ADAM17 up-regulation in renal transplant dysfunction and non-transplant-related renal fibrosis

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Abstract

Background. Interstitial fibrosis and tubular atrophy (IF/TA) is an important cause of renal function loss and ischaemia–reperfusion (I/R) injury is considered to play an important role in its pathophysiology. The aim of the present study was to investigate the role of a disintegrin and metalloproteinase 17 (ADAM17) in human renal allograft disease and in experimental I/R injury of the kidney.

Methods. We studied the expression of ADAM17 messenger RNA (mRNA) in IF/TA and control kidneys by reverse transcription–polymerase chain reaction and in situ hybridization. Moreover, we assessed ADAM17-mediated heparin-binding epidermal growth factor (HB-EGF) shedding in immortalized human cells. Finally, we studied the effect of pharmacological ADAM17 inhibition in a model of renal I/R injury in rats.

Results. ADAM17 mRNA was up-regulated in IF/TA when compared to control kidneys. In normal kidneys, ADAM17 mRNA was weakly expressed in proximal tubules, peritubular capillaries, glomerular endothelium and parietal epithelium. In IF/TA, tubular, capillary and glomerular ADAM17 expression was strongly enhanced with de novo expression in the mesangium. In interstitial fibrotic lesions, we observed co-localization of ADAM17 with HB-EGF protein. In vitro, inhibition of ADAM17 with TNF484 resulted in a dose-dependent reduction of HB-EGF shedding in phorbol 12-myristate 13-acetate-stimulated cells and non-stimulated cells. In vivo, ADAM17 inhibition significantly reduced the number of glomerular and interstitial macrophages at Day 4 of reperfusion.

Conclusions. In conclusion, HB-EGF co-expresses with ADAM17 in renal interstitial fibrosis, suggesting a potential interaction in IF/TA. Targeting ADAM17 to reduce epidermal growth factor receptor phosphorylation could be a promising way of intervention in human renal disease.

Keywords: ADAM17; EGF receptor; fibrosis; IF/TA; ischaemia–reperfusion injury

Introduction

Interstitial fibrosis and tubular atrophy (IF/TA), a non-specific entity, characterized by renal structural deterioration and progressive renal function loss, is an important cause of morbidity and mortality worldwide [1]. Patients with IF/TA may ultimately develop end-stage renal disease (ESRD), leading to the need for kidney replacement therapy. The incidence and prevalence of ESRD and IF/TA have increased dramatically over the last decades due to ageing of the population, reduced cardiovascular mortality of patients with IF/TA and an increasing incidence of type 2 diabetes and obesity [2]. Within the pathophysiology of IF/TA, ischaemia–reperfusion (I/R) injury, an inevitable event in kidney transplantation, affects both short-term and long-term allograft outcome [3, 4]. The initial event in I/R injury is acute ischaemia, which activates the endothelium, thereby leading to increased vascular permeability, expression of adhesion molecules, complement activation and transcription of pro-inflammatory genes [5–7]. Reperfusion inflicts additional damage, as attracted leucocytes adhere to the endothelium and release reactive oxygen species as well as additional pro-inflammatory cytokines, thereby enhancing the inflammatory response. At later stages, pro-fibrotic processes join the inflammatory environment. Despite its intention to maintain kidney integrity, reparative fibrosis contributes to loss of functional tissue. The balance between reparative fibrosis and loss of functional tissue determines the state of renal allograft outcome [8, 9].

