Mechanisms of Scarring in Focal Segmental Glomerulosclerosis

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Keywords

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Abstract

Background: Focal segmental glomerulosclerosis (FSGS) is a histologic pattern characterized by focal glomerular scarring, which often progresses to systemic and diffuse glomerulosclerosis. Previous studies have emphasized that the initiation of classic FSGS occurs in podocytes. The dysfunction and loss of podocytes have been associated with the development of proteinuria and the progression of various diseases. In addition, primary, secondary, and genetic FSGS are caused by different mechanisms of podocyte injury. **Summary:** The potential sources and mechanism of podocyte supplementation are the focus of our current research. Increasing attention has been paid to the role played by parietal epithelial cells (PECs) during the progression of FSGS. PECs are not only the primary influencing factors in glomerulosclerosis lesions but also have repair abilities, which remain a focus of debate. Notably, other resident glomerular cells also play significant roles in the progression of this disease. **Key Message:** In this review, we focus on the mechanism of scarring in FSGS and discuss current and potential therapeutic strategies.

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Introduction

Focal segmental glomerulosclerosis (FSGS) is a type of pathological syndrome characterized by sclerosis in segmental ($\leq 50\%$ of the glomerular tuft affected) and focal ($\leq 50\%$ of all glomeruli affected) glomeruli [1]. The clinical manifestations of FSGS include massive proteinuria and nephrotic syndrome, which are primarily resistant to hormone therapy [2]. Approximately 30–40% of FSGS patients experience recurrence after renal transplantation, which presents as early nephrotic syndrome and the disappearance of foot processes that rapidly develops into dominant sclerosis [3]. The Columbia classification of FSGS identified 5 morphologic patterns [4], and patients with the collapsing variant have the worst prognosis, whereas those with the tip variant have a favorable curative effect and are more sensitive to immunosuppressive therapies [5–7].

FSGS describes a renal histologic lesion with diverse etiologies and pathogenicities that have been linked to podocyte injury and depletion [8]. In addition, parietal epithelial cells (PECs) are considered to play a key role in the development of glomerulosclerosis lesions. Activated PECs (aPECs) undergo migration and proliferation and are characterized by a crescent formation or the deposition of extracellular matrix proteins, contributing to FSGS progression [9]. Complex interactions involving

Fig. 1. Podocytes and PECs' role in repairing versus scarring after glomerular injury. EMT, epithelial-mesenchymal transition; PECs, parietal epithelial cells; ECM, extracellular matrix; GBM, glomerular basement membrane; BC, Bowman's capsule.

resident glomerular cells participate in the various processes underlying FSGS. In this review, we will discuss the mechanism of scarring and potential therapeutic strategies for the disease.

Clinical Setting

FSGS lesions can be broadly subdivided into primary, genetic, and secondary forms. Primary FSGS is thought to be caused by a circulating factor, possibly a cytokine that is secreted by extrarenal sources, causing generalized injury to podocytes [10]. Secondary FSGS often has a definite etiology, such as virus-induced FSGS, drug-induced FSGS, or maladaptive forms, caused by a reduction in the number of functioning nephrons or when the normal nephron population is subjected to abnormal stress [11]. The genetic causes of FSGS may present as a sporadic or familial disease, with autosomal dominant, autosomal recessive, X-linked, or mitochondrial (matrilineal) inheritance patterns [12]. Genetic FSGS may be either limited to the kidney or present as part of a broader syndrome with extrarenal involvement. Despite sharing certain

clinical and histological features, these subclasses differ noticeably in response to treatment and prognosis. We will discuss the pathophysiological mechanisms and therapeutic strategies associated with each type in the later parts of this review.

