The Podocyte Protease Web: Uncovering the Gatekeepers of Glomerular Disease

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Abstract

Proteases regulate glomerular physiology. The last decade has revealed a multitude of podocyte proteases that govern the glomerular response to numerous physical and biological cues. These proteases form a protein signaling web that generates stress stimuli and serves as a key controller of the glomerular microenvironment. Furthermore, both the extracellular and intracellular proteolytic networks are perturbed in focal segmental glomerulosclerosis, as well as hypertensive and diabetic nephropathy. The highly-intertwined podocyte protease web is accordingly an integrative part of the podocyte's damage response and is reactive to a variety of chemical, mechanical, and metabolic cues. Novel mass-spectrometry based technologies will help to untangle this proteolytic network: functional readouts acquired from deep podocyte proteomics, single glomerular proteomics, and degradomics may expose unprecedented protease activity. Future efforts should characterize the interdependency and upstream regulation of key proteases, along with their role in promoting tissue heterogeneity in glomerular diseases. These efforts will not only illuminate the machinery of podocyte proteostasis, but also reveal avenues for therapeutic intervention in the podocyte protease web.

1. Introduction

The renal filtration barrier is localized in the glomeruli and consists of three layers: the fenestrated endothelia, the glomerular basement membrane (GBM), and the podocytes. Glomeruli also contain mesangial cells. In the last decade, podocytes have emerged as the key cell type maintaining the renal filtration barrier. Podocytes are post-mitotic, neuron-like epithelial cells with a limited capacity for regeneration. They interdigitate via their foot processes and form specialized cell-cell contacts called slit diaphragms. Proteinuria, nephrotic syndrome, and focal segmental glomerulosclerosis (FSGS) result from podocyte injury and detachment (4). Therefore, the podocyte is an important target for diabetic and hypertensive nephropathy and glomerular damage (19)(25)(16).

Proteolytic processing of proteins is a crucial mechanism governing physiological and pathophysiological processes, such as differentiation, development, apoptosis, and cancer. Proteases are increasingly regarded as tailored therapeutic targets (38). It has been known for more than a decade that intracellular protease activity is essential for physiological podocyte maintenance (17)(43)(1)(32). The advent of novel mass-spectrometry based technologies unveiled the interdependence of various proteolytic systems in multiple fields, such as cancer and immunology. These advances gave rise to the concept of a “protease web” that dramatically controls cellular fate (9). The aim of this mini-review article is to provide an update on the physiological roles and functions of podocyte proteases (Fig. 1), identify the cues that alter the podocyte protease web, and highlight novel technologies for the study of podocyte protease function.

2. Intracellular proteases

Cathepsin proteases have long been implicated as disease drivers (17)(43)(1)(32). Increased abundance of Cathepsin L, an intracellular cysteine protease, is

observed in a number of pathological glomerular states (35)(11) and promotes Cd2ap and synaptopodin proteolysis to drive podocyte damage, leading to massive podocyte effacement (43). Cathepsin abrogation is protective from a variety of external stimuli. Mechanistically, Cathepsin L cleaves Dynamin, which affects the regulation of actin cytoskeleton in foot processes (32). More recently, it was found that Cathepsin L knockout mice do not develop streptozotocin induced glomerular damage (although they develop diabetes), a finding that could also be related to protection of the endothelia (11). Tissue cathepsin L can also be induced by cyclosporine in podocytes from cyclosporine A-treated patients (35) and correlates with proteinuria (6). However, cathepsin inhibition is not a general treatment in all conditions of glomerular disease. Blass and colleagues showed that using E-64, a broad and irreversible cysteine cathepsin inhibitor, did not reduce blood pressure or other aspects of glomerular injury that were induced by salt-sensitive hypertension. This suggests that under hypertensive conditions, cathepsin inhibition may not be a sufficient strategy for reducing glomerular damage (3). On the other hand, Cathepsin D, an aspartic protease, is important for maintaining podocyte integrity, since Cathepsin D deficient mice develop slit diaphragm fragmentation, proteinuria, and end stage renal disease (44). While loss of Cathepsin L has a protective effect in mice, decreased Cathepsin D appears to have a destructive effect. These studies underline the importance of distinguishing the roles of different cathepsins. Specific inhibitors that target particular cathepsins can further understanding and lead to more effective therapies. Cathepsins contribute to both maintenance and acute injury response, and their widely-studied functions place them at a pivotal node in the podocyte protease web.

Usp40, and Uchl1, two members of the family of ubiquitin proteases, are involved in sustaining podocyte integrity and link proteases to podocyte proteostasis. Usp40 is an intermediate filament-associated ubiquitin protease (36). Its expression is reduced in the puromycin aminonucleoside (PAN)-treated rat minimal change model, and zebrafish lacking the Usp40 orthologue demonstrate disruption of the renal filtration barrier (36). Uchl1 is a ubiquitin protease that is normally absent from the kidney, but expressed in damaged human podocytes (24). Uchl1 deficiency, in contrast, leads to a strong

glomerular phenotype that includes increased proteasome activity, a clear link to proteostasis (28). Therefore, de novo expression of Uchl1 in injured podocytes appears to help restore proteasomal proteostasis. These studies thus far indicate the diverse capacities of ubiquitin proteases, and their ability affect the cytoskeletal and proteomic composition of podocytes.

