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The emerging role of coagulation proteases in kidney disease

Thati Madhusudhan¹, Bryce A. Kerlin², and Berend Isermann¹

¹Institute of Clinical Chemistry and Pathobiochemistry, Medical Faculty, Otto-von-Guericke-University, Magdeburg, Leipziger Strasse 44, Magdeburg D-39120, Germany

²Center for Clinical and Translational Research, Nationwide Children's Hospital, 700 Children's Drive, W325 Columbus, Ohio 43205, USA

Abstract

A role of coagulation proteases in kidney disease beyond their function in normal haemostasis and thrombosis has long been suspected, and studies performed in the past 15 years have provided novel insights into the mechanisms involved. The expression of protease-activated receptors (PARs) in renal cells provides a molecular link between coagulation proteases and renal cell function and revitalizes research evaluating the role of haemostasis regulators in renal disease. Renal cell-specific expression and activity of coagulation proteases, their regulators and their receptors are dynamically altered during disease processes. Furthermore, renal inflammation and tissue remodelling are not only associated, but are causally linked with altered coagulation activation and protease-dependent signalling. Intriguingly, coagulation proteases signal through more than one receptor or induce formation of receptor complexes in a cell-specific manner, emphasizing context specificity. Understanding these cell-specific signalosomes and their regulation in kidney disease is crucial to unravelling the pathophysiological relevance of coagulation regulators in renal disease. In addition, the clinical availability of small molecule targeted anticoagulants as well as the development of PAR antagonists increases the need for in-depth knowledge of the mechanisms through which coagulation proteases might regulate renal physiology.

Tight regulation of coagulation proteases is required for normal haemostasis in the kidney. Coagulation is intimately associated with inflammation and the functions of the haemostatic system extend beyond maintenance of vascular integrity and prevention of excessive blood loss. This crosstalk is reflected phylogenetically by the haemocyte or amoebocyte, the sole circulating blood element of the horseshoe crab (*Limulus polyphemus*)¹, which simultaneously fulfils the functions of platelets and phagocytic cells. Vertebrates do not have a single cell type that unifies the coagulation system and innate inflammatory regulators, but soluble coagulation proteases regulate haemostasis as well as inflammation and tissue remodelling².

Correspondence to B.I. berend.isermann@med.ovgu.de.

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The close association of inflammation and tissue remodelling with renal disease has prompted researchers to address the functions of haemostatic regulators in this setting. The discovery of protease-activated receptors (PARs), the pivotal cellular receptors for coagulation proteases, provided new impetus leading to novel pathogenic concepts in various organ systems. Moreover, the increasing clinical utilization of anticoagulants targeting specific coagulation proteases and the advent of PAR antagonists warrant additional investigation to gain deeper insights into the mechanisms through which the coagulation system modulates renal health^{3,4} (TABLE 1). Such knowledge might enable nephrologists to exploit the beneficial effects of such interventions while simultaneously avoiding the detrimental aspects. In this Review we summarize current knowledge regarding the role of coagulation proteases in renal disease.

The coagulation system

The coagulation system is traditionally viewed as a cascade-like system with two activation pathways: the tissue factor (TF; also known as extrinsic) pathway and the contact (also known as intrinsic) pathway⁵ (FIG. 1). TF is a type 1 transmembrane receptor that shares substantial structural homology with type II cytokine receptors. Full length TF is a 263 amino acid, single-chain polypeptide with a 219 amino acid extracellular N-terminus, a 23 amino acid transmembrane domain and a 21 amino acid intracellular C-terminus⁶. As TF is physiologically expressed by perivascular cells but not by resting endothelial cells, leucocytes or other cells in direct contact with blood (with the exception of placental trophoblasts and cancer cells), it comes into contact with blood only upon vascular injury or following its induction (for example by inflammatory cytokines) on endothelial cells or leucocytes.

Key points

- In addition to their pivotal and well-established functions in haemostasis, coagulation regulators and receptors mediate non-haemostatic functions in the kidney
- Derangements of the coagulation system and altered coagulation-protease-dependent signalling in renal disease might alter disease progression
- Coagulation proteases alter the function of a variety of renal cell types via distinct protease-activated receptors (PARs) and co-receptors
- Activated protein C has nephroprotective effects that are at least partly independent of its anticoagulant function
- The new drug classes of target-specific oral anticoagulants and PAR inhibitors might interfere with the functions of coagulation proteases in renal disease, with potential beneficial or adverse effects

TF is induced in diverse renal diseases and modulates renal function through various mechanisms. It lacks intrinsic proteolytic activity, but can bind factor VII or factor VIIa in a Ca^{2+} -dependent manner, promoting factor VII activation or enhancing the catalytic activity

of factor VIIa⁷. The TF/VIIa complex interacts with and activates factor IX and factor X. Activated factor X enables formation of the prothrombinase complex (comprised of factor Xa, factor Va, calcium and negatively charged phospholipids of activated cells such as platelets). The prothrombinase complex promotes the conversion of prothrombin to thrombin (also known as factor IIa). Although the initial quantity generated is small, thrombin initiates an amplification loop via factor XIa generation, activates the non-proteolytic cofactors V and VIII (resulting in an ~1,000-fold acceleration in thrombin generation) and recruits activated platelets, thus ensuring the localized production of haemostatic thrombin concentrations (FIG. 2). Subsequently, thrombin activates fibrinogen and platelets (via PARs), generating fibrin-platelet aggregates known as blood clots.

The role of the contact pathway in coagulation has been redefined in the past decade⁸. A series of elegant studies have established a factor XII-dependent mechanism in the pathophysiology of thrombo-occlusive diseases, even though factor XII activation is dispensable for haemostasis⁸. These studies demonstrate that haemostasis (prevention of excessive blood loss) and thrombosis (excess thrombus formation) are mechanistically separable, suggesting that pharmaceutical interventions that target pathological thrombosis without interfering with normal haemostasis are feasible⁹.

The dependence of factor XII activation on negatively charged surfaces has been known for almost a century¹⁰, but the importance of this phenomenon was elucidated only in the past decade, with the discovery that polyphosphates have the ability to enhance factor XII activation^{8,11,12}. This effect and additional polyphosphate-dependent effects on the coagulation system, such as promoting factor V activation and enhancing the anti-fibrinolytic activity of thrombin activable fibrinolysis inhibitor (TAFI, also known as carboxypeptidase B2), depend on the polymer length, which varies depending on the source (for example, very long polymers present in microorganisms and shorter polymers present in platelets)^{11,13}.

Several physiological anticoagulant mechanisms prevent excessive coagulation activation. Tissue factor pathway inhibitor (TFPI) is a Kunitz-type protease inhibitor that constrains the activity of the TF/factor VIIa and prothrombinase complexes, thus restricting initiation of coagulation. The serine protease inhibitor antithrombin (also known as antithrombin III) inhibits multiple coagulation proteases, including its primary target thrombin and factors VIIa, IXa, Xa, XIa and XIIa. Antithrombin also inhibits other serine proteases, such as kallikrein, plasmin and trypsin as well as the complement lectin pathway. The inhibitory activity of antithrombin is enhanced ~2,000–4,000 fold in the presence of heparin¹⁴.

A more specific and equally important anticoagulant mechanism is initiated by endothelial thrombomodulin, which modulates the function of thrombin. When bound to thrombomodulin, thrombin loses its pro-coagulant function but efficiently activates protein C¹⁵. Activated protein C (aPC) in turn inactivates cofactors Va and VIIIa, thus efficiently dampening amplification of thrombin generation. In addition, the thrombomodulin–thrombin complex activates TAFI, which removes C-terminal lysine and arginine residues from peptides. In the case of fibrin these C-terminal residues are required for tissue-type

plasminogen activator (tPA)-mediated activation of plasminogen. All of these anticoagulant regulators have functions in renal diseases.

The formation of a fibrin–platelet aggregate is not the final step in haemostasis and is succeeded by the equally well-controlled process of fibrinolysis. This proteolytic process is not restricted to fibrin but might also target the extracellular matrix. Plasmin-mediated fibrinolysis and degradation of extracellular matrix is highly relevant in the context of fibrotic renal diseases. In addition, the proteases involved in fibrinolysis might either activate or disarm PARs and can directly modulate cellular function through urokinase plasminogen activator receptor (uPAR)^{16,17}. The roles of fibrinolysis and uPAR in renal disease have been reviewed previously^{17–19}.

Protease activated receptors

Beyond its indispensable haemostatic functions, the coagulation system has non-haemostatic functions, for example in inflammation and tissue remodelling. The discovery of PARs provided insight into the non-haemostatic functions of coagulation proteases. Although well accepted in basic research, knowledge of these non-haemostatic functions has not yet been translated into clinical applications.

PARs are members of the class A family of rhodopsin-like G protein-coupled receptors (GPCRs) and are predominantly organized within lipid rafts²⁰. Four distinct PARs have been identified (PAR1, PAR2, PAR3 and PAR4). Unlike the metabotropic members of the class C family of GPCRs, which are obligate dimers, PARs can function as protomers as well as heterodimers. This ability enables PARs to activate diverse signalling pathways in tissue, context and temporally specific fashions. PARs transmit cellular responses initiated by a number of coagulation regulators, for example factor IIa (PAR1, PAR3 and PAR4), aPC (PAR1 and PAR3), factor Xa (PAR2) or plasmin (PAR1 and PAR4), as well as non-coagulation proteases, for example matrix metalloproteinase-1 (PAR1), tryptase or matriptase (PAR2), cathepsin G (PAR4) and cathepsin S (PAR2)^{15,16,21–28} (TABLE 2).

The functions of individual PAR protomers and downstream signalling cascades have been extensively studied, but the mechanisms of heterodimerization and their pathophysiological relevance remain incompletely explored. PARs have a unique activation mechanism: following cell surface localization the protease cleaves the extracellular N-terminus, unmasking a cryptic N-terminal sequence, which acts as a tethered ligand inducing a conformational rearrangement and irreversible activation of heterotrimeric G proteins^{4,26} (FIG. 3). This mechanism is in contrast to that of most GPCRs, which are reversibly activated.

The expression of PARs is highly heterogeneous and more than one PAR is expressed on many cell types. In addition, expression of PARs in some cell types is species specific. For example, human platelets express PAR1 and PAR4 and human podocytes predominately express PAR2 and PAR3, whereas murine platelets express PAR3 and PAR4²⁹ and murine podocytes predominately express PAR1 and PAR3³⁰. The diversity of protease-dependent signalling is furthermore exemplified by the TF/VIIa/Xa complex and the serine proteases

thrombin and aPC. The TF/VIIa/Xa complex can activate PAR2, but the cytoplasmic domain of TF (which can be phosphorylated but is dispensable for coagulation activation) independently regulates cellular functions such as adhesion or migration³¹. Thrombin directly interacts with PAR1, PAR3 and PAR4, whereas aPC interacts with PAR1 and PAR3. Despite activation of PAR1 and PAR3 by both thrombin and aPC, these proteases convey opposing cellular effects²⁰. Biased (that is, functionally selective) and cell-specific PAR-mediated signalling is thought to result from distinct cleavage sites, which result in distinct neo-N-termini, and cell-specific supramolecular receptor complexes (that is, PAR signalosomes)^{15,23–25}. PARs can form homodimers or heterodimers either constitutively or upon activation, depending on the cell type, extracellular protease, specific PAR that is activated and the presence of co-receptors. In addition to PAR–PAR interactions, PARs can interact with other receptors (for example sphingosine 1-phosphate receptor 1 [S1P1], epidermal growth factor receptor [EGFR] and P2Y purinoceptor 12), emphasizing the involvement of cell-specific signalosomes^{23–26} (FIG. 3).

Coagulation regulators and receptors

Coagulation regulators and receptors for coagulation protease signalling are widely expressed in renal cell types (FIG. 4). For example, thrombomodulin and endothelial protein C receptor (EPCR) are expressed on endothelial cells. Expression of thrombomodulin and EPCR is reduced in acute inflammatory states (for example sepsis) or chronic diseases (for example diabetes mellitus)^{32–34}. In a model of endotoxaemia, genetically modified mice with reduced EPCR expression (10% of wild-type levels) displayed enhanced albuminuria and renal haemorrhage, demonstrating a crucial role of EPCR in the regulation of vascular permeability in the kidney³⁵. EPCR was likewise required for vascular protection in lung and brain, but to a lesser extent in other organs — an observation that is consistent with the concept of organ-specific regulation of coagulation^{35,36}.