ADAM17 (a disintegrin and metalloproteinase 17; also known as TACE) is a key mediator of cellular processes by means of its function in protein ectodomain shedding, which is the proteolytic release of cytokines, growth factors and receptors from their cell membrane-bound precursors. First discovered as the sheddase of tumour necrosis factor-α, ADAM17 was considered a positive regulator of
inflammation [10]. Later studies revealed that ADAM17 is also responsible for shedding epidural growth factor receptor (EGFR) ligands such as transforming growth factor-α (TGF-α) and heparin-binding epidural growth factor (HB-EGF) [11], which links ADAM17 to proliferation and fibrosis via EGFR signalling [12]. Together, ADAM17 is implicated in both pro-inflammatory and pro-fibrotic processes, which positions ADAM17 as a possible target of intervention in a variety of diseases. Intriguingly, in vivo pharmacological ADAM17 inhibition was already shown to attenuate hepatic I/R injury in rats [13]. Moreover, ADAM17 inhibition reduced renal fibrosis in angiotensin II-induced kidney disease in mice [14] and reduced cardiac fibrosis in angiotensin II-induced heart disease in rats [15]. Furthermore, we recently showed that absence of HB-EGF and blockade of the EGFR protects against renal damage, supporting a modulating role for the HB-EGF/ADAM17/EGFR axis in early I/R injury [16].

Here, we investigated the extent and site of expression of ADAM17 in human renal allograft and non-transplant-related renal fibrosis. We furthermore assessed ADAM17-mediated growth factor shedding in cultured human kidney (HK2) cells. We finally tested the hypothesis that ADAM17 activity is detrimental in the process of I/R injury, by studying the effect of pharmacological ADAM17 inhibition in a model of renal I/R injury in rats.

Materials and methods

Human tissue

Human renal tissue was collected from the tissue archive at the Department of Pathology of the University Medical Center Groningen, the Netherlands. Frozen kidney tissue for RNA extraction was available from patients with IF/TA (nephrectomies, n = 13) and from control kidneys (unaffected part of kidneys with renal cell carcinoma and unused donor kidneys, n = 10). Paraffin-embedded biopsy tissue was available from patients with a renal allograft without histological abnormalities (Tx-NA) (n = 24) (biopsies were taken under clinical suspicion of rejection), acute allograft rejection (AR) (n = 28), IF/TA (n = 32) and non-transplant interstitial fibrosis (non-Tx IF) (n = 12). Paraffin-embedded control kidney tissue (n = 14) was obtained from the unaffected part of kidneys with renal cell carcinoma or from unused donor kidneys. Diagnosis was performed on the basis of histological alterations according to the Banff ’97 classification of renal allograft pathology and updates [17, 18]. All procedures and use of anonymized tissue were performed according to national ethical guidelines.

Real-time reverse transcription-polymerase chain reaction

RNA was extracted from frozen HK2 biopsies using the TRIZOL method (Invitrogen, Carlsbad, CA). DNase treatment was performed using Turbo DNase-free (Ambion, Austin, TX). Complementary DNA (cDNA) was synthesized using Superscript II RT and random hexamer primers (Invitrogen). A relative quantification polymerase chain reaction (PCR) was performed to determine gene expression (Applied Biosystems, Foster City, CA). HPRT was used as housekeeping gene, primer forward: 5'-GGGAGG-TATAATCCAAAGATGGTCAA-3'; primer reverse: 5'-GTTCTGGCT- TTATATCCAACACTTGCT-3' (Invitrogen) and probe: 6-FAM 5'-CAAGCTTGCTGTTGGAAGGACCTCC-3' TAMRA (Eurogentec, Liege, Belgium). For ADAM17, we used the gene expression assay (Applied Biosystems). PCR was performed in a total volume of 20 µL containing 10 ng cDNA template and 10 µL PCR-mastermix (Eurogentec). The thermal profile was 15 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. The average Ct values for the target gene ADAM17 were subtracted from the average housekeeping gene Ct values to yield the delta Ct. Results were finally expressed as 2-ΔΔCt, which is an index of the amount of gene expression in each group.

