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Biologically active ADAMTS13 is expressed in renal tubular epithelial cells

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Abstract

ADAMTS13 mRNA, encoding the von Willebrand factor-cleaving protease, has been detected in a variety of tissues including the kidney. The present study aimed to characterize tubular expression and bioactivity of ADAMTS13. *ADAMTS13* mRNA was detected in cultured primary human renal tubular epithelial cells (HRTEC) and in A498 cells, a human renal carcinoma cell line, by real-time PCR. Protein was detected using immunofluorescence and immunoblotting. Immunoblots demonstrated that the protein was secreted. The protease was proteolytically active in both cell lysates and cleaved the FRETS-VWF73 substrate. ADAMTS13 was demonstrated *in situ* in the renal cortex by immunohistochemistry. Protease was detected in both the proximal and distal renal tubules in normal renal tissue (n=3) as well as in patients with tubular disorders (n=3). Using immunoblotting ADAMTS13 was demonstrated in urine from patients with tubulopathy (n=5) but not in normal urine. ADAMTS13 in urine had a molecular size similar to that in plasma, which would indicate that the protease originates in the tubuli because such large proteins do not normally pass the glomerular filter. In conclusion, human renal tubular epithelial cells synthesize biologically active ADAMTS13 which may, after release from tubuli, regulate hemostasis in the local microenvironment.

Key words: ADAMTS13, kidney, pediatrics, tubular epithelial cells, urine

Introduction

ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type-1 motifs) is the physiological von Willebrand factor (VWF) cleaving protease [1, 2]. Severe ADAMTS13 deficiency (<5% of normal plasma activity) is associated with thrombotic thrombocytopenic purpura (TTP) [3] and may occur due to mutations in the *ADAMTS13* gene (congenital TTP) [4, 5] or due to auto-antibodies against the protease (acquired TTP) [6].

TTP is a thrombotic microangiopathy characterized by the occurrence of disseminated VWF and platelet-rich thrombi in the microcirculation of multiple organs, including the kidneys [7, 8]. The function of VWF is to induce formation of platelet thrombi at sites of vascular injury and high-shear stress and it is dependent on the size of the VWF multimers, i.e. the larger the VWF multimer the more biologically active it is in inducing platelet aggregation. ADAMTS13 regulates the size and thereby the activity of VWF multimers by rapid cleavage upon their release from endothelial cells [9]. Deficient VWF cleavage, as occurs in TTP, allows ultra-large VWF (ULVWF) to accumulate in the circulation and contribute to thrombus formation [10].

ADAMTS13 normally circulates in plasma and has a mass of approximately 150 kD under non-reducing conditions and 190 kD under reducing conditions [1, 11]. ADAMTS13 antigen is present in the plasma of patients with acquired TTP, where it appears to be in complex with auto-antibodies [12], and is absent in congenital TTP [11, 13]. The cellular origin of ADAMTS13 in plasma has not been completely elucidated but the protease has been shown to be synthesized in liver stellate cells and in endothelial cells [14, 15]. *ADAMTS13* mRNA has been detected in a variety of tissues including the kidneys [4, 16, 17]. The kidneys are one

of the organs mainly affected in TTP and localization of renal ADAMTS13 expression may therefore be relevant for understanding the pathophysiology. We have previously shown ADAMTS13 expression in the renal cortex in podocytes, glomerular endothelium, basement membrane and tubuli [16].

The ADAMTS13 protease has been well characterized and consists of a signal peptide, a short propeptide, the metalloprotease domain, a disintegrin-like domain, a thrombospondin-1 repeat, a Cys-rich domain, a spacer domain, seven additional thrombospondin-1 (TSP-1) repeats and two CUB domains [2]. In the present study we characterized ADAMTS13 expression in cultured renal tubular epithelial cells by real-time PCR, immunoblotting and immunofluorescence using monoclonal and polyclonal antibodies directed to distinct domains in the protease. Bioactivity was demonstrated using the FRETS-VWF73 assay. *In situ* tubular cell expression was studied by immunohistochemistry and the pattern of expression in tubular damage as well as secretion into the tubular lumen, and ultimately into urine, was investigated.

Methods and subjects

Cell culture

In order to investigate ADAMTS13 expression cells of proximal tubular origin were cultured. HRTEC (human renal tubular epithelial cells) are primary human renal cortical cells previously isolated in our laboratory and characterized as tubular epithelial cells [18]. The A498 cell line originates from human renal epithelial carcinoma of tubular origin [19]. HRTEC and A498 cells were cultured as previously described [18, 20]. HRTEC were grown in Advanced D-MEM F-12 medium (Invitrogen GmbH, Karlsruhe, Germany) supplemented with 20 U/ml heparin (Leo Pharma, Malmö, Sweden), 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 15% fetal bovine serum (all from Invitrogen GmbH). A498 cells were cultured in RPMI-1640 medium (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) supplemented with 1 mM non-essential amino acids (PAA Laboratories GmbH, Cölbe, Germany), 1 mM sodium pyruvate (PAA Laboratories GmbH), 100 U/mL penicillin, 100 μg/mL streptomycin and 10% fetal bovine serum (all from Invitrogen GmbH). Cells were grown in culture flasks (Göteborgs Termometerfabrik, Västra Frölunda, Sweden) to collect cell lysate, medium and RNA, or on chamber slides (Nunc, Roskilde, Denmark) for immunofluorescence staining. HRTEC were used at passage 3-4.

