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See related article, "A Patient-Centered Vision of Care for ESRD: Dialysis as a Bridging Treatment or as a Final Destination?," on pages 1647–1651.

Soluble Urokinase-Type Plasminogen Activator Receptor in FSGS: Stirred but Not Shaken

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The soluble urokinase-type plasminogen activator receptor (suPAR) has been proposed as a candidate circulating factor causing FSGS.[1] In this issue of *JASN*, Cathelin *et al.* further examine the short-term effects of two different types of suPAR on the kidney filtration barrier.[2] Although the authors show deposition of suPAR in the glomerular capillary wall of their experimental models, they do not find changes in albumin permeability. The activation of the suPAR target on podocytes, $\alpha v\beta 3$ integrin, is not examined, leaving the question of target engagement unanswered. Nevertheless, this study provides some additional insights into the complexity of suPAR-derived signals in kidney disease and offers a potential explanation as to why patients with elevated acute phase–associated suPAR may not readily develop nephrotic syndrome.

The debate regarding the existence of a serum factor that causes FSGS is certainly glorified, heated, and polarizing. Since Shalhoub first suggested the existence of such a factor in 1974,[3] the quest to find such molecules is ongoing and is in line with the ever-growing need for definitive treatments that eradicate pretransplant and post-transplant FSGS. Savin *et al.* are credited for demonstrating that serum and plasma from patients with FSGS induce kidney filter permeability changes.[4] Savin *et al.* also proposed that the FSGS factor is a protein with a molecular mass between 20 and 50 kD.[4] Studies in our laboratory showed that suPAR is a permeability factor in native and recurrent FSGS.[1] suPAR is a multidomain protein that is heavily glycosylated and precisely fits the suggested size range expected for the putative circulating factor. The proposed pathogenic role of suPAR is based on three observations: (*1*) variants of suPAR produced proteinuria in several mouse models, (*2*) total levels of glycosylated suPAR were elevated in the majority of patients with FSGS, and (*3*) suPAR can bind to and activate podocyte $\beta 3$ integrins allowing for activation of Rac-1 and podocyte motility (a surrogate for podocyte foot process effacement).

Several follow-up studies confirmed increased total suPAR serum levels in FSGS, which were validated in patients with normal or mildly reduced renal function compared with other glomerular diseases[5] but not necessarily in advanced renal failure in which suPAR accumulation may occur.[6] Furthermore, it should be noted that in certain recent studies, serum suPAR did not differentiate FSGS from other glomerulopathies in the setting of relatively preserved renal function.[7] However, healthy control patients in this study also had elevated suPAR levels at baseline, which is atypical and might be a confounder of the cohort. Nevertheless, these discrepancies around single-value suPAR testing in different cohorts with the current ELISA imposes an obstacle for bulk suPAR measurements in clinical practice.[6] Development of a more specific FSGS-suPAR ELISA and/or cell-based testing systems that can detect different forms of suPAR with strong podocyte integrin activation capacities is needed.[8]

suPAR is the cleaved product of the cell-bound urokinase-type plasminogen activator receptor (uPAR), a multifunctional receptor that binds both the protease urokinase and the adhesion protein vitronectin.[9] uPAR also functionally and physically interacts with integrins both directly and indirectly through signaling, with the latter in some circumstances due to uPAR vitronectin binding.[10] suPAR is normally heavily glycosylated and can be cleaved into various shorter molecules that determine variability in suPAR's cell signaling function and stability in body fluids, including serum. Cathelin *et al.*

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Table 1. Effects of various suPAR types on podocyte integrin activation and kidney filter function

suPAR Type	Source	Accession	Recipient	Route	Dose (μg)	Time	Glomerular β3 Integrin Activity	Kidney Phenotype	Reference
Full length, isoform 1, mouse	*Drosophila* S2 cells	NM_01111	B6 or 129 mice	Intravenous	Up to 100	24 h	Not studied	No proteinuria, no podocyte FP effacement	2
Full length, isoform 1, Fc chimera, mouse	Mouse NS0 cells	Q545X5	B6 or 129 mice	Intravenous	Up to 100	24 h	Not studied	No proteinuria, no podocyte FP effacement	2
Full length, isoform 1, mouse	*Drosophila* S2 cells	NM_01111	B6 mice	Osmotic pump	200	1 wk	Not studied	No proteinuria, no FP effacement	2
Full length, isoform 1, Fc chimera mouse	Mouse NS0 cells	Q545X5	uPAR KO mice 129/B6	Intravenous	20	24 h	Increased	Proteinuria	1
Endogenous	LPS induced	—	B6 mice	Endogenous	N/A	24 h	Increased	Serum and urinary suPAR increased, proteinuria, podocyte FP effacement	1
Endogenous	LPS induced	—	uPAR null kidney engrafted into B6 mice	Host suPAR circulating into the engrafted uPAR null kidney	N/A	24 h	Increased	suPAR deposits into uPAR null kidney, podocyte FP effacement	1
Secreted, isoform 2	Plasmid DNA	BC010309	B6 mice	Intradermal electroporation	80 DNA weekly	1 mo	Increased	FSGS-like changes, proteinuria	1, 11

