Chapter 10
The Glomerular Endothelium in Diabetic Nephropathy: Role of Heparanase

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Glomerular endothelial cells are highly specialized cells covering the inner side of glomerular capillaries. Glomerular endothelial cells are highly fenestrated, and under normal conditions the fenestrated area constitutes up to 50% of the entire endothelial surface, which facilitates the passage of water and small solutes [1]. The maintenance and formation of the fenestrae depends on vascular endothelial growth factor (VEGF) that is produced by podocytes [2].

The non-diaphragmed fenestrae of the glomerular endothelium traverse the cytoplasm and have a diameter of 50–80 nm [3]. Although the size of the fenestrae seems relatively large in relation to the size of circulating proteins, such as albumin with a diameter of 3.6 nm, it has been clearly shown that the glomerular endothelial glycocalyx contributes to the glomerular filtration barrier function. Removal of non-covalently bound components of the endothelial glycocalyx by infusion of hypertonic sodium chloride into the renal artery has been shown to cause a 12-fold increase in the fractional clearance of albumin without detectable changes in endothelial morphology [4].

Dysfunction of the (glomerular) endothelium is associated with the development and progression of diabetic vascular complications, including diabetic retinopathy and diabetic nephropathy [5, 6]. There are many factors and mechanisms that determine endothelial health and function, such as nitric oxide (NO), which is produced by the endothelial cells via endothelial nitric oxide synthase (eNOS) and which is crucial for endothelial health, integrity, and function [5, 7]. Importantly, eNOS has an anti-inflammatory effect, which is relevant for diabetic nephropathy, since diabetic nephropathy can be considered as an inflammatory glomerular disease [8]. It is therefore not surprising that a decreased NO production and bioavailability are observed in diabetes, thereby contributing to endothelial dysfunction (Fig. 10.2) [5].

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The glomerular endothelium cells are covered by, and fenestrae are filled with, a carbohydrate-rich layer, the glycocalyx, which represents a barrier that restricts passage of proteins from blood to urine [2]. The endothelial glycocalyx is a gel-like structure that is composed of a network of cell membrane-bound glycoproteins and proteoglycans, such as syndecans, glypicans, perlecan, and versican. The proteoglycans form the structural and functional backbone of the glycocalyx. In particular, the glycosaminoglycan (GAG) side chains of the proteoglycans contribute to the glomerular charge barrier function due to the overall highly negative charge of GAGs, whereas in addition GAGs can bind soluble molecules such as albumin, orosomucoid, and lumican and many other factors [1, 3, 9].

Heparan sulfate (HS) is one of the main sulfated GAGs in the glomerular endothelial glycocalyx. In addition to the contribution of HS to the glomerular filtration barrier function, HS is also a key player in cellular and tissue homeostasis, due to its capacity to selectively bind growth factors, chemokines, and many other factors. Heparanase is the only known mammalian endoglycosidase that is capable of degrading heparan sulfate. Chondroitin sulfate (CS) is another sulfated GAG that is typically present in the glycocalyx at a ratio of 1:4 compared to HS, although this ratio is variable depending on, for example, endothelial cell activation status. The major non-sulfated GAG in the glycocalyx is hyaluronan (HA). HA is a very efficient water binder, 1 gram of HA is able to bind 1000 gram of water, and therefore HA is responsible for the gel-like structure of the glycocalyx, whereas HA plays an important role in structural maintenance of vascular integrity [10]. Degradation of hyaluronan by hyaluronidase causes both a reduced glycocalyx thickness and an increased passage of albumin across the endothelium, illustrating the importance of an intact glycocalyx in barrier function [10, 11].

Due to the many functions and complexity of the glycocalyx, any disruption or damage to the glycocalyx contributes toward various vascular pathologies, including diabetic nephropathy [9, 12]. For instance, upon endothelial activation through inflammation, both the glycocalyx thickness and composition are changed, which is mainly due to action of the heparan sulfate-degrading enzyme heparanase [10]. In animal models for diabetes, a threefold increase in permeability of the negatively charged albumin can be observed, which is correlated to a reduced endothelial glycocalyx thickness and a reduced presence of glycocalyx proteins, such as versican and decorin [3, 13]. In contrast to the increased permeability for albumin, the permeability for Ficoll, with a similar size to albumin, but neutral in charge, is not affected in these animal models for diabetes. Therefore, it can be concluded that the charge barrier function, rather than the size barrier function is affected in these animal models for diabetes. Therefore, it may be suggested that endothelial damage, in particular a compromised glycocalyx, seems to precede damage to the podocyte/glomerular basement membrane [3].

