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Changes in podocyte TRPC channels evoked by plasma and sera from patients with recurrent FSGS and by putative glomerular permeability factors

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ABSTRACT

Primary forms of focal and segmental glomerulosclerosis (FSGS) are driven by circulating factors that cause dysfunction or loss podocytes. Rare genetic forms of FSGS can be caused by mutations in *TRPC6*, which encodes a Ca^{2+}-permeable cationic channel expressed in mesangial cells and podocytes; and *NPHS2*, which encodes podocin, a TRPC6-binding protein expressed in podocyte slit diaphragm domains. Here we observed that exposing immortalized mouse podocytes to serum or plasma from recurrent FSGS patients for 24 h increased the steady-state cell-surface abundance of TRPC6, accompanied by an increase in currents through endogenous TRPC6 channels evoked by a hypoosmotic stretch stimulus. These effects were mimicked by the soluble urokinase receptor (suPAR) and by tumor necrosis factor (TNF), circulating factors implicated in nephrotic syndromes. Most but not all of the recurrent FSGS plasma samples that we examined also caused a loss of podocin over a period of several hours. The loss of podocin was also seen following exposure to suPAR but not TNF. However, TNF increased the effects of suPAR on TRPC6 and podocin, and TNF and suPAR are required for the full effects of one of the recurrent FSGS plasma samples. The actions of FSGS plasma, suPAR and TNF on surface abundance of TRPC6 were blocked by cilengitide, an inhibitor of αvβ3-integrin signaling. These data suggest that primary FSGS is a heterogeneous condition mediated by multiple circulating factors, and support TRPC6 and αvβ3-integrin as potential therapeutic targets.

1. Introduction

Focal and segmental glomerulosclerosis refers to lesions in which an accumulation of extracellular matrix obliterates varying portions of glomerular capillary tufts. While these lesions have several etiologies, many cases of histologically verified FSGS are primary and idiopathic. Primary FSGS patients who present with nephrotic levels of proteinuria and who do not respond to glucocorticoids have an especially high risk of progressing to renal failure. Moreover, a substantial portion of patients who receive a kidney allograft as a result of steroid-resistant primary FSGS will experience early recurrence of nephrotic range proteinuria and are at high risk for graft failure [1,2].

It is now generally accepted that recurrence of FSGS in an allograft recipient is caused by circulating factors that affect podocytes [3,4]. The identity of the circulating factor(s) that drive primary and recurrent FSGS has been elusive and controversial. One candidate is the soluble urokinase and plasminogen activator receptor (suPAR) [5], a term that encompasses a class of 22–50 kD glycoproteins shed from many cell types, including hematopoietic cells, endothelial cells, fibroblasts, and smooth muscle cells, as a result of proteolytic or phospholipase-mediated cleavage of a glycosylphosphatidylinositol-anchored glycoprotein [6]. It was originally reported that total plasma suPAR levels are elevated in a subset of patients with FSGS, especially patients with recurrent forms of FSGS [5], and this association was subsequently found to be stronger when urine levels of suPAR were measured [7–9]. Beyond the context of FSGS, large longitudinal studies have found that elevated blood suPAR levels in people with normal baseline renal function are associated with future chronic kidney disease and declines in estimated glomerular filtration rate [10,11]. Moreover, there is evidence that elevated serum suPAR levels predict future

Abbreviations: BUN, blood urea nitrogen; Cyto D, cytochalasin D; DAPI, 4,6-diamidino-2-phenylindole; FSGS, focal and segmental glomerulosclerosis; LDL, low density lipoprotein; suPAR, soluble urokinase receptor; TNF, tumor necrosis factor; TRPC6, canonical transient receptor potential-6 channel

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Table 1
Clinical features of patients whose serum or plasma samples were used in this study.

Patient	Diagnosis and features	Renal function tests at time of sample	Source of sample
101	Plasma from 44 year old female with recurrent FSGS. Samples were taken before and after transient remission induced by plasma exchange.	NA	Bristol, UK
048	Serum from patient with recurrent FSGS	NA	Johns Hopkins University, Baltimore, MD
054	Serum from patient with recurrent FSGS	NA	Johns Hopkins University, Baltimore, MD
356	Plasma from male child with recurrent FSGS, with initial nephrotic syndrome diagnosis at age of 16 months. Samples taken before and after plasma exchange	Proteinuria noted after transplant and transiently reduced after plasma exchange.	Bristol, UK
021	Serum from patient with recurrent FSGS	NA	Rush University, Chicago, IL
022	Serum from patient with recurrent FSGS	NA	Rush University, Chicago, IL
004	Plasma from adult female with recurrent FSGS at time of sampling. Currently back on dialysis.	BUN = 49 mg/dl	Cologne, Germany
DH	Plasma from male child with recurrent FSGS. Samples were taken before and after transient remission was induced by three sessions of LDL apheresis.	BUN = 99 mg/dl during relapse. BUN = 78 mg/dl after LDL apheresis	Cologne, Germany
011	Plasma from female with recurrent FSGS, has received two kidney transplants to date.	BUN = 114 mg/dl	Cologne, Germany

Coded samples of serum or plasma were sent to us from clinician scientists at different university medical centers as indicated. All of these patients have had recurrence of FSGS after a kidney transplant. In some cases we received samples from the same patients at different times in which the severity of the disease appeared different based on clinical tests. We have provided laboratory values where we could obtain the information.

microalbuminuria in patients at risk for or with newly manifested type 2 diabetes mellitus [12]. In mice there is evidence that the circulating suPAR that drives kidney disease is derived from immature myeloid cells in bone marrow, and that transplantation of myeloid cells secreting high levels of suPAR can induce kidney disease in recipient mice [13].

