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High Expression of Human Uroplakin Ia in Urinary Bladder Transitional Cell Carcinoma

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Uroplakins (UPs) Ia, Ib, II, and III are tissue-specific and differentiation-dependent transmembrane proteins of the urothelium. We assessed the usefulness of human UP Ia as a histological marker by examining its expression in urinary bladder transitional cell carcinoma (TCC). A polyclonal antibody against human UP Ia was raised using a synthesized polypeptide. We applied our antibody to various organ tissues, including urothelium, and observed no crossreactivity. Analysis by RT-PCR of normal urothelium, TCC and other organ tissues indicated that the human *UP Ia* gene expression is highly specialized to urothelium, and is conserved in TCC. Using immunohistochemistry, we investigated the expression of UP Ia in TCC from patients who had undergone radical cystectomy and from autopsy cases. Positive staining (10% or more positive cancer cells) was noted in primary lesions from 61 of 63 (96.8%) cystectomy patients. Depending on pathological grade, high expression (50% or more positive cancer cells) was observed in 17 of 18 (94.4%) moderately- to well-differentiated TCC and in 36 of 45 (80.0%) poorly differentiated TCC. With regard to tumor invasion, high expression was noted in 20 of 22 (90.9%) superficial and 33 of 41 (80.5%) muscle-invasive TCC. Cause-specific survival rates were 68.6% and 75.0% in high- and low-expression patients, respectively (log-rank test, *P*=0.855, mean follow-up; 65.0 months). In metastases, positive reactions were observed in 13 of 18 (72.2%) lesions. UP Ia may represent a specific histological marker judging from the stable expression, although its value as a prognostic factor remains undetermined.

Key words: Uroplakin Ia — Bladder cancer — Immunohistochemistry

Bladder cancer is a common urological neoplasm in many countries. For example, in the United States and Japan, there are approximately 53 200 and 10 600 newly diagnosed cases per year, respectively.[1)§] Transitional cell carcinoma (TCC) represents the majority (75–90%) of bladder cancer, with squamous cell carcinoma (2–15%), mixed carcinoma (4–6%) and adenocarcinoma (<2%) comprising the remainder of cases.[2)] Histopathological diagnosis of TCC metastases mainly relies on the morphological features of specimens. When tumors are poorly differentiated or obtained specimens are very small, diagnosis becomes complicated. The establishment of antibodies that react specifically to TCC would be valuable.

Since the 1960's it has been known that the specific structure of the urothelial apical surface is an asymmetric unit membrane (AUM). However, despite much effort, AUM extraction was not accomplished until 1990, when Yu *et al.* succeeded in purifying AUM proteins using a monoclonal antibody (AE31) against bovine bladder epithelium plasma membrane.[3)] They designated these proteins as uroplakins (UPs). Four integral proteins were found, UPs Ia (molecular weight, 27 kDa in non-reduced SDS-PAGE), Ib (28 kDa), II (15 kDa) and III (47 kDa). Immunohistochemical studies revealed that UPs are highly specific for mammalian urothelium; they are present in the apical surface membrane of umbrella cells and are considered as differential antigens. It has been hypothesized that the role of UPs is to protect against bladder wall distention.

Recently, we determined the nucleotide sequences of human UPs Ib and III using a PCR-based method.[4)] We also identified *UP* gene-expressing cancer cells in the peripheral blood of patients with metastatic TCC by nested RT-PCR and suggested the possibility that UPs might be TCC-specific markers.[4–6)]

Several monoclonal or polyclonal anti-bovine UPs II and III antibodies have been raised, and immunohistochemical studies were performed.[7–10)] The expression of UPs II and III was observed not only in normal transitional cells, but also in TCC.[7–9)] However, anti-human UP Ia antibodies have not been produced, nor has UP Ia expression been examined in TCC. Therefore, its clinical usefulness remains unknown.

In the present study, we produced a polyclonal antibody against human UP Ia and assessed its clinical significance by examining expression in TCC specimens.

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[§] http://www.ncc.go.jp/jp/statistics/1999/index_i.html (The last date of access was November 1st 2001.) Cancer Statistics in Japan 1999. The Foundation for Promotion of Cancer Research.

