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Word count: 1091

Abstract: The pathogenic mechanisms of acquired focal and segmental glomerulosclerosis are only partially known and represent a medical challenge in nephrology. The article by May et al. sheds additional light on previous data indicating the key role of the protease activated receptor 1. The new evidence is based on *in vivo* studies in relevant animal models and on patient biopsies and represents a significant step forward in the understanding of this pathological condition.

Keywords: FSGS, glomerulopathies, glomerulus, podocyte, TRPC6, idiopathic nephrotic syndrome

Primary focal and segmental glomerulosclerosis (FSGS) is generally acknowledged as resulting from the presence of one of more pathogenic factors in circulation. The latter are hypothesized to be secreted by immune cells, and their identity remains to be confirmed. The canonical mechanism would require the interaction of the putative circulating factor with a receptor at the surface of podocytes, ultimately resulting in foot process effacement and dysfunction of the permeability barrier.

Harris et al showed in a seminal study that FSGS recurrence plasma was able to induce phosphorylation of VASP (vasodilator stimulated phosphoprotein) in both cultured podocytes and in human glomeruli freshly isolated from biopsies [1]. VASP is a key regulator of cytoskeleton actin polymerization. The authors found VASP phosphorylation abrogated by inhibition of plasma proteases, and this led to identifying the protease activated receptor 1 (PAR-1, not to be confused with suPAR, "soluble urokinase plasminogen activator receptor") activation as an upstream event to VASP phosphorylation and podocyte dysfunction. As a result PAR-1, which is activated through cleavage by serine proteases (figure), was identified as a mediator in the pathogenic mechanisms occurring in the podocyte in response to FSGS plasma. Likewise, VASP phosphorylation was suggested as a marker of FSGS disease activity.

The article by May et al. [2] explores further this hypothesis, and by thorough characterization of several animal models adds up *in vivo* relevance and additional mechanistic insight. Two mouse models have been used in the study: one is a transgenic mouse overexpressing PAR-1; the other one is a TRPC6 (Transient Receptor Potential Cation Channel Subfamily C Member 6) podocyte-specific knock out. TRPC6 is a calcium channel that has been involved in several glomerulopathies, including FSGS (reviewed in [3]). TRPC6, whose mutations are included among the causes of genetic FSGS, along with TRPC5 participates in the fine-tuning regulation of actin cytoskeleton dynamics [4] (figure). In the models studied in May's work, expression of a constitutive active form of PAR-1 induces proteinuria and glomerular sclerosis. This is prevented in animals where TRPC6 has been invalidated. Therefore, the

authors show this way a two wheeled mechanism gearing PAR-1 and TRPC6 at the center of podocyte injury in FSGS. This represents the main novelty of the study.

Unequivocally, these new findings further clarify the general picture of FSGS pathophysiology, and open direct implications in FSGS therapy. Hence, the same group states that the response of podocytes to nephrotic plasma is blocked by an approved PAR-1 inhibitor, which could become an interesting option in the treatment of recurrent FSGS. Likewise, PAR-1 inhibition has been suggested as kidney protective in other pathophysiological settings. Indeed, a very recent publication shows that PAR-1 invalidation protects against the transition to chronic kidney disease in a mouse ischemia-reperfusion model [5].

This additional evidence also prompts several questions. First, how specific of FSGS is the described mechanism? Specificity was already evoked in the seminal article by the same group, as plasma from HUS, ANCA vasculitis, and lupus nephritis patients did not induce the observed changes in cultured podocytes [2]. Nevertheless, it must be considered that PAR-1 mediated glomerular injury has also been described in crescentic glomerulonephritis [6], and in diabetic nephropathy [7]. Consequently, PAR-1 inhibition has been suggested as a potential therapeutic strategy in the former. An interesting result in the original work by Harris et al. was the fact that the effect of FSGS plasma on VASP phosphorylation was mimicked by puromycin in the absence of disease plasma and the putative factor [1]. Puromycin is a toxic a compound to the podocyte and is commonly used to induce *in vivo* FSGS in rodents. This strongly suggests that the described mechanism could be common to different types of podocyte injury. Yet, this does not undermine the therapeutic perspectives raised.

