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EDITORIAL

Beyond the RAAS: dissecting the antifibrotic effects of vitamin D analogues

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The worldwide prevalence of end-stage renal disease, the final stage of chronic kidney disease (CKD), where dialysis or preferably kidney transplantation is required, is increasing.[1] At the histological level, renal fibrosis is the final common outcome of progressive kidney disease, irrespective of the initial injury. Numerous studies have shown that the extent of renal fibrosis, particularly tubulointerstitial fibrosis, is the strongest determinant of the rate of renal function loss. The development of novel antifibrotic treatments is therefore warranted.

Angiotensin II (AngII), one of the main effector molecules of the renin–angiotensin–aldosterone system (RAAS), is considered a crucial mediator of progressive renal injury. This vasoactive peptide mediates blood pressure via vasoconstriction, stimulation of aldosterone release, and regulation of salt and water reabsorption. However, the detrimental role of AngII in the development of renal fibrosis extends beyond its hemodynamic effects. AngII regulates the expression and activity of proinflammatory and profibrotic pathways, resulting in deregulation of cellular growth and proliferation, extracellular matrix accumulation, epithelial to mesenchymal transformation and inflammation.[2,3]

Pharmacological inhibition of AngII by angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) is the most powerful renoprotective strategy currently available. However, even optimal RAAS blockade with ACE inhibitors and ARBs, although beneficial in terms of proteinuria and blood pressure reduction, does not translate into full renoprotection.[4] Moreover, our group previously demonstrated a clear dissociation between the effects of RAAS blockade on glomerulosclerosis and the effects on interstitial fibrosis.[5,6] Novel (adjunct) strategies to target interstitial fibrosis are required.

Transforming growth factor β (TGF-β) has been recognized as a key fibrogenic growth factor mediating progressive renal fibrosis.[7] Under physiological conditions, TGF-β is produced by a variety of renal cells and secreted in the form of an inactive precursor.[8] Tissue damage activates TGF-β, functioning in a paracrine/autocrine manner to activate downstream pathways. Regulation of TGF-β/Smad signaling is depicted in Figure 1. In short, binding of TGF-β to its receptors results in phosphorylation of Smad2 and Smad3, which form a complex with Smad4, followed by translocation into the nucleus and regulation of target gene expression. Dephosphorylation of p-Smad2/3 by phosphatase PPM1A followed by dissociation of the Smad complex and subsequent nuclear export of Smad2/3 leads to termination of TGF-β signaling.[9] Activation of Smad2/3 also results in upregulation of Smad7, which negatively regulates Smad2/3, thus providing a negative feedback loop. Progressive interstitial fibrosis results in a significant decrease in Smad7 protein[10] and overactivity of Smad2/3. Importantly, activation of Smad3 might lead to amplification of injury-induced TGF-β through a positive-feedback auto-inductive loop.[11] In addition, AngII may activate Smad3 also independently of TGF-β (Figure 1).[12] In the context of diabetic kidney injury, advanced glycation end products may also induce Smad3 independently of TGF-β.[13]

Specific inhibition of TGF-β signaling has been performed in animal models of CKD with encouraging results, particularly when using drug targeting to avoid systemic side effects and increased drug efficacy.[14] Although these

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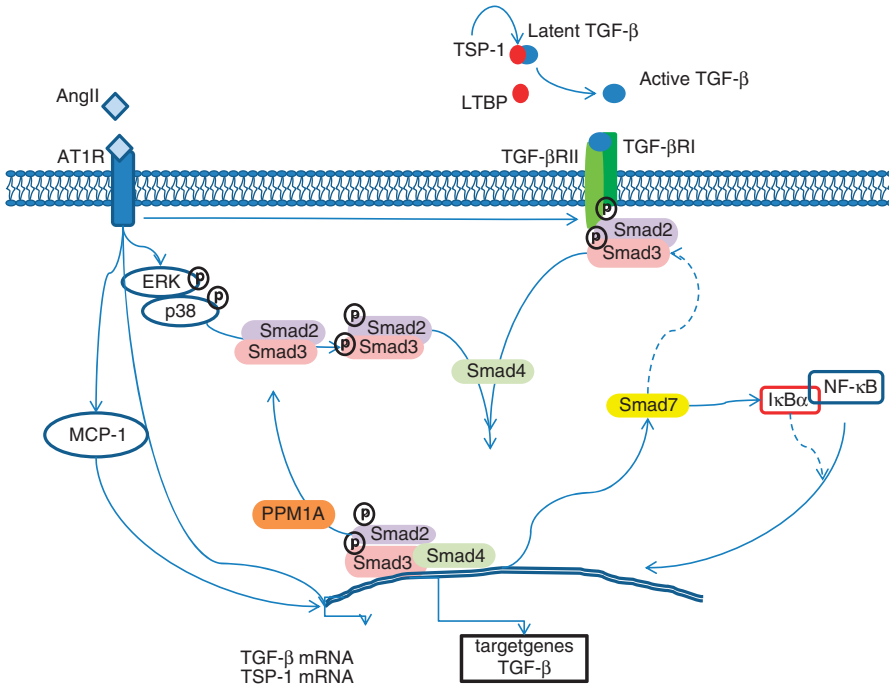