For experiments cells at approximately 95% confluence were washed and incubated with serum-free medium (as above for each cell type but without serum). After 24 hours media were collected and supplemented with Complete EDTA-free protease inhibitors (Complete Mini, EDTA-free; Roche Diagnostics GmbH, Mannheim, Germany). Media were centrifuged to remove cell debris and concentrated 20 to 30-fold using Centricon concentrators (Millipore Corp., Bedford, MA). Cells were washed and trypsinized. Detached cells were washed and

lyzed in lysis buffer: 0.01 M Tris 0.15 M NaCl pH 7.4 supplemented with protease inhibitors. Approximately 0.7-1 x 10^6 cells/100 µl buffer were lyzed by repeated freezing and thawing (in liquid nitrogen and 37ºC water bath, respectively) followed by sonication in an ultrasonic bath (Grants Instruments (Cambridge) Ltd., Royston, England). Cell debris was removed by centrifugation. Total RNA was isolated as previously described [16]. For immunofluorescence experiments cells grown on chamber slides were washed, fixed with 2% paraformaldehyde (Sigma Aldrich Chemie GmbH), 4% sucrose (Acros, Geel, Belgium) in PBS and permeabilized with 0.3% Triton X-100 (ICN Biomedical Inc., Aurora, OH) in PBS as previously described [21].

Real-time PCR

RNA was reverse transcribed and ADAMTS13 gene transcripts were detected by real-time PCR (ABI Prism 7000, Applied Biosystems, Foster City, CA) with a probe against exons 28-29 (translating into part of the second CUB domain; Applied Biosystems, assay ID Hs00260148_m1) [16]. We have previously shown that kidney contains *ADAMTS13* mRNA [16] and normal kidney RNA pooled from 14 individuals (BD Biosciences Clontech, Palo Alto, CA) was therefore used as the positive control. In addition, normal liver RNA (BD Biosciences) was used as a positive control. No template control was used as the negative control. 18S ribosomal RNA transcript (Applied Biosystems, assay ID Hs99999901_s1) was used as the reference gene to standardize the data.

Anti-ADAMTS13 antibodies

Two polyclonal rabbit anti-human ADAMTS13 antibodies generated in our laboratory were used. SU19 against the second CUB domain was previously described [11] and SNO357 directed against the metalloprotease and disintegrin-like domains, specifically at amino acids 281-296 of the protease. The peptides against which these antibodies were raised were synthesized by Eurogentec, Seraing, Belgium. In addition, one mouse monoclonal antibody, A10 (a gift from Y. Fujimura, Department of Blood Transfusion Medicine, Nara Medical University, Nara and H. Hiura, Japan Clinical Laboratories, Kyoto, Japan) directed against the disintegrin domain [14] was used. The SU19 polyclonal antibody recognizes ADAMTS13 under reducing and non-reducing conditions [11] whereas the SNO357 antibody recognizes ADAMTS13 only under reducing conditions and the A10 antibody recognizes only the non-reduced form [11].

Immunofluorescence

ADAMTS13 was detected using the A10 monoclonal antibody at 5 µg/ml or SU19 polyclonal anti-ADAMTS13 $F(ab')_2$ at 15 µg/ml as previously described [16]. Signal was detected using goat-anti-mouse IgG $F(ab')_2$ FITC 1:20 (DakoCytomation, Carpinteria, CA) or goat-anti-rabbit (H+L) $F(ab')_2$ Alexa Fluor 488 1:150 (Molecular Probes, Invitrogen GmbH, Karlsruhe, Germany). Normal mouse $IgG_{2b-\kappa}$ was used as the isotype control antibody for A10. Specificity of the polyclonal antibody was tested by preincubation with a 50-fold molar surplus of blocking peptide [16]. Specificity of the secondary antibodies was tested by omitting the primary antibody.

Immunoblotting for detection of ADAMTS13

HRTEC and A498 cell lysates and media (1:2) were subject to SDS-PAGE and immunoblotting under reducing conditions as previously described [11]. ADAMTS13 was detected with the SNO357 antibody at 3.6 µg/ml. Purified recombinant ADAMTS13 1:2 was used as the positive control. For this purpose HEK293 cells transfected with the pIRESneo2 VCP HIS-vector containing the human *ADAMTS13* gene (a kind gift from Professor Reinhard Schneppenheim, University Medical Center Hamburg-Eppendorf, Department of Pediatric Hematology and Oncology, Hamburg, Germany) were cultured. The His-tagged protease was purified from HEK293 cell medium on Ni Sepharose His SpinTrap columns (GE Healthcare, Buckinghamshire, UK). Specificity of the antibody was tested by preincubation with 50x molar surplus of blocking peptide prior to the immunoblotting procedure as previously described [11].

Urine samples were subject to SDS-PAGE and immunoblotting conditions as previously described [11]. Samples were concentrated 20-fold with Centricon concentrators (Millipore). Immunoblotting with samples diluted 1:2 under non-reducing conditions was carried out using the A10 (0.55 µg/ml) monoclonal antibody. Normal plasma was used as the positive control.

