glomerular disease

Revisiting the determinants of the glomerular filtration barrier: what goes round must come round

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The glomerular filtration barrier (GFB) is characterized by a very high hydraulic permeability, combined with a marked permselectivity that excludes macromolecules such as albumin. Thus, the GFB retains most of the plasma proteins, with only 0.06% of albumin getting across the basement membrane. The GFB consists of 3 layers: fenestrated endothelial cells, the glomerular basement membrane, and podocytes. Injury to any of these components can result in the development of proteinuria. The contribution of the major components of the GFB has recently been reexamined and is discussed in the context of our past and present understanding.

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Glomerular filtration barrier (GFB) function and morphology were established with the application of micropuncture and electron microscopy techniques in the 1960s and early 1970s. The fenestrated glomerular endothelium, the multilayered glomerular basement membrane (GBM), and the podocyte, with its intricate interdigitating foot processes separated by the interposed slit diaphragm, were described by Farquhar *et al.* as well as Rodewald and Karnovsky.[1,2] Farquhar *et al.* performed a sophisticated functional analysis of the GFB with the use of size-selective proteins (albumin and dextrans) and charge-selective tracers (ferritin). They showed that the GBM represented the major permselective barrier for both molecular size and charge, excluding molecules with the size and negative charge of albumin from glomerular filtration under normal conditions. The slit diaphragm was identified as a structure connecting foot processes of adjacent podocytes, but its functional role in the GFB remained controversial.

The glomerular basement membrane as a component of the filtration barrier

Over the ensuing decades the meshwork of extracellular matrix proteins constituting the GBM was analyzed in detail. The major components of the GBM include type IV collagens with alpha 3, 4, and 5 chains, laminin β2, and negatively charged heparin-sulfate proteoglycans such as agrin and nidogen. These components generate the molecular scaffold essential for the adult GBM. The GBM provides not only structural support for the glomerular capillaries, but also contributes to cell-matrix and cell-cell cross-talk, which are essential for the maintenance of the functional unit of the glomerulus.

The contribution of individual components of the GBM to its permselectivity was elucidated in large part through genetic studies demonstrating that mutations in different components of the GBM lead to albuminuria and progressive renal failure.[3] For example, mutations in collagen IV α3 in Alport syndrome and in laminin β2 in Pierson syndrome both result in albuminuria, proof of the importance of the normal GBM as a major barrier for proteins. Laminins represent not only important links for the collagen meshwork of the GBM, but also for cell-cell and cell-matrix signaling. The podocyte-matrix signaling system depends on interactions with podocyte transmembrane integrins, specifically α3 β1 integrin, as well as with the dystroglycans.[3]

The changes in collagen or laminin composition resulting from mutations in collagen IV α3 and in laminin β2 were also

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visualized by disruption of the normal ultrastructural architecture of the GBM. Furthermore, in the GBM of patients with Alport syndrome and mutations in collagen IV α3, ectopic deposition of laminin α1 and increased levels of laminin α5, 6, and 7 have been noted.[3] These observations suggest that the laminin and collagen networks are intrinsically connected as major determinants of the GBM permselectivity barrier.

Based on these observations, a model for the permselectivity of the GBM was proposed, with the interwoven molecular architecture generating a fine-pore mesh filter with negative charge, which excluded molecules such as albumin based on size and to some extent also on charge. The Nobel laureate Oliver Smithies then proposed, based on his intricate knowledge of gel electrophoresis for size separation of proteins, that the GBM acts as a size-selective gel for diffusion of molecules, excluding macromolecules such as albumin from entering the GBM.

He stated: "My permeation diffusion hypothesis depends on 2 main assumptions: (i) that the GBM is a gel having size-selective properties determined by permeation and diffusion, not by filtration; and (ii) that the slit diaphragm is essential for normal glomerular structure but does not act as a molecular sieve, even though it introduces considerable resistance to hydrodynamic flow."[4] Nearly 15 years later, the concept of the GBM as a size-selective gel is widely accepted.

The endothelial glycocalyx as part of the filtration barrier

The glomerular endothelial layer was initially thought to only exclude cellular components of the blood from filtration as a result of the fenestrations. More recently, it has been recognized that a fine meshwork of glycosaminoglycans covers the entire luminal glomerular endothelial layer, even bridging the fenestrations.[5] Recognition of the endothelial glycocalyx required special fixation and staining techniques, including the use of specific lectins for visualizing the glycocalyx, that are not routinely performed on kidney biopsies. By using these tools, the contribution of the endothelial glycocalyx to the permeability properties of the GFB is now well established, as are alterations in its components as contributors to albuminuria in various glomerular diseases.[5] Thus, the endothelial glycocalyx is now considered the initial, coarse barrier for macromolecular exclusion from ultrafiltration.

