The EP4 Receptor for Prostaglandin E₂ in Glomerular Disease: A Good Receptor Turned Bad?

Matthew A. Sparks and Thomas M. Coffman
Division of Nephrology, Department of Medicine, Duke University and Durham VA Medical Centers, Durham, North Carolina

Prostaglandins generated by the metabolism of arachidonic acid through the cyclooxygenase (COX) pathway have diverse functions in health and disease.1 The kidney is prominent among the physiologic systems affected by these ubiquitous mediators. Along with effects to modulate renal blood flow, GFR, and sodium excretion, prostanooids affect the function of the glomerular filtration barrier. This is illustrated by clinical studies showing that nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit COX enzymes, attenuate the levels of proteinuria in patients with glomerular diseases.2,3 Because NSAIDs block production of all prostanooids, the specific prostanooids influencing glomerular function in this setting cannot be discerned. Moreover, because NSAIDs frequently cause adverse effects in patients with kidney disease, including acute deterioration in GFR and exacerbation of hypertension, this approach to antiproteinuric therapy has limited applications.

In experimental models of glomerular disease, a number of studies have indicated beneficial effects of more specific pharmacologic inhibitors that block individual prostanooid synthetic pathways or receptors. More recently, this issue has been explored using modern molecular genetics. For example, Harris and associates4,5 showed recently, this issue has been explored using modern molecular genetics. For example, Harris and associates4,5 showed that overexpression of the inducible COX isoform, COX2, in podocytes of transgenic mice enhances susceptibility to glomerular injury caused by adriamycin or puromycin. This was due, at least in part, to exaggerated production of thromboxane A₂ and activation of the thromboxane-prostanoid (TP) receptor.6 These findings are consistent with older studies showing efficacy of thromboxane synthase inhibitors and TP receptor antagonists in experimental models such as adriamycin nephropathy7 and murine lupus nephritis.8

In this issue of JASN, Stitt-Cavanagh et al.9 implicate another prostanooid pathway in glomerular disease: Prostaglandin E₂ (PGE₂) acting through its EP4 receptor. This finding is surprising from at least two perspectives. First, in the studies of Harris and associates, deletion of EP4 receptors from podocytes had no effect on proteinuria and kidney injury in mice also overexpressing COX2.6 Second, the EP4 receptor has long been considered to have beneficial and protective effects in kidney disease and hypertension.

Once COX and PGE synthases form PGE₂ through the successive metabolism of arachidonic acid, it elicits its biological effects through a family of G protein–coupled receptors. These receptors, by convention designated EP (for E-prostanoid) receptors, are divided into four distinct pharmacologic classes: EP1 through 4.10,11 The EP4 receptor signals through Gs proteins and adenylyl cyclase.11 Because of the linkage between enhanced formation of cAMP and relaxation of bronchial and vascular smooth muscle, the EP4 receptor has been classically considered a relaxant receptor.11 In the kidney, vasodilator actions of PGE₂, likely mediated by the EP4 receptor, are linked to protection of renal blood flow during states of compromise, such as congestive heart failure, volume depletion, or renal parenchymal disease. Inhibition of this protective, vasodilator pathway is an underlying mechanism of NSAID-associated acute renal failure.

To examine the role of EP4 receptors in glomerular disease, Stitt-Cavanagh et al.9 used two approaches. First, they generated transgenic mice (EP₄gpod⁺) expressing a modified version of the EP4 receptor in podocytes using nephrin-specific promoter. The 5/6 nephrectomy model of renal ablation was used to induce proteinuria and glomerular injury. After partial renal ablation, levels of proteinuria were increased fourfold and mortality was dramatically enhanced in the EP₄gpod⁺ mice. Although renal function was not measured, the extent of glomerular pathology in the EP₄gpod⁺ mice was also accelerated. Because there were no differences in BP, at least as measured by tail-cuff monometry, the authors attribute the worsening of glomerular injury to consequences of increased activation of EP4 receptors on podocytes. There are, however, some potential interpretive problems with this part of the study. First, because the EP₄gpod⁺ mice express a truncated version of the EP4 receptor that is resistant to agonist-induced desensitization, the specific applicability of these findings to the native EP4 receptor is not clear. In addition, concerns about potential nonspecific consequences