Second, still dealing with specificity, can it be assumed that the described mechanism is common to all primary FSGS cases? As said in the discussion of May's article their conclusions would help in the stratification of FSGS patients, which remains a prominent challenge [2]. In the same way as FSGS is a histological finding present in more than one disease, it could be hypothesized that post-transplant recurrence of FSGS may also correspond to different pathogenic processes. The suggested PAR-

1/TRPC6 mechanism was identified in all the biopsies included in the study. Although this suggests a general feature it could be present only in one subpopulation of recurrent FSGS patients. If the latter were the case, could recurrent FSGS patients be further stratified based on this? Assuming that the former option is true -a general idiopathic FSGS mechanism- then it could be argued that PAR-1 activation could be the response to one single circulating factor, or a convergent response to different factors. In all cases a study on a larger cohort would help answer the question.

Third, and following the previous reflection: is this a real opening to the identification of pathogenic circulating factors? The circulating factor hypothesis has prompted a rich body of research, but the results so far have been either partial or inconclusive and have not resulted in any therapeutic improvement to date. It could be argued that, if PAR-1 inhibition is enough to establish an efficient therapeutic protocol, then it might not be necessary to keep searching for elusive molecules in the circulation. The results by May et al. suggest circulating serine proteases as the main suspects, but the real mechanism could be much more complex than supposed, involving several sequential steps, diverse cell types and multiple molecules, including inhibitors. Here, the participation of a protease or a protein activating PAR-1 is strongly suggested, though this could represent the final link of a long chain of events. The authors suggest, based on their previous results, Th17 as the T cell population responsible for a factor secretion that would eventually result in eliciting a PAR1-mediated response in the podocyte [8]. This is a very reasonable and thrilling hypothesis to be further explored.

Finally, can this finding explain, at least in part, the result of decades of research on FSGS-inducing circulating factors? In general, concerning the candidate circulating pathogenic molecules reported to date, no direct link has been described between any of them and PAR-1 activation. Interestingly, TRPC6 membrane localization and function was found increased in podocytes by recurrent FSGS plasma and by the putative and controversial circulating factor suPAR [9].

In conclusion, confirmation of PAR-1 activation as an intermediate step of podocyte damage in recurrent FSGS, and its link to TRPC6-mediated regulation of cytoskeleton structure, highlights the way for further research and warrants a therapeutic strategy to be tested.

Disclosure: The authors declare no conflict of interest.