RNA in situ hybridization

ADAM17 messenger RNA (mRNA) was detected using RNA in situ hybridization (ISH). ADAM17-FR PCR product of 518 bp was subcloned in pCRII-TOPO vector (Invitrogen). Insert lengths were routinely checked by [1] amplification with M13 forward and M13 reverse primers and [2] restriction enzyme analysis with EcoR1. Orientation of the PCR product was determined by PCR using ADAM17-specific primers in combination with vector specific M13F and M13R primers, confirmed by sequence analysis of the ADAM17 construct [ADAM17 primer sequence: AGT.TCT.TGT.GTT.TGGA.CC (forward); ACT.GGC.TAC.CAT.GTA.TAG.CC (reversed)]. Deparaffinized sections were air-dried, treated with Triton X-100, followed by proteinase K (5 µg/mL in Tris-buffered saline) (Roche, Woerden, The Netherlands) at 37°C for 20 min, washed with phosphate-buffered saline (PBS) and incubated with 10 ng/µL DIG-labelled probe (anti-sense Sp6 or sense T7) in a hybridization solution consisting of 100 µL 50X Denhardt’s solution, 1 mL 20X sodium saline citrate (SSC), 1 mL 50X Dextran sulfate, 2.5 µL formamide, 200 µL (25 mg/mL) transfer RNA, 49 µL 1 M dithiorthiol and 125 µL (10 mg/mL) salmon sperm DNA overnight at 55°C. After washing, slides were treated with 2 U/mL RNase T1 (Sigma–Aldrich, Zwijndrecht, The Netherlands) in 1 mM ethylenediaminetetraacetic acid (pH 8.0) and 2X SSC at 37°C for 30 min. Positive cells were visualized with anti-DIG labelled alkaline phosphatase (Roche) for 1 h at 37°C in 0.1 M maleic-acid buffer containing 0.15 M NaCl, 1% blocking buffer and 2% normal sheep serum. Staining reaction was performed with NBT and BCIP (Roche) in 1 mL AF buffer (pH = 9.0) containing 50 mM MgCl2 and 0.01 M levamisole for 1 h at 37°C, followed by 16 h at 4°C.

Renal ADAM17 mRNA localization and staining intensity as obtained by ISH was scored semi-quantitatively (0–4) as described previously [19], with 0 indicating no staining, 1 indicating weak staining, 2 indicating moderate staining, 3 indicating strong staining and 4 indicating very strong staining.

Immunofluorescence staining

Formalin-fixed paraffin-embedded or frozen tissue sections (4 µm) from control kidney (n = 4), IF/TA (n = 4) and non-Tx IF (n = 3) tissues were used. For detection of endogenous HB-EGF protein, tissue sections were fixed in acetone for 10 min and incubated with goat anti-human HB-EGF antibody (R&D Systems, Wiesbaden-Nordstadt, Germany; 1:50; dilution in PBS/1% bovine serum albumin (BSA)) for 1 h, followed by sequential incubations of 30 min with rabbit anti-goat FITC antibody (DAKO, Glostrup, Denmark; 1:25) and goat anti-rabbit FITC antibody (DAKO; 1:25), diluted in PBS with 1% normal human serum. Slides were then incubated with 5% normal goat serum (Sigma–Aldrich) for 30 min. ADAM17 was detected using a rabbit anti-human ADAM17 polyclonal antibody (Chemicon International, Temecula, CA, US; 1:200; dilution in PBS/1% BSA) after incubation for 1 h, followed by goat anti-rabbit-TRITC antibody (DAKO; 1:25, diluted in PBS) for 30 min. Nuclei were stained with 4,6-diamidino-2-phenylindole and sections mounted with citifluor (Agar Scientific, Stansted, UK). PBS replacing the primary antibodies was used as a control of staining specificity. Fluorescence microscopy was performed using a DMLB microscope, DC300F camera and Qwin 2.8 software (Leica Microsystems).