An organ-specific function has likewise been identified for the cytoplasmic domain of TF³⁷. Mice that lack this domain express tumour necrosis factor (TNF) on their glomeruli from the age of 6 weeks and spontaneously develop albuminuria, podocyte effacement and loss³⁸. They are sensitive to glomerular injury, but resistant to lipopolysaccharide-induced systemic inflammation or arthritis^{38–40}. The spontaneous phenotypes of these mice exemplify the non-haemostatic function of the coagulation system, as the cytoplasmic domain is not required for the pro-coagulant function of TF, but is involved in TF signalling. The renal pathology is exacerbated in a glomerulonephritis model independent of leucocyte TF expression, suggesting a pathogenic function of renal TF expression^{38,41}. Although the pathophysiological relevance of TF and the cytoplasmic domain has been shown in murine models, the expression pattern of TF in renal cell types remains to be characterized in mice^{42–44}. Conversely, in humans and rabbits, glomerular TF expression has been demonstrated in podocytes, parietal epithelial cells and possibly also in mesangial cells^{45–48}. Notably, increased TF expression has been detected in acute and chronic kidney diseases (CKDs) in humans and rodents^{49–53}. As TFPI is expressed in interstitial blood vessels, but not in glomeruli, glomerular TF expression is not opposed by concomitant TFPI expression⁵⁴.

Intriguingly protein C and protein C inhibitor (PCI), which were initially thought to be specific to the liver, are also expressed in renal cells⁵⁵. Moreover, renal protein C expression is reduced in mice with systemic inflammation or diabetes⁵⁵. PCI co-localizes with urokinase in proximal tubular cells (PTCs) and urokinase–PCI complexes are readily detectable in urine⁵⁶. Proinflammatory stimuli induce glomerular factor V expression, whereas ischaemia–reperfusion injury (IRI) induces tubular fibrinogen expression^{53,57–59}. Although endogenous expression of coagulation regulators such as TFPI, protein C, PCI, and factor V within renal cell types has been demonstrated, their pathophysiological relevance in regulating local coagulation and protease signalling in kidney disease remains unknown and warrants additional investigation.

Direct effects of coagulation proteases such as factor IIa or factor Xa on glomerular cells have long been known, implying receptor-dependent modulation of intracellular signalling pathways^{53,60}. A physiological function of PARs was first demonstrated in 1991 (REF. 61); renal PAR1 activation conveys vasoconstriction and reduces glomerular filtration rate (GFR), whereas PAR2 activation causes vasodilation, partially reverses the vasoconstriction induced by PAR1 agonists or angiotensin II, and increases GFR⁶². Renal endothelial cells express PAR1, PAR2 and EPCR, whereas podocytes express PAR3, but lack EPCR, demonstrating the presence of distinct cell-specific receptor complexes³⁰ (FIG. 3). Intriguingly, podocyte-specific expression of PAR1, PAR2 and PAR4 varies across species, whereas PAR3 expression is conserved across humans, mice and rats³⁰. In human podocytes PAR3 dimerizes with PAR2 following aPC-mediated PAR3 activation, whereas in murine podocytes the cytoprotective effect of aPC depends on PAR3/PAR1 heterodimerization³⁰. Murine mesangial cells express PAR1 and PAR2^{30,63} and murine and human tubular cells express PAR1, PAR2 and EPCR; reports regarding the expression of PAR3 and PAR4 are conflicting, possibly reflecting different cell sources^{34,64,65}. Integrins (for example α M β 2, β 1 and β 3) and other receptors such as apolipoprotein E receptor 2, platelet glycoprotein Ib α chain and S1P1²³ also interact with coagulation proteases, but little if anything is known about these interactions in the kidney.

Considering the wide expression of coagulation regulators, their receptors and potential co-receptors by renal cells, autocrine and paracrine effects of coagulation proteases on renal function seem likely. This concept is supported by the experimental data discussed below. Endothelial dysfunction resulting in altered coagulation activation might, therefore, not only promote thrombus formation, but also impact other renal cell types, such as podocytes and tubular cells^{66–68}.

Role of the glycocalyx

The glycocalyx regulates both capillary permeability and activation of coagulation^{69,70}. Although glycocalyx function remains incompletely characterized (reflecting largely unmet analytical challenges), important insights have been gained for the endothelial glycocalyx, including that of glomerular endothelial cells⁷⁰.

The glycocalyx consists of covalently linked structures such as membrane-bound glycoproteins (for example, syndecan-1 and thrombomodulin) and attached negatively

charged glycosaminoglycans⁷¹. The membrane-bound, multifunctional, anticoagulant protein thrombomodulin contains a chondroitin sulphate side chain, to which glycosaminoglycans are attached⁷¹. This loosely attached coat consists of secreted proteoglycans, including perlecan and hyaluronan. The glycocalyx has important roles in the transduction of shear stress, regulation of leucocyte–endothelial cell interactions, selective permeability, growth-factor binding, and complement and coagulation regulation^{71,72}. Some of the endothelium and plasma-derived soluble molecules bound to the glycocalyx are important regulators of the glomerular filtration barrier and/or the coagulation system (for example vascular endothelial growth factor A [VEGFA], protein C and antithrombin)^{71–73}.

Coagulation regulators that bind to the glycocalyx include thrombin, antithrombin, heparin cofactor 2 and TFPI. Heparan sulphates provide a scaffold for the interaction of antithrombin with procoagulant enzymes (such as thrombin, factor Xa and factor IXa), thus enhancing the anticoagulant effect several thousand-fold. TFPI is thought to bind to the glycocalyx via heparan sulphates, but other proteins could also be involved⁷⁴. Uptake and degradation of TFPI–factor Xa complexes also depends on heparan sulphates in the glycocalyx⁷⁵. Heparin cofactor 2 is a thrombin-specific protease inhibitor, which is activated by dermatan sulphate in the glycocalyx⁷¹. Likewise PCI inhibition of aPC requires binding to heparan sulphate⁷¹. All of these anticoagulant molecules within the glycocalyx contribute to the thrombo-resistant nature of healthy endothelium. Quantitative and qualitative alterations of the glycocalyx, for example following toxic stimuli such as IRI or hyperglycaemia, modulate the bioavailability of these proteins^{69,76}. Loss of endothelial glycocalyx during acute hyperglycaemia coincides with endothelial dysfunction and coagulation activation *in vivo*⁶⁹. Importantly, damage to the endothelial glycocalyx has been demonstrated in patients with type 1 diabetes mellitus, the severity of which is increased in the presence of microalbuminuria⁷⁷. These studies highlight the crucial pathophysiological relevance of the endothelial glycocalyx. Further investigation is warranted to gain insight into the mechanistic relevance of glycocalyx components in the regulation of coagulation activation and glomerular permeability.

Modulation of haemostasis in renal disease

Thrombotic disease is a significant morbidity of renal diseases, especially in the settings of nephrotic syndrome and haemodialysis^{78,79}. The likelihood of thrombosis is also heightened in other kidney diseases, including diabetic nephropathy, hypertensive nephropathy, glomerulonephritis, interstitial nephritis and polycystic kidney disease⁷⁸. In general the mechanisms underlying this increased risk remain ill-defined^{80–86}.

In CKD, hypercoagulability is associated with markers of endothelial dysfunction (for example, increased levels of soluble thrombomodulin and changes in flow-mediated dilatation) and inflammation (such as IL-6)^{87,88}. Factor VIII and von Willebrand factor have been reported to contribute to venous thromboembolism in patients with CKD, but both of these coagulation regulators are acute phase reactant proteins, hence this observation might simply reflect an association of endothelial dysfunction (and secondary hypercoagulability) with inflammation⁸⁴. In patients with advanced CKD, plasma levels of soluble thrombomodulin are elevated, reflecting endothelial dysfunction, whereas levels of aPC are

reduced, providing a potential link between CKD, endothelial dysfunction and hypercoagulability^{87,89–91}. These clinical observations provided the rationale for mechanistic studies of the endothelial thrombomodulin–protein C system in renal disease. Following kidney transplantation, markers of hypercoagulability and endothelial dysfunction (such as soluble thrombomodulin and EPCR) normalize, suggesting that impaired renal function is causally related to hypercoagulability, potentially via endothelial dysfunction^{92–94}.

In addition to hypercoagulability, fibrin clots in patients with end-stage renal disease (ESRD) are markedly altered and characterized by a reduced porous structure, resistance to fibrinolysis and increased overall fibre thickness^{95,96}. These characteristics are associated with increased mortality and with a pro-inflammatory state, but not with azotaemia^{95,96}. The increased frequencies of cardiovascular disease and cardiovascular death in patients with CKD have been postulated to be partly accounted for by coagulation activation and altered blood clot structure, but causality has not yet been established.

Effect of anticoagulation on renal function

In acute renal diseases, such as newly-diagnosed nephrotic syndrome, hypercoagulability is an important co-morbidity^{97–99}, but prophylactic anticoagulation remains controversial^{100,101}. A similar situation exists for chronic renal diseases. In the context of diabetic nephropathy, researchers have evaluated whether heparins or other glycosaminoglycans improve renal function^{102,103}. In general these studies did not address the role of altered coagulation protease activity or signalling, but rather evaluated whether glycosaminoglycans can reconstitute negative charges within the glomerular filtration barrier — a concept that remains unproven¹⁰⁴. Direct effects of anticoagulants on kidney homeostasis and renal disease progression remain undefined in acute and chronic renal diseases, but given the wide use of these therapies, such effects might have important clinical implications. The first study to evaluate the effect of the novel direct coagulation protease inhibitor rivaroxaban on renal function is now underway in patients with CKD and atrial fibrillation¹⁰⁵.

Both pharmacological anticoagulation and naturally occurring anticoagulants have been demonstrated to positively affect nephron health^{106–115}. By contrast, supratherapeutic anticoagulation has been associated with accelerated loss of GFR^{116–118}. These effects might potentially result in a vicious cycle, as many anticoagulants are dependent on renal clearance and might accumulate with worsening renal function. Although glomerular haemorrhage and tubular obstruction, particularly in patients with pre-existing CKD, has been proposed to be causative, gross haematuria has only rarely been observed in these patients¹¹⁸. Other mechanisms of anticoagulant-mediated glomerular injury should, therefore, be considered.

Interestingly, in rats PAR1 inhibition and the direct thrombin inhibitor dabigatran have similar detrimental effects on renal function to supratherapeutic anticoagulation with warfarin^{116,119}. These findings demonstrate that these adverse effects are not specific to warfarin and — as rat platelets do not express PAR1 — might be dependent on PAR1

signalling in renal cells^{116,119}. In this context the protective effect of low but sustained thrombin activation observed in diabetic nephropathy, and the cytoprotective effect of low thrombin concentrations *in vitro* on endothelial cells, podocytes and tubular cells are noteworthy, as they support the hypothesis that a low level of coagulation activation might be nephroprotective.^{65,120} Such protective effects might be lost following supratherapeutic anticoagulation or direct thrombin inhibition.

The effects of coagulation protease inhibitors have been investigated in several preclinical studies^{121,122}. Notably, in a mouse model of sickle cell disease, the factor Xa inhibitor rivaroxaban had a greater anti-inflammatory effect (characterized by a reduction in plasma levels of IL-6) than the thrombin inhibitor dabigatran, despite a less potent anticoagulant dose, indicating specific differences between these inhibitors in relation to blood clotting and cytoprotective effects¹²³. Mechanistically, direct suppression of the thrombin-induced negative-feedback system by inhibiting thrombomodulin–thrombin-mediated protein C activation might underlie these differential effects of factor Xa versus thrombin inhibition^{124,125}. In addition to their essential role in blood clotting, both factor Xa and thrombin can elicit multiple cellular effects via PARs and their co-receptors. Thrombin activates PAR1, whereas factor Xa can also signal via PAR2, directly or together with the TF–factor VIIa complex^{122,123,126}. Consistent with these observations, inhibition of factor Xa but not of thrombin suppressed expression of the proinflammatory cytokine IL-6, emphasizing the beneficial effects of protease-specific inhibition in a mouse model of sickle cell disease¹²³. Determining whether direct anticoagulants differ in regard to their efficacy and safety in the context of renal function and disease will be of great clinical interest. Furthermore, elucidating the molecular mechanisms of coagulation protease signalling in renal cells might lead to new therapies that target the involved receptors and pathways without increasing the risk of glomerular haemorrhage.

Coagulation proteases in renal disease

Acute glomerular disease

The implications of glomerular coagulation activity and signalling have been thoroughly studied in the setting of rapidly progressive glomerulonephritis (RPGN). A key feature of RPGN is the formation of a fibrin matrix in the Bowman capsule, which forms the basis for the pathognomonic crescent-shaped scar seen in kidney biopsy samples. Fibrinogen-deficient mice are partially resistant to antibody-mediated (anti-glomerular basement membrane) RPGN¹²⁷.