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Correspondence: Dr. Thomas M. Coffman, Duke University Medical Center, Room 2028 MSRB2, 106 Research Drive, Durham, NC 27710. Phone: 919-684-9788; Fax: 919-684-3011; E-mail: tcoffman@duke.edu

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of high levels of transgene expression in podocytes have been raised previously.\(^\text{12}\)

In view of these potential confounding features, the authors’ second experimental approach was more compelling. In this case, they used \textit{Cre-LoxP} technology to delete EP4 receptors specifically from podocytes, and the results nicely complemented the findings in the mice that overexpressed EP4. Proteinuria and glomerular pathology were reduced in mice lacking EP4 receptors only in podocytes, and these beneficial effects were achieved despite only partial excision of the floxed EP4 receptor gene locus.

In the context of previous studies,\(^\text{13}\) these findings suggest that a mechanistic pathway of increased expression of COX-2 in the injured glomerulus triggers local generation of PGE\(_2\) with activation of the EP4 receptor in podocytes, causing exaggerated proteinuria and glomerular damage. This pathway may work in parallel with enhanced thromboxane production and TP receptor activation.\(^8\)

The findings of Stitt-Cavanaugh \textit{et al.}\(^9\) raise several questions for future exploration and scrutiny. For example, which signaling pathways linked to the EP4 receptors are responsible for its action in glomerular disease? Other podocyte G protein–coupled receptors with established actions to enhance proteinuria, such as the AT\(_1\) angiotensin or TP receptors, couple to G\(_q\) proteins, whereas the EP4 receptor is linked to Gs and cAMP. Nonetheless, it is possible that cAMP–associated signaling may have distinct detrimental actions in podocytes. Conversely, stimulation of EP4 but not EP2 receptors leads to phosphorylation of the extracellular signal–regulated kinases through a phosphatidylinositol-3 kinase–dependent mechanism.\(^14,15\) In this regard, phosphatidylinositol-3 kinase plays a role in various pathologic disorders, including angiotensin II–dependent hypertension and end-organ damage,\(^16\) and might be expected to have similar detrimental consequences in podocytes.

The specific effects of EP4 receptors to influence the cell biology of the podocyte are also of potential interest. In a limited experiment, the authors suggested that enhanced expression of EP4 receptors in cultured podocytes affects their adaptation to mechanical stretch. This could be relevant because perturbation of the podocyte cytoskeleton is a common feature of the inherited glomerulopathies.\(^17\) While enhanced podocyte apoptosis is also identified as another key pathway in pathogenesis of proteinuria, studies of other models suggested that EP4 receptor activation actually promotes cell survival.\(^18,19\)

The final and perhaps most intriguing possibility raised by these observations is whether specific EP4 receptor antagonism could be a useful therapeutic strategy in glomerular diseases. There is an obvious need for new therapies in this area, and the actions of NSAIDs to reduce proteinuria indicate a potential capacity for this approach. The critical issue is whether an EP4 receptor antagonist could improve glomerular permeability defects without causing the adverse renal effects associated with global prostanooid inhibition by NSAIDs. Because of the putative role of EP4 receptors to mediate vasodilation in the renal circulation, this seems unlikely but could be readily tested in animal models using available small molecular EP4 receptor antagonists.

**DISCLOSURES**

None.

