Urol Res (1987) 15:191–194

Urological Research

Tissue Polypeptide Antigen (TPA), Some Cytokeratins and Epithelial Membrane Antigen (EMA) in Normal, Inflamed and Malignant Urothelium

B. E. Carbin, V. P. Collins and P. Ekman

Departments of Urology and Tumor Pathology, Karolinska Hospital, Stockholm, Sweden

Accepted: January 1, 1987

Summary. The distribution of tissue polypeptide antigen (TPA), epithelial membrane antigen (EMA) and various cytokeratins was studied with immunohistochemical techniques in malignant, normal and inflamed urothelium. TPA and cytokeratins 8, 18 and 19 were similarly distributed and there was no difference between benign and malignant conditions. All keratins were most abundant in the outer cell layers of the urothelium. EMA was present primarily in the so called umbrella cells. Increased levels of TPA in urine and serum are found in patients with urothelial carcinoma. TPA is reported to be related to the cytoskeletal keratins and its presence definitely does not imply malignant transformation as we have demonstrated it in both benign and malignant urothelium. The increased levels of TPA in urine and serum are probably due to a more rapid turnover and autolysis of cells and may parallel a more aggressive tumor behaviour.

Key words: Bladder cancer, Tumor markers, TPA, Cytokeratin.

Introduction

Histopathology alone offers a crude assessment of the aggressiveness of a tumor. Well differentiated cancer of the bladder usually follows a benign course and rarely becomes invasive whereas poorly differentiated cancers rapidly progress with early invasion [5]. The clinical course of the intermediate group, moderately well differentiated cancer, is far more variable and unpredictable. The tumor cell DNA-content as measured by flow-cytometry has given additional information of prognostic value [7]. The loss of ABO(H)-antigen on the tumor cell has also been correlated to aggressive tumor behaviour [9].

Despite the prognostically useful information obtained from these methods the identification of a substance signaling tumor recurrance prior to the development of clinically evident disease is awaited. The ideal would be the presence in serum and urine of a readily measurable specific marker substance reflecting the recurrence of the tumor. So far, no tumor markers have been found specific for transitional cell carcinoma.

Tissue polypeptide antigen (TPA) was first isolated in 1957 from "membranes" of various human carcinomas [2]. TPA is a nonspecific tumor marker and serum content of TPA has been found to be elevated in a number of different malignant conditions [3]. Also several "benign" disorders like inflammatory bowel disease and autoimmune disorders occasionally induce a rise in circulating TPA [10, 11]. Moreover, the number of false "negative" cases in malignant conditions is high, and, therefore, its value for screening of absence or presence of cancer is limited.

More recently, elevation of TPA in serum has been reported to parallel progression of urothelial cancer [1], and as such may reflect the aggressiveness of an individual tumor. In addition Lewenhaupt and collaborators found a highly significant difference in survival rates in patients with prostatic carcinoma; the prognosis being more favorable when the serum content of TPA was normal at the time of diagnosis [8]. A clear relation between urinary content of TPA and the clinical course of human bladder cancer has been found. In our own laboratory, we have recently also been able to confirm a correlation between TPA levels in voided urine and progression of bladder carcinoma [4].

The purpose of the present study was to compare the tissue distribution of TPA in normal, inflamed and malignant urothelium. The urothelial carcinomas were of varying histological grades of malignancy.

As TPA has been reported to be related to the cytoskeletal keratins it was of interest to compare its distribution with a number of cytokeratins. Epithelial membrane antigen (EMA) which is an epithelial marker was also included in the study.