Heterogeneity of Renal Endothelial Cells

Endothelial cells from renal arteries, arterioles, capillaries, venules, veins, and glomerular capillaries are phenotypically distinct, which may include differences in glycocalyx composition. The renal endothelium has several functions, such as

oxygen/nutrient delivery and/or charge-selective filtration, whereas the endothelial cells in different renal compartments perform different functions to maintain kidney homeostasis [14–16]. Endothelial cells derived from different renal compartments display varying chemokine receptor expression patterns, thereby mediating renal compartment-specific immune cell recruitment under inflammatory conditions [14]. Regarding reported changes in the renal endothelial glycocalyx in diabetic nephropathy, the majority of published research has focused on the glomerular endothelium. Changes in glycocalyx, in, for example, peritubular capillaries and other renal vasculature, and their corresponding effects on endothelial function have to our knowledge not been adequately addressed yet. It can be hypothesized that a better understanding of glycocalyx composition and function in different renal vascular beds may contribute to a better understanding of endothelial cell dysfunction in progressive kidney injury, including diabetic nephropathy.

Glomerular Endothelial Abnormalities in Diabetic Patients

Morphological abnormalities of the glomerular endothelium in diabetic patients have been reported in several studies. For instance, in patients with type 1 diabetes, a reduction in the amount of fenestrated glomerular endothelium was observed, ranging from 41% in controls to 32% in normo- and microalbuminuric patients and to 25% in macroalbuminuric patients [17]. The reduced glomerular endothelial cell fenestration was related to typical histological diabetic nephropathy lesions and a reduced renal function.

A similar decrease of glomerular endothelial fenestration was reported for type 2 diabetes patients [18]. Moreover, this latter study revealed that endothelial damage and podocyte damage occur simultaneously, which seems in contradiction with the idea of some researchers that in diabetic nephropathy podocyte injury is the primary event and endothelial damage occurs as a secondary effect. Nevertheless, aforementioned findings support the concept that the glomerular filtration barrier is only functional when all layers are intact [18, 19].

Heparan Sulfate as Part of the Extracellular Matrix

The glycocalyx in fact is part of the extracellular matrix (ECM). Constituents of the glycocalyx are, among others, the heparan sulfate proteoglycans (HSPGs) consisting of the polysaccharide HS linked to a proteoglycan core protein, such as syndecans and glypicans, the polysaccharide hyaluronan as well as other glycosylated proteins, and proteins that bind to these polysaccharides and glycosylated proteins [10]. HSPGs are involved in cell-cell and cell-ECM interaction via their HS chains. Furthermore, HS domains mediate binding of specific proteins, which is essential for the organization of cell surface protein-receptor interactions and for the creation of chemotactic gradients of growth factors and chemokines. For example, HS mediates the interaction of fibroblast growth factor-2 (FGF-2), vascular endothelial

growth factor (VEGF), and heparin-binding epidermal growth factor-like growth factor (HB-EGF) in conjunction with their corresponding receptors [20, 21], thereby underscoring a crucial role for HSPGs in physiological and pathological processes in the kidney.

Biosynthesis and Regulation of Heparan Sulfate

HS biosynthesis occurs in the Golgi apparatus via a complex multistep process, which is characterized by chain initiation, polymerization, and several modifications, such as N-deacetylation and O-sulfation [22–24]. All these different modifications give rise to an enormous structural diversity of HS, which dictates binding and modulation of growth factors, complement factors, chemokines, cytokines, selectins, enzymes, and other proteins [22, 24]. HS is degraded by heparanase, a β(1–4)-endoglucuronidase that cleaves HS via hydrolysis [25]. The recognized cleavage site is a tetrasaccharide that is accommodated within the heparanase-binding cleft. Heparanase is encoded by the HPSE1 gene, which is located on chromosome 4 at 4q21.3. Alternative splicing leads to expression of two mRNA transcripts that have the same open reading frame and encode the same 543 amino acid polypeptides, i.e., HPSE1a and HPSE1b. Heparanase is synthesized as a 68 kDa pre-proheparanase, which is targeted to the endoplasmic reticulum, via its signal peptide, and after cleavage of the signal peptide, the 65 kDa proheparanase is formed. The proheparanase is then targeted to the Golgi apparatus where it will be secreted in vesicles (Fig. 10.1) [21, 22, 26].

Heparanase is regulated in multiple ways. Heparanase activity is negatively regulated by heparanase 2, which lacks enzymatic activity and thus is an inactive heparanase variant, although the exact mechanism needs to be identified [27]. Furthermore, heparanase expression is regulated by methylation of the HPSE1 promotor and by the actions of cellular tumor antigen p53, inducing constitutively silencing of the gene. Additionally, members of the SP1 and ETS transcription factor families can induce HPSE1 expression. Moreover, HPSE1 expression can be induced by inflammatory mediators such as TNFα and IL-1β via their downstream transcription factor NF-κβ, as well as factors involved in cell growth like transcription factor early growth response protein 1 (EGR1). Factors involved in diabetic conditions also induce glomerular heparanase expression, such as components of the renin-angiotensin-aldosterone system (RAAS), advanced glycation end products, high glucose, reactive oxygen species (ROS), and endothelin-1 (Fig. 10.2) [21]. On the other hand, cellular quiescence induced by nitric oxide that is produced by eNOS and anti-inflammatory intracellular signaling through the vitamin D receptor suppress heparanase expression. The regulation of heparanase expression through vitamin D signaling was demonstrated by an increased heparanase expression and the development of proteinuria in knockout mice incapable of converting vitamin D into its active form and by reduction of heparanase expression and proteinuria after treatment with the active form of vitamin D (Fig. 10.2) [21, 28].