MATERIALS AND METHODS

Patients Urinary bladder TCC tissues were obtained from 63 patients according to institutional guidelines. All had undergone radical cystectomy and simultaneous bilateral pelvic lymph node dissection at the Department of Urology of Shiga University of Medical Science between 1979 and 1995.[11] Clinical and pathological features are shown in Table I. For statistical analysis, we divided the patients into two categories according to histological grading: those with a well- to moderately-differentiated tumor (18 patients, grades 1–2) and those with a poorly differentiated tumor (45 patients, grade 3). We also divided the patients into two other categories by depth of invasion: superficial tumor (22 patients, Ta-1) and muscle-invasive tumor (41 patients, T2-4). The extent of disease was classified according to TNM staging criteria (4th Ed.).[12] Pelvic lymph node metastases were confirmed pathologically with dissected specimens; 9 patients had lymph node involvement. Fifteen patients underwent cisplatin-based chemotherapy as pre- or post-operative adjuvant therapy. No patient had neoadjuvant irradiation. The follow-up period ranged from 1 to 186 months (mean±standard deviation [SD] 65.0±48.1 months; median 56). Fifteen of the 63 patients died of disease, and the cumulative cause-specific survival rate was 69.7%.

Eighteen metastatic tissue samples were obtained from 5 patients who had undergone lymph node dissection and 9 autopsy cases who had died of systemically advanced TCC. The sites of metastases were pelvic lymph nodes (n=6), lung (4), liver (4), bone marrow (2), pancreas (1) and colon (1).

Tissues were appropriately processed immediately after surgery or autopsy and embedded in paraffin blocks.

Synthesized polypeptide Based on DNA sequence data of human UP Ia registered in GenBank (Lamerdin, AC002115 and our original data[5]), we synthesized a polypeptide corresponding to amino acid residues 141 to 152 (DQGQELTRLWDR). This amino acid sequence is located in the extracellular loop domain and has no similarity to any other sequence in the protein database except that of bovine UP Ia.

Generation of antibody We raised a rabbit polyclonal antibody using the synthetic UP Ia polypeptide as the immunogen. Immunization was performed by injection of keyhole limpet hemocyanin-conjugated polypeptide six times, followed by one booster injection of free polypeptide.

Immunohistochemistry Immunohistochemical staining was performed by the streptavidin-biotin-peroxidase complex method (Histofine SAB-PO(R) kit; Nichirei, Tokyo) as described previously.[13] It should be noted that the specimens were incubated with 0.1% trypsin (Nacalai Tesque,

Table I. Patients' Characteristics and Statistical Analysis of UP Ia Expression in Primary Bladder TCC Tissue and Clinicopathological Features

		Total (n=63)	UP Ia high-exp. (n=53)	UP Ia low-exp. (n=10)	P value
Age	mean (SD)	65.1 (9.7)	65.6 (9.6)	62.5 (10.2)	0.358
Sex	male	51	42	9	0.671
	female	12	11	1	
Configuration	papillary	18	17	1	0.257
	non-papillary	45	36	9	
Number of tumors	solitary	31	25	6	0.509
	multiple	32	28	4	
Grade	well-mod.	18	17	1	0.257
	poor	45	36	9	
Depth of invasion	superficial	22	20	2	0.472
	invasive	41	33	8	
Lymph node involvement	neg.	54	46	8	0.626
	pos.	9	7	2	
Lymphatic invasion	neg.	16	13	3	0.705
	pos.	33	28	5	
	unknown	14	12	2	
Venous invasion	neg.	40	34	6	0.628
	pos.	9	7	2	
	unknown	14	12	2	
Follow-up (mo.)	mean (SD)	65.0 (48.1)	65.2 (47.8)	64.3 (52.3)	0.957

Inc., Kyoto) in 0.01 *M* phosphate-buffered saline (PBS) at room temperature for 60 min for the purpose of antigen retrieval after deparaffinization and rehydration. The slides were incubated with anti-UP Ia polyclonal antibody diluted 1:200 at room temperature for 60 min.

To determine organ specificity, our antibody was tested not only on normal bladder tissues, but also on those of various organs (n=27), including the heart, lung, stomach, small intestine, colon, liver, pancreas, spleen, kidney, thyroid, adrenal gland and prostate. To investigate the expression of UP Ia in primary bladder TCC and metastatic lesions, we applied the antibody to specimens according to the above procedure.