Despite beneficial effects in pre-clinical and early non-randomized clinical observational studies, therapies aimed at reducing fibrin deposition have not been successfully translated into the clinic^{128,129}. This failure might reflect the inherent haemorrhage risk of such therapies and the now well-established multifaceted functions of the coagulation system, which are partially independent of fibrin formation. Once formed, fibrin is regulated by the plasmin system (FIG. 5). Mice with plasminogen or tPA deficiency are prone to exacerbated RPGN¹²⁹. Interestingly, deficiency of urokinase plasminogen activator (uPA), or its receptor uPAR, reduced glomerular macrophage infiltration, but did not significantly alter the RPGN disease course¹³⁰. The proinflammatory cytokine IL-1 β , which is released from infiltrating

mononuclear cells in experimental RPGN, upregulates glomerular expression of tPA, but not uPA, and downregulates the tPA inhibitor plasminogen activator inhibitor-1 (PAI-1) in cultured mesangial cells^{131–133}. Mice deficient in PAI-1 are partially protected from experimental RPGN, whereas those that transgenically overexpress PAI-1 have exacerbated disease, suggesting that compensatory changes in tPA and PAI-1 expression are inadequate to control experimental RPGN¹³⁴. A function of the fibrinolytic system nephroprotection is further supported by a study in which pharmacological inhibition of TAFI conveyed protective effects in mice with acute glomerulonephritis by promoting fibrinolysis¹³⁵.

During RPGN, coagulation activation and subsequent fibrin deposition seem to be dependent on TF expressed by infiltrating mononuclear cells^{47,136}. An association of increased TF expression with glomerular fibrin deposition and renal failure has been shown in human RPGN and in rabbit models⁴⁷. Administration of anti-TF antibody in rabbits ameliorated these changes without affecting macrophage infiltration, indicating that TF is the major *in vivo* initiator of fibrin deposition and renal failure in RPGN⁴⁷. Specific thrombin inhibition using the naturally occurring anticoagulant hirudin ameliorated crescent formation, glomerular T-cell and macrophage infiltration, and serum creatinine elevation, emphasizing the pathophysiological relevance of thrombin in this disease model¹³⁷. Similarly, factor Xa inhibition ameliorated the disease phenotype in rats with experimental mesangioproliferative glomerulonephritis in a manner that was suggested to be dependent on mesangial cell PAR2 signalling^{53,106,138}. Indeed, signalling by procoagulant proteases that are activated downstream of TF might contribute to RPGN independent of fibrin formation and dissolution. Thus, PAR1 or PAR2 knockout diminished progression of experimental glomerulonephritis in mice, demonstrating that PAR signalling drives this immune-cell-mediated glomerular disease^{137,139}. Although these studies clearly establish a pathogenic role of coagulation proteases and PARs in acute glomerular diseases, the dynamics of receptor activation and interaction remain unknown.

In a mouse model of thrombotic microangiopathy (TMA) induced by various murine monoclonal and human antiphospholipid antibodies, glomerular TF expression was markedly enhanced and TF seemed to be a common mediator of glomerular injury⁴³. Following exposure to these antibodies, which had antiphospholipid activity, both TF expression and complement activation (that is, glomerular C3 deposition) were increased; C5a receptor (C5aR) deficiency partially protected against these effects. Importantly, genetic or pharmacological (using pravastatin) reduction of TF expression prevented renal injury irrespective of whether the antibody-induced glomerular injury, and increased TF expression occurred via a complement-dependent or complement-independent pathway⁴³. These studies established a causal relationship of TF in regulation of acute glomerular injury, which is partially potentiated by complement activation.

Induction of TF expression following C5aR signalling has also been shown in human and murine neutrophils^{140,141} and the functional interaction of TF and complement might reflect the well-established communication between these systems¹⁴². In addition to inducing TF expression, the complement C5b-7 complex might activate TF by oxidizing its disulphide bond (via protein-disulphide-isomerase-dependent thiol-disulphide exchange), resulting in optimal TF folding and oxidation and thus contributing to TF activation^{7,143}. Intriguingly,

thrombomodulin, the functional opponent of TF in coagulation activation, dampens complement activation¹⁴⁴ and thrombomodulin polymorphisms are associated with atypical haemolytic uraemic syndrome (HUS)¹⁴⁵. Thrombomodulin dampens complement activation via activation of TAFI (via removal of carboxyterminal arginines from C3a and C5a) and through a poorly defined mechanism involving the lectin-like domain^{144,146}. Deficiency of the lectin-like domain of thrombomodulin worsened Shiga-toxin-induced experimental HUS in mice; these mice showed enhanced renal impairment and increased glomerular complement and fibrin deposition compared with wild-type controls¹⁴⁷. Whether thrombomodulin functionally interacts with TF via complement regulation in the context of glomerular disease has not yet been addressed.

The renin–angiotensin–aldosterone system (RAAS) is integrally involved in the progression of many kidney diseases¹⁴⁸. Signalling through angiotensin II type 1 and angiotensin IV receptors upregulates expression of PAI-1¹⁴⁸. In addition, aldosterone stimulates renal PAI-1 expression and PAI-1-deficient animals are protected from aldosterone-induced glomerulosclerosis^{149,150}. These effects are thought to occur via amelioration of PAI-1-mediated suppression of plasmin and matrix-metalloproteinase-mediated degradation of extracellular matrix proteins that are involved in renal fibrosis¹⁴⁸. Interestingly, plasmin can either disarm or activate PAR1 by proteolytic cleavage and might also signal via PAR4 (TABLE 2). The implications of PAI-1 alterations on plasmin-mediated cell signalling in the kidney remain unexplored in the contexts of RAAS-mediated renal fibrosis and glomerulonephritis^{151,152}.

Nonspecific coagulation inhibition with heparin or antithrombin reduces proteinuria in the puromycin aminonucleoside and adriamycin experimental models of nephrotic syndrome^{153–156}. Unfortunately, owing to the nonspecific nature of these inhibitors it remains unclear which protease(s) are involved in persistent proteinuria. Circulating protease activity from patients with nephrotic syndrome has, however, been demonstrated to injure cultured podocytes in what seems to be a PAR1-dependent manner, implicating PAR1 cleaving proteases as promising targets for further investigation^{151,157}.

Acute tubular disease

The effects of coagulation proteases on renal tubular health have been addressed in several studies. In the context of IRI, limited fibrin formation is required for tissue repair; complete fibrinogen deficiency severely impairs renal function, whereas reduced fibrinogen expression protects against ischaemic injury in heterozygous fibrinogen-knockout mice (FIG. 4)^{57,158}. The fibrin-derived peptide B β 15–42 protects kidneys from IRI, potentially by competitively blocking binding of fibrin to integrins, ICAM-1 or VE-cadherin, thus altering cellular signalling and reducing apoptosis^{57,159,160}. A protective effect of the B β 15–42 peptide is apparent even if applied post-injury and might involve preservation of endothelial and vascular integrity^{57,161}. The observation that urinary fibrinogen might be a marker of acute kidney injury (AKI) is also of potential clinical relevance¹⁶².

The coagulation system clearly also has functions that are independent of fibrin formation in AKI. Thus, deficiencies in TF or PAR1 are protective in renal IRI and impaired thrombomodulin-dependent protein C activation worsens disease outcomes^{34,52}. In renal

IRI, aPC — independent of its anticoagulant function — sustains intracellular levels of the cold-shock protein YB-1 by restricting its ubiquitination and degradation through PAR1 — EPCR signalling in tubular cells³⁴. These studies have identified a new mechanism through which coagulation proteases modulate cellular function in AKI.

Additional roles for coagulation signalling in tubular epithelial cells might influence renal outcomes. A prominent example is the discovery that plasmin activates sodium and calcium channels of PTCs during nephrotic-range proteinuria^{163,164}. These protease-mediated alterations in ion channel function might at least partly explain oedema formation in nephrotic syndrome. Furthermore, thrombin stimulates proliferation and pro-inflammatory responses in cultured human PTCs^{64,165}. These responses can be recapitulated with PAR2 activating peptides but not with other PAR agonists; this finding is interesting because thrombin is not known to cleave PAR2^{16,151}. When cleaved, however, the PAR1-tethered ligand might transactivate PAR2 that is heterodimerized to PAR1, potentially explaining these data²⁷. These responses to thrombin seem to be TGF- β -dependent. PAR1 cleavage by aPC bound to EPCR seems to counteract the effect of thrombin by downregulating TGF- β -mediated production of profibrotic extracellular matrix proteins by PTCs^{64,65}.

The fibrinolytic system might also be involved in tubular injury. Mice that overexpress PAI-1 develop significantly aggravated renal fibrosis in response to ureteral obstruction, compared with wild-type mice¹⁶⁶. These effects might be mediated through a downstream reduction in extracellular matrix degradation, as proposed to occur for the RAAS in the glomerulus¹⁴⁸. The effects of PAI-1 modulation on downstream plasmin — PAR1 signalling have not yet been investigated.

Together the available data suggest that coagulation derangements and egress of coagulation proteins into the renal interstitium and/or urinary space might influence disease progression. The implications of coagulation protease signalling in acute kidney diseases likely extend beyond those described in this Review. For example, coagulation is known to be activated in complement (HUS) and non-complement (TTP) mediated thrombotic microangiopathy (TMA)⁴³. Notably thrombomodulin has been shown to regulate complement in atypical HUS¹⁴⁵. Renal injury in the setting of TMA is thought to be mediated, at least in part, by ischaemic effects secondary to small artery thrombosis. However, the implications of downstream cellular injury mediated by activated coagulation protease signalling remains unexplored in these settings. Anticoagulation with heparin might modulate complement system activity, reflecting the close interaction of the complement and coagulation systems and suggesting that it might be possible to pharmaceutically target and utilize these systems in AKI¹⁶⁷.

Chronic kidney disease

CKD (defined as estimated GFR <60 ml/min/1.73 m²) is an increasing medical problem, which afflicts ~26 million people in the USA alone. As outlined above, hypercoagulability in CKD is well documented, but the underlying mechanisms remain poorly defined. Hypercoagulability might be linked to altered clearance or inactivation of coagulation regulators, uraemic toxins, electrolyte and acid–base abnormalities and/or endothelial dysfunction. The loss of renal thrombomodulin expression and other procoagulant and

proinflammatory regulators during ageing might depend on NF- κ B signalling, consistent with a role of this transcription factor in the regulation of coagulation regulators^{32,168}. Fibrinolysis regulators are also activated in CKD¹⁶⁹ and both impaired fibrinolytic activity and hyperfibrinolysis have been described^{88,170,171}. Plasma levels of PAI-1 are increased in patients with CKD, including those with diabetic nephropathy¹⁸. To date, most existing studies of the role of haemostatic regulators in CKD have focused on diabetic nephropathy — a leading cause of ESRD.

Diabetic nephropathy

Fibrin deposition is increased in diabetic glomeruli and is associated with glomerular extracellular matrix accumulation (FIG. 6)^{172,173}. Thrombin-induced mesangial TGF- β expression and an increased peripheral blood PAI-1 to tPA ratio in diabetic patients might contribute to these changes^{174,175}. The function of the endothelial thrombomodulin–protein C system is impaired in patients with diabetic nephropathy, as reflected by increased plasma levels of soluble thrombomodulin and reduced levels of aPC^{176,177}.

Animal studies have been instrumental in deciphering the role of coagulation proteases in diabetic nephropathy. TF expression is increased in diabetic mice (db/db and streptozotocin models), along with increased expression of PAR2 (db/db mice show a transient increase at 20 weeks) and factor V^{51,58}. Increased TF expression has been observed in tubular cells and glomerular parietal cells⁵¹, whereas glomerular thrombomodulin expression is reduced in diabetic mice³³. Studies involving genetic modification of the thrombomodulin–protein C system established that loss of thrombomodulin-dependent protein C activation aggravates diabetic nephropathy, whereas compensation for impaired protein C activation protects against the disease³³. Exogenous application of aPC likewise ameliorated diabetic nephropathy in mice, demonstrating that the underlying mechanism can, in principle, be pharmacologically targeted^{178,179}. As the nephroprotective effect of aPC is independent of its anticoagulant properties, the possibility exists that the underlying mechanism could be utilized without interfering with the haemostatic system^{30,179}. aPC engages distinct receptor complexes (PAR1–EPCR on endothelial cells and PAR3 in podocytes) to ameliorate glucose toxicity. In podocytes aPC signalling via PAR3 transactivates PAR2 in humans or PAR1 in mice³⁰. Given the species-specific expression of PARs in podocytes, results obtained in mice, for example when evaluating new therapeutic strategies or the risk of adverse effects, cannot generally be extrapolated to humans.

Association of the PAR3/PAR2 dimer with caveolin-1 is also required for the cytoprotective effect of aPC *in vitro*³⁰. aPC reduces caveolin-1 Tyr-14 phosphorylation in a time-dependent manner and this aPC-mediated dephosphorylation enables the dissociation of caveolin-1 from PAR2 and PAR3, effectively inhibiting podocyte apoptosis³⁰. The mechanistic importance of PAR–caveolin interactions has likewise been established in endothelial cells, in which compartmentalization of PAR1 into caveolar microdomains determines the barrier protective versus barrier disruptive effects of aPC and thrombin, respectively²⁰. Whether these observations made in HUVEC–derived EA.hy926 cells can be extrapolated to glomerular endothelial cells remains unknown. Based on the available data, however, it can be concluded that proteases convey their effects in renal cells through cell-specific PAR

signalosomes¹⁸⁰. The characterization of these signalosomes and the downstream signalling events might facilitate the identification of small molecules that target these protease-induced cytoprotective mechanisms.