Materials and Methods

Biopsies from the bladder mucosa of 32 patients were studied. From some of the patients repeated biopsies were taken; alogether 40 biopsies were included in the series. 14 of these contained malignat

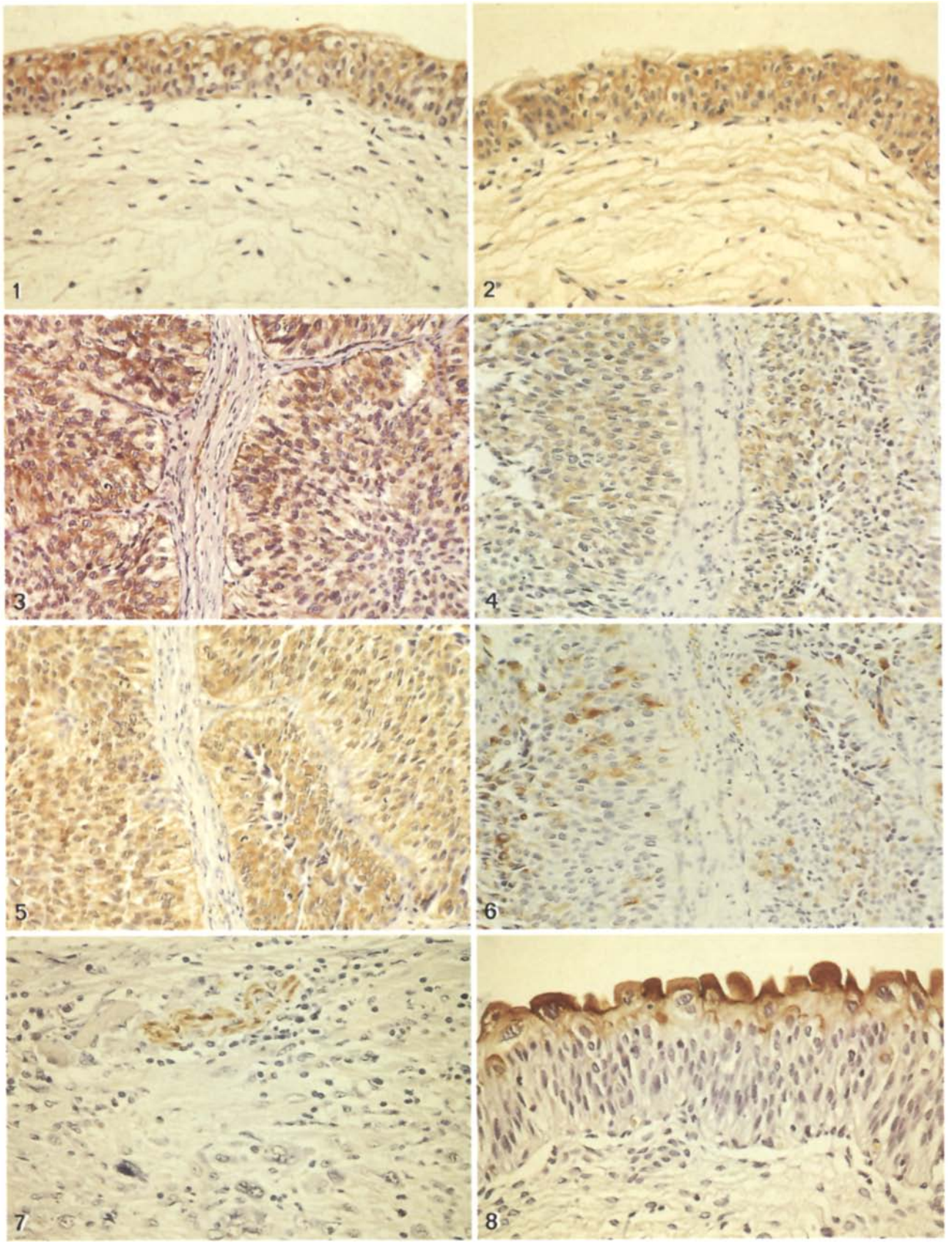

Fig. 1. Normal urothelium showing immunoreactivity for TPA in all layers, particularly the umbrella cells. The section has not been blocked for endogenous peroxidase as can be seen by the stained red blood cells. Control sections showed no evidence of endogenous peroxidase activity in the urothelium (x50)

Fig. 2. The same specimen as shown in Fig. 1 but immuno-histochemistry using the monoclonal antibody to Cytokeratin (recognises keratin nos. 8, 18 and 19). Note the similarity to Fig. 1 in distribution of the reaction product in the urothelium (x50)

Table 1.

