suPAR and focal segmental glomerulosclerosis

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In recent years, the nephrological community has witnessed very important discoveries in renal diseases, and within them, in glomerulonephritis. Good examples are the identification of the pathogenic mechanism in more than 70% of membranous nephropathy patients (recognition of the phospholipase A2 receptor as a podocyte antigen against which specific antibodies are formed)[1] or the increasingly more extensive and precise knowledge of mutations causing genetic forms of focal segmental glomerulosclerosis (FSGS).[2] Nevertheless, one of the main subjects that needs clarification in this area is the identification of the permeabilizing circulating factor, which causes the nephrotic syndrome in primary or idiopathic FSGS. Despite the efforts of many laboratories in recent decades, we have not yet managed to capture this (or these) intriguing pathogenic agent(s).

As is well-known, several clinical arguments, with good reason, make us suspect the existence of this permeabilizing factor: 1) the known possibility of massive proteinuria recurring within a few hours or even minutes of a renal transplantation in patients with primary FSGS,[3] 2) the disappearance of this proteinuria whenever kidneys with proteinuria recurrence are retransplanted into a patient without FSGS,[4] 3) induction of proteinuria in rats in which patient serum with primary FSGS is injected,[5] and 4) the efficacy of plasmapheresis in cases of proteinuria recurrence in transplanted kidneys.[6]

Several candidates for this mysterious and evasive permeabilizing factor have been proposed over this period, but without any reliable clinical demonstration being achieved. In 2011, a new candidate was proposed: soluble urokinase-type plasminogen activator receptor or suPAR.[7] In this study, it was demonstrated that patients with FSGS recurring in kidney transplants had significantly higher suPAR levels than those with minimal lesions or healthy controls or those with non-recurring FSGS. After performing plasmapheresis in recurring cases and in parallel with nephrotic syndrome remission, suPAR levels dropped significantly. Furthermore, the authors reported highly interesting experimental data that showed how high suPAR levels activated podocyte ß3 integrins, causing diffuse pedicel fusion and complete nephrotic syndrome. Knockout animals for the urokinase-type plasminogen activator receptor (uPAR) coding gene showed notable resistance to proteinuria measured by lipopolysaccharides and puromycin and the administration of a specific antibody against uPAR markedly decreased the severity of renal damage.[7] The molecular size of suPAR (20-50kDa) was similar to that which studies carried out years ago attributed to the circulating factor responsible for FSGS nephrotic syndrome. Subsequent studies by the same group that proposed suPAR as an agent responsible for primary FSGS demonstrated that suPAR levels were significantly higher in two FSGS patient cohorts.[8]

These studies stirred up great expectation, given that they presented solid data that indicated that suPAR could be the permeabilizing factor in a majority of primary FSGS cases and also because they demonstrated potential new therapeutic avenues on blocking this factor. Nevertheless, subsequent studies have cast many doubts on the real significance of suPAR in the pathogenesis of FSGS.

The structure of uPAR and its participation in many cell functions have been known for some time (reviewed in Maas et al.[9]). uPAR exists in very diverse types of cell area, including endothelial, mesangial and podocyte cells. uPAR remains attached to cell surfaces by means of an anchor composed of glycosylphosphatidylinositol. Various enzymes may free this anchorage, releasing uPAR into circulation in

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its soluble form (suPAR).[10] suPAR has a known capacity to activate vitronectin and various types of integrins, particularly podocyte ß3 integrins. The previously known fact that serum of FSGS patients can activate podocyte ß3 integrins, causing massive pedicel fusion, would suggest the possibility that suPAR may play a role in FSGS pathogenesis. Nevertheless, various other factors, such as the tumour necrosis factor, may perform similar actions on podocyte integrins and indeed, they exert profound changes on the cellular expression of uPAR. Moreover, suPAR levels have been studied in very diverse diseases and it has been observed that they are characteristically high in sepsis, tumours, liver diseases and generalised arteriosclerosis. In sepsis and systemic diseases, such as systemic erythematosus lupus, a relationship between suPAR levels and survival or disease activity has been noted. These data indicate a certain lack of specificity of suPAR levels, highly linked to inflammatory processes of a different kind. Another important piece of evidence, which would go against the proposed permeabilizing role of suPAR, is that in the abovementioned pathological conditions associated with very high suPAR levels, the presence of significant proteinuria was not ascertained.