Contributions of the podocyte and its slit diaphragm to the filtration barrier

During the last decades an impressive number of proteinuric glomerular diseases have been ascribed to mutations in podocyte-expressed genes.[6] This association has led to the proposal that podocytes and their slit diaphragm directly contribute to the GFB, especially as a number of mutated genes contributing to nephrotic syndrome, most notably nephrin, are integral components of the slit diaphragm. Other genes mutated in hereditary glomerular diseases include molecules involved in cell-cell and cell-matrix interaction and signaling, as well as cytoskeletal organization. Disruptions in any of these can be associated with proteinuric kidney diseases. Furthermore, podocyte injury occurs in a wide variety of acquired proteinuric disorders, such as diabetic nephropathy and immune-mediated glomerulonephritis. In all of these conditions the architecture of the slit diaphragm is disturbed, resulting in what renal pathologists refer to as "foot process effacement;" as a result, the conclusion that the slit diaphragm is an important component of the GFB appeared logical.

Questions about the role of the slit diaphragm as a direct contributor to the permselectivity of the GFB were raised as early as 1975 by Farquhar, and more recently by Smithies and colleagues[4,7] and Grahammer *et al.*[8] Nonetheless, even if the slit diaphragm itself does not directly contribute to glomerular permselectivity, it may indirectly contribute to the function of the GFB. This might be due to functions of the slit diaphragm in connecting foot processes from adjacent podocytes, and thereby in cell-cell signaling. The slit diaphragm also separates the podocyte cell membrane in the urinary space from that facing the basement membrane, which may be important in maintaining the GFB through cell-matrix/GBM interactions and integrin signaling.

What is the role of the special glycocalyx membrane domain between podocytes and the basement membrane in glomerular permselectivity?

The base of the podocyte foot processes directly interacting with the GBM is covered by a special and unique glycocalyx, which is different from that covering the luminal surface of podocytes.[9] It has been speculated that this glycocalyx between podocyte and GBM may be part of the charge-dependent GFB. If and how changes in this unique glycocalyx secondary to alterations in the slit diaphragm, podocyte injury, or inside-out signaling might alter the GFB remain to be examined.

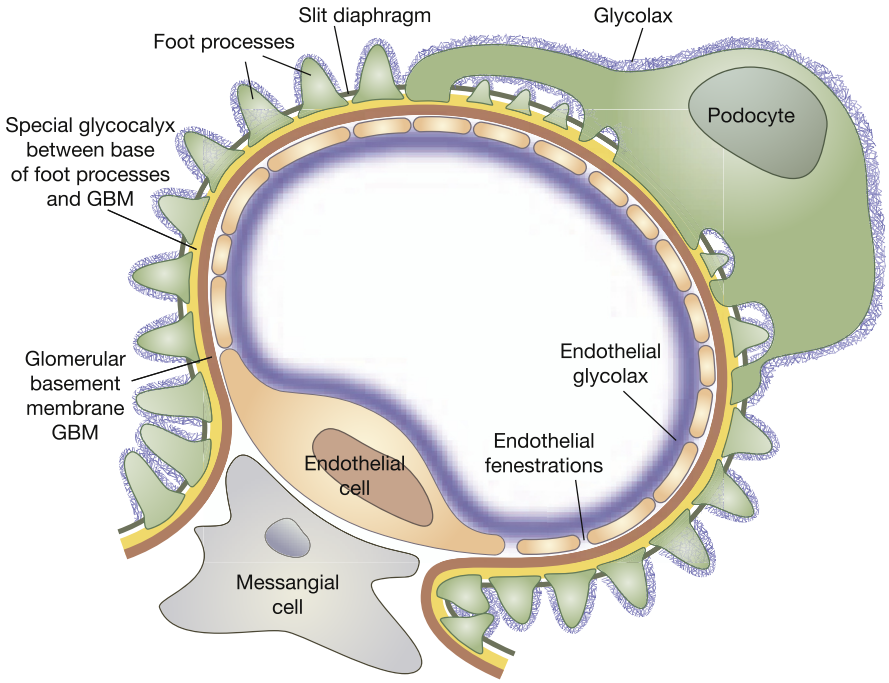

Figure 1 | Schematic drawing of a glomerular capillary. The elements considered as part of the glomerular filtration barrier include the following: (i) the endothelial glycocalyx, (ii) the fenestrated endothelium, (iii) the basement membrane, (iv) the specialized glycocalyx between the foot processes and basement membrane, and (v) the podocyte and slit diaphragm. GBM, glomerular basement membrane.

