Update on Recurrent Focal Segmental Glomerulosclerosis in Kidney Transplantation

Jun Shoji^a Akiko Mii^b Mika Terasaki^c Akira Shimizu^c

^aDivision of Transplant Nephrology, University of California San Francisco, San Francisco, CA, USA; ^bDepartment of Nephrology, Nippon Medical School, Tokyo, Japan; ^cDepartment of Analytic Human Pathology, Nippon Medical School, Tokyo, Japan

Keywords

Circulating permeability factor · Focal segmental glomerulosclerosis · Kidney transplantation · Pathology · Recurrent focal segmental glomerulosclerosis

Abstract

Background: Focal segmental glomerulosclerosis (FSGS) is a clinicopathological syndrome characterized by nephrotic-range proteinuria with high incidence of progression to end-stage renal disease (ESRD). In primary FSGS, 40–60% of patients develop ESRD within 10–20 years. **Summary:** Recurrence of FSGS after kidney transplantation is frequent and is associated with poor allograft survival. The risk factors for recurrent FSGS include onset of FSGS during childhood, rapid progression of primary FSGS to ESRD, history of recurrent FSGS in previous allograft, and diffuse mesangial hypercellularity or collapsing variant of FSGS in the native kidney. The early histological findings of recurrent FSGS consist of unremarkable glomerular changes on light microscopy but significant podocyte effacement on electron microscopy; the loss of foot processes with eventual dropout of podocytes leads to the development of segmental lesions in the glomerulus. Experimental and clinical data suggest the existence

of circulating permeability factors, such as soluble urokinase-type plasminogen activator receptor (suPAR), cardiotrophin-like cytokine factor-1 (CLCF-1), CD40 axis, and apolipoprotein A-Ib (ApoA-Ib), in the pathogenesis of recurrent FSGS. These biomarkers including circulating permeability factors may facilitate earlier diagnosis of FSGS post-transplant and may guide in the development of novel therapies that may be more effective and improve long-term outcomes in kidney transplantation. **Key Messages:** Several studies have suggested the possible circulating permeability factors, such as suPAR, CLCF-1, CD40 axis, and ApoA-Ib, in the pathogenesis and disease progression of FSGS and recurrent FSGS. Further studies should be performed to elucidate the true essential biomarker(s) associated with the onset and progression of FSGS as well as recurrent FSGS.

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Introduction

Idiopathic focal segmental glomerulosclerosis (FSGS) is the most common cause of nephrotic syndrome in adults that often leads to end-stage renal disease (ESRD) requiring dialysis or renal transplant [1, 2]. Unfortunate-

ly, FSGS frequently recurs in kidney transplant and is associated with poor allograft survival. Primary FSGS has a 30–80% risk of recurrence in kidney transplant, leading to accelerated graft loss. Patients who lose their allograft to recurrent FSGS are usually not retransplanted since the risk of recurrence in the subsequent allograft is approximately 80–100% [2].

Plasmapheresis has been the mainstay treatment for recurrent FSGS. Full remission is defined as a urine protein-to-creatinine ratio (UPCR) of ≤ 0.5 grams per gram after treatment. Partial remission is a decrease in UPCR by at least 50%, and treatment failure is persistently elevated UPCR of 50% or above the initial value after receiving plasmapheresis. However, response to plasmapheresis treatment has not been consistent. Typically, approximately 60% achieve full remission, 20% with partial remission, and 20% do not respond to treatment. In addition, a third of the patients who achieve full remission relapse, requiring additional plasmapheresis therapy. Factors that may predict response to treatment have not been well defined [1].

Glomerular Filtration Barrier and the Pathophysiology of FSGS

Podocytes are the site of damage in FSGS. These highly differentiated epithelial cells of renal glomeruli consist of a cell body and foot processes which make contact with the glomerular basement membrane. The foot processes form an interdigitating pattern with the foot processes of neighboring podocytes and form the glomerular filtration barrier with the slit diaphragm.