Therefore, despite the attractive aspects of suPAR as a potential permeabilizing factor, there are still many aspects that need to be clarified before attributing the predominant pathogenic role to it as initial studies suggested. Moreover, we do not have rational hypotheses on mechanisms or factors that may precipitate an increase in suPAR levels in FSGS patients.

Despite these doubts, the determination of suPAR levels in patients with FSGS could theoretically be a useful biomarker, both for the differential diagnosis of primary FSGS with other causes of nephrotic syndrome and for the evaluation of the disease's activity or the risk of recurrence following renal transplantation. In the previous edition of this journal, Segarra and collaborators presented two very interesting studies in this regard.[11,12] In one of them,[11] they studied suPAR levels using a commercially available ELISA in 60 patients affected with the three diseases that most commonly cause complete nephrotic syndrome: minimal change disease (27 patients), membranous nephropathy (24) and FSGS (20). suPAR levels were significantly higher in FSGS (3938±849pg/ml) compared with patients with minimal change disease (2668±625pg/ml, $p<.001$), although there were no differences between FSGS and membranous nephropathy (3373±1073pg/ml). The authors carried out a careful analysis of the potential distinguishing value of suPAR levels and found that values greater than 3531pg/ml would have a high specificity (although a low sensitivity) for distinguishing between idiopathic FSGS and minimal change disease. Another interesting finding was the significant influence that renal function and age have on suPAR levels (which increase with age and with a decrease in renal function). However, the statistical specificity of elevated suPAR with respect to minimal change disease was obtained after adjusting for age and renal function.

The results of this study suggest that suPAR determination could help us know whether patients who had an inconclusive renal biopsy have FSGS or minimal change disease (for example, patients with a normal histology in whom sclerotic glomeruli may be attributed to unspecific sclerosis due to age or arteriosclerosis), once membranous nephropathy and other glomerular pathologies have been ruled out. It is necessary to bear in mind, however, that these same doubts in the histological categorisation of patients with nephrotic syndrome are also a limiting factor in the reliability of this study's results, as indicated by the same authors: those who were classified as minimal change disease patients may in reality have FSGS in which lesions were not detected due to the small number of glomeruli, and therefore, suPAR values may have been attributed to the wrong disease. Furthermore, as illustrated by cases of nephrotic syndrome recurrence in transplant patients with FSGS, patients with FSGS have a period of time (whose duration has not been defined, although it is probably highly variable) with massive proteinuria and complete nephrotic syndrome in which the biopsy only shows data typical of minimal change disease: normal in the optical microscope, negative immunofluorescence and pedicel fusion in the electron microscope. Only after weeks or months in this situation do lesions typical of glomerulosclerosis begin to appear. Although the results of this study are partially consistent with the two previous studies,[7,8] their results must fit with those of other critical studies with validity of suPAR determination in kidney patients. Bock and collaborators[13] found in 110 children that suPAR levels were higher in cases with non-glomerular renal diseases than in FSGS, although there were no significant differences. Neither were differences found with healthy controls. In this study, the presence of proteinuria was associated with lower levels of suPAR. Given the disparity of data and the absence of specifically higher values in idiopathic FSGS, the authors concluded that it is unlikely that suPAR is another factor causing FSGS and that its determination is not clinically useful. Other authors have also expressed their criticisms about the usefulness of measuring suPAR for the differential diagnosis of nephrotic syndromes.[9,14]

In the other study, Segarra and collaborators[12] analysed suPAR values in two cohorts of patients diagnosed with primary FSGS (35 patients) or secondary FSGS (48). As in the previous study, diagnoses of primary or secondary FSGS were based on histological and clinical criteria. suPAR levels were significantly higher in primary than in secondary FSGS. The authors proposed, in accordance with their data, that values greater than 4000pg/ml would be highly specific to primary FSGS. However, when values of patients with primary FSGS were analysed in accordance with the presence of nephrotic syndrome or its remission, no significant differences were found (4088±1019 versus 4079±1329pg/ml). This absence of correlation with the disease's clinical activity casts even

more doubt on the pathogenic significance of suPAR in primary FSGS. Furthermore, Huang and collaborators[15] presented data on suPAR levels in patients with primary and secondary FSGS. Although suPAR levels were significantly higher in FSGS than in minimal change disease, membranous nephropathy and healthy controls, there were no differences between primary and secondary forms of the disease (mean and interquartile range 2923, 2205-4360pg/ml in primary forms and 2639, 1945-3166pg/ml in secondary forms). As in the studies by Segarra, renal function significantly influenced suPAR levels.

