Title: Role of ADAM17 in kidney disease.

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ABSTRACT

It is known that the renin angiotensin system (RAS) plays a major role in the pathophysiology of cardiovascular disease and renal injury. Within the RAS, angiotensin converting enzyme 2 (ACE2) cleaves the angiotensin (Ang) II to generate the Ang1-7 peptide which counteracts the adverse effects of AngII accumulation. ACE2 can undergo cleavage or shedding to release the catalytically active ectodomain into the circulation by a disintegrin and metalloprotease (ADAM)17, also known as TNF-α converting enzyme (TACE). ADAM17 is involved in many pathological processes such as cancer, inflammatory diseases, neurological diseases, cardiovascular diseases, atherosclerosis, diabetes and hypertension. Clinical and experimental studies have shown that ADAM17 has been involved in chronic kidney disease (CKD) with a pro-inflammatory and pro-fibrotic role suggesting that it could be an important mediator of CKD progression. ADAM17 inhibition attenuates fibrosis and inflammation suggesting that its inhibition may be a possible new valuable therapeutic tool in fibrotic kidney disease treatment. In addition, in renal disease, some experimental studies demonstrated that ADAM17 is differently expressed in the kidney. Thus, ADAM17 is highly expressed in distal renal tubules and increased in the whole kidney in diabetic models. In this paper we will review the role of ADAM17 under physiologic and pathological conditions. We will mainly focus in the importance of ADAM17 in the context of CKD.

INTRODUCTION

Disintegrins and Metalloproteases (ADAMs) are membrane-anchored cell surface and secreted proteins containing a disintegrin and metalloproteinase domains. The ADAMs play an important role in the regulation of cell phenotype via their effects on cell adhesion, migration and proteolysis and in modulating cell signaling and biological responses (6, 17). In humans, only 13 of the 21 genes in the family encode functional proteases. The functional ADAMs are involved in ectodomain shedding of diverse growth factors, cytokines, receptors and adhesion molecules (17). ADAMs enzymatic activities are controlled by post-transcriptionally tissue inhibitors of metalloproteases (TIMPs) (61). There are some members of ADAMs family associated to different diseases. Among them, ADAM8, ADAM9, ADAM10, ADAM15, ADAM17 or ADAM33 are altered in atherosclerosis (81). ADAM12 has been related to late-onset Alzheimer's disease and breast and bladder cancer. ADAM10 and ADAM17 are the most studied members of the ADAMs family and play important roles in Alzheimer's disease, diabetes, atherosclerosis, inflammatory diseases, cancer, and sepsis (81). ADAM17 is a type I transmembrane protein with an N-terminal signal sequence followed by a prodomain with a cysteine switch-like region (CysSL), a metalloproteinase domain with Zn-binding domain region (Zn-BR) catalytically active, a disintegrin cysteine-rich domain, an EGF-like domain, a single transmembrane domain and a cytoplasmic tail (25, 53, 54, 94). ADAM17 was identified predominantly in two forms, as a full-length inactive protein (100kDa) and as a mature form lacking the pro-domain, the active form (80kDa) (22) (Figure 1).

The ADAM17 zymogen is enzymatically inactive because of the pro-domain interaction with the active site making substrates inaccessible to the catalytic domain. The pro-domain inhibits ADAM17 activity. Maturation of ADAM17 takes place in lipid rafts

that are present in the medial Golgi where furin cleaves ADAM17 pro-domain (79). The cleavage of ADAM17 pro-domain by furin is needed for its activation together with other molecular processes such as phosphorylation events (98). Activation of the ERK or p38-MAPK pathways increases phosphorylation in Thr^{735} at the cytoplasmic domain, whereas growth factor stimulation increases phosphorylation of Ser^{819} and dephosphorylation of Ser^{791} of the pro-ADAM17 (15). Mutation or dephosphorylation of Ser^{791} enhance phosphorylation at Thr^{735} (95). Phosphorylation of Thr^{735} by p38 MAPK or ERK is necessary for ADAM17 maturation and regulates its cell surface levels (95) (Figure 2).