RT-PCR To examine the expression of the *UP Ia* gene we performed RT-PCR for several tissues. Total RNA was obtained from normal bladder urothelium, TCC tissue of cancer patient and other organ tissues (brain, heart, lung, liver, kidney, appendix, testis, ovary and peripheral blood lymphocytes) of non-TCC patients. Single-strand cDNA was synthesized from 5 μg of total RNA with 20 U of RAV-2 reverse transcriptase (Takara Biochemicals, Kyoto) using random primers. We also synthesized the sense and antisense primers for human UP Ia mRNA as described by Yuasa *et al.*: 5′-ACGTCCTACACCCACCGTGA-3′ and 5′-ACCCCACGTGTAGCTGTCGAT-3′, respectively.[5] Sense and antisense primers for GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA were used as controls: 5′-GGATTTGGTCGTATTGGGCGCCT-3′ and 5′-AGTGAGCTTCCCGTCTAGCTCAG-3′, respectively.[13] The PCR reaction included 0.4 μl of template cDNA, 2.5 U of KOD dash polymerase (Toyobo Biochemicals, Osaka), 1 m*M* $MgCl_2$, 10 m*M* KCl, 6 m*M* $(NH_4)_2SO_4$, 0.1% Triton X-100, 10 μg/ml bovine serum albumin, 0.2 m*M* each of deoxynucleotide triphosphates and 4 pmol of primers in 20 μl of 120 m*M* Tris-HCl buffer (pH 8.0). Amplification was performed with 30 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 2 s and elongation at 74°C for 30 s. The PCR products were analyzed by electrophoresis on 2% agarose gel with ethidium bromide.

Immunoblotting For immunoblotting, we used recombinant human UP Ia, which was produced by the pET System that was developed for expression of recombinant proteins combined with thioredoxin in *Escherichia coli* based on the T7 promoter-driven system (pET TRX Fusion System 32, Novagen, Darmstadt, Germany). One microgram of the lysate dissolved in sample buffer, including 62.5 m*M* Tris-HCl, pH 6.8, 5% 2-mercaptoethanol, 2% SDS and 10% glycerol, was loaded onto each lane. Proteins were separated by 18% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and then transferred onto a polyvinylidine difluoride membrane (Immobilon PVDF; Millipore Co., Bedford, MA) with transfer buffer containing 25 m*M* tris-base, 192 m*M* glycine, 0.1% SDS and 10% methanol. The membrane was incubated overnight at 4°C in Super Block in TBS (Pierce, Rockford, IL) to block nonspecific binding sites. Anti-UP Ia antibody (diluted 1:10 000) was incubated with the membrane for 1 h at room temperature. Preimmune serum from the same rabbit was used as a negative control. Peroxidase-labeled anti-rabbit IgG goat antibody (Vector Laboratories, Inc., Burlingame, CA) diluted 1:10 000 was applied as the second antibody. The immunoproducts were visualized with 3,3′-diaminobenzidine (DAB) and H_2O_2.

We also performed an absorption test in immunoblot analysis. Recombinant human UP Ia (0.1 and 10 μg) or synthesized polypeptide (10 μg) used as the immunogen was incubated in 100 μl of the anti-UP Ia antiserum

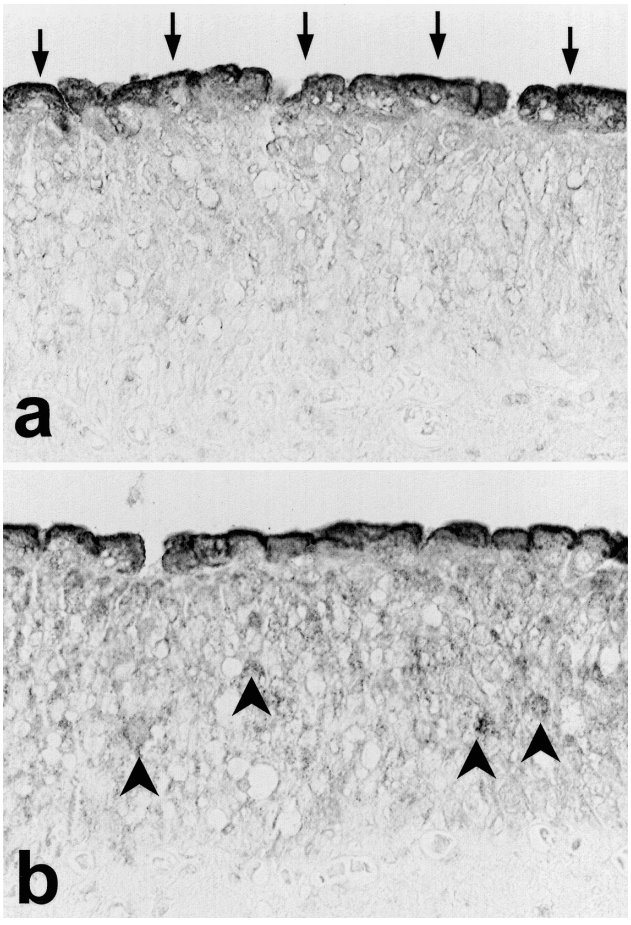

Fig. 1. UP Ia staining in normal transitional cells without nuclear staining. (a) Staining without antigen unmasking. Arrows indicate strong apical membrane in umbrella cells. (b) Staining with antigen retrieval treatment with 0.1% trypsin. Granular stains in the intermediate layer of the epithelium can be seen (arrowheads). ×400.

