Has the circulating permeability factor in primary FSGS been found?

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Abstract

A circulating permeability factor has long been implicated in the pathogenesis of primary focal segmental glomerulosclerosis (FSGS). Recent evidence in animal models, and now in several cohorts of patients with primary FSGS, suggest that the soluble urokinase-type plasminogen-activator receptor (suPAR) might fulfill at least a role as biomarker and perhaps even as contributing factor. Although ongoing studies are needed, confirmation of these findings might lead to new diagnostic and therapeutic strategies for this often resistant glomerular disease, as well as a better understanding of podocyte dysfunction.

Linking circulating permeability factors to primary FSGS

FSGS, a common cause of nephrotic syndrome, is considered a disorder of glomerular visceral epithelial cells called podocytes. Although the word “primary” denotes that the cause is not known to date, a circulating permeability factor has long been suspected. The term “permeability” refers to the increased leakiness of the glomerular filtration barrier leading to proteinuria. The evidence for a “circulating” component of permeability factor in FSGS includes: (1) primary FSGS can recur very rapidly after kidney transplantation (~30% of cases in adults, >50% in children). Conversely, FSGS can often be prevented or delayed in high risk patients with pre-transplant plasmapheresis, which presumably removes the factor(s) from the circulation; (2) injection of plasma or plasma fractions from patients with FSGS into rats causes proteinuria, (3) sera from patients with FSGS increase albumin permeability in an isolated glomerulus model ex vivo, (4) and a transient nephrotic syndrome has been transmitted to a newborn from a mother with FSGS [reviewed in (1)]. Together, these data have suggested that primary FSGS is likely a systemic disorder, where a factor(s) present in the circulation is the pathogenic culprit, but has its target effects on glomerular podocytes.

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Identification of a circulating FSGS factor has proven elusive, although several candidates have been proposed including cardiotrophin-like cytokine 1 (1). After decades of failed attempts, recent evidence implicates the soluble urokinase plasminogen-activator receptor (suPAR) (2–4). However, when considering if a candidate is a circulating permeability factor for FSGS, we need to ask if the putative factor is a mediator of disease, a biomarker, or both? We will address these aspects in this commentary based on current experimental and clinical peer-reviewed published data.

**Is suPAR a mediator of Primary FSGS?**

In order for a circulating protein/factor to be considered a mediator of disease requires that it is likely formed at a site beyond the target organ, circulates, and has reproducible biological activity on the target organ. The identified factor should have similar effects on that organ in a different host, and the biological effects inhibited/reduced by selectively removing the factor from the circulation, or by specifically inhibiting its action on the target cell(s). Let us examine suPAR in this context.

**Pre-Clinical studies on suPAR as a “mediating” circulating permeability factor**

Podocytes are under considerable mechanical stress, and adhere tightly to the underlying glomerular basement membrane (GBM) primarily via interactions between the actin cytoskeleton, integrins α3β1 and αvβ3, and the GBM components laminin 521 and type IV collagen. Within podocytes, Wei et al. showed that enhanced αvβ3 integrin signaling is associated with foot process effacement and the development of proteinuria, hallmarks of primary FSGS. (3) While seeking mechanisms for β3 integrin activation, they showed that membrane bound urokinase-type plasminogen activator receptor (uPAR) on podocytes activates this pathway. These seminal studies placed uPAR at center stage as a potential mediator for some of the podocyte’s responses to injury in FSGS. However, uPAR is a glycosyl-phosphatidylinositol (GPI) anchored membrane protein present on multiple cells, including podocytes, and does not circulate, thereby excluding it as a circulating factor candidate. Of relevance to primary FSGS is that proteolytic cleavage of uPAR can release several circulating protein fragments collectively known as soluble urokinase plasminogen-activator receptor (suPAR) from cells within the circulation such as neutrophils. (5)

suPAR is by definition a “circulating” protein, and is not expressed on podocytes. This begged the question that if uPAR injures podocytes, can suPAR do so too? Pre-clinical studies by Wei and Reiser showed suPAR deposits in mice kidneys along podocytes which was associated with an increase in β3 integrin activity. (2) Elegant proof of principle experiments showed that in uPAR null mice, chronic suPAR overexpression or administration resulted in a glomerulopathy with foot process effacement, proteinuria and other features of FSGS, which could be ameliorated with a uPAR-specific monoclonal antibody (2) Taken together, these pre-clinical studies provide initial support that circulating suPAR may induce changes in podocytes similar to that of primary FSGS, and therefore is a prominent candidate for being a circulating mediator of primary FSGS. Future studies will be needed to strengthen this concept.
Clinical Studies on suPAR as a “mediating” circulating permeability factor

Following the discovery that suPAR alters the biology, morphology and function of podocytes in cell culture and in animals, the next question is does suPAR mediate podocyte injury clinically in man? Currently we have limited clinical data to directly support this concept. The source of suPAR in the circulation is unknown, and biopsy studies in humans to detect suPAR in the glomerulus have not been reported at the time of this commentary, although activated β3 integrin staining in FSGS biopsies has been demonstrated (3).