In explaining the lack of concordance found between the different studies carried out, some authors have expressed serious doubts that the commercial test available for measuring suPAR is sufficiently reliable.[16] suPAR circulates as different fragments of various sizes and we currently do not know which of them are active in podocytes.

In conclusion, it is obvious that the suPAR podocyte ß3 integrin activation pathway is a very interesting area for exploring the pathogenesis of primary or idiopathic FSGS, but we are still far from being able to affirm that suPAR is the proteinuric circulating factor responsible for nephrotic syndrome in these patients. Despite the fact that most studies found high suPAR levels in FSGS, there is a notable overlapping between different glomerular diseases and we have not been able to reproduce a correlation between suPAR and the disease's activity. High suPAR levels are detected in many infectious and inflammatory diseases, without triggering proteinuria and, on the contrary, in many patients with a clear diagnosis of primary FSGS, there are normal and even low circulating levels of suPAR. All of these data call into question the central pathogenic role of suPAR and its potential usefulness as a diagnostic biomarker. The data of Segarra and collaborators are interesting, since they propose precise limits on its potential diagnostic usefulness: a suPAR level >3531pg/ml to distinguish FSGS from minimal change disease and >4000pg/ml to distinguish primary FSGS from secondary forms. However, further studies are required in larger cohorts, in order to establish conclusions that can be applied to clinical practice in patients with nephrotic syndrome.

In summary, we could say that the expectations created by the first studies on suPAR have been lowered by subsequent studies, whose conflicting results we have summarised. However, we must not forget that the potential beneficial effects of blocking suPAR and/or ß3 integrins, which experimental models clearly demonstrated, should continue to be studied with an eye to future application in humans. In this regard, as an example of the clinical significance that this research may have, positive results have been published very recently on treatment with abatacept, a B7-1 (CD80) inhibitor, a T-cell costimulatory molecule, in five patients with nephrotic syndrome due to FSGS.[16] Four of the patients

KEY POINTS

1. Experimental studies have shown that suPAR, through activation of podocyte β3 integrins, causes massive pedicel fusion and nephrotic syndrome. These data, along with the fact that anti-uPAR antibodies have a clearly positive effect, resulted in suPAR being proposed as the potential circulating factor causing primary FSGS.
2. Patients with FSGS have higher suPAR levels than those with other forms of glomerulonephritis, although there is a considerable overlap and not all studies agree. Age and reduction of renal function increase suPAR values.
3. suPAR levels are high in various clinical conditions (sepsis, tumours, liver disease, lupus), which reduces its specificity. Very high levels of suPAR in these conditions are not associated with proteinuria, which calls into question its pathogenic role as a proteinuric circulating factor.
4. suPAR levels above 3531pg/ml would support the diagnosis of FSGS versus minimal change disease in cases of dubious histologies. Levels greater than 4000pg/ml would support the diagnosis of primary glomerulosclerosis versus the secondary form.
5. Given the lack of homogeneity of the clinical results obtained, further studies are required in order that we may recommend suPAR levels as a useful biomarker in clinical practice.
6. Likewise, more experimental studies are required in order to establish the role of suPAR in FSGS genesis and that of other glomerular processes.

had recurrences following renal transplantation and the other was a case of corticosteroid resistance in the native kidney. All of these patients had complete or partial remission of proteinuria. A high urinary excretion of CD80 has been found in patients with minimal change disease in comparison with FSGS, while serum suPAR levels were significantly higher in FSGS than in minimal change disease in the same study.[17] However, all these data, many of them which are still disjointed and even contradictory, indicate that there will be more specific and effective treatments for primary FSGS in the near future.

Conflicts of interest

The authors declare that they have no conflicts of interest related to the contents of this article.

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