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Collecting Duct Pro(Renin) Receptor Contributes to Unilateral Ureteral Obstruction-Induced Kidney Injury via Activation of the Intrarenal RAS

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Abstract

Background: Although the concept of the intrarenal renin-angiotensin system (RAS) in renal disease is well-described in the literature, the precise pathogenic role and mechanism of this local system haven't been directly assessed in the absence of confounding influence from the systemic RAS. The present study employed novel mouse models of collecting duct (CD)-specific deletion of (pro)renin receptor (PRR) or renin together with pharmacological inhibition of soluble PRR (sPRR) production to unravel the precise contribution of the intrarenal RAS to renal injury induced by unilateral ureteral obstruction (UUO).

Methods: We examined the impact of CD-specific deletion of PRR (CD PRR KO), CD-specific deletion of renin (CD renin KO) and site-1 protease (S1P) inhibitor PF429242 treatment on renal fibrosis and inflammation and the indices of the intrarenal RAS in a mouse model of UUO.

Results: After 3 days of UUO, the indices of the intrarenal RAS including the renal medullary renin content, activity and mRNA expression, and angiotensin II (Ang II) content in obstructed kidneys of floxed mice were all increased. That effect was reversed with CD PRR KO, CD renin KO, and PF429242 treatment, accompanied with consistent improvement in renal fibrosis and inflammation. On the other hand, renal cortical renin levels were unaffected by UUO, irrespective of the genotype. Similar results were obtained via pharmacological inhibition of S1P, the key protease for the generation of soluble PRR (sPRR).

Conclusions: Our results reveal that PRR/sPRR-dependent activation of CD renin represents a key determinant of the intrarenal RAS and thus obstruction-induced renal inflammation and fibrosis.

Introduction

Chronic kidney disease (CKD) is increasingly recognized as a major public health problem worldwide, leading to end-stage renal disease (ESRD) and increased cardiovascular

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Disclosures

None.

morbidity and mortality. The incidence of ESRD worldwide represents a growing clinical and economic burden[1]. Renal tubulointerstitial damage is a common feature of all chronic progressive renal diseases, a final common pathway mediating progression from CKD to ESRD[2]. Additionally, there is a growing body of evidence indicating that that CKD is accompanied by inflammatory responses in the kidney, characterized by increased inflammatory cytokines, infiltration of inflammatory cells and accumulation of myofibroblasts. Those conditions can in turn lead to tubulointerstitial injury and kidney fibrosis[3]. However, the molecular mechanisms underlying the initiation of inflammatory responses in CKD are poorly understood. What is known is that the inflammatory response results from a highly coordinated network involving multiple cell types: activated macrophages, monocytes, and other cells mediate local responses to tissue damage and infection[4]. Renal tubular epithelial cells are likely important promoters of renal inflammation by secreting a variety of inflammatory cytokines in response to both immune and non-immune factors[5]. Traditionally, the renal collecting duct (CD) plays an important role in the control of fluid and electrolyte homeostasis[6]. However, increasing evidence suggests a potential role of CD cells in interstitial fibrosis. For example, the CD cells may function as sentinel cells that can sense danger signals and promote inflammasome activation[7]. In addition, CD cells actively recognize uropathogenic bacteria, such as Escherichia coli, and triggers the innate immune system as part of the anti-bacterial defense response[8]. Furthermore, the transcription factor, Krüppel-like factor-5, is mainly expressed in CD cells critically involved in inflammatory responses to unilateral ureteral obstruction (UUO)[9]. Whether or how CD cells contribute to the initiation and progression of tubulointerstitial inflammation and fibrosis warrants further investigation.

The renin–angiotensin system (RAS) plays a crucial role in the pathogenesis of CKD[10]. Apart from this well-known systemic RAS, the intrarenal RAS is also well known to contribute to the pathogenesis of CKD[11]. Renin is primarily synthesized in the juxtaglomerular (JG) cells, whereas increasing evidence demonstrates that renin is also synthesized and secreted by the CD principal cells as a major component of the intrarenal RAS[12–14]. (Pro)renin receptor (PRR) was also considered as an important component of the intrarenal RAS by facilitating the conversion of angiotensinogen to angiotensin I[15]. Within the kidney, PRR expression is predominantly expressed on the apical membrane of CD intercalated cells albeit with lower expression in a few other cell types[16]. A large body of experimental evidence demonstrates that CD PRR plays a critical role in physiological regulation of fluid and electrolyte homeostasis as well as the pathogenesis of hypertension[17–19]. The soluble PRR (sPRR) is produced by protease-mediated cleavage of PRR and is elevated under certain pathological conditions[20,21]. Increasing evidence suggested that sPRR exerts a series of biological functions[22–24]. Two independent studies using CRISP-Cas9 strategy to mutagenize the cleavage site of PRR to abrogate Ang II-induced hypertension in mice[25,26]. However, the mechanism of action of sPRR, particularly during kidney disease, remains incompletely understood. Previous studies have found that sPRR can bind with renin or prorenin to activate the tissue RAS[27]. However, whether sPRR regulates CD renin and intrarenal RAS in CKD remains unknown.

Evidence is available to suggest a potential role of the intrarenal RAS in pathogenesis of renal disease[28,29]. However, the precise intrarenal sites of RAS activation during renal injury

and the detailed regulatory mechanisms still largely remain elusive. Furthermore, due to the confounding influence from the systemic RAS, the precise contribution of the intrarenal RAS to renal disease needs to be determined by functional studies, particularly those using mice with genetic manipulations of RAS components in a tissue-specific manner without perturbing the systemic RAS[29]. Herein, we addressed this issue by using novel mouse models of CD-specific deletion of PRR and renin, the two key components of the intrarenal RAS. We further examined the role of sPRR by using a S1P inhibitor PF429242.

Materials and methods

Data availability.