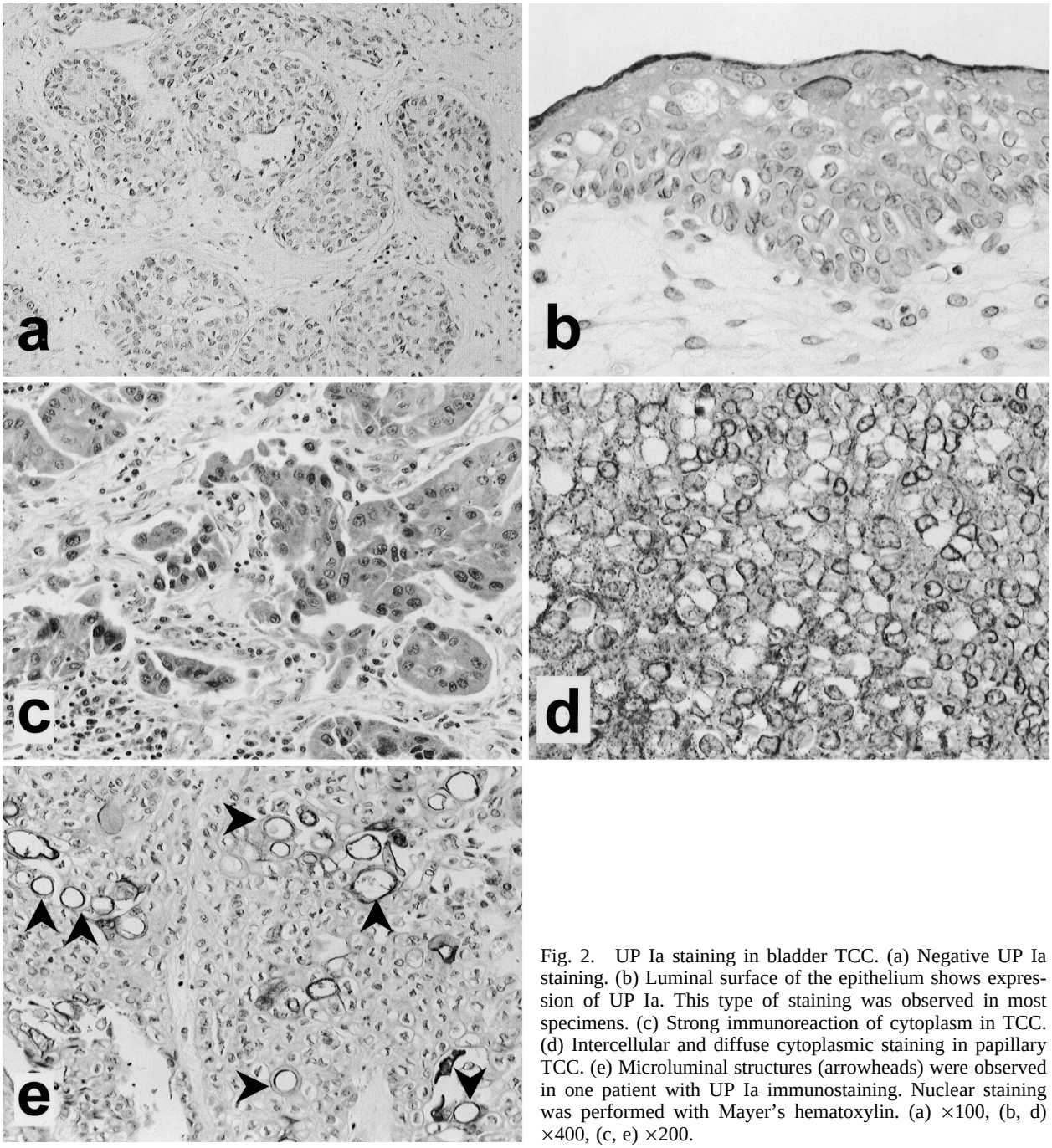

Fig. 2. UP Ia staining in bladder TCC. (a) Negative UP Ia staining. (b) Luminal surface of the epithelium shows expression of UP Ia. This type of staining was observed in most specimens. (c) Strong immunoreaction of cytoplasm in TCC. (d) Intercellular and diffuse cytoplasmic staining in papillary TCC. (e) Microluminal structures (arrowheads) were observed in one patient with UP Ia immunostaining. Nuclear staining was performed with Mayer's hematoxylin. (a) ×100, (b, d) ×400, (c, e) ×200.

(diluted 1:50) for 30 min at room temperature, and then further diluted (1:10 000) and applied to the membranes as the first antibody.