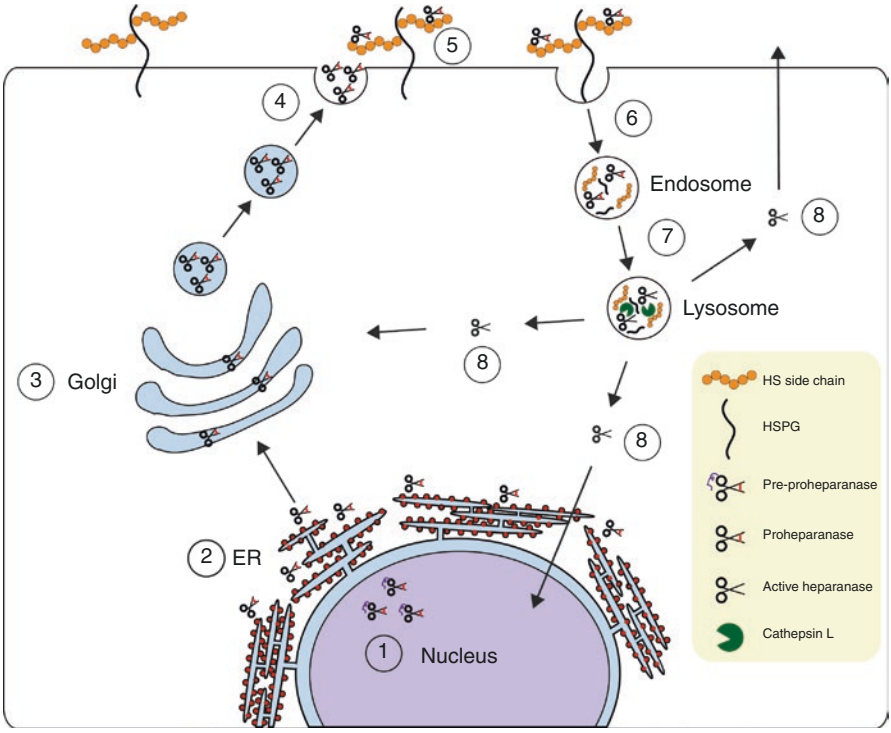

Fig. 10.1 Schematic overview of heparanase biosynthesis and trafficking. (1) Heparanase is synthesized in the nucleus as pre-proheparanase and subsequently trafficks to the endoplasmic reticulum (ER). (2) In the ER the signal peptide of pre-proheparanase is cleaved off resulting in proheparanase. (3) Proheparanase trafficks to the Golgi apparatus, where proheparanase is packaged into vesicles. (4) Subsequently, proheparanase is secreted into the extracellular matrix. (5) Once proheparanase is located in the extracellular matrix, proheparanase can bind to cell-associated HSPGs (in particular to syndecan). (6) Binding of proheparanase to HSPGs then causes internalization of the complex consisting of HSPGs and proheparanase by endocytosis. (7) As endosomal maturation takes place, the endosomes will become more acidic and thus convert into lysosomes, which will activate cathepsin L. Cathepsin L will cleave out an internal linker domain of proheparanase thereby processing proheparanase into the active heparanase heterodimer. (8) Upon activation, heparanase can be transported back to the Golgi apparatus, where heparanase will cause further remodeling of the intracellular HS structures. Furthermore, heparanase can be transported to the nucleus, where it is involved in the process of chromatin remodeling, probably by affecting histone acetyltransferase activity. Finally, heparanase can be transported back to the cell surface where it will be secreted into the ECM and degrade HS of the glycocalyx

Heparan Sulfate in Charge-Selective Filtration

It has been thought for decades that negatively charged HS in the glomerular basement membrane (GBM) was essential for the charge-selective permeability of the glomerular filtration barrier (GFB). HS in the GBM appears to be decreased in many glomerular diseases, like diabetic nephropathy. This decreased HS expression