VWF-cleaving activity

ADAMTS13 VWF-cleaving activity in HRTEC and A498 cell lysates was assayed using the FRETS-VWF73 substrate as previously described [22]. Briefly, 10 µl of sample was added to each well, followed by addition of 100 µl each of assay buffer (5 mmol/l Bis–Tris, 25 mmol/l $CaCl_2$, 0.005% Tween-20, pH 6.0) and substrate solution (2 µM final concentration of FRETS-VWF73) [22]. A dilution series of normal pooled plasma was used for assay

calibration. Lysis buffer was used as the negative control. In order to test specificity cell lysates were preincubated with the A10 monoclonal antibody at 50 µg/ml final concentration, which has previously been shown to block ADAMTS13 activity [14].

Immunohistochemistry

Renal tissue was formaldehyde-fixed, paraffin-embedded, sectioned (3,5 µm on glass slides) and prepared as previously described [23]. ADAMTS13 expression in renal tissue was detected by immunohistochemistry with the A10 monoclonal antibody at 3 µg/ml as previously described [16]. Signal was detected using EnVision+ System anti-mouse HRP (DakoCytomation). Normal mouse $IgG_{2b-\kappa}$ was used as the isotype control and specificity of the secondary antibody was tested by omission of the primary antibody. Positive signal stained brown.

Urine samples and renal tissue from patients and controls

Patients

Urine samples were available from 5 patients and renal biopsies from 3 patients with tubular disorders. All patients were treated at the Division of Pediatric Nephrology of the Department of Pediatrics, Lund University Hospital. The patients are described in Table 1. Sufficient renal tissue for immunohistochemical analysis was available from patients 4, 5 and 6.

Controls

Positive controls for the presence of ADAMTS13 in urine:

Urine from a 16-year old female patient with nephrotic syndrome was available. The patient had nephrotic syndrome (with nephrotic-range proteinuria at sampling) due to focal segmental glomerulosclerosis, as diagnosed by renal biopsy in which no tubular damage was detected.

Urine from a 3-year old girl with acute post-infectious glomerulonephritis was also available. The diagnosis was based on the simultaneous presence of macroscopic hematuria, hypertension and low complement C3. At debut the patient had non-nephrotic range albuminuria as detected by albumin/creatinine index of 116 (reference value < 3.8 g/mol). No evidence of streptococcal infection was found but the patient made a full recovery including normalization of complement levels.

Pediatric controls for ADAMTS13 in urine:

Urine samples were available from four pediatric controls treated at the outpatient clinic of the Division of Pediatric Nephrology, Department of Pediatrics, Lund University Hospital. These patients, all boys aged 3, 6, 8 and 16 years, were followed for reflux nephropathy (2 patients), posterior urethral valve and hypertension, respectively.

Normal controls:

Urine samples from seven healthy adults (of which three were women) were used as normal controls. None of the negative controls, both healthy adults and pediatric controls, had glomerular or tubular proteinuria (assayed as in Table 1).

Control renal tissue was obtained from two adult patients (one male and one female) nephrectomized due to renal cancer. The tissue was taken from areas unaffected by the cancer and deemed normal by the pathologist. The patients had not received chemotherapy or radiotherapy prior to surgery. Normal renal tissue was also obtained from a renal biopsy taken from a 10-year old girl investigated for hematuria in which the renal biopsy was deemed normal by light microscopy and immunofluorescence [23]. The study was conducted with the

approval of the ethics committee of Lund University and samples were taken with the written informed consent of the participants or their parents.

Results

ADAMTS13 mRNA is expressed in cultured tubular epithelial cells

ADAMTS13 mRNA was detected in HRTEC and A498 cells using the probe against exons 28-29. Human liver and human kidney have been shown to express *ADAMTS13* mRNA [4, 16, 17] and were therefore used as the positive controls. The negative control (no template control) showed no amplification (data not shown). The housekeeping gene 18S was expressed at comparable levels in all samples (data not shown) and was used as an endogenous control to standardize the data. Results are presented as the ratio of ADAMTS13/18S (Figure 1).

ADAMTS13 protein is expressed in cultured tubular epithelial cells

ADAMTS13 expression was detected in primary HRTEC cells (Figure 2, upper panel) using the A10 monoclonal antibody against the disintegrin domain, and the SU19 polyclonal antibody against the second CUB domain of the protease (Figures 2A and 2B). Incubation with the mouse control antibody resulted in lack of signal (Figure 2C), while preincubation of the polyclonal antibody with blocking peptide markedly reduced staining (Figure 2D). When primary antibodies were omitted no signal was detected (Figures 2E and 2F).

The renal carcinoma A498 cell line showed similar results (Figure 2, lower panel). ADAMTS13 expression was observed using both the monoclonal and polyclonal antibody (Figures 2G and 2H). Specificity experiments were carried out as for the HRTEC cells and resulted in similar observations (Figures 2I-L).

ADAMTS13 expression was further investigated by immunoblotting of cell lysates and media. Purified recombinant human ADAMTS13 was used as the positive control and exhibited a band at 190 kD under reducing conditions using the SNO357 antibody against the metalloprotease/disintegrin-like domains (Figure 3). Immunoblots of HRTEC cell lysates and media revealed bands at approximately 170 and 150 kD, respectively (Figure 3). Preincubation of the antibody with the blocking peptide which it was raised against, prior to the immunoblotting procedure, abolished all bands (data not shown). No bands were detected in the cell lysates and media of A498 cells (data not shown).