Statistical analysis The relationship of the staining reaction to various clinical parameters was analyzed using Student's *t* test or Fisher's exact probability test (2-sided, *P* value <0.05). Kaplan-Meier analysis was used to estimate the cumulative cause-specific survival rate, and the log-rank test was employed to assess differences in survival distribution for patients with staining intensity of UP Ia immunohistochemistry. For multivariate analysis, the Cox proportional hazards model was used to examine associa-

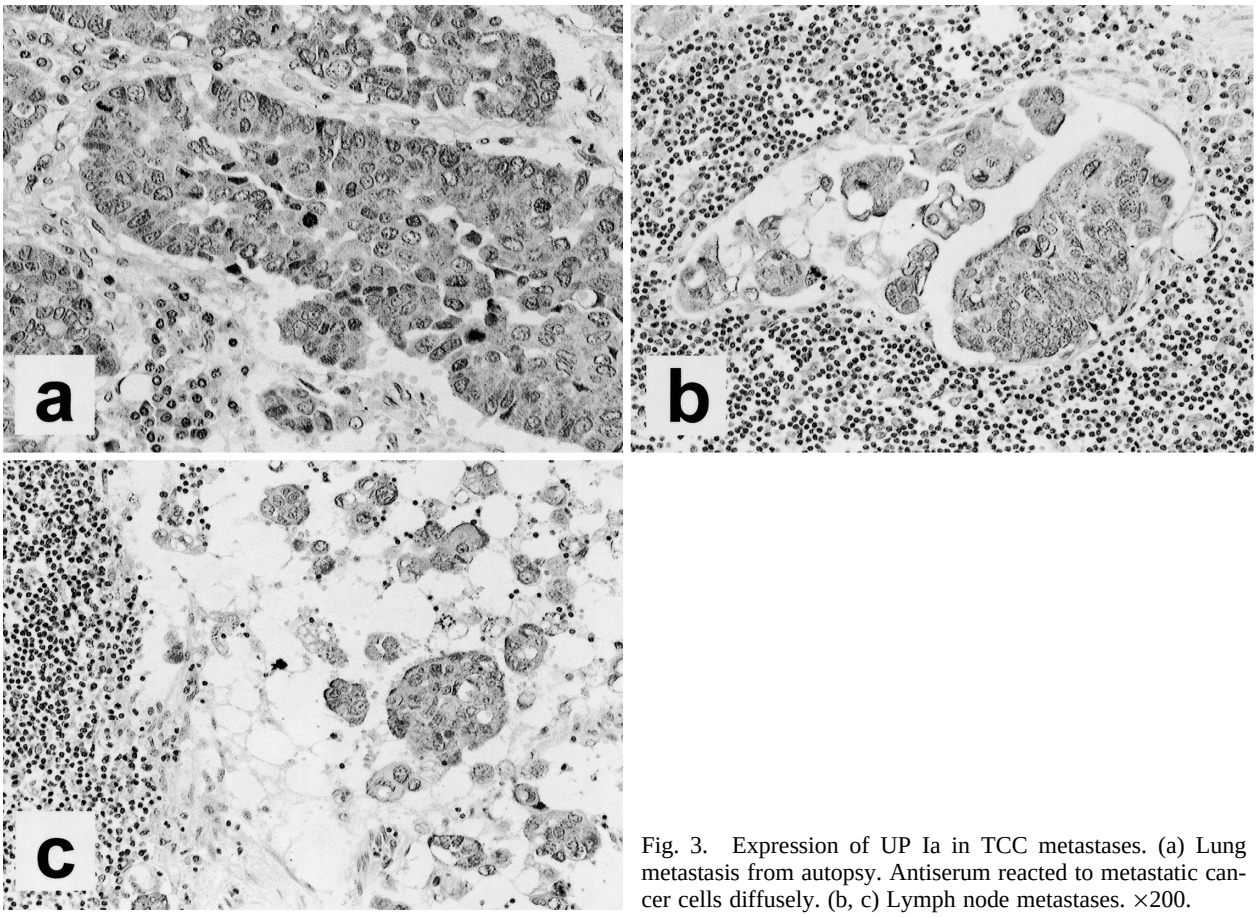

Fig. 3. Expression of UP Ia in TCC metastases. (a) Lung metastasis from autopsy. Antiserum reacted to metastatic cancer cells diffusely. (b, c) Lymph node metastases. ×200.

tions between potential prognostic factors (histological grade, depth of invasion, tumor configuration, number of tumors, lymph node involvement, perioperative chemotherapy and staining intensity of UP Ia) and survival time. We used StatView version 4.5 (Abacus Concepts, Inc., Berkeley, CA) as statistical software.