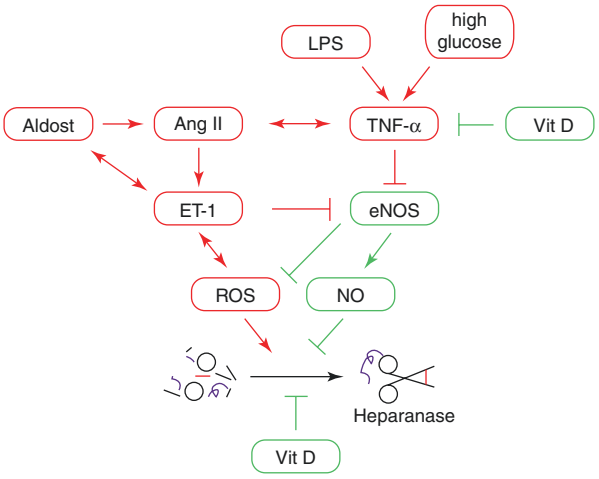

Fig. 10.2 Factors involved in the regulation of heparanase expression. In inflammatory conditions (such as in the presence of LPS) or under conditions of high glucose, TNF-α is induced. TNF-α will subsequently reduce eNOS expression, while it induces angiotensin II expression. Angiotensin II induces endothelin-1 expression, which induces aldosterone expression; creating a positive feedback loop as aldosterone further increases angiotensin II expression. Furthermore, endothelin-1 stimulated ROS production and reduces eNOS expression. eNOS reduces heparanase expression via reducing the ROS production and increasing NO production. Finally, vitamin D reduces heparanase expression either directly or indirectly via reduction of TNF-α expression. The abbreviations used are standing for LPS (lipopolysaccharide), Vit D (vitamin D), TNF-α (tumor necrosis factor-α), Aldost (Aldosterone), Ang II (angiotensin II), ET-1 (endothelin-1), ROS (reactive oxygen species), eNOS (endothelial nitric oxide synthase), and NO (nitric oxide). The factors that reduce heparanase expression are depicted in green, while the factors that increase heparanase expression are shown in red

is inversely correlated with the level of urinary protein excretion [29]. However, the primary role of HS in the GBM in charge-selective filtration was questioned by studies that applied several genetically engineered mouse models with disturbed HS(PG) expression in the GBM. Mice lacking the most abundantly expressed core protein agrin, or both agrin and perlecan, lack the majority of anionic sites in the GBM, but did not develop proteinuria or glomerular abnormalities [30, 31]. Furthermore, mice deficient for the cell surface HSPG syndecan-1 or endothelial NDST-1 also did not show albuminuria [32]. Mice lacking the essential HS-polymerizing enzyme EXT1 in their podocytes clearly showed glomerular ultrastructural abnormalities like foot process effacement, but only mild albuminuria was observed [33]. Finally, overexpression of heparanase in mice displayed a fivefold reduction of GAG-associated anionic sites in the GBM, but no severe albuminuria or ultrastructural abnormalities were observed [34]. Taken together, several mouse models targeting HS expression in the GBM do not display proteinuria, whereas a reduced HS expression in the capillary filter is associated with proteinuria in proteinuric patients. The primary role of HS, in the GBM, in determining charge-selective filtration can be rejected based on aforementioned studies. However,

limitations of the aforementioned studies were that targeting of HS in the GBM was performed under non-pathological conditions, which still warrants a possible role of reduced HS in the GBM under pathological conditions. Importantly, aforementioned studies shifted focus to the glomerular endothelial glycocalyx as the primary barrier in charge selectivity of the capillary filter under normal conditions [2].

Tight Control of Heparanase Activity Under Healthy Conditions

Heparanase facilitates HS turnover and recycling, thereby also contributing to remodeling of HS within the glycocalyx that may be important for disruption of barrier function and/or inflammatory processes. Because heparanase can disrupt the ECM and cell surface signaling processes, it is of great importance that heparanase activity is tightly controlled under healthy conditions. It has, for instance, been shown that heparanase can modulate HS within the glycocalyx, thereby facilitating binding of chemokines and growth factors and leukocytes [35]. Furthermore, heparanase is involved in autophagy, a cellular defense mechanism that generates metabolic precursors and ATP, clears cell debris and misfolded proteins, and is important for cell survival under stressful conditions. Autophagy deficiencies in podocytes lead to accelerated diabetes-induced podocytopathy in mice with streptozotocin-induced diabetes mellitus. Both HS and heparanase can influence autophagy. HS can constitutively inhibit autophagy, while heparanase positively stimulates the autophagy process through a nonenzymatic mechanism [21, 36].

The enzymatic activity of heparanase relies on an acidic environment. Raising the lysosomal pH by administration of substances such as chloroquine and bafilomycin A1 can therefore block the heparanase enzymatic activity. Under normal conditions, heparanase acts typically intracellular. Secreted proheparanase is quickly bound and taken up by the cells, mediated by the low-density lipoprotein receptor-related proteins and mannose-6 phosphate receptors, after which it is transferred to late endosomes and lysosomes [21]. Cathepsin L subsequently cleaves proheparanase into the mature active enzyme (Fig. 10.1) [37]. The proteoglycan syndecan-1 seems to be critical for proheparanase processing after its internalization [38].

Upon heparanase activation in the endosome, heparanase can be taken up by the Golgi system again, although its enzymatic activity will be relatively low due to the neutral pH in the Golgi (Fig. 10.1). Furthermore, heparanase can be transported from the endosome to the nucleus where it may facilitate chromatin remodeling, thereby increasing transcriptional activity of specific genes, which is mechanistically still poorly understood (Fig. 10.1). HS in the nucleus may inhibit histone acetylation, whereas heparanase can relieve this HS-mediated inhibition. Therefore, loss of nuclear heparanase is associated with tumor progression and tumor cell dedifferentiation [39, 40].