ADAMTS13 activity

Activity of cultured HRTEC and A498 cells was tested as the ability to cleave the FRETS-VWF73 substrate (Figure 4). Experiments revealed VWF-cleaving activity in both cell types. HRTEC lysates showed a mean activity value of 6.24% (range 4.90-9.16%), while A498 cell lysates had a mean activity of 10.12% (range 6.05-13.50%). To test the specificity of the assay lysates were incubated with the A10 monoclonal antibody which has been shown to inhibit ADAMTS13 activity [14]. Following incubation with the monoclonal antibody activity values decreased to 1.93% for HRTEC lysates and 3.44% for A498 lysates.

ADAMTS13 expression in damaged renal tubuli

The ADAMTS13 expression pattern was examined in normal renal tissue as well as in biopsies from patients 4, 5 and 6 with tubular damage. ADAMTS13 antigen expression was detected in all tubular cells of the renal cortex, suggesting its presence in both proximal and distal tubuli (Figure 5A normal, 5B patient 4). In the normal tissue a diffuse intracellular expression pattern was exhibited (Figure 5C). In renal tissue from patients 4 and 5 ADAMTS13 was either expressed diffusely or concentrated apically in damaged tubular cells

(Figures 5D-E). The tissue from patient 4 also showed desquamated tubular epithelial cells containing ADAMTS13 (Figure 5E). Labelling of the renal biopsy from patient 6 showed similar results (data not shown). The control antibody did not exhibit staining (Figure 5F). Similarly, experiments in which the primary antibody was omitted were negative (data not shown).

ADAMTS13 detection in urine samples

Urine samples were investigated with respect to ADAMTS13 expression with the A10 monoclonal antibody using the immunoblotting procedure under non-reducing conditions. Results are shown in Figure 6. ADAMTS13 was not detected in urine from seven healthy individuals (Figure 6, lane 1 shows urine from one normal control) or in the urine samples from the four pediatric controls (data not shown). The A10 monoclonal antibody has previously been shown to detect an ADAMTS13 band in plasma at 150 kD [11]. Normal plasma was used as the positive control and showed a strong immunoreactive band at 150 kD (Figure 6, lane 2). Urine from the two positive controls (with unselective proteinuria) were run for comparison and exhibited a strong immunoreactive band at 150 kD (urine from the patient with nephrotic syndrome is shown in Figure 6, lane 3, data not shown for the other patient). An immunoreactive band at approximately 150 kD was observed in the urine samples from patients 1 to 5 with tubular damage (Figure 6, lanes 4 to 8).

Discussion

In the kidney ADAMTS13 is found in glomerular endothelial cells, podocytes, glomerular basement membrane, as well as in tubular epithelial cells [16]. In the present study we investigated ADAMTS13 expression and activity in renal tubular epithelial cells. Both primary HRTEC and a renal carcinoma cell line of tubular epithelial origin (A498) [19] expressed ADAMTS13 mRNA (detected by real-time PCR) and protein (detected by immunofluorescence and immunoblotting). Furthermore, ADAMTS13 expressed by both cell types showed biological activity and was able to cleave the FRETS-VWF73 substrate [22]. ADAMTS13 was detected in the renal cortex in tubuli and showed a diffuse staining pattern in normal renal tissue. ADAMTS13 in renal tissue from patients with tubular damage presented a diffuse pattern of staining or apical accumulation. Furthermore, ADAMTS13 was detected in the urine of patients with tubular damage but not in normal individuals. Taken together, these results indicate that tubular cells synthesize biologically active ADAMTS13.

Urine samples from patients with tubular damage revealed detectable ADAMTS13 by immunoblotting. The size of the ADAMTS13 band detected in these urine samples was comparable to that in the plasma, indicating that tubular cells are capable of synthesizing and secreting full-length ADAMTS13. No ADAMTS13 was detected in the urine from the normal adult controls or from four pediatric controls. Such a large protein would not be expected to be filtered into the urine of individuals with healthy glomeruli, explaining why it is not found in normal urine. Its presence in tubular injury suggests that ADAMTS13 originated from the tubular cells as this large protein (150 kD) should not normally pass the glomerular barrier. Albumin (66 kD) and low molecular weight proteins may be filtered in small amounts but undergo reabsorption in healthy tubular cells. The presence of minimal albuminuria in

patients with tubulopathy was indicative of defective tubular reabsorption. In the positive controls with nephrotic syndrome and post-infectious glomerulonephritis the ADAMTS13 band was much stronger due to the fact that these patients leak plasma proteins unselectively into the urine.

ADAMTS13 VWF cleaving activity was measured in the cell lysates as the ability to cleave the FRETS-VWF73 peptide, which is the smallest known substrate for ADAMTS13 [22, 24]. Activity was detected in both cell types and was markedly decreased when cell lysates were preincubated with an inhibitory antibody, further indicating specific ADAMTS13 activity. The presence of ADAMTS13 in the medium of HRTEC cells suggests that the protease is secreted from tubular cells. ADAMTS13 was not detected in the medium and lysates of A498 cells. This discrepancy between HRTEC and A498 cells may be attributed to the fact that HRTEC are primary cells, thereby more closely resembling the normal cell physiology, whereas A498 is a cell line. HRTEC medium and lysate exhibited a band at 150 and 170 kD, respectively. An ADAMTS13 band at 170 kD has previously been described in endothelial cell lysates [25]. The slightly lower band detected in the medium may represent a breakdown product.