In diabetic nephropathy, aPC epigenetically suppresses the redox enzyme p66^{Shc} and thereby limits sustained generation of mitochondrial reactive oxygen species¹⁷⁹. These effects are dependent on thrombomodulin-mediated protein C activation on endothelial cells and aPC/ PAR3 signalling in podocytes, establishing an endothelial-to-podocyte crosstalk mechanism¹⁷⁹. This coagulation-protease-dependent crosstalk complements the VEGF and angiopoietin crosstalk at the glomerular filtration barrier, which is controlled by podocytes¹⁸¹. In the diabetic milieu this coagulation-protease-dependent crosstalk is disturbed, impairing the glomerular filtration barrier and podocyte function, thus contributing to diabetic glomerulopathy.

Consistent with the *in vitro* concentration-dependent effects of thrombin in tubular cells and podocytes, mice with low but sustained thrombin generation owing to the factor V Leiden (FVL) mutation are partially protected from diabetic nephropathy¹²⁰. This effect is lost following anticoagulation with the direct and irreversible thrombin inhibitor hirudin, indicating a protective role of thrombin generation at low levels¹²⁰. Whether low thrombin concentrations directly convey cytoprotective signalling, as suggested by *in vitro* studies, or whether indirect effects such as protein C activation convey cytoprotection, remains unknown^{120,182}. Importantly, the FVL mutation is associated with reduced albuminuria in diabetic and pre-diabetic individuals, demonstrating the relevance of this phenomenon in patients^{120,183}.

The apparent nephroprotective effect of low thrombin concentrations might relate to controversial data obtained from the use of anticoagulants in animal models of diabetic nephropathy. Initially, heparins were thought to replenish negatively charged glycosaminoglycans in the glomerular filtration barrier, thus restoring its function^{184,185}. This possibility cannot be completely refuted, but other mechanisms for the nephroprotective effect should be considered. Efficient thrombin inhibition is expected to impair both protein C activation and direct cytoprotective signalling. Furthermore, thrombin and factor Xa activate different receptors; hence anticoagulation with direct thrombin or factor Xa inhibitors might have distinct consequences. Thus, fondaparinux, a selective factor Xa inhibitor, conveys partially protective effects in db/db mice (that is, it has a minor effect on albuminuria and slightly reduces glomerular size), contrasting with the disadvantageous effects of hirudin^{58,120}. Considering the increasing availability and use of small molecule anticoagulants, and the recent introduction of the first PAR-antagonist for clinical use, studies deciphering the effects of these new drugs on glomerular function and renal disease are needed.

As diabetic nephropathy is characterized by extracellular matrix accumulation, a number of studies have addressed the role of fibrinolysis regulators in this disease. Expression of PAI-1 is induced in diabetic nephropathy¹⁸⁶. Loss of PAI-1 expression in mice reduces extracellular matrix accumulation, which is associated with increased plasmin and matrix metalloproteinase-2 activity, but seems to also involve signalling via uPAR and the ERK/

MAPK pathway^{187–190}. Notably loss or gain of PAI-1 expression resulted in tubulointerstitial injury in aged non-diabetic mice, illustrating a crucial role of PAI-1 in renal homeostasis in health and disease¹⁸⁷.

Similar to acute renal diseases (such as glomerulonephritis, haemolytic–uraemic syndrome and other complement-mediated diseases), in diabetic nephropathy thrombomodulin suppresses complement activation in addition to providing a functional switch between thrombin and aPC signalling. The lectin-like domain of thrombomodulin inhibits complement activation through a poorly characterized mechanism in glucose-stressed endothelial cells and podocytes and ameliorates diabetic nephropathy in mice independent of blood coagulation¹⁹¹.

Tubulointerstitial fibrosis

In diabetic patients on dialysis, levels of TAFI and activated TAFI are elevated, indicating impaired fibrinolysis¹⁹². A potential role of activated TAFI in tubular injury is supported by its induction by glucose-stressed tubular cells *in vitro*, resulting in reduced plasmin activity and an increase in extracellular matrix¹⁹³. Moreover, inhibition of TAFI reduced tubulointerstitial and glomerular fibrosis following subtotal nephrectomy in mice¹⁹⁴, consistent with observations made in TAFI-deficient mice challenged with chronic glomerulonephritis¹⁹⁵. Regarding the possible involvement of PARs in tubulointerstitial fibrosis, the increased expression of tubular PAR2 in human chronic renal disease (IgA nephropathy) and in the murine model of unilateral ureteral obstruction is noteworthy^{196,197}. Congruent with its profibrinogenic role in other organs (such as the liver and lung), PAR2 promotes early renal tubular injury and fibrosis in the murine unilateral ureteral obstruction model¹⁹⁷. Analyses of human PTCs *in vitro* suggest that the profibrinogenic effects of PAR2 are mechanistically linked to TGF- β and EGFR transactivation¹⁹⁷. The role of coagulation proteases in PAR-activation, modulation of receptor transactivation or tubulointerstitial fibrosis in chronic renal disease models remains unresolved.

Renal transplantation

Given the roles of coagulation proteases in chronic renal diseases their impact on transplant nephropathy is of potential interest. Enhanced PAI-1 expression, which seems to be reduced by rapamycin treatment, might promote chronic allograft nephropathy^{198,199}. Increased vascular expression of TF has been observed in chronic, but not in acute allograft nephropathy²⁰⁰. In addition, acute ciclosporin-induced nephropathy was associated with increased TF immunoreactivity in the tubular brush border, suggesting that TF expression might be a useful diagnostic marker for this condition²⁰⁰. The relevance of this observation remains to be established. In models of xenotransplantation, inhibition of coagulation activation during the ischaemic period with either antithrombin or aPC markedly reduced chronic kidney graft fibrosis²⁰¹. This observation implies that excessive coagulation activation during the ischaemic period harms the transplant and that *ex vivo* anticoagulation of the transplant would be beneficial without increasing the risk of haemorrhage in the recipient. Deciphering the mechanism of such long-lasting effects, including the potential involvement of PARs¹⁹⁹, might lead to novel clinical interventions.

Unresolved questions

Although studies evaluating the role of the haemostatic system in renal disease have provided novel pathophysiological insights, a number of questions remain. For example the kinetics of altered expression of coagulation regulators and their receptors in renal disease remain largely undefined. Whether coagulation proteases gain access to extravascular renal cell compartments through controlled mechanisms and the role of locally expressed coagulation regulators also need to be defined. Some insights into cell-type-specific signalosomes have been gained, but further studies are warranted to characterize the interaction of PARs with other receptors, such as Toll-like receptors and receptor tyrosine kinases (for example EGFR or VEGFR), in the context of renal diseases^{28,197}. Such insights might enable the design of kidney-specific therapeutic approaches. Studies addressing these questions will likely provide new insights into the regulation of intracellular signalling pathways, tissue remodelling and sterile inflammation in renal disease. While these questions are being experimentally addressed, clinical studies evaluating novel anticoagulants or the first clinically approved PAR antagonist (voraxapar), which might alter coagulation signalling in the kidney, should include renal end points to provide additional insight into their renal benefits and/or adverse effects. Such clinical studies have been initiated and data might become available in the near future¹⁰⁵.

Conclusions

Considerable advances have been made in understanding the physiology and pathophysiology of coagulation proteases, their regulators and their receptors in renal disease. Knowledge now exists of the functions of the haemostatic system conveyed via intravascular blood clotting, fibrin formation and fibrinolysis, but also through direct modulation of renal cell function through protease-dependent signalling. The identification of cell-specific, PAR-dependent signalosomes in renal tissue has provided new pathogenic concepts and, importantly, identified potential therapeutic targets. The translation of these new insights into the clinic, however, requires further mechanistic studies. In addition, surveillance of renal function in clinical studies evaluating new anticoagulants or therapeutics targeting PARs is required to gain insights into the function of coagulation proteases and their receptors in humans. Based on the available data, the possibility exists, but remains to be explored, that targeting specific coagulation proteases, their receptors or underlying mechanisms might enable clinicians to improve the treatment of renal disease.

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References

1. Hoess A, Watson S, Siber GR, Liddington R. Crystal structure of an endotoxin-neutralizing protein from the horseshoe crab, *Limulus* anti-LPS factor, at 1.5Å resolution. *EMBO J.* 1993; 12:3351–3356. [PubMed: 8253062]

2. Esmon CT. The interactions between inflammation and coagulation. *Br J Haematol.* 2005; 131:417–430. [PubMed: 16281932]
3. Benmira S, Banda ZK, Bhattacharya V. Old versus new anticoagulants: focus on pharmacology. *Recent Pat Cardiovasc Drug Discov.* 2010; 5:120–137. [PubMed: 20337579]
4. Ramachandran R, Noorbakhsh F, Defea K, Hollenberg MD. Targeting proteinase-activated receptors: therapeutic potential and challenges. *Nat Rev Drug Discov.* 2012; 11:69–86. [PubMed: 22212680]
5. Adams RL, Bird RJ. Review article: coagulation cascade and therapeutics update: relevance to nephrology. Part 1: Overview of coagulation, thrombophilias and history of anticoagulants. *Nephrology (Carlton).* 2009; 14:462–470. [PubMed: 19674315]
6. Eisenreich A, Rauch U. Regulation and differential role of the tissue factor isoforms in cardiovascular biology. *Trends Cardiovasc Med.* 2010; 20:199–203. [PubMed: 22137642]
7. Monroe DM, Key NS. The tissue factor–factor VIIa complex: procoagulant activity, regulation, and multitasking. *J Thromb Haemost.* 2007; 5:1097–1105. [PubMed: 17567444]
8. Bjorkqvist J, Nickel KF, Stavrou E, Renne T. *In vivo* activation and functions of the protease factor XII. *Thromb Haemost.* 2014; 112:868–875. [PubMed: 25187064]
9. Kenne E, Renne T. Factor XII: a drug target for safe interference with thrombosis and inflammation. *Drug Discov Today.* 2014; 19:1459–1464. [PubMed: 24993156]
10. Griffin JH. Role of surface in surface-dependent activation of Hageman factor (blood coagulation factor XII). *Proc Natl Acad Sci USA.* 1978; 75:1998–2002. [PubMed: 273926]
11. Smith SA, et al. Polyphosphate modulates blood coagulation and fibrinolysis. *Proc Natl Acad Sci USA.* 2006; 103:903–908. [PubMed: 16410357]
12. Muller F, et al. Platelet polyphosphates are proinflammatory and procoagulant mediators *in vivo*. *Cell.* 2009; 139:1143–1156. [PubMed: 20005807]
13. Smith SA, et al. Polyphosphate exerts differential effects on blood clotting, depending on polymer size. *Blood.* 2010; 116:4353–4359. [PubMed: 20709905]
14. Jordan RE, Oosta GM, Gardner WT, Rosenberg RD. The kinetics of hemostatic enzyme–antithrombin interactions in the presence of low molecular weight heparin. *J Biol Chem.* 1980; 255:10081–10090. [PubMed: 6448846]
15. Griffin JH, Zlokovic BV, Mosnier LO. Activated protein C: biased for translation. *Blood.* 2015; 125:2898–2907. [PubMed: 25824691]
16. Adams MN, et al. Structure, function and pathophysiology of protease activated receptors. *Pharmacol Ther.* 2011; 130:248–282. [PubMed: 21277892]
17. Zhang G, Eddy AA. Urokinase and its receptors in chronic kidney disease. *Front Biosci.* 2008; 13:5462–5478. [PubMed: 18508599]
18. Malgorzewicz S, Skrzypczak-Jankun E, Jankun J. Plasminogen activator inhibitor-1 in kidney pathology (review). *Int J Mol Med.* 2013; 31:503–510. [PubMed: 23314920]
19. Luft FC. uPAR signaling is under par for the podocyte course. *J Mol Med (Berl).* 2012; 90:1357–1359. [PubMed: 23160536]
20. Russo A, Soh UJ, Paing MM, Arora P, Trejo J. Caveolae are required for protease-selective signaling by protease-activated receptor-1. *Proc Natl Acad Sci USA.* 2009; 106:6393–6397. [PubMed: 19332793]
21. Elmariah SB, Reddy VB, Lerner EA. Cathepsin S. signals via PAR2 and generates a novel tethered ligand receptor agonist. *PLoS ONE.* 2014; 9:e99702. [PubMed: 24964046]
22. Boire A, et al. PAR1 is a matrix metalloprotease-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. *Cell.* 2005; 120:303–313. [PubMed: 15707890]
23. Weiler H. Multiple receptor-mediated functions of activated protein C. *Hamostaseologie.* 2011; 31:185–195. [PubMed: 21826371]
24. Shahzad K, Isermann B. The evolving plasticity of coagulation protease-dependent cytoprotective signalling. *Hamostaseologie.* 2011; 31:179–184. [PubMed: 21691672]
25. Bouwens EA, Stavenuiter F, Mosnier LO. Mechanisms of anticoagulant and cytoprotective actions of the protein C pathway. *J Thromb Haemost.* 2013; 11:S242–S253.