Upon activation, heparanase can also be transported from the endosome back to the cell surface where it can degrade HS in the ECM including the glycocalyx of

endothelial cells and podocytes (Fig. 10.1). The degradation of HS in the ECM and glycocalyx impairs barrier function of the glomerular capillary filter and causes release and modulation of the HS-bound chemokines and growth factors and generates potentially bioactive HS fragments. Under physiological healthy conditions transport of activated heparanase from endosome back to the cell surface does not occur often, except in placental trophoblasts and blood-borne immune cells due to the requirement of heparanase activation for physiological tissue remodeling and cell invasion. However, under inflammatory conditions, as in diabetes mellitus, extracellular heparanase activity is increased [21, 41].

Glomerular Endothelium in Kidney Disease: Role of Heparanase

Dysfunction of the endothelium due to, for instance, disturbance of metabolism of the endothelium by hyperglycemia in diabetic nephropathy is defined by impaired endothelium-dependent vasodilation and endothelial activation. This impaired vasodilation and endothelial activation is associated with an environment that promotes initiation and complications of atherogenesis due to its proinflammatory, proliferative, and procoagulatory effects [6]. The observed vasodilation is mainly due to the key role the endothelium plays in vascular homeostasis and damage to the endothelium will therefore disturb the balance between vasoconstriction and vasodilation, thereby initiating a cascade of events promoting or exacerbating atherosclerosis [6].

The endothelial glycocalyx exerts a key function in many physiological processes, including vascular permeability, attenuation of blood cell-vessel wall interactions, mechanotransduction, signaling, and vascular protection. Glycocalyx damage may disturb these physiological processes, potentially causing several vascular pathologies, such as development of proteinuria and inflammation, including diabetic nephropathy [2, 9, 12].

Increased glomerular heparanase activity has been demonstrated in human proteinuric glomerular diseases, including diabetic nephropathy [42]. In general, both podocytes and glomerular endothelial cells show increased heparanase expression associated with proteinuric glomerular disease, whereas heparanase levels in tubular cells are typically high also under healthy conditions [22, 43]. Increased expression of glomerular heparanase corresponds to loss of glomerular HS in rats, while the onset of proteinuria and loss of glomerular HS can be prevented by administration of a neutralizing antibody against heparanase or the heparanase inhibitor PI-88, thereby directly linking the development of proteinuria to loss of HS as mediated by heparanase [27, 44].

Heparanase Is Crucial for the Pathogenesis of Diabetes Mellitus and Diabetic Nephropathy

Heparanase has been implicated in the pathogenesis of various inflammatory kidney diseases, in acute kidney injury caused by sepsis [45], and has a potential role in the development of diabetes and diabetes complications. Increased extracellular heparanase activity is associated with pancreatic β-cell failure. Islet-specific autoreactive T cells can produce heparanase that promotes the migration of leukocytes going from the pancreatic blood vessels into the islets, which causes an immune response while simultaneously depleting islet β-cells of the intracellular HS necessary for cell survival [46]. Moreover, heparanase is overexpressed in the pancreas under obese conditions, causing polarization of islet-infiltrating macrophages toward a damaging inflammatory M1 phenotype, leading to pancreatic β-cell failure. On the other hand, heparanase can be associated with immunomodulatory protective effect, as it stimulates regulatory T helper type 2 cells production in some nondiabetes mouse models. This indicates that heparanase-mediated effects are model-dependent and that our understanding of heparanase is incomplete [21].

A ground breaking study demonstrated that heparanase is crucial for the development of experimental diabetic nephropathy. In contrast to wild-type mice, heparanase-deficient mice are completely resistant to streptozotocin-induced diabetic nephropathy [47]. Heparanase-deficient mice failed to develop proteinuria, and their urinary albumin excretion rate was normal, while a fivefold increase in urinary excretion rate was observed in the wild-type mice after streptozotocin-induced diabetic nephropathy. The role of heparanase under pathological conditions is supported by the fact that SST0001, a heparanase inhibitor, lowers albuminuria in type 1 and type 2 diabetic mice [47].

The diabetic milieu is one of the strongest inducers of heparanase expression. Patients with type 1 or type 2 diabetes show both a reduction in systemic glycocalyx volume and initially microalbuminuria that is associated with urinary heparanase levels [22]. High glucose levels alter the biosynthesis of sulfated GAG domains, in particular that of HS, indicating a possible role of hyperglycemia in systemic glycocalyx reduction. Furthermore, both hyperglycemia and glycated serum proteins contribute to heparanase upregulation in certain cell types, among others, endothelial cells and podocytes, whereas other factors such as ROS, aldosterone, and angiotensin II are amplifying factors as well [22, 48, 49].

