

# Differential expression of trypsinogen and tumor-associated trypsin inhibitor (TATI) in bladder cancer

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**Abstract.** Tumor-associated trypsin inhibitor (TATI) is a marker of mucinous ovarian carcinoma, but it is also widely expressed in other malignant tumors and normal human tissues. Elevated serum concentrations of TATI are of prognostic value in ovarian, kidney, and bladder cancer. Tumor-associated trypsin is co-expressed with TATI in many malignancies and is thought to be involved in tumor invasion. TATI mRNA has been shown to be overexpressed in bladder cancer. We therefore studied whether trypsinogen expression also can be detected in bladder cancer and how this and TATI expression are associated with the clinicopathological characteristics of the tumors. We used RT-PCR, *in situ* hybridization and immunohistochemistry to detect trypsinogen- and TATI mRNA and protein in tissue samples from 28 bladder cancer patients and ten benign urothelia. TATI expression was detected in all benign tissues and non-invasive tumors. However, the expression was lower in the muscle-invasive tumors (pT2; n=5), whereas trypsinogen expression was seen in all but one non-invasive tumor. We conclude that trypsinogen is expressed in both malignant and benign bladder epithelium, whereas TATI expression decreases with increasing stage and grade of the tumor. This may suggest that a balanced expression of TATI and trypsinogen is required in normal tissue and that this balance is disrupted during tumor progression.

## Introduction

Tumor-associated trypsin inhibitor (TATI) is a 6 kDa peptide that was originally isolated from the urine of an ovarian cancer patient (1). TATI has been found to be identical to the pancreatic secretory trypsin inhibitor (PSTI) (2), also called

serine proteinase inhibitor Kazal-type 1 (SPINK1) (3). TATI is also often expressed in many tumors of the gastrointestinal and urogenital tracts (4,5). Increased serum concentrations of TATI have been observed in many types of cancer (6), and clinically serum TATI is useful in the monitoring of mucinous ovarian cancer. In ovarian (7,8), kidney (9) and bladder cancer (10) an elevated serum TATI is a prognostic marker independent of stage and grade. We have recently shown that protein expression of TATI in ovarian cancer tissue also predicts adverse outcome (11).

Trypsin is the major pancreatic proteinase, but it is also expressed in other normal gastrointestinal and urogenital tissues (12) and in several tumor types. Trypsinogen is expressed together with TATI both in the pancreatic acinar cells and in malignant tumors, and is thought to play a role in tumor invasion by degrading a wide variety of extracellular matrix proteins and by activating other tumor-associated proteinases, e.g. urokinase-type plasminogen activator (uPA) and several collagenases (13-17).

We have shown that trypsinogen is locally produced in the seminal vesicle, epididymis and the prostate (12), and that an increased trypsinogen expression is associated with high Gleason grade in prostate carcinoma (18). The expression of TATI mRNA has been shown to be up-regulated in urothelial tumors and TATI protein expression has been detected in bladder epithelia (5). Our aim was to study whether trypsinogen also is expressed in bladder cancer, how this correlates with TATI expression and whether the expression is associated with the clinicopathological characteristics of the tumor.

## Patients and methods

**Patients and samples.** Tissue samples were collected from 28 bladder cancer patients (8 females and 20 males) with a mean age of 67 years (range 40-85 years), and from 3 patients undergoing examinations for hematuria or recurrent lower urinary tract infections. Twenty-five of the patients had a primary tumor and three had relapsing disease. Of the patients 21 developed at least one relapse, stage progression occurred in three cases, and grade progression in another three cases. Mean time to relapse was 257 days (range 33-1658 days; median 140).

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Tissue sections (n=28) used for the immunohistochemical studies were obtained by transurethral resection or by open surgery. The tumor tissues were fixed in 4% buffered formaldehyde for 12-48 h, processed and embedded in paraffin. Tissue samples used to study mRNA expression were immediately frozen by immersion in liquid nitrogen and stored at -80°C until RNA isolation. Benign renal pelvic urothelial tissue of nephrectomy specimens (n=7) were used as controls in RT-PCR. The study was approved by the ethics committee of Helsinki University Hospital and all patients participated after informed consent.

Tumor staging was performed according to the TNM-classification (19) and grading according to the WHO/ISUP classification (20).