Role of the Podocyte in FSGS

Podocyte Injury Is the Primary Target of FSGS

Podocyte injury is a characteristic feature of FSGS, characterized by cellular hypertrophy, foot process disappearance, pseudocyst formation, and microvilli changes. The dysfunction and loss of podocytes are closely related to the development of proteinuria and disease progression [13]. Due to the weak proliferative capacity of podocytes, the number of cells decreases after consequent cell death or separation, leading to a mismatch between podocyte coverage and the surface area of the glomerular basement membrane (GBM) and the development of uncovered GBM areas. Due to the lack of the structural support

Table 1. Different causes of FSGS

Primary	Circulating podocyte-toxic factor (e.g., anti-CD40 antibody, CLCF1, and suPAR)
Secondary	
Virus-induced	HIV-1, CMV, Epstein-Barr virus, cytomegalovirus, and hepatitis C
Drug-induced	Anthracyclines (Adriamycin), direct-acting antiviral therapy, calcineurin inhibitors, etc.
Maladaptive	Reduced number of functioning nephrons/abnormal stress on an initially normal nephron population (e.g., diabetes, hypertension, and obesity)
Genetic	Gene mutations (e.g., NPHS1 and NPHS2, ACTN4, WT-1)

FSGS, focal segmental glomerulosclerosis; CLCF1, cardiotrophin-like cytokine factor 1; suPAR, serum urine-type plasminogen activator receptor.

typically provided by podocytes at these sites, the capillary loop is able to expand into Bowman's capsule (BC), forming an initial junction between PECs and the podocyte-free areas of the GBM (Fig. 1). Furthermore, increased proteinuria may have direct toxic effects on tubular epithelial cells, inducing the epithelial-mesenchymal transition, resulting in a decrease in cellular adhesion and increased invasive ability, disrupting the basement membrane of renal tubules [14].

The initiation of classical FSGS occurs in podocytes, and classical FSGS animal models are typically generated through the induction of podocyte damage, either directly or indirectly, through nephrectomy, podocyte-specific toxins, or targeted genetic mutations [15]. In recent years, animal models of FSGS have primarily concentrated on genes that regulate podocyte metabolism and maintain the filtration barrier, and new genes have been identified that may participate in the initial podocyte injury mechanisms associated with FSGS, such as CD2-associated protein, atypical protein kinase C, and Wilms tumor 1 (WT1) [16].

Mechanisms of Podocyte Injury in Different Forms of FSGS

Primary FSGS is thought to be caused by circulating factors, which rapidly recur in 30–40% of transplant patients [5]. Several circulating factors may contribute to podocyte injury, such as anti-CD40 antibodies, cardiotrophin-like cytokine factor 1, and serum urine-type plasminogen activator receptor (suPAR; Table 1). Anti-CD40 antibodies are highly toxic to cultured podocytes, and their production is an important predictor of FSGS recurrence, suggesting that CD40 may be involved in FSGS damage [17]. Savin et al. [18] demonstrated that cardiotrophin-like cytokine factor 1-induced podocyte injury was mediated by the Janus kinase/signal transducer

and activator of transcription signaling pathway. The role of suPAR remains controversial, and several cohort studies have demonstrated that serum suPAR levels are negatively correlated with renal function, indicating that FSGS is difficult to distinguish from other glomerular diseases [19–21]. However, other in vivo studies have shown that the upregulation of suPAR did not induce proteinuria or podocyte injury [22, 23]. One current theory is that another form of suPAR may be involved in this process that cannot currently be detected using existing enzyme-linked immunosorbent assays. Circulating factors might be valid biomarkers for FSGS diagnosis, in addition to serving as effective therapeutic targets for primary and recurrent FSGS, although additional research remains necessary.

Secondary FSGS often has a definite etiology, such as virus-induced FSGS, drug-induced FSGS, or FSGS associated with systemic diseases. FSGS associated with infection is commonly caused by viruses. Human immunodeficiency virus is closely related to FSGS, particularly the collapsing glomerulopathy type, and the virus can cause damage to podocytes through direct infection or through the release of inflammatory cytokines [24]. Chandra and Kopp [25] reviewed other viruses known to cause FSGS, including Epstein-Barr virus, cytomegalovirus, and hepatitis C virus (Table 1). In cases of medication-associated FSGS, anthracycline medications have been strongly associated with FSGS development, and Adriamycin has been widely used in the establishment of an FSGS mouse model [26]. However, the mechanisms of podocyte injury that occur during medication-associated FSGS remain unclear. Systemic diseases, such as diabetes, hypertension, and obesity, cause glomerular filtration hypertension, which places mechanical pressure on podocytes, leading to FSGS [27]. In addition, the activation of angiotensin II and transforming growth factor-beta appears to

be relevant pathways that promote podocyte hypertrophy, disappearance, and detachment from the GBM, eventually leading to podocyte depletion.

