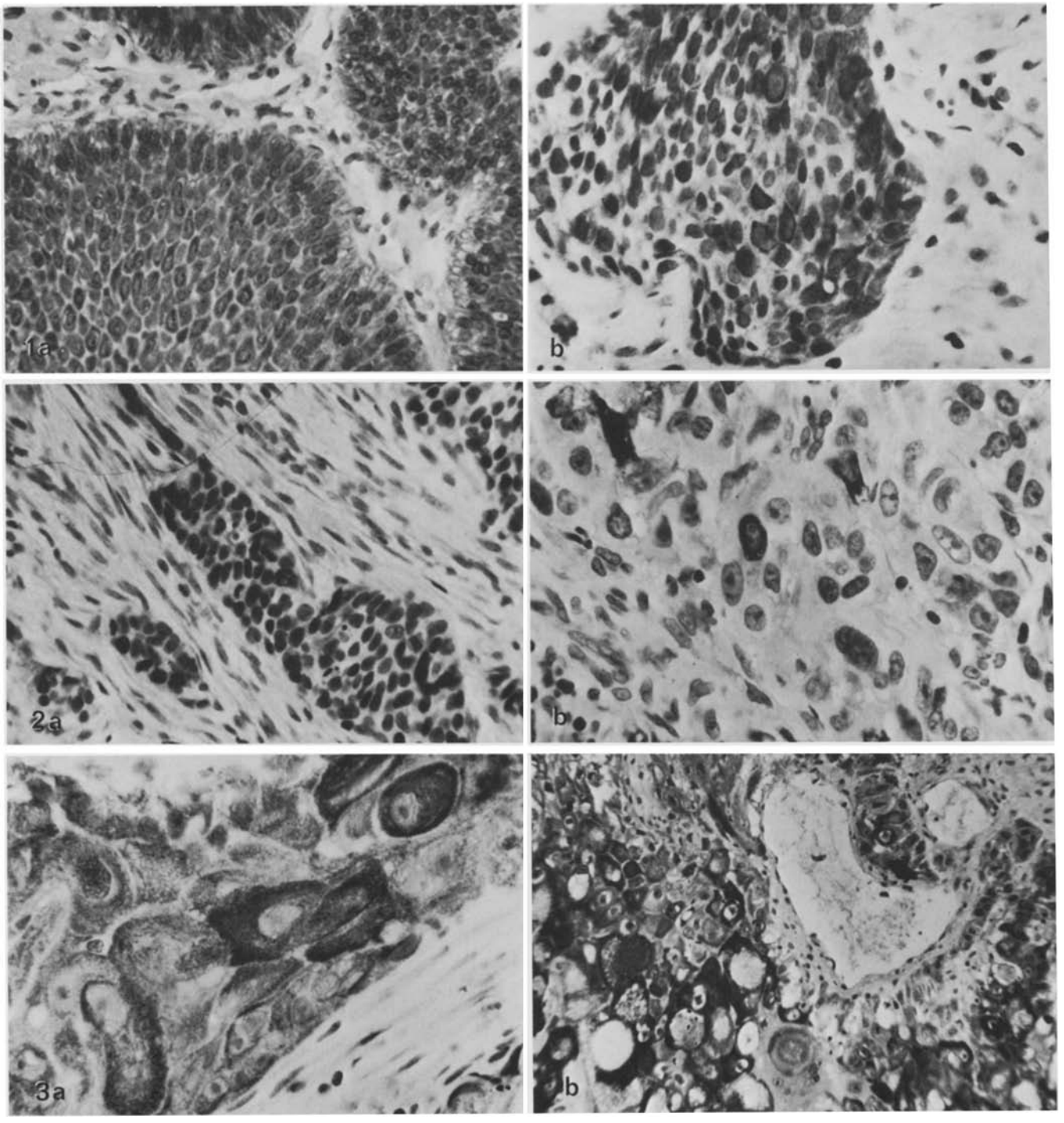


**Fig. 1a, b.** Moderately differentiated bladder carcinoma (G2) **(a)**, tumor cells with various degrees of *keratin* staining, x100. **b** Poorly differentiated transitional cell carcinoma (G3), isolated cells strongly positive for *keratin* together with regressive tumor cells exhibiting a negative reaction, x250