Encouraging results in a few patients with recurrent FSGS after transplantation are described where suPAR levels decreased after plasmapheresis to a level resulting in reduced podocyte β3 integrin activation which in turn was associated with an improvement in proteinuria. However, to our knowledge no specific inhibitors of suPAR exist to determine a direct causal association in man. A recent case report describes the resolution of transient proteinuria in a newborn where suPAR was likely transmitted from the mother with FSGS. (6)

Is suPAR a biomarker for Primary FSGS?

The FDA uses the term ‘biomarker’ to describe any measurable diagnostic indicator that is used to assess the risk or presence of disease. Thus, an ideal biomarker should be easily measurable with an accurate and reproducible assay, with results consistent across gender and ethnic groups. A biomarker should be highly sensitive (positive in nearly all subjects with the disease), highly specific (most subjects without the disease should have negative values) and therefore have strong predictive value. While a majority of patients with FSGS have elevated suPAR serum levels, the specificity for suPAR as a biomarker for primary FSGS is somewhat offset by the absence of similar histological and clinical findings in patients with very high suPAR levels in other conditions such as inflammation.

To date, data has been reported on 327 patients with primary FSGS, summarized in Table 1. Reiser and colleagues were the first to describe elevated suPAR levels in 70% of a diverse group of patients with FSGS (2). In order to validate these novel data, an important confirmatory study was undertaken by the same group in two well-characterized and diverse cohorts of patients from the North American FSGS-CT clinical trial (n=70), and the European FSGS consortium, PodoNet (n=94). (4) Using 3000 pg/ml as a cutoff, Wei and Reiser demonstrated elevated suPAR levels in 84% and 55% of subjects respectively.

In this Journal, Huang et al. also demonstrate elevated levels of suPAR (mean 2923 pg/ml) in a cohort of 74 patients with primary FSGS compared to a control group consisting of kidney donors (mean 1739 pg/ml) and patients with other podocyte disorders including minimal change disease (mean 2050 pg/ml) and membranous nephropathy (mean 2028 pg/ml). (7) Furthermore, they show that within the primary FSGS group, higher levels are associated with worse pathology (increased interstitial fibrosis, tubular atrophy and glomerular crescents) and worse renal function. They also showed that the tip variant, which typically has a better prognosis, had lower levels of suPAR than the not otherwise specified (NOS) or cellular variants of FSGS. Using the definitions of elevated suPAR levels by the authors in these combined studies, these data show that high levels of suPAR are indeed
detected in a majority of patients with primary FSGS, and thus provide support for suPAR as a potential biomarker for primary FSGS.

What are the limitations of this data?

Although four cohorts of primary FSGS patients are now described that show elevated levels of suPAR (2, 4, 7), not all patients in each group showed elevated levels (range from 50 to 80%). One small single center study (n=11) showed only a few primary FSGS patients with elevated suPAR levels (8). In the positive studies, the sensitivity of the assay was low, as a significant number of patients with primary FSGS (16–45%) did not have elevated suPAR levels. This may reflect the heterogeneity of this disease (some patients may have had familial FSGS due to mutations in podocyte expressed genes that were not tested), the time course of the disease (suPAR may not remain persistently elevated, or was elevated earlier in the course of their disease), or that the assay may not be specific enough to detect elevation in the biologically active suPAR fragment(s) compared to total suPAR levels (see below). Increased circulating suPAR is also not specific to FSGS as levels may be markedly increased (up to 10,000 pg/ml) in inflammatory disorders that are not typically associated with proteinuria such as systemic infection (human immunodeficiency virus, tuberculosis, malaria, bacteremia), atherosclerotic disease, myocardial infarction, decompensated cirrhosis, systemic lupus erythematosus and certain cancers.