Genetic FSGS is associated with many different gene mutations, which primarily encode proteins that promote cytoskeletal dynamics, septal junction integrity, and signal transduction in podocytes (Table 1). *NPHS1* and *NPHS2* encode nephrin and podocin, respectively, and the interaction between these 2 proteins forms the critical structural components of gap junctions [28]. Mutations in *NPHS1* and *NPHS2* are frequently detected in children with steroid-resistant nephrotic syndrome [29]. The *ACTN4*-encoded protein α -actinin-4 interacts with the actin cytoskeleton in podocytes, participating in a variety of transcriptional activation pathways [30]. FSGS-linked mutations in *ACTN4* can eliminate nuclear receptor activity in response to estrogen and retinoic acid, block the adaptability of the cytoskeleton to cyclic tension, and promote podocyte detachment from the GBM [31]. Moreover, *WT-1* is a crucial podocyte marker, and specific mutations in this gene can lead to Frasier syndrome, which is characterized by the histological appearance of FSGS during early childhood [32]. Not surprisingly, those genes are associated with therapeutic response.

The Role Played by PECs in FSGS

PECs Are the Primary Influencing Factors in Glomerulosclerosis and Proliferative Lesions

Recently, increasing attention has been paid to the role played by PECs in the development and progression of FSGS. Previous studies have shown that PECs participate in the development of glomerular sclerotic lesions in FSGS [33–35]. Lineage tracing studies have indicated that aPECs, which are characterized by increased migration, proliferation, and matrix deposition, can migrate along BC to the vascular stalk, invading segments of the glomerular tuft and promoting the deposition of the extracellular matrix [36]. In addition, aPECs show the de novo expression of CD44, which is considered to represent a specific aPEC marker, which allows for the invasion of the glomerular tuft during scarring glomerular diseases [35, 37]. Recently Eymael et al. [38] found that CD44 plays an important role in the pathogenesis of collapsing FSGS, demonstrating that CD44 deficiency leads to decreased glomerular cell proliferation and albuminuria. Furthermore, recent studies have found that CD74 expression is upregulated in PECs in FSGS mouse models, indicating that CD74 may serve as a marker of PEC activation [39]. Importantly, aPECs can only be detected in the glomeru-

lar sclerosing segment and do not express any known podocyte markers [36]. Recently, Kuppe et al. [40] indicated that novel PEC subpopulations contribute to the development of glomerulosclerosis and tip lesions. Interestingly, they investigated 2 neglected PEC subgroups, proximal tubular epithelial-like cells (termed cuboidal PECs, cPECs) and intermediate PECs (iPECs). Using a transgenic *Pax8-rtTA* mouse, in which cPECs and iPECs are labeled, in addition to a *PEC-rtTA* mouse, in which all 3 PEC subpopulations are labeled, they were able to determine that cPECs and iPECs participated in sclerotic FSGS lesions. In addition, the cells associated with tip lesions in FSGS human samples were found to express iPEC markers. Furthermore, iPECs became more numerous and expressed the activation marker CD44 in FSGS model mice. These data strongly indicated that novel PEC subgroups (especially iPECs) were involved in glomerulosclerosis and were more easily activated than conventional flat PECs in FSGS.