Reanalysis of the GBM and podocyte contributions to the glomerular filtration barrier

Oliver Smithies and his group have recently reanalyzed the contribution of the GBM and podocytes or slit diaphragm to the permselectivity of the GFB in an elegant paper published posthumously.[7] The authors used unfixed whole-mount mouse kidneys to examine the permeation into the GBM of fluorescent-tagged proteins and neutral dextrans of different molecular size. Their results were in general agreement with the expected size-selective permeation of molecules into gels. Next, the authors performed electron-microscopic analyses of kidneys after injecting gold-tagged albumin, negatively charged gold nanoparticles, and stable oligoclusters of gold nanoparticles. Extending the original observations by Farquhar *et al.*,[1] these experiments confirmed that the lamina densa of the GBM acts as a barrier for nanoparticles the size of IgG and albumin. Slightly smaller-sized particles (12 kDa parvalbumin or 45 kDa ovalbumin) permeated somewhat into the lamina densa, and accumulated upstream of the podocyte glycocalyx covering the base of the foot processes adjacent to the GBM. Thus, the unique glycocalyx located between the "soles" of the foot processes and the GBM, but upstream of the slit diaphragm, provided an additional barrier for the filtration of molecules smaller than albumin. In contrast, no particles aggregated upstream of the slit diaphragm, arguing against a molecular-size barrier function of this structure. Any smaller nanoparticles present in the primary ultrafiltrate were removed by proximal tubule cell endocytosis.

The authors then used proteinuric mouse models of Pierson and Alport syndromes with mutations in the GBM structural proteins laminin β2 or collagen IV α3, respectively. As expected, the GBM in these models was irregularly swollen, the lamina densa was absent, and the size selectivity of the GBM was lost to a major extent.[7]

Conclusion

Taken together, these results suggest a model of the GFB as a sequential barrier comprising first the endothelial glycocalyx, then the basement membrane as a size-selective gel, and finally the unique glycocalyx of podocyte foot processes adjacent to the GBM (Figure 1). These findings also challenge the concept of the slit diaphragm as a size-sensitive filter, but not its essential role in maintaining podocyte foot process architecture and integrity. Further studies are needed to determine whether podocyte injury, alterations in the slit diaphragm, or mesangial and endothelial cell cross-talk alter the GFB through alteration of the basal podocyte glycocalyx.

Essentially nothing is known about factors modulating the specialized surface domain of the

basal podocyte glycocalyx, and only a bit more is known about factors influencing that of the endothelial glycocalyx. Our present concept of the GBM may also be much too static, and perhaps cells anchored to the GBM (i.e., endothelial cells, mesangial cells, and podocytes) can acutely influence the arrangement of the molecular components of the GBM and thereby its size-selective properties. Inside-out and outside-in cell-matrix signaling through integrins can influence the podocyte cytoskeleton, which in turn affects the GFB. Similar considerations may apply to the distinct glycocalyx layers. For example, both the glycocalyx of the capillary luminal endothelia and the free urinary space podocyte surface contain N-acetyl glucosamines, whereas the glycocalyx at the base of podocyte foot processes contains nonreducing N-acetyl-D-galactosamine.[9] Future experiments to address the respective contribution of the endothelial and podocyte glycocalyx to glomerular permselectivity will not only have to employ cell-specific (endothelial vs. podocyte) modulation of genes involved in the generation of glycocalyx, but should also focus on genes encoding for the specific enzymes involved in the generation of either N-acetyl glucosamines or N-acetyl-D-galactosamine. Similar considerations apply to disease-associated changes in glycocalyx, such as those observed in diabetic nephropathy, as these may occur in 1 or both layers. Certainly future research will be required to determine whether and how cell-cell and cell-matrix signaling pathways may influence the permselectivity of the basal podocyte glycocalyx and of the GBM. Elucidation of these signaling pathways as potentially "druggable" targets may yet prove to be a worthwhile endeavor for future treatment of proteinuric kidney diseases.

DISCLOSURE

All the authors declared no competing interests.

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hypertension

How low can you go? Achieved blood pressure and cardiovascular outcomes

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The benefits of controlling blood pressure to levels < 140/90 mm Hg are well established, but the risks and benefits of further reductions in blood pressure are less clear. A recent observational study using pooled data from 2 large randomized trials of renin-angiotensin system blockers suggested no added benefit and some increased risk of cardiovascular events with achieved blood pressures < 120 mm Hg systolic or 70 mm Hg diastolic. Caveats of observational studies notwithstanding, these results add to the ongoing controversy over the optimal blood pressure target for high-risk individuals.

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