**Immunohistochemistry.** The alkaline phosphatase anti-alkaline phosphatase (APAAP) method was used to detect TATI and trypsinogen immunoreactivity (18). We used monoclonal antibodies (mAb) to detect trypsinogen (mAb against trypsinogen-1, code 6D11, concentration 2 µg/ml) (21) and TATI (6E8, concentration 4 µg/ml) (22). In addition, a biotinylated rabbit anti-mouse (code Z259), swine anti-rabbit IgG conjugated with alkaline phosphatase (code D306) and a non-immune mouse mAb diluted 1:200 (code X0931, all from Dako A/S, Glostrup, Denmark) were used.

After deparaffinization and rehydration, sections were subjected to antigen retrieval by incubation with SSC (10 mM, pH 6.0) and heated in a microwave oven at 750 W for 2x3 min prior to immunodetection of TATI. For detection of trypsinogen, tissues were digested with 0.4% pepsin in 0.2 M HCl for 15 min at 37°C. The sections were then thoroughly rinsed in distilled water and immersed in Tris buffer (0.05 M Tris, pH 7.6) with 0.05% Tween-20 for 2x10 min and incubated overnight at RT with primary antibody diluted in Tris-buffer 0.05% Tween-20 and 2% BSA, and subsequently with rabbit anti-mouse or IgG for 45 min. After washing, the sections were incubated with the APAAP complex for 30 min at RT. The sections were developed in 0.05 M Tris-buffer (pH 8.4) containing 0.13 M NaCl, 0.3 mg/ml naphthol phosphate (code no. N-4875) diluted in dimethyl formamide, 1.5 mg/ml fast red and 5 mM levamisol (Sigma-Aldrich) for 30 min. The sections were then washed in distilled water, counterstained with Mayer's hematoxylin solution for 30 sec and coverslips applied with Faramount™ mounting medium (Dako A/S). All other reagents used in this process were purchased from Merck.

As negative controls, adjacent tissue sections were processed by replacing the primary anti-trypsinogen antibody with non-immune mouse IgG1. Pancreatic tissue was used as a positive control. All sections were reviewed and scored by three persons (A. Bjartell, A. Sankila and K. Hotakainen), who were blinded to clinical and outcome data. The antigen staining was categorized in 4 classes: negative (0) denoting no staining; faintly positive (1) when a few cells stained faintly; unequivocally positive (2) when most but not all of the cells were faintly to strongly stained, and strongly positive staining (3) when all cells showed strong staining. Staining in various parts of the tumor tissue and in adjacent benign epithelium was recorded separately.

**Probe synthesis for *in situ* hybridization of trypsinogen and TATI.** *In vitro* transcription of sense and antisense probes for trypsinogen were made by fluorescein-UTP riboprobe synthesis using the RNA colour kit (Amersham Pharmacia Biotech, Uppsala, Sweden) as described (12). As a template, a 627-bp long trypsinogen-2 cDNA fragment [corresponding to nucleotides 42-688, accession number M27602 (23)], was cloned from COLO 205 cells by RT-PCR using the TA Cloning Kit (Invitrogen, San Diego, CA) and the following primers: 5'TGCTGTTGCTGCCCCCTTTG'3 (sense) and 5'GCACAGCCATAGCCCCAGGAG'3 (antisense). The integrity and length of the probes was determined by gel electrophoresis. The antisense probe is presumed to recognize both trypsinogen-1 and trypsinogen-2 transcripts in ISH experiments as judged from extensive similarity (92%) in cDNA nucleotide sequences (23).

Oligodeoxynucleotide probes for TATI were constructed corresponding to nucleotides 381-410 of the published sequence for TATI mRNA (24); 5'AGGTCACCGCGAGGC CTGACTGGCCTTATT'3 (sense) and 3'TCCAGTGGCGCT CCGGACTGACCGGAATAA'5 (antisense). An oligodeoxythymidine (oligo-dT) probe (30-mer) was used as a positive control for successful tissue preparation and hybridization. All probes were purchased from DNA Technology (Aarhus, Denmark) and labeled with digoxigenin (Dig)-dUTP using a 3'-end-labeling kit for tailing (Roche), as previously described (25).