Calpain, a calcium-dependent cysteine protease, was originally identified as a protease that cleaved talin, an important focal adhesion molecule in the podocyte (37). Recent evidence shows that it may be proteolytically processing calcineurin in vitro and potentially in vivo - a smaller calcineurin fragment with unknown specificity and function was described (7). However, increased calpain and calcineurin activity with reduced expression of the calpain target Talin-1 was observed in a rat model of FSGS: increased glomerular and urinary calpain activity was associated with lowered Talin-1 abundance, enhanced calcineurin activity, and increased proteinuria. Treatment with the calpain inhibitor, calpeptin, prevented these effects (39). This suggests that proteolytic turnover of focal adhesion proteins is a key process driving FSGS .

The caspase system is commonly used as a readout of glomerular injury, particularly in *in vitro* studies. For instance, inhibiting PAR2 using the FSLLRY-amide peptide reduced levels of damage markers caspase 9 and desmin in an *in vitro* model of podocyte damage (40). High expression of TMEM16A, a transmembrane protein in the P38/JNK signaling pathway, activates caspases 3 and 9 in cultured podocytes, implicating it as an agent of podocyte apoptosis in diabetic neuropathy (21). At least in diabetic nephropathy, an alternative role for caspase 1 emerged *in vivo*. Caspase 1 is an intracellular cysteine-aspartic protease that regulates the NLRP3 inflammasome, which is implicated in podocyte injury (33). The caspase 3 inhibitor had no effect, whereas the caspase 1 inhibitor was able to block the progression of diabetic nephropathy. This suggests that the caspase 1-dependent inflammasome has a stronger part in promoting diabetic nephropathy than mere apoptosis-related caspase signaling (33). Interleukin-17 also activates caspase 1 (45). These data show that caspase activity determines the balance between inflammasome and apoptosis--two

fate-determining events in glomerular biology--and that the inflammasome is a particular driver of diabetic nephropathy.

3. Extracellular proteases

Extracellular matrix metalloproteases, thrombin, and complement form a microenvironment that is altered in glomerular disease states. Matrix metalloprotease 9, a mesangial cell expressed protease, controls podocyte differentiation (20). Knockout of the membrane-bound metalloproteases meprin α and β leads to exacerbation of streptozotocin-induced diabetic nephropathy (5). Angiotensin-II reduces expression of MMP-2 and 9, and this can be reversed by Notch inhibition in vitro (46). A focal increase in gelatinase activity and MMP-9 protein was observed in the glomeruli of diabetic rats (Zucker rats). Albumin administration resulted in a dose-dependent increase in MMP-9 protein and activity in culture supernatants of parietal epithelial cells in vitro (47). These studies illuminate the first cues that regulate matrix metalloproteinases in kidney disease, but their role in modulating the glomerular extracellular matrix requires further studies.

Thrombin is a serine protease that interacts with protease-activated receptors (PARs), and is associated with podocyte differentiation and pathogenesis. PARs are expressed throughout the body; in the kidney, these G-protein coupled receptors have been found in mesangial cells and podocytes (31). Palygin et al. recently reviewed the function of PAR receptors activated by thrombin proteolysis (27) (23). In the kidney, thrombin regulates glomerular filtration rate and renal hemodynamics via PARs. Moreover, elevated urinary thrombin is associated with glomerulonephritis and leads to PAR overstimulation, increased intracellular calcium levels, proteinuria, and deterioration of the glomerulus (18) (8). Inhibition of thrombin with hirudin reduced proteinuria in two rat nephrosis models (34). PAR-1 antagonist Q94 (negative allosteric modulator of the PAR-1 receptor) protects from doxorubicin-inflicted podocyte damage (12). Future studies should continue to clarify the emerging function of thrombin and

PARs in podocytes. Furthermore, important signaling molecules with direct relevance for podocyte biology, such as suPAR (42) and Notch (26), are proteolytically processed.

Taken together, the interplay between extracellular proteases the microenvironment is not yet comprehensively characterized, but initial *in vivo* studies already show great promise for further interventional studies in proteinuric conditions.

4. Novel technologies generated insights for the podocyte web.

Recent technological advances have enabled the fast interrogation of molecular machinery within podocytes. A proteome and transcriptome atlas of the native podocyte identified 9000 proteins and determined numerous novel podocyte enriched proteins, including several disease candidates (29). Among the podocyte-enriched proteins were Glutamyl aminopeptidase (Enpep), Ubiquitin carboxyl-terminal hydrolase 13 (Usp13), Carboxypeptidase Q (Cpq), Neprilysin (Mme), Pyroglutamyl-peptidase 1 (Pgpep1), Rhomboid-related protein 3 (Rhbdl3), Dipeptidyl peptidase 4 (Dpp4), Secernin-2 (Scrn2), Serine protease HTRA1 (Htra1), Mucosa-associated lymphoid tissue lymphoma translocation protein 1 homolog (Malt1), Secernin-3 (Scrn3), OTU domain-containing protein 7B (Otud7b), Probable aminopeptidase NPEPL1 (Npepl1), Disintegrin and metalloproteinase domain-containing protein 10 (Adam10), Carboxypeptidase D (Cpd), and Nardilysin (Nrd1). Some of these proteases have already been found in podocytes, e.g. Otud7b (also known as Cezanne), which is induced by ischemia in the kidney and in podocytes (22). However, the majority of these proteases are functionally not characterized.