All supporting data and detailed methods including animal preparation, ELISA, histology, Western blot, immunostaining, and qRT-PCR are available within the article and its Supplemental Material.

Statistical analysis.

Data are summarized as means ± SEM. Prespecified hypotheses were proposed before the start of the experimental series. The sample size was determined by power analysis. Two-way ANOVA, followed by Bonferroni correction, was used to analyze means between more than two groups, and unpaired Student's t-test was used to analyze when two groups were present. GraphPad Prism 6 software was used for statistical analyses. $p < 0.05$ was considered statistically significant.

Results

CD PRR KO attenuates renal fibrosis and inflammation in obstructed kidneys.

Unilateral ureteral obstruction (UUO) is associated with interstitial fibrosis and inflammatory response in obstructed kidneys. According to a previous study[30], early renal fibrosis can be observed in UUO rats from 3 days after UUO surgery. To investigate the effect of CD PRR on early stage of renal fibrosis induced by UUO, we subjected floxed and CD PRR KO mice to UUO and analyzed the phenotype at day 3. We examined renal fibrosis by detecting the protein expression of fibrosis makers in obstructed kidneys. As shown in Figure 1A–C, fibronectin (FN) and α-smooth muscle actin (α-SMA) protein expression assessed by Western blotting analysis increased in the obstructed kidneys of floxed mice compared with sham controls. Along this line, the improvement of obstruction-induced fibrosis by CD PRR KO was also validated by measurement of hydroxylproline (Figure 1D) and Masson's trichrome analysis of the tubulointerstitial lesions (Figure 1E&F). By qRT-PCR, the mRNA expression of several fibrosis/sclerosis-related genes including FN, α-SMA, collagen I (COL I), collagen III (COL III) and transforming growth factor-β (TGF-β) in the whole kidneys was all increased in the obstructed kidneys of floxed mice. In contrast, the protein or mRNA expression of the above-mentioned fibrotic markers was all blunted in the obstructed kidneys of CD PRR KO mice ($p < 0.05$) (Figure S1A–E). Similar results were obtained when kidney sections were immunostained for COL I (Figure S1F). Meanwhile, CD PRR KO blunted the elevated plasma TGF-β1 level induced by UUO (Figure S1G). CD PRR KO also blunted the increase of interleukin 6 (IL-6) mRNA expression in obstructed

kidneys ($p < 0.05$) (Figure S1H). Besides, we also detected mRNA levels of FN, α-SMA, COL I, COL III, TGF-β and IL-6 in the renal medulla and cortex in floxed and CD PRR KO mice with UUO (Figure S6). We found that CD PRR KO similarly attenuated renal medullary and cortical mRNA expression of the inflammation and fibrosis markers in the obstructed kidneys. Taken together, these results strongly suggest that activation of CD PRR promoted renal fibrosis and inflammation induced by UUO.

CD PRR KO inhibited the activation of the intrarenal RAS of obstructed kidneys.

Increasing evidence suggests that CD PRR functions as an important regulator of the intrarenal RAS to control fluid and electrolyte homeostasis as well as Ang II-induced hypertension[17–19]. We therefore examined a possibility that CD PRR may determine activation of the intrarenal RAS in the UUO model. As shown in Figure 2A&B, renal medullary PRR expression was increased in obstructed kidneys of floxed mice, which was blunted by CD PRR KO. Furthermore, CD PRR KO significantly decreased renal medullary renin mRNA expression ($p < 0.05$) (Figure 2C) and renin activity ($p < 0.05$) (Figure 2D) and active renin content ($p < 0.05$) (Figure 2E) in obstructed kidneys. However, CD PRR KO had no effect on the renal cortical renin mRNA (Figure S2A), renin activity (Figure S2B), active renin content (Figure S2C) in obstructed kidneys. These results indicated that CD PRR acts via the intrarenal RAS to promote renal fibrosis in the UUO model.

Effects of PF429242 on renal fibrosis and inflammation in obstructed kidneys.

Our previous study demonstrated that site-1 protease is required for the generation of sPRR[20]. Circulating sPRR is an important biomarker of CKD[31,32]. To test whether sPRR mediates profibrotic action of CD PRR, we administered PF429242 to C57/BL6 mice with UUO. Plasma sPRR was increased in UUO mice and this increase was blocked by PF429242 treatment ($p < 0.05$) (Figure 3A). The content of renal hydroxylproline in obstructed kidneys were increased as compared with sham controls and this increase was blunted by PF429242 treatment ($p < 0.05$) (Figure 3B). We further examined renal fibrosis by determining the protein expression of fibrosis makers in the kidney. As shown in Figure 3C–E, FN and α-SMA protein expressions were increased in obstructed kidneys compared with sham kidney. PF429242 treatment prevented the increase of α-SMA and FN protein expression. In addition, the mRNA expression of FN, α-SMA, Col I, Col III, TGF-β and IL-6 was upregulated in obstructed kidneys. PF429242 treatment induced a dramatic suppression of expression of all of these genes except for Col III ($p < 0.05$) (Figure 3F). One can conclude that inhibition of endogenous sPRR production by PF429242 attenuates renal fibrosis and inflammation in obstructed kidneys.

Effects of PF429242 on the activation of the intrarenal RAS in obstructed kidneys.

Previous studies demonstrated that sPRR increased renin activity of prorenin in vitro[33]. We therefore examined the possibility that sPRR may control the activity of intrarenal RAS in obstructed kidneys. As shown in Figure 4A, renal medullary sPRR was increased in obstructed kidneys, which was blocked by PF429242 ($p < 0.05$). PF429242 blocked the obstruction-induced increases of renal medullary renin mRNA level (Figure 4B), renin activity (Figure 4C), active renin activity (Figure 4D) and Ang II content (Figure 4E) ($p < 0.05$). However, PF429242 has no effect on renal cortical renin mRNA level (Figure S3A),

renin activity (Figure S3B) and active renin content (Figure S3C). Interestingly, PF429242 blocked the increase of renal cortical Ang II content (Figure S3D) in obstructed kidneys. We conclude that sPRR promotes the activation of the intrarenal RAS in obstructed kidneys.