***In situ* hybridization of trypsinogen and TATI.** All reagents were purchased from Sigma-Aldrich and Amersham Pharmacia Biotech. Tissue specimens were fixed, paraffin-embedded, sectioned (4 µm), dried for 2 h at 65°C and mounted on SuperFrost™ plus slides (Menzel-Gläser) under RNase free conditions. The sections were deparaffinized in xylene, rehydrated, and processed as described (12), pretreated with 0.2 M HCl to abolish endogenous enzyme activity, and digested with proteinase K (20 µg/ml in 20 mM Tris-HCl, 2 mM CaCl<sub>2</sub>, pH 7.5) for 25 min at 37°C. After pre-hybridization with 40 µl of hybridization buffer containing 50% (v/v) formamide, 10 mM Tris-HCl pH 7.6, 1X Denhardt's solution (BSA, polyvinylpyrrolidone and Ficoll, all at 0.2 mg/ml), 2X SSC, and 0.4 µg/ml salmon sperm DNA at 55°C for 1 h, the slides were hybridized with 40 µl of 250 ng/l antisense or sense probe in hybridization buffer first for 8 min at 85°C and then for 16 h at 55°C. After hybridization, the slides were washed in 0.1X SSC at 60°C (4x15 min) and then equilibrated in TBS (100 mM Tris-HCl, 0.4 M NaCl, pH 7.5). For detection of hybridization signals, tissue sections were first incubated in blocking reagent and subsequently incubated with anti-fluorescein alkaline phosphatase conjugate (Amersham Pharmacia Biotech) diluted 1:1000 in TBS containing 5 g/l BSA for 2 h at RT. Hybridization signals were visualised with levamisol, NBT and BCIP. The color reaction was stopped after 2-8 h and the slides were coverslipped using Faramount mounting medium (Dako A/S).

*In situ* hybridization of TATI was performed as previously described (25). Tissue sections were hybridized with 30 µl probe (200 ng/ml) overnight at 30°C and washed in 1X SSC (150 mM NaCl and 15 mM sodium citrate, pH 7.0) at RT for 10 min, 0.2X SSC at 43°C for 4x15 min, and 1X SSC

Table I. An overview of the Materials and methods used in the study.

	Description
Immunohistochemistry; antibodies	
6E8	MAb anti-TATI (22)
6D11	MAb anti-trypsinogen-1 (21)
RT-PCR; primers	
5'TCAGCCATGAAGGTAACAG'3	TATI outer sense
5'CAAGGCCAGATTTTTGA'3	TATI outer antisense
5'GGTAACAGGCATCTTTCTTCTC'3	TATI nested sense
5'TTTCCATCAGTCCCACAG'3	TATI nested antisense
5'CATGAATCTACTCCTGATCC'3	Trypsinogen-1, 2 outer sense
5'TGTCATTGTCCAGAGTCC'3	Trypsinogen-1, 2 outer antisense
5'CCCCTTTGATGATGATGAC'3	Trypsinogen-1, 2 nested sense
5'AACTGTTTCATTCCTCC'3	Trypsinogen-1, 2 nested antisense
<i>In situ</i> hybridization; probes	
Nucleotides 42-688; M27602	Trypsinogen-1 and -2 riboprobe
5'AGGTCACCGCGAGGCCTGACTGGCCTTATT'3	TATI sense oligoprobe
3'TCCAGTGGCGCTCCGGACTGACCGGAATAA'5	TATI antisense oligoprobe
Statistical analysis	
	Mann Whitney U test
	Kruskal-Wallis test
	Kaplan-Meier and the log-rank test

for 10 min at RT. The sections were incubated with alkaline phosphatase-conjugated Fab fragments of anti-Dig IgG for 3 h at RT, and developed in detection buffer containing nitroblue tetrazolium chloride (NBT), 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and levamisole. The staining reaction was stopped in EDTA buffer after 15 to 20 h. The slides were coverslipped with Faramount mounting medium (Dako A/S). All other reagents were purchased from Sigma-Aldrich, Merck or Amersham Pharmacia Biotech. For control purposes, adjacent sections were hybridized with Dig-labeled oligo-dT probe to assure successful fixation, whereas sense probes and buffer were used as negative controls. Pancreatic tissue was used as a positive control for both trypsinogen and TATI mRNA (Table I).