ADAM17 gene is approximately 50kb and contains 19 exons. Chromosomal mapping places ADAM17 on human chromosome 2p25 and mouse chromosome 12 (29, 58). Mouse ADAM17 has a 92% amino-acid identity with the human protein and was ubiquitously expressed (58). ADAM17 was initially described by Black and colleagues to specifically cleave the precursor of tumor necrosis factor α (pro-TNFα) (5). Currently, it is known that ADAM17 can also release ectodomains of a diverse variety of membrane-anchored cytokines, cell adhesion molecules, receptors, ligands, and enzymes, such as, transforming growth factor α (TGFα), L-selectin, and angiotensin-converting enzyme 2 (ACE2) (23, 25, 45, 54, 69). Several substrates for ADAM17 have been involved in many pathological processes such as cancer, inflammatory diseases, neurological diseases, cardiovascular (CV) diseases, atherosclerosis, diabetes and hypertension (45, 54, 57, 67, 68, 92, 97).

Clinical and experimental studies have shown that ADAM17 is involved in chronic kidney disease (CKD) with a pro-inflammatory and pro-fibrotic role suggesting that it could be an important mediator on CKD progression (20, 32, 56, 74). For this reason, in

this review, we aimed to summarize the importance of ADAM17 under physiologic and pathological conditions related to renal function.

THE IMPORTANT ROLE OF ADAM17 IN RAS REGULATION

The renin angiotensin system (RAS) has a crucial role in the regulation of the cardiovascular system and renal function (65). Thus, dysregulations of this system are associated to the development of cardiovascular pathologies, including kidney injury (9). Angiotensin (Ang) II is the main molecular effector in RAS interacting with angiotensin II receptor 1 (AT1R) at the cell surface (43). AngII induces vascular contractility, cellular transport, hypertrophy, growth factors synthesis and ROS generation (96). Angiotensin converting enzyme (ACE) forms AngII from AngI, whereas ACE2 cleaves AngII to form Ang1-7 (33)counterbalancing the AngII-promoting effects of ACE by preventing AngII accumulation in tissues (12, 70). The suppression of the ACE/AngII axis of the RAS minimizes cardiovascular and renal complications (42).

ACE2 is a type I transmembrane glycoprotein with its catalytic site exposed to the extracellular surface (18, 80). When ADAM17 catalyzes ACE2 ectodomain shedding, the compensatory axis of the RAS mediated by ACE2 is compromised (Figure 3). Increased ACE2 ectodomain into the urine has been found in diabetic mice (11, 63, 66, 91) and in patients with chronic kidney disease, in diabetic nephropathy (DN), and in diabetic renal transplant patients (47, 82, 93). Interestingly, experimental studies showed that ACE2 and ADAM17 colocalize in the apical side of the proximal tubule brush-border from diabetic Akita mice (66). Several investigators demonstrated increased renal ADAM17 expression and urinary ACE2 excretion in diabetic mice. These levels were reversed by insulin treatment or insulin sensitizer rosiglitazone

administration (11, 63, 66). Our group demonstrated, in non-obese diabetic (NOD) mice, a model of type 1 diabetes, that ADAM17 expression and circulating ACE2 activity were both increased in diabetic mice as compared to non-diabetic mice. In addition, paricalcitol administration, could be a negative regulator of RAS system exerting a protective role in the diabetic kidney (63).

Moreover, in type 2 diabetic patients, urinary ACE2 activity and protein levels have been found increased as compared with healthy controls (40). The authors speculate that high glucose could increase ADAM17 expression leading to ACE2 shedding from tubular epithelial cells in type 2 diabetic patients.

Different studies demonstrated that ADAM17 was unable to cleave ACE ectodomain suggesting that ADAM17 may be involved only in the ectodomain shedding of ACE2 (26, 35, 38, 66, 84). Using tissue inhibitors of metalloproteinases (TIMPs) proteins, specific ADAM17 inhibitors, Lambert and colleagues have seen that ACE2 shedding in human embryonic kidney (HEK) cells was only inhibited after 15 minutes of incubation with TIMP-3. However, no inhibition was observed in the presence of TIMP-1 (35). This differential effect may be ascribed to the fact that TIMP proteins can inhibit diverse ADAMs metalloproteases. Thus, whereas TIMP-3 is a potent inhibitor of ADAM17 and ADAM10, TIMP-1 can only inhibit ADAM10 activity (1, 38). Other studies conducted by Salem and colleagues demonstrated ACE2 shedding by ADAM17 using TAPI-1, a synthetic ADAM17 inhibitor, in human kidney-2 (HK-2) cells. By Western Blot, they demonstrated that ACE2 shedding into the cell medium was significantly decreased after incubating cells with TAPI-1 (66). In concordance with these two studies, Brobe and colleagues have also demonstrated that ADAM17 gene silencing in COS7 cells significantly reduced ACE2 protein expression in the medium in comparison with control cells (26). However, there was no significant difference in

ACE2 protein expression in lysates from silenced COS7 cells as compared with control cell lysates (26).