CD renin KO attenuates renal fibrosis and inflammation in obstructed kidneys.

The above-described results demonstrated that CD PRR KO or S1P inhibition attenuated obstruction-induced renal fibrosis and inflammation associated with suppressed renal medullary renin levels. Therefore, we speculated that the pathogenic role of CD PRR/sPRR in the UUO model might be mediated by CD renin. To address this possibility, we subjected CD renin KO mice and their floxed controls to UUO for 3 days and analyzed the resulting renal fibrotic and inflammatory phenotype. As shown in Figure 5A–C, the protein expression of FN and α-SMA was markedly increased in obstructed kidneys of floxed mice and this increase was significantly inhibited in CD renin KO mice. qRT-PCR confirmed similar patterns of changes in the mRNA expression of FN (Figure 5D) and α-SMA (Figure 5E) and a few other fibrosis markers such as COL I (Figure 5F), COL III (Figure S4A), and TGF-β1 (Figure 4SB). Similar results were obtained when kidney sections were immunostained for COL I (Figure 4SC). Meanwhile, CD renin KO reduced the elevated plasma TGF-β1 level in mice with UUO (Figure S4D). Furthermore, attenuation of obstruction-induced fibrosis in CD renin KO mice was validated by Masson's trichrome analysis of the tubulointerstitial lesions (Figure S4F&G) and measurement of hydroxylproline (Figure S4E). In addition to renal fibrosis, UUO features heightened inflammatory responses. As shown in Figure S4H, changes in renal mRNA expression of IL-6 followed the pattern of renal fibrosis. Together, these results suggest that CD renin KO largely recapitulates the phenotype of CD PRR KO as well as the effect of PF429242 in the UUO model.

CD renin KO blocked obstruction-induced activation of the intrarenal RAS.

The RAS is known to play a role in regulation of the hemodynamic parameters and tubular function in the UUO model[34]. The intrarenal RAS has been shown to contribute to the pathogenesis of CKD induced by UUO. To determine the role of CD renin in obstruction-induced activation of the intrarenal RAS, we examined mRNA expression of renin (Figure 6A and Figure 5SA), renin activity (Figure 6B and Figure 5SB) and active renin content (Figure 6C and Figure 5SC) in the renal medulla and cortex among the four groups. In floxed controls, obstruction induced robust increases in renal medullary renin mRNA expression (Figure 6A), renin activity (Figure 6B) and active renin content (Figure 6C), and Ang II content (Figure 6D), contrasting to no change in renin mRNA expression and renin activity, and modest elevations of renin content and Ang II content in the renal cortex (Figure S5A–D). As expected, CD renin KO reduced baseline renin level in the renal medulla but not the renal cortex. CD renin KO remarkably blocked obstruction-induced activation of renal medullary renin at all levels without a significant effect on renal cortical renin (Figure 6A–D and Figure 5SA–D). These results suggest that CD renin is an essential determinant of the intrarenal RAS in the UUO model.

Discussion

The concept about the role of the intrarenal RAS in renal disease is supported by a large number of clinical and animal studies[28,29,35–37]. However, the majority of aforementioned studies are correlative or reliant on systemic approaches that confoundingly influenced the systemic RAS. The intrarenal RAS plays is known to play an important role in cellular homeostasis in the kidney. Local RAS activation and its components lead to structural and functional changes in renal cells[38]. To the best of our knowledge, the present study is the first to definitely address this issue by using novel mouse models of CD-specific deletion of renin and its receptor PRR, the two key components of the intrarenal RAS, in the UUO model. We consistently observed similar levels of attenuation of obstruction-induced renal fibrosis and inflammation in the two strains of null mice, paralleled with suppressed activation of the intrarenal RAS. We provide further *in vivo* evidence to support sPRR as an important mediator of PRR in the UUO model.

Experimental evidence is available to demonstrate the presence of prorenin and renin in the CD principal cells[13,14]. Furthermore, the expression of CD renin is elevated by Ang II treatment *in vitro* and *in vivo*[39] and it is also upregulated in 2-Kidney, 1-Clip Goldblatt hypertensive rats[40]. Functional evidence suggests that activation of CD renin contributes to Ang II-induced hypertension[41]. However, less is known about the potential role of CD renin in renal disease. In the present study, we found that renal medullary renin expression and activity were increased but renal cortical renin expression and activity remained unchanged in obstructed kidneys of mice with UUO for 3 days. Figueroa SM et al. showed that UUO modestly (approximately 10%) downregulated renal medullary PRR expression, which is inconsistent with our observation of UUO-induced upregulation of renal medullary PRR expression[42]. The reason for this discrepancy is unknown but could be related to differences in durations of UUO or other aspects of the experimental protocols. The durations of UUO were 7 days in this study but 3 days in ours. These results may suggest a time-dependent regulation of renal medullary PRR expression in UUO with a rapid induction at day 3 and a downregulation thereafter. Future studies are needed to fully evaluate the time course of renal medullary PRR expression during UUO and the underlying mechanism.

CD renin KO blocked the increase of renal medullary Ang II content and intrarenal RAS activation, which likely confers renoprotection against renal inflammation and fibrosis. These results indicated that renal CD is involved in kidney injury induced by UUO and CD renin contributed to this process via mediating activation of intrarenal RAS.