**Fig. 2a, b.** Moderately differentiated transitional cell carcinoma (G2) **(a)**, isolated *CEA*-positive cells, x250. **b** Poorly differentiated transitional cell carcinoma (G3), isolated *CEA*-positive cells, x400

**Fig. 3a, b** Squamous cell carcinoma of the bladder **(a)**, very stron *keratin* staining, x400. **b** Squamous cell carcinoma of the bladder, distinctly positive *cytokeratin* staining, x400

Table 1a. Semiquantitative immunohistochemical staining of cytokeratin and keratin in transitional cell carcinomas of the urinary bladder of various histological differentiations (G1–G3)

| | *n* | cytokeratin | keratin |
|---|---|---|---|
| G1 | 15 | 12 ( 80%) | 14 (93.3%) |
| G2 | 15 | 15 (100%) | 14 (93.3%) |
| G3 | 20 | 19 ( 95%) | 19 (95.5%) |

Table 1b. Semiquantitative immunohistochemical staining of cytokeratin and keratin in transitional cell carcinoma of the urinary bladder of various histological differentiations (G1–G3)

| | *n* | cytokeratin | keratin |
|---|---|---|---|
| G1 | 15 | + – + + | + + |
| G2 | 15 | + + | + + |
| G3 | 20 | + + | + + – + + + |

+: low intensity of staining
++: moderate intensity of staining
+++: high intensity of staining

Table 2. Immunohistochemical identification of tumor-associated antigens in transitional cell carcinomas of the urinary bladder of various histological differentiations (G1–G3)

| | *n* | keratin | cytokeratin | CEA |
|---|---|---|---|---|
| G1 | 15 | 14 (93.3%) | 12 ( 80%) | 3 (20%) |
| G2 | 15 | 14 (93.3%) | 15 (100%) | 6 (40%) |
| G3 | 20 | 19 (95.0%) | 19 ( 95%) | 8 (40%) |

The *monoclonal anti-Ca1 antibody* was supplied by Wellcome (London, UK) (1 : 200 dilution).

We obtained the second antiserum (peroxidase-conjugated goat F $(ab)_2$ anti-mouse IgG antibody) from Medac (Hamburg, FRG) (1 : 50 dilution).

Aminoethylcarbazol (AEC) was used to identify the peroxidase. We obtained *positive controls* by incubating squamous cell carcinomas of the skin and colon. The *negative controls* were prepared by replacing the primary antiserum by Tris buffer.

*Polyclonal Antibodies*

*Anti-keratin* (1 : 200 dilution), anti-CEA (1 : 200 dilution) and anti-PSA (1 : 400 dilution) were supplied by Dakopatts (Hamburg, FRG). *Anti-PAP* was provided in kit form by Immulok (Santa Barbara, USA). The *second antibody* (swine-anti-rabbit, Dakopatts) was diluted 1 : 60 and the peroxidase-antiperoxidase complex (Dakopatts). 1 : 60.

The peroxidase was identified using aminoethylcarbazol (AEC). We prepared positive controls by incubating epidermis, carcinoma of the colon and normal prostate tissue and *negative controls* by replacing the primary antiserum with Tris buffer.

Table 3. Semiquantitative immunohistochemical staining of cytokeratin and keratin in squamous cell carcinomas of the urinary bladder

| E-Nr. | cytokeratin | keratin |
|---|---|---|
| 2680/79 | + | + + + |
| 10596/80 | + – + + | + + |
| 7774/82 | + | + + + |
| 11447/82 | + | + + + |
| 2205/83 | + – + + | + + + |
| 3910/83 | + – + + | + + + |
| 9530/83 | + – + + | + + + |

+: low intensity of staining
++: moderate intensity of staining
+++: high intensity of staining

## Results

*Transitional Cell Carcinoma*

*Keratin and the cytokeratin demonstrated with the aid of monoclonal antibodies proved to be the most reliable tumor markers* (Table 1a). 14 of the 15 G1 carcinomas were positive for keratin and 12 for cytokeratin. Both markers were detected in the 15 G2 carcinomas, in 14 and 15 cases respectively. 19 of the 20 G3 tumors were positive for keratin and 19 for cytokeratin (Figs. 1a and 1b). *The intensity of the keratin and cytokeratin staining increased with increasing dedifferentiation* (Table 1b).