In addition, Kuruppu and colleagues have demonstrated that ADAM17 also participates in Neprilysin (NEP) shedding. Using specific inhibitors for ADAM17 on Ea.hy926 endothelial cells they observed that NEP activity in the cell medium was decreased (34).

Gutta and colleagues have described urinary ACE2, NEP and ADAM17 protein levels in diabetic type 2 patients with normo-, micro- and macroalbuminuria (27). Interestingly, fragmented ACE2 and NEP bands in urine were only seen in all diabetic patients. In contrast, no ADAM17 either ACE2 protein expression were detected in urine from non-diabetic patients (27). These results suggest that the detection of ACE2 and NEP degradation products in the urine might be used as a biomarker of kidney disease early on in diabetic nephropathy, before the onset of microalbuminuria. Moreover, the authors speculate that ADAM17 could be responsible of ACE2 and NEP shedding from the kidney to the urine (27). These results suggest that ADAM17 would be implicated on the effectiveness of RAS hampering ACE2/Ang1-7 axis and stimulating AngII accumulation.

ADAM17 IN DIABETES

Active protein C (APC) has been shown to have endothelium-protective functions including anti-inflammatory and cytoprotective effects (19). Protein C binds to the endothelial protein C receptor (EPCR) to be active. Overexpression of APC reduces hyperglycemia-induced glomerular apoptosis, prevents endothelial dysfunction and apoptosis, glomerular capillary injury and DN development in mice (30). ADAM17 is known to cleave membrane-bound EPCR, releasing soluble EPCR into the circulation. In DN, higher levels of circulating and urine EPCR were observed in diabetic patients

169 as compared with non-diabetic patients (36). In addition, increased ADAM17
170 glomerular levels were found in diabetic patients. These results confirmed that as
171 expected, in diabetic patients, increased ADAM17 levels contributed to EPCR shedding
172 increasing its levels in circulation and urine. Moreover, this study demonstrated that DN
173 patients presented higher cardiovascular risk factors as compared with patients without
174 DN (36).Rizza and colleagues demonstrated that increased circulating levels of VCAM-
175 1, ICAM-1, IL6R and TNFR1, other ADAM17 substrates, were associated to a
176 significant higher rate of second cardiovascular events in a cohort of 298 patients with
177 established vascular atherosclerosis. They have demonstrated that the increase of its
178 substrates in the circulation depends on increased ADAM17 enzymatic activity at local
179 inflammatory sites (73). Renal ADAM17 activation leads to the release of pro-
180 inflammatory and pro-fibrotic markers such as cytokines, TNFα, ICAM-1, and VCAM-
181 1 stimulating renal inflammation and CV damage (25, 48).
182 *In vitro* experimental studies have demonstrated that high concentrations of glucose
183 mimicking the diabetic milieu increased ADAM17 protein expression in glomerular
184 epithelial cells or podocytes, glomerular endothelial cells, mesangial cells, and proximal
185 tubular epithelial cells (20, 36, 78). Moreover, glucose activates ADAM17 and
186 epidermal growth factor (EGF) receptor and regulates pro-fibrotic TGFβ and the
187 accumulation of matrix proteins (87–90) (Figure 4).

188 Salem and colleagues have demonstrated increased renal and urinary ACE2 activity in
189 Akita type 1 diabetic mice. The highest urinary ACE2 levels were associated with
190 increased ACE2 and ADAM17 renal protein expression demonstrating that in
191 experimental diabetes, ADAM17 increases the ectodomain shedding of ACE2
192 increasing its levels in urine. Furthermore, insulin treatment in those mice normalizes

hyperglycemia and attenuates urinary ACE2 shedding, albuminuria and renal ADAM17 expression (66).

Within the kidney, increased ADAM17 expression and activity was found in the renal cortex from OVE26 mice, a model of type 1 diabetes. Pharmacological inhibition of ADAM17 with TMI-005 reduced collagen IV, fibronectin, Nox4 protein expression, and NADPH activity in the glomeruli and tubule/interstitium from OVE26 mice (20). These results demonstrated that ADAM17 is upregulated in the diabetic kidney contributing to matrix accumulation through upregulation of Nox4 and increasing oxidative stress whereas its inhibition downregulated Nox4 protein expression, NADPH oxidase activity and reduced fibronectin expression.