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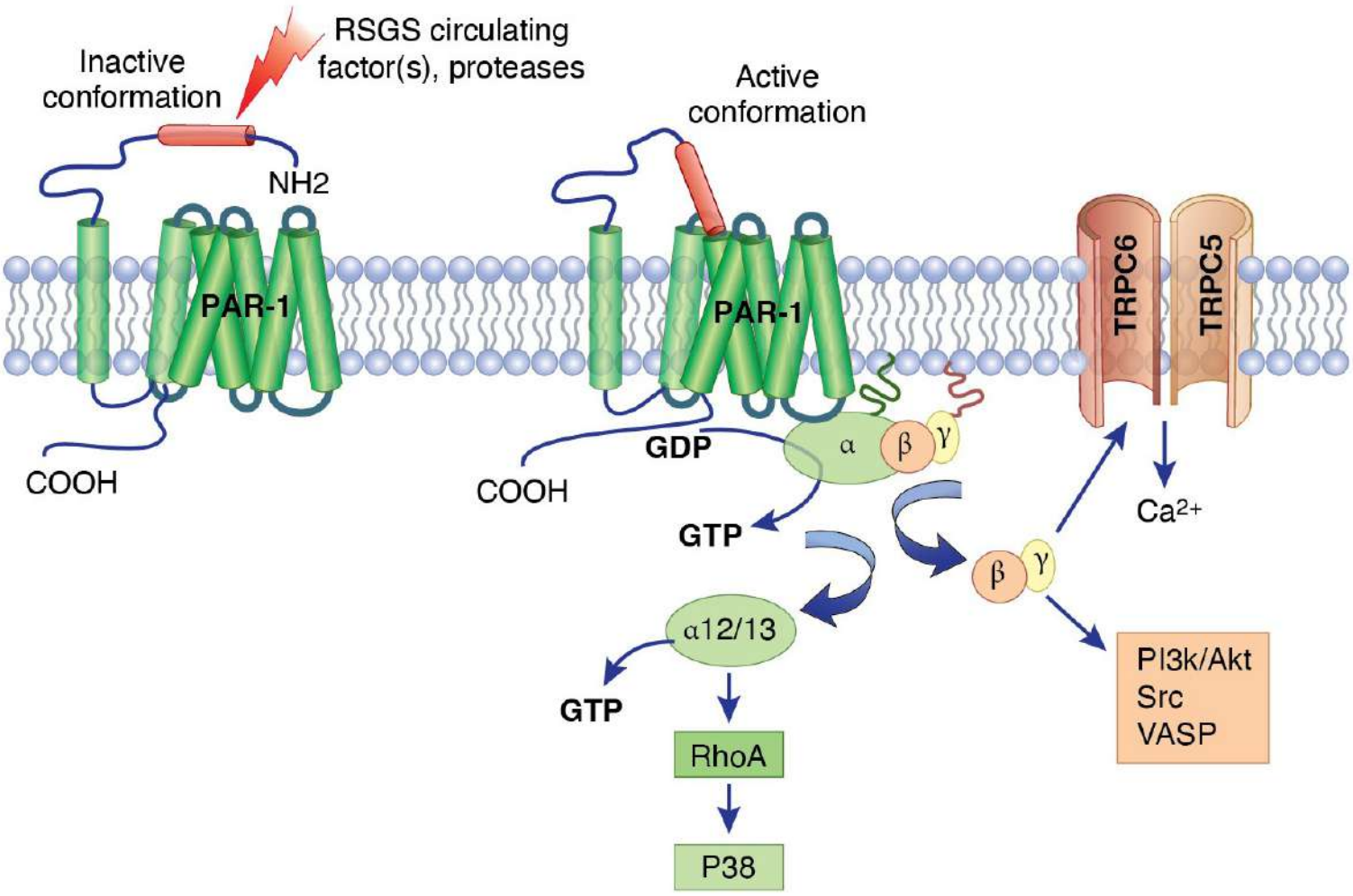
Figure legend

Activation and signaling by protease-activated receptor-1 (PAR1). PAR-1 is a seven transmembrane domain G protein-coupled receptor which is activated by ligand-mediated cleavage at the N-terminal site, generating a new N-terminal peptide that binds intramolecularly and initiates a conformational change, triggering activation of G proteins. The latter exchange guanosine diphosphate (GDP) for guanosine triphosphate (GTP), causing dissociation of the GTP-bound activated Ga from Gb/g subunits and initiate downstream signaling pathways. PAR-1 activates a variety of effectors involved in cell shape changes, cellular growth and motility, and secretion of vasoactive factors. The hallmark of this receptor family (PAR-1-4) is to generate distinct signaling responses through the activation of the same PAR (named-biased agonism or functional selectivity). This unique property could explain how different pathophysiological entities, including extracapillary proliferative glomerulopathies, Goodpasture syndrome, diabetes, FSGS, MCNS, are found associated with activated PAR-1 signaling in the glomeruli. The mechanism regulating functional selectivity seems related to cleavage at distinct N-terminal sites by agonists/proteases/FSGS circulating factor(s). Specifically, activation of PAR-1 by a putative FSGS factor triggers an Ca^{2+} influx through recruitment of the ion channel TRPC6/TRPC5, resulting in RhoA and p38 kinase activation.


RSGS circulating
factor(s), proteases
Inactive
conformation
NH2
PAR-1
COOH
Active
conformation
PAR-1
COOH
GDP
α
β
γ
GTP
α12/13
GTP
RhoA
P38
TRPC6
TRPC5
Ca2+
β
γ
Pl3k/Akt
Src
VASP