Antiserum/antibody	Normal	Inflammatory	Cancer-oumatous
TPA (Sangtec, Bromma)	12/12	14/14	13/14
Cytokeratin (Labsystem)	12/12	14/14	13/14
Keratin Z 655 (Dako)	12/12	14/14	14/14
Keratin A 575 (Dako)	11/12	02/14	14/14
EMA M 613 (Dako)	12/12	12/14	14/14
NRIF (Dako)	00/12	00/14	00/14
Tp-NFP1A3 (Zheng & Collins)	00/12	00/14	00/14

urothelium. Of these 6 were classified as WHO grade I, 4 as grade II, 3 as grade III and one carcinoma in situ. Twelve specimens of normal urothelium served as controls and another 14 specimens were studied from patients with cystitis. Formalin fixed specimens were embedded in paraffin (64 °C melting point). Serial sections were cut and placed on slides. A minimum of two sections was placed on each slide, one of which was counterstained with Haematoxylin after the immunocytochemical procedure. The sections of 4 μm were dewaxed and hydrated. Nonspecific antigen sites were blocked using a 10 min incubation with 5% Bovine serum albumin in PBS (Phosphate Buffered Saline). After a rinse the sections were incubated with the antisera/antibodies for 12–18 h. All sections were incubated with one of the following:

1. Rabbit antiserum to TPA (Sangtec, Bromma, Sweden).

2. Monoclonal antibody (IgG_1) to Cytokeratin (Labsystem OY), recognizes keratins 8, 18 and 19 according to the nomenclature of Moll et al. [12].

3. Rabbit antiserum to Keratin Z 622 (Dako Inc.), animal immunized with bovine muzzle epidermal keratin. The product information states that the antiserum recognizes keratins of 58, 56 and 52 kD; less abundant 60, 51 and 48 kD. These probably correspond to keratins 5, 6 and 8; 4, 14 and 16 according to the nomenclature of Moll et al. [12].

4. Rabbit antiserum to Keratin A 575 (Dako Inc.), animal immunized with human stratum corneum keratins. The products information states that the antiserum recongizes keratins of molecular weight 56 and 64 kD. These probably correspond to keratins 6 and 9 according to the nomenclature of Moll et al. [12].

5. EMA (Epithelial Membrane Antigen) M 613 (Dako Inc.). A coctail of two monoclonal antibodies to EMA.

6. NRIF (Normal Rabbit Immune Fraction) (Dako Inc.).

7. A monoclonal antibody Tp-NFP1A3 (IgG_1) to NFP (Neuro Filament Protein, 200 kD) forms a control for the IgG_1 monoclonals to keratin and EMA (Zheng and Collins 1985).

Sections incubated with TPA, Z 622, A 575 and NRIF were, following rinsing 3 x 10 min in TBS (Tris Buffered Saline), incubated in biotinised goat-anti-rabbit IgG antiserum and then the procedure was continued according to the Vectastain Avidine Biotine Complex protocol (Vectastain®).

Sections incubated with IgG_1 to Cytokeratin were treated in a similar manner with the exception that biotinised goat-anti-mouse IgG antiserum (Vectastain®) was used as the second layer.

In sections incubated with EMA and NFP were, following rinsing, exposed to peroxidase conjugated goat-anti-mouse IgG (Dako) which was followed by peroxidase conjugated sheep-anti-goat Ig antiserum. All specimens were then developed using Diaminobenzidinetetrahydrocloride (DAB) as the chromogen.

Controls without reagents were included for each of the immunohistochemical steps and they gave appropriate negative results. As immunohistochemical enzyme technique can only be assessed semiquantitatively under strictly standardised conditions, no attempts were made to separate different intensities of staining. Only qualitative assessments are reported.