Sominemi and colleagues were focused on studying the effect of exercise and metformin administration in murine models of type 2 diabetes. They reported that in db/db mice renal injury was associated with enhanced renal ADAM17 protein-mediated shedding of ACE2 (71). ADAM17 and ACE2 colocalized in diabetic kidney. Moreover, ADAM17 protein expression was upregulated in the kidney from db/db mice and ACE2 levels were increased in the urine of diabetic mice. Based on these data, they postulated that after early stages of kidney damage, due to the action of ADAM17, ACE2 was shed into the urine in db/db mice. Thus, the loss of renoprotective enzyme ACE2 could contribute to renal damage in diabetes (11). In addition, they demonstrated that renal ACE2 activity in 8-week-old db/db mice, was significantly higher than in 31-week-old db/db mice. These results suggested that during the progression of the disease, the kidneys are unable to keep ACE2 levels due to the increase of ADAM17 protein levels (11).

ADAM17 increases and leads to ACE2 shedding in db/db mice. This was associated with higher glomerular area, glomerular and tubular basement membrane thickening, expanded mesangial matrix, and collagen deposition (71). Exercise training attenuates renal ADAM17 protein levels in the kidneys of these mice suggesting a possible renoprotective mechanism where decreased ADAM17 levels attenuate ACE2 shedding into urine. Furthermore, exercise training alone or in combination with metformin administration reduced blood glucose, albuminuria, glomerular and tubular thickening and collagen depositions in db/db mice. Of note that metformin administration alone did not attenuate renal ADAM17 and ACE2 shedding and did not exert any effect on kidneys from diabetic mice (71).

ADAM17 AND ACUTE KIDNEY INJURY

Acute kidney injury (AKI) occurs in about 13.3 million people per year, with around 85% from developing countries population and is associated with high mortality and morbidity (50, 64). Ischemia-reperfusion injury is the leading cause of AKI which results from generalized or localized impairment of oxygen and nutrients delivery to, and waste products removal from, kidney cells. The impairment of local tissue oxygen supply with the accumulation of waste products of metabolism injure the tubular epithelial cells and cause death by apoptosis and necrosis (8, 28).

Renal ADAM17 is elevated in acute and chronic kidney injury, and can activate TNFα and EGFR ligands leading to the synthesis and release of inflammatory and pro-fibrotic factors (20, 31, 32, 56, 74). Experimental studies have demonstrated that EGFR inhibition attenuates renal fibrosis and renal disease progression (10, 41, 75).

ADAM17 participates in the regulation of neutrophil migration out of blood vessels at sites of inflammation. ADAM17 overactivation downregulates interleukin 8 receptor

beta (CXCR2) and induces neutrophil dysfunction (46). These findings suggest that ADAM17 could be used as a therapeutic target to beef up neutrophil infiltration and pathogen clearance during inflammation (46). In addition, TNFα cleavage was reduced in the kidney of ADAM17 knockout mice and correlated with a reduction in pro-inflammatory markers, macrophage, and neutrophil infiltration in the kidney after AKI or in unilateral ureteral obstruction (UUO) model (32). These results demonstrated the important protective effect that ADAM17 inhibition could exert on kidney fibrosis after acute kidney injury suggesting a central role of ADAM17 in fibrotic and inflammatory kidney diseases through EGFR signaling pathway. TNFα and HB-EGF, ADAM17 substrates, have been related to kidney damage in lupus nephritis (LN) stimulating EGFR signaling pathway and mediating kidney inflammation (7, 59). Qing and colleagues have seen that in *Fcgr2b*$^{-/-}$ mice, a mouse experimental model of LN, iRhom2 and ADAM17 are upregulated. Interestingly, the deficiency of iRhom2 protects the lupus-prone *Fcgr2b*$^{-/-}$ mice from renal inflammation by simultaneously blocking TNFα and HB-EGF/EGFR signaling in the kidney. *Fcgr2b*$^{-/-}$ mice lacking iRhom2 did not present proteinuria, ECM depositions, infiltrated inflammatory cells or glomerular damage (59). These results demonstrated that iRhom2 is necessary for ADAM17 activity to induce TNFα and EGFR substrates shedding implicated in renal inflammation (39).