Immunohistochemical studies of normal renal cortex showed that ADAMTS13 is diffusely expressed in renal tubular cells. However, in the renal tissue from patients with tubular damage the ADAMTS13 antigen was also detected apically in the cells. It is as yet unclear if there is a shift in the localization of ADAMTS13 in damaged tubular cells and whether this is due to a passive mechanism as a result of damage to the tubular cells, or an active one by which ADAMTS13 is transported to the lumen. This remains to be elucidated in future studies. Furthermore, desquamated tubular cells containing ADAMTS13 were found in

patient tissue. We propose that the ADAMTS13 detected in urine from the patients with tubular damage is possibly a combination of ADAMTS13 originating from the desquamated cells as well as ADAMTS13 released from damaged tubular cells.

Besides their role in regulating water and electrolyte balance renal tubular cells seem to possess an important function in regulating coagulation/thrombosis and fibrinolysis in the kidney, as indicated by their ability to express a variety of proteins involved in these systems. Tubular epithelial cells have been shown to express both protein C and protein C inhibitor [26, 27], tissue factor and tissue factor inhibitor [28, 29] as well as urokinase-type plasminogen activator [30]. Chronic or acute kidney disease characterized by tubular and interstitial damage such as ischemic tubular necrosis and experimental lupus nephritis are often associated with fibrin deposition in the peritubular capillaries and along the tubular basement membrane [29]. Protein C expression in kidneys from mice with lupus nephritis revealed decreased levels of protein C mRNA in renal tubuli and lipopolysaccharide injected intraperitoneally in healthy mice lead to a transient decrease in protein C mRNA in tubular epithelial cells [27]. Furthermore, Sappino et al [30] demonstrated that murine tubular epithelial cells produced urokinase-type plasminogen activator that, besides its function in fibrinolysis, participated in extracellular proteolysis in tissue remodelling and cellular invasion. It is possible that ADAMTS13 expression by tubular epithelial cells may play a part in regulation of hemostasis, however, recent studies have shown that tubular cells also produce and release matrix metalloproteinases, and their inhibitors, found to be active in tissue remodelling [31]. Thus ADAMTS13 may have an alternate function, other than cleavage of VWF, possibly related to glomerular or tubular remodelling.

In a previous study [16] we demonstrated ADAMTS13 expression in podocytes and glomerular endothelial cells. Podocytes were found to secrete bioactive protease. We suggested that podocyte-derived ADAMTS13 may provide a mechanism by which secretion of the protease into the glomerular basement membrane protects glomeruli from the formation of platelet thrombi under local conditions of high shear stress and endothelial cell injury. We speculate that tubular cells could possibly secrete ADAMTS13 basally into peritubular capillaries providing a local protective mechanism against formation of thrombi.

The findings observed in the present study indicate that tubular epithelial cells are able to express proteolytically active ADAMTS13. Immunohistochemical studies of ADAMTS13 protein expression *in situ* in renal tubuli revealed that the pattern of expression may be altered with apical concentration in renal tissue from patients with tubular damage as compared to a more diffuse expression in normal tissue. In patients with tubular damage ADAMTS13 is released into the urine. Thus tubuli synthesize biologically functional ADAMTS13 possibly regulating hemostasis in the local vascular microenvironment.

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Table 1: Patients included in this study

Patient number	Sex/ Age (yrs)	Diagnosis	Evidence of tubular damage	Urine Albumin	Renal function	Biopsy finding	Urine sample available
1	M/ 10	Mild chronic tubulopathy of unknown etiology	Urine-NAG[1]: 2.2 urine α1- microglobulin[2]: 32	0.02[3]	Normal	NA	Yes
2	M/ 7	Fanconi's syndrome induced by chemotherapy and radiotherapy	Urine NAG[1]: 4	17[4]	Reduced	Tubular interstitial damage without glomerular pathology	Yes
3	F/ 4	Sodium valproate-induced tubulopathy, Fanconi's syndrome	Urine α1- microglobulin[2]: 79	0	Reduced	NA	Yes
4	F/ 18	Acute tubular damage of unknown etiology	Urine α1- microglobulin[2]: 3.7	38[4]	Reduced	Acute tubular necrosis without glomerular pathology	Yes
5	F/ 2	Small dysplastic kidneys, Fanconi's syndrome autosomal dominant family history of tubular affection	Urine α1- microglobulin[2]: 36	78[4]	Reduced	Chronic tubular necrosis, dilated tubuli and desquamated tubular epithelial cells, interstitial fibrosis. Minimal mesangial proliferation	Yes
6	F/ 9	Ifosfamide-induced tubular toxiciy	Glucosuria and tubular proteinuria	0	Reduced	Prominent tubulo-interstitial changes with inflammatory infiltrates and edema	No

NA; not available. 1; NAG: N acetyl β-*D*-glucosamine, reference value < 0.6 u/mmol creatinine. 2; reference value of urine α1- microglobulin < 0.7 g/mol. 3; assayed by albumin/creatinine clearance, reference value < 0.01 x 10^3. 4; assayed by albumin/creatinine index, reference value <3.8 g/mol.