In the present study, we found that CD PRR KO prevented the increase of renal medullary renin expression, renin activity and active renin content and attenuated kidney injury in obstructed kidneys. These results represented strong *in vivo* evidence for a role of CD PRR in the regulation of intrarenal RAS in obstructed kidney following UUO. Consistent with this notion, a large number of previous studies favor PRR as a potential regulator of the intrarenal RAS during Ang II-induced hypertension as well as renal disease[43–47]. However, under some circumstances, PRR/sPRR can act in renin-independent manner. For example, activation of PRR/sPRR contributes to pathogenesis of renin-independent hypertension and renal injury induced by deoxycorticosterone-salt (DOCA-salt)[48] or aldosterone-salt

treatment[49]. Along this line, evidence is available to suggest that PRR may serve as an amplifier of Wnt/ β-Catenin signaling in kidney injury, which involves vacuolar H^+-ATPase activity but not renin[36].

Evidence is emerging to support the pleiotropic roles of S1P-derived sPRR in renal handling of electrolytes and fluid, renal pathophysiology, as well as insulin sensitivity and energy metabolism[20,50]. In particular, circulating sPRR levels have been shown to be inversely correlated with the estimated glomerular filtration rate in patients with CKD caused by hypertension and diabetes[51]. Our earlier study additionally found that sPRR promotes the fibrotic response in renal proximal tubule epithelial cells *in vitro* via the Akt/beta-catenin/Snail signaling pathway[52]. However, the *in vivo* role of sPRR in fibrosis response of CKD model remains unknown. More importantly, whether sPRR contributes to renal fibrosis in CKD model by regulating CD renin and intrarenal RAS hasn't been directly tested by any prior study. In the present study, we found that circulating and renal medullary sPRR was increased in UUO mice, which was blocked by PF429242 treatment. This result supports the idea that S1P is a major PRR cleavage enzyme during UUO. Furthermore, obstruction-induced sPRR appears to mainly derive from the CD. We noted that PF429242 inhibited the increase of renal medullary renin and attenuated renal inflammation and fibrosis. There results provided strong *in vivo* evidence that sPRR contributed to the initiation and progression of CKD through the intrarenal RAS.

The renal CD played an important role in the control of fluid and electrolyte homeostasis. However, increasing evidence suggests a potential role of CD in the development of renal tubulointerstitial fibrosis in response to tubular injury[53]. Chassin et al. reported that CD epithelial cells actively recognize uropathogenic bacteria, such as Escherichia coli, and initiate inflammatory responses, indicating the role of antibacterial defenses of kidney CD epithelial cells[54]. Similarly, Fujiu et al. showed that the transcription factor, Krüppel-like factor-5, whose expression is mainly found in CD epithelial cells, is essential for inflammatory responses to UUO[9].

Accumulating evidence indicates that inflammation in the absence of pathogens, also called sterile inflammation, is mediated through the inflammasome, a large cytosolic multiple protein complex regulating proinflammatory cytokine IL-1β production[55]. The inflammasome contains NOD-like receptors (NLRs) associated with apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), which recruits caspase-1 and induces its activation. Caspase-1 then processes pro-IL-1β into its mature form IL-1β, causing inflammation and tissue damage. Komada et al. reported that ASC in CD epithelial cells contributed to inflammation and injury after UUO[56]. In agreement with these studies, we demonstrated that activation of CD renin/PRR determines activity of the intrarenal RAS to induce obstruction-induced tubulointerstitial inflammation and fibrosis. Besides, our previous study found direct renin inhibitor aliskiren preventing NLRP3 inflammasome activation in obstructed kidneys[57]. This may indicate that CD renin/PRR contributes to UUO-induced kidney injury via intrarenal RAS-NLRP3 inflammasome activation.

Given the fact that PRR and renin are localized to intercalated and principal cells in the CD, respectively, it seems reasonable to speculate that sPRR may mediate the communication

between the two cell types and that sPRR derived from intercalated cells acts in a paracrine fashion to regulate renin expression/activity in the neighboring principal cells. We have previously shown that sPRR signals via β-catenin-signaling to upregulate aquaporin-2 expression in the CD cells[22]. Future investigation is needed to test whether β-catenin signaling is responsible for the renin-activating action of sPRR during UUO.

Until the very recent development of sodium-glucose cotransporter-2 (SGLT2) inhibitors, the anti-RAS regimen was almost the only therapy for the past several decades to slow the CKD progression as evidenced by the wide use of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) for treatment of this disease[58]. Nearly 3 decades ago, clinical trials demonstrated that ACE inhibitor slows the onset of renal complications in diabetic patients by 50%[59]. The benefit of losartan (RENAAL trial)[60] and irbesartan (IDNT trial)[61] in patients with type 2 diabetes and albuminuric CKD has been demonstrated to reduce the risk of renal failure. However, the anti-RAS regimen fails to halt the CKD progression and is also limited by common side effects of hyperkalemia and acute decline in renal function among others[62,63]. The current study fills the knowledge gap by elucidating a novel mechanism of PRR/sPRR-dependent activation of the intrarenal RAS in the pathogenesis of obstruction-induced renal fibrosis. We anticipate that targeting components of this pathway may be advantageous over existing RAS inhibitors in terms of efficacy and safety profiles. Inhibiting the intrarenal RAS, the root cause of CKD progression may be more effective and also may be devoid of the toxicity seen with non-selective anti-RAS therapy. Preclinical studies have demonstrated that administration of a PRR decoy peptide PRO20 protects against renal injury induced by cyclosporin A[64], albumin overload[46], adriamycin[43], 5/6 nephrectomy[47] and angiotensin II infusion[17] associated with suppressed intrarenal RAS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

CD	Collecting duct
CD PRR KO	CD-specific deletion of PRR
CD renin KO	CD-specific deletion of renin
RAS	The renin-angiotensin system
UUO	Unilateral ureteral obstruction
PRR	(Pro)renin receptor

sPRR	Soluble PRR
CKD	Chronic kidney disease
FN	Fibronectin
α-SMA	Alpha smooth muscle actin
Col I	Collagen I
Col III	Collagen III
TGF-β1	Transforming growth factor-β1
IL-6	Interleukin-6
S1P	Site-1 protease

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Perspectives

Renal tubulointerstitial fibrosis is a final common pathway mediating progression from CKD to ESRD. There is unmet need in understanding the molecular mechanism and developing novel therapeutics to treat this disease. Although anti-RAS therapies are widely used for the management of CKD and hypertension, they are limited by the partial efficacy and the class toxicities of hyperkalemia and acute decline of renal function. The present study for the first time applied the UUO model to CD PRR KO and CD renin KO mice together with pharmacological inhibition of S1P to demonstrate an important role of interaction between PRR/sPRR and renin in the distal nephron in promoting renal fibrosis. Targeting this local mechanism may hold promise in treating this devastating disease.