RESULTS

Organ specificity of anti-UP Ia polyclonal antibody In the normal bladder, without using the antigen retrieval procedure, slides revealed strong apical membrane staining and weak granular cytoplasmic reactivity in umbrella cells (Fig. 1a). Through the use of antigen-unmasking treatment with 0.1% trypsin, the staining intensity became stronger, and a positive reaction was detected not only in umbrella cells, but also in the intermediate layer of the urothelium (Fig. 1b). The heat-induced epitope retrieval procedure had little effect (data not shown).

All other normal organ tissues (n=27), including those from the heart, lung, stomach, small intestine, colon, liver, pancreas, spleen, kidney, thyroid, adrenal gland and prostate yielded no reaction. We, therefore, concluded that our polyclonal antibody was specific for urinary transitional cells.

UP Ia expression in transitional cell carcinoma Tissue sections exhibiting immunostaining in 10% or more tumor cells were classified as having “positive expression,” whereas tissue sections with less than 10% of such cells were classified as having “negative expression.” Among primary lesions, UP Ia was detected in 61 of 63 (96.8%) bladders; negative staining was found in only two specimens (Fig. 2a). In most tissues, markedly linear and continuous positive staining was observed in the superficial membrane of the tumor cells (Fig. 2b). Strong granular staining in the cytoplasm and distinct intercellular space were recognized (Fig. 2, c and d). In one patient, many microluminal structures reacted with anti-UP Ia antibody (Fig. 2e). Of 18 metastases 13 (72.2%) showed positive expression of UP Ia (Fig. 3, a–c).

We defined two subgroups for cystectomy specimens according to the intensity of UP Ia immunoreactivity; tis-

sue of patients in the "high-expression" group revealed positive staining in 50% or more of the cancer cells while those of the "low-expression" group had either a negative reaction or positive staining in fewer than 50% of the cancer cells. There were 53 (84.1%) and 10 (15.9%) patients in the high- and low-expression groups, respectively.

We also applied our antibody to some urological tumors of histological types other than TCC, but they did not show positive staining (data not shown).

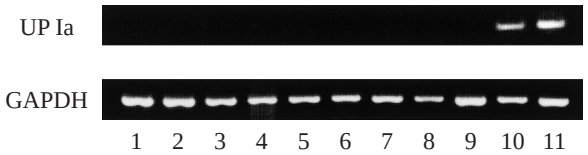

Fig. 4. RT-PCR analysis of human *UP Ia* gene expression in normal urothelium, TCC, and other organ tissues. Expression of GAPDH was used as a control. Lane 1, brain; lane 2, heart; lane 3, lung; lane 4, liver; lane 5, kidney; lane 6, appendix; lane 7, testis; lane 8, ovary; lane 9, peripheral blood lymphocytes; lane 10, normal urothelium; lane 11, TCC.

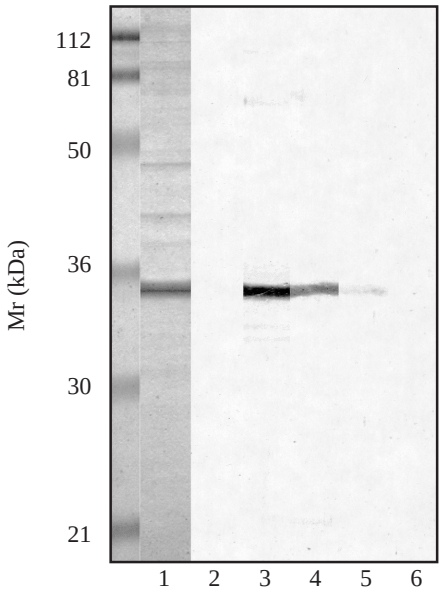

Fig. 5. Immunoblotting. Recombinant human UP Ia protein linked with thioredoxin (approximate molecular weight, 35 kDa) was subjected to reduced SDS-PAGE and immunostained with anti-human UP Ia antibody. Lane 1, SDS-PAGE visualized by Coomassie brilliant blue R-250; lane 2, negative control with preimmune rabbit serum; lane 3, anti-human UP Ia antiserum; lane 4, anti-human UP Ia antiserum absorbed by recombinant human UP Ia (0.1 μg); lane 5, anti-human UP Ia antiserum absorbed by recombinant human UP Ia (10 μg); lane 6, anti-human UP Ia antiserum absorbed by synthetic polypeptide, DQGQELTRLWDR (10 μg).

RT-PCR In RT-PCR analysis of normal urothelium, TCC and other organ tissues, amplified bands corresponding to the human *UP Ia* gene were obtained only from normal urothelial and TCC samples. This result indicates that human *UP Ia* gene expression is highly specialized to urothelium, and is conserved in TCC (Fig. 4).