*Reverse transcriptase-polymerase chain reaction (RT-PCR) and sequencing of the product.* Total RNA was extracted from frozen tissues using the RNeasy kit (Qiagen, MD). The oligonucleotide primers were constructed on the basis of published sequences for trypsinogen-1 and -2 (23) and TATI (24): 5'CATGAATCTACTCCTGATCC'3 (trypsinogen outer sense), 5'TGTCATTGTCCAGAGTCC'3 (trypsinogen outer antisense), 5'CCCCTTTGATGATGATGAC'3 (trypsinogen nested sense), 5'AACTGTTTCATTCCTCC'3 (trypsinogen nested antisense) 5'TCAGCCATGAAGGTAACAG'3 (TATI outer sense), and 5'CAAGGCCAGATTTTTGA'3 (TATI

outer antisense), 5'GGTAACAGGCATCTTTCTTCTC'3 (TATI nested sense), 5'TTTCCATCAGTCCCACAG'3 (TATI nested antisense). The outer trypsinogen primers produce a fragment of 323 bp and those for TATI a fragment of 243 bp. The products obtained with the nested primers are 213 bp for trypsinogen and 150 bp for TATI. The RT-PCR for both TATI (26) and trypsinogen (12) were performed as previously described. Briefly, total RNA (1 µg) was transcribed into cDNA using SuperScript II-RT (Gibco-BRL, Paisley, UK) according to the manufacturer's instructions. Contamination of RNA samples with DNA was excluded with control reactions without RT. The RT product (1 µl) was amplified in a 40-µl reaction volume in 1X PCR buffer, 20 pmol of both sense and antisense primers, 0.25 mM of each dNTP and 1.6 U of Dynazyme DNA polymerase (Finnzymes). The amplification conditions for cDNA with the outer primers were 30 cycles at 95°C for 1 min and 60°C for 1 min for trypsinogen and 30 cycles at 95°C for 1 min and 53°C for 1 min for TATI. One microliter of the product was further amplified with the nested primers in a 50-µl reaction volume for 30 cycles at 95°C for 1 min and 53°C for 1 min (trypsinogen) and 35 cycles at 95°C for 1 min and 55°C for 1 min (TATI). Water was used as a negative control and cDNA from COLO 205 colon adenocarcinoma cells as a positive control in all experiments. Agarose gel electrophoresis and sequencing of the PCR products were carried out as described (12).

Table II. TATI and trypsinogen mRNA and protein detected by RT-PCR and immunohistochemistry in tissue samples according to tumor stage.<sup>a</sup>

	TATI		Trypsinogen	
	Antigen	mRNA	Antigen	mRNA
Benign urothelia	3/3 (100)	10/10 (100)	3/3 (100)	9/10 (90)
Ta	12/12 (100)	12/14 (86)	11/12 (92)	11/14 (79)
T1	7/8 (88)	8/9 (89)	8/8 (100)	7/9 (78)
T2	1/5 (20)	1/5 (20)	5/5 (100)	3/5 (60)
All carcinomas	20/25 (80)	21/28 (75)	24/25 (96)	21/28 (75)

<sup>a</sup>The benign control samples were from a patient with hematuria of unknown origin and two patients with recurrent lower urinary tract infections. Seven urothelial samples were obtained from the renal pelvis of nephrectomy specimens.