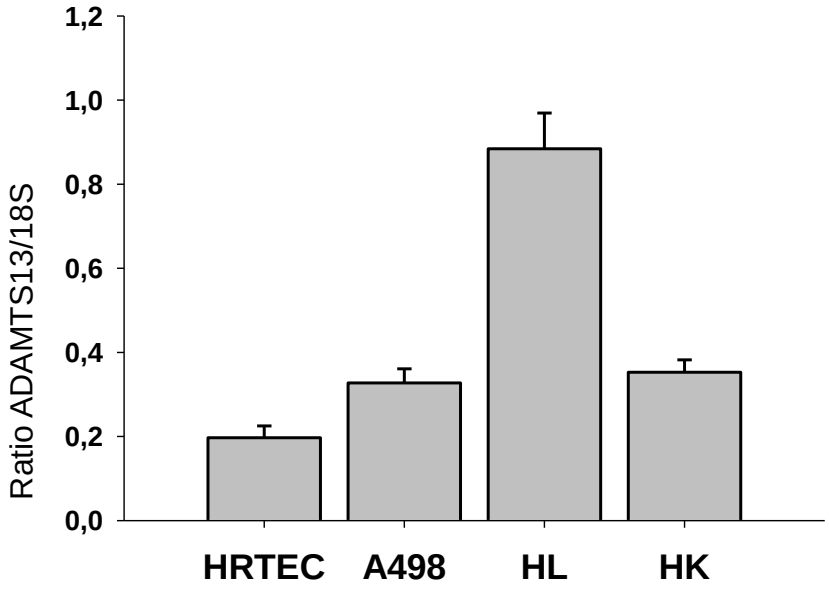

Figure 1

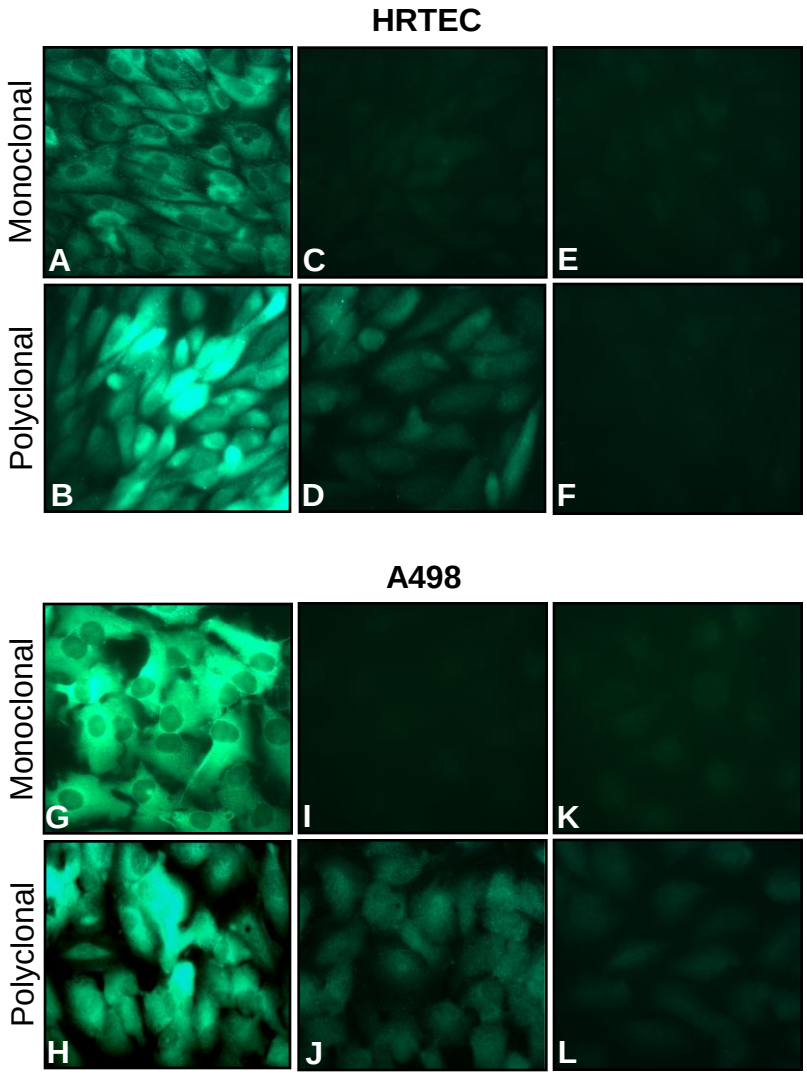
HRTEC
Monoclonal
Polyclonal
A
C
E
B
D
F
A498
Monoclonal
Polyclonal
G
I
K
H
J
L