The actin-based cytoskeleton is critical in maintaining an effective glomerular filtration barrier to prevent proteinuria. The disruption of podocyte cytoskeleton and slit diaphragm with subsequent foot process effacement, podocyte hypertrophy, detachment from the glomerular basement membrane, and the loss with migration into the Bowman space leads to the development of FSGS [3].

Histological Findings of Recurrent FSGS

The early histological findings of recurrent FSGS consist of unremarkable glomerular changes on light microscopy but significant podocyte effacement on electron microscopy. The loss of foot processes eventually evolves to podocyte dropout and development of segmental lesions in those who are unable to achieve remission [4]. Podocytes in recurrent FSGS undergo profound changes in ad-

hesion molecules and transdifferentiate into macrophage-like cells. These dysregulated podocytes have reduced expression of $\alpha 5$ integrin (fibronectin receptor), $\alpha 3\beta 3$ integrin (vitronectin receptor), and actin leading to the disturbance of cell polarity. In advanced stages of FSGS, there is a disappearance of both $\alpha 5$ integrin and $\alpha 3\beta 3$ integrin. With the reduction in adhesion molecules, podocyte detachment is observed [5, 6].

The relevance of Columbia classification of the histological variants of FSGS is equivocal since studies have not shown a consistent pattern of recurrence posttransplant. The Columbia classification of FSGS includes (1) tip lesion variant, (2) cellular variant, (3) collapsing variant, (4) perihilar variant, and (5) FSGS not otherwise specified (NOS) [7, 8].

The main histological feature at the time of recurrence is minimal change disease, and subsequent biopsies show the increase in the incidence of FSGS variants (Fig. 1). The FSGS variant in the native kidneys has not been shown to predict the recurrence or the FSGS variant type seen in the renal transplant [8].

Circulating Permeability Factors of FSGS

Experimental and clinical data have strongly suggested the existence of circulating permeability factors that likely contribute to podocyte injury and development of proteinuria in idiopathic FSGS [9]. The recurrence of proteinuria posttransplant in recurrent FSGS and improvement of proteinuria after treatment with plasmapheresis also support the existence of circulating permeability factor in the pathophysiology of primary FSGS. Although the circulating permeability factor has not been clearly identified, studies have suggested the role of the soluble urokinase-type plasminogen activator receptor (suPAR), cardiotrophin-like cytokine factor-1 (CLCF-1), CD40 axis, and apolipoprotein A-Ib (ApoA-Ib) in the pathogenesis of recurrent FSGS.

suPAR and FSGS

One of the first circulating permeability factors that have been identified as a possible culprit in FSGS is suPAR. It is generated by the cleavage of the glycosylphosphatidylinositol (GPI) anchor of urokinase receptor (uPAR) or secreted directly from cells as an alternative transcript [10]. High levels of suPAR have been shown to cause podocyte damage by binding and activating the integrin pathway [11–13]. In clinical studies, the elevated suPAR levels have been described in patients with FSGS [13, 14]. In addition, high

Fig. 1. Recurrent FSGS after kidney transplantation. **a** This is a case of a 23-year-old female who developed proteinuria 5 days after kidney transplantation. Initial renal biopsy at the onset of proteinuria showed minimal change disease on light microscopy. FSGS NOS (Columbia classification) with mild epithelial hypercellularity and segmental glomerulosclerosis is noted here on 4-month posttransplant biopsy (PAS stain, $\times 200$). **b** Hypertrophy of podocytes with diffuse effacement of foot processes and irregular dense network of microfilament bundles is noted on electron microscopy. Podocyte injury due to FSGS is demonstrated here by the dark-staining protein droplets in the podocyte cytoplasm, pseudocyst formation (star), and microvillous transformation. **c** Collapsing variant of FSGS (Columbia classification) in a 36-year-old male who is 2-months post-transplant: collapsing sclerotic lesion in the

glomerulus consists of global occlusion of the capillaries and prominent overlying podocyte hypertrophy and hyperplasia (PAM stain, $\times 400$). **d** Segmental cellular glomerular lesion that is characterized by engorgement of glomerular capillary lumen with infiltration of foam cells and mild overlying podocyte hyperplasia (arrow) is consistent with cellular variant of FSGS (Columbia classification) in a 27-year-old male who is 3.5-months after kidney transplantation. (Masson stain, $\times 600$). **e** This is a case of a 42-year-old female who is 6-months post-transplant with segmental glomerular sclerosis with mild epithelial hypercellularity and adherence lesion to Bowman's capsule, which is indicative of FSGS NOS (Columbia classification) (PAS stain, $\times 600$). FSGS, focal segmental glomerulosclerosis; NOS, not otherwise specified.

blood levels of suPAR could be predictive of FSGS recurrence in transplanted kidneys [13, 14].