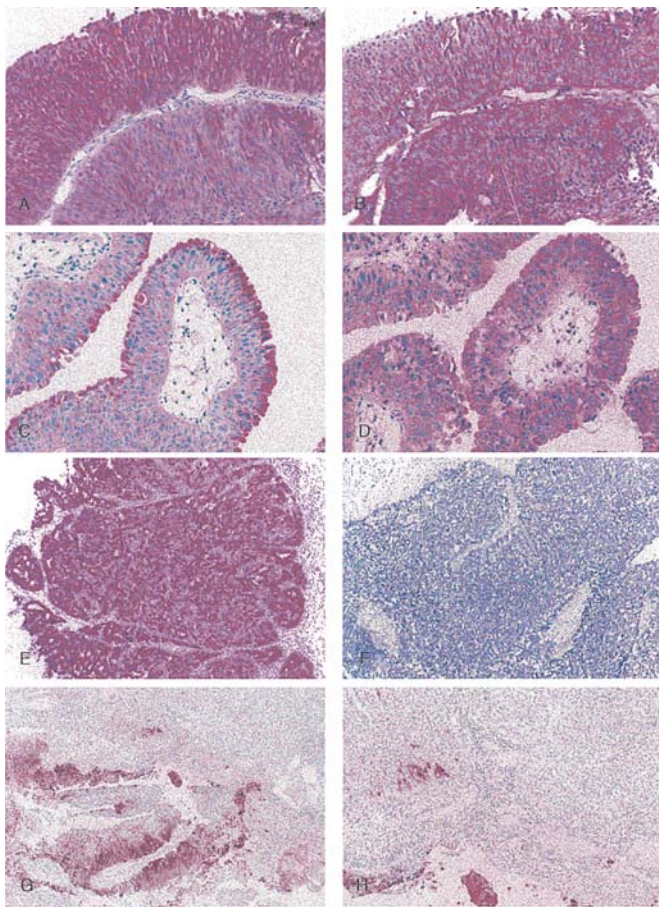


Figure 1. Immunohistochemical expression of TATI (A, C, E, F, G and H) and trypsinogen (B and D). Paraffin sections from stage pTa grade 1 (A and B), pTa grade 2 (C and D), pT1 grade 3 (E and F) and pT2a grade 2 (G and H) bladder carcinoma. The superficial parts of the pT1 grade 3 tumor in (E) is staining strongly positive for TATI, whereas the invasive part of the same tumor is completely negative (F). (G) shows the expression of TATI in a pT2a grade 2 tumor, of which the invasive part (G and H; top right) is negative. Original magnifications x200 (A-D); x100 (E, F and H), and x40 (G).

tests were two-sided and p-values <0.05 were considered significant. The disease-free survival of the patients was compared using the Kaplan-Meier and log-rank tests.

**Results**

*TATI and trypsinogen mRNA in bladder tumors and benign urothelia.* TATI mRNA was detected with the outer primers in most (87%) pTa and pT1 tumors and in all benign samples (n=3), as well as in all control urothelia from nephrectomy specimens (n=7) (Table II). However, all five muscle-invasive tumors were negative after one PCR round but one turned positive in the nested PCR (Table II). Twelve of thirteen grade 1 tumors (all pTa), eight of nine (89%) grade 2 tumors and three of six (50%) grade 3 tumors were positive for TATI mRNA, respectively. The disease-free interval was significantly longer in the TATI mRNA-positive cases (mean 758, median 207 days) than in the negative ones (mean 242, median 69 days; p=0.028). Five of the positive ones (24%) never relapsed during the mean follow-up period of 1716 days, whereas all seven negative ones did.

Trypsinogen mRNA was also detected by RT-PCR in most of the non-invasive tumors (78%), but only after nested PCR in 8 of the 11 positive pTa-tumors, in 2 of the 7 pT1-tumors, and in one of three pT2-tumors (Table II). Sequencing of the product revealed that it consisted predominantly of trypsinogen-1 cDNA, but a weak expression of trypsinogen-2 could not be excluded. None of the ten benign control urothelia were positive with the outer primers, but seven were positive by nested PCR. There was no significant difference in the disease-free interval between the trypsinogen mRNA-positive (mean disease-free interval 645 days; median 202) and negative cases (mean 383 days; median 122, p=0.22).

By *in situ* hybridization, trypsinogen mRNA was detected in all four samples tested; two of these were pT1 grade 2 tumors, one was pT2a grade 2 and one a non-invasive grade 1 tumor. TATI mRNA was also detected in all of the six samples studied; three of these were non-invasive (pTa) and three invasive (pT1) grade 2 tumors (Fig. 2). In these, positivity was seen also in the benign parts of the section corresponding to those staining positively in immunohistochemistry. In two

*Statistical analysis.* Differences between groups were analyzed by the Mann Whitney U test or the Kruskal-Wallis test. All