**REFERENCES**

VEGF Receptors and Glomerular Function

Samir M. Parikh and Martin R. Pollak
Division of Nephrology, Department of Medicine, Beth Israel Deaconess Medical Center, and Harvard Medical School, Boston, Massachusetts


In this issue of JASN, Sison et al.1 build on previous work from their group demonstrating the necessity of intact podocyte expression of vascular endothelial growth factor (VEGF) in preventing progressive glomerular endothelial damage and proteinuria in mice.2,3 This study adds to these findings by using compound transgenic mice to ask which glomerular cell and which VEGF receptors (VEGFRs) mediate this critical role of VEGF in maintaining normal glomerular function.1

With two major VEGFRs (R1 and R2) and three glomerular cell types (endothelium, mesangium, and podocyte), six possible combinations exist for creating tissue-specific knockouts of single VEGFRs. The authors narrow this considerable experimental complexity by observing no thrombotic microangiopathy in whole-body, VEGFR1-null mice. By contrast, whole-body, VEGFR2-null mice show renal changes mimicking thrombotic microangiopathy after excision of VEGF in the adult podocyte. The two most likely hypotheses to align these findings are (1) a paracrine effect of podocyte-secreted VEGF on mesangium or glomerular endothelium or (2) an autocrine effect whereby the podocyte secretes VEGF, which then activates VEGFR2 expressed by itself or nearby podocytes. Observing a lack of glomerular pathology in the podocyte-VEGFR2-null mouse, the authors then conclude, by process of elimination, that glomerular derangements arising from systemic VEGFR2 deletion are attributable to VEGFR2 expression in the glomerular endothelium (or possibly the mesangium) but not the podocyte. Although this line of reasoning leaves some room for dissent—for example, what role do the thyroid and liver derangements in the total-body VEGFR2 knockout play in the renal changes—the authors use two sensitive tools, a VEGFR2-reporter mouse and quantitative PCR, to assert further that VEGFR2 is not even expressed at appreciable levels in murine podocytes in vivo.

Examples of autocrine VEGF action exist in endothelium and hematopoietic stem cells,4,5 but the more conventional observation is that VEGF is produced by perivascular mural cells (vascular smooth muscle cells and pericytes) and provides a local trophic signal for nearby endothelial cells expressing VEGFR2. In this context, VEGFR2 is considered a modulatory receptor because it avidly binds VEGF but is phosphorylated only weakly.6,7 Endothelial cells, in turn, secrete pro-survival mitogens, such as PDGF, to recruit support cells and aid their survival. Indeed, global deletion of either PDGF-β or its receptor results in rudimentary glomeruli lacking mesangial cells.8,9 Although the identity of endothelium-derived, podocyte-supportive factors remains a mystery, this model posits a remarkable symbiosis in which glomerular endothelia and their support cells are engaged in a tonic molecular dialogue to maintain the unique microanatomy necessary for efficient filtration.

Evidence against an autocrine VEGF signaling loop in podocytes in vivo is at odds with previous data from independent groups that used cultured human and mouse podocytes.10–12 Of those reports, only one identified VEGFR2,10 whereas the other two implicated VEGFR1 as the critical receptor for VEGF responsiveness.11,12 Sison et al.1 note unpublished data that their total-body VEGFR1-null mouse did not copy the phenotype of podocyte-VEGFR2 knockouts and use this observation to support the idea that VEGFR2, not VEGFR1, is essential for VEGF signaling in the glomerulus.

The authors next show that excess podocyte-derived VEGF is also injurious to the filtration barrier, repriming their previous findings2 but with more detailed kinetics. These data are also consistent with a more recent report from a different group.13 Thus, it is probably safe to conclude that either deficient or excess podocyte-derived VEGF is bad for the filtration barrier. Both perturbations lead to proteinuria but by dichotomous pathobiological routes. Loss of VEGF leads to endothelial swelling and formation of microthrombi, hallmarks of endothelial injury. Conversely, excess glomerular VEGF induces a hypertrophic phenotype characterized by glomerular basement membrane thickening, accumulation of mesangial matrix, and, possibly, inflammatory infiltrates. Just as the proteinuria after bevacizumab therapy14 or soluble VEGF receptor 1 elevation15 seems to recapitulate intraglomerular VEGF depletion, it is attractive to speculate that sclerotic renal diseases characterized by high matrix deposition may, too, have roots in local VEGF imbalance.16,17


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