26. Hollenberg MD, et al. Biased signalling and proteinase-activated receptors (PARs): targeting inflammatory disease. *Br J Pharmacol.* 2014; 171:1180–1194. [PubMed: 24354792]
27. Lin H, Liu AP, Smith TH, Trejo J. Cofactoring and dimerization of proteinase-activated receptors. *Pharmacol Rev.* 2013; 65:1198–1213. [PubMed: 24064459]
28. Gieseler F, Ungefroren H, Settmacher U, Hollenberg MD, Kaufmann R. Proteinase-activated receptors (PARs) — focus on receptor–receptor-interactions and their physiological and pathophysiological impact. *Cell Commun Signal.* 2013; 11:86. [PubMed: 24215724]
29. Coughlin SR. Thrombin signalling and protease-activated receptors. *Nature.* 2000; 407:258–264. [PubMed: 11001069]
30. Madhusudhan T, et al. Cytoprotective signaling by activated protein C requires protease-activated receptor-3 in podocytes. *Blood.* 2012; 119:874–883. [PubMed: 22117049]
31. Ruf W, Disse J, Carneiro-Lobo TC, Yokota N, Schaffner F. Tissue factor and cell signalling in cancer progression and thrombosis. *J Thromb Haemost.* 2011; 9:S306–S315.
32. Song D, Ye X, Xu H, Liu SF. Activation of endothelial intrinsic NF- κ B pathway impairs protein C anticoagulation mechanism and promotes coagulation in endotoxemic mice. *Blood.* 2009; 114:2521–2529. [PubMed: 19620400]
33. Isermann B, et al. Activated protein C protects against diabetic nephropathy by inhibiting endothelial and podocyte apoptosis. *Nat Med.* 2007; 13:1349–1358. [PubMed: 17982464]
34. Dong, W., et al. The protease aPC ameliorates renal I/R-injury by restricting YB-1 ubiquitination. *J Am Soc Nephrol.* <http://dx.doi.org/10.1681/ASN.2014080846>
35. von Drygalski A, Furlan-Freguia C, Ruf W, Griffin JH, Mosnier LO. Organ-specific protection against lipopolysaccharide-induced vascular leak is dependent on the endothelial protein C receptor. *Arterioscler Thromb Vasc Biol.* 2013; 33:769–776. [PubMed: 23393392]
36. Weiler H, et al. Characterization of a mouse model for thrombomodulin deficiency. *Arterioscler Thromb Vasc Biol.* 2001; 21:1531–1537. [PubMed: 11557684]
37. Apostolopoulos J, et al. The cytoplasmic domain of tissue factor in macrophages augments cutaneous delayed-type hypersensitivity. *J Leukoc Biol.* 2008; 83:902–911. [PubMed: 18180372]
38. Apostolopoulos J, Moussa L, Tipping PG. The cytoplasmic domain of tissue factor restricts physiological albuminuria and pathological proteinuria associated with glomerulonephritis in mice. *Nephron Exp Nephrol.* 2010; 116:e72–e83. [PubMed: 20664298]
39. Sharma L, et al. The cytoplasmic domain of tissue factor contributes to leukocyte recruitment and death in endotoxemia. *Am J Pathol.* 2004; 165:331–340. [PubMed: 15215187]
40. Yang YH, et al. Reduction in arthritis severity and modulation of immune function in tissue factor cytoplasmic domain mutant mice. *Am J Pathol.* 2004; 164:109–117. [PubMed: 14695325]
41. Cunningham MA, Kitching AR, Tipping PG, Holdsworth SR. Fibrin independent proinflammatory effects of tissue factor in experimental crescentic glomerulonephritis. *Kidney Int.* 2004; 66:647–654. [PubMed: 15253718]
42. Mackman N, Sawdey MS, Keeton MR, Loskutoff DJ. Murine tissue factor gene expression *in vivo* Tissue and cell specificity and regulation by lipopolysaccharide. *Am J Pathol.* 1993; 143:76–84. [PubMed: 8317556]
43. Seshan SV, et al. Role of tissue factor in a mouse model of thrombotic microangiopathy induced by antiphospholipid antibodies. *Blood.* 2009; 114:1675–1683. [PubMed: 19535796]
44. Osterud B, Bjorklid E. Sources of tissue factor. *Semin Thromb Hemost.* 2006; 32:11–23. [PubMed: 16479458]
45. Drake TA, Morrissey JH, Edgington TS. Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am J Pathol.* 1989; 134:1087–1097. [PubMed: 2719077]
46. Flossel C, Luther T, Muller M, Albrecht S, Kasper M. Immunohistochemical detection of tissue factor (TF) on paraffin sections of routinely fixed human tissue. *Histochemistry.* 1994; 101:449–453. [PubMed: 7960944]
47. Erlich JH, Holdsworth SR, Tipping PG. Tissue factor initiates glomerular fibrin deposition and promotes major histocompatibility complex class II expression in crescentic glomerulonephritis. *Am J Pathol.* 1997; 150:873–880. [PubMed: 9060825]

48. Ettelaie C, Su S, Li C, Collier ME. Tissue factor-containing microparticles released from mesangial cells in response to high glucose and AGE induce tube formation in microvascular cells. *Microvasc Res.* 2008; 76:152–160. [PubMed: 18725234]
49. Wiggins RC, Njoku N, Sedor JR. Tissue factor production by cultured rat mesangial cells. Stimulation by TNF α and lipopolysaccharide. *Kidney Int.* 1990; 37:1281–1285. [PubMed: 2345426]
50. Ono T, et al. Coagulation process proceeds on cultured human mesangial cells via expression of factor V. *Kidney Int.* 2001; 60:1009–1017. [PubMed: 11532095]
51. Sommeijer DW, et al. Renal tissue factor expression is increased in streptozotocin-induced diabetic mice. *Nephron Exp Nephrol.* 2005; 101:e86–e94. [PubMed: 15990447]
52. Sevastos J, et al. Tissue factor deficiency and PAR-1 deficiency are protective against renal ischemia reperfusion injury. *Blood.* 2007; 109:577–583. [PubMed: 16990608]
53. Nomura K, et al. Roles of coagulation pathway and factor Xa in rat mesangioproliferative glomerulonephritis. *Lab Invest.* 2007; 87:150–160. [PubMed: 17179958]
54. Cunningham MA, et al. Tissue factor pathway inhibitor expression in human crescentic glomerulonephritis. *Kidney Int.* 1999; 55:1311–1318. [PubMed: 10200995]
55. Yamamoto K, Loskutoff DJ. Extrahepatic expression and regulation of protein C in the mouse. *Am J Pathol.* 1998; 153:547–555. [PubMed: 9708814]
56. Song Z, et al. Intracellular localization of protein C inhibitor (PCI) and urinary plasminogen activator in renal tubular epithelial cells from humans and human PCI gene transgenic mice. *Histochem Cell Biol.* 2007; 128:293–300. [PubMed: 18193533]
57. Sorensen-Zender I, et al. Role of fibrinogen in acute ischemic kidney injury. *Am J Physiol Renal Physiol.* 2013; 305:F777–F785. [PubMed: 23804451]
58. Sumi A, et al. Roles of coagulation pathway and factor Xa in the progression of diabetic nephropathy in db/db mice. *Biol Pharm Bull.* 2011; 34:824–830. [PubMed: 21628879]
59. Tillet S, et al. Kidney graft outcome using an anti-Xa therapeutic strategy in an experimental model of severe ischaemia-reperfusion injury. *Br J Surg.* 2015; 102:132–142. [PubMed: 25402331]
60. Xu Y, et al. Constitutive expression and modulation of the functional thrombin receptor in the human kidney. *Am J Pathol.* 1995; 146:101–110. [PubMed: 7856719]
61. Vu TK, Hung DT, Wheaton VI, Coughlin SR. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell.* 1991; 64:1057–1068. [PubMed: 1672265]
62. Gui Y, Loutzenhiser R, Hollenberg MD. Bidirectional regulation of renal hemodynamics by activation of PAR1 and PAR2 in isolated perfused rat kidney. *Am J Physiol Renal Physiol.* 2003; 285:F95–F104. [PubMed: 12644441]
63. D'Andrea MR, et al. Characterization of protease-activated receptor-2 immunoreactivity in normal human tissues. *J Histochem Cytochem.* 1998; 46:157–164. [PubMed: 9446822]
64. Vesey DA, et al. Thrombin stimulates proinflammatory and proliferative responses in primary cultures of human proximal tubule cells. *Kidney Int.* 2005; 67:1315–1329. [PubMed: 15780084]
65. Bae JS, Kim IS, Rezaie AR. Thrombin down-regulates the TGF- β -mediated synthesis of collagen and fibronectin by human proximal tubule epithelial cells through the EPCR-dependent activation of PAR-1. *J Cell Physiol.* 2010; 225:233–239. [PubMed: 20506163]
66. Molitoris BA, Sutton TA. Endothelial injury and dysfunction: role in the extension phase of acute renal failure. *Kidney Int.* 2004; 66:496–499. [PubMed: 15253696]
67. Nawroth PP, Isermann B. Mechanisms of diabetic nephropathy—old buddies and newcomers part 2. *Exp Clin Endocrinol Diabetes.* 2010; 118:667–672. [PubMed: 20658437]
68. Nawroth PP, Isermann B. Mechanisms of diabetic nephropathy—old buddies and newcomers part 1. *Exp Clin Endocrinol Diabetes.* 2010; 118:571–576. [PubMed: 20658438]
69. Nieuwdorp M, et al. Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation *in vivo*. *Diabetes.* 2006; 55:480–486. [PubMed: 16443784]
70. Haraldsson B, Nystrom J, Deen WM. Properties of the glomerular barrier and mechanisms of proteinuria. *Physiol Rev.* 2008; 88:451–487. [PubMed: 18391170]

71. Xu D, Esko JD. Demystifying heparan sulfate–protein interactions. *Annu Rev Biochem.* 2014; 83:129–157. [PubMed: 24606135]
72. Salmon AH, Satchell SC. Endothelial glycocalyx dysfunction in disease: albuminuria and increased microvascular permeability. *J Pathol.* 2012; 226:562–574. [PubMed: 22102407]
73. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. *Pflugers Arch.* 2007; 454:345–359. [PubMed: 17256154]
74. Kato H. Regulation of functions of vascular wall cells by tissue factor pathway inhibitor: basic and clinical aspects. *Arterioscler Thromb Vasc Biol.* 2002; 22:539–548. [PubMed: 11950688]
75. Ho G, Broze GJ Jr, Schwartz AL. Role of heparan sulfate proteoglycans in the uptake and degradation of tissue factor pathway inhibitor–coagulation factor Xa complexes. *J Biol Chem.* 1997; 272:16838–16844. [PubMed: 9201990]
76. van Golen RF, et al. The mechanisms and physiological relevance of glycocalyx degradation in hepatic ischemia/reperfusion injury. *Antioxid Redox Signal.* 2014; 21:1098–1118. [PubMed: 24313895]
77. Nieuwdorp M, et al. Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. *Diabetes.* 2006; 55:1127–1132. [PubMed: 16567538]
78. Christiansen CF, et al. Kidney disease and risk of venous thromboembolism: a nationwide population-based case-control study. *J Thromb Haemost.* 2014; 12:1449–1454. [PubMed: 25040558]
79. Kerlin BA, Smoyer WE, Tsai J, Boulet SL. Healthcare burden of venous thromboembolism in childhood chronic renal diseases. *Pediatr Nephrol.* 2014; 30:829–837. [PubMed: 25487668]
80. Burton JO, et al. Elevated levels of procoagulant plasma microvesicles in dialysis patients. *PLoS ONE.* 2013; 8:e72663. [PubMed: 23936542]
81. Hrafnkelsdottir T, Ottosson P, Gudnason T, Samuelsson O, Jern S. Impaired endothelial release of tissue-type plasminogen activator in patients with chronic kidney disease and hypertension. *Hypertension.* 2004; 44:300–304. [PubMed: 15249548]
82. Jeong JC, Kim JE, Ryu JW, Joo KW, Kim HK. Plasma haemostatic potential of haemodialysis patients assessed by thrombin generation assay: hypercoagulability in patients with vascular access thrombosis. *Thromb Res.* 2013; 132:604–609. [PubMed: 24060191]
83. Kourtzelis I, et al. Complement anaphylatoxin C5a contributes to hemodialysis-associated thrombosis. *Blood.* 2010; 116:631–639. [PubMed: 20424189]
84. Ocak G, et al. Role of hemostatic factors on the risk of venous thrombosis in people with impaired kidney function. *Circulation.* 2014; 129:683–691. [PubMed: 24211824]
85. Schlegel N. Thromboembolic risks and complications in nephrotic children. *Semin Thromb Hemost.* 1997; 23:271–280. [PubMed: 9255908]
86. Zwaginga JJ, Koomans HA, Sixma JJ, Rabelink TJ. Thrombus formation and platelet–vessel wall interaction in the nephrotic syndrome under flow conditions. *J Clin Invest.* 1994; 93:204–211. [PubMed: 8282789]
87. Dubin R, et al. Kidney function and multiple hemostatic markers: cross sectional associations in the multi-ethnic study of atherosclerosis. *BMC Nephrol.* 2011; 12:3. [PubMed: 21269477]
88. Adams MJ, Irish AB, Watts GF, Ostryck R, Dogra GK. Hypercoagulability in chronic kidney disease is associated with coagulation activation but not endothelial function. *Thromb Res.* 2008; 123:374–380. [PubMed: 18486198]
89. Adams GN, et al. Murine prolylcarboxypeptidase depletion induces vascular dysfunction with hypertension and faster arterial thrombosis. *Blood.* 2011; 117:3929–3937. [PubMed: 21297000]
90. Bao YS, et al. Characterization of soluble thrombomodulin levels in patients with stage 3–5 chronic kidney disease. *Biomarkers.* 2014; 19:275–280. [PubMed: 24854597]
91. Faioni EM, et al. Low levels of the anticoagulant activity of protein C in patients with chronic renal insufficiency: an inhibitor of protein C is present in uremic plasma. *Thromb Haemost.* 1991; 66:420–425. [PubMed: 1796390]
92. Nampoor MR, et al. Hypercoagulability, a serious problem in patients with ESRD on maintenance hemodialysis, and its correction after kidney transplantation. *Am J Kidney Dis.* 2003; 42:797–805. [PubMed: 14520631]