These studies suggest that the activation, migration, and proliferation of PECs have deleterious biological effects that may contribute to the pathogenesis of glomerulosclerosis. Therefore, targeting the activation of PECs has been viewed as a promising therapeutic avenue for FSGS. Recent research performed by Lazareth et al. [41] reveals the underlying mechanism through which PECs invade the glomerular capillaries, enhance cell proliferation, and form cellular and sclerosing lesions. Their study found that the expression of the tetraspanin CD9 in PECs was significantly increased in mice and humans with FSGS. Importantly, CD9 deficiency blocked the directed migration of PECs to glomeruli, preventing CD44 and β 1 integrin expression, resulting in improved renal function and proteinuria. Moreover, both podocytes and PECs can express CD9 in human pathologies, whereas CD9 depletion not only improved PEC-induced sclerotic lesions but also maintained podocyte numbers. These results emphasize the key roles played by the expression of CD9 as a common pathogenicity switch that drives the PEC phenotype in FSGS, providing an underlying treatment target for this disease.

PECs Serve as Podocyte Stem or Progenitor Cells, Enhancing Regeneration

Podocytes are terminally differentiated cells with limited regenerative capacity, and the loss of podocytes results in increased protein filtration in the glomeruli, decreasing renal function. The potential source and mechanism of podocyte supplementation and rescue is a current focus of research studies. Clinicians have long searched

for renal progenitors that may be involved in podocyte regeneration. Importantly, some PEC subgroups appear to be promising candidates for podocyte restoration, despite the presence of several stem and progenitor cells in the kidney.

A growing body of evidence supports a role for PECs in podocyte regenerative properties. First, both PECs and podocytes originate from the metanephric mesenchyme during normal kidney development [42]. During the s-shaped stage of glomerulogenesis, mesenchymal cells differentiate separately to form PECs and podocytes. Because these cells share a common ancestor, the possibility exists that PECs could transdifferentiate into podocytes upon activation. Second, several PEC subpopulations express progenitor markers and podocyte markers, including CD133 and CD24, which are often used to characterize cells that have stem or progenitor properties in humans. CD24 and CD133 are coexpressed in the PEC subgroup on the BC of human kidneys near the tubule/glomerular junction. A second subpopulation, CD133⁺CD24⁺Podocalyxin⁺PECs, was also discovered, located between the urinary pole and the vascular pole [43, 44]. In vitro, this subgroup of cells could be differentiated according to their expression of various podocyte-associated proteins, such as WT-1 and nephrin [44]. Thus, CD133⁺CD24⁺Podocalyxin⁺PECs could represent podocyte progenitor cells in humans and mice. Moreover, Prochnicki et al. [45] demonstrated that SOX9 was another potential PEC marker also expressed by progenitors. SOX9 was expressed in a subpopulation of PECs, and SOX9 is relatively well conserved among different species [46].

Despite these findings, whether PEC can transdifferentiate into podocytes under conditions of podocyte depletion remains under debate. Notably, little evidence has been presented to suggest that the transdifferentiation of PECs can directly ameliorate disease prognosis or progression. Interestingly, several studies have presented evidence to counter the regenerative potential of PECs as podocyte progenitors in adult rodents. Lineage tracing studies performed by Wanner et al. [47] and Berger et al. [48] indicated that no genetic-labeled PECs were recruited onto the capillary tuft, and no significant regeneration of podocytes was observed in adult FSGS animals.

Recently, Kaverina et al. [49] performed a thorough dual-lineage tracing experiment to label PECs and podocytes, which indicated that PECs could transdifferentiate toward an adult podocyte phenotype after podocyte depletion. Notably, they found that PECs migrated to the

glomerular tuft in the presence of primary, secondary, and minor processes, demonstrating that transdifferentiated PECs could acquire the morphological and ultrastructural characteristics of podocytes. This study provided strong evidence that PECs can be regarded as a source of podocyte renewal in adult mice, contrasting with the results of 2 previous studies. One possible explanation may be that podocyte loss was not a feature of the animal models examined in the Wanner et al. [47] and Berger et al. [48] studies, which were examining renal ablation and glomerular hypertrophy.