It was less often possible to demonstrate keratin with monoclonal antibodies than with polyvalent antisera and the staining was not as strong.

We were able to identify *CEA* in transitional cell carcinomas. *The incidence of CEA-positive cells was higher at higher degrees of anaplasia* (Table 2). It was striking that in several cases only single cells whose HE staining did *not* differ from that of the other tumor cells were positive Figs. 2a and 2b).

*Ca1* was *not* demonstrated in *any* of the urothelial carcinoma cells.

The prostate-specific epithelial markers PSA and PAP were also not identifiable.

*Squamous Cell Carcinomas*

*Keratin proved to be the most reliable marker* (Table 3). *All 7 samples showed strong positive cytoplasmic staining* (Figure 3a). The cytokeratin identified with monoclonal antibodies showed a positive reaction in all cases, however, the staining was distinctly weaker (Fig. 3b and Table 3).

## Discussion

No specific markers have yet been found for carcinoma of the bladder.

Numerous clinical investigations and studies in pathological anatomy have been conducted in order to define the prognostic value of *CEA* [4, 6, 8, 9, 11, 12, 16–24, 26, 28, 29, 32, 35, 38, 39, 41, 42, 44, 45]. This oncofetal antigen is normally found in the epithelial cell membranes in the gastrointestinal tracts of fetuses. Although in isolated cases it is also detectable in healthy adults CEA is mainly found in adenocarcinomas of endodermal origin [10, 13, 14]. Since the bladder is also of endodermal origin in 1972 Hall et al. [17] used the determination of urinary CEA as a method of screening and follow-up in cases of transitional cell carcinoma.

In addition to being detectable by means of raised CEA levels in the serum and urine the antigen can also be identified immunocytochemically in tumor cells using urine exfoliative cytology and in histological sections [15, 41]. *In the material we investigated only 34% of the bladder carcinomas were positive for CEA.* Reports in the literature fluctuate between 11% [15] and 57% [24]. We found that CEA staining was stronger in dedifferentiated Grade 2 and 3 carcinomas than in Grade 1 tumors. In contrast Shevchuk et al. [35] found a decrease in CEA intensity with increasing anaplasia.

However, the following facts must be taken into account when comparing our histochemical findings with the urinary CEA values reported by other authors: with the immunoperoxidase technique it is possible to identify CEA in specific single cells (Fig. 2b) and determine quantitative differences in CEA content [35]. Urinary levels of CEA, on the other hand, are affected by several variables:

1. the quantity of the CEA produced by individual urothelial cells;

2. the total number of cells producing CEA;

3. the cell regeneration rate;

4. both the concentration in the urine and the intervals between micturation times.

*In this study keratin and cytokeratin proved to be the most reliable markers of bladder carcinoma. The origins of dedifferentiated tumors are particularly difficult to determine conclusively on the basis of clinical and histological indicators and this is where the detection of keratin is an additional pointer in the direction of primary urothelial carcinoma of the bladder.* Schlegel et al. [33, 34] were able to identify keratin immunohistochemically in normal cell layers on squamous epithelium, ductal glandular epithelium and in the epithelium of the respiratory and urinary systems. Recently Ramaekers et al. [30] were able to detect keratin in 64 different adenocarcinomas of varying histogenesis. This marker is also found in areas of squamous metaplasia in various organs [33, 34]. In our study we were able to identify keratin in metaplastic squamous epithelium from poorly differentiated prostate carcinomas [36].

*In the squamous cell carcinomas of the bladder we investigated in this study keratin exhibited a strong reaction in the cytoplasm of the tumor cells* (Fig. 3a). Our results show that *it is less often possible to detect keratin with monoclonal than with polyclonal antibodies.* This is understandable, since, owing to their high specificity, although monoclonal antibodies can be used to identify specifically the smallest components of antigens they are not as efficient as the polyvalent antisera in detecting the whole antigen [27]. *We were not able to demonstrate the Ca1 antigen with the monoclonal antibody Ca1 first described in 1982, which is reported to occur in malignant tumors [5, 25], in any of our samples.* As expected it was not possible to identify the prostate-specific epithelial markers PAP and PSA in any of the transitional cell carcinomas.

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