The finding in the Huang study of suPAR levels in patients with secondary FSGS (n=14) being similar to the primary FSGS group was surprising and does question the specificity of suPAR for primary FSGS. It is possible that some of these patients may have been misclassified as secondary FSGS (here defined by non-diffuse effacement of podocyte foot processes). Alternatively, one might speculate that increased suPAR levels may represent a non-specific mechanism of podocyte injury, or merely a biomarker resulting from podocyte and/or glomerular injury (although the source of the protein is not known). It is notable that even some patients with membranous nephropathy, another podocyte disorder, have elevated suPAR levels. (2)

Is the commercial assay reliable as a biomarker for primary FSGS?

Nephrologists recall the saga of “fragment assay problems” with circulating parathyroid hormone. SuPAR circulates as multiple fragments of different sizes (20–55kDa), and importantly it is unclear which of these fragments is biologically active on the podocyte. (9). The R&D suPAR Elisa used in all published studies does not differentiate between these, as it is based on a capturing antibody that recognizes the glycosylated form(s) of suPAR. SuPAR has up to five glycosylation sites. Thus, high circulating levels could stem from an abundance of the low glycosylated protein forms, and/or low amounts of the highly glycosylated form of suPAR. There may also be variability in suPAR levels according to age and ethnic group, and notably, suPAR is small enough to be filtered at the glomerulus, and a decrease in renal function might be associated with higher levels. (4, 7) Wei et al. demonstrated that suPAR in FSGS is not associated with high CRP, and therefore might be different from the forms of suPAR present in response to inflammation (2). Future studies are needed to describe the FSGS-suPAR forms or fragments as refined tests become
available. Until then, it is recommended to measure suPAR in FSGS patients concomitantly with CRP, as the latter levels should be low.

So, has a circulating permeability factor in primary (and recurrent) FSGS been found?. The pre-clinical data support that the (su)PAR-β3 integrin signaling cascade can cause damage to podocytes akin to primary FSGS. Specific removal of suPAR from native and post-transplant recurrent FSGS patients would give definitive proof, as well as the development of specific inhibitors in humans to confirm a causative role of suPAR as a mediator of primary FSGS. It is not clear however that the current assay for suPAR is a suitable biomarker for this disease. Further efforts defining the sensitivity and specificity of this assay are essential, and indeed, a more precise assay that can identify the biologically active fragments is required to better answer this question.

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Clinical studies of suPAR in primary FSGS.

| Patient Cohort | Primary FSGS cases (n) | Demographics (race, sex, age) | Renal function (creatinine in mg/dl) | suPAR levels (pg/ml)* R&D assay | Control Groups (n, mean suPAR level where available) | Comments |
|----------------|------------------------|--------------------------------|--------------------------------------|---------------------------------|--------------------------------------------------------|----------|
| **Wei et al. (2)** | 78                     | Mean age 27yrs W-60%; B-17%; H-17%; A-6% M-60% | ESRD (n=54) CKD (n=23, mean Cr 1.9) | 71% > 3000                     | MCD (n=25) MN (n=11) Pre-eclampsia (n=7) | • Multi-center cohort  
• 54 of primary FSGS group reached ESRD and received kidney transplant.  
• 4/11 patients with membranous nephropathy had elevated suPAR levels. |
| **FSGS-CT (4)** | 70                     | Mean age 19yrs B-33% M-55% | Mean Cr 1.1 | 4588 ± 203 | Healthy (n=40) (age 16-52 yrs) | • Multi-center cohort  
• CRP normal |
| **PodoNet (4)** | 94                     | Pediatric (<18yrs) n/a | Cr range 0.69–0.91 | 3497 ± 195 | Healthy (n=110) (age < 18 yrs) | • Multi-center cohort  
• Genetic forms of FSGS had higher suPAR levels than non-genetic forms  
• CRP normal |
| **Huang et al. (7)** | 74                     | Median age 29yrs A-100% M-68% | Median Cr 1.1 | 2923 (2205–4360) | 2nd FSGS (n=14, 2639) MCD (n=14, 2450) MN (n=29, 2029) Normal (n=56, 1793) | • Multi-center cohort  
• Secondary FSGS had similar levels to primary FSGS |
| **Maas et al. (8)** | 11                     | n/a | n/a | 2392 | MCD (n=7, 2482) 2nd FSGS (n=5, 2716) | • Single-center cohort  
• Small number of patients, clinical details unclear |

*Mean (interquartile range) or mean ± standard deviation.

Abbreviations: yrs-years of age, Cr – creatinine, W-White, B-Black, A-Asian, H-Hispanic, M-male, F-female, n/a-not available, ESRD- end stage renal disease, FSGS-focal segmental glomerulosclerosis, MCD-minimal change disease, MN-membranous nephropathy.