Results

The antibodies denoted TPA, Cytokeratin, Keratin Z 622 and A 575 recognized antigen in the normal, inflamed and neoplastic urothelium of all specimens but one. The blocks from this tumor consisted mainly of necrotic material and many sections contained no truly well preserved tissue. Otherwise the brown reaction product was seen in the cytoplasm of most but not all urothelial cells. There was no specific pattern of distribution for the normal, neoplastic or inflamed urothelium (Figs. 1–3).

TPA and Cytokeratin-antibodies recognized antigens with a similar and broad distribution in all specimens. All urothelial cells, whether normal, neoplastic or inflamed contained these antigens.

The keratin antibodies Keratin A 575 recognized antigens in all cancer specimens and in most specimens from normal urothelium. The most positive cells were found in the superficial layers of the normal and neoplastic urothelium, the umbrella-cells being particularly positive. Only two specimens of the fourteen cases with inflamed urothelium showed positivity for this antibody (Fig. 4). The antigens recognized by Keratin Z 622 were present in practically all urothelial cells giving a clearcut cytoplasmic reaction product (Fig. 5). EMA, a cell-surface antigen, was found mainly on the umbrella-cells. The two EMA-negative cases in the inflammatory group did not have any umbrella-cells in the sections studied (Fig. 6).

NRIF served as a negative control for the antiseras and NFP was used as a negative control for the IgG_1 monoclonal antibodies with the advantage that it recognized all nerve

◀ **Fig. 3.** Immunoreactivity for TPA in a urothelial cancer grade 3 (x50)

Fig. 4. Same area of same cancer (see Fig. 3) showing immunoreactivity for Cytokeratin (recognises keratin nos. 8, 18 and 19 (x50)

Fig. 5. As Figs. 3, 4 showing immunoreactivity for the polyclonal antiserum K 622 (probably recognises keratin nos. 4, 5, 6, 8, 14 and 16) (x50)

Fig. 6. As Figs. 3–5 showing immunoreactivity for EMA (x50)

Fig. 7. Immunoreactivity for EMA in normal urethelium, note the strong reaction product in the umbrella cells (x50)

Fig. 8. As Figs. 3–6. A control slide with a nerve showing immuno-reactivity for Neurofilament Proteins. Note the absence of reaction product in the surrounding tissue. Slight nuclear staining (x50)

branches and made recognition of nerve invasion simple (Fig. 7). Both of the latter did not stain urothelium following the immunocytochemical procedure.

Discussion

The immunohistochemical technique used does not permit any quantitation of TPA and therefore nothing can be said quantitively about the TPA content of the cells.

Normal urothelium contains cytokeratins No 7, 8, 19 and in smaller amounts 5, 13 and 18 [12]. As reported by Weber et al. [14] TPA seems related to keratins 9, 18 and 19. This study would confirm these observations. If the product information is correct and also our assumption that antiserum A 575 (Dako Inc.) *only* recognizes keratins 6 and 9 it would seem that these keratins appear during terminal differentiation in the outer layers of the urothelium. However, one must be very careful in drawing conclusions of this sort on the basis of studies with antisera of uncertain character but the brown reaction product was seen mainly in the superficial layer of the urothelium and that layer was not present in specimens with inflammatory urothelium.

The present study failed to demonstrate any difference in the distribution of TPA in normal, inflammatory and carcinomatous urothelium.

The data presented herein shows a striking resemblance between TPA and cytokeratin with regard to the immunohistochemical distribution patterns.

The fact that TPA was seen in both normal and cancerous urothelium shows that this is in no way a specific marker for neoplastic urothelial cells. The elevated TPA-levels in serum and urine thus require some explanation. TPA seems to be related to the cytoskeletal keratins and therefore less likely to be excreted from the cell. The urothelial cells proliferate at the basal membrane and then become post-mitotic as they differentiate and move superficially. Finally they are desquamated and are lost into the urine. Where there is a large cellular turnover the amounts of autolytic urothelial products found in the urine will increase. If the urothelial carcinoma cells are invasive on the other hand, the urothelial cells disintegrate in the tissue, and cannot be lost into the urine. The autolysed cellular components are thus retained in the body resulting in elevated serum-TPA in patients with invasive bladder cancer. Elevated serum levels of TPA are also seen in patients with hepatitis, Crohns disease, ulcerative colitis [3] thus in all conditions which include a degree of epithelial degeneration.