An endothelial peptide, involved in the progression of diabetic nephropathy, called endothelin-1, activates podocytes to release heparanase. Furthermore, the endothelin-1-induced heparanase expression in podocytes causes an increase of transendothelial albumin passage. Moreover, the diabetes-induced upregulation of glomerular heparanase expression and corresponding reduction in HS expression, endothelial glycocalyx thickness, and development of proteinuria, which was

observed in wild-type mice, can be prevented by podocyte-specific deletion of the endothelin receptor [50].

Besides directly affecting endothelial glycocalyx, and thus barrier function, heparanase-mediated effects on inflammation provide an additional mechanism that contributes to the development of diabetic nephrology. Patients with diabetic nephropathy show increased levels of monocyte chemoattractant protein-1 (MCP-1) in their renal tissue and urine, thereby suggesting that macrophages have a pathogenic role in the development of proteinuria and glomerular damage and the progression of renal disease in humans [51]. This association between renal macrophages and pathological lesions in human diabetic nephropathy was indeed confirmed. The lysosomal cysteine protease cathepsin L, which facilitates processing and activation of enzymes, such as proheparanase in the extracellular matrix, can be secreted by macrophages [44, 52]. Furthermore, it was shown that macrophages are more prone to activation by, for instance, LPS or INF-γ, after they have been pretreated with heparanase, resulting in, among others, an increased TNF-α production (Fig. 10.3) [42, 53]. The mechanism behind this sensitization of macrophages, and also other cell types, by heparanase is only poorly understood, but it has been suggested that toll-like receptors (TLRs) 2 and 4 mediate cellular hyperactivation by binding of heparanase-generated HS fragments [54, 55]. Additionally, increased cellular activation can be due to heparanase-mediated shaving of cells, which may improve the accessibility of cytokine receptors for their specific ligands. A recent study even showed that heparanase-mediated sensitization is not limited to macrophages, because heparanase-mediated hypersensitivity for insulin was shown in breast cancer cells [56], thereby postulating the possibility of sensitization of other cell types involved in diabetic nephropathy such as glomerular endothelial cells but also podocytes.

Pathological events mediated by podocytes are also related to cleavage of cytoskeleton-associated proteins such as dynamin and synaptopodin by cathepsin L, which leads to impairment of the actin cytoskeleton and effacement of the podocyte foot process, for example, by cathepsin L-mediated cleavage of synaptopodin [57]. Besides macrophages, also podocytes in response to injury can secrete cathepsin L into the extracellular space. It has been shown that cathepsin L expression is increased in many glomerular diseases, like in diabetic nephropathy, and treatment of this increased cathepsin L expression with a cathepsin L inhibitor results in reduction of proteinuria [52, 58]. Cathepsin L-deficient mice with streptozotocin-induced diabetes do not lose their renal function and do not develop albuminuria, mesangial matrix expansion, tubulointerstitial fibrosis, podocyte injury, or renal macrophage influx, which is most likely caused by the inability of cathepsin L-deficient mice to activate heparanase [52]. Furthermore, MCP-1 inhibition results in decreased albuminuria and is associated with a shift in tissue macrophage phenotype toward alternatively activated M2 macrophages, resulting in a reduced glomerular cathepsin L and heparanase expression and restoration of the glomerular glycocalyx barrier function [21].

One of the hallmarks of glomerular lesions in diabetic nephropathy is destabilization of glomerular capillaries and corresponding mesangiolysis and glomerular