Other factors have been proposed to mediate recurrent FSGS. A handful of case reports have documented remissions of recurrent FSGS following *anti*-TNF therapy in pediatric patients [14–16]. In addition, there are reports that circulating TNF is elevated in patients with primary nephrotic syndromes [17], and monocytes isolated from these patients secrete TNF at an order of magnitude greater rate than monocytes from healthy controls [18–20]. Moreover, changes in podocyte cytoskeletal organization evoked by plasma from patients with recurrent FSGS were blocked by inhibitors of TNF signaling [16]. Sustained TNF infusion induces glomerular pathology [21]. It has also been reported that TNF increases the albumin permeability of isolated glomeruli [22]. Other circulating factors, such as cardiotrophin-like cytokine 1, which activates transduction cascades that overlap those of TNF, may also drive recurrent FSGS [23]. It is possible that primary and recurrent FSGS may be driven by different patterns of circulating factors in different patients [24].

Genetic studies have identified a number of genes that are mutated in familial forms of FSGS. Two of these genes encode proteins that are the focus of the present study. Mutations in the *NPHS2* gene encoding the hairpin loop protein podocin give rise to severe and typically early-onset autosomal recessive nephrotic syndromes [25]. Most *NPHS2* mutations result in non-functional proteins [26–28]. The *TRPC6* gene encodes a Ca^{2+}-permeable cation channel (TRPC6) expressed in many different cell types, including mesangial cells and podocytes. FSGS associated with *TRPC6* mutations often presents with an adult onset, with an autosomal dominant mode of inheritance, and the mutant proteins frequently have a gain of function or increased surface expression when examined in heterologous expression systems [29–31]. It should be noted, however, that loss of activation by at least some gating modes has been seen with some TRPC6 mutations [32,33], including a dominant-negative mutation that resulted in complete loss of activation by G protein cascades and that occurred with an unusually early disease onset [33].

TRPC6 and podocin are expressed at the slit diaphragm domains of podocytes [30,34,35] and there are functionally significant biochemical interactions between these two proteins [30,35,36]. We have previously reported that podocin differentially regulates the sensitivity of podocyte TRPC6 channels to various activating stimuli [36]. Specifically, podocin enhances activation of TRPC6 induced by G protein signaling pathways or by diacylglycerol [36–38] or eicosanoids [39]. Conversely, podocin suppresses activation of podocyte TRPC6 channels induced by membrane stretch, a process that occurs by poorly understood transduction mechanisms [36]. Therefore, processes that result in a loss in glomerular podocin expression would be expected to exert complex functional effects on TRPC6, but in particular should increase activation by membrane stretch or other mechanical stimuli.

It has been reported that patients with primary FSGS have elevated glomerular expression of TRPC6 [40] as well as reduced expression of podocin [41–43]. Moreover, a loss or internalization of podocin can be recapitulated *in vitro* by exposing cultured podocytes to serum from patients with recurrent FSGS [44–46]. Therefore, it is possible that dysfunction in one or both of these proteins could contribute to disease progression in “acquired” as opposed to genetic forms of kidney disease.

The primary purpose of the present study is to explore the effects of putative glomerular permeability factors, as well as serum and plasma from patients with recurrent forms of FSGS, on TRPC6 channels in a widely used immortalized podocyte cell line. Since podocin is a TRPC6-interacting protein that affects TRPC6 function, we have also examined this protein. The results support a model in which functionally significant changes in TRPC6 surface expression and increases in its activation occur in response to circulating factors in FSGS patients, in many but not all cases accompanied by a loss of podocin. These data are also consistent with multiple circulating factor models of primary FSGS, in which suPAR, TNF, and probably several other circulating factors produce additive or synergistic effects that converge on podocytes. We will also present evidence that these circulating factors utilize integrin signaling pathways to increase the abundance of TRPC6 channels on the cell surface. If this multiple-factor model is correct, correlations between circulating levels of any one circulating factor and clinical status in primary FSGS (or other glomerular diseases) may not always be seen, even in cases when that factor contributes to the pathology. However, it is possible that downstream targets shared by these circulating factors, such as TRPC6 channels or integrins, might represent effective therapeutic strategies for acquired forms of FSGS.

2. Material and methods

2.1. Podocyte cell culture and glomerular isolation

An immortalized mouse podocyte cell line (MPC-5) was provided by Dr. Peter Mundel of Harvard Medical School and propagated and maintained as described previously [36,47]. Podocyte differentiation