Pathophysiological Novelty and Relevance

What Is New?

- The present study is the first to demonstrate that overactivation of CD PRR to contribute to obstruction-induced renal fibrosis.
- The pathogenic role of CD PRR in the UUO model largely relies on the intrarenal but not systemic renin and via releasing sPRR.
- The CD is well-known to regulate fluid and electrolyte balance but not traditionally thought to play a role in renal fibrosis.

What Is Relevant?

- Non-specifically targeting the RAS in CKD is only partially effective and also limited by class toxicities of hyperkalemia and an acute decline in renal function. Therefore, there is unmet need in understanding the molecular mechanism and developing more effective and safer therapies for this disease.
- Our results shed light on the interaction between PRR/sPRR and renin in the distal nephron as a key mechanism of renal fibrosis and thus as a potential therapeutic target for development of renoprotective and anti-fibrotic therapies.

Clinical/Pathophysiological Implications?

- Despite the recognized benefits of anti-RAS modality and several other renoprotective therapies, currently CDK treatment still remains a clinical challenge.
- PRR/sPRR interact with renin in the CD, representing a key driver of the intrarenal RAS to mediate obstruction-induced renal inflammation and fibrosis.

The local mechanism in the CD may help design future therapies for treatment of CKD.

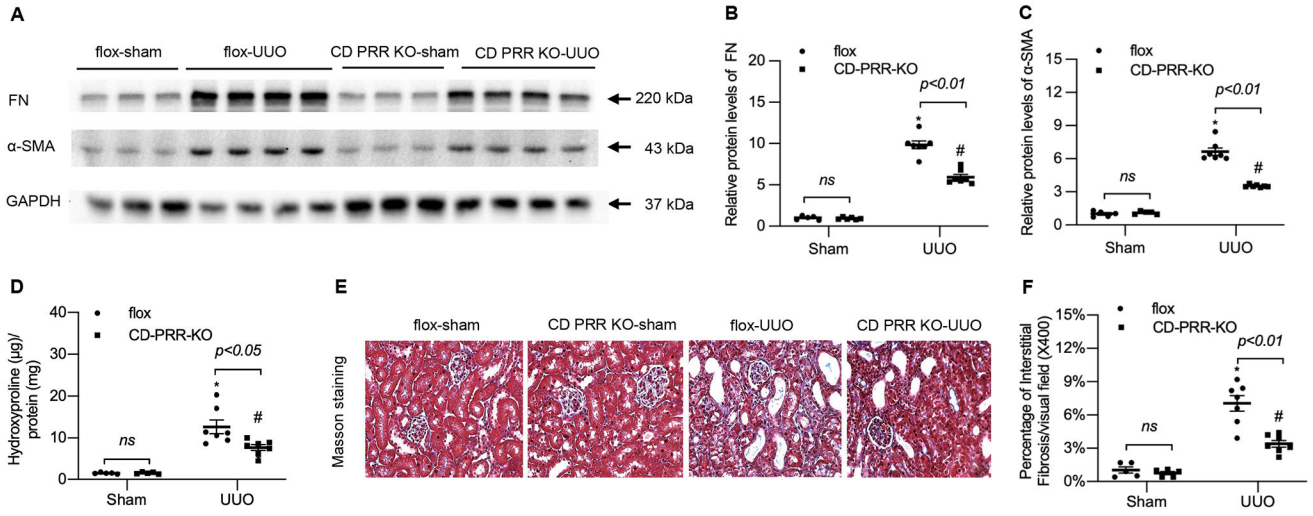

Figure 1.

Effect of CD PRR KO on renal fibrosis and inflammation in obstructed kidneys of mice with UUO. (A-C) Immunoblotting analyses of FN and α-SMA in the kidney. (D) Renal hydroxyproline content. (E) Masson staining. (F) Percentage of interstitial fibrosis/visual field (×400). *, $p < 0.05$ vs. Sham group; #, $p < 0.05$ vs. flox-UUO group. flox-Sham: n = 5; flox-UUO: n = 7; CD PRR KO-Sham: n = 5; CD PRR KO-UUO: n=7. Values are means ± SEM.