To detect binding of exogenously added HB-EGF, formalin-fixed paraffin-embedded renal tissue sections were deparaffinized and rehydrated and incubated with recombinant human HB-EGF (2.5 µg/mL; R&D Systems) for 2h at room temperature (RT), followed by anti-human basic FGF (Sigma–Aldrich) for 1 h and AlexaFluor-labelled anti-mouse IgG secondary antibody for 45 min at RT. Isotype-matched non-relevant antibodies served as background controls and proved to be negative. Sections were examined using a Nikon Eclipse E800 fluorescence microscope with digital camera using identical microscopy conditions and exposure time for different samples [20].

Cell-based ADAM17 shedding activity

The shedding activity of ADAM17 was studied in immortalized human mesangial cells [21]. Cells were seeded in 24-well plates until 90% confluence. Culture medium was then refreshed, and cells were stimulated with phorbol 12-myristate 13-acetate (PMA) (100 ng/mL) for 4 h. Before stimulation, cells were pre-incubated for 10 min with or without the pharmacological compound TNF484 (described as compound 5 in [22]); a kind gift from Novartis Pharma, Bern, Switzerland). The conditioned media
were harvested and a commercially available enzyme-linked immunosorbent assay (ELISA) was performed according to manufacturer’s guidelines to quantify the amount of HB-EGF (R&D systems, Minneapolis, MN; sensitivity 8.4 pg/mL). Experiments were performed at least three times in triplo.

**Rat model of renal I/R**

Male Wistar rats (n = 36) (Harlan, Horst, The Netherlands; 250–300 g) with free access to water and rodent chow were subjected to unilateral renal I/R as follows. Anaesthesia with isoflurane (3%) enabled a left-flank incision with dissection of the left renal vessels, which were clamped with twoatraumatic clips for 45 min (ischaemia time). After removing the clips, reperfusion of the kidney was confirmed visually and the muscle and skin layers were sutured. Rats were randomly assigned to six study groups (six rats per group), relating to time of sacrifice (after 1, 4 or 14 days of reperfusion) and treatment (with or without TNF484 for each time point). The treatment group received a daily dose of 30 mg TNF484 per kilogram bodyweight in a solution of 700 mL, which was administered via oral gavage. Animals in the control group received water only. Treatment was initiated 1 day before induction of ischaemia. Animals were sacrificed by exsanguination under anaesthesia with isoflurane 3%. The kidneys were then perfused with saline and harvested. A mid-coronal slice of the kidneys was fixed in 4% paraformaldehyde and processed for paraffin embedding for immunohistochemical usage. The responsible ethics committee of the University Medical Center Groningen approved animal experiments.

**Assessment of I/R injury**

To assess the extent of renal ischaemia, Periodic Acid-Schiff staining was performed on the ischaemic–reperfused kidneys. Using light microscopy, the ischaemic area of the renal cortex was measured in relation to the total area of renal cortex (length of ischaemia/total length). For each kidney, we started to measure from the inner cortex outward in eight directions and used the mean of eight measurements to express one ischaemia score per kidney. To further characterize the histological changes in the ischaemic–reperfused kidneys, we performed immunohistochemical staining for ED-1 (Serotec Ltd, Oxford, UK) as marker for macrophages, α-smooth muscle actin (SMA) as marker of profibrosis (Sigma Chemical Co, St Louis, MO) and collagen III (Biogenesis Inc., Sandown, NH) as marker of fibrosis. Sections (4 μm) were deparaffinized in xylol and progressively hydrated. Antigen retrieval was performed by overnight incubation in 0.1 M Tris/HCl buffer, pH 9.0 at 80°C.

**Results**

**Patient and renal tissue characteristics**

Patient clinical characteristics and renal tissue histological changes [for ISH analysis and reverse transcription–polymerase chain reaction (RT–PCR)] are presented in Table 1. When compared to patients with normal renal tissue, patients with Tx-NA, AR and IF/TA were significantly older (P < 0.05). IF/TA patients had significantly higher levels of serum creatinine and IF/TA score between the different renal patients groups and the control group. IF/TA patients had significantly higher blood pressure levels compared to the control group (P < 0.05). All patient groups had significantly elevated levels of serum creatinine (P < 0.05) when compared to normal renal tissue. IF/TA score were significantly higher in AR, IF/TA and non-Tx IF (P < 0.05) compared to control group.