Bertram and colleagues analyzed circulating ADAM17 activity of serum samples by using the InnoZyme TACE Activity kit (3). They demonstrated that circulating ADAM17 levels are increased in patients with active ANCA-associated vasculitis, suggesting a model in which increased plasma ADAM17 correlates with enhanced proteolytic activity and disease progression. The increase in ADAM17 observed in the setting of active ANCA-associated vasculitis and in advanced CKD may be in part

related to its direct role in inflammation (3). In concordance, different studies demonstrated that ADAM17 is involved in inflammatory responses playing an important role in the regulation of neutrophil influx into the peritoneal cavity (77), participating in monocytes transmigration (24) and regulating macrophages uptake of apoptotic cells (15).

Previous studies demonstrated that ADAM17 can also shed kidney injury molecule-1 (KIM-1) who is responsible of transforming proximal tubular epithelial cells (PTEC) into phagocytes for clearance of apoptotic cells (2, 21). The efficient clearance by PTEC is inhibited by excess of soluble KIM-1 (21). Ganghi and colleagues have found higher levels of soluble KIM-1 and higher cell-surface expression of ADAM17 after AKI. They speculate that excess of soluble KIM-1 might occur after AKI and could limit kidney recovery (21). Moreover, it has been shown that after AKI, ADAM17 mRNA expression, EGF ligands, TNFα substrates and their receptors were increased. As a consequence of ADAM17 hyperactivation, EGFR is persistently activated leading to the synthesis and release of pro-inflammatory and pro-fibrotic factors such as TGFβ resulting in macrophages and neutrophil infiltration and fibrosis (32) (Figure 4).

ADAM17 AND CHRONIC KIDNEY DISEASE

Chronic kidney disease is defined as an alteration in kidney structure and function with variable clinical presentation, severity and rate of progression of the disease that is present for more than three months. It can result from a variety of diseases but the most commonly are diabetes and hypertension (62). CKD is characterized by progressive nephron loss involving tubulointerstitial fibrosis and glomerulosclerosis, leading to end stage renal disease (ESRD) and need for renal replacement therapy or kidney transplantation (13).

ADAM17 has been studied in experimental models of renal diseases and in kidney biopsies from CKD patients, suggesting an important role on kidney fibrosis, inflammation and disease progression (31, 32). Our group demonstrated that in plasma samples from CKD patients without previous history of cardiovascular disease from the NEFRONA study, circulating ADAMs activity was increased in CKD3-5 patients and was consistently increased in CKD5D patients. Interestingly, circulating ADAMs activity in CKD patients progressively increases as CKD progresses. Moreover, circulating ADAMs activity was identified as a risk factor for CKD progression only in male patients and as an independent risk factor for CV events after 48 months of follow-up (55).

Several studies have demonstrated that ADAM17 is increased in glomerular sclerosis and interstitial fibrosis and correlated positively with fibrotic markers in kidney biopsies (32, 44, 51). In normal human kindeys, by RNA *in situ* hybridation it has been shown that ADAM17 staining was predominantly negative in the proximal tubules, glomerular mesangium and endothelium, few staining was seen in glomerular parietal and podocytes, moderate staining in renal arterial endothelial cells and smooth muscle cells and high ADAM17 expression was detected in distal tubules (44, 51). In the same line, experimental studies have shown that the release of ADAM17 substrates such as TGFα contributes to proteinuria, glomerulosclerosis, mononuclear cell infiltration, and fibrosis in the kidney (16, 44). In addition, high ADAM17 mRNA staining was observed in glomerular parietal epithelium and podocytes from several renal diseases such as diabetic nephropathy, focal segmental glomerulosclerosis, intersititial fibrosis and tubular atrophy, acute allograft rejection, and non-transplant intersitical fibrosis, among others (44, 51). ADAM17 mRNA was up-regulated in glomerular mesangium, endothelium, proximal tubules, and peritubular capillaries in the kidneys of CKD