Figure 2

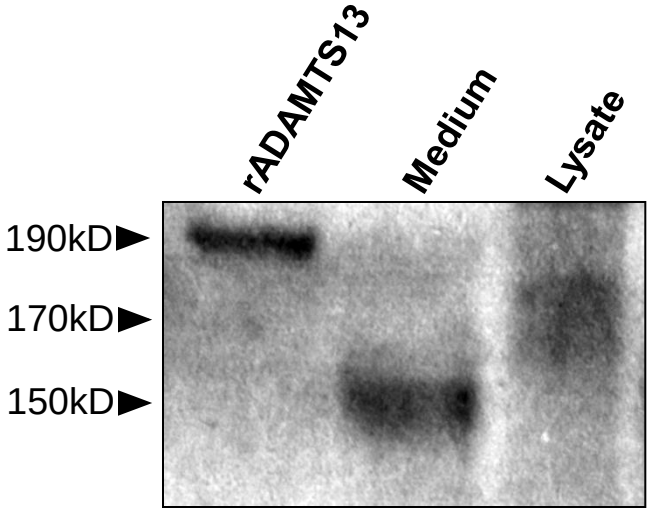

Figure 3

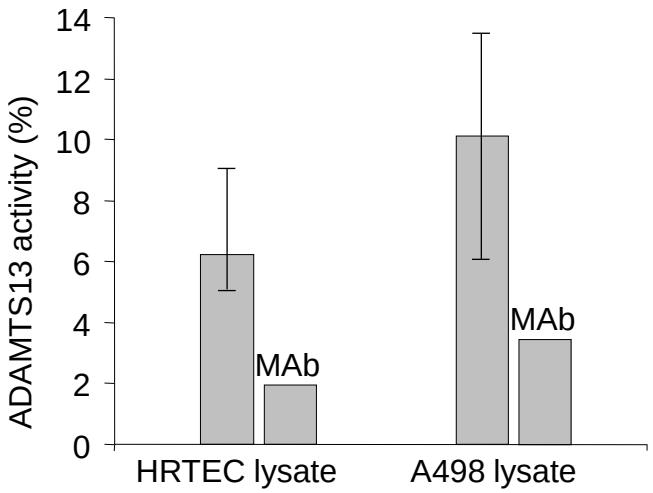
ADAMTS13 activity (%)
14
12
10
8
6
4
2
0
MAb
MAb
HRTEC lysate
A498 lysate