The IF/TA patients (frozen renal tissue used for RT–PCR) had significantly higher levels of serum creatinine compared to control patients (P < 0.01). Furthermore, IF/TA patients showed significant higher IF/TA scores when compared to patients with normal kidney tissue (P < 0.01).

<table>
<thead>
<tr>
<th>Renal tissue</th>
<th>n</th>
<th>Age (years)</th>
<th>Sex</th>
<th>MAP (mmHg)</th>
<th>Serum creatinine (mg/L)</th>
<th>Proteinuria (g/day)</th>
<th>IF/TA score</th>
<th>Banff score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>68 (29–84)</td>
<td>9/3</td>
<td>87 (78–90)</td>
<td>91 (61–303)</td>
<td>0.6 (0–0.8)</td>
<td>0 (0–3)</td>
<td>97–07</td>
</tr>
<tr>
<td>TX-NA</td>
<td>24</td>
<td>51 (18–70)**</td>
<td>19/5</td>
<td>100 (74–120)</td>
<td>223 (105–1229)*</td>
<td>0.3 (0.0–1.7)</td>
<td>1 (0–1)</td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>28</td>
<td>45 (25–66)**</td>
<td>16/12</td>
<td>100 (67–137)</td>
<td>252 (127–1021)**</td>
<td>0.4 (0.0–11.3)</td>
<td>1 (0–2)*</td>
<td>3 (0–5)</td>
</tr>
<tr>
<td>IF/TA</td>
<td>32</td>
<td>45 (11–69)**</td>
<td>20/12</td>
<td>103 (80–153)*</td>
<td>276 (125–730)**</td>
<td>1.6 (0.0–9.4)</td>
<td>2 (1–3)**</td>
<td></td>
</tr>
<tr>
<td>Non-Tx IF</td>
<td>12</td>
<td>60 (20–79)</td>
<td>6/6</td>
<td>102 (84–133)</td>
<td>394 (80–632)**</td>
<td>1.1 (0.3–11.6)</td>
<td>2.5 (1–3)**</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>53 (5–73)</td>
<td>5/5</td>
<td>109 (87–150)</td>
<td>176 (44–762)##</td>
<td>N/A</td>
<td>0 (0)##</td>
<td></td>
</tr>
<tr>
<td>IF/TA</td>
<td>13</td>
<td>43 (27–55)</td>
<td>7/6</td>
<td>116 (93–153)</td>
<td>608 (294–1043)##</td>
<td>8.0 (8.0–18.0)</td>
<td>2.5 (1–3)</td>
<td></td>
</tr>
</tbody>
</table>

*Values are expressed as median and range. m/f, male–female ratio; MAP, mean arterial pressure; IF, interstitial fibrosis; TX-NA, functioning renal allograft without histological abnormalities; AR, acute allograft rejection.

**P < 0.05, **P < 0.01, ***P < 0.001 compared to control (for ISH analysis).

##P < 0.001, ###P < 0.001 compared to control (for RT–PCR).
No significant differences were found in other clinical characteristics.

**ADAM17 mRNA expression and localization in normal kidneys and allograft disease**

ADAM17 mRNA as assessed by RT–PCR was almost 7-fold up-regulated in renal IF/TA when compared to normal kidney tissue (8.104 ± 1.884 versus 1.277 ± 0.285, respectively, P < 0.001). In normal kidneys, ADAM17 mRNA was absent in the glomerular mesangium and weakly expressed in the glomerular endothelium and parietal epithelium as assessed by ISH (Figure 1A). In contrast, these renal structures showed strong ADAM17 mRNA expression in IF/TA (Figure 1B), Tx-NA, AR and non-Tx IF. The glomerular visceral epithelium stained very strongly positive for ADAM17 mRNA in both control kidney tissue and in renal disease states (Figure 1A and B), as did the distal tubular cells (Figure 1C–F). Proximal tubular cells and peritubular capillaries were weakly positive in control kidney tissue (Figure 1C), whereas strong expression was detected in IF/TA (Figure 1D) as well as in Tx-NA, AR (Figure 1E) and non-Tx IF (Figure 1F).