Figure 1 AngII and TGF-β/Smad pathway in renal fibrosis and inflammation. AngII, abundantly present in conditions of renal damage, stimulates transcription of the TGF-β gene, either directly or through chemokines such as MCP-1. AngII also upregulates the expression of TGF-βRII and thrombospondin-1 (TSP-1), which activates TGF-β via proteolytic cleavage of latency-associated peptide (LAP) and dissociation from latent TGF-β binding proteins (LTBP). Activation of TGF-β receptors leads to phosphorylation of Smad2/3, which form a complex with Smad4 to regulate the transcription of target genes including TGF-β itself, and including the negative regulator Smad7. Smad7, in turn, also upregulates the inhibitory unit of NF-κB (IkBα). Importantly, AngII can activate Smad2/3 directly via the ERK/p38 pathway. On the other side, PPM1A deactivates the Smads by dephosphorylation, leading to their translocation from the nucleus. As illustrated by the left side of the figure, AngII may regulate TGF-β/Smad3 signaling at multiple levels. Nevertheless, as is clear from the right side of the figure, TGF-β/Smad3 signaling does not require AngII but can also be auto-induced. Activation is designated by solid lines and inhibition by dashed lines.

preclinical results may be promising, specific TGF-β inhibitors are not yet available for clinical application. Still, Inoue *et al* report in this issue of *Lab Invest* that we might already have what is needed.[15]

Vitamin D has long been recognized as an important regulator of calcium/phosphate metabolism. Over the past two decades it has, however, become increasingly clear that the vitamin D system has important protective effects on the kidney and cardiovascular system beyond mineral metabolism.[16] Most vitamin D is activated in the kidney, and progression of CKD is strongly associated with vitamin D deficiency.[17] On the other hand, vitamin D deficiency might contribute to progression of renal function loss.[18] VDR knockout ($VDR^{-/-}$) mice develop more severe renal damage than their wild- type counterparts.[19,20] Moreover, vitamin D analogs have demonstrated impressive renoprotective effects in several animal models of kidney damage, either when given as monotherapy or in combination with RAAS blockade.[21] Results from clinical trials are also encouraging,[22,23] although the antifibrotic effects of vitamin D analogs cannot be easily addressed in humans.

Vitamin D exerts beneficial effects through attenuation of inflammatory responses, modulation of profibrotic processes and direct protection of cells such as podocytes.[24] However, the molecular mechanisms underlying these effects are far less understood; currently these are believed to include negative regulation of the RAAS, and inhibition of the NF-κB and wnt/β-catenin pathways.

The concept of vitamin D as a negative regulator of the RAAS came from studies on $VDR^{-/-}$ mice that demonstrated higher renin expression and angiotensin II production.[25] More detailed studies revealed that active vitamin D suppresses CRE-mediated transcriptional activity by blocking CREB binding, thereby disrupting the formation of a CRE–CREB–CBP/p300 complex, leading to reduction in prorenin gene expression.[26] $VDR^{-/-}$ mice develop more severe renal injury, most probably

due to an overactivated RAAS, induced by hyperreninemia.[19,20] Consequently, treatment with an AT1 receptor blocker attenuated renal damage to the same level in both VDR$^{-/-}$ and wild-type mice.[19] Studies with the vitamin D analog paricalcitol confirmed that the renoprotective effects of vitamin D are, at least in part, mediated by suppression of the RAAS.[27] Vitamin D analogs have also important anti-inflammatory properties through an interaction between the VDR and the p65 NF-κB subunit, repressing NF-κB-mediated gene transcription.[28] However, as AngII is also a potent inducer of NF-κB activity, the anti-inflammatory effects of paricalcitol probably also partially depend on its ability to downregulate the RAAS. Finally, in a model of proteinuria-driven nephropathy, paricalcitol prevented the development of proteinuria and subsequent renal interstitial damage through inhibition of the Wnt/β-catenin pathway.[29] Again, the renoprotective effects observed in this study cannot be easily dissociated.

Taking all this into consideration, we can pose several questions. Are there any more pieces of the puzzle of renoprotection by vitamin D? Are all renoprotective effects of vitamin D mediated by the RAAS or are other pathways independently involved? In this issue of *Lab Invest*, Inoue *et al* report on the antifibrotic effects of the vitamin D analog maxacalcitol in the rat model of unilateral ureteral obstruction.[15] In their study, treatment with maxacalcitol attenuated inflammation and tubulointerstitial fibrosis by inhibition of the TGF-β pathway. Interestingly, these protective effects were not accompanied by any effect on renin expression or angiotensin II production. The authors went further to explain the mechanism behind TGF-β downregulation and found that maxacalcitol blocked auto-induction of TGF-β by recruiting the PPM1A/VDR complex to Smad3.

What are the implications of these findings? First of all, to our knowledge, this study is the first to show a direct antifibrotic effect of a vitamin D analog through suppression of Smad3 signaling, a pathway crucially involved in the development of interstitial fibrosis.[11] The lack of effect on the RAAS suggests that maxacalcitol has different downstream effects than other vitamin D analogs, such as paricalcitol. This, in turn, raises the question if maxacalcitol in combination with a RAAS blocker would provide even more powerful renoprotection than either monotherapy, possibly even beyond the at least additive effects already observed for paricalcitol.[30] A head-to-head comparison of the renoprotective potential of different vitamin D analogs under RAAS blockade may be an important next step towards optimization of renoprotective therapy. Vitamin D analogs may become a clinically meaningful add-on therapy, continuing where RAAS-mediated renoprotection ends.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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