Published in final edited form as:

Curr Opin Nephrol Hypertens. 2008 July ; 17(4): 386–392. doi:10.1097/MNH.0b013e32830464f4.

Therapeutic targets in focal and segmental glomerulosclerosis

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Abstract

Purpose of review—Focal and segmental glomerulosclerosis occurs due to a defect in the glomerular filtration barrier. This review highlights contributions from the past year that have enhanced our understanding of the pathophysiology of focal and segmental glomerulosclerosis with emphasis on discoveries which may lead to the identification of therapeutic targets.

Recent findings—Slit diaphragm proteins have become increasingly important in signal transduction and in mediating downstream events. Actin polymerization occurs after the podocin–nephrin–Neph-1 complex is phosphorylated by Src kinase and Fyn. Recent studies of angiotensin receptor antagonists, corticosteroids and erythropoietin unravel new mechanisms that ameliorate proteinuria by targeting the cell cycle within the podocyte. The discovery that an N-acetylmannosamine kinase (MNK) mutant mouse has glomerulopathy is suggestive that human sialylation pathways may represent therapeutic targets. Proteinuria before podocyte effacement demonstrated in laminin- β 2 null mice highlights the importance of the glomerular basement membrane. Interferon- β reduced proteinuria in three models of kidney injury, showing greatest effect on glomerular endothelial cells *in vitro*.

Summary—Basic research has illuminated mechanisms by which classic therapies have antiproteinuric effects directly on the podocyte. As knowledge expands with improved molecular techniques, understanding signaling pathways in health and proteinuric states should lead to potential therapeutic targets in focal and segmental glomerulosclerosis.

Keywords

apoptosis; cell cycle; glomerular basement membrane; glomerular endothelial cells; podocyte; slit diaphragm

Introduction

Focal and segmental glomerulosclerosis (FSGS) is a common cause of nephrotic syndrome, both in children, in whom it accounts for 7–20% of cases, and in adults, in whom it accounts for up to 35% of cases [1]. Histologically, the lesion is characterized by focal glomerulosclerosis or tuft collapse, segmental hyalinosis, occasionally IgM staining on immunofluorescence and effacement of foot processes on electron microscopy. The clinicopathologic syndrome may be classified as primary (idiopathic), secondary or familial. The familial forms may be sub-classified into those with autosomal dominant and recessive patterns of inheritance as well as those associated with congenital syndromes such as Laurence–Moon–Biedl and Charcot–Marie–Tooth [2,3].

The primary defect in FSGS lies in the filtration barrier of the glomeruli. This barrier is made up of the fenestrated endothelium, the glomerular basement membrane, and podocytes, with a slit diaphragm between interdigitating foot processes. Disruption of the filtration barrier results in loss of permselectivity and macromolecules such as albumin in urine. There is debate over the relative importance of each component of the filtration barrier in maintaining its integrity [4]. This review will focus on contributions in the last year that have enhanced our understanding of FSGS and proteinuric kidney disease and their implications for potential therapeutic targets.

The role of podocyte injury in focal and segmental glomerulosclerosis

Podocytes are highly specialized, terminally differentiated epithelial cells of mesenchymal origin [5]. Podocyte injury, with disruption of their specialized functions [forming a size and charge selective barrier to protein, maintaining capillary loop shape, opposing intraglomerular pressure, producing and maintaining glomerular basement membrane (GBM), synthesizing and secreting vascular endothelial growth factor to maintain glomerular endothelial cells] leads to proteinuria and foot process effacement. This can progress to glomerulosclerosis if repair and resolution of the effacement does not occur [6]. Foot process effacement is not pathognomonic of a specific disease but is a generalized response to podocyte injury. Podocyte injury may occur as a result of disruption of the slit diaphragm complex, actin cytoskeleton, podocyte-glomerular basement membrane interface as well as apical negative charge barrier. Alteration in podocyte number can also lead to proteinuria and FSGS.