In the glomerular parietal epithelium, endothelium (Figure 2A), mesangium (Figure 2B), peritubular capillaries (Figure 2C), proximal tubules (Figure 2D) and vascular endothelium, ADAM17 mRNA, as assessed by semi-quantitative scoring, showed up-regulated expression in Tx-NA, AR, IF/TA and non-Tx IF when compared to normal kidneys. In the glomerular visceral epithelium and distal tubules, no differences were detected between the kidney diagnosis groups.

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**Fig. 1.** Photographs are representative for the various renal diseases, magnification ×400. In normal kidneys, the glomerular endothelium (closed arrow) and parietal epithelium showed weak ADAM17 mRNA expression, whereas the mesangium (open arrow) was negative (A). In contrast, these renal structures showed strong ADAM17 mRNA expression in IF/TA (B). The glomerular visceral epithelium stained strongly for ADAM17 mRNA in all tissue samples of normal kidneys (A) and IF/TA (B). The distal tubules of normal kidneys (C) and of Tx-NA, IF/TA (D), AR (E) and non-Tx IF (F) were strongly stained for ADAM17 mRNA. Proximal tubular cells and peritubular capillaries (white closed arrow) were weakly positive in control kidney tissue (C), whereas strong expression was detected in Tx-NA, IF/TA (D), AR (E) and non-Tx IF (F).
ADAM17 co-localizes with HB-EGF in interstitial fibrotic lesions

HB-EGF protein was not detected in control kidneys (Figure 3A) but was detected in interstitial fibrotic lesions in IF/TA and co-localized with ADAM17 protein (Figure 3B), confirming the ADAM17 mRNA expression pattern as identified by RNA ISH. Binding of exogenously added HB-EGF was extensive in interstitial matrix of control kidneys and in the expanded interstitial matrix in IF/TA, in addition to a number of interstitial cells (Figure 3C). A similar binding pattern was observed in non-Tx IF biopsies (data not shown). These data demonstrate that ADAM17 co-localizes with HB-EGF and free HB-EGF can be bound in the interstitial matrix of renal tissue to exert its function (data not shown).

ADAM17 mediates HB-EGF shedding in cultured human mesangial cells

We next examined whether shedding of HB-EGF could be inhibited by TNF484, a known inhibitor of ADAM17 activity. In cultured human mesangial cells, PMA stimulation for 4 h induced a 4-fold increased shedding of HB-EGF when compared to baseline shedding. Inhibition with TNF484 resulted in a dose-dependent reduction of shedding, up to 82% in PMA-stimulated cells (P < 0.005) and up to 53% in non-stimulated cells (P < 0.005) (Figure 4). HB-EGF was not detected in cell culture medium, confirming the cellular source of HB-EGF. No TGF-α shedding from human mesangial cells was detected under any of the studied conditions (data not shown).

ADAM17 inhibition does not attenuate ischaemia or fibrosis in rat I/R injury

To confirm tissue TNF484 availability after daily administration in the rat model of unilateral I/R injury, we analysed all serum samples and kidneys (obtained at time of sacrifice) for TNF484. Consistent with our experimental set-up, all rats that had received TNF484 showed detectable levels of TNF484 in serum and kidney tissue at levels comparable to a previous study [22], while no TNF484 was detected in the vehicle-treatment group (data not shown).