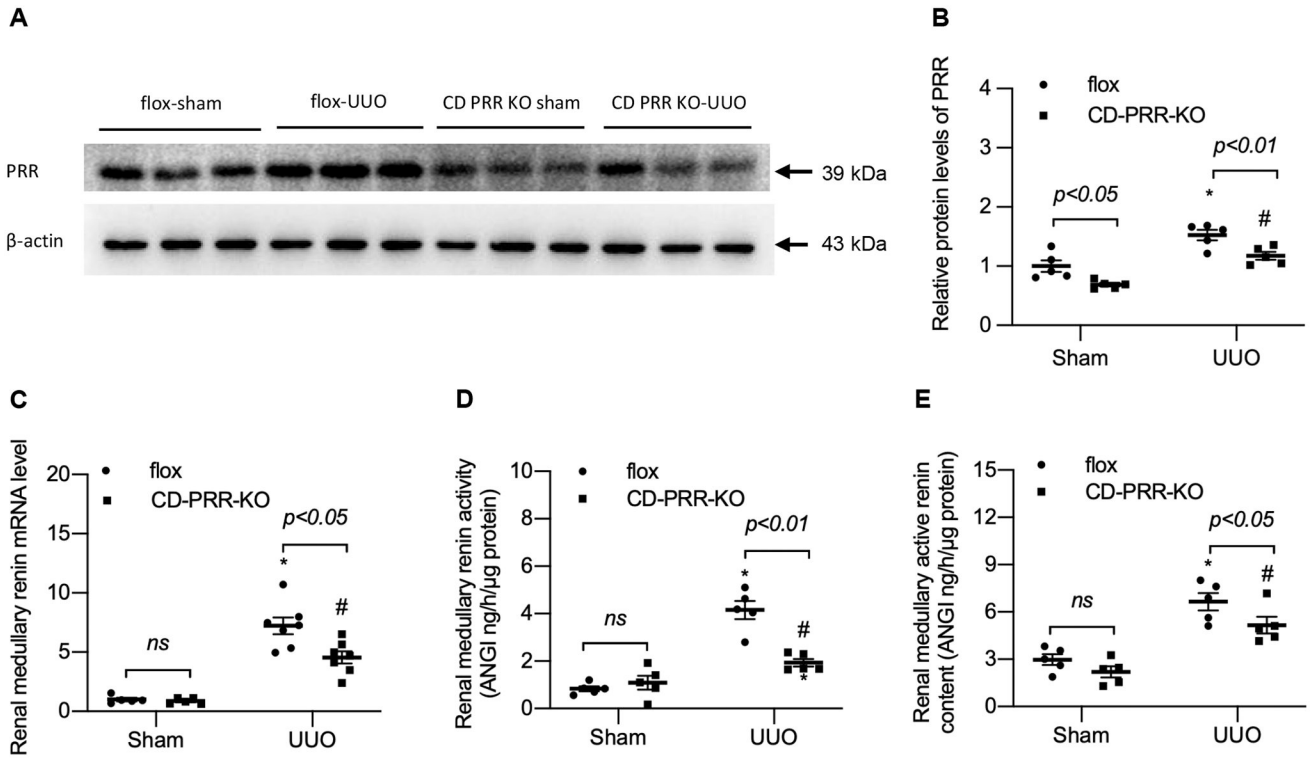

Figure 2.

Effect of CD PRR KO on the activation of the intrarenal RAS of the obstructed kidneys. (A-B) Immunoblotting analyses of renal medullary PRR. (C) mRNA expression of renal medullary renin. (D) Renal medullary renin activity. (E) Renal medullary active renin content. *$p < 0.05$ vs Sham group; # $p < 0.05$ vs flox-UUO group. flox-Sham: n = 5; flox-UUO: n = 5; CD PRR KO-Sham: n = 5; CD PRR KO-UUO: n = 5. Values are means ± SEM.

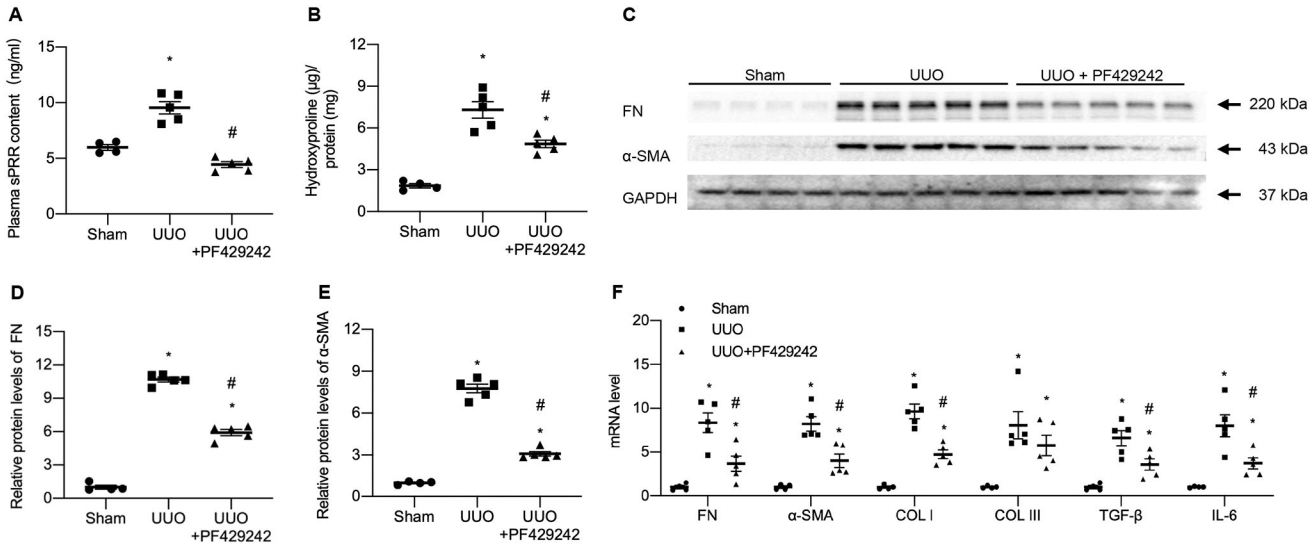

Figure 3.

Effects of PF429242 on renal fibrosis and inflammation in obstructed kidneys. (A) Plasma sPRR content. (B) Renal hydroxyproline content. (C-E) Immunoblotting analyses of FN and α-SMA in the kidney. (F) mRNA level of FN, α-SMA, COL I, COL III, TGF-β, and IL-6. *$p < 0.05$ vs Sham group; # $p < 0.05$ vs UUO group. Sham: n = 4; UUO: n = 5; UUO+PF4292942: n = 5. Values are means ± SEM.