Figure 4

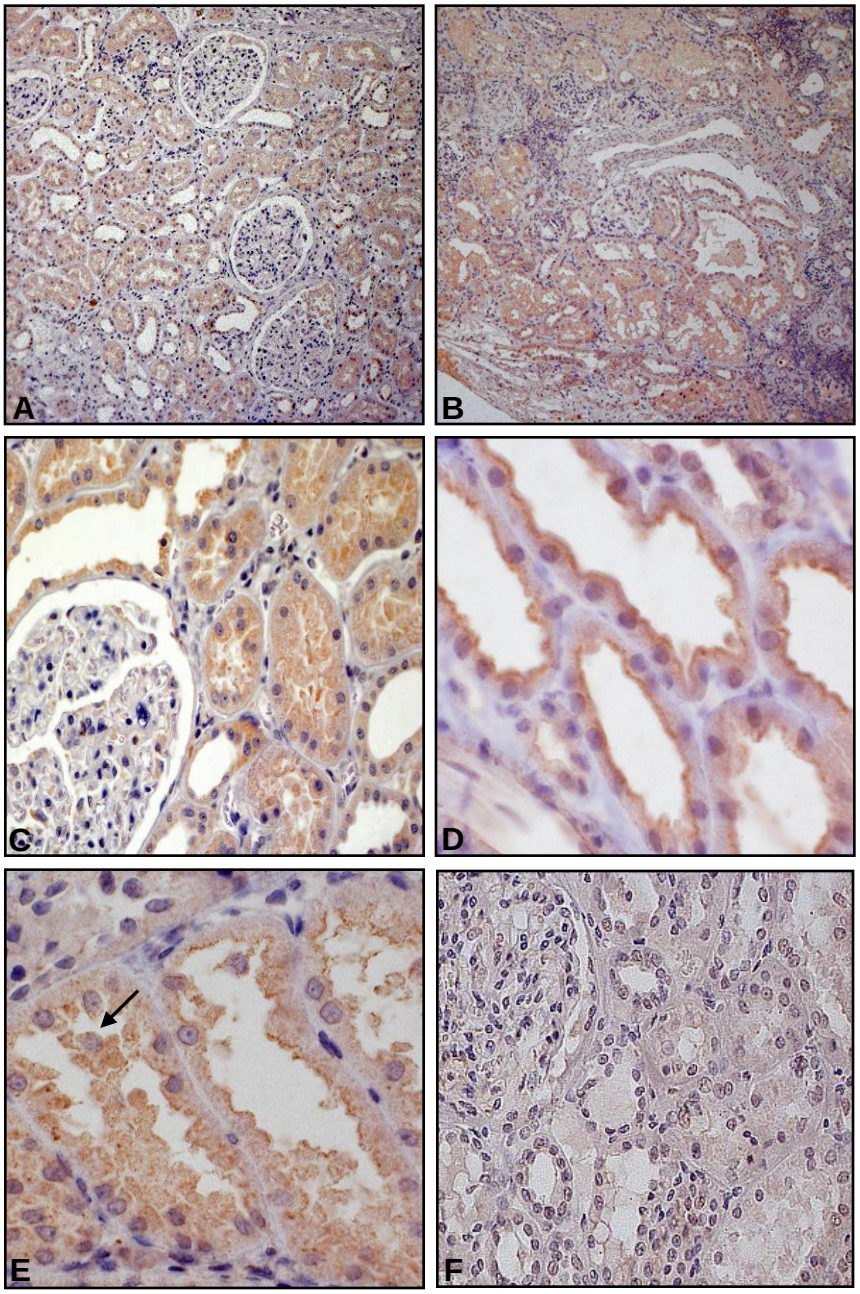

Figure 5

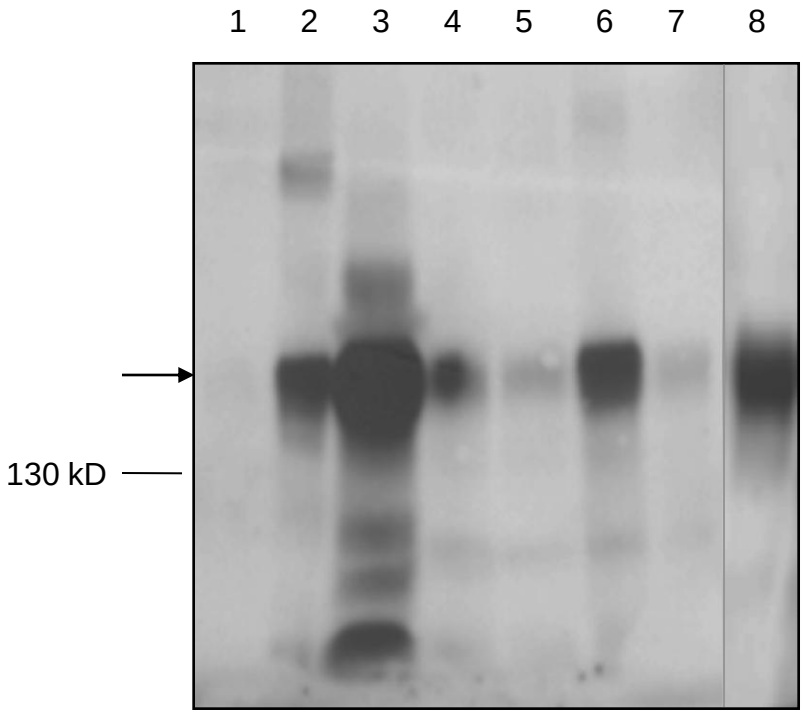

Figure 6

Figure legends

Figure 1. *ADAMTS13* mRNA detected in cultured renal tubular epithelial cells

mRNA from HRTEC and A498 cells was available from two separate cell experiments. Human liver and human kidney were used as the positive controls. The data was standardized against the housekeeping gene 18S, which was expressed at comparable levels in all samples. Results were run three times using cells from two experiments and are presented as mean and range. HL: human liver. HK: human kidney.

Figure 2. ADAMTS13 protein expressed in cultured renal tubular epithelial cells

ADAMTS13 protein was detected in HRTEC cells (upper panel) using the A10 monoclonal antibody (A) and the SU19 polyclonal antibody (B). The mouse control antibody (C) resulted in lack of signal and preincubation of the polyclonal antibody with blocking peptide resulted in marked decrease in signal intensity (D). The secondary antibodies alone were negative (E) and (F). The A498 cell line showed comparable results (lower panel). Both the A10 monoclonal antibody and the SU19 polyclonal antibody revealed a positive signal (G) and (H). The mouse control antibody was negative (I). Blocking of the polyclonal antibody with the peptide it was raised against resulted in marked decrease in signal intensity (J). Omission of the primary antibodies resulted in lack of signal (K) and (L). Reproducible results were obtained in at least three experiments performed on slides coated with cells from two separate experiments with each cell type. All images at 600x magnification.

Figure 3. ADAMTS13 detected in HRTEC lysates and media.

Purified recombinant human ADAMTS13 was used as the positive control and revealed a band at 190 kD under reducing conditions as expected [11]. Lysate and medium from HRTEC cells were run on the same gel and exhibited a band at 170 and 150 kD, respectively. Experiments were carried out three times on samples from four cell experiments, with reproducible results.

Figure 4. HRTEC and A498 cells exhibit ADAMTS13 VWF-cleaving activity

HRTEC and A498 cell lysates, respectively, were incubated with FRETS-VWF73 and revealed mean activity values of 6.24 % (range 4.90 - 9.16 %) for HRTEC cells and 10.12 % (range 6.05 - 13.50 %) for A498 cells. Experiments were carried out twice using cells from two separate experiments with each cell type. Normal plasma was used as the reference standard. Specificity of the assay was tested by preincubation with the A10 monoclonal antibody [29] which has been shown to inhibit ADAMTS13 activity [14]. Activity values decreased to a mean of 1.93 % (range 0 - 3.86 %) for HRTEC cells and 3.44 % for A498 cells.

Figure 5. ADAMTS13 expression in normal renal cortex and in patients with tubulopathy.

ADAMTS13 antigen was detected in all tubular cells (both proximal and distal) in normal renal cortex (A) and renal cortex from patient 4 with a tubular disorder (B). Tubular epithelial cells in normal renal tissue expressed ADAMTS13 diffusely in the cell cytoplasm (C). In renal tissue from patients with tubulopathy the signal was either concentrated apically (D and E; patients 4 and 5, respectively) or expressed diffusely in the tubular cells (E; patient 5). The tissue from patient 5 also exhibited desquamated tubular cells staining positively for

ADAMTS13 (E, see arrow). The control antibody incubated with normal kidney did not exhibit staining (F). Magnifications: A - B at 100x, C and F at 400x and D-G at 600x.

Figure 6. ADAMTS13 in urine

ADAMTS13 protein was detected in urine from patients with tubular damage by immunoblotting with the A10 monoclonal antibody. Normal urine was negative (lane 1), normal plasma used as the positive control (lane 2) showed an immunoreactive band at approximately 150 kD. Urine from a patient with nephrotic syndrome was used for comparison and exhibited a strong immunoreactive band (lane 3). In addition, a weaker band at 150 kD was detected in the urine sample from patients 1 - 5 with tubular damage (patient 1, lane 4; patient 2, lane 5; patient 3, lane 6; patient 5, lane 7; patient 4, lane 8). The urine sample from patient 4 was run on a separate gel depicted by the separating line.