Unilateral ischaemia for 45 min resulted in ischaemic damage of the renal cortex, as evidenced by widespread tubular necrosis. After Day 1 of reperfusion, no difference in ischaemia (length of cortical ischaemia: total cortical length) was detected between vehicle- and TNF484-treated rats (data not shown). The number of glomerular and interstitial macrophages, serving as marker for renal inflammation, did not change between treatment groups on Day 1 and Day 14 after of reperfusion. However, a significantly lower number of glomerular macrophages [0.84 (0.78–1.11) versus 1.14 (0.95–1.18); P < 0.05] (Figure 5A–C) and interstitial macrophages [45.4 (40.9–58.2) versus 65.3 (42.4–75.2); P < 0.05] (Figure 5D–F) was detected at Day
of reperfusion in the ADAM17 inhibition group. The α-SMA-positive area, serving as marker for profibrosis, and the interstitial collagen III-positive area, serving as marker for fibrosis, did not significantly change between the groups receiving TNF484 or vehicle after any of the reperfusion times (data not shown).

**Discussion**

In the present study, we detected marked ADAM17 up-regulation in human IF/TA when compared to normal human kidneys. Tissue expression analysis revealed increased ADAM17 in the glomerular endothelium, mesangium, proximal tubules and peritubular capillaries of Tx-NA, AR IF/TA and non-Tx IF kidneys. Additionally, ADAM17 co-localized with HB-EGF in renal fibrotic lesions, and ADAM17 inhibition reduced HB-EGF shedding from cultured human mesangial cells. In vivo, daily pharmacological ADAM17 inhibition in a rat model of renal I/R injury decreased inflammation, yet renal pro-fibrotic changes were not attenuated at these early time points.

In our analysis of ADAM17 expression in human renal allografts without histological abnormalities, acute allograft rejection, IF/TA and non-Tx IF, we found no clues towards specific ADAM17 regulation in relation to any of the diagnosis groups. Instead, ADAM17 was generally up-regulated in diseased tissue, such as the glomerular mesangium, proximal tubules and peritubular capillaries. These cell types contribute to fibrogenesis and are key players in renal pathophysiology. A similar observation was reported for ADAM family member ADAM19, which was up-regulated in human renal disease and was associated with glomerular and interstitial fibrosis throughout renal disease [19].

The postulated mechanism to explain ADAM17's contribution to renal pathophysiology relates to EGFR signalling. Being responsible for the proteolytic release of the majority of EGFR ligands, ADAM17 coordinates the binding to and activation of autocrine, juxtacrine and paracrine EGFRs. Downstream signalling of activated EGFRs, via the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI-3K) signalling pathways, has the potential to guide cellular behaviour towards proliferation, migration and fibrogenesis [23]. ADAM-mediated EGFR activation and downstream signalling occurs in the kidney in vitro in glomerular mesangial cells [24], visceral epithelial cells [25] and proximal tubular cells [26]. In vivo inhibition of individual
components of the signalling pathway may result in an anti-proliferative or anti-fibrotic outcome for the kidney, which has been demonstrated for ADAM17 [14] and the EGFR [27]. In addition, rats treated daily with PKI-166, an EGFR inhibitor, showed an early reduction in inflammation and pre-fibrosis 1 and 4 days after I/R injury [16].

HB-EGF was recognized to promote smooth muscle cell migration and proliferation [28]. Its expression in glomerular mesangial cells was shown to induce the production of fibronectin, which is a critical component of glomerulofibrotic lesions [24]. In patients with various forms of glomerulonephritis, glomerular mesangial HB-EGF was correlated with the grade of mesangial proliferation [29]. Moreover, ADAM17-mediated HB-EGF shedding in mesangial cells induced cellular proliferation following phosphorylation of ERK, a transcription factor downstream of the EGFR [30]. HB-EGF was up-regulated after I/R injury in rats [16, 31], after hypoxia/re-oxygenation in vitro and in human renal living and deceased brain dead transplant donor biopsies [16]. In addition, urinary HB-EGF was increased after kidney transplantation compared to control urine from healthy subjects [16]. In human IF/TA tissue, we identified HB-EGF staining in interstitial fibrotic lesions in co-localization with ADAM17. Moreover, we showed that exogenously added HB-EGF could be bound locally in the interstitial matrix of IF/TA tissue, indicating that the released form of HB-EGF can be stored in the matrix of IF/TA tissue. Supportive of a functional interaction between ADAM17 and HB-EGF, we showed that HB-EGF shedding from human mesangial cells was inhibited by TNF484 in vitro. In cultured human visceral epithelial cells and proximal tubular cells, pharmacological ADAM17 inhibition reduced shedding of TGF-α. Addition of TNF484 to the cell culture media reduced proliferation below the level of non-stimulated proximal tubular cells [32].