Immunoblotting Immunoblotting with anti-UP Ia antibody revealed a band of approximately 35 kDa, which corresponded to the recombinant human UP Ia (24 kDa in reduced condition) fusion protein with thioredoxin (11 kDa). In the absorption test with recombinant UP Ia and synthesized polypeptide, the 35 kDa band was weakened in intensity or became undetectable. These findings indicated that our antibody specifically recognized epitopes existing in human UP Ia (Fig. 5).

Relationship between expression of UP Ia and clinicopathological factors in radical cystectomy patients We sought differences in clinicopathological features and survival rates between the high-UP Ia expression and low-expression groups. Information was obtained from medical records and is summarized in Table I. Age distribution and sex ratio were similar in both groups. Histological grade, depth of invasion, tumor configuration, number of tumors, lymph node involvement and microvessel invasion were not statistically significantly different (*P* values for each are shown in Table I).

Cause-specific survival rates of UP Ia high-expression cases (alive, 40; cancer deaths, 13) and low-expression cases (alive, 8; cancer deaths, 2) were 68.6% and 75.0%, respectively (Fig. 6). There were no significant differences between groups by the log-rank test (P=0.855). On the other hand, patients with a muscle-invasive tumor (P=0.034) or lymph node involvement (P=0.001) had a sta-

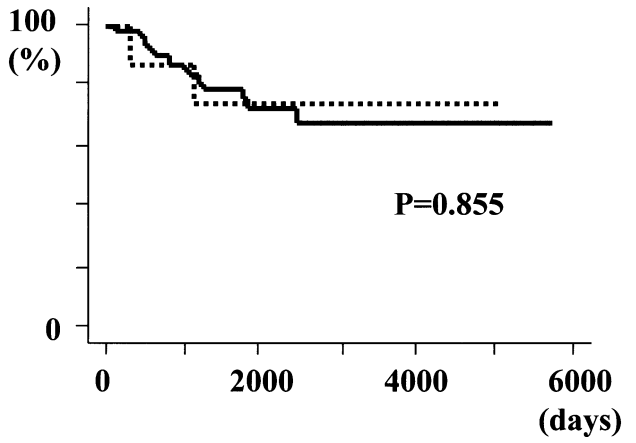

Fig. 6. Cumulative cause-specific survival curves according to UP Ia expression in immunohistochemistry. (---------) low expression of UP Ia, (———) high expression of UP Ia.

Table II. Results of Cox Proportional Hazards Analysis

		Hazard ratio	95% confidence interval	*P* value
Grade	well-mod.	1		
	poor	0.73	0.20–2.69	0.638
Depth of invasion	superficial	1		
	invasive	2.83	0.54–14.85	0.218
Configuration	papillary	1		
	non-papillary	2.7	0.55–13.35	0.224
Number of tumors	solitary	1		
	multiple	1.21	0.41–3.61	0.731
Lymph node involvement	neg.	1		
	pos.	5.08	1.28–20.41	0.021
Perioperative chemotherapy	no	1		
	yes	1.22	0.30–5.02	0.780
UP Ia expression	low	1		
	high	1.69	0.32–9.01	0.536

tistically significantly poorer outcome than those without such factors.

We calculated prognostic factors by means of Cox proportional hazards analysis using histological grade, depth of invasion, tumor configuration, number of tumors, lymph node involvement, perioperative chemotherapy and UP Ia expression intensity (Table II). Lymph node involvement was the only statistically significant prognostic factor, with a hazard ratio of 5.08 (95% confidence interval 1.28–20.41, P=0.021). UP Ia expression intensity did not differ between the two groups.

DISCUSSION

Several antibodies reacting to each of the UP proteins have been produced.[3, 7, 8, 14–16] However, only a small number of antibodies binding to UP Ia have been reported, and all were generated against bovine UP Ia.[3, 14, 15] Therefore, to our knowledge, this is the first report of the establishment of an anti-human UP Ia polyclonal antibody. We chose amino acid residues 141 to 152 (DQGQELTRLWDR) as the immunogen because this sequence is located in the extracellular loop domain and has high specificity for UP Ia. The corresponding amino acid sequence of bovine UP Ia has 83.3% homology and may be preserved in other mammalian urothelium. Wu *et al.* generated two kinds of anti-bovine UP Ia antisera that responded to amino acid sequences 41 to 59 (VIADQYRIYPLMGVSGKDD) and 139 to 152 (DNSQGRELTRLWDR).[15] They found crossreactivity of their anti-bovine UP Ia antisera to urothelium of eight mammalian species (human, monkey, sheep, pig, dog, rabbit, rat and mouse) by immunoblotting and concluded that the UP structure is highly conserved. Although we proved that our antibody had no crossreactivity with many human tissues other than urinary transitional cells, we have not yet examined immunoreactions with other mammalian bladders, including bovine bladder.