Glomerular diseases are heterogenous (14). In a heterogenous disease, all glomeruli can be assumed to be at a different disease state, as demonstrated by emerging single cell studies. Very recently, ultrasensitive proteomic analyses has been developed for single glomeruli--one glomerulus at a time (13). Quantifying approximately 50 glomeruli from two different FSGS models, the lysosomal protein

LAMP1 was positively correlated with markers of glomerular damage, including Desmin, Col4a3, and intraglomerular albumin. Moreover, the abundance of Cathepsin B, L, and Z were associated with LAMP1 abundance, and all of these proteases were increased. Genetic deletion of Cathepsin B and L, but not Z, ameliorated proteinuria induced by nephrotoxic serum. This suggests that the individual intra-renal correlation of proteome profiles can improve the prioritization of disease-associated proteases across the population of glomeruli (13).

How can proteases be functionally characterized and linked to their substrates? The key technology to analyze podocyte function is mass spectrometry-based degradomics. Proteolytic cleavage generates new neo-N and neo-C termini in substrate proteins, but these are typically missed by standard proteomics approaches. Similar to other post-translational modifications, dedicated protocols for enrichment of protein N- and/or C-terminal peptides have been developed to enable their identification and quantification on a proteome-wide scale (15). Our recent N-degradomic analysis of podocytes revealed proteolytic processing of a variety of cytoskeletal and slit diaphragm proteins, including ACTN4, nephrin, podocin, and the intermediate filament protein vimentin (30). Several of these novel proteoforms were dynamically altered by PAN-mediated podocyte injury *in vitro* and *in vivo* in WT1 heterozygous and PAN treated rats. Analysis of cleavage sequence motifs and known interactions in the protease web provides insights into protease hierarchies (10), which confirmed caspase activation in PAN-induced injury *in vitro* but not *in vivo* and revealed reduced processing by Arg-specific enzymes (30). Furthermore, differential proteolytic processing was recently shown to correlate with the degree of kidney damage after cisplatin treatment in connection with protective preconditioning by hypoxia treatment or calory restriction (Späth et al).

5. Conclusion.

Taken together, podocyte proteases are important for the physiological filtration barrier and autonomous integration of multiple cues. However, the network character of

the proteases, as presented here (Fig. 1), is not yet commonly acknowledged. Various sites appear to be specifically controlled, novel podocyte proteases have emerged in the proteome, and tight links exist to proteostasis and the microenvironment in diabetic, inflammatory, and sclerotic glomerular diseases. Comprehensive characterization of this podocyte protease web is not only informative, but also necessary to decode the upstream metabolic and physiological cues of these programs. This should include both large-scale profiling of proteolytic processes and their (mis)regulation in kidney disease, as well as more detailed mechanistic studies of the role of individual proteases. Finally, spatial relationships need to be considered and analyzed. Collectively, this will complement ongoing efforts to model podocyte signaling networks (2, 41). Therefore, it is fair to state: the function of the podocyte protease web is just beginning to be deciphered.

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8. Figure Legends

Fig. 1. An initial outline of the podocyte protease web. All proteases are in white boxes, and known proteolytic relationships are described as arrows. Suggested proteolytic processes (based on degradomic data) are depicted as dashed arrows. Compounds are depicted in grey boxes. The left side comprises largely uncharacterized proteases, while the right side contains proteases related to lysosomal and inflammatory processes in FSGS and diabetic nephropathy.

extracellular

intracellular

cross-section of foot process

slit diaphragm

Uncharacterized Proteases

Pgpep1	Dpp4	Npepl1
Otud7b	Scrn2	Adam10
Enpep	Htra1	disintegrin
Usp13	Malt1	Cpd
Cpq	Scrn3	Nrd1
Mme	Rhbdl3	

meprin

coagulation cascade

thrombin

hirudin

Q94

PAR

TRPC6

Ca^{2+}

apoptosis

caspase cascade

calmodulin

calcineurin

CsA

NFAT

P

synaptopodin

P P

calpeptin

calpain

actin

dynamin

IL-17

caspase 1

inflammasome

complement cascade

E-64

cathepsin B

cathepsin Z

LAMP1

lysosome

cathepsin L

E-64

cathepsin D

Notch

γ-secretase

USP40

proteasome proteostasis

UCH-L1

?

ACTN4

podocin

vimentin

nephrin

CD2AP

talin-1

intermediate filaments

MMP-2

MMP-9

Ang II

albumin

mesangial cell

induces dedifferentiation

PM

GBM

fenestrated endothelia

$\alpha_3\beta_1$ integrin

GBM remodeling

protease

compound

known cleavage

putative cleavage

association

podocytes

foot processes