A possible link between ADAM17 and fibrosis was established when it was shown that inhibition of ADAM17 reduced angiotensin II-induced renal fibrosis in mice [14]. And, deficiency of TIMP-3, the natural inhibitor of ADAM17, resulted in increased ADAM17 activity with a concomitant increase of renal fibrosis in a model of unilateral ureteral obstruction [33]. Mice lacking HB-EGF that were subjected to I/R injury [16] or TGF-α-deficient mice, that were infused with AngII [14], exhibit largely reduced renal lesions. Daily pharmacological ADAM17 inhibition using the synthetic inhibitor WTACE2 reduced angiotensin II-induced renal fibrosis in mice, which resulted in attenuated renal function [14]. In hepatic I/R injury, ADAM17 inhibition using a recombinant form of TIMP-3 reduced liver histological abnormalities [13]. ADAM17 inhibition with TNF484 has been successfully used to reduce systemic inflammation and associated histological changes after induction of pneumococcal meningitis in mice [34]. Additionally, it was demonstrated that knockdown of ADAM17 by siRNA protected mice from AngII-induced cardiac fibrosis and hypertrophy in models of hypertension [15]. Interestingly, we observed that ADAM17 inhibition in rat I/R injury decreased the number of glomerular and interstitial macrophages significantly at Day 4 of reperfusion. Although macrophages are central denominators of tissue remodelling, it remains to be established whether such an effect is relevant in the context of ADAM17 inhibition. Specifically in renal I/R injury, macrophages have been shown to play a crucial role in mediating persistent inflammation and fibrosis, leading to development of chronic kidney disease [35, 36]. Nevertheless, the reduction in macrophages in ischaemic–reperfused kidneys on Day 4 after I/R did not change renal fibrotic lesions on Day 4 or Day 14 after I/R. It could be that the extent of I/R injury, being maximal in covering the whole renal cortex at
Day 1 after I/R, has been too severe for establishing a positive treatment effect. On the other hand, TNF484 was administered as performed in a previous intervention animal model, and tissue availability of TNF484 was confirmed in kidneys and plasma of treated animals. It must be remarked that TNF484 is a very potent ADAM17 inhibitor at sub-nanomolar concentrations, yet TNF484 could also inhibit activity of some matrix metalloproteinases [22], which should be taken into account when interpreting effects from TNF484 inhibition.

In conclusion, the marked induction of ADAM17 in IF/TA suggests involvement of this protein in interstitial renal damage associated with various conditions related to transplantation and non-transplant-related ischaemic kidney injury. In line with earlier reports linking ADAM17 to fibrosis, ADAM17 and HB-EGF may interact to serve EGFR signalling. The treatment value of targeting ADAM17 within the pathway of early I/R injury was partly corroborated in rat renal I/R injury because daily pharmacological inhibition. The treatment value of targeting ADAM17 within the pathway of early I/R injury was partly corroborated in rat renal I/R injury because daily pharmacological ADAM17 inhibition did have anti-inflammatory effects. Since EGFR signalling plays a role in the development of renal fibrosis, targeting ADAM17 to reduce EGFR phosphorylation could be a promising way of intervention in human renal disease.

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Conflict of interest statement. None declared.

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