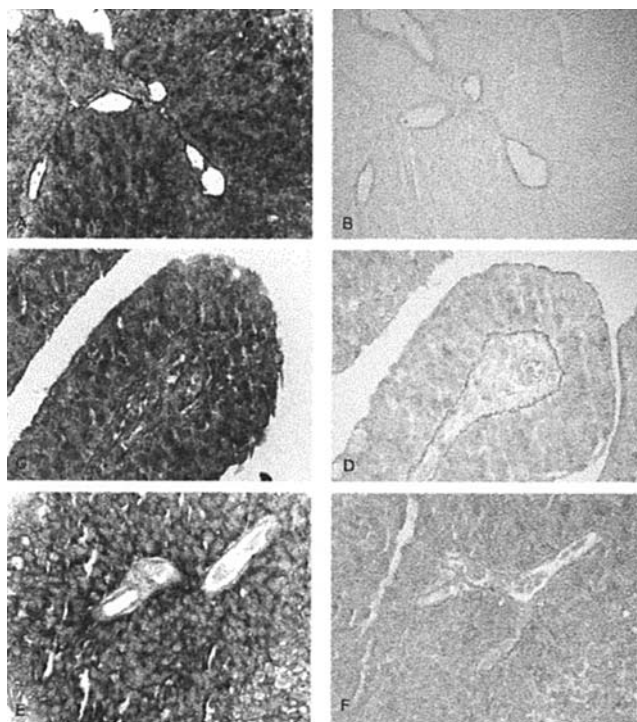


Figure 2. *In situ* hybridization analysis for TATI (A-D) and trypsinogen (E and F) mRNA in bladder cancer. Paraffin sections from a pTa grade 1 (A) and pTa grade 2 (C) tumor were hybridized with the antisense TATI oligo-probe and the corresponding sense probe (B and D). To visualize the expression of trypsinogen mRNA, the sections from the pTa grade 1 tumor were hybridized with a 627-base long trypsinogen-2 antisense riboprobe construct (E) as well as the corresponding sense riboprobe (F). Original magnification x400.

of the tumors the superficial areas were TATI-positive by both immunohistochemistry (Fig. 1E-H) and *in situ* hybridization, whereas the invasive part of the same tumor was negative by both methods.

#### *Immunohistochemical staining of TATI and trypsinogens.*

Twenty of the 25 tumors and the three samples from patients with benign diseases studied by immunohistochemistry were positive for TATI (Table II and Fig. 1). In most tumors the staining was strong in all cells and throughout the epithelium. Five tumors were only faintly positive and staining was seen in less than 10% of the cells, whereas the adjacent normal epithelium stained strongly positive. In most cases, the staining in benign epithelium occurred predominantly in the umbrella cells while the basal cell layers were negative. Four of the five muscle-invasive tumors (pT2) were negative, and all of these were also negative for TATI mRNA. In two of the invasive tumors (one pT1 and one pT2) the superficial parts of the tumor were unequivocally positive, whereas the invasive areas were negative (Fig. 1). Only one of the 24 tumors studied was negative for trypsinogen; this was a non-invasive (pTa, grade 3) tumor (Table II). In two of the invasive tumors squamous differentiation was seen and these areas were negative for TATI, but diffusely positive for trypsinogen.

The disease-free interval tended to be longer in the cases with positive staining for TATI (mean 681 days) than in those with weak or negative staining (mean 451 days,  $p=0.36$ ).

Furthermore, all of the negative or weakly staining tumors relapsed ( $n=10$ ), whereas 40% (7 of 18) of the strongly positive tumors did not relapse at all during the mean follow-up period of 1716 days.

#### **Discussion**

We found co-expression of TATI and trypsinogen on the tissue level in both benign and malignant urothelia, but while TATI expression was observed most frequently in benign urothelium, the trypsinogen expression was similar in normal and malignant tissues and in tumors it was not associated with stage or grade. TATI mRNA was readily demonstrated by RT-PCR with outer primers only, whereas that of trypsinogen could be detected only after nested RT-PCR in half of the cases. Both by immunohistochemistry and RT-PCR, TATI expression was most prominent in non-invasive tumors while lack of tissue expression was associated with invasive disease, more frequent relapses and a shorter disease-free interval. Furthermore, TATI expression was weaker or absent in muscle-invasive and high-grade tumors whereas all of the benign control urothelia were positive. Recently, Paju *et al* (11) described a similar inverse correlation between tumor stage and grade and tissue expression of TATI in ovarian carcinoma, but in spite of this TATI expression in tumor tissue was a strong marker of adverse prognosis and it was independent of stage and grade.