93. Ghisdal L, et al. Thrombophilic factors in stage V chronic kidney disease patients are largely corrected by renal transplantation. *Nephrol Dial Transplant*. 2011; 26:2700–2705. [PubMed: 21285127]
94. Keven K, et al. Soluble endothelial cell protein C receptor and thrombomodulin levels after renal transplantation. *Int Urol Nephrol*. 2010; 42:1093–1098. [PubMed: 19802701]
95. Undas A, Kolarz M, Kopec G, Tracz W. Altered fibrin clot properties in patients on long-term haemodialysis: relation to cardiovascular mortality. *Nephrol Dial Transplant*. 2008; 23:2010–2015. [PubMed: 18156458]
96. Sjoland JA, et al. Fibrin clot structure in patients with end-stage renal disease. *Thromb Haemost*. 2007; 98:339–345. [PubMed: 17721616]
97. Kerlin BA, Ayoob R, Smoyer WE. Epidemiology and pathophysiology of nephrotic syndrome-associated thromboembolic disease. *Clin J Am Soc Nephrol*. 2012; 7:513–520. [PubMed: 22344511]
98. Kerlin, BA., et al. Disease severity correlates with thrombotic capacity in experimental nephrotic syndrome. *J Am Soc Nephrol*. <http://dx.doi.org/10.1681/ASN.2014111097>
99. Loscalzo J. Venous thrombosis in the nephrotic syndrome. *N Engl J Med*. 2013; 368:956–958. [PubMed: 23465106]
100. Glasscock RJ. Prophylactic anticoagulation in nephrotic syndrome: a clinical conundrum. *J Am Soc Nephrol*. 2007; 18:2221–2225. [PubMed: 17599972]
101. Lee T, et al. Personalized prophylactic anticoagulation decision analysis in patients with membranous nephropathy. *Kidney Int*. 2014; 85:1412–1420. [PubMed: 24336031]
102. Gambaro G, van der Woude FJ. Glycosaminoglycans: use in treatment of diabetic nephropathy. *J Am Soc Nephrol*. 2000; 11:359–368. [PubMed: 10665944]
103. Lewis EJ, Xu X. Abnormal glomerular permeability characteristics in diabetic nephropathy: implications for the therapeutic use of low-molecular weight heparin. *Diabetes Care*. 2008; 31:S202–S207. [PubMed: 18227486]
104. Li J, Wu HM, Zhang L, Zhu B, Dong BR. Heparin and related substances for preventing diabetic kidney disease. *Cochrane Database Syst Rev*. 2010:CD005631. [PubMed: 20824845]
105. Suzuki M, et al. Rationale and design of the efficacy of rivaroxaban on renal function in patients with non-valvular atrial fibrillation and chronic kidney disease: the X-NOAC study. *Int J Cardiol*. 2015; 188:52–53. [PubMed: 25889327]
106. Floege J, Eng E, Young BA, Couser WG, Johnson RJ. Heparin suppresses mesangial cell proliferation and matrix expansion in experimental mesangioproliferative glomerulonephritis. *Kidney Int*. 1993; 43:369–380. [PubMed: 8441232]
107. van der Pijl JW, et al. Danaparoid sodium lowers proteinuria in diabetic nephropathy. *J Am Soc Nephrol*. 1997; 8:456–462. [PubMed: 9071714]
108. Ikeguchi H, et al. Effects of human soluble thrombomodulin on experimental glomerulonephritis. *Kidney Int*. 2002; 61:490–501. [PubMed: 11849389]
109. Matsuyama M, et al. Tissue factor antisense oligonucleotides prevent renal ischemia-reperfusion injury. *Transplantation*. 2003; 76:786–791. [PubMed: 14501854]
110. Gupta A, et al. Activated protein C ameliorates LPS-induced acute kidney injury and downregulates renal INOS and angiotensin 2. *Am J Physiol Renal Physiol*. 2007; 293:F245–F254. [PubMed: 17409278]
111. Ozaki T, et al. Intrarenal administration of recombinant human soluble thrombomodulin ameliorates ischaemic acute renal failure. *Nephrol Dial Transplant*. 2008; 23:110–119. [PubMed: 17804460]
112. Sharfuddin AA, et al. Soluble thrombomodulin protects ischemic kidneys. *J Am Soc Nephrol*. 2009; 20:524–534. [PubMed: 19176699]
113. Thuillier R, et al. Thrombin inhibition during kidney ischemia-reperfusion reduces chronic graft inflammation and tubular atrophy. *Transplantation*. 2010; 90:612–621. [PubMed: 20865816]
114. Abdel-Bakky MS, Hammad MA, Walker LA, Ashfaq MK. Silencing of tissue factor by antisense deoxyoligonucleotide prevents monocrotaline/LPS renal injury in mice. *Arch Toxicol*. 2011; 85:1245–1256. [PubMed: 21327618]

115. Rajashekhar G, et al. Soluble thrombomodulin reduces inflammation and prevents microalbuminuria induced by chronic endothelial activation in transgenic mice. *Am J Physiol Renal Physiol.* 2012; 302:F703–F712. [PubMed: 22129968]
116. Ryan M, et al. Warfarin-related nephropathy is the tip of the iceberg: direct thrombin inhibitor dabigatran induces glomerular hemorrhage with acute kidney injury in rats. *Nephrol Dial Transplant.* 2014; 29:2228–2234. [PubMed: 24009280]
117. van Blijderveen JC, et al. Overanticoagulation is associated with renal function decline. *J Nephrol.* 2013; 26:691–698. [PubMed: 23843045]
118. Brodsky SV, et al. Warfarin-related nephropathy occurs in patients with and without chronic kidney disease and is associated with an increased mortality rate. *Kidney Int.* 2011; 80:181–189. [PubMed: 21389969]
119. Arachiche A, de la Fuente M, Nieman MT. Platelet specific promoters are insufficient to express protease activated receptor 1 (PAR1) transgene in mouse platelets. *PLoS ONE.* 2014; 9:e97724. [PubMed: 24830314]
120. Wang H, et al. Low but sustained coagulation activation ameliorates glucose-induced podocyte apoptosis: protective effect of factor V Leiden in diabetic nephropathy. *Blood.* 2011; 117:5231–5242. [PubMed: 21389321]
121. Borensztajn K, Spek CA. Blood coagulation factor Xa as an emerging drug target. *Expert Opin Ther Targets.* 2011; 15:341–349. [PubMed: 21250873]
122. Ruf W. FXa takes center stage in vascular inflammation. *Blood.* 2014; 123:1630–1631. [PubMed: 24627521]
123. Sparkenbaugh EM, et al. Differential contribution of FXa and thrombin to vascular inflammation in a mouse model of sickle cell disease. *Blood.* 2014; 123:1747–1756. [PubMed: 24449213]
124. Furugohri T, et al. Different antithrombotic properties of factor Xa inhibitor and thrombin inhibitor in rat thrombosis models. *Eur J Pharmacol.* 2005; 514:35–42. [PubMed: 15878322]
125. Furugohri T, Sugiyama N, Morishima Y, Shibano T. Antithrombin-independent thrombin inhibitors, but not direct factor Xa inhibitors, enhance thrombin generation in plasma through inhibition of thrombin-thrombomodulin-protein C system. *Thromb Haemost.* 2011; 106:1076–1083. [PubMed: 22012070]
126. Rana S, Yang L, Hassanian SM, Rezaie AR. Determinants of the specificity of protease-activated receptors 1 and 2 signaling by factor Xa and thrombin. *J Cell Biochem.* 2012; 113:977–984. [PubMed: 22034092]
127. Drew AF, et al. Crescentic glomerulonephritis is diminished in fibrinogen-deficient mice. *Am J Physiol Renal Physiol.* 2001; 281:F1157–F1163. [PubMed: 11704568]
128. Hariharan S, Pollak VE, Kant KS, Weiss MA, Wadhwa NK. Diffuse proliferative lupus nephritis: long-term observations in patients treated with ancrad. *Clin Nephrol.* 1990; 34:61–69. [PubMed: 2225555]
129. Craciun FL, et al. Pharmacological and genetic depletion of fibrinogen protects from kidney fibrosis. *Am J Physiol Renal Physiol.* 2014; 307:F471–F484. [PubMed: 25007874]
130. Kitching AR, et al. Plasminogen and plasminogen activators protect against renal injury in crescentic glomerulonephritis. *J Exp Med.* 1997; 185:963–968. [PubMed: 9120402]
131. Arai KI, et al. Cytokines: coordinators of immune and inflammatory responses. *Annu Rev Biochem.* 1990; 59:783–836. [PubMed: 1695833]
132. Wilson HM, Haites NE, Reid FJ, Booth NA. Interleukin-1 β up-regulates the plasminogen activator/plasmin system in human mesangial cells. *Kidney Int.* 1996; 49:1097–1104. [PubMed: 8691730]
133. Sedor JR, Nakazato Y, Konieczkowski M. Interleukin-1 and the mesangial cell. *Kidney Int.* 1992; 41:595–599. [PubMed: 1573834]
134. Kitching AR, et al. Plasminogen activator inhibitor-1 is a significant determinant of renal injury in experimental crescentic glomerulonephritis. *J Am Soc Nephrol.* 2003; 14:1487–1495. [PubMed: 12761249]
135. Muto Y, Suzuki K, Iida H, Ishii H. EF6265, a novel plasma carboxypeptidase B inhibitor, protects against renal dysfunction in rat thrombotic glomerulonephritis through enhancing fibrinolysis. *Nephron Exp Nephrol.* 2007; 106:e113–e121. [PubMed: 17622739]