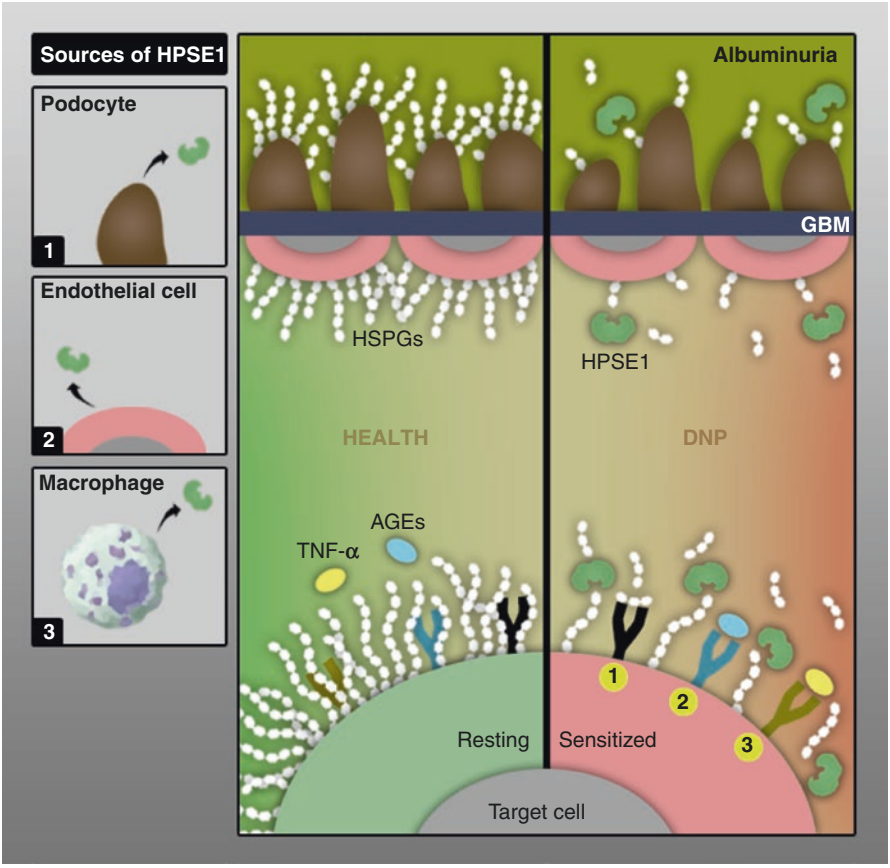

Fig. 10.3 The interplay between glomerular and immune cells leading to increased glomerular heparanase activity and albuminuria. During the development of diabetic nephropathy (DNP), glomerular heparanase activity is increased, thereby degrading heparan sulfate (HS) in the glomerular filtration barrier, which leads to proteinuria. Heparanase may be derived from immune cells such as macrophages and glomerular cells, such as podocytes and glomerular endothelial cells (left panel). The local inflammatory cytokine milieu acts on both immune and glomerular cells, thereby further enhancing heparanase and cathepsin L expression (not shown), which is required to activate pro-heparanase. Exciting recent data show that active heparanase can sensitize cells by degrading HS at the cell surface, as depicted in the lower part of the figure. Heparanase-generated HS then binds to TLR2 and TLR4 (depicted by 1), thereby increasing cellular activation. Additionally, heparanase-mediated shaving of cells may improve the accessibility of cytokine receptors (depicted by 2 and 3) for their specific ligands, which also leads to increased cellular activation. Overall, the increased glomerular heparanase activity shaves the glycocalyx from both endothelial cells and podocytes, thereby facilitating albuminuria

hypertrophy [59]. Induction of increased HS turnover and O-sulfation by increased activity of extracellular heparanase results in enlarged angiogenic growth factor binding. In case of sustained heparanase activity, heparanase in the ECM is released together with its bound growth factors, thereby facilitating angiogenesis and vessel

destabilization [60]. Moreover, angiogenesis is boosted by upregulated intracellular heparanase expression via direct enhancement of cellular VEGF production [61]. Finally, immunocytes are recruited due to the action of heparanase, which constitutes another important role in the angiogenesis process [21].

It can be concluded that heparanase activity, most likely in relation to reduction in HS expression levels, plays a crucial role in pathogenesis of proteinuria in experimental diabetic nephropathy at several levels.

Heparanase as a Pharmacological Target

Intracellular heparanase expression and activity plays a key role in cell survival and communication and should therefore not be targeted for therapy, except in cases where cells have to be killed like in cancer. As outlined, extracellular heparanase activation can cause inflammation, vessel destabilization, and fibrosis. A promising treatment strategy in kidney disease would thus be targeting of the extracellular heparanase activity. Some compounds that are aiming to inhibit heparanase expression or reduce heparanase activation are being developed and are already being tested for their therapeutic benefit. However, those compounds are thus far mainly used and developed in the context of cancer therapies and might target both intracellular and extracellular heparanase.

One approach is the development of drugs that compete with natural HS substrate by binding to the HS substrate-binding domain of heparanase, and this class of drugs are called HS mimetics [21, 62, 63]. One drawback of HS mimetics is that due to the structural resemblance to natural HS, these compounds can bind to many other HS-binding proteins, increasing the possibility of off-target effects. There are hundreds of proteins known to possess the capacity to interact with HS, together called the heparan sulfate interactome, or heparanome. This interactome includes proteins that are involved in various cellular and biological processes such as cell attachment, migration, invasion and differentiation, morphogenesis, organogenesis, blood coagulation, lipid metabolism, inflammation, and responses to injury [64]. For example, HS mimetics can modulate HS-mediated interactions between thrombin, antithrombin III, and protein C inhibitor, thereby influencing coagulation [64]. Furthermore, HS mimetics can be taken up by cells and modify the intracellular regulatory function of heparanase, as outlined. Another downside of HS mimetics is their probability to provoke an inflammatory response since HS can serve as ligand for TLR-2 and TLR-4 on macrophages and epithelial cells [54].

In addition to HS mimetics, such as SST0001 [62], synthetic HS tetrasaccharides containing unsubstituted glucosamine residues, like GP545, are under development. These synthetic HS structures are resistant to heparanase activity and can therefore be applied as a heparanase inhibitor [65]. Furthermore, multiple oligosaccharides that are derived from marine algae are currently tested for their possible ability to modify HS-heparanase interactions. Sulfated polysaccharides that resemble glycosaminoglycans are present in different algae species in the marine environment. One

compound that has been tested thus far is λ-carraheptaose, which is a highly sulfated polysaccharide derived from red algae. The λ-carraheptaose seems to act simultaneously as competitive inhibitor of heparanase, and thus as HS-mimetic, and as inhibitor of FGF-2 signaling. Another compound, a fucosylated form of chondroitin sulfate, which is extracted from marine echinoderm, was shown to reduce heparanase expression in the glomerulus thereby protecting rats from streptozotocin-induced diabetic nephropathy [21].