Slit diaphragm complex

Foot processes from adjacent podocytes interdigitate, forming a highly specialized gap junction, the slit diaphragm, which forms the principal size selective barrier to protein passage across the glomerular filtration barrier. Identification of proteins and the nature of their interactions at the slit diaphragm have enhanced our understanding of the molecular mechanisms responsible for the maintenance of the glomerular filtration barrier. Nephrin, podocin and Nephrin form a protein complex that acts as a transmembrane receptor at the slit diaphragm. Loss of function mutations in the genes coding for these proteins have been shown to result in nephrotic syndrome in humans [7,8] and proteinuria with perinatal lethality in mice [9].

Phosphorylation of the nephrin protein by Fyn kinase has recently been shown to mediate outside-in signaling mediated via the adapter protein Nck that induces actin polymerization resulting in change in podocyte structure [10,11] (Fig. 1) [10,12*,13,14*-16*,17*,18-20]. Similarly when Nephrin is phosphorylated by Fyn kinase, Grb2 is recruited which induces actin polymerization [12*] (Fig. 1). Grb2 (growth factor receptor-bound protein 2) is an adaptor protein involved in signal transduction that binds the epidermal growth factor receptor. When Grb2 is inhibited, cellular processes such as proliferation are blocked. Nephrin phosphorylation also leads to inactivation of the pro-apoptotic factor Bad via the AKT signaling pathway. CD2-associated protein (CD2AP), which is localized to the slit diaphragm is known to inhibit apoptosis and interacts with Fyn and synaptopodin. Elegant bigenic heterozygous mouse models of CD2AP with Fyn or synaptopodin resulted in spontaneous proteinuria [21]. Modulation of these pathways in the setting of proteinuria could represent a promising therapeutic target.

Transient receptor potential cation channel 6 (TRPC6), a channel protein found at the slit diaphragm, which co-localizes with nephrin, podocin and CD2AP [22], has recently been shown to play a novel role in the pathogenesis of FSGS (Fig. 1). A missense mutation, changing a highly conserved proline in the first ankyrin repeat of TRPC6 to a glutamine at amino acid 112 (P112Q), was identified in a large kindred with autosomal dominant FSGS [13]. The

TRPC6^{P112Q} mutation augments intracellular calcium influx into the podocyte, leading to FSGS through unclear mechanisms. This intracellular calcium influx was exaggerated in the presence of angiotensin II (Ang II). Additional mutations were identified in this gene, upon screening of other pedigrees with familial FSGS, some of which were also associated with increased calcium transients [22]. TRPC6 expression has also been shown to be upregulated in acquired human proteinuric kidney diseases [23]. Blocking the TRPC6 channel would be an attractive option to modulate proteinuric kidney disease, particularly as it is closely associated with nephrin and also because TRPC6 dependent calcium influx is augmented by Ang II. The 75% sequence homology of TRPC6 with TRPC3 and TRPC7, however, may make creating a specific blocker a formidable task.

Urokinase plasminogen activator receptor (uPAR) is a glycosylphosphatidylinositol (GPI) protein anchored to the lipid raft at the slit diaphragm. Wei *et al.* [14*] showed that induction of uPAR leads to foot process effacement and proteinuria through lipid dependent activation of $\alpha\beta3$ integrin. They also demonstrated that uPAR expression in podocytes was a prerequisite for the development of proteinuria in the lipopolysaccharide (LPS) injury model. Activated $\alpha\beta3$ integrin promotes cell motility by activation of the small GTPases, Cdc42 and Rac1. Inhibition of $\alpha\beta3$ integrin resulted in reduced podocyte motility and proteinuria. Specific inhibition of $\alpha\beta3$ integrin in acquired proteinuric disease would be an attractive therapeutic target.

Actin cytoskeleton

Podocyte function is dependent on the plasticity of its cytoskeletal architecture. Proteins which regulate this structure such as Rho GDIalpha, synaptopodin, α -actinin-4, podocalyxin, cadherin, FAT1 and Nck are vital to maintenance of the function of the glomerular filtration barrier. Recently, synaptopodin has been shown to orchestrate actin organization and cell motility via activation of RhoA *in vitro* [24]. Further studies in conditionally immortalized mouse podocytes found that synaptopodin directly binds insulin receptor substrate p53 (IRSp53), thereby blocking the interaction of IRSp53 with the small GTPases, Mena and Cdc42 and preventing filopodia formation. Although yet to be confirmed as functional by in-vivo studies, this could represent an antiproteinuric pathway involving synaptopodin as a potential therapeutic target [15*].