316 patients (44, 51). Moreover, high ADAM17 mRNA expression was also found in
317 intestitial inflammatory cells, in the renal arterial endothelium and smooth muscle cells
318 in the kidneys of different renal diseases (44). In addition, ADAM17 was highly
319 expressed in fibrotic areas and in sclerosed glomeruli from Wegener glomerulomatosis
320 patients, suggesting its important role in fibrosis and advanced CKD (44).
321 *In vitro* studies by Melenhorst and colleagues demonstrated a 300-fold increase in
322 TGFα shedding by ADAM17 after phorbol 12-myristate 13-acetate (PMA) stimulation
323 in podocytes. Pharmacological ADAM17 inhibition with TNF484, a TNF-alpha
324 converting enzyme inhibitor, reduced TGFα shedding in human podocytes and proximal
325 tubular cells (44). In concordance, Lautrette and collegues demonstrated that AngII
326 induces and redistributes ADAM17 to the apical membranes of distal tubules improving
327 TGFα cleavages. The blockage of this pathway by overexpressing a negative isoform of
328 EGFR, using a TGFα inhibitor or an ADAM17 inhibitor prevented the injury
329 development in the kindey from AngII-treated mice (37). In concordance, Mulder and
330 colleagues have demonstrated that ADAM17 induces HB-EGF and TGFα shedding in
331 cultured human mesangial cells upon PMA stimulation. ADAM17 shedding was
332 inhibited up to 82% in PMA-stimulated cells after ADAM17 inhibition with TNF484
333 (51). Moreover, Morgado-Pascual and colleagues have demonstrated that aldosteron
334 stimulation activated EGFR signalling pathway due to TGFα shedding by ADAM17 in
335 cultured human tubular epithelial cells (HK-2). Aldosterone increased EGFR
336 phosphorilation leading to its activation and resulting in upregulation of pro-
337 inflammatory factors such as chemokine (C-C motif) ligand (CCL) 2 and CCL5 (49).
338 These results suggested that ADAM17 targeting could become a therapeutic tool to
339 reduce EGFR signaling and subsequently protecting kidney from renal fibrosis and
340 inflammation.

EFFECT OF ADAM17 DOWNREGULATION

An efficient way to study ADAM17 function is working with knockout animal models and to assess its effect on the animal phenotype. ADAM17 knockout mice die between embryonic day 17.5 and the first day of birth due to defects in heart development and show epithelial abnormalities in several organs (94). Thereby, Adam17$^{-/-}$ mice nearly mimic EGFR knockout mice, lacking the EGFR ligands TGF-α, HB-EGF, or amphiregulin (AREG), suggesting an *in vivo* relevance of ADAM17 in EGFR signaling pathway(4, 6, 85). For this reason, the generation of conditional tissue-specific mutants of ADAM17 has given the opportunity to study the important role of ADAM17 in different diseases.

In ADAM17 hypomorphic mice fed with a semisynthetic diet for 16 weeks to obtain a mice model with a moderate degree of hypercholesterolemia, an increase in atherosclerotic plaque size at the aortic root was seen as compared to controls. However, only in ADAM17 deficient male mice, LDL cholesterol was lower than in controls. As expected, ADAM17 substrates such as sTNFR1, sTNFR2, and sILR6 were reduced in ADAM17 hypomorphic mice as compared with wild-type mice (52). TNFRs are related to apoptosis and proliferation; whereas TNFR1 increases apoptosis, TNFR2 attenuates apoptosis and increases cell proliferation. In macrophages isolated from ADAM17 hypomorphic mice, they observed that TNFR2 levels were increased and caspase-3 activity was reduced demonstrating that ADAM17 deficiency attenuates apoptosis signaling pathways. TNFR2 deletion in ADAM17-deficient macrophages restored caspase-3 activity leading to apoptotic and cell proliferative normal levels. The authors conclude that ADAM17 exert a protective role in atherosclerosis controlling apoptosis and cell proliferation levels (52).

Shedding of ADAM17 substrates leads to the release of pro-inflammatory cytokines, pro-fibrotic TGFβ, and neutrophil and macrophage infiltration. After kidney injury EGFR activation is necessary in the early phases of tubular repair. However, sustained activation of the EGFR is linked to kidney fibrosis increasing TGFβ production (10, 41, 75, 76, 83). ADAM17 releases the ectodomains of most EGFR ligands, tumor necrosis factor-like weak (TWEAK) and TNFα (23, 25, 45, 54, 69). As expected, ADAM17, EGF ligand, TWEAK, and TNFα substrates have been found increased after a kidney injury in mice (32). Rayego-Mateos and colleagues demonstrated that TWEAK stimulates renal inflammation by EGFR signaling pathway and ADAM17 induces TWEAK-EGFR pathway that leads to the up-regulation of pro-inflammatory factors and increasing inflammatory cell infiltration in the kidney (60). ADAM17 hypomorphic mice showed reduced levels of TGF-β, MCP1 expression, pro-inflammatory cytokine levels, decreased macrophage ingress into the kidney, and decreased neutrophil infiltration as compared to wild-type mice with UUO injury. Moreover, mice with ADAM17 specific deletion on proximal tubular cells also presented reduced levels of macrophage and neutrophil infiltration as compared to wild-type mice after ischemia reperfusion injury (32). Taken together, ADAM17 downregulation or specific proximal ADAM17 knockout exert a protective effect against kidney fibrosis reducing EGFR activation.