S2, Schneider 2; KO, knockout; FP, foot process.

use two different types of suPAR. *Drosophila melanogaster* Schneider 2 cells produced monomeric three-domain mouse suPAR, which is structurally well characterized,[11] and recombinant mouse myeloma produced uPAR Fc chimera (three domains).[1] The former exhibits a macroheterogeneity in its glycosylation profile that is comparable with that of wild-type suPAR but the attached carbohydrate chains are generally smaller and more homogenous, which might account for some of the functional differences in integrin binding and activity generation. Cathelin *et al.* injected both types of suPAR or infused three-domain mouse suPAR and did not find significant albuminuria in 24 hours or over 1 week despite glomerular deposits of suPAR.[2] These experiments are informative because they are different with respect to suPAR type and experimental models compared with studies by Wei *et al.*[1] Wei *et al.* used (s)uPAR null mice to study the role of exogenous or endogenous suPAR deposition and proteinuria in concordance with podocyte integrin activation. By contrast, the experiments of Cathelin *et al.* omit analysis of podocyte integrin activation and instead rely on the presence of glomerular suPAR deposits, which does not allow the conclusion that delivered suPAR either binds and/or activaties podocyte $\beta3$ integrin (unclear target engagement). Assuming that the delivered suPAR reaches podocytes, one has to question why there is no development of proteinuria. This could have several reasons. For example, the physical forms of suPAR are not all equivalent and subtle variations in structure decisively influence its capacity for integrin activation. Perhaps the delivered three-domain suPAR represents a physiologic acute phase reactant that carries low integrin activating capacity and as such would not harm podocytes, at least not over a short time period. Another possibility is that the activation of podocyte $\alpha v\beta3$ is actually mediated by a vitronectin/suPAR complex. $\alpha v\beta3$ is a known vitronectin receptor but its activation by vitronectin bound to suPAR is unstudied. This interpretation is in line with the experiments by Wei *et al.*, who also used an alternative splice variant of suPAR containing parts of suPAR domains I and II (IMAGE cDNA clone 3158012; Table 1),[1] with a reduced number of glycosylation sites and preserved capacity for integrin activation. The vitronectin binding site on uPAR maps to residues in domain I and the domain I/II linker region.[12] When this suPAR type, which is expected to still be capable of vitronectin binding, is delivered over several weeks, it causes podocyte effacement and proteinuria with features of FSGS but only if the integrin activating moiety was left intact.[1] Although Cathelin *et al.* hypothesize that this form of suPAR may not fold properly, it is important to note that the suPAR plasmid expressing IMAGE cDNA clone 3158012 was successfully used to generate specific antibodies by utilizing gene-based immunization strategies.[12] Furthermore, this type of suPAR also produces albuminuria in transgenic mice that drive suPAR expression from adipocytes (our own unpublished results). Given the absence of proteinuria in their suPAR infusion studies, Cathelin *et al.* challenged wild-type mice with LPS and discovered increased glomerular

uPAR/suPAR expression[2] as previously described by Wei *et al.*[1] The resultant proteinuria in this study set could not be further exacerbated by coinjection of exogenous physiologic suPAR forms. These experiments argue that the glomerular suPAR that is generated or deposited in the kidney in response to LPS is different from the recombinant one administered by Cathelin *et al.*[2] An appealing possibility is that the vitronectin/suPAR complex is disrupted by acute phase rises in plasminogen activator inhibitor-1. Plasminogen activator inhibitor-1 competes with uPAR for vitronectin binding[13]; thus, one might expect less vitronectin/suPAR during an acute phase reaction. This scenario favors vitronectin/suPAR as the active principle in integrin activation.

Overall, the study by Cathelin *et al.* lets us appreciate that we currently do not fully understand which suPAR forms or associated proteins (*e.g.*, vitronectin) represent the most podocyte pathogenic ones. Given this shortcoming in knowledge regarding which of the suPARs is most relevant to FSGS, none of the animal experiments using suPAR may precisely reflect what occurs in human FSGS. From a scientific perspective, these will be very interesting questions to answer. From a clinical perspective, the removal of all suPAR forms in patients' circulation using a specific suPAR-immunoadsorption device may provide an approach in addressing the relevance of suPAR for human FSGS, and, if successful, may suggest a therapeutic consideration in this and potentially other suPAR-mediated disorders.

DISCLOSURES

J.R. has pending or issued patents on novel kidney protective drug therapies. He stands to gain royalties from their commercialization.

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Cytomegalovirus and Anemia: Not Just for Transplant Anymore

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Cytomegalovirus (CMV) is one of the most important viruses in renal transplantation that causes significant morbidity and mortality, even in the current era of effective prophylaxis and treatment. The CMV syndrome is characterized by fever, malaise, and transplant dysfunction that manifests as AKI, leukopenia, monocytosis, and anemia. Less than 20 years ago, in the absence of effective prophylactic or treatment agents, immunosuppression reduction was the only treatment and was commonly associated with death and/or transplant failure. The introduction of effective antiviral medications, such as

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