The Role of PECs in the Repair of Scarring after Glomerular Injury

PECs appear to play a crucial role in both podocyte regenerative properties and pathogenic activation in glomerular disease (Fig. 1). On the one hand, the pathogenic role of PECs has been well accepted by the scientific community, and recent studies have provided further insights into the molecular mechanisms that underlie the pathogenic proliferation, migration, and extracellular matrix synthesis of PECs in FSGS, indicating that the targeting of PEC activation may represent a promising therapeutic approach in FSGS. On the other hand, controversies regarding the characteristics of PEC transdifferentiation into podocytes remain. One challenge will be the enhancement of podocyte regenerative properties among PECs and the development of strategies to increase the efficiency of PEC-mediated glomerular repair. Furthermore, previous observations have indicated that PECs display the potential for self-renewal [50, 51]. Lineage tracing and the genetic labeling of PECs in aging mice were examined, which showed that the labeling was not diluted over time, which indicated that PECs are capable of self-renewal throughout the lifetime of mice [52].

The different roles played by PECs in glomerular disease may be associated with the different functions of PEC subpopulations. During the process of renal development, different subsets of PECs were detected in both healthy and diseased kidneys using both ultrastructural and immunostaining techniques. Shankland et al. [53] have categorized several PEC subpopulations based on their characteristics and their potential functions, including classical PECs, parietal podocytes, aPECs, and podocyte-committed progenitor cells. With new discoveries in the field, the definitions and terminologies used to describe various PEC subpopulations are likely to evolve over time. Future studies may develop additional strategies for the purification and separation of different PEC subpopulations.

Table 2. Current therapies for different forms of FSGS [57–69]

Different forms of FSGS	Treatment	Corresponding mechanism
Primary FSGS	Immunosuppressive agents (glucocorticoids, CNIs)	Directly modulate the podocyte phenotype [57]
	Immune modulation (rituximab, ACTH)	Rituximab: directed against the CD20 antigen expressed in human B cells [58] ACTH: direct MCR-mediated immunomodulation, direct MCR-mediated protection of kidney cells, as well as non-MC1R-mediated protection of podocyte [59–61]
	Circulating factors (plasmapheresis, galactose)	Remove the permeability factor [62]
	Antifibrotic agents (adalimumab, FG-3019, fresolimumab, pirfenidone)	Adalimumab: a human anti-TNF monoclonal antibody [63] FG-3019: a human monoclonal antibody against CTGF [64] Fresolimumab, a human monoclonal antibody directed against human TGF- β [64] Pirfenidone: decrease TGF- β 1, TNF- α , PDGF, and COL1A1 expression [65]
Secondary FSGS		
Virus-induced FSGS	Antiretroviral therapy [66]	
Drug-induced FSGS	Stop the offending medication	Target-specific etiology
Caused by system disease	Renin-angiotensin system inhibitors [67] Glucose control [68] Lose weight [69]	
Family FSGS	Gene testing and individualized treatment	Target gene mutation
FSGS, focal segmental glomerulosclerosis; CNIs, calcineurin inhibitors; MCR, melanocortin receptors.		

Roles of Other Resident Glomerular Cells in FSGS

FSGS is a morphological pattern of injury, which can be caused by diverse injuries in various glomerular cells. After podocytes experience an initial damaging event, other cellular events and detrimental crosstalk among resident renal cells participate in the progression of FSGS. Previous studies have shown that podocyte injury in FSGS contributes to endothelial dysfunction [54]. By contrast, endothelial dysfunction can precede podocyte damage [55]. Lim et al. [56] demonstrated that pre-existing tubulointerstitial injury caused the glomerulus to become more responsive to subsequent podocyte damage. The proximal tubule cells are particularly vulnerable to injury, and crosstalk between tubular injury and interstitial fibrosis is quite interesting. Tubular injury often presents as a series of mild injuries, accompanied by transient dedifferentiation and mitochondrial disorder, resulting in growth stagnation and impaired repair functions. Tubular cells can also produce extracellular matrix, leading to tubulointerstitial fibrosis. Interstitial fibrosis can play a variety of roles in tubular injury. On the one hand, the increased stroma can increase the pressure on the tubular basement membrane. On the other hand, tubular injury

can activate fibroblasts, resulting in the secretion of growth factors to perpetuate tubular injury. Therefore, understanding the specific cellular perturbations that occur in FSGS is important for identifying new therapeutic targets.