However, several studies have not confirmed these findings of elevated suPAR in patients with FSGS or recurrent FSGS [15–18]. The recent study of suPAR levels in Japanese patients with glomerular diseases showed that the suPAR levels were inversely correlated with the eGFR and did not appear to be a useful biomarker for FSGS [19, 20]. Recently published data using 2 methods for measuring intact and fragments of suPAR have found no correlation with recurrent FSGS [21]. Two studies in mice did not demonstrate podocyte injury or proteinuria with elevated levels of suPAR [15, 17]. suPAR levels and their correlation with response to treatment have also been investigated. After treatment with plasmapheresis and rituximab, suPAR levels were noted to decrease. However, since the suPAR level reduction is transient and rebounds after treatment, it is not shown to predict the clinical outcome in recurrent FSGS [22, 23].

Although these studies have shown ambivalent results, it is unlikely that FSGS and suPAR are entirely unrelated [24, 25]. Despite suPAR or anti-CD40 antibody alone has not caused podocyte damage and proteinuria, the coadministration of suPAR and anti-CD40 antibody in mice has led to podocyte damage and proteinuria. This indicates that circulating suPAR and CD40 autoantibodies synergistically cause glomerular damage. Further study is warranted to assess the role of suPAR in the pathogenesis and disease progression of FSGS.

CLCF-1 in FSGS

Another potential permeability factor in primary FSGS that has been isolated via galactose affinity chromatography and mass spectrometry is CLCF-1, a member of the IL-6 family of B-cell-stimulating cytokine with a predicted molecular weight of 22 kDa [26]. In patients with recurrent FSGS, levels of CLCF-1 were found to be 100 times higher than in controls.

CLCF-1 likely causes FSGS due to destabilization of the actin cytoskeleton of podocytes. One study has noted that CLCF-1 decreased nephrin expression in isolated rat glomeruli and cultured murine podocytes [27]. Incubation with CLCF-1 also caused marked changes in the configuration of the actin cytoskeleton of cultured murine podocytes. These changes progressed with time and concentration in a dependent manner and are likely a consequence of activation of the cell toward a more motile phenotype that may be more vulnerable to detachment under mechanical or metabolic stress [27]. In addition, recombinant CLCF-1 increased permeability of albumin in iso-

lated rat glomeruli through the phosphorylation of signal transducer and activator of transcription 3 (STAT3) as well as in mice after acute and chronic infusion [27].

Anti-CLCF-1 antibody blocked the CLCF-1-induced or FSGS serum-induced increase in albumin permeability [28]. CLCF-1 is secreted and forms heterodimers with either cytokine receptor-like factor 1 (CRLF1) or soluble ciliary neurotrophic receptor alpha (sCNTFR α), and the heterodimer composed of CLCF-1 and CRLF1 attenuated the increase in albumin permeability caused by CLCF-1 or FSGS serum. Furthermore, the Janus kinase 2 (JAK2) inhibitor or STAT3 inhibitor significantly blocked the effect of CLCF1 or FSGS serum on permeability of albumin [28]. This suggests that antibodies targeting CLCF-1 or its receptors and the inhibitor of JAK/STAT pathway may be novel therapeutic targets in the treatment of primary FSGS and recurrent FSGS.

