Review


# Glomerular basement membrane heparan sulfate in health and disease: A regulator of local complement activation

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## Abstract

The glomerular basement membrane (GBM) is an essential component of the glomerular filtration barrier. Heparan sulfate proteoglycans such as agrin are major components of the GBM, along with α345(IV) collagen, laminin-521 and nidogen. A loss of GBM heparan sulfate chains is associated with proteinuria in several glomerular diseases and may contribute to the underlying pathology. As the major determinants of the anionic charge of the GBM, heparan sulfate chains have been thought to impart charge selectivity to the glomerular filtration, a view challenged by the negligible albuminuria in mice that lack heparan sulfate in the GBM. Recent studies provide increasing evidence that heparan sulfate chains modulate local complement activation by recruiting complement regulatory protein factor H, the major inhibitor of the alternative pathway in plasma. Factor H selectively inactivates C3b bound to surfaces bearing host-specific polyanions such as heparan sulfate, thus limiting complement activation on self surfaces such as the GBM, which are not protected by cell-bound complement regulators. We discuss mechanisms whereby the acquired loss of GBM heparan sulfate can impair the local regulation of the alternative pathway, exacerbating complement activation and glomerular injury in immune-mediated kidney diseases such as membranous nephropathy and lupus nephritis.



## Introduction

The glomerular basement membrane (GBM) is an amorphous layer of extracellular matrix separating glomerular endothelial cells and podocytes. Together, these components form the glomerular filtration barrier, which acts as a semi-permeable sieve allowing the relatively unimpeded flow of water and small solutes while restricting the passage of proteins such as albumin from blood into the primary urine. The GBM forms during the glomerular development through the fusion of the basement membranes of glomerular endothelial cells and podocytes, and both kinds of cells contribute components to the mature GBM [1,2]. Reflecting adaptions to a specialized role in the glomerular filtration, the GBM is thicker (250–350 nm in humans) than other basement membranes and has a distinctive biochemical composition.

The major components of the mature GBM are α345(IV) collagen, laminin-521, nidogens, and heparan sulfate proteoglycans (HSPG), including agrin and perlecan [3]. Several GBM components have been identified as essential for normal glomerular structure and function, based on the phenotype of mutations associated with human disease. In Alport syndrome, mutations in COL4A3, COL4A4 or COL4A5 genes (encoding the α3, α4 and α5 chains of type IV collagen, respectively) impair the normal assembly of α345(IV) collagen molecules [4,5], causing abnormal GBM deposition of α12(IV) collagen, GBM thickening and splitting, progressive proteinuria with onset in childhood, and eventually renal failure. In Pierson syndrome, mutations in LAMB2 gene (encoding laminin β2 chain) impair the GBM deposition of laminin-521 and cause congenital nephrotic syndrome [6].

More enigmatic are the roles of HSPG in the GBM under physiological and pathological conditions—the focus of this mini-review. An acquired loss of GBM heparan sulfate chains is associated with proteinuria in several human and experimental

kidney diseases, providing circumstantial evidence that GBM HSPG are important for normal glomerular function and homeostasis. As the major determinants of the anionic charge of the GBM, heparan sulfate chains have been presumed important for the charge selectivity of the glomerular filtration, a view challenged by recent studies. An emerging role of heparan sulfate is the local regulation of complement activation in tissues, including in the kidneys [7–9]. Heparan sulfate chains function as so-called "self-associated molecular patterns" [10] recognized by complement regulatory proteins, which enable the complement system to discriminate between host and pathogens. We will discuss in detail how this self-recognition function may be compromised in kidney disease with detrimental results.

## Heparan sulfate proteoglycans in the normal GBM

Heparan sulfate proteoglycans (HSPG) consist of one or more heparan sulfate chains attached to a core protein [11]. Heparan sulfate chains are linear polysaccharides consisting of 50–200 repeating disaccharide units. Their biosynthesis is initiated by the assembly of a tetra-saccharide structure (xylose–galactose–galactose–glucuronic acid) onto specific serine residues of the core protein, followed by the addition of one N-acetylglucosamine unit (catalyzed by ExtL3), which signals the heparan sulfate copolymerase complex Ext1/Ext2 to assemble the heparan sulfate chain by adding alternating glucuronic acid and N-acetylglucosamine units [12]. The chains are further modified by N-deacetylation/N-sulfation of the glucosamine residue (catalyzed by an N-deacetylase/N-sulfotransferase), epimerization of glucuronic to iduronic acid (catalyzed by a C5-epimerase), and addition of sulfate groups to the 2-O, 3-O and 6-O positions (catalyzed by various O-sulfotransferases). These modifications are incomplete and vary along the chain, creating a mosaic of high N-sulfated domains and high N-acetylated domains separated by transition zones with intermediary composition [13]. As a result, heparan sulfate chains exhibit considerable structural diversity within and among tissues. After biosynthesis, the structure of heparan sulfate can be further edited by enzymes such as the endoglucuronidase heparanase (discussed below), and Sulf-1 and Sulf-2, extracellular sulfatases that remove 6-O sulfate groups, which may fine-tune function and biological activity.

Within the kidney glomerulus, several types of HSPG are found with distinct distribution: syndecans and glypicans are associated with cell surfaces, while perlecan, agrin, and type XVIII collagen are components of basement membranes [14,15]. Perlecan is the prominent HSPG in most basement membranes, though in the mature GBM it is confined to the subendothelial side [16]. It consists of a 467 kDa core protein comprising five domains, which bears three heparan sulfate chains within the amino-terminal domain I [17]. Perlecan is anchored in basement membranes by interactions with other constituents, binding to nidogen via its core protein and to laminin and collagen IV via its heparan sulfate chains [18]. Another HSPG often co-localized with perlecan is collagen XVIII [19], which is prominently expressed in the mesangial matrix and Bowman capsule basement membrane, but is a minor component of the normal GBM.

Agrin is the most abundant HSPG in the GBM [20]. It consists of a 212 kDa core protein, to which two or three heparan sulfate chains are attached in the amino terminal half [21]. Full length agrin is restricted to the GBM while truncated isoforms lacking C-terminal epitopes are more broadly found in other kidney basement membranes, possibly reflecting alternative splicing or post-translational modifications [22]. Agrin may play a role in cell–matrix adhesion because its N-terminal end binds to the coiled coil domain of laminin γ1 chain while its C-terminal end binds to cell surface receptors such as integrins or α-dystroglycan [23–25]. The ultrastructural localization of agrin in the GBM has been recently determined by ultra-high resolution STORM imaging correlated with electron microscopy [26]. In the mouse GBM, agrin is localized in two layers and is oriented perpendicular or slightly oblique to the plane of the GBM, with its C-terminal end near adjacent cell membranes and its N-terminus toward the center of the GBM. In the human GBM, significantly more agrin (as detected with antibodies to its C-terminal domain) is present in a subepithelial layer, adjacent to podocytes, which may reflect differences in expression or alternatively spliced isoforms [26].

The phenotype of known mutations affecting HSPG core proteins in human disease affords no inferences about the role of HSPG in the GBM. Whereas missense mutations in the genes encoding agrin [27,28] or perlecan [29,30] do occur in congenital myasthenic syndrome and Schwartz–Jampel syndrome, respectively, no kidney dysfunction has been reported in these patients, presumably because these mutations have limited, localized effect on protein function. Nonetheless, as discussed in the next section, the expression of heparan sulfate in the GBM is altered in various kidney diseases, suggesting a functional role under pathologic conditions.

## Alterations of glomerular heparan sulfate chains in kidney diseases

A reduction of GBM anionic sites (presumably heparan sulfate) in several human and experimental