Wilson and colleagues studied the effect of endothelial specific ADAM17 knockout and global ADAM17 knockout in mice with excision in exon 5. Global knockout mice were studied at day 18.5 (E18.5) of embryogenesis due to its pour survival. They observed that endothelial ADAM17 knockout mice presented cardiomegaly postnatally but not at E18.5. Instead, global knockout mice presented cardiomegaly at E18.5. The cardiomegaly in endothelial knockout mice was consequence of enlargement of the left

ventricle whereas in the global knockout model, the hearts presented perinatal enlargement of the right and left ventricle. Moreover, they have seen that in the aortic cusps of endothelial knockout mice there were changes in the levels of glycosaminoglycan and proteoglycans. By transmission electron microscopy they found that the glycosaminoglycan/proteoglycan-rich layer in cusps was enlarged with increased space between the granules compared with control mice (86). In contrary, studies by Xia et al. on experimental hypertension demonstrated that knockdown of ADAM17 by siRNA attenuated DOCA-salt hypertension. These results showed that ADAM17 contributes to the maintenance of hypertension by increasing the ACE2 shedding, reducing the membrane-bound carboxypeptidase levels and increasing its soluble form (92).

CONCLUDING REMARKS

ADAM17 is increased in diabetic, hypertensive patients and in experimental models of acute and chronic kidney disease. Several substrates for ADAM17 are implicated in the main pathways involved in kidney disease progression (Figure 5). Studies in humans demonstrated its role in acute and chronic kidney disease with pro-inflammatory and pro-fibrotic effects. ADAM17 could be an important mediator on CKD progression. Interestingly, ADAM17 deletion has been shown to be beneficious in some diseases by attenuating fibrosis, hypertension and inflammation. Although the relevance of ADAM17 is clearly stated in this review, more studies focusing on the role of ADAM17 in the progression of renal diseases are needed.

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754 **FIGURE LEGENDS**

755 **Figure 1: Representation of the ADAM17 domains.** The inactive pro-ADAM17
756 contains a pro-domain, a catalytic domain, a disintegrin domain, an EGF like domain, a
757 transmembrane domain, and a cytoplasmatic tail. Furin is needed for ADAM17
758 maturation and activation. Briefly, furin cleaves the ADAM17 pro-domain and T^{735} at
759 the cytoplasmatic tail is phosphorylated by mitogen-activated protein kinases (p-38
760 MAPK) or extracellular signal-regulated kinase (ERK) stimulation.

761 **Figure 2: Schematic representation of ADAM17 phosphorylation**. Activation of
762 mitogen-activated protein kinases (p38 MAPKs) leads to ADAM17 phosphorylation in
763 Thr^{735} and activation. After its activation, ADAM17 catalyzes pro-TNFα shedding by
764 releasing soluble TNFα. TNFα binds to its receptors (TNFR) and activates apoptosis
765 pathways and inflammatory pathways. Adapted from Xu P et al., Mol. Cell, 2010 (95).

766 **Figure 3: A simplified representation of the RAS pathway.** Whereas ACE converts
767 AngI to AngII, ACE2 converts AngII to Ang1–7. ACE2 is cleaved by the
768 metalloprotease ADAM17, producing a soluble form of ACE2.

769 **Figure 4: Schematic representation of TNFα and TGFβ shedding after ADAM17**
770 **upregulation during kidney injury.** Kidney injury increases AngII levels leading to
771 ADAM17 upregulation. ADAM17 contributes to TNFα and TGFβ shedding increasing
772 fibrosis, inflammation, protein matrix accumulation, glomerulosclerosis, and
773 macrophages and neutrophil infiltration by TNFR and EGFR signaling pathway.

774 **Figure 5: ADAM17 substrates.** The diagram shows ADAM17 substrates confirmed by

775 *in vivo* and *in vitro* studies.

776