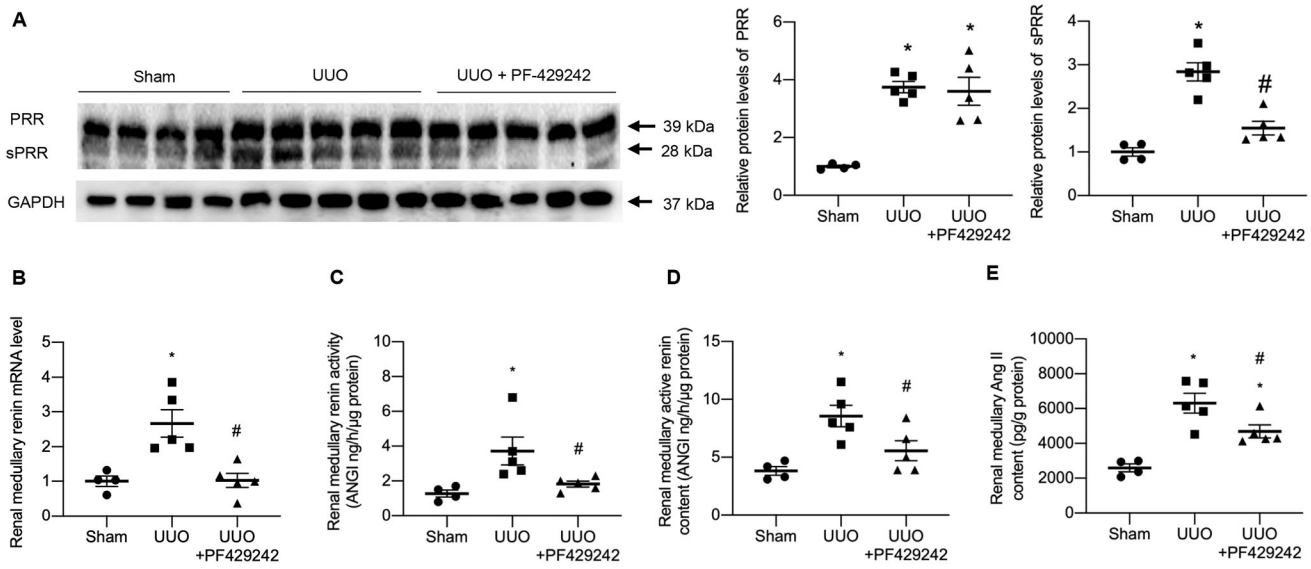

Figure 4.

Effects of PF429242 on the activation of intrarenal RAS in obstructed kidneys. (A) Immunoblotting analyses of renal medullary PRR/sPRR. (B) mRNA expression of renal medullary renin. (C) Renal medullary renin activity. (D) Renal medullary active renin content. (E) Renal medullary Ang II content. *$p < 0.05$ vs Sham group; # $p < 0.05$ vs UUO group. Sham: n = 4; UUO: n = 5; UUO+PF4292942: n = 5. Values are means ± SEM.