Current Therapies and Potential Therapeutic Targets for FSGS

The objective of FSGS treatment is the induction of complete proteinuria remission, slowing the progression of renal disease and significantly improving kidney function. Understanding the pathogenesis of the disease can promote the detection of potential therapeutic strategies for the future. Current therapies for different forms of FSGS have been described by Rosenberg [2] and Chen and Liapis [1] (Table 2). The current KDIGO (Kidney Disease: Improving Global Outcomes) guidelines recommend that primary FSGS first be treated with high-dose prednisone for 4–16 weeks until complete remission [70]. Calcineurin inhibitors are recommended for steroid-resistant patients, which should be administered for at least 1 year if patients respond to treatment. Several new treatments have been developed based on different mecha-

nisms, most of which remain under investigation (Table 2). The management of secondary FSGS involves targeting the underlying etiology. To a large extent, this strategy involves the use of renin-angiotensin system inhibitors as the first-line treatment to reduce systemic and glomerular pressure. For the treatment of familial FSGS, the identification of specific gene mutations and exploring the underlying mechanisms can provide tremendous potential for highly targeted therapy. Ashraf et al. [71] indicated that supplementation with coenzyme Q10 attenuated proteinuria in steroid-resistant nephrotic syndrome patients who harbor mutations in genes associated with the CoQ10 biosynthetic pathway, such as aarF domain-containing kinase 4 (*ADCK4*).

When considering treatment from the perspective of pathogenesis, specific cells should be targeted. Current therapeutic strategies focus primarily on podocytes, the role of therapeutic agents on PECs, mesangial cells, and other cells that are less well known in FSGS. Based on our previous discussion, the targeting of PEC activation has emerged as a promising therapeutic approach in FSGS. Therefore, future studies should explore the clinical outcomes of FSGS patients treated with CD9/CD44 antagonists to block the activation of PECs. Fibroblasts also represent an important potential target for the treatment of renal fibrosis. Recent studies performed by Fu et al. [72] have indicated that tenascin-C, an extracellular matrix glycoprotein, represents the primary component of the fibrotic niche in the fibrotic kidney, accelerating the progression of acute kidney injury to chronic kidney disease by damaging the integrity of tubular cells [73]. Importantly, small interfering RNA-mediated tenascin-C knockdown repressed the *in vivo* induction of fibroblast expansion and renal fibrosis following injury [72]. Furthermore, the level of urinary tenascin-C in chronic kidney disease was associated with kidney insufficiency and renal fibrosis [73]. Thus, blocking tenascin-C signaling may represent a new therapeutic strategy for treating kidney fibrosis in FSGS. Our lab has generated a renal medullary interstitial cell-specific tenascin-C-CreER2 knock-in mouse line [74], which may represent the most specific animal model for studying the role played by tenascin-C in FSGS.

Conclusion

Further understanding of the underlying mechanisms of FSGS could lead to significant advances in enhanced interventions targeting podocytes and other damaged

nephron components, which could slow or reverse the progression of FSGS. Podocyte depletion patterns play critical roles in the pathogenesis of classical FSGS. Therefore, identifying potential sources and mechanisms to support podocyte replacement and rescue remains a primary focus of current research. Recent studies have begun to reveal the roles played by PECs and other resident cells in disease etiology. These processes occur during the process of glomerular injury, but different PEC subsets have demonstrated functional heterogeneity during the processes of regeneration and pathogenicity. In conclusion, we suggest that the pathogenesis of FSGS represents a complicated interaction among glomerular resident cells and propose that the regulation of the complex crosstalk that occurs among these cells could represent a potential future target for the treatment of FSGS and other causes of CKD.

Conflict of Interest Statement

Dr. Chua-Ming Hao is an Associate Editor of “Kidney Diseases.”

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Author Contributions

Ke Sun and Qionghong Xie drafted the manuscript. Chuanming Hao revised and approved the final manuscript.

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