Serum concentrations of TATI have previously been found to be potentially useful in monitoring the disease activity and response to treatment in advanced bladder cancer (27) and we have shown that serum TATI is an independent predictor of adverse prognosis in bladder cancer even when the patient has no evidence of tumor in cystoscopy or cytology (10). In the present study we also determined serum and urine levels of TATI and trypsinogens, but no significant correlation between the protein levels and tumor stage and grade or disease-free interval was observed (data not shown). However, the urinary concentrations of TATI tended to be higher in the mRNA-positive cases and those staining strongly in immunohistochemistry, but these associations were not statistically significant. This is probably explained by the small number of cases in each category.

Trypsinogen expression was detected at all tumor stages and grades (96%) by immunohistochemistry, and in most tumors by RT-PCR (75%). The disease-free interval tended to be longer in cases with trypsinogen mRNA expression, but the protein expression was strong in most tumors and any potential prognostic significance of the tissue expression could not be demonstrated in this study. Trypsinogen can contribute to tumor invasion by degrading the extracellular matrix and activating other proteinases (13-17). Tissue expression of trypsinogen is strongly associated with recurrence and poor prognosis of colorectal cancer (28) and squamous cell carcinoma of the esophagus (29). It is also a characteristic of aggressive cancers of the ovary (30). Trypsin in circulation is inactivated by  $\alpha$ -2-macroglobulin and  $\beta$ -1-proteinase inhibitor (API), and the serum concentration of the trypsin-API complex is a strong independent prognostic factor in epithelial ovarian cancer (11). Increased trypsinogen-1 expression has been demonstrated in the first stages of entero-

cytic differentiation of cultured colon carcinoma cell lines, whereas this did not occur in cells remaining undifferentiated (31). Interestingly, Yamashita *et al* found that trypsinogen expression was silenced through DNA methylation in a large proportion of esophageal squamous cell carcinomas and gastric adenocarcinomas. In gastric carcinomas this was associated with deeper invasion and higher stage (32) and the authors suggested a tumor suppressive role of trypsin in cancer progression. In serous ovarian carcinoma, a strong tissue expression of trypsinogen-2 is also associated with a better prognosis, but trypsinogen expression was not correlated with stage or grade (11). Thus, trypsin expression has been found to be associated both with poorly differentiated aggressive tumors and tumor progression, and on the other hand, differentiation of tissues and tumor suppression. Trypsinogen mRNA expression was equally frequent in benign and malignant tissues but the protein expression tended to be stronger in the invasive tumors, all of which were positive for trypsinogen antigen. On the contrary, TATI expression was strongest on both mRNA and protein level in the more superficial tumors and absent in the most invasive ones. This may suggest that a balanced expression of TATI and trypsinogen is required in normal tissue but the balance may be disrupted during tumor progression. In ovarian tumors, trypsinogen expression has been detected more frequently in malignant than benign tumors by immunohistochemistry (33), and TATI concentrations have been found to be higher in benign than in malignant cyst fluids of mucinous ovarian tumors. Furthermore, the molar ratios of trypsinogens to TATI were higher in the cyst fluids of serous than mucinous ovarian carcinomas (16) and serous carcinomas have a worse prognosis than the mucinous ones (34). This could be associated with an excess of trypsinogen compared to TATI. The fact that TATI expression is weaker in invasive bladder tumors at both mRNA and protein level supports the hypothesis of a disturbed balance between proteinase and proteinase-inhibitor. Recently, the expression of urokinase-type plasminogen activator and matrix metalloproteinase (MMP)-14 have been shown to be progressively up-regulated with advanced stage in bladder tumors (35), and trypsin has been shown to activate urokinase and several MMPs (MMP 1, 3, 7, 8, 9, 13) (14,34,17).

In conclusion, TATI and trypsinogen are co-expressed in bladder cancer, but the balance between them changes during tumor progression. Tissue expression of TATI is strongest in early-stage and low-grade disease, whereas trypsinogen expression is less clearly associated with tumor stage or grade. Expression of TATI and trypsinogen in bladder cancer warrants further studies to elucidate the biochemical background and to evaluate potential clinical applications.

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