The same type of cytokeratins that have been found in the urothelium have also been identified in cells from the liver, small intestine and colon [12].

Thus despite the lack of differences in the distribution of TPA in normal vs cancerous urothelium, TPA forms a useful marker. Elevated levels of TPA in serum and/or in urine in patients must be explained and recurrence of urothelial carcinoma must be excluded.

The intermediate filament (IF) of many celltypes are built up by specific IF protein combinations and during cell transformation and tumor development this specificity if often preserved [6]. In order to give a more exact histological diagnosis, analysis of the IF protein pattern can be a valuable parameter [13]. If, in the future, it is possible to produce monoclonal antibodies specific for each cytokeratin, analysis of the pattern of the elevated cytokeratin-levels could give us more specific information.

Acknowledgements. Supported by Swedish Cancer Society, Stockholm's Cancer Society and The Maud & Birger Gustavssons Foundation.

References

1. Adolphs HD, Oehr P (1984) Significance of plasma tissue polypeptide antigen determination for diagnosis and follow-up of urothelial bladder cancer. Urol Res 12:125–128
2. Björklund B, Björklund V (1957) Antigenicity of pooled human malignant and normal tissues by cyto-immunological technique: presence of an insoluble, heat-labile tumor antigen. Int Arch Allergy 10:153–184
3. Björklund B (1980) On the nature of clinical use to tissue polypeptide antigen (TPA). Tumor Diagn 1:9
4. Carbin BE, Eneroth P, Ekman P (1986) Significance of urine-TPA (Tissue Polypeptide Antigen) as a prognostic marker for urothelial carcinoma (submitted for publication)
5. Collan Y, Mäkinen J, Heikkinen M (1979) Histological grading of transitional cell tumors of the bladder. Value of histological grading (WHO) in prognosis. Eur Urol 5:311–318
6. Franke WW, Eber K, Osborn M, Schmid E, Freundstein C (1978) Antibody to prekeratin. Cecoration of tonofilament-like arrays in various cells of epithelial character. Exp Cell Res 116:429–445
7. Gustafson H, Tribukait B, Esposti PL (1982) DNA pattern, histological grade and multiplicity related to recurrance rate in superficial bladder tumors. Scand J Urol Nephrol 16:135
8. Lewenhaupt A, Ekman P, Eneroth P, Nilsson B, Nordström L (1985) TPA-S, prognostic aid in human prostatic carcinoma. Prostate 6:285–291
9. Limas C, Lage PH, Fraley EE, Vessela R (1979) A, B, H antigens in trasitional cell tumors of the urinary bladder. Cancer 44:2099
10. Lundström R, Björklund B, Eklund G (1973) A tissue-derived polypeptide antigen: its relation ot cancer and its temporary occurrance in certain infectious disease. In: Björklund B (ed) Immunological techniques for detection of cancer. Bonniers, Stockholm, p 243
11. Menendez-Botet CJ, Oettgen HF, Pinsky CM, Schwartz A (1978) A preliminary evaluation of tissue polypeptide antigen in serum or urine (or both) of patients with cancer or benign neoplasms. Clin Chem 24:868
12. Moll R, Franke WW, Schiller DL (1982) The catalogue of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24
13. Ramaekers FCS, Puts JJG, Moesker O, Kant A, Huysmans A, Haag D, Jap PHK, Herman CJ, Vooijs GP (1983) Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumors: An overview. Histochem J 15:691–713
14. Weber K, Osborn M, Moll R, Wiklund B, Lüning B (1984) Tissue polypeptide antigen (TPA) is related to the non-epidermal keratins 8, 18 and 19 typical of simple and non-squamous epithelia: re-evaluation of a human tumor marker. EMBO J 3:2707–2714

B. E. Carbin, M.D.
Department of Urology, Karolinska Hospital, Box 60500
S-10401 Stockholm, Sweden