136. Erlich JH, et al. Renal expression of tissue factor pathway inhibitor and evidence for a role in crescentic glomerulonephritis in rabbits. *J Clin Invest.* 1996; 98:325–335. [PubMed: 8755641]
137. Cunningham MA, et al. Protease-activated receptor 1 mediates thrombin-dependent, cell-mediated renal inflammation in crescentic glomerulonephritis. *J Exp Med.* 2000; 191:455–462. [PubMed: 10662791]
138. Tanaka M, et al. Role of coagulation factor Xa and protease-activated receptor 2 in human mesangial cell proliferation. *Kidney Int.* 2005; 67:2123–2133. [PubMed: 15882255]
139. Moussa L, Apostolopoulos J, Davenport P, Tchongue J, Tipping PG. Protease-activated receptor-2 augments experimental crescentic glomerulonephritis. *Am J Pathol.* 2007; 171:800–808. [PubMed: 17640968]
140. Redecha P, et al. Tissue factor: a link between C5a and neutrophil activation in antiphospholipid antibody induced fetal injury. *Blood.* 2007; 110:2423–2431. [PubMed: 17536017]
141. Ritis K, et al. A novel C5a receptor-tissue factor cross-talk in neutrophils links innate immunity to coagulation pathways. *J Immunol.* 2006; 177:4794–4802. [PubMed: 16982920]
142. Amara U, et al. Molecular intercommunication between the complement and coagulation systems. *J Immunol.* 2010; 185:5628–5636. [PubMed: 20870944]
143. Langer F, et al. Rapid activation of monocyte tissue factor by antithymocyte globulin is dependent on complement and protein disulfide isomerase. *Blood.* 2013; 121:2324–2335. [PubMed: 23315166]
144. Conway EM. Thrombomodulin and its role in inflammation. *Semin Immunopathol.* 2012; 34:107–125. [PubMed: 21805323]
145. Delvaeye M, et al. Thrombomodulin mutations in atypical hemolytic-uremic syndrome. *N Engl J Med.* 2009; 361:345–357. [PubMed: 19625716]
146. Weiler H, Isermann BH. Thrombomodulin. *J Thromb Haemost.* 2003; 1:1515–1524. [PubMed: 12871287]
147. Zoja C, et al. Lack of the lectin-like domain of thrombomodulin worsens Shiga toxin-associated hemolytic uremic syndrome in mice. *J Immunol.* 2012; 189:3661–3668. [PubMed: 22942429]
148. Fogo AB. The role of angiotensin II and plasminogen activator inhibitor-1 in progressive glomerulosclerosis. *Am J Kidney Dis.* 2000; 35:179–188. [PubMed: 10676714]
149. Brown NJ, et al. Aldosterone modulates plasminogen activator inhibitor-1 and glomerulosclerosis *in vivo*. *Kidney Int.* 2000; 58:1219–1227. [PubMed: 10972684]
150. Ma J, et al. Plasminogen activator inhibitor-1 deficiency protects against aldosterone-induced glomerular injury. *Kidney Int.* 2006; 69:1064–1072. [PubMed: 16528256]
151. Soh UJ, Doros MR, Chen B, Trejo J. Signal transduction by protease-activated receptors. *Br J Pharmacol.* 2010; 160:191–203. [PubMed: 20423334]
152. Kuliopulos A, et al. Plasmin desensitization of the PAR1 thrombin receptor: kinetics, sites of truncation, and implications for thrombolytic therapy. *Biochemistry.* 1999; 38:4572–4585. [PubMed: 10194379]
153. Baroni EA, Costa RS, da Silva CG, Coimbra TM. Heparin treatment reduces glomerular injury in rats with adriamycin-induced nephropathy but does not modify tubulointerstitial damage or the renal production of transforming growth factor- β . *Nephron.* 2000; 84:248–257. [PubMed: 10720896]
154. Benchetrit S, et al. Low molecular weight heparin reduces proteinuria and modulates glomerular TNF- α production in the early phase of adriamycin nephropathy. *Nephron.* 2001; 87:155–160. [PubMed: 11244311]
155. Diamond JR, Karnovsky MJ. Nonanticoagulant protective effect of heparin in chronic aminonucleoside nephrosis. *Ren Physiol.* 1986; 9:366–374. [PubMed: 3602582]
156. Yamashita J, et al. Protective effects of antithrombin on puromycin aminonucleoside nephrosis in rats. *Eur J Pharmacol.* 2008; 589:239–244. [PubMed: 18541230]
157. Harris JJ, et al. Active proteases in nephrotic plasma lead to a podocin-dependent phosphorylation of VASP in podocytes via protease activated receptor-1. *J Pathol.* 2013; 229:660–671. [PubMed: 23436459]

158. Ajay AK, Saikumar J, Bijol V, Vaidya VS. Heterozygosity for fibrinogen results in efficient resolution of kidney ischemia reperfusion injury. *PLoS ONE*. 2012; 7:e45628. [PubMed: 23029147]
159. Devarajan P. Update on mechanisms of ischemic acute kidney injury. *J Am Soc Nephrol*. 2006; 17:1503–1520. [PubMed: 16707563]
160. Krishnamoorthy A, et al. Fibrinogen β -derived B β _{15–42} peptide protects against kidney ischemia/reperfusion injury. *Blood*. 2011; 118:1934–1942. [PubMed: 21685370]
161. Urbschat A, et al. The small fibrinopeptide B β _{15–42} as renoprotective agent preserving the endothelial and vascular integrity in early ischemia reperfusion injury in the mouse kidney. *PLoS ONE*. 2014; 9:e84432. [PubMed: 24392138]
162. Hoffmann D, et al. Fibrinogen excretion in the urine and immunoreactivity in the kidney serves as a translational biomarker for acute kidney injury. *Am J Pathol*. 2012; 181:818–828. [PubMed: 22819533]
163. Svenningsen P, et al. Plasmin in nephrotic urine activates the epithelial sodium channel. *J Am Soc Nephrol*. 2009; 20:299–310. [PubMed: 19073825]
164. Tudpor K, et al. Urinary plasmin inhibits TRPV5 in nephrotic-range proteinuria. *J Am Soc Nephrol*. 2012; 23:1824–1834. [PubMed: 23024298]
165. Grandaliano G, et al. Regenerative and proinflammatory effects of thrombin on human proximal tubular cells. *J Am Soc Nephrol*. 2000; 11:1016–1025. [PubMed: 10820165]
166. Matsuo S, et al. Multifunctionality of PAI-1 in fibrogenesis: evidence from obstructive nephropathy in PAI-1-overexpressing mice. *Kidney Int*. 2005; 67:2221–2238. [PubMed: 15882265]
167. Zaferani A, et al. Heparin/heparan sulphate interactions with complement—a possible target for reduction of renal function loss? *Nephrol Dial Transplant*. 2014; 29:515–522. [PubMed: 23880790]
168. Wiggins JE, et al. NF κ B promotes inflammation, coagulation, and fibrosis in the aging glomerulus. *J Am Soc Nephrol*. 2010; 21:587–597. [PubMed: 20150534]
169. Pawlak K, Ulazka B, Mysliwiec M, Pawlak D. Vascular endothelial growth factor and uPA/suPAR system in early and advanced chronic kidney disease patients: a new link between angiogenesis and hyperfibrinolysis? *Transl Res*. 2012; 160:346–354. [PubMed: 22683425]
170. Ito T, Niwa T, Matsui E. Fibrinolytic activity in renal disease. *Clin Chim Acta*. 1972; 36:145–151. [PubMed: 4257416]
171. Mezzano D, et al. Hemostatic disorder of uremia: the platelet defect, main determinant of the prolonged bleeding time, is correlated with indices of activation of coagulation and fibrinolysis. *Thromb Haemost*. 1996; 76:312–321. [PubMed: 8883263]
172. Farquhar A, MacDonald MK, Ireland JT. The role of fibrin deposition in diabetic glomerulosclerosis: a light, electron and immunofluorescence microscopy study. *J Clin Pathol*. 1972; 25:657–667. [PubMed: 4561948]
173. Nakagawa T, et al. Diabetic endothelial nitric oxide synthase knockout mice develop advanced diabetic nephropathy. *J Am Soc Nephrol*. 2007; 18:539–550. [PubMed: 17202420]
174. Yamabe H, et al. Thrombin stimulates production of transforming growth factor- β by cultured human mesangial cells. *Nephrol Dial Transplant*. 1997; 12:438–442. [PubMed: 9075121]
175. Ito Y, et al. Kinetics of connective tissue growth factor expression during experimental proliferative glomerulonephritis. *J Am Soc Nephrol*. 2001; 12:472–484. [PubMed: 11181795]
176. Mormile A, et al. Physiological inhibitors of blood coagulation and prothrombin fragment F 1 + 2 in type 2 diabetic patients with normoalbuminuria and incipient nephropathy. *Acta Diabetol*. 1996; 33:241–245. [PubMed: 8904933]
177. Matsumoto K, et al. Inverse correlation between activated protein C generation and carotid atherosclerosis in Type 2 diabetic patients. *Diabet Med*. 2007; 24:1322–1328. [PubMed: 17971179]
178. Gil-Bernabe P, et al. Exogenous activated protein C inhibits the progression of diabetic nephropathy. *J Thromb Haemost*. 2012; 10:337–346. [PubMed: 22236035]
179. Bock F, et al. Activated protein C ameliorates diabetic nephropathy by epigenetically inhibiting the redox enzyme p66Shc. *Proc Natl Acad Sci USA*. 2013; 110:648–653. [PubMed: 23267072]

180. Bock F, Shahzad K, Vergnolle N, Isermann B. Activated protein C based therapeutic strategies in chronic diseases. *Thromb Haemost.* 2014; 111:610–617. [PubMed: 24652581]
181. Fu J, Lee K, Chuang PY, Liu Z, He JC. Glomerular endothelial cell injury and cross talk in diabetic kidney disease. *Am J Physiol Renal Physiol.* 2015; 308:F287–F297. [PubMed: 25411387]
182. Kerlin BA, et al. Survival advantage associated with heterozygous factor V Leiden mutation in patients with severe sepsis and in mouse endotoxemia. *Blood.* 2003; 102:3085–3092. [PubMed: 12869495]
183. Peter A, et al. Lower plasma creatinine and urine albumin in individuals at increased risk of type 2 diabetes with factor v leiden mutation. *ISRN Endocrinol.* 2014; 2014:530830. [PubMed: 24729885]
184. Pugliese F, Mene P, Cinotti GA. Glomerular polyanion and control of cell function. *Am J Nephrol.* 1990; 10:S14–S18.
185. Gambaro G, Kong NC. Glycosaminoglycan treatment in glomerulonephritis? An interesting option to investigate. *J Nephrol.* 2010; 23:244–252. [PubMed: 20155726]
186. Hagiwara H, et al. Expression of type-1 plasminogen activator inhibitor in the kidney of diabetic rat models. *Thromb Res.* 2003; 111:301–309. [PubMed: 14693179]
187. Lassila M, et al. Plasminogen activator inhibitor-1 production is pathogenetic in experimental murine diabetic renal disease. *Diabetologia.* 2007; 50:1315–1326. [PubMed: 17415547]
188. Nicholas SB, et al. Plasminogen activator inhibitor-1 deficiency retards diabetic nephropathy. *Kidney Int.* 2005; 67:1297–1307. [PubMed: 15780082]
189. Kenichi M, et al. Renal synthesis of urokinase type-plasminogen activator, its receptor, and plasminogen activator inhibitor-1 in diabetic nephropathy in rats: modulation by angiotensin-converting-enzyme inhibitor. *J Lab Clin Med.* 2004; 144:69–77. [PubMed: 15322501]
190. Cheng H, Chen C, Wang S. Effects of uPA on mesangial matrix changes in the kidney of diabetic rats. *Ren Fail.* 2014; 36:1322–1327. [PubMed: 25010090]
191. Wang H, et al. The lectin-like domain of thrombomodulin ameliorates diabetic glomerulopathy via complement inhibition. *Thromb Haemost.* 2012; 108:1141–1153. [PubMed: 23014597]
192. Malyszko J, Malyszko JS, Hryszko T, Mysliwiec M. Thrombin activatable fibrinolysis inhibitor (TAFI) and markers of endothelial cell injury in dialyzed patients with diabetic nephropathy. *Thromb Haemost.* 2004; 91:480–486. [PubMed: 14983223]
193. Atkinson JM, Pullen N, Johnson TS. An inhibitor of thrombin activated fibrinolysis inhibitor (TAFI) can reduce extracellular matrix accumulation in an *in vitro* model of glucose induced ECM expansion. *Matrix Biol.* 2013; 32:277–287. [PubMed: 23369837]
194. Atkinson JM, Pullen N, Da Silva-Lodge M, Williams L, Johnson TS. Inhibition of thrombin-activated fibrinolysis inhibitor increases survival in experimental kidney fibrosis. *J Am Soc Nephrol.* 2015; 26:1925–1937. [PubMed: 25411467]
195. Bruno NE, et al. Immune complex-mediated glomerulonephritis is ameliorated by thrombin-activatable fibrinolysis inhibitor deficiency. *Thromb Haemost.* 2008; 100:90–100. [PubMed: 18612543]
196. Grandaliano G, et al. Protease-activated receptor-2 expression in IgA nephropathy: a potential role in the pathogenesis of interstitial fibrosis. *J Am Soc Nephrol.* 2003; 14:2072–2083. [PubMed: 12874461]
197. Chung H, Ramachandran R, Hollenberg MD, Muruve DA. Proteinase-activated receptor-2 transactivation of epidermal growth factor receptor and transforming growth factor- β receptor signaling pathways contributes to renal fibrosis. *J Biol Chem.* 2013; 288:37319–37331. [PubMed: 24253040]
198. Pontrelli P, et al. Rapamycin inhibits PAI-1 expression and reduces interstitial fibrosis and glomerulosclerosis in chronic allograft nephropathy. *Transplantation.* 2008; 85:125–134. [PubMed: 18192922]
199. Grandaliano G, et al. Protease-activated receptor 1 and plasminogen activator inhibitor 1 expression in chronic allograft nephropathy: the role of coagulation and fibrinolysis in renal graft fibrosis. *Transplantation.* 2001; 72:1437–1443. [PubMed: 11685117]

200. Osterholm C, Veress B, Simanaitis M, Hedner U, Ekberg H. Differential expression of tissue factor (TF) in calcineurin inhibitor-induced nephrotoxicity and rejection—implications for development of a possible diagnostic marker. *Transpl Immunol.* 2005; 15:165–172. [PubMed: 16412961]
201. Ramackers W, et al. Recombinant human antithrombin prevents xenogenic activation of hemostasis in a model of pig-to-human kidney transplantation. *Xenotransplantation.* 2014; 21:367–375. [PubMed: 24807299]

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Figure 1. The coagulation system

The tissue factor (TF) and contact activation pathways lead to a common pathway that generates thrombin. In the tissue factor pathway, tissue injury or inflammatory cytokines induce cell-surface expression of TF. The TF/FVIIa complex activates FX, which converts FII to thrombin. In the contact activation pathway, negatively charged surfaces (such as phospholipids and polyphosphates from activated platelets) activate FXII, initiating a cascade leading to FX activation and thrombin generation. Thrombin triggers formation of blood clots, provides feed-back amplification or inhibition of the coagulation activation process and engages in receptor-dependent signalling. Localization of the coagulation cascade to cell surfaces ensures a spatial constraint on thrombin generation. Feed-back amplification is provided by activation of the non-catalytic cofactors FV and FVIII, platelet activation and thrombin-mediated activation of FXI. Excess coagulation activation is averted through several anticoagulant mechanisms, including inhibition of the TF/FVIIa/FXa complex by tissue factor pathway inhibitor (TFPI), inhibition of several coagulation factors by antithrombin (AT) and proteolytic inactivation of FVa and FVIIIa by activated protein C (aPC). As aPC is generated by the FIIa/thrombomodulin complex on undisturbed endothelial cells, local generation of FIIa following endothelial or vascular injury triggers aPC formation in a spatially and temporally limited fashion. Thrombin, aPC and other coagulation proteases interact with protease activated receptors, initiating cellular signalling that regulates inflammation and tissue remodelling. a, activated; F, factor; PC, protein C.