CD40-CD40L Axis in the Pathogenesis of Recurrent FSGS

CD40 autoantibodies and CD40 ligand have been shown to be present in clinical samples of patients with recurrent FSGS and have produced podocyte injury in vitro [3, 11, 25]. In renal pathophysiology, CD40-CD40L signaling pathway blockade has been shown to be protective in multiple models of glomerulonephritis. Soluble CD40L levels were noted to be significantly higher in patients with FSGS compared to healthy controls [3]. Among the panel of 7 autoantibodies (CD40, PTPRO, CGB5, FAS, P2RY11, SNRBP2, and APOL2) that have been identified to possibly predict posttransplant FSGS recurrence with 92% accuracy, pretransplant elevation of CD40 autoantibody is shown to have the highest correlation with posttransplant recurrence of FSGS [25]. CD40 likely has a direct role in maintaining the integrity of the podocyte architecture. suPAR infusion followed by the infusion of CD40 autoantibody isolated from patients with recurrent FSGS has led to proteinuria in mice, and podocyte effacement was seen on electron microscopy [25]. Furthermore, activation of CD40 on podocytes with soluble CD40L has shown to cause rearrangement of the actin cytoskeleton and loss of slit diaphragm proteins including nephrin and podocin [3]. Thus, although there has been equivocal evidence of suPAR's role in recurrent FSGS, this is likely due to the complex pathway in the pathogenesis of FSGS that involves suPAR and the CD40 axis.

ApoA-Ib in Recurrent FSGS

Another biomarker that has been investigated as possibly correlating with disease activity in recurrent FSGS is

ApoA-Ib [29, 30]. ApoA-I is a component of HDL particle that is absent in the urine of healthy population. Amongst the several modified ApoA-I forms, ApoA-Ib is noted to be present in the urine of recurrent FSGS. ApoA-Ib is a high molecular weight form of ApoA-I that may be associated with a misprocessed form of ApoA-I precursor with no genetic variations in the ApoA-I gene [31]. One study performed a proteomic analysis of serum and urine samples of transplant patients with history of primary FSGS; the results showed the existence of ApoA-Ib exclusively in patients with relapsing FSGS [29]. A separate study confirmed this finding of increased level of ApoA-Ib in the urine of relapsing patients. This elevation was not found in nonrelapsing FSGS and non-FSGS nephrotic patients [29, 30]. Urinary ApoA-Ib predated the recurrence of FSGS when levels were followed longitudinally for 1 year in patients with idiopathic FSGS [30]. Furthermore, several cases of relapsing FSGS with elevated ApoA-Ib preceded any histological damage from recurrent FSGS, suggesting its use in earlier diagnosis of FSGS [30]. This urinary biomarker may serve as both diagnostic and prognostic marker for recurrent FSGS.

The biomarkers including the circulating permeability factors may facilitate earlier diagnosis of FSGS and recurrent FSGS after transplantation, mediate the clarification of pathogenesis of FSGS, and may lead to the development of effective and novel therapies of FSGS as well as recurrent FSGS. Further studies should be performed to elucidate the true essential biomarkers associated with the onset and progression of FSGS and recurrent FSGS.

Conclusion

Recurrent FSGS is a primary glomerular disease with significant impact on allograft and patient survival. As a disease that is due to the disruption of the podocyte cyto-

skeleton, treatments have targeted culprits that destabilize this structure. Identifying biomarkers that may serve to diagnose and predict outcomes of recurrent FSGS not only will facilitate rapid diagnosis of FSGS posttransplant but may also guide the development of novel therapies that may be more effective and improve long-term outcomes in kidney transplantation. Based on the recent results, further studies should be performed for searching the involvement of possible circulating permeability factor(s) in pathogenesis and disease progression of FSGS.

Acknowledgements

The authors express special thanks to Mr. Takashi Arai, Ms. Mitue Kataoka, Ms. Kyoko Wakamatsu, Ms. Arimi Ishikawa, and Ms. Naomi Kuwahara (Department of Analytic Human Pathology, Nippon Medical School) for their expert technical assistance.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study did not receive any funding.

Author Contributions

Akira Shimizu supervised the study; Jun Shoji and Akiko Mii wrote the manuscript; Mika Terasaki and Akira Shimizu provided Figure 1. All authors reviewed the final manuscript.

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