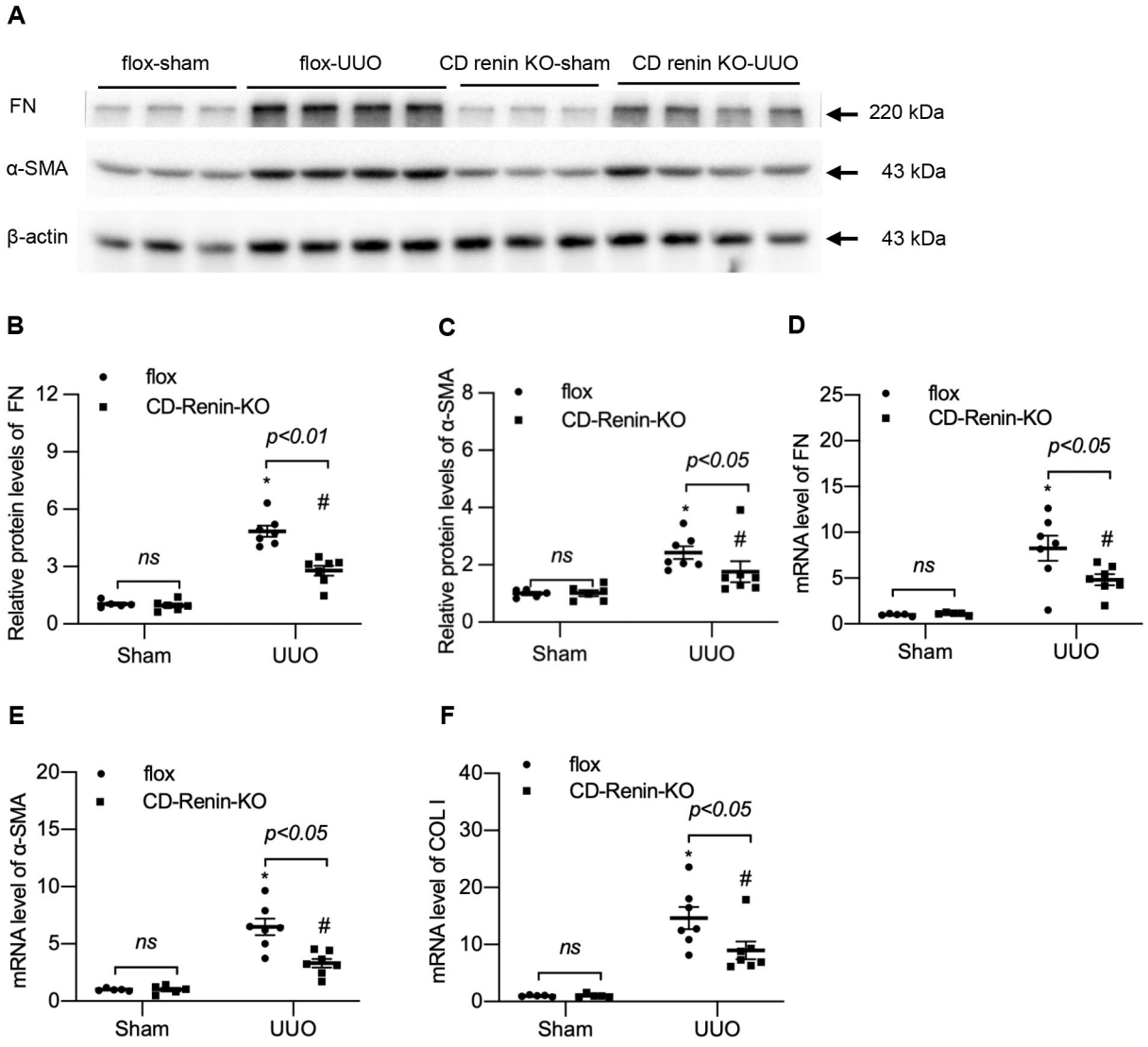

Figure 5.

Effect of CD renin KO on renal fibrosis and inflammation in obstructed kidneys of mice with UUO. (A-C) Immunoblotting analyses of FN and α-SMA. (D-F) mRNA level of FN, α-SMA, and COL I. *$p < 0.05$ vs Sham group; # $p < 0.05$ vs flox-UUO group. flox-Sham: n = 5; flox-UUO: n = 7; CD renin KO-Sham: n = 5; CD renin KO-UUO: n = 7. Values are means ± SEM.

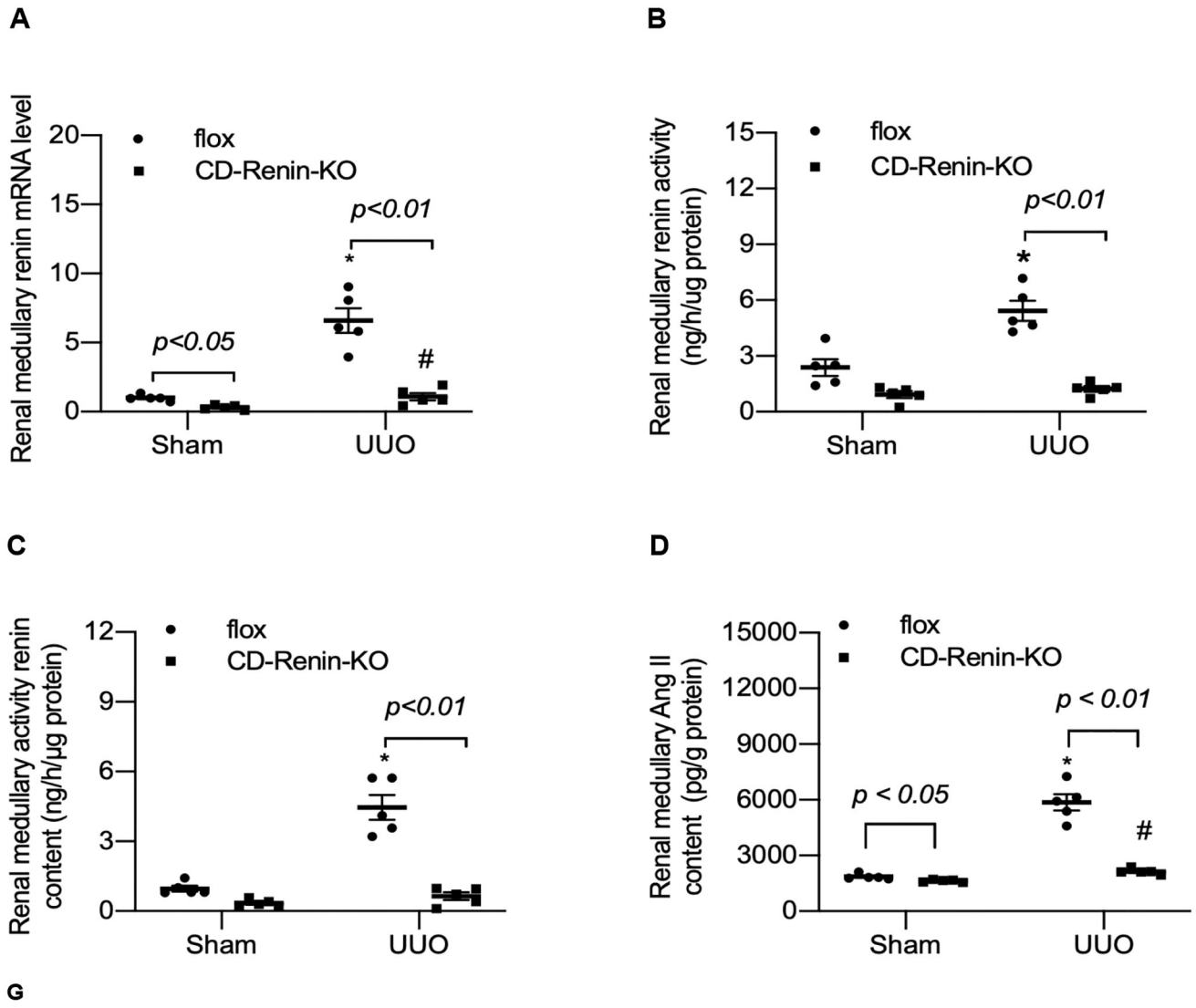

Figure 6.

Effect of CD renin KO on the activation of intrarenal RAS of the obstructed kidneys. (A) mRNA expression of renal medullary renin. (B) Renal medullary renin activity. (C) Renal medullary active renin content. (D) Renal medullary Ang II content. *$p < 0.05$ vs Sham group; # $p < 0.05$ vs flox-UUO group. flox-Sham: n = 5; flox-UUO: n = 5; CD renin KO-Sham: n=5; CD renin KO-UUO: n = 5. Values are means ± SEM.