Figure 2. Initiation, amplification and propagation of coagulation

Upon vessel wall injury and/or activation of endothelial cells, tissue factor (TF) is exposed to blood and binds to FVII or FVIIa, promoting FVII activation or enhancing its catalytic activity. The TF–FVIIa (extrinsic tenase) complex activates small amounts of FIX and FX. FXa associates with FVa to form the prothrombinase complex, which cleaves FII to generate a small amount of thrombin. This initiation phase is followed by the amplification phase, in which thrombin activates cell-surface (predominately platelet) bound FV and FVIII and platelet-bound FXI. FIXa binds to FVIIIa on negatively charged surfaces (predominately platelet-derived phospholipids), activating FX (intrinsic tenase) and initiating a burst of thrombin generation—the propagation phase. Thrombin has pleiotropic functions including feedback inhibition, fibrin formation, platelet activation and signalling through protease activated receptors. a, activated; F, factor.

Figure 3. Potential mechanisms of PAR activation by thrombin and aPC

An example scheme of protease activated receptors (PARs) and the N-terminal sequences of human PAR1 and PAR3 depicting distinct cleavage sites for thrombin and activated protein C (aPC; arrows). The qualitatively distinct signalling mechanisms of thrombin and aPC can be attributed to the distinct proteolytic activation mechanisms of the G protein-coupled receptor (GPCR) N-terminus, resulting in protease-specific tethered ligands (shown in red for thrombin and blue for aPC) or induction of distinct protease-specific signalling complexes. PAR3 is not considered to be signalling competent and the function of the tethered ligands remains incompletely resolved. Activation of PARs might elicit protease-specific classical GPCR signalling by activation of individual PAR receptors (that is protomers) or ligand-specific PAR–PAR heterodimers. In addition, coagulation-protease-dependent signalling might engage non-PAR receptors, enabling biased signalling and thus leading to signalling diversity. Other proteases can cleave PARs at different sites, for example matrix metalloproteinase-1 cleaves PAR1 at Asp39 and neutrophil elastase cleaves PAR-1 at Leu45.

Figure 4. Expression of coagulation protease receptors in renal cells

Protease activated receptor (PAR) 2 is expressed on all types of human renal cells but is not expressed on murine podocytes, whereas PAR3 expression is predominantly restricted to podocytes. Endothelial protein C receptor (EPCR), which promotes protein C activation and activated protein C signalling, is expressed by glomerular endothelial and tubular epithelial cells. Tissue factor (TF) is expressed by podocytes and mesangial cells in humans, whether it is expressed on these cells in other species remains unproven. In various disease models (for example sepsis and diabetic nephropathy) TF expression is induced, but expression of thrombomodulin and EPCR is impaired.

Figure 5. Coagulation regulators in acute kidney injury

a | In acute glomerular injury, inflammation induces tissue factor (TF) expression on inflammatory cells recruited into the glomeruli or potentially on glomerular cells themselves, resulting in coagulation activation. FVa and the FXa complex assemble on these cells and enhance thrombin generation. FXa and thrombin induce glomerular cell dysfunction via protease activated receptor (PAR) 2 and PAR1, respectively. Thrombin signalling via PAR1 might involve transactivation of PAR2. Increased fibrin deposition contributes to the formation of glomerular crescents, which is inhibited by plasmin-mediated fibrinolysis. Inhibition of plasmin by plasminogen activator inhibitor-1 (PAI-1), which might be induced via the renin–angiotensin–aldosterone system (RAAS) or activated thrombin activatable fibrinolysis inhibitor (TAFIa), abolishes this effect. **b** | In acute tubular injury inflammation, for example in the context of ischaemia–reperfusion injury (IRI), induces TF expression on inflammatory cells or potentially on tubular epithelial cells themselves, triggering coagulation activation within the tubular compartment. Thrombin signalling via PAR1 leads to transforming growth factor β (TGF- β)-dependent tissue remodelling and tubular injury, whereas activated protein C (aPC) signalling via PAR1–endothelial protein C receptor (EPCR) inhibits TGF- β -dependent tubular fibrosis and preserves expression of YB-1 by restricting its ubiquitin-dependent degradation, thereby preventing tubular injury. Excess fibrin formation induces tubular injury, whereas moderate fibrin generation triggers fibrinolysis, leading to extracellular matrix (ECM) degradation and generation of the fibrin-derived peptide B β 15–42, which blocks the interaction of fibrin with intercellular adhesion molecule 1 (ICAM-1). Moderate fibrin deposition, therefore, contributes to renal recovery. Green inhibitory arrows indicate that inhibition promotes repair; red inhibitory arrow

indicates that inhibition promotes injury. a, activated; AT, antithrombin; F, factor; TM, thrombomodulin.

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Figure 6. Coagulation regulators in chronic kidney injury

a | In chronic kidney disease, subclinical inflammation induces expression of tissue factor (TF) on glomerular cells or potentially on inflammatory cells recruited into the glomeruli, thus triggering coagulation activation. FXa and thrombin induce glomerular cell dysfunction via protease activated receptor (PAR) signalling, fibrin induction and extracellular matrix (ECM) deposition. In chronic diabetic kidney disease, thrombomodulin (TM)-dependent protein C activation is impaired resulting in exacerbated glucose toxicity and glomerular cell dysfunction. Impaired protein C activation also triggers mitochondrial localization of Bax and p66Shc, resulting in mitochondrial dysfunction in glomerular cells. Reconstitution of activated protein C (aPC) signalling, for example by exogenous administration, restores cellular function via endothelial protein C receptor (EPCR)–PAR1 signalling in endothelial cells and PAR3-mediated signalling in podocytes, thus preventing diabetic nephropathy. Independent of aPC generation, thrombomodulin inhibits complement activation via its lectin-like domain and ameliorates diabetic nephropathy. **b** | Chronic inflammation triggers fibrin generation in the tubulointerstitial compartment, contributing to tubular injury. Plasmin-mediated degradation of fibrin and ECM inhibits this process, but this tissue-protective mechanism is inhibited by thrombin-mediated activation of thrombin activable fibrinolysis inhibitor (TAFI). Green inhibitory arrow indicates that inhibition promotes repair; red inhibitory arrow indicates that inhibition promotes injury. a, activated; EC, endothelial cells; F, factor; P, podocytes; PAI-1 plasminogen activator inhibitor-1; ROS, reactive oxygen species; TGF-β, transforming growth factor β; tPA, tissue-type plasminogen activator.

Table 1

Coagulation regulators with known functions in renal physiology and pathophysiology

Coagulation regulator (alternative name(s))	Function	Expression	Effects in renal physiology and pathophysiology	Refs
<i>Procoagulant</i>				
Tissue factor (thromboplastin, CD142, factor III)	Initiator; cofactor for factor VIIa in factor IX and factor X activation	Subendothelium	Induced in CGN, TMA, DN and endotoxaemia Inhibition protects against CGN and TMA	33–38,40,41, 43–45,98, 102, 190
Factor Xa	Enzyme	Plasma	Inhibition is protective in DN, endotoxaemia, MsPGN and IR	49–51,97,136
Factor VIII (antihemophilic factor)	Cofactor for activated factor IX in factor X activation	Plasma	Links impaired kidney function with risk of venous thrombosis	75
Factor V (labile factor)	Cofactor for factor Xa in prothrombin activation	Platelets and plasma	Factor V-dependent initiation of coagulation in human mesangial cells <i>in vitro</i> FV Leiden mutation conveys protective effects in DN	42,112
Prothrombin/thrombin (factor IIa)	Zymogen and protease	Plasma	Elevated in AKI, nephrotic syndrome, CKD, CGN and other renal diseases Inhibition protects against tubular atrophy, AKI and proinflammatory and proliferative responses in proximal tubular cells and mesangial cells	55,56,90,106, 108,135, 54, 165
Fibrinogen (factor I)	Fibrin precursor	Plasma, platelets	Plasma levels are elevated in diabetic patients and after renal IR Heterozygous but not homozygous depletion is protective in AKI, CGN and IR B β 15–42 peptide protects against renal IR injury	48,119,147, 149–151
<i>Anticoagulant</i>				
Thrombomodulin	Cofactor for thrombin in protein C activation	Endothelial surface	Shedding of thrombomodulin and elevated plasma levels of soluble thrombomodulin in diabetes and CKD Soluble thrombomodulin is	81,86,101, 103–105,131, 133,181

Coagulation regulator (alternative name(s))	Function	Expression	Effects in renal physiology and pathophysiology	Refs
			protective in experimental glomerulonephritis and IR Thrombomodulin lectin-like domain deficiency exacerbates HUS and DN	
Protein C	Zymogen and protease	Plasma	Renal-specific expression is reduced in various kidney diseases Activation is impaired in animals and patients with diabetes and in renal insufficiency Treatment with activated protein C is protective in experimental DN, IR and endotoxaemia	25,28,29,46, 82,100,167, 168,172
Antithrombin (heparin cofactor)	Protease inhibitor	Plasma	Deficient in experimental nephrotic syndrome Protects against tubular atrophy, AKI and PAN- induced nephrosis	145,191
Tissue factor pathway inhibitor (extrinsic pathway inhibitor)	Protease inhibitor	Endothelial surface via GPI, platelets, plasma	Induced in CGN Elevated levels partially compensate coagulation activation in diabetes Deficiency inhibits experimental glomerulonephritis Inhibition is protective in experimental kidney fibrosis	125,182, 184,185

AKI, acute kidney injury; CGN, chronic glomerulonephritis; CKD, chronic kidney disease; DN, diabetic nephropathy; GPI, glycosylphosphatidyl inositol; HUS, haemolytic uraemic syndrome; IR, ischaemia–reperfusion; MsPGN, mesangioproliferative glomerulonephritis; PAN, puromycin aminonucleoside; TMA, thrombotic microangiopathy.

Table 2Activating and disarming (inactivating) proteases of PARs^{15,16,26–28}

PAR	Activating proteases		Disarming proteases	
	Coagulation	Other	Coagulation	Other
PAR1	Thrombin, aPC, EPCR, activated factor X, plasmin	Granzyme A, trypsin IV, KLK1, KLK4, KLK6, KLK14, MMP-1, elastase, cathepsin G, proatherocytin [*] , Pen C 13 [‡]	Plasmin	KLK1, KLK14, ADAM17, protease 3, trypsin, cathepsin G, elastase
PAR2	Tissue factor, activated factor X, factor VIIa	Trypsin, mast cell tryptase, acrosin, matrilysin/MT-SP1, HAT, trypsin IV, granzyme A, TMPRSS2, chitinase [§] , KLK2, KLK4, KLK5, KLK6, KLK14, bacterial gingipains, Der P1, P2, P3 ^{//} , Pen C 13 [‡]	Plasmin	Protease 3, calpain, cathepsin G, elastase
PAR3	Thrombin, aPC, activated factor X	NA	NA	Cathepsin G, elastase
PAR4	Thrombin, plasmin, activated factor X	Trypsin, cathepsin G, trypsin IV, MASP-1, bacterial gingipains, KLK1, KLK14	NA	NA

Functional relevance of only a few proteases and corresponding PAR signalling has been demonstrated in renal physiology and pathophysiology. ADAM17, disintegrin and metalloproteinase domain-containing protein 17; aPC, activated protein C; EPCR, endothelial protein C receptor; HAT, human airway trypsin-like protease; KLK, kallikrein-related peptidase; MASP-1, mannan-binding lectin serine protease 1; MMP-1, matrix metalloproteinase-1; NA, not applicable.

^{*} Isolated from snake venom.

[‡] Mould allergen.

[§] From *Streptomyces griseus*.

^{//} House dust mite cysteine (P1) or serine (P2, P3) proteases.