Administration of HS, heparin, low-molecular-weight heparin or the heparinoid danaparoid all are, in potential, able to reduce albuminuria in patients with diabetes mellitus, but these compounds have some off-target effects. One example is sulodexide, which is a highly purified mixture consisting of 80% of low-molecular-weight heparin and of 20% of dermatan sulfate [66], for which there are some conflicting studies. Sulodexide was shown to be effective in restoring the glycocalyx thickness and showed a trend toward normalization of systemic albumin clearance in a study of type 2 diabetes mellitus patients, but in two other studies, no such effect was observed in type 2 diabetes mellitus and diabetic nephropathy patients [21]. These contradicting findings might be explained by the use of sulodexide derived from different animal sources, and therefore the presence of different biological active structures in different preparations. Due to the lack of insight into the specific structures within different sulodexide preparations that are responsible for heparanase inhibition and subsequent anti-proteinuric effects, wrong conclusions may have been drawn from aforementioned studies.

Another strategy to inhibit heparanase-mediated HS breakdown is by directly blocking the HS-binding site on heparanase. There are three potential HS-binding domains in heparanase identified that could serve as a target for heparanase inhibition. A peptide directed against Lys158-Asp171 domain of heparanase physically interacts with HS and heparin and inhibits heparanase activity [67]. Moreover, a polyclonal antibody and two monoclonal antibodies raised against this region may provide a new class of drugs leading to a reduced heparanase activity [67, 68]. This new class of drugs, yet to be tested in models of kidney disease, might become an appealing treatment option for patients with diabetic nephropathy or other heparanase-mediated nephropathies.

Some drugs that are currently used in renal medicine have been shown to suppress glomerular heparanase expression and activity, such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), and vitamin D [28, 49]. These drugs have been associated with reduced albuminuria in clinical trials, which might be explained by their ability to reduce heparanase activity. Endothelin-A (ETa)-receptor blockade is currently under exploration in randomized clinical trials for its renoprotective potential, since it reduces albuminuria clinically. Selective ETa-receptor blockade facilitates preferential ETb-receptor stimulation by endothelin-1 and increased nitric oxide production by the endothelium. Moreover, ETa-receptor blockade causes suppression of heparanase activity associated with restoration of the glomerular glycocalyx, restoration of the barrier function, and reduction of albuminuria [21].

Several drugs currently tested or used for treatment of renal dysfunction target pathways that reduce heparanase activity both in immunocytes and podocytes and

might therefore be adequate to resolve residual albuminuria treatment. Clinical studies that have therapeutically targeted monocytes by blocking their chemokine receptor CCR2 (also known as CD192) or by blocking the CCR2 ligand, MCP-1, support the idea that albuminuria can be reduced by targeting immunocyte activation as both approaches led to reduced albuminuria in patients with diabetic nephropathy [69, 70]. The obtained reduction in albuminuria due to blockage of the CCR2 ligand was associated with a reduced cathepsin L release by tissue macrophages [21]. As outlined, cathepsin L is important in heparanase activation, and reduction of cathepsin L expression/activity was therefore further associated with reduced heparanase activity and restoration of the glycocalyx filtration barrier. Due to its key role in heparanase activation, cathepsin L could also be considered a potential therapeutic target. Furthermore, cathepsin L is shown to be required for the development of albuminuria and diabetic nephropathy [52]. Only several non-specific cathepsin L inhibitors have been tested and showed reduction of proteinuria in experimental models of anti-glomerular basement membrane glomerulonephritis [21, 71, 72]. To date, more specific cathepsin L inhibitors have been developed; however, their therapeutic effects in glomerular diseases have not yet been tested.

Another promising treatment strategy includes the use of heparanase 2, which is an inactive heparanase variant as it shares 44% identity and 59% similarity with heparanase but lacks enzymatic activity [27]. It has been shown that heparanase 2 inhibits heparanase activity [27, 73]. The mode of action of heparanase 2 may rely on its higher affinity to HS compared to heparanase, thereby blocking the binding of heparanase to HS. As heparanase cannot bind to HS on the cell surface, it fails to get internalized and will therefore remain inactive. In addition, heparanase 2 may physically interact with heparanase, thereby preventing the cleavage of HS chains. Furthermore, heparanase 2 is not likely to activate macrophages, which is problem in case of HS mimetics aiming to inhibit heparanase activity. However, the potential of heparanase 2 as an inhibitor of heparanase activity in glomerular diseases remains to be elucidated in experimental models.

Besides application of heparanase as a target for therapy, heparanase activity might also serve as a suitable biomarker for risk stratification and treatment titration as urinary heparanase excretion is increased in patients with diabetes, especially in case of albuminuria, whereas no urinary heparanase activity is present in healthy individuals.

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