Concerning tumor tissue other than TCC, Ogawa *et al.* reported positive staining in ovarian Brenner tumors by immunohistochemical analysis using anti AUM antibody that reacts with all four UPs, and concluded that the Brenner tumor represents urothelial differentiation.[10] We could not confirm immunoreactivity in Brenner tumors because of lack of appropriate specimens, but we observed no crossreaction in several tumor specimens of other histological types, including squamous cell carcinoma and adenocarcinoma (data not shown).

We found that the antigen retrieval procedure using trypsin in paraffin sections was effective for immunohistochemistry of UP Ia. Previous reports have described antigen-unmasking treatments including trypsin and microwaves.[7, 8, 10, 17–22] We also used a heating method employing a microwave oven, but could not achieve stronger staining than that with untreated slides (data not shown). In our study, the immunoreactivity of the intermediate layer in urothelium was enhanced by the antigen retrieval technique. An electron microscopic study by Wu *et al.* on the ultrastructural localization of UPs revealed some small vesicles beneath the luminal surface of umbrella cells.[23] The vesicles had small patches that reacted with UP antibodies, and they speculated that these patches might have been immature UPs. Antigen retrieval treatment might enhance the immunoreactivity for these immature UPs.

Immunohistochemical examination of UP Ia in TCC has not been reported. Our study clarified that TCC, as well as normal transitional cells, expressed UP Ia. Positive stain-

ing in primary cancer lesions was detected in 61 of 63 (96.8%) patients. This positive rate was much higher than formerly reported for UPs II and III.[7–9] Using anti UP II antibodies, Wu *et al.* reported that UP II was detected in 17 of 43 (39.5%) bladder TCCs. Moll *et al.* and Kaufmann *et al.* detected UP III in 43 of 71 (61%) and 21 of 35 (60%) bladder TCCs, respectively. Therefore, UP Ia might be superior to UPs II or III as a histological marker.

UPs were thought to be more frequently detected in well-differentiated than in poorly differentiated TCC as a result of their upregulation upon urothelial differentiation.[24] Moll *et al.* reported positive reactions for UP III in 14 of the 16 (88%) papillary non-invasive TCCs that they examined, which included many differentiated tumors, and 29 of 55 (53%) invasive TCCs, which included a large proportion of high-grade tumors.[7] However, Kaufmann *et al.* reported no difference in positive staining rates for UP III between grade 2 (6 of 11, 54%) and grade 3 (15 of 24, 62%) primary bladder tumors.[8] In our study, we also obtained a greater high-expression rate in well- to moderately-differentiated tumors (17 of 18, 94.4%) than in poorly differentiated tumors (36 of 45, 80.0%), but the difference was not statistically significant (P=0.257). The relationship between the expression of UPs and tumor histological grade remains uncertain.

Our antibody was able to identify cancer cell foci in metastatic lesions; the detection rate was 72.2% (13 of 18 metastases). At present, pathologists base their diagnosis mainly upon the morphological features of TCC metastases and have no helpful antibodies to specify tissues of origin. Our RT-PCR analysis revealed no mRNA expression of the *UP Ia* gene in other organs except normal and malignant transitional cells. This antibody, therefore, may become a powerful tool for identifying metastatic cells of uncertain origin.

Expression of UP Ia was not useful as a prognostic factor. In cause-specific survival analysis, there was no statistically significant difference in UP Ia expression in primary lesions according to the log-rank test and Cox proportional hazards model. Unfortunately, the usefulness of UP Ia as a prognostic factor could not be demonstrated. We speculate that UPs are so highly conserved in TCC as differential products that we could not distinguish between good- and poor-outcome populations. A study employing a larger number of subjects will be required to clarify this issue.

There are at least two interesting possibilities for exploiting our antibody in clinical practice. The first is as a histodiagnostic tool for cancer metastases of uncertain origin, as mentioned above. The second is as an assay system for detection of TCC. The quantity of UP Ia, as well as BLCA-4, in urine may increase in TCC cases owing to the rapid disruption of cancer cells.[25] For urologists and oncologists, whether TCC patients have tumor cells after local (transurethral resection, radical cystectomy, irradiation, and bladder instillation therapy) or systemic treatment is an important issue. We have already proved the existence of TCC cells in peripheral blood using RT-PCR for UPs.[4–6] If an assay for detecting epitopes of UPs in blood or urine is further developed, it may become a very informative clinical test for screening and surveillance of TCC.

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