Recently Sever *et al.* [16*] found that cathepsin L was upregulated in acquired proteinuric kidney disease and in cultured human podocytes subjected to stressful conditions. Cathepsin L, normally restricted to lysosomes, was found throughout the cytoplasm in these settings. The authors also showed that cathepsin L cleaved dynamin resulting in podocyte effacement and proteinuria. This proteinuria was ameliorated in mice using podocyte specific delivery of a mutant form of dynamin, which is resistant to cleavage by cathepsin L. Further in-vivo studies may elucidate a possible role for modulation of cathepsin L in proteinuric kidney disease.

Apical domain proteins

The anionic apical proteins podocalyxin and podoplanin confer a negative charge barrier to prevent the passage of anionic proteins through the glomerular filtration barrier. Podocalyxin, a sialomucin, is a heavily sialylated and sulfated membrane protein. Neutralization of the anionic charge with protamine sulfate leads to foot process effacement [25].

A recent study by Galeano *et al.* [17**] showed that decreased availability of sialic acid in mice due to a mutation in the bifunctional enzyme uridine diphospho-N-acetylglucosamine 2-epimerase (UDP-GlcNAc)/MNK was associated with severe proteinuria, segmental splitting of the glomerular basement membrane and podocyte foot process effacement as well as neonatal lethality. Supplementation with N-acetyl mannosamine rescued these mice with

marked improvement in the integrity of the GBM, reduced effacement of the podocytes and increased sialylation of renal podocalyxin. The discovery of this gene-targeted knock in mouse with a severe podocytopathy was quite serendipitous as the model was initially designed to study hereditary inclusion body myopathy (HIBM), an adult-onset neuromuscular disorder. As expected, this renal phenotype is not seen in humans with HIBM as humans rely on 5-N-acetylneuraminic acid as their main sialic acid, whereas most other mammals (including mice) utilize N-glycolylneuraminic acid. Nonetheless this study will prompt researchers to search for other sialylation defects in both acquired and inherited forms of FSGS with the possibility of therapy with sialic acid supplementation.

Phospholipase C ϵ 1 (PLC ϵ 1) has been localized to the apical domain and throughout the cytoplasm in the podocyte [18]. Recently mutations in this gene have been shown to cause early onset nephrotic syndrome. The authors speculate that it plays a significant role in glomerular development. Interestingly, two patients with PLC ϵ 1 variations responded to therapy with complete remission of proteinuria and symptoms. Thus the presence of a PLC ϵ 1 mutation in early onset nephrotic syndrome may represent the potential to respond to therapy.

The podocyte–glomerular basement membrane junction

Podocytes are anchored to the underlying GBM via α 3 β 1 integrins and α - β dystroglycans. Kojima *et al.* [26] showed reduction in the basal density of α - β dystroglycans with associated podocyte effacement in two distinct injury models; the reactive oxygen species dependent puromycin aminonucleoside (PAN) model and perfusion of kidneys with protamine sulphate. A podocyte selective dystroglycan knockout mouse, however, shows no phenotype despite all dystroglycan being abolished (personal communication, S. Quaggin, SLRI, University of Toronto). A number of groups have found that viable podocytes are shed in the urine in diabetic and nondiabetic glomerular disease [27-29], suggesting that podocyte detachment may be an early rather than a terminal event. Taken together these studies suggest that podocyte detachment may be an early rather than terminal event in FSGS. Modulation of this phenomenon may be a useful therapy in FSGS. With advances in urinary proteomics there is potential to identify early podocyte specific markers of disease in early stages of FSGS [30].

Enhanced understanding of antiproteinuric mechanisms of known therapeutic agents

Total podocyte number is dependent on the balance between proliferation and loss. Kim *et al.* [31] showed that glomerulosclerosis is related to podocyte number following a 20% reduction in number in the PAN injury model in Sprague–Dawley rats. This initially raised the possibility that therapies aimed at reducing podocyte apoptosis could be useful in ameliorating the progression of FSGS. Recently it has been shown that cyclin I protects podocytes from apoptosis, with preliminary data that this is mediated by stabilization of p21^{Cip1/Waf1} [32]. Corticosteroids which are a mainstay of treatment for many proteinuric diseases have been shown to have direct effects on podocyte survival. Exposure to dexamethasone prevented PAN induced apoptosis, by preventing upregulation of the pro-apoptotic factor p53 while maintaining levels of the pro-survival factor Bcl-xL [33].

Erythropoietin receptors have been identified on podocytes in fixed tissue specimens [34]. Darbopoetin α has been shown to reduce podocyte apoptosis occurring in response to low dose ultraviolet (UV) irradiation or transforming growth factor β (TGF- β) [35]. Both UV irradiation and TGF- β cause caspase-3-dependent apoptosis. Initial studies by this group suggest that the reduced podocyte apoptosis was associated with phosphorylation of Janus family protein kinase-2 (JAK2) and AKT. Further studies examining pathway redundancy are underway. Interestingly, darbopoetin α treatment had no effect on PAN-induced podocyte apoptosis that occurs in a p53 dependent manner.

While the podocyte is conventionally considered a terminally differentiated cell with disease resulting from decreased podocyte number, there are a number of situations when increased proliferation and poorly differentiated podocytes are seen in disease states; notably collapsing or cellular FSGS and HIV associated nephropathy [36]. Selective inhibition of CDK2 with roscovitine, which inhibits DNA synthesis in the S phase of the cell cycle, has resulted in stabilization of proteinuria and improvement in the collapsing glomerulopathy in HIV associated nephropathy [37], and improvement in renal function and crescent formation in experimental crescentic glomerulonephritis [38].

There is evidence to suggest a specific role of the renin–angiotensin system (RAS) within human [39] and mouse [40] podocytes. Ang II was shown to increase staurosporine induced apoptosis in cultured human podocytes [39] leading the authors to speculate that angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers may protect against podocyte apoptosis. Recently Hiramatsu *et al.* [41] developed a conditional transgenic mouse model of HIV associated nephropathy by expressing the HIV accessory gene, *Vpr*, specifically in podocytes using a podocin promoter. They observed nephrotic range proteinuria and podocyte dedifferentiation and dysregulation in these mice, as represented by reduced expression of nephrin, synaptopodin and Wilms' tumor-1 protein. Treatment with olmesartan reduced proteinuria and preserved podocyte differentiation. A significant effect was seen even with delayed treatment with olmesartan. Another group showed ACE inhibition ameliorated development of collapsing FSGS in Thy 1.1 transgenic mice injected with anti-Thy 1.1 antibodies [42]. These studies when taken together suggest that inhibition of the RAS can protect against both podocyte apoptosis and dedifferentiation.

Glomerular basement membrane defects cause focal and segmental glomerulosclerosis

While the majority of research in FSGS is focused upon aberrant podocyte function, architecture and alignment, there is an increasing body of evidence supporting GBM pathology as another primary cause of proteinuria. Recently, Jarad *et al.* [43] demonstrated that absence of laminin- β 2 in the GBM leads to proteinuria in mice. This is consistent with the observation that a mutation of the laminin- β 2 gene causes early onset nephritic syndrome in humans [44]. They also demonstrated that the permeability of the GBM preceded any podocyte effacement using electron dense tracer ferritin, a large molecule with a molecular weight of 480 kDa. Significantly, the authors showed that the situation was different in CD2AP null mice, which have a primary podocyte defect. This study compels even the staunchest podocyte biologists to consider that proteinuria can arise from other components of the glomerular filtration barrier. This study would concur with Smithies' gel permeation/diffusion hypothesis that supports the concept that size-selectivity of the glomerulus resides in the GBM and the epithelial slits impose substantial resistance to liquid flow across the glomerulus without acting as a molecular sieve [45]. As more information is gleaned about the permeability and physiology of the GBM, therapeutic targets may be identified.

Glomerular endothelial cells and proteinuria

Glomerular endothelial cells are considered to be highly permeable and have numerous fenestrae, which may play a role in that permeability. If glomerular endothelial cells were to provide no barrier to circulating plasma proteins, there would be massive convective movement of proteins into the GBM and one would expect it to become obstructed [45]. It has been noted that patients with preeclampsia [46] and hemolytic-uremic syndrome [47] develop proteinuria, both disorders causing injury to glomerular endothelial cells. Recently Satchell *et al.* [48**] showed that treatment with interferon- β reduces proteinuria in experimental glomerulonephritis. The authors studied the effect of interferon- β in three separate models of

glomerular injury: nephrotoxic nephritis in WKY rats (a model of acute inflammation and progressive renal scarring), Thy 1.1 nephritis (a model of hypercellular FSGS) and PAN nephritis (direct injury to podocytes). In all of these models there was significant reduction in proteinuria with treatment without significant improvement in inflammation or histology. This suggests that the action of interferon- β was directly on the glomerular filtration barrier and independent of its anti-inflammatory effect. Further study revealed that treatment of monolayers of cultured glomerular endothelial cells and podocytes with interferon- β caused increased electrical resistance and retarded the passage of labeled bovine serum albumin across these cells. This in-vitro phenomenon was more pronounced in cultured glomerular endothelial cells than podocytes. Though preliminary this exciting study raises the possibility of using interferon- β as a therapy for proteinuria of various causes.

Conclusion

Insight into the pathophysiology of FSGS has increased exponentially over the last decade as molecular techniques have evolved. The discovery of mutations in key podocyte genes causing nephrotic syndrome have been pivotal starting points to the study of signaling pathways involving the slit diaphragm proteins. We now understand that phosphorylation of the nephrin-podocin-Neph1 complex at the slit diaphragm can affect podocyte morphology and proteinuria by inducing polymerization of the actin cytoskeleton and inactivate pro-apoptotic pathways. Modulation of these pathways and other cell cycle pathways may be beneficial in treatment of FSGS. TRPC6, a channel protein also localized to the slit diaphragm, if amenable to specific blockade, may represent a novel therapeutic target. Modulation of the apical negative charge barrier of the podocyte may become a focus of research following the description of a glomerulopathy in an MNK mutant mouse.

While the majority of focus has been on podocyte biology, the importance of the other components of the glomerular filtration barrier has been highlighted by demonstration of proteinuria preceding podocyte effacement with a primary defect in the GBM. Another very interesting aspect of recent advances has been increased knowledge about podocyte specific actions of classical antiproteinuric therapies. Modulation of the renin-angiotensin system can prevent podocyte apoptosis and prevent podocyte dedifferentiation. Dexamethasone and erythropoietin have been shown to have direct antiapoptotic effects on podocytes. It would appear that further research along the avenues described in this article may yield therapeutic targets which may be translated from bench to bedside.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 437).

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Figure 1.

(1) Phosphorylation of the nephrin protein by Fyn kinase via the adapter protein Nck that induces actin polymerization [10]. (2) Neph1 phosphorylation by Fyn kinase and recruitment of Grb2 induces actin polymerization [12*]. (3) Specific blockade of the TRPC6 channel may be a useful therapy in FSGS [13]. (4) Identification of mutations in PLCε1 may identify a subset of patients who are amenable to standard therapy [18]. (5) Modulation of sialylation pathways may represent therapeutic targets with the discovery that an MNK null mouse has a glomerular phenotype [17**]. (6) Lysosomal cathepsin (CATL), present in podocyte cytoplasm in acquired proteinuric kidney disease, cleaves dynamin leading to filopodia formation, podocyte effacement and proteinuria [16*]. Modulation of CATL or dynamin may be a useful in therapy of FSGS. (7) Synaptopodin binds insulin receptor substrate p53 (IRSP3) preventing its binding with the small GTPases (Rac1, Cdc42 and Mena) thereby preventing filopodia formation and proteinuria [15*]. (8) Induction of urokinase plasminogen activator receptor (uPAR) causes proteinuria through lipid dependent activation of αvβ3 integrin. Inhibition of αvβ3 integrin

resulted in reduced podocyte motility and proteinuria [14*]. ACTN4, α -actinin-4; CAS, p130Cas, an adaptor protein (Crk-associated substrate); CD2AP, CD2-associated protein; FAK, focal adhesion kinase; Fat1, cadherin Fat1; Grb2, growth factor receptor-bound protein 2; Nck, Nck adapter protein; TPV, talin, paxillin, and vinculin complex; ZO-1, zonula occluden-1. Adapted